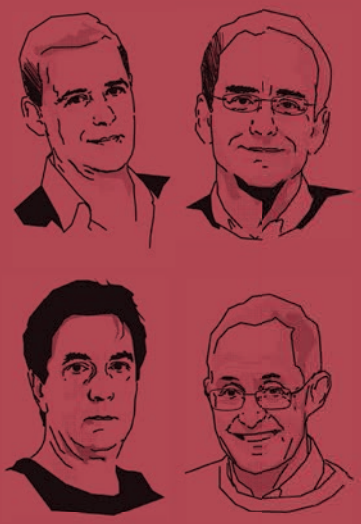
BOTÍN FOUNDATION.

28 STORIES OF SCIENCE AND BIOMEDICAL INNOVATION IN SPAIN_



BOTÍN FOUNDATION.

28 stories of science and biomedical innovation in Spain

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28 STORIES OF SCIENCE AND BIOMEDICAL INNOVATION IN SPAIN

Science is one of the key vectors of wealth creation and social progress in Spain.

The Botín Foundation has been aware from the outset that one of its fundamental roles is to support science. A specific concern of ours is technology transfer. We see before us the opportunity and challenge of transforming research excellence – in the biotech field particularly – into economic and social wealth.

Over the past ten years, the Botín Foundation has, I hope, grasped the opportunity and risen to the challenge by means of its technology transfer programme. We work alongside some of Spain's finest scientific minds to turn their discoveries into development and highly skilled jobs.

This book tells the stories of the researchers who have worked with us over this period. Viewed as a whole, their narratives help us understand the recent history of Spanish science. They condense hope and ambition, enthusiasm, grit, disappointment and success, as told by people who have decisively contributed to the international role that our biomedicine community plays today.

It falls to me now to thank the scientists, and their teams and host institutions, for their commitment to knowledge and to the development of our country. I am honoured by the generosity and trust implied in their long-standing partnership with us, which has entailed a complex process of rethinking the way they do science to turn it into wealth and wellbeing. And their example has helped Spanish society to discover and acknowledge that its scientists are one of its most valuable assets.

It is fitting that I should also mention Emilio Botín, who chaired this Foundation from 1993 to 2014. His personal engagement, founded on his admiration and respect for our scientists, has underpinned the success of our technology transfer programme. Over these years, the Foundation assessed more than 300 proposals, which ultimately led to 47 patents, 27 licence agreements and four start-ups, while directly creating jobs and providing training to 447 young researchers and lab technicians.

These past ten years are only the beginning. Our bid to support science is a long-distance race. The Botín Foundation will continue in this endeavour, exploring new ways of turning scientific talent into social progress.

Javier Botín
Chairman of the Botín Foundation

ANTECEDENTS

BIOGRAPHIES

Review of an Ambitious Idea

The Botín Foundation Science Programme

Pedro R. García Barreno
Science Programme Coordinator

Mr Emilo Botín,
in memoriam.

The prosperity of a nation, the welfare of its people and the prospects for their future lie within the strength of its educational system, the soundness of its democratic structures and the vitality of its scientific and technological initiative. In our times, the fabric created by science and technology affects every aspect of policy, from national security to people's welfare.

However, despite academia's desire to put its practical applications to use, real difficulties must be overcome when analysing their potential, intellectual property and conflicts of interest, involving both financing and rights. Similarly, insofar as the products put out by academic institutions become available under reasonable terms in differing ways, above all through the development of pathways for the creation of value, this must not interfere with the academic foundation that allows for the cultivation of innovative knowledge.

Within this context, we must create a road map that clarifies a strategy for achieving a series of objectives, in which cooperation among the various public and private role players in the world of science and technology predominates, in a country whose highest priorities do not include research and development. Universities, public research entities (OPIs), science and technology parks, financial bodies and investors form the foundation for innovation, but to achieve innovation it is essential that lawmakers facilitate an understanding that forges a path towards cooperation so that Spain may grow and become competitive. The lack of communication is a challenge, but at the same time an excellent opportunity to be dealt with in the context of Spain's technology transfer system. The executive and legislative branches of power must devise a coherent administrative framework, perhaps a single agency that does away with stifling, ineffective bureaucracy, excessive officialdom, lack of oversight and the need for standardization of procedures.

Efforts made in science and technology are profitable due to the social repercussions of the results they produce, since they are a driving force behind development and increase our country's prestige. Today we are overwhelmed with examinations, agreements, notices and reports about the current situation, but we lack many practical initiatives that offer solutions.

Various authors have advised against making predictions. Even so, for better or worse, one can draw analogies between physics and biology and the twentieth century, which has now finished playing out. Physics contributed two factors over the past century: power over nature and a wide range of developments through technology. Though it is still too soon to determine whether biology will be the most decisive factor in the twenty-first century, many indicators seem to be pointing in that direction. A large number of the great problems that humankind faces are biological or subject to biological intervention. In any case, research does save lives and resources while producing wealth.

It all began with a letter, the invitation to take part in a conference series titled "The Third Culture", organized by the Botín Foundation. It was signed by Ms Esperanza Botella Pombo, the Foundation's assistant director. So, what has happened over these last twenty years?

Well, the discussion titled "Between Hope and Fear: Recent Research in Biomedicine" was held in the events hall at the founding site in Pedrueca, late in the afternoon of 31 January 1995. Then a dinner was held with Doña Esperanza and Mr Enrique Martínez Berro, the Foundation's managing director (appointed in January 1989). The talk after dinner lasted until well past midnight, but was then followed by months of silence. Perhaps upon returning from the summer holiday that year, I could get away to Santander to resume the conversation with my two fellow diners. I returned to Madrid but received no further news until one year later, when I got a telephone call from Doña Esperanza proposing we have lunch in Madrid. We did so at the Lur Maytea restaurant on 12 September 1996. The same people attended the meal. After dessert, Don Enrique remarked that the time had come to make a few changes in the Foundation's general approach, for which purpose the Board of Trustees had decided to create two areas of action (humanities and science) in order to spread the Foundation's area of influence beyond just the region of Cantabria. After that introduction came their offer, and my acceptance of the position at the helm of the Science Area.

From then on, my visits to Santander became frequent. In the spring of the following year, the first, highly anticipated meeting with Don Emilio took place: suffice it to say that this is now a deeply missed relationship. And the minutes of the Board of Trustees meeting held on 20 June 1997 include the "appointment of Pedro García Barreno as a member of the Scientific Committee of the Botín Foundation". Along with José María de Prada, a public notary, and Francisco Jarauta, a professor of Philosophy, the Committee was thus formed. Since then there have been proposals and counterproposals. Recorded in the minutes of the Board of Trustees meeting held on 3 December 1998 is the debate over potential areas of interest in biomedicine. After rejecting some of them, information was finally requested on gene therapy – then an incipient field within our area – from Dr García Barreno, who expressed that he was in favour of supporting the creation of an integrated gene therapy centre "from laboratory to clinic". After members of the Board of Trustees set off for the United States, Enrique Martínez Berro informed García Barreno of the decision that had been reached by the Foundation's Board of Trustees, at its meeting held on 2 November, to name him coordinator of the *Gene Therapy* project. A few months earlier – on 11 March –, the Board of Trustees had named Mr Federico Ysart the director general of the Foundation, while Don Enrique was to continue holding the position of managing director.

Fate had it that one week later the first Nature Biotechnology-Gene Therapy Symposium was held in Washington DC. Furthermore, Francisco J. Ayala, then secretary of the Science and Technology Advisory Commission for President W. J. Clinton, was also in that city. The trip was a "must". Professor Ayala paved the way. After several meetings with Theodore Friedmann (the "father of gene therapy"), Helen Blau and David Curiel – all heavyweights within the field –, I returned with one name alone: José Luis Jorcano Noval. I did not know him personally. He led the Biology group at the CIEMAT (Research Centre

for Energy, Environment and Technology). On 26 November the Board of Trustees was sent the report drafted by García Barreno of the meeting. Shortly afterwards, on 3 December, an agreement was signed by the Botín Foundation and the CIEMAT, lasting nine years. The Centre was run by Professor Félix Ynduráin.

That proposal was somewhat surprising. It was always backed with the Foundation's support as well as that of Francisco J. Ayala, of the University of California (Irvine), and Frederick F. Becker, vice-president of Research, as well as José Trujillo, director of the Biochemistry and Molecular Biology Laboratory of the M. D. Anderson Cancer Center in Houston. They were the earliest advisers in terms of that decision. There was a steadfast commitment to the group – of which Juan Bueren was a member – led by Dr Jorcano and to the Biology Laboratory, which vastly improved the facilities.

After a rewarding initial experience, several factors made it necessary to change the programme strategy, which caused major personal and institutional disappointment. The happy smiles seen at the beginning turned into stern frowns. This marked the beginning of the end of what could be considered the "first stage" of the Botín Foundation's Science Programme. The initial momentum stayed alive due to the unconditional support of Mr Emilio Botín, who gave the project a second chance, and to the naming of a new director general, Mr Rafael Benjumea, in March 2002, when he took over the Foundation's direction and management. Just a few days after he joined the team, we held a long conversation. I described the idea we had started out with: "bench-to-bedside", or translational biomedicine. He agreed with it but pointed out that we should turn the strategy in a new direction. We visited the new director general of the CIEMAT, Mr Juan Antonio Rubio, but did not obtain the expected results. The idea of an integrated gene therapy centre went up in smoke and the agreement that had been signed was definitively brought to an end.

The "second stage" of the programme, as it had been conceived in general, was based on a model that had come into existence as far back as 1954, at the Howard Hughes Medical Institute (HHMI): "The primary purpose and objective of the HHMI shall be to promote human knowledge in the field of basic sciences (mainly in the fields of medical research and education) and their effective application to the benefit of mankind".

Adapting this model to our own environment was no minor task. Selecting "basic" researchers and, while respecting their way of working, at least orienting a part of their work towards the creation of added value would require, and continues to require, resolving a multifaceted bottleneck: though irregularity of financing and restrictions on use remained important, of equal or even greater importance was a mindset constructed upon the absolute priority of publishing results, along with a very "fuzzy" concept of whether those results could be patented, a mistrust towards the presence of outside role players and a lack of tools to perform effective technology transfer despite the large number of technology transfer offices (TTOs). The imperative need to locate technology transfer professionals was, and is, as important as achieving economic stability. However, at the end of implementation throughout the entire pipeline we ran up against yet another difficulty, which, in part, contradicted the attempt to somewhat relegate the problem of financing.

In order to attain validation, to carry out the concept testing on a preliminary product for the purposes of presenting it to the market with the required sales handbook and business plan, some capital was needed: just a small amount, but financing nonetheless, which was non-existent in our environment. The idea was brought up to create a fund to deal with this problem should some research result manage to reach this stage. This became the seed for the *Mind the Gap* programme.

The first long year of this new direction was oriented towards training a group of professional "transferologists". The requirements were that they had to possess a doctorate in one of the experimental sciences and have experience in the business world and knowledge of the international market. So, where were we to seek out such rare individuals?

Quite a few years earlier, a recent graduate in veterinary medicine had showed up at the laboratory I was running at the time. I was in no way familiar with him, and nobody had spoken to me about him, but I hired him immediately. In the end, he completed the work for his doctoral thesis under my direction, on inositol phosphates in the arterial wall. An exceptional individual and a great professional, a series of reasons led him to drop his research and plan a future in the business world. There, at what was then an amazing multinational pharmaceuticals firm, he triumphed. I watched over his progress, though he always took the initiative. Every time we ran into each other, he would say, "Don Pedro, we'll have to get together to do something". During the now blurry times of that earliest era, I asked him several questions about the translational pipeline. Without a doubt, he was and is the right person to ask. Don Rafael spoke with him, and they swiftly reached an understanding. It was not difficult to get him to join the project, first as a consultant (in 2003) and finally as a full member of the team in January 2007. At that time Francisco Moreno took over the direction of what was to become the Technology Transfer Unit and Pedro García Barreno the programme's general coordination. Paco had his hands free to put a team together. Each member was required to, and still must, meet a series of well-established conditions, in addition to possessing the personal ability to fit in with the research group, while never losing sight of the fact that the transfer group's relationship with investors and companies is equally essential.

The current and former members of the Unit, under the direction of Francisco Moreno, are Lala Aguadé, Myriam Asunción, Pablo Cironi, Ángel Durán, Simone Frieiro, Pepa Limeres, Usue Martínez Stuyk, María Paniagua, Daniella Piazzolla, Marisol Quintero, Michael Tadros, Ana Villafaina and Miriam Zeini. Some of them have left the Foundation to take jobs in research and development management at different institutions, which has always been a reason for satisfaction and has served as a stimulus. Improving the training of competent professionals who are able to go out into the market successfully is one of the project's "collateral" objectives, you might say; these are professionals capable of turning around the problem of losing ideas in translation during the knowledge transfer process.

So, what about our relationship with institutions? We introduce ourselves using language that is somewhat distanced from the norm. Speaking of neither subsidies nor sponsorship, we use the word "investment". Nor do we accept a percentage for expenses or general costs (overheads); our intention is to create a sort of temporary joint venture that we refer

to as a temporary joint venture for technology transfer, or "UT3". If any profits are made, the stake in them is discussed; the agreed percentage belonging to the Foundation, always a minority, is to be reinvested in the programme. The centres must act as scientific-technological niches with a sufficient critical mass and internationally accepted scientific oversight mechanisms.

The institutions, with which the Foundation has collaborated through its R&D management units, can be divided into two groups. One is made up of those institutions to which the programme's researchers belong or have belonged: Centre for Genomic Regulation (CRG), Biocat, Barcelona; "Severo Ochoa" Molecular Biology Centre (CBM), CSIC, Madrid; Cancer Research Centre, University of Salamanca; Research Centre for Energy, Environment and Technology (CIEMAT), State Secretariat for Research, Development and Innovation, Madrid; National Biotechnology Centre (CNB), CSIC, Madrid; National Cancer Research Centre (CNIO), the Carlos III Health Institute, Madrid; Institute for Bioengineering of Catalonia (IBEC), University of Barcelona; Catalan Institute of Nanoscience and Nanotechnology (ICN2); Bellvitge Biomedical Research Institute (IDIBELL), Barcelona; Institute for Research in Biomedicine (IRB), University of Barcelona; Biomedicine Institute of Seville (IBiS), University of Seville; Institute of Neurosciences (IN) of UMH/CSIC; Complutense University of Madrid; Polytechnic University of Madrid; University of Oviedo; University of Santiago de Compostela; University of Valencia; and Pompeu Fabra University, Barcelona. The other group includes those which, though they do not currently implement them, have expressed an interest in introducing the premises of the Foundation's programme into their R&D management procedures, or at least to discuss them: CIDAUT Foundation for Transport and Energy Research Development, Valladolid; the Research Units at the Hospital Clnic, the Vall d'Hebron Hospital and the San Pau Hospital, in Barcelona; the La Fe Hospital, in Valencia; La Paz, Puerta de Hierro, Ramón y Cajal and Gregorio Marañón hospitals, in Madrid; Xeral Hospital, in Galicia; Hospital General, in Oviedo; and the National Institute for Agricultural and Food Research and Technology (INIA). In both of these groups, a degree of understanding has been achieved first, followed by collaboration, though results and outcomes are difficult to predict.

And what about the researchers? Because they are the main role players in any "ambitious idea". The Foundation supports researchers and, in principle, not the institutions in which they perform their work (in the language of the HHMI, this translates into "people, not projects"). The programme does not finance infrastructures. The profile of a Howard Hughes researcher is that of a "turbo-charged version of the system used to promote scientists up the road at the NIH," someone with an "uncommon curiosity" involved in "transforming discoveries". In accordance with these premises and assuming the factors implicit in their work (high-risk, high-reward research), the Foundation contributes, or rather invests, a significant amount so that the financial support of the basic work will remain unaffected by the new commitment, with the researcher being the direct manager of this aid (flexible funding). Another programme feature is non-interference in the research work (few constraints), and, perhaps most importantly, the assistance provided by technology transfer professionals.

There is no publicly announced competitive process, and no CVs or projects are requested. The selection is carried out by an observatory that is responsible for gathering overall informa-

tion about potential candidates. Choosing researchers with the predetermined profile occurs in four phases. The first is based on information obtained from high-profile entities: EMBO, the European Research Council, the Zero Project at Harvard University, distinctions awarded by scientific societies or peer "recommendations", and so forth. Moreover, all the candidates must successfully pass a screening process and periodic scientific evaluation by the centres where they are located and be "tracked" for at least two years by the programme coordinator so as to verify their full integration upon "returning" to their new work. In the second and third phases, the candidates are analysed by peers: the head researchers of the groups integrated into the programme. The initial screening points out the ten highest rated, and second, the top five. Then, the programme coordinator gathers information about these five "finalists" for an external committee that establishes an order of preference. Once the candidates are selected, they apply for final approval to the Foundation's director and Board of Trustees. Once their conformity has been obtained, the coordinator carries out a series of talks with the objective of explaining the programme's characteristics to the candidates. In certain cases, the selected candidate has rejected the programme's "conditions". In all, ninety-six researchers have been evaluated in this manner. Then the corresponding institution is contacted, and once an agreement has been reached, the administrative arrangements culminating in the signing of an agreement between the Foundation, the researcher and the researcher's centre are executed.

The contracts have a duration of two years, though they may be extended up to five years and are renewable from one year to the next. The end of the second year is a true turning point at which the researcher's commitment must be justified if he or she has failed to achieve the predetermined objectives, or a new phase of collaboration is begun for another three years. Regardless of the annual tracking, an external economic audit is performed when the programme ends, at the same time as a technical audit of the project. To complete all this, a structured tracking and oversight system based on quantifiable intermediate and final objectives is established with the researcher. Despite the necessary planning that such a focus requires, the basic aspects of the programme spirit are always honoured: freedom of action for the researcher, a close relationship and a lack of bureaucracy. To achieve this, each manager works side-by-side with the researcher and his or her institution in order to adapt the Foundation's intervention to each specific case.

As for researchers and their institutions, the management of technology transfer is the responsibility of the Foundation's experts, in close collaboration with the proper TTO, of course. Within this context, the relationship between researcher and transferologist is crucial. The latter's role is to detect an innovative idea and make sure it is placed into the evaluation chain, in a daily relationship between these two professionals whose objectives include relying on mutual trust as a primary tool in their work.

Researchers must also be persuaded to adapt their experimental procedures to the requirements that all products with industrial aspirations must meet (Good Laboratory Practices) and achieve a balance between scientific priorities and the idea's socio-economic value. They must be vaccinated against what is known as "patentability syndrome": one must only patent something that at least presumably has a reasonable chance of "success". Plus, there are other ways to defend intellectual property.

Those currently and formerly responsible for the scientific research groups are: Jesús Ávila, Eduard Batlle, Carlos Belmonte, María Antonia Blasco, Juan Bueren, Ángel Carracedo, María Domínguez, Mariano Esteban, Manel Esteller, Isabel Fariñas, Óscar Férnández-Capetillo, Gustavo Guinea, Joan J. Guinovart, José L. Jorcano, Laura Lechuga, José López Barneo, Carlos López Otín, Raúl Méndez, María Teresa Miras, Modesto Orozco, Juan Ortín, Francesc Posas, Josep Samitier, Eugenio Santos, Luis Serrano, Manuel Serrano, Ricard Solé and Juan Valcárcel. In early 2015, Salvador Aznar-Benitah of the IRB joined the team. The first inclusion of a new researcher into the programme, during this second stage, took place in late 2004.

Some of them, after completing the five-year period established in the agreement included in the signed by-laws, have shifted into a special status, becoming "Botín Researchers", whereby they maintain a long-term relationship with the Foundation under the same terms established in their initial contracts. This is the case of José López Barneo, Carlos López Otín, Francesc Posas, Manuel Serrano and Juan Valcárcel. In any case, the support of the Foundation's Technology Transfer Group is maintained *sine die* for any of the groups that request it. In January 2015, María A. Blasco and Ricard Solé became new beneficiaries of this programme.

The fact that several researchers have been promoted within their institutions to hold positions of responsibility in R&D management – having done so in accordance with the spirit of the Foundation programme – is a matter that is worth analysing. Could this be a direct result of the programme? Last of all, we must draw attention to a topic that proves quite important: it is not possible, in principle, to identify the researchers' behaviour with respect to the programme's objectives. The "performance" of each of the researchers who have joined this programme is ascertained gradually as they travel down this path together. Investment and weighted risk go together hand-in-hand.

During this second stage, a tracking group made up of Cristina Garmendia, Antonio Garrigues, Ricardo Martí-Fluxá and Regina Revilla remained operative. The first of them left the group when she was named Minister of Science and Innovation in the Spanish Government. Some of the researchers would also attend the meetings of the Foundation's Board of Trustees quite frequently, which made it possible for the trustees to gain first-hand knowledge of their personality, work and opinion. Furthermore, the decision was reached to hold a yearly meeting, on the first Monday of each February, for all the programme's researchers.

Two special activities were also carried out during this time period. The first was the result of a fire at the laboratories inside the School of Sciences at the Austral University of Chile. A cooperation agreement was reached with this Chilean university, thanks to which seven doctoral candidates – Romina P. Bertinat, Rodrigo Gática, Víctor Olavarría, Fabián Pardo, Alejandro Pérez, Carlos Spichiger and Zahaday Velásques –, whose work was interrupted, joined Spanish groups in order to complete that work for periods lasting from six to twenty-four months, from June 2008 through August 2010. Jesús Ávila (CBM, CSIC), Carme Caelles (Univ. Barcelona), Ramón Gomis (Univ. Barcelona), Joan J. Guinovart (Univ. Barcelona), María Pilar Lostao (Univ. Navarre) and Victoriano Mulero (Univ. Murcia) were in charge of welcoming them, supervising their work and co-directing their theses.

The corresponding collaboration agreements were signed with the Spanish institutions (in certain cases, such as those of J. Ávila and J. Guinovart, this was unnecessary because they already formed part of the Foundation programme). These agreements included the arrival of the corresponding Chilean directors – Jaime Figueroa, Alejandro M. Reyes, Juan C. Slebe and Alejandro J. Yáñez – to establish scientific relationships in person.

The second activity was aimed at high-quality researchers involved in research on diseases. One researcher was signed on to the agreement with the Chilean university – Professor Gomis, of the Clinic foundation of Barcelona; another belonging to the University of Extremadura – Dr Ana María Mata; and another from the University of Granada – Dr Rafael Saltó; in all three cases, the agreement lasted for a term of five years.

Mr Rafael Benjumea handed his position over to Mr Íñigo Sáenz de Miera in October 2009. What was remarked on further above about the possible creation of a flexible financing fund aimed at taking action with other possible partners necessary to consolidate the initial process for evaluating a result that would make it possible to enter into the business world with a consistent business plan was the commitment made by this new director. The idea took shape in the *Mind the Gap* programme, which created an identifying trademark for the "third stage of the programme", which is what we are working on at present, supporting research and remaining committed to the effective transfer of results.

First of all, *Mind the Gap* specifically deals with the gap in defining technological production. It is the riskiest stage of development, but, without it, there is no way to power the engine of technological innovation. In order to fill this gap, the Botín Foundation contributes financing wherever all others fail to do so, adding value to the capacity for management by the scientist and research institution in their relationships with third parties, above all in the world of business and capital. Of course there are other gaps to be filled as we continue to move forward in product development, but, unlike this first gap, the Botín Foundation would cease to contribute the value of its management in filling later gaps, exiting its active role as a truly dynamic catalyst for socio-economic development.

The financing of the *Mind the Gap* projects was designed to meet the needs of those activities required to complete a product's definition. This encompasses a wide range of activities, which include technical verification, market studies, analysing its industrial property status and the strategy for strengthening that status. Likewise, the potential business opportunities and models must be explored, which may include, depending on each case, a certain degree of validation of the business model in the market. The goal is to be able to prepare a sales portfolio that is appealing to potential investors or industrial partners who will be taking over the project. To achieve this, a maximum time period of two years has been established, with a predetermined economic limit.

Mind the Gap is proposed as a platform for inter-institutional cooperation. In other words, the programme brings together a group of entities that share their finest technologies with a view to creating a portfolio of projects able to attract investors' attention. In order to ensure the quality of the projects and optimize their likelihood of success, these institutions agree to ad-

here to a strict selection process performed by a committee of independent experts. Last of all, the programme defines a business management model that establishes efficiency and profitability criteria for the projects. However, calling this "entrepreneurial management" does not mean creating a specific company for each project. Nonetheless, our country currently lacks technology transfer structures able to contribute the management skills required by these projects. Because of this, the Foundation will in many cases find itself forced to create new companies, at least during the earliest stage of the programme. This situation is expected to change as the programme grows stronger and the viability of the model is demonstrated, creating the need for new management structures. In any case, it is important to remember that the creation of companies in *Mind the Gap* is a means and not an end in and of itself.

Mind the Gap is, therefore, emerging as a programme with the ability to create a point of reference within the "third sector". If the proposed model is confirmed, it would allow us to demonstrate that momentum can be created and a social impact made in the long term through investment in science and technology transfer. Through *Mind the Gap*, the Botín Foundation continues to play and consolidate its role coherent with the objective of supporting technology transfer, through its capabilities in science and innovation management and, at the highest level, its founding mission of contributing to our country's socio-economic development.

The programme-tracking group convened to take on the form of an Advisory Committee, and in January 2014 Banco Santander joined the programme through its Global Santander Universities Division. *Mind the Gap* and the programme's global management are responsibilities belonging to the Foundation, whereas the Bank assumes financing of the research groups. A couple of months ago, Mr Javier Botín O'Shea took over the presidency of the Botín Foundation Board of Trustees, where he has experienced the ups and downs in its history as a trustee since it was first created.

The increasingly important repercussions of science and technology on a wide range of fields in human activity – new ways to access natural resources, climate change, the use of renewable energies, urban planning, food production, medical care and so on –, coupled with the difficulty and complication of the associated social and ethical factors, will mark the path towards the future we are able to create, and this requires greater scientific preparation from us all. Politicians must understand the basics of scientific evidence, and society as a whole must remain informed enough to be able to participate in the debate over the complex effects of advancement in scientific research and the constant technological development and innovation taking place. This sensitivity towards knowledge must begin in schools, where arousing curiosity and providing scientific education should hold an important place in our culture.

Innovative technologies have made Western societies the most advanced in history. They have made a more competitive economy possible, creating millions of jobs while providing a foundation for our global standard of welfare. They have led to improvements in our health and lengthened our expected lifespan. However, these achievements did not just appear

from one day to the next. They are the result of a commitment maintained for decades, with the policies of the most developed nations having the constant goal of promoting scientific discovery and the development of new technologies. This has been possible thanks to a two-sided strategy, the public administration's support for academic research programmes as a vital investment for the future of their countries, and the role of industry in driving new technologies towards the marketplace. This commitment made between resources for education and scientific research at universities and centres for research, the financing provided by governments and technology transfer by industry have been the main factors in upholding technological leadership based on constant innovation.

However, innovation is not something simple; it is not a linear system in which "basic" research ends up turning into technology, and that technology into innovation, even though this is the model used worldwide to introduce technology transfer. Innovation is a complex ecosystem subject to a series of interdependent factors and feedback systems that are not yet well understood and, above all, are not easy to quantify. With time, the innovation ecosystem, placing value on ideas, technology transfer and "bench-to-bedside" will be better understood. In the meantime, researchers, institutions, financiers, benefactors, companies, economists and analysts must continue to learn how to cooperate for long enough without wasting their efforts on short-term, uncoordinated actions that are costly, amount to nothing but spending and are useless because they fail to draw any conclusions, or even a basic outline of the real situation. Scientific research does not produce "wealth" in the short and medium term. Its current benefits are the result of scientific achievement from no less than two decades ago. The next generation of drugs or instruments, or the understanding of diseases currently out of control, depend upon the knowledge of today and tomorrow. The "Reserve Fund" required for the purpose of financial stability should be coupled with another "Reserve Fund" essential for consolidating the other, for education, training, mathematics, science and technology.

May the following reflections serve as a closing statement for this chapter and an introduction to the next (titled "Assessment of the Botín Foundation's Science Programme"). They are words taken from the introduction to another idea – *The Wisconsin Idea* – written in 1912 by President Theodore Roosevelt:

> *As Professor Simon Nelson Patten [1852–1922] says, "Without means of attainment and measures of result an ideal becomes meaningless. The real idealist is a pragmatist and an economist. He demands measurable results and reaches them by means made available by economic efficiency. Only in this way is social progress possible" [...] The Wisconsin reformers have accomplished the extraordinary results for which the whole nation owes them so much, primarily because they have not confined themselves to dreaming dreams and then to talking about them. They have had power to see the vision, of course; if they did not have in them the possibility of seeing visions, they could accomplish nothing; but they have tried to make their ideals realizable, and then they have tried, with an extraordinary measure of success, actually to realize them. As soon as they decided that a certain object was desirable they at once set to work practically to study how to develop the constructive machinery through which it could be achieved This is not an easy attitude to maintain. Yet every true reformer must maintain it.*

My gratitude to Mr Emilio Botín, who listened, trusted and allowed us to work

Programme Assessment of the Botín Foundation Science Programme (2005-14)

Taking up the thread of the preceding article, we would do well to remember the words of Linus C. Pauling (1901–84), who won the Nobel Prize for Chemistry in 1954 and the Nobel Peace Prize in 1962: "The best way to have a good idea is to have a lot of ideas." Or of Johann von Goethe (1749–1832): "Knowing is not enough; we must apply. Willing is not enough; we must do." And, if we do, then, in the words of William Thomson, Lord Kelvin (1824–1907): "When you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind."

1. Conception and aims: why have an assessment model at all?

Since its conception in 2005, the Science Area of the Botín Foundation has been aware of the need to use indicators to measure progress towards targets and compare management effectiveness in relation to peer institutions in the field of technology transfer. In 2012 we undertook an in-depth review of the impact of our science programmes, driven by two distinct needs. Firstly, we wanted to find out whether invested funds were having a real impact on Spain's wealth and quality of life. Secondly, we wanted to obtain data that would enable us to be accountable to society at large and stakeholders in particular. We designed an assessment model and applied it to the 2005–11 period. Next, in order to introduce final management measures and support future assessment and follow-up, in 2013 we adapted our procedures and updated our assessment framework with a proprietary IT application. We are continuing to move forward to specify the best way of communicating our results, which are regularly reviewed and updated in light of the different stakeholders that interact with the Science Area.

2. The Science Area's science and technology ecosystem

The Science Area works within a clear framework based on supporting principal investigators and their teams. These investigators continue to conduct their research within their affiliated institutions, and receive consultancy and guidance from the Foundation to ensure that their work methodologies and research facilitate the transfer of research results to the community.

The main focus is to provide guidance and support to principal investigators, who represent the cornerstone of the science and technology ecosystem. Support functions as a catalyst of the transfer process, helps identify potentially commercial ideas at an early

stage and provides necessary resources to assess and channel them to market through development and market interest assessment. As such, a particularly important role of the Foundation is to screen research initiatives for ideas and identify those that may potentially become assets. This aids the effective allocation of resources and enhances efficiency within the ecosystem.

3. Methodological features

Having regard to our scope of concern, we have designed the Science Area's contribution model on the basis of a reflective process that aims to answer three key questions. (fig.1)

The three elements underlying this reflective process are: rigour – it is based on a comprehensive assessment of the Foundation's Science programmes since 2005; involvement – it is put together by the Science Area's director in partnership with technology transfer specialists who are aware of researchers' activities; and iteration – the method is predicated on continuing reflection, review and adaptation of the metrics underpinning the model.

4. Fields of contribution

As a result, our defined and evaluated model has enabled our achievements to be visualized along five major axes within the ecosystem in which we operate: research culture, knowledge, economic activity, community and people.

The Science Area has helped bring about a shift in mindset towards developing economically valuable science during the corresponding analysis period, in addition to encouraging the professionalization of technology transfer processes that underpins the creation of economic value. The Foundation has also supported innovations that provided benefit to the community through value creation in the form of market recognition and collaboration in the development of products and services. We have also fostered public-private partnerships and networked efforts to optimize the resources that public administrations and the wider community allocate to research, development and innovation, in addition to the creation of products and services in the biomedical field to improve standards of health across society at large. (fig.2)

5A. Assessment conclusions: overview

This sets out the long-term results that revert to the science and technology ecosystem in general and to the Foundation in particular. These results represent society's recognition and value towards the Foundation's activities.

5B. Assessment conclusions: direct impact

The Science Area's contribution to the aforementioned axes (research culture, knowledge, economic activity, community and people) translates into direct and

Figure 1

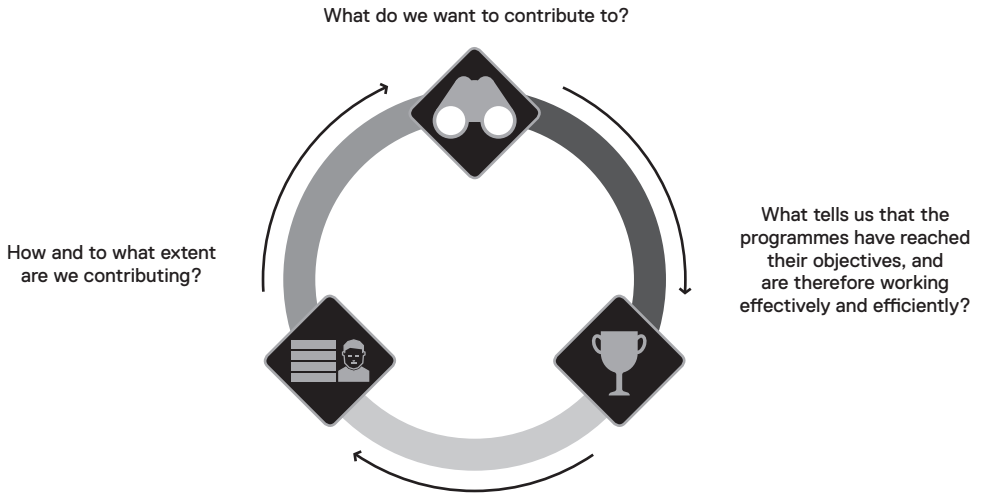
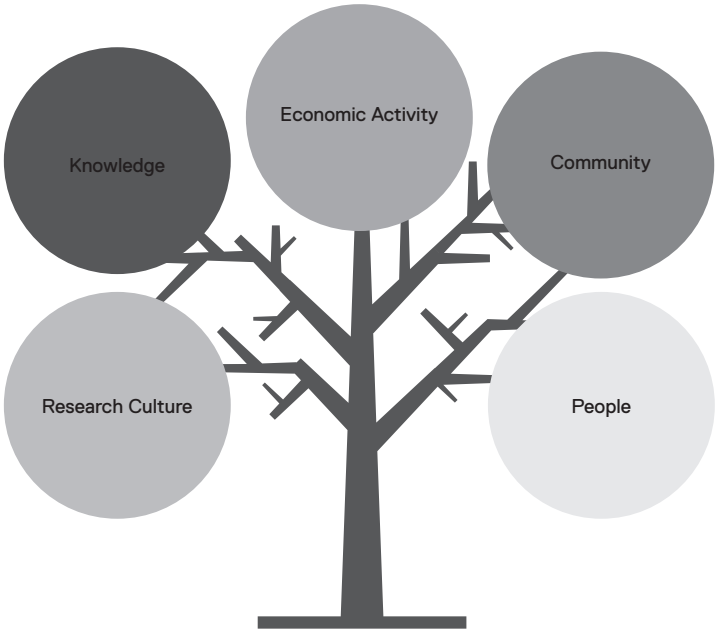
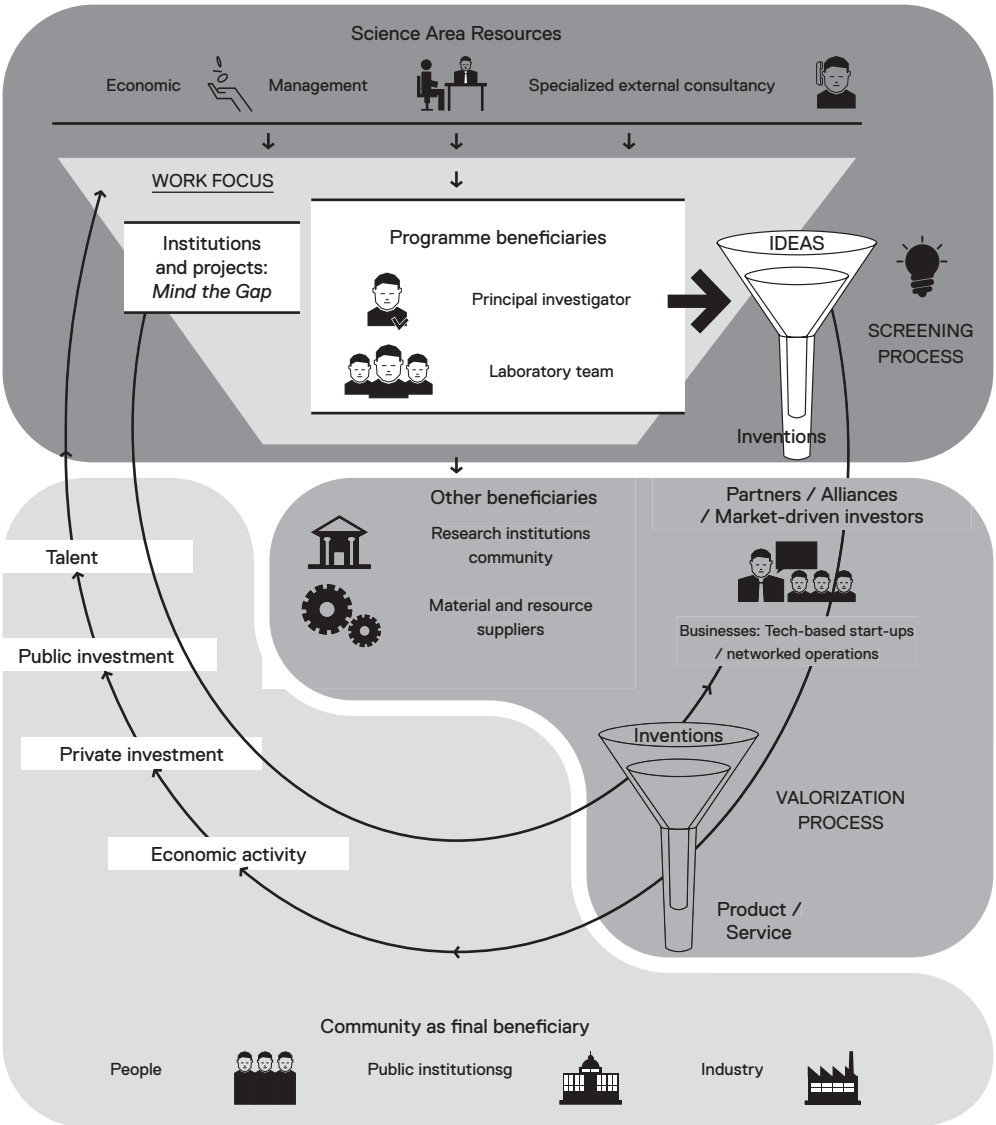


Figure 2



Science Area tree of contribution



Direct impacts
Results obtained by direct action by the Science Area. Performance is translated into short-term metrics in target fields: research scientists and their activities.

Indirect impacts
Indirect results arising in the medium and long term as a consequence of action by other entities and support at each link of the technology transfer chain.

Induced impacts
This sets out the long-term results that revert to the science and technology ecosystem in general and to the Foundation in particular. These results represent society's recognition and value towards the Foundation's activities.

measurable short-term impacts in target fields: research scientists and their activities. Our assessment conclusions encompass the direct impacts arising in different contribution areas:

Support for research

• Since 2005 the Foundation has worked with twenty-eight national researchers in the forefront of their respective fields in terms of national and international recognition. For instance, 37% of the principal investigators we have supported have been awarded grants by the European Research Council (ERC).

• During this period the Foundation has allocated €27.8 million in direct support for research scientists and an additional €20 million to managing the Science Area, the *Technology Transfer* and the *Mind the Gap* programmes. This amounts to an average annual support of €4.7 million.

• In 2014 the contribution represented 23% of the total funds received by research teams within the programmes.

• This support has enabled research scientists to undertake riskier projects and enjoy wider flexibility in the scope of their fields of interest and specialization.

Highly qualified jobs

• The Foundation has contributed to the direct employment of 447 people during the 2005–14 period, representing an average of eighty-two direct jobs per year.

• In addition, the Science Area itself has maintained six jobs per year.

Scientific talent

• The Science Area helps retain, develop and attract talent, and as a consequence maintains and reinforces research teams.

• Since the start of the programme the Foundation has enabled 447 young research scientists to pursue their research careers within twenty-eight leading laboratories:

- retaining scientists who would otherwise have had to leave Spain;
- recruiting promising young researchers at the start of their careers;
- attracting outstanding foreign researchers to Spain-based teams;
- enabling scientists at the early stages of their careers to undertake research assignments at world-class institutions.

Commitment to the transfer process

• Each research team has received an average of thirty-seven hours per year of face-to-face support. The close support of the Foundation's technology transfer specialists and the mutual trust that is developed among them nurtures communication of ideas.

• During the 2005–14 period the Foundation has provided specialized external consultancy worth €150,000 on average per year, in addition to direct funding.

• These efforts have garnered a number of accolades, such as the recent Premio Nacional de Mecenazgo Científico de la Fundació Catalana per a la Recerca i la Innovació, and the Premio Corresponsables: in 2013 the Botín Foundation was selected from among over four hundred candidatures from eighteen different countries.

Community awareness and shift in mindset

• A key element of the research process is the diffusion of scientific knowledge. Scientific publications add value to the research progress achieved and allow knowledge to be shared with the scientific community.

• The Science Area programmes are intended to nurture a cultural shift in research scientists, whereby research leading to publications should be coupled with a prior reflection on the usefulness to the wider community through discoveries. In this process of reflection the role of the Science Area is essential.

• The data shows that, in 2013, 98% of ideas that were identified with commercial potential were communicated prior to publication. This fact reflects the real influence of the Science Area's programmes on research priorities: they are now concerned not only with creating scientific knowledge per se, but also with its usefulness to the community.

Useful and innovative knowledge

• The Science Area identifies and works with ideas at very early stages of maturation. This implies distinguishing useful and innovative ideas with potential economic value from basic scientific knowledge. From 2005 to 2014 the Foundation has evaluated over three hundred ideas with application possibilities, of which 28% led to inventions. In 2014 the Foundation devoted 14% of its time to evaluating ideas and inventions.

• This means speeding up the technology transfer process by acting as a catalyst and selecting those ideas with the strongest market potential, thus optimizing time and resources by channelling them towards ideas with commercial potential.

5C. Assessment conclusions: indirect impact

In addition to the Foundation's direct impacts through its Science programmes, a range of mid- and long-term indirect outcomes have been identified as a consequence of our support. Assessment of the Science programmes reveals the following indirect impacts:

Value creation from knowledge

The process of value creation starts with the identification, screening and evaluation that may lead to applications giving rise to inventions. For each idea the Foundation determines the best intellectual property protection strategy, which might lead to patents, competitive agreements with businesses (under licence, transfer, partnership and so on) and other forms of value creation.

• During the 2005–14 period the Foundation supported the creation of eighty-eight inventions and the application of forty-seven patents, leading to an average of three inventions every four months and one patent every annual quarter.

• The Foundation's science team annually devotes 18% of its time to evaluating ideas and providing advice on intellectual property protection.

• In addition, the Foundation has supported the creation of four tech-based start-ups that have developed five products or services that continue to form part of their current portfolios.

Mobilizing private initiative: "drag-along" effect

• Eighty per cent of the investment needed to set the tech-based start-ups in motion came from private sources.

• This means that each euro of direct funding provided by the Foundation "dragged along" or mobilized four additional euros from private sources.

Economic catalyst

• The costs of the Science Area itself and of mobilizing private initiative come to an annual average of €1.9 million, which generate €3 million of GDP, hence each euro generates €1.5 of GDP.

• In addition, as a result of the economic activity generated, beneficiaries (researchers, departments and institutions) can make returns from technology transfer licences.

Job maintenance and creation

• As a whole, the Science Area's activity has contributed to the maintenance or creation of ninety-two indirect jobs: fifty-nine jobs throughout Spain as a result of the Area's own spending, and twenty-one jobs associated with the creation of tech-based start-ups.

Efficiency and transformation in institutions

• The Foundation has worked in partnership with fifteen institutions, which account for 16% of Spain's TTOs (technology transfer offices) during the 2005–14 period.

• Most of these institutions share the Foundation's technology transfer methodologies. Two of them, namely the National Cancer Research Centre (CNIO) and the Institute for Research in Biomedicine (IRB), have created specific innovation departments that work on a similar basis to that of the Botín Foundation. The cultural change driven by the Science Area includes a total of over 760 research scientists through its collaborating research teams, who collaborate through the method and approach introduced by the Foundation.

• The Foundation's support helped collaborating investigators acquire and maintain research infrastructure and equipment. From 2005 to 2013, over €2.3 million was allocated for such purposes, representing 9% of the total grant budget directly supporting collaborating researchers.

• In addition, in that same period institutions received €1.7 million as part of the funding from the Foundation (6.8% of the total budget).

• In total, institutions have received funds worth €4 million, making for an average income of €266,000 per institution.

Talent transfer

• Seven principal investigators are now in senior management positions and have incorporated innovation to their institutional strategic goals by creating at least two innovation or technology transfer structures.

• Two technology transfer specialists of the Foundation's Science Area have joined collaborating institutions as heads of their respective innovation units, therefore amplifying the transforming effect of the Area.

• Moreover, the start-ups supported by the Foundation stem from four research scientists who have collaborated in the *Technology Transfer Programme*. In practical terms this means bringing science closer to business and making organizations aware of the importance of research, development and innovation.

Creating and spreading knowledge

• The volume of scientific publications from collaborating investigators exceeded 1335 papers during the 2005–13 period (an average of fifty-five published papers per research scientist), and the number of citations amounted to 22,337 (an average of 930 citations per researcher and eighteen citations per paper).

• Of the fifteen research scientists who by 31 December 2013 had completed their five-year collaboration with the Foundation, 80% were above the world average of citations in their research area. In addition, six out of ten researchers had improved their average number of citations with respect to the five years prior to their collaboration with the Foundation.

5D. Assessment conclusions: induced impact

Finally, "induced impact" embraces the long-term results that revert to the science and technology ecosystem in general and to the Foundation in particular. These results represent recognition of, and value creation from, the Foundation's activities by the wider community and its stakeholders. Our analysis detected the following induced impacts as a result of the Science Area's activities:

Health and people: improved treatments and diagnostic approaches

• Over twelve areas of research are funded by the Science Area programmes, such as cancer (to which research teams devote 35% of their time), ageing (9%) and neurodegenerative diseases such as Alzheimer's and Parkinson's (6%).

• The potential beneficiaries of this progress exceed 2.3 million people in Spain alone. This signifies an average research investment of €12 per potential beneficiary (with regards to 2005–13 investments).

Optimizing resources: public administration

• The annual average investment through the Science Area programmes is equivalent to 0.15% of Spain's Ministry of Economy and Competitiveness 2013 expenditure in science and technology research programmes, and equivalent to 0.5% of the budget expenditure in biotechnology research. This shows how the Foundation plays a key role in supporting research and technology transfer as a supplement to the role of government, and even more so in current times of economic downturn, when government is inclined to cut back its expenditure in research.

• The economic activity induced by the Science Area's investment resulted in government revenue of almost €300,000 through relevant income taxes. This means that each euro invested by the Foundation yielded €0.18 for government.

Induced economic activity

• The Science Area has enhanced public-private partnerships and networked efforts among the various participants within the science and technology ecosystem. As a result, these participants are structuring consortia including members from research, businesses and government realms with the aim of attracting European funding. When these projects achieve success, they typically attract foreign investment for further development, and contribute on an induced basis to bolstering research and technology transfer and economic growth.

• The potential from the ideas and inventions supported by the Foundation can be demonstrated, for example, through the public funds secured to support tech-based start-ups, which amounted to €330,000 during the 2011–13 period. This means that each euro contributed by the Foundation to tech-based start-ups "dragged along" 23% of additional financing from public funds.

Returns on talent

• Collaborating research scientists and their laboratory members maintain ties with the Foundation, which may garner returns in the form of further cooperation (communication of ideas, candidatures, collaborations and sponsoring entities and so on), which will allow for using and incorporating the knowledge acquired throughout their professional careers.

6. Facing the future: from transfer to exchange

Our achievements are the outcome of a sustained effort to pursue a clear model that focuses on the main assets of the research process, and a strong focus on targeting those results that bring real benefits to the wider community. Our success reinforces a twofold belief:

1. Science has a real positive impact on the society that it serves, and that impact can and ought to be regularly measured and assessed. Our assessment has been internalized and incorporated as one of the Area's management tools.

2. Support for science involves a long-term commitment. The Science Area implements its support as a catalyst. This enables us to provide higher value to the community by driving and accelerating the technology transfer processes (mainly by accelerating the maturity of ideas). This requires dedication, continuity and know-how. Our goal is to improve the efficiency of the system as a whole without interrupting the development of ideas.

From transfer to exchange. Over the years since its onset, the Foundation's Science Area has found itself undergoing a change in paradigm to allow for an open innovation perspective in the future and to foster collaborative spaces that accelerate the time-to-market

of products and services intended to raise standards of health and life throughout the wider community. We intend to work on the following areas to reinforce the impact that our support for science has demonstrated so far:

1. Maintain a commitment to create value from science to ensure that knowledge and technology leads to new products and services that support social and economic development and progress. The Botín Foundation relies on the skills and expertise of its team to identify and assess promising ideas – this is the key distinctive feature of the Science Area.

2. Encourage and reinforce the role of businesses as major stakeholders who give tangible form and market recognition to research. To achieve this, collaboration agreements will establish the existing network of knowledge exchange and talent, and help maximize impact by appropriately directing resources within the technology transfer chain.

The Botín Foundation's operating model aspires to transcend the traditional linear process of invention-patent-licence-commercialization by developing an iterative and multidirectional scheme where each stakeholder contributes the best of their knowledge, experience and resources for mutual benefits. Research saves lives, reduces costs and creates wealth.

Francisco Moreno
Director of the Science Area

Pedro R. García Barreno
Science Programme Coordinator

JOSÉ LUIS JORCANO NOVAL

THE LONG AND WINDING ROAD – THE BEATLES

1

As far back as my memory reaches, I was always drawn to science. Although I am quite forgetful, I have two very vivid memories from my youth. On the one hand, the figure of Albert Einstein as the archetypal scientist, pure and capable of changing your world view through reasoning, almost like a magician taking a dove out of his hat. At the age of fifteen, I attempted to understand the theory of relativity, without managing to do so, of course – I do not know how far I have progressed in this undertaking up to the present time either. However, the figure of Einstein, the model scientist that he represented, greatly impressed me. Similarly, a book I was given by a very beloved aunt of mine also had a huge impact on me. It was titled *Gods, Tombs and the Wise*, and it dealt with the archaeological discoveries made in Egypt, Mesopotamia and Central America. The discovery of Tutankhamun's "lost" tomb, thanks to the tireless efforts of Howard Carter, and the wonders found there, which I had the opportunity to see years later at the Archaeological Museum in Cairo until an usher "kicked me out" because I refused to leave, seemed more fascinating to me than an Agatha Christie novel. And then there was the way in which Jean-François Champollion deciphered the hieroglyphics! Pure adventure. So, I suppose I was born for this. It is a matter of genetics.

I am fundamentally a product of the public education system: I completed my primary education at the Jovellanos elementary school in the city of Gijón, and later I finished my baccalaureate studies at the Jovellanos secondary school, also in Gijón. At a time when political and religious ideologies were passed on in the classroom, I had the luck that not much of an emphasis was placed on such indoctrination at these two institutions. In fact, the Jovellanos secondary school had a staff of teachers who gave me a well-rounded education, not only in terms of the technical aspects of the subjects they taught, but also the culture of effort, work well done and responsibility. I believe that all of us who went through this secondary school basically share the same opinion as well as agreeing that this school provided a service to Gijón society and has not been given the acknowledgement that it truly deserves.

In the final years of the baccalaureate and the university preparatory course (then known as the "*Preu*"), I was fortunate enough to be in the same class as a group of people who also left their mark on me in many aspects of my life; some of them form part of that core group of lifelong friends who I continue to see as often as possible. Sometimes it seems as if time has not even gone by, as if we had just seen each other yesterday. From them I learned a great deal as a person, as a youth: about "modern" music and the rebellion that it represented (rock and roll, the Beatles, long hair, Bob Dylan...), the advanced literature of that era (Sartre, Beauvoir, Marcuse...), acting out against the powers that be – suffocating during that era –, being part of a group and, of course, winning over my first girlfriends...

All this came to form part of my *Weltanschauung*, which is why I have become increasingly aware of its influence on my "scientific career".

Moreover, I was born into a family of humble means, in a period, that of Spain's post-Civil War era, in which neediness was obvious, and I did not have to be shown that this was so, or be asked to strive harder and devote myself to my studies, because people's material needs were a reality faced in everyday life. I think I was the first member of my family to go to university. My parents had to make major personal and economic sacrifices so that I could become a student; I am what I am thanks to them. They also instilled me with a series of ethical, social and behavioural values that have marked my whole life, including my work in science. At the same time, they never placed any pressure on me to study a more economically or socially advantageous subject at university. Instead, they afforded me full freedom to pursue my own personal interests, which is remarkable considering the climate of economic scarcity. Because of all this, it is evident that those times required a much greater effort and harder work to earn high marks, with honours if possible, so as to bring down tuition costs and allow me to win scholarships. By the way, to be awarded a scholarship one had to earn much higher marks than those required today. I was able to fulfil that commitment and get high grades during the baccalaureate. Despite this, the courses I took in the humanities did not interest me as much, whereas I was highly interested in physics, chemistry, geology and biology. One of the things I already noticed back then, which would finally determine my path on a career in science, is that I was unable to get a proper grasp of mathematics: I was able to get high marks and do everything my teachers asked of me, but I could not gain a truly in-depth understanding of the subject.

It was not all a cakewalk, though. For instance, I remember my fourth year in the elementary baccalaureate, when my Latin professor said to me one day: "Mr Jorcano, you will be receiving a mark of absolute zero, or rather minus 273, I should say". By the end of the year, however, I had learned to translate Latin without a dictionary.

When I had to reach a decision about what course of study to follow at university, I was fraught with doubts like almost everyone else, in my case between physics and biology. I ended up opting for physics, due to a number of circumstances, and I left to study this major at the University of Zaragoza, which had a young, quite prestigious faculty at that time. That is where I completed my second and third years at university, then transferring for my fourth and fifth years to study fundamental physics at the Complutense University in Madrid, a city where I had some family.

In both Zaragoza, and above all in Madrid, very much to my parents' chagrin, I took part not as a ringleader but as a pretty active member of the events that took place in May '68 and the later confrontation between the university establishment and Franco's dictatorship. In large part, these events also helped build my character, my view of the world and my ability to commit to ideas. Later on, it has also been reflected in my way of viewing scientific activity. In my fourth year of physics, I came into contact with modern physics, atomic and nuclear physics, quantum mechanics and so forth, and I began to realize that those subjects were not my cup of tea. On the one hand, the daunting, convoluted math-

ematical formalism was suffocating to me and, secondly, quantum mechanics just seemed too abstract and distant from my common sense, with models that I could not easily wrap my head around. I was, along with Einstein, one of those who thought that "God doesn't play dice". That is why I decided to finish my university studies and then attempt to get a foothold in the world of biology, if possible.

On finishing my Physics degree, I was fortunate enough to find out – I cannot remember how – that at the institution now known as the CIEMAT (Research Centre for Energy, Environment and Technology), which at that time was called the Nuclear Energy Board (known as the JEN or "the *Junta*" back then), they were looking for students who could use techniques from physics, such as viscosymmetry and sedimentation velocity, to study DNA. I was selected, and that is how I became a fellowship holder, in the beginning under an "honorary" status, meaning I was paid nothing, a concept that already existed back in that day. Later I received a fellowship of 10,000 pesetas (60 euros!) per month awarded by the Nuclear Energy Board itself, and finally I signed an employment contract that formed part of what was then called the "Fourth Development Plan", in which I was paid the vast sum of 30,000 pesetas (180 euros) per month. I duly celebrated this in style. Were we to use the proper monetary conversion rates, one could probably conclude that I was now what Spaniards today call a *mileurista*, or "thousand-euro earner"! For five years, thanks to the work on my end-of-university project (then known as the *Tesina*) and my doctoral thesis, I came into contact with experimental science, the scientific method, the world of authoring articles and reading research journals and so on. I was lucky enough to have Francisco Mingot, aka Paco, as my counsellor. He was a young scientist, also a physicist from Gijón, to whom I owe in large part the fact that I have devoted my life to science. He taught me to be a thorough experimentalist, to be creative, to discuss my ideas with others, to produce scientific writings and to have an enthusiastic, passionate view of science. Furthermore, at very personally and professionally difficult times, of which there were several, he always supported me.

When I finished my thesis at the JEN in 1976, I was forced to make a choice at my first major crossroads. I could no longer continue there, and I had to think up possible destinations. Our laboratory practiced a policy of little outside communication, and therefore I did not have many contacts or a clear idea of what a researcher's career was like. Through friends of my parents, I was fortunate enough to get a meeting with Margarita Salas, who worked at the recently created CSIC Centre of Molecular Biology (CBM). She informed me that many young scientists left to spend periods of time at foreign research centres after completing their doctorates.

At that time I was engaged – though I am not sure whether she would agree to that definition – with the woman who ended up becoming, and is still, my wife. Her mother was Spanish, but her father was from Berlin and still lived in that city. Though, at that time, most young scientists interested in biology set off for American or English institutes, because of the relationship with my wife, I decided to head for Germany. I was lucky to receive fellowships, first from EMBO and then from the Max Planck Society, and I was able to work at a centre ranking among those which today we would call "centres of scientific excellence": the Max Planck Institute for Molecular Genetics in Berlin, where I remained

for three years. Moreover, I was paid 2000 marks per month, about 725 euros, a fortune compared with my earnings in Spain. Clearly, "we were making progress". Speaking very little English – in my day, people studied French at school – and with practically no knowledge whatsoever of German, I headed for Berlin. When I recall all this, I think I must have been oblivious to what awaited me there, though at the same time I realize our students and young scientists today are now infinitely better prepared than we were at the time to set off on this adventure, which I recommend to them all: going abroad to receive training.

In all truth, our country has improved its scientific system notably in recent decades, though not as much as we would like. Let us hope that the cutbacks imposed under the aegis of the nearly endless economic crisis assailing us do not cause us to turn the clock back too far.

At the Max Planck Institute I soon became aware of two things: the little I knew, and that I would have to work very hard to forge a path outside Spain that might allow me to return some day. Though I did not have a clear idea of how long I would stay in Germany, most postdoctoral researchers only remained outside Spain for two to three years, and then they returned. I stayed for nearly twelve years. In Berlin I took steps towards research more closely related with biology. I worked on the structure of chromatin, mainly on the structure of nucleosomes, which at that time was one of the "hot" fields in biology; in fact, Aaron Klug, whom I met personally and in whose laboratory I was on the verge of working, won the Nobel Prize for Chemistry in 1982, for proposing the currently accepted structure of nucleosomes. This work allowed me to come into contact with the world and techniques involved in proteins and their interactions with DNA. It also allowed me to stay at a centre whose policy was to attract young, promising scientists with whom I had the privilege of collaborating. One example was Ken Timmis, one of the creators of plasmids, and therefore I was one of the first people who could make use of this type of molecules; another was Günther Schütz, who later played a very important role in German molecular biology, and whom I later encountered in Heidelberg; and then colleagues from my work group, such as Manolo Perucho, who was to become one of the co-discoverers of oncogenes, and Pere Puigdomènech, who stood out for his brilliant work in Catalan and Spanish biology. Therefore, in Berlin I came into contact with the essence of competitive science, with the pressure to get published in journals that we would now call "high impact", and I succeeded in placing myself in what was the forefront of research in biology at that time. Three years later I was given the opportunity to continue my postdoctoral training in the United States and Canada, but in the end I decided to stay in Germany, on receiving a good offer to go and work at another centre of excellence, the Max Planck Biology Institute in the city of Tübingen, which was also in the environs of another three Max Planck centres. There I took one step further towards better understanding the complexity of biological systems, given that I worked on the structure of chromosomes; in other words, I shifted from the relatively simple, linear structure of DNA packaged into nucleosomes to dealing with the structures of a higher order in chromosomes.

During the two years I stayed in Tübingen I realized that the topic on which I was working was of horrific complexity and that, at that time, there were no techniques available to

achieve progress on it, so I decided to change to a new field of study. I came into contact with molecular biology, which was emerging with great momentum at that time. I also had the luck to meet another of the people who marked me scientifically and personally: a man from León, Ángel Alonso, who had been heading a research group at the German Cancer Research Centre (DKFZ) for quite a while in the city of Heidelberg. Ángel was interested in discovering the role played by *small nuclear RNAs* (snRNAs), a mysterious set of recently discovered RNA molecules, which do not encode proteins, and which we now know are the ones that carry out the important task of *splicing*, the processing of introns from the mRNAs in eukaryotes. Thanks to our collaboration and Ángel's generosity (he took me in at his laboratory), I learned the basic techniques of molecular biology: working with RNA, cDNA, cloning and identifying genes, sequencing them and so on.

This knowledge and the major scientific contact and friendship that I have maintained with Ángel were what allowed me to take my next step, which was probably the one that most profoundly marked my scientific career: joining the Werner Franke group at the DKFZ, which was then very actively working in the field of intermediate filaments, and more specifically on keratins, the third cytoskeletal system of eukaryotic cells. This had been discovered quite recently and was one of the budding fields of the moment in biology. My time at the DKFZ was one of major productivity. It was a great centre, in terms of both size and quality, and very well financed. You could find experts wanting to collaborate on the widest range of state-of-the-art technologies. Franke's group itself was quite large (about sixty people), and they used all the techniques necessary for analysing proteins and cells. He was both an excellent cellular biologist and a person with great "political" power within the world of science. A true German *Panzer*. In other words, this was a place where I could learn a great deal very quickly, and if you wanted to work hard, you could be very productive. And I did want to work and learn. I was delighted by the work I did and the facilities that were available there. For the first time ever, I had my own work group. I had reached the maturity required to do so, and Franke gave me quite a bit of freedom within my subject area. I therefore attained high productivity levels and got published in the finest biology journals. Moreover, I was able to gain first-hand experience of what it means to work within the leading group within a specific scientific field worldwide.

In addition to that, Heidelberg was a very pleasant city to live in, with a wonderful cultural scene. Bearing in mind the intense scientific activity at the city's university and the fact that EMBO's laboratories were located nearby, it was the ideal place to live and to work in molecular and cellular biology. Therefore, despite being quite a rough period personally, because my closest family members suffered from a whole series of extremely serious illnesses, I look back on it as the best in my life. To top it all off, shortly before returning to Spain, my only son was born there.

Oddly enough, the first offer Franke made to me was to clone the ribosomal RNA genes, a topic of no appeal to me whatsoever. It was Ángel who proposed an idea to me: "Tell him you want to clone keratin genes. That is a very hot topic." And Franke responded to my proposal: "Three highly qualified people in my group have tried that in the last two years unsuccessfully. Why do you think you can achieve it?" I responded to him, "Well,

I'm just a *postdoc* researcher, so you're not taking as much of a risk. Let me work alone, and I'll pass the bill on to you if I'm successful." With Ángel's help, I achieved success in less than a year, and that changed the whole direction taken by the laboratory because, from that moment on, in addition to proteins, we were able to work with nucleic acids. This demonstrates that, despite the shortcomings existing at our universities and in our research system, which we must certainly correct, we Spaniards have no reason to envy anyone. When we end up somewhere and are given the proper means and organization, we are just as capable of competing as the best researchers.

In collaboration with my now great friend Ángel Alonso, whose laboratory I worked at prior to having my own – that was the bill Franke had to pay in the end –, I studied the sequence, structure, function and specific tissue expression of the keratin gene superfamily (approximately sixty members, all of which are related in terms of their sequencing, though they express themselves in differing manners in different epithelia, which indicated that they most likely played a role with significant nuanced effects in epithelial biology) as well as their relationship with a wide range of human afflictions. They had been discovered shortly before this and were a focal point of active research, as I have mentioned. At that time this was a problem of great technical complexity and intellectual innovation that required me to combine the most advanced techniques in molecular and cellular biology of the moment, leading me to discover two fundamental aspects in the development of my later research: firstly, skin as a magnificent subject of experimental study, pretty much unexplored at that time, and secondly, the need to apply basic discoveries in biology to the creation of better diagnostic and therapeutic methods for human diseases.

After nearly six years in Heidelberg I was offered another permanent position at the DKFZ in 1986, which made me think over my future plans for the first time throughout that entire long stay in Germany. Paradoxically, the enticing proposal made me realize that, if I accepted, there was no way I would be able to leave Germany until I retired, or perhaps even until I died. For strictly family-related reasons – I am an only child and had to take care of my parents, who were reaching an elderly age – and social reasons – despite feeling very at ease in Germany, a country to which I am hugely grateful for everything it generously offered me, I still felt foreign –, I began to think about coming home. After discussing it with my wife, whose fifty per cent Spanish genes helped her to accept the idea, we decided to return to Spain, and at the same time carry out the best of my experiments before we were past our prime: having our son.

During those times friends from the "Spanish Club" in Heidelberg, who had come from the CSIC, informed me that some positions were opening up at that institution, and that I was eligible to apply. I did so in the status of a "researcher" and was awarded the position. I soon discovered that this only ensured me a salary, but that to meet the rest of my needs as a researcher (space, laboratory equipment, doctoral students, technicians and so on), I had to "fend for myself". In other words, I had to convince some friend of mine to lend me enough space in his laboratory, and obtain other such similar favours. By then, informal talks that I had been holding with the former Nuclear Energy Board, which had now become the CIEMAT, took shape in a contract offer to take over and promote the activity

of the laboratory in which I had completed my doctoral thesis, now named the Molecular Biology Unit. Though modern molecular and cellular biology were not very well developed at the CIEMAT, they offered me enough space, doctoral candidates, lab technicians and funding to begin work, as I sought out external financing for my projects.

Although the position at the CSIC was that of a civil servant and, in the long run, it seemed more appropriate to attempt to forge my way at one of the elite CSIC centres, such as the Centre of Molecular Biology (CBM), at that time probably the best Spanish centre in biology, or at one of those which later began to open their doors (CNB, IIB and so on), I felt that I could not waste several years attempting just to get established, because that would probably force me to have to give up my highly competitive subject area of work, in which I had managed to earn a certain level of international prestige. So I accepted the CIEMAT's offer. Although my homecoming did not go as smoothly as I had expected, including the breakup of certain personal relationships from the times of my doctorate, which led to great discouragement and frustration for me, and the fact that scientific life at the CIEMAT, as at all other Spanish centres, was quite different from the ambience at the DKFZ, I must admit that the centre supported biological research coherently across time. I promoted the research in this field in such a way that, at present, the initial Unit has been transformed into two Divisions, at which approximately one hundred people work doing competitive science in fields as stimulating as gene therapy and regenerative medicine. Furthermore, some of the people to whom I provided guidance, or who I directly trained, are now directors and acclaimed scientists leading these research projects. In fact, I continue to be the director of one of these Divisions (Epithelial Biomedicine). At present, this Division possesses two Units (Regenerative Medicine and Skin Disease Models) led by two individuals with a well-grounded scientific career and great national and international recognition: Dr Marcela del Río and Dr Fernando Larcher, respectively.

During this period, my laboratory's interest shifted towards concentrating on the role of keratins in both normal and pathological proliferation and differentiation processes in epithelia and, in particular, the skin. Our group received international acknowledgement as a pioneer and leader in applying molecular and cellular biology and producing transgenic models for studying skin and cutaneous diseases. We identified the factors that regulate the expression of various keratin genes, which made it possible to direct the expression of genes of interest towards specific cell types in the skin and other stratified epithelia in transgenic mice, in both a constitutive and inducible manner. Many laboratories from around the world have asked us for these regulatory regions for their use. Our own laboratory has generated over forty transgenic lines aimed at researching the role of growth factors and their receptors (such as IGF-1, IGF1-R and EGFR), angiogenic factors (for example, VEGF), cellular cycle molecules (such as E2F-1, D1 cycline), hormones and hormone receptors (such as leptin and the glucocorticoid receptor), molecules related with inflammation (members of the NF-kB pathway), oncogenes and tumour-suppressing genes (such as PTCH, Ha-Ras, Akt) in skin homeostasis and tumorigenesis. We have also produced and performed widespread distribution of transgenic animals that express Cre recombinase in skin in a constitutive or inducible manner. This allows for the activation/deactivation of genes in this tissue, in a specific way.

Later, with the creation of the Epithelial Biomedicine Division based on the Molecular Biology Unit, and taking advantage of the extensive experience in basic research acquired on skin, our laboratory was oriented towards more translational research: developing innovative therapies (cellular, tissue-based and genetic) for the treatment of skin diseases, in particular those related with scarring (major burn victims, chronic ulcers [vascular, diabetic, pressure-related]), and later the diagnosis and treatment of rare genetic skin diseases (epidermolysis). In collaboration with Dr Álvaro Meana of the Asturias Regional Blood and Tissue Centre (CCST-PA), we developed a new system for *in vitro* culturing and expansion of human skin. Using a small biopsy (1-2 cm^2), within a term of three weeks we can obtain two square meters of skin, which makes it possible to cover the entire surface of a person's body. Using this product, originally designed for the treatment of major burn victims, more than 150 such patients have been treated at the most important major burn units in Spain, the pioneer among them being the University Hospital of Getafe. Saving the life of the first patients inspired great satisfaction and allowed me to realize that so much effort, both mine and my colleagues', beyond just doing good science, actually "served some purpose". I should point out that this collaboration with Álvaro was another milestone within the advancement of my scientific career. As a doctor, he had patients' needs very clear as well as how to develop products that would be of therapeutic use. In other words, his contribution was essential in helping us make the move from basic to translational research.

In collaboration with Álvaro Meana and his team at the CCST-PA as well as several hospitals, we have applied this skin culturing to other widespread skin pathologies with high hospital costs, including chronic vascular and diabetic skin ulcers: more than one hundred patients have been treated in compassionate use trials. At the same time, the Regenerative Medicine Unit in our Division, under the direction of Dr Del Río, is using this skin culturing and other new variants thereof in the treatment of patients with dystrophic epidermolysis bullosa, with the cooperation of the patients' association (DEBRA Spain). Though these are only palliative treatments for the time being, the hope for a time when our current research might allow us to correct this genetic defect, being able to improve patients' quality of life with our "bioengineered skin", above all children with this terrible disease, is also a great source of satisfaction.

Moreover, we have licensed the patent for this product, firstly to the company Cellerix (now known as TiGenix), and more recently to BioDan, which has received authorization from the Spanish Agency of Medicines and Health Care Products (AEMPS) to perform commercial production and distribution to hospitals of skin and oral epithelium, both autologous, for their use in various types of treatments: for major burn victims, wounds with loss of substance, skin defects and reconstructions, maxillofacial surgery and reconstructions of the genito-urinary system. We will apparently be among the first in Europe and throughout the world to get a tissue-engineering product onto the market, which demonstrates that, despite the difficult conditions existing in our country to create companies based on technology, it is possible. We hope it will become increasingly frequent as well. Our future depends on it.

At the same time, if produced using cells from patients with skin diseases, these organotypic cultures, when transplanted into immunodeficient mice, reproduce the original

patient's disease, as expected. This has allowed our laboratory, under the direction of Dr Fernando Larcher, to develop a wide range of "mice with humanized skin" models for human skin diseases, including various types of epidermolysis, lamellar ichthyosis, Netherton's syndrome, xeroderma pigmentosum, scleroderma and psoriasis. These animals, though complex to develop, are the best preclinical human models today and constitute an excellent platform on which we can design and test new therapies, generally of tissue engineering or genetic sort, but also new medicines, before they are authorized for use with patients, which is why we are constantly asked for them from domestic and foreign laboratories.

Obviously, our research is financed in large part by national and international public funding (National Plan, EU, National Institutes of Health [NIH] and so on) and, to a lesser degree, by private companies, almost always pharmaceutical multinationals. However, the aforementioned transition from basic research to more translational research was made possible thanks to the fact that our laboratory has been very generously and flexibly financed by the Botín Foundation for several years. This allowed us to have the staff, equipment and fungible materials necessary to take on much more ambitious projects. As if that were not enough, thanks to this initiative by the Botín Foundation, I met Professor Pedro García Barreno, who was its director. Since then I have shared much excitement and frustration with him, always able to rely on his support, experience and opinion, which is very sincere, but not necessarily flattering to hear. That is what makes it particularly valuable.

From July 2002 to May 2009, after pondering over it a great deal due to the potential consequences, my scientific work underwent a considerable change when I accepted the position of director general of the Genome Spain Foundation. Genome Spain, the Foundation for the Development of Research on Genomics and Proteomics, was created by the Spanish Ministry of Health and Consumer Affairs and the Ministry of Science and Technology in 2002, as a reaction to the obvious fact that the recently published human genome sequence would be leading to major technological and conceptual change in the work performed in the fields of biology and medicine. Its goal was to promote and facilitate research on genomics and proteomics in our country as well as biotechnology in general, so as to improve people's health and standard of living. This type of research required making it possible for our researchers to gain access to very new, expensive, rapidly changing technologies as well as achieving the organization and financing of multidisciplinary projects, almost always multinational, with high budgets and always oriented towards generating economic and social value as well as good science.

To demonstrate how much importance was being placed on this, the Genome Spain Board of Trustees was chaired by the Spanish Minister of Health Care and Consumer Affairs and the Minister of Science and Technology. Its trustees included state secretaries from both of those ministries, the president of the CSIC, the director of the Carlos III Health Institute as well as representatives of the Ministry of Industry, Tourism and Trade, the Ministry of the Environmental and Rural and Marine Affairs, and five autonomous regions. In other words, it was like walking on eggshells. Also forming part of the Board of Trustees was

Antoni Esteve, who was the president of Laboratorios Esteve. He provided me with extremely valuable scientific, technical and personal support at all times.

I was responsible for putting the Foundation in motion as of its creation. It had approximately twenty-five highly qualified staff members and managed a budget of about 20 million euros per year, of which 11.5 came from its Board of Trustees, and the rest consisted of resources obtained by the Foundation. During my seven years as its director, the Foundation carried out intensive work aimed at establishing a useful scientific and technological tool in the field of genomics and its biotechnological applications, acting as a catalyst and nexus joining together all the stakeholders in the system (government, autonomous regions, research groups, companies, private capital and civil society).

The following were some of its main achievements:

- starting up eleven large multidisciplinary, public-private cooperation projects of an international dimension in the fields of health (five projects), the agro-food industry (four projects), bioenergy (one project) and aquaculture (one project), with a total investment of over 32 million euros;

- the creation of four scientific and technological platforms (National Centre for Genotyping, National DNA Bank, National Biocomputing Institute and the National Proteomics Institute);

- through its Technological Portfolio programme: creation of thirteen spin-offs (2.8 million euros invested and more than 13 million euros of outside capital obtained); financing for more than 120 patent applications; evaluation of more than two hundred project applications, of which thirty-one were supported economically, leading to the creation of nineteen patents and twenty-five jobs and the development of eighteen new products and technologies;

- through the Bioentrepreneurs Training programme: 177 bioentrepreneurs were trained, 143 of whom created a Business Plan, with sixty-five new biotechnology companies established;

- publishing of more than thirty studies and strategic reports on technological prospecting, technological oversight, divulgation and so on;

- aid for the internationalization of more than 150 companies.

All these achievements led Genome Spain to be considered "the entity that most actively supports and promotes biotechnology in Spain".

Though this activity forced me to restructure my scientific work, it also allowed me to develop in-depth knowledge of the science-technology-business system in the fields of biomedicine and biotechnology, at both the national and international levels.

After my return to the CIEMAT, I applied for a publicly offered position at the Carlos III University of Madrid (UC3M) in 2010. The reason behind this was that a new degree was being organized and started up in Biomedical Engineering, with three fields of specialization, one of which was Tissue Engineering. The university was hoping to attract research groups with a good standing in this field that could contribute their experience and knowledge to the UC3M. There were more than sixty applicants from different countries, and the selection process was headed by a committee whose members included prestigious Spanish and American scientists in the fields of biomedicine and bioengineering. I ranked at the top of the list in the selection process, and in February 2011 I joined the recently created UC3M Department of Bioengineering and Aerospace Engineering, as a visiting professor, accompanied by several members of the Epithelial Biomedicine Division of the CIEMAT.

The CIEMAT and the UC3M signed an agreement that allows us to continue performing our scientific activities at the former, thanks to the creation of the Mixed UC3M-CIEMAT Biomedical Engineering Research Unit, which I now direct. However, beyond its direct utility alone, the ultimate objective of this agreement is to carry out scientific research, development and technological innovation projects and programmes in the field of biomedical engineering, creating synergies between the various facilities and the abilities of both institutions. It pays particular attention to promoting cooperation among biomedical engineers and researchers, in line with the currently widespread trend in the most technologically advanced countries of creating multidisciplinary teams in order to deal more successfully with the complex problems brought up by biomedicine. This has led to a final-hour change in my science career, which is in a certain way allowing me to return to my origins as a physicist, thereby coming full circle.

In collaboration with engineers from the Polytechnic School at our university, with members of the Polytechnic University and the Complutense University of Madrid as well as bioengineers from the University of California, San Diego, part of my team has embarked on two types of projects. Those of the first type are very technological. On the one hand, we have developed a 3D bioprinter prototype for the automation, standardization and decrease in cost of skin production – currently having reached the patenting and publishing stage –, which we hope will eventually include the bioprinting of other tissues. On the other hand, we are interested in developing microbiosensors and other detection systems that make it possible to determine the skin's physio-pathological functioning parameters in a minimally invasive or non-invasive manner.

The projects of the second type are of a more basic nature and fall within the realm of what is known as "bio-inspired or biomimetic engineering". By combining our biological experience in skin with microfluidics and microfabrication, similar to those that are used to build the microchips in our computers and mobile phones, following the path forged by some pioneering centres, we are producing microsystems known as "tissue-on-a-chip" (or in our case, "skin-on-a-chip"). These systems make it possible to analyse and at the same time monitor a large number of these microdevices to better understand the way normal human skin and skin pathologies work, and above all their response to new drugs. This

last problem is of great interest to the pharmaceutical industry, because the tests completed on animals often lead to results that are not reproduced in humans.

At the same time, we are very much interested in understanding the role played by mechanical forces in the development and functioning of our tissues and organs. Though the current description of how our cells and tissues work is essentially of a genetic and biochemical nature, we have recently begun to understand that, in the functioning of living tissues, intercellular forces and the internal (intracellular) pressures to which they give rise play a basic role in the regulation of not just cell proliferation (cellular division), but also differentiation, migration and cellular apoptosis (programmed cell death). Despite their importance, the mechanisms that produce the cells' biochemical response to these mechanical forces continue to be poorly understood. This is due in part to the lack of adequate methods for accurate measurement of cellular forces in three-dimensional tissues with high resolution in terms of time and space. So, this is precisely our objective: using microfabrication techniques and sophisticated three-dimensional skin cultures, we intend to develop new experimental and computational methodologies to measure the changes in intercellular forces across time and space throughout this tissue's three-dimensional development. The influence of mechanical forces in the behaviour of skin is especially clear. To provide some examples, our skin grows in synchronicity with the rest of our bodies largely because it responds to the mechanical tension produced by the growth of bones and muscles (as well as adipose tissue, for those of us with a tendency to gain this!). Likewise, skin grows as the bellies of pregnant women grow larger.

This proposal is very innovative from a scientific perspective because it could lead to a better understanding of the pathogenesis of diverse diseases, thereby opening the doors to new diagnostic methods and treatments. To put it bluntly: will we be able to heal using forces instead of or along with medicines in the future? If we can, nothing would be more gratifying to a physicist like me, who began his career by "betraying" physics to become a molecular and cellular biologist, then becoming interested in applying my developments from basic science to the solution of biomedical problems and, in the end, attempting to integrate biomedical knowledge with physics and engineering. This would be really cool. My story might give you the sensation that this journey through the world of science was reasonably calm and well planned out. However, this was not the case. Life is not like that. It was filled with difficult times, decisions reached with uncertain consequences and mistaken roads travelled. I was able to meet some truly brilliant people, though, and do creative, exciting work, and I believe that is all I ever really wanted. Like I said at the beginning: "As far back as my memory reaches, I was always drawn to science".

Last of all, I would like to express my gratitude to many people, including family members, friends, teachers, mentors and collaborators whom I could not mention explicitly due to space limitations, though without a doubt they will recognize themselves in the stories I tell. The best thing we can do in life is to surround ourselves with people who are wiser and better than ourselves.

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JUAN A. BUEREN

STEM CELLS: BACK TO THE FUTURE!

2

Introduction

I find it particularly hard to write about my own life. Anything in it that might be of interest I owe to the many colleagues with whom I have had the pleasure to work throughout my research career. Nevertheless, I feel I should write about myself for two reasons. Firstly, I have been asked to do so by Professor García Barreno. My admiration and respect for him prevent me from doing anything less than what he asks. Secondly, it is my hope that in this article I may give some measure of recognition to those who work in the Division I am honoured to lead, and to all the many research scientists who have helped us move forward in the fascinating journey on which we are embarked.

Early days in radiobiology

My joining the Junta de Energía Nuclear (JEN – Spain's Nuclear Energy Board), was largely thanks to Dr Julio Petrement, the head of the Board's Environment and Radiological Protection Department. In 1979, when I was in my final year of my Pharmacy degree, Don Julio introduced me to Dr Manuel Nieto, who would later become my thesis supervisor. From the outset, my conversations with Don Manuel impressed upon me that his was a highly distinctive personality. He was on a quest for the unknown; he had a penchant for art and for restoring antiques.

At the time, aided by a small coterie of researchers, Manuel Nieto was working in radiobiology. Among other things, he wanted to find out how and to what extent the thermal increase in the reservoirs receiving cooling water from nuclear power plants had an influence on the generation kinetics of haematopoietic progenitors of indigenous fish species. In the basement building 7 at the CIEMAT he had installed a number of aquariums kept at different temperatures, populated by carp specimens – most of which had been caught in the pond of Madrid's Casa de Campo woodland reserve. For the park's many casual visitors, the process of catching the carp must have seemed an intriguing and unexplained spectacle.

The research model I was beginning to develop at the time was based on using diffusion chambers containing haematopoietic cells obtained from carp kidneys, which were later inserted into the carp's peritoneal cavity. One of the difficulties we came up against with this *in vivo* cultivation approach was micro-organisms contamination. So we chose to switch from carp to mice. It was tough getting approval because my contract was attached to an agreement between Spain and the USA to study the thermal contamination of inland waters. But the move was necessary because the results would be far more meaningful.

Effective cultivation of haematopoietic progenitors in diffusion chambers implanted in the abdominal cavity of mice enabled me to complete my doctoral thesis in 1982. My theme was to analyse the behaviour of these cells in the face of ionizing radiation and hyperthermia. My findings turned out to be significant in the development of antitumoural therapies.

In 1986 JEN was renamed Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT – Research Centre for Energy, Environment and Technology). This is the name by which that body is known today. The change of name was coupled with new research aims. But Manuel Nieto's lab was part of the CIEMAT's Environment and Radiological Protection Department. So our work continued along its same path, focusing on the response of haematopoietic system cells to ionizing radiation and other environmental pollutants.

For a number of years I worked in collaboration with Dr Bryan Lord, Dr Jolyon Hendry and Dr Nydia Testa from the Paterson Institute in Manchester (UK). In 1990 they invited me to take part for the first time in the European Commission research project *Dysfunctions and Neoplasias of Haematopoietic and Osteogenic Tissue Following External Irradiation or Contamination in utero or During Neonatal Development*, which was then within the Second Framework Programme. At the time the European Commission was interested in determining the late effects of irradiation on the haematopoietic system. Sporadic cases of leukaemia had turned up in areas near nuclear fuel processing facilities.

As a result of this project we proved the existence of late effects on the bone marrow of mice exposed to moderate doses of ionizing radiation.[1] We then joined a collaborative research venture titled *The European Late Effects Project (EULEP)*, which ran from 1991 to 1993. The aim was to evaluate the long-term effects on the haematopoietic system of ionizing radiation and contamination by heavy radionuclides.

From 1992 to 1995 we took part in a European project entitled *Dosimetry and Effects of Parental, Foetal and Neonatal Exposure to Incorporated Radionuclides and External Radiation*. From then on, funding for radiobiology research was restricted to low or very low doses of radiation. This seriously compromised our work in the field of stem-cell radiobiology.

Progress in experimental haematology

In the mid 1990s public opinion swerved towards limiting animal experiments in biomedical research, particularly in toxicological studies. However, lab techniques for *in vitro* cultivation of haematopoietic progenitors were significantly improving.[2] This enabled us to join an international programme to validate the cultivation of granulocyte-macrophage progenitors (CFU-GM) as an alternative to haematoxicological studies performed *in vivo* in various animal species within the rules for authorizing drugs and other chemicals. In 1997 and 1999 we took part in a programme promoted by the European Centre for Validation of Alternative Methods (ECVAM) titled *Validation of the Relevance and Reliability of Non-Animal Test Procedures for Assessing the Potential Toxicity of Chemicals and Hematotoxic*

Products for Establishing the Efficacy of Hematopoietic Active Products. This project enabled us to validate *in vitro* cultivation of CFU-GMs to refine *in vivo* research as required by the European Pharmacopoeia.[3]

Our early laboratory studies on *ex vivo* expansion of haematopoietic progenitors[4] drew the attention of Professor Gerard Wagemaker, a widely recognized authority on the biological effects of ionizing radiation. Wagemaker visited our laboratory to assess our findings in the field of haematopoietic stem cells. He then asked me to make a formal proposal for a European project to develop new ways of reactivating stem cells in organisms that – whether by accident or in the course of therapy – had been exposed to ionizing radiation. This led to a new project (conducted from 2002 to 2004) titled *Novel Approaches for the Management of Radiation Injury*. I was honoured to be the lead researcher. The members of my team included research scientists affiliated with the Erasmus Hospital, the University of Ulm, Genopole, and two distinguished French institutions that were closely involved in examining the biological effects of ionizing radiation, the CEA (the French Atomic Energy Commission) and the IRSN (Radioprotection and Nuclear Safety Institute).

Our achievement was the *in vitro* expansion of haematopoietic progenitors in such a way that they could be transplanted into animals exposed to potentially lethal irradiation to significantly reduce the aplasia period when compared to transplants using unmanipulated bone marrow cells. However, we were unable to demonstrate the expansion of authentic stem cells. A wealth of research papers has been published on this topic, but it continues to be a debated issue. After Dr Francisco Mingot was appointed to head the Environment Department at the CIEMAT, he encouraged me to continue to engage in international research partnerships, particularly in the European Union. I have always been grateful to "Paco" – as he is known to his friends – for his support and advice.

Viruses, viral vectors and stem cells

In the 1990s I did some very interesting work with a researcher attached to the "Severo Ochoa" Centre of Molecular Biology (CBM), Professor José María Almendral, known as "Pepe", who is a good friend of mine to this day. Members of our team included doctoral students such as José Carlos Segovia and Guillermo Güenechea. After successfully defending their doctoral theses and undertaking related training, they have become – along with other scientists I shall mention below – key researchers in our group. During those busy years of lab work, we were also joined by the postdoctoral researcher Antonio Bernad, who had just completed his doctoral thesis – on polymerases – at the Centre of Molecular Biology under the supervision of Professor Margarita Salas. With Pepe Almendral our focus turned to interactions between viruses and stem cells. Throughout the work that would later become the basis of a doctoral thesis submitted by José Carlos Segovia we provided the earliest description of the interaction of the parvovirus minute virus of mice with haematopoietic stem cells.[5]

During this period we came under pressure to explain the whys and wherefores of our work. The quality of our research was not in question; rather, we were criticized for

departing from the fields traditionally targeted by the CIEMAT, such as the environment and radio protection. Then Professor Félix Ynduráin was appointed as the head of the CIEMAT. This meant our lab work was placed on a surer footing. A number of highly qualified and insightful external committees – including Professors Margarita Salas and Jesús Ávila – took a favourable view of what we were doing. This enabled us to concentrate on our work without having to constantly persuade people of its worth and value.

The main challenges our laboratory wanted to address were the discoveries published in the late 1980s by Dr Lemischka, Dr Mulligan and Dr Williams about gene insertion in haematopoietic stem-cell genomes. Our team – which now included the members I have mentioned – undertook a comprehensive series of experiments designed to transfer genes into haematopoietic stem cells derived from mice. In the course of the work forming the basis of the doctoral thesis of Florencio Varas – which he successfully defended in 1995 – we demonstrated various aspects of the kinetics and mobilization of haematopoietic stem cells using retroviral vector genetic marker techniques.[6] This experimental approach became a mainstay of all our research projects.

Early contact with Fanconi anaemia research

The laboratory gradually acquired experience in handling haematopoietic stem cells derived from mice and humans – which we were able to graft into immunodeficient mice, thus significantly raising the visibility of our work.[7,8] Having achieved these new forms of xenogenic transplant we were invited by Dr Odile Cohen-Haguenauer to take part in a new European Consortium focusing on research and therapy surrounding a disease I had not heard of before: Fanconi anaemia. Ever since, however, this disorder has been one of the focuses of my career. Scientifically, it is of great interest, but, over and above that, I and many other members of the lab were touched by the experiences of sufferers of the disease and their families.

In the course of this project we interacted with highly accomplished researchers, such as Professor Eliene Gluckman – who achieved the first umbilical cord blood cell transplant, in a patient who, as it happened, had Fanconi anaemia – and Professor Hans Joenje, who had discovered a large number of Fanconi-related genes. Joenje introduced me to a number of Spanish clinical experts and geneticists with experience in diagnosing and treating Fanconi anaemia sufferers. This was how I met Dr Juan José Ortega and Dr Luis Madero, the respective heads of the paediatric Oncohaematology Departments of the Vall d'Hebrón Hospital (Barcelona) and the Niño Jesús Hospital (Madrid), and Dr Jordi Surrallés, with whom I have maintained a close and friendly research partnership ever since.

The European project did not lead to the results we had hoped for. However, it enabled me to address a paediatric disease that remains very difficult to cure. In future we may be able to apply what we know about stem cells and gene transfer. It was during this period that the lab welcomed several new pre-doctoral students to work on new gene transfer research projects. One of them was Paula Río, who was writing her doctoral thesis on a preclinical

model for gene therapy in mice having mutations in the *Fanca* gene.[9] Since the successful defence of that thesis in 2002, we have carried on an unrelenting effort to achieve gene therapy for sufferers of Fanconi anaemia.

Cooperation with the Botín Foundation and CIBERER (Centre for Biomedical Network Research on Rare Diseases)

In 2001 Professor García Barreno, a scientific adviser to the Botín Foundation, took an interest in our work, which turned out to be a very fortunate development. Several meetings were held with Félix Ynduráin, the director of the CIEMAT. As a result, we were one of the first teams selected to set in motion an ambitious project that reflected an unprecedented undertaking by a Spanish private foundation to support technology transfer in the field of biotechnology. Working with the Botín Foundation's in-house team, the breadth of our research goals became wider than had ever seemed imaginable before. In partnership with a company called Cellerix, our work with adipocyte-derived mesenchymal stromal cells led to a United States patent for a graft-versus-host disease therapy frequently associated with the allogenic transplant of haematopoietic progenitors.[10] We also engaged in a wide range of partnerships with the technology sector and learned how to achieve highly meaningful "value-added" in our laboratory work.

In addition to the support offered by the Botín Foundation, in 2007 we became a member of CIBERER (ISCIII Centre for Biomedical Network Research on Rare Diseases). This further fostered our cooperative research efforts and took us beyond Fanconi anaemia to other disorders I shall mention below.

Thanks to the support of the Botín Foundation and CIBERER, and to the confidence placed in us by the various management teams of the CIEMAT – including Professors Félix Ynduráin, César Dopazo, Juan Antonio Rubio and, today, Cayetano López – we were able to re-hire some of the research staff who had trained in our laboratory as well as scientists from other institutions. We created a multidisciplinary team of senior researchers, which today includes Elena Almarza, José Antonio Casado, Oscar Quintana, María García-Bravo, Marina Garín, Guillermo Güenechea, Mercedes López-Santalla, Maruja Lamana, Susana Navarro, Paula Río, José Carlos Segovia and Rosa Yáñez. Our research team, our highly specialized technicians, and our pre-doctoral staff have placed us in an ideal position to undertake major biomedical research projects that in the absence of all this support would have been unthinkable.

Setting in motion the Spanish network for Fanconi anaemia research and European projects on gene therapy and cell reprogramming

Our increasing contact with other research teams who also wanted to set in motion an ambitious project in the field of Fanconi anaemia prompted us to host the first Fanconi anaemia research meeting at the CIEMAT on 10 September 2001.

Our guest speaker at the event was Professor Manuel Buchwald. In 1992 he had discovered the first Fanconi gene – *FANCC* – using cell fusion and gene supplementation techniques. As a result of the meeting, in 2002 we formally applied to the ISCIII (Carlos III Institute of Health) Cooperative Research Networks Programme for support for a research project titled *Applications of Molecular and Cell Biology to the Diagnosis and Treatment of Fanconi Anaemia Sufferers*. The project was led first by Dr Luis Madero; I took over from him in 2006. The venture planted the seed of a research network that has been very active ever since. Our goal was to ensure that Spanish patients with Fanconi anaemia should receive the best treatment available anywhere in the world, and that the laboratories within the network should carry on the most effective preclinical and clinical research imaginable at that time. One feature of the project was to reach out to the families of Fanconi anaemia sufferers. We urged them to form an association – today, that body is one of the world's leading Fanconi anaemia entities (www.asoc-anemiafanconi.es).

From 2007 to 2011 – with support from a Genoma España programme financing a small selection of projects on rare diseases – we undertook a research venture titled *Application of Modern Biology in the Development of Improved Diagnostic Tools and More Efficient Therapies for Patients with Mutated Fanconi Anemia/BRCA Genes (FANCOGENE)*. In addition to the CIEMAT, the participating institutions included the CNIO (Spanish National Cancer Research Centre), the University Clinic of Navarre, the Marqués de Valdecilla University Hospital, the Niño Jesús Hospital, the Autonomous University of Barcelona, the Catalan Institute of Oncology, the Spanish Association of Fanconi Anaemia (AEAF), Pharmamar and the Botín Foundation. This project provided robust underpinnings for Spanish gene transfer research on Fanconi anaemia.

As to our European collaboration efforts, from 2004 to 2008 we were involved in the European FP6 project titled *Concerted Safety and Efficiency Evaluation of Retroviral Transgenesis in Gene Therapy of Inherited Diseases* within the Programme of Life Sciences, Genomics and Biotechnology for Health (CONSERT), coordinated by Dr Gerard Wagemaker, with a focus on gene therapy for monogenic blood diseases. In the course of this research we witnessed the first successes of gene therapy for the severe combined immunodeficiencies SCID-X1 and ADA, achieved by the teams led by Dr Alain Fisher and Dr Marina Cavazzana at the Necker Hospital in Paris, Dr Adrian Thrasher at University College London, and Dr Luigi Naldini and Dr Alessandro Aiuti at the San Raffaele Institute in Milan. But another key event was the emergence of the first lymphocytic leukaemias in some of the SCID-X1 patients who had been treated using gene therapy. These were tough years for gene therapy in Europe and the rest of the world because, in the light of the side effects of insertional mutagenicity, many funding entities ceased to regard gene therapy as a priority target for research.

Following on from the CONSERT project, in 2008 and 2009 we were invited to take part in *Persisting Transgenesis* (PERSIST; FP7), coordinated by Professor Luigi Naldini of the San Raffaele Institute in Milan. As a postdoctoral researcher in California, with Dr Didier Trono, Naldini had developed the first lentiviral vectors derived from the HIV-I virus. Unlike their gamma-retroviral counterparts, these vectors were capable of stable transduction in both dividing cells and quiescent ones – such as haematopoietic stem cells. Using a cooperative

research framework, research scientists affiliated to these two consortia took only a few years to show that the reason why SCID-X1 patients had developed leukaemia lay in two distinctive features of gamma-retroviral vectors: their high capacity to transactivate adjacent genes, and their preferential integration in the early stages of gene transcription. While these two projects were underway, new gamma-retroviral and lentiviral vectors were developed to treat a range of monogenic blood diseases, which enabled us to transfer this knowledge to our own laboratory work. During the research that led to the thesis successfully completed by Dr África González – now employed at the Niño Jesús Hospital in Madrid – we created a family of safer and more effective lentiviral vectors for the treatment of Fanconi anaemia.[11] In 2010 the European Commission designated one of these vectors as an "Orphan Medicinal Drug", making it far easier for us to find funding for a gene therapy clinical trial to be conducted in subtype A Fanconi anaemia patients.

In October 2011 the efforts of the Spanish Research Network (Red Española de Investigación) were recognized by the Fanconi Anemia Research Fund (FARF) when it granted me a Distinguished Service Award for "commitment to research and creation of a cooperative research environment in the field of Fanconi anaemia".

Not long after Professor Shin'ya Yamanaka published his results on cell reprogramming, I got a call from Professor Juan Carlos Izpisúa, of the Salk Institute. He told me that Dr Ángel Raya and his co-researchers at the Centre of Regenerative Medicine in Barcelona (CMRB) had also managed to reprogramme differentiated cells, thus creating induced pluripotent stem cells that were similar in phenotype to embryonic cells. Given that the trigger for bone marrow aplasia in Fanconi anaemia patients is the gradual loss of haematopoietic stem cells in their bone marrow, we thought that coupling cell reprogramming techniques to gene therapy might generate healthy blood cells based on reprogrammed skin cells. Close cooperation among the CMRB, the CIEMAT and the Autonomous University of Barcelona enabled us to demonstrate for the first time that healthy blood cells can be generated from the skin of patients with a genetic disorder.[12]

Following up this work, in partnership with Professor Naldini's team, we proved – very recently – that new targeted gene therapy and cell reprogramming approaches can generate disease-free blood cells, but this time by inserting a therapeutic gene in a defined region of the genome, so minimizing the risks of insertional mutagenicity.[13]

Implications of developing an orphan medical product; the formation of the Hybrid Unit for Cell Therapy in partnership with the Health Research Institute of the Jiménez Díaz Foundation; and other activities of the Division

The fact that the vector developed in our laboratory had been designated an "Orphan Medicinal Drug", coupled with the track record of the Spanish Research Network in Fanconi anaemia, enabled us to attract funding to set in motion two closely related research projects to address gene therapy for Fanconi anaemia sufferers. Funded by the Spanish Ministry of Health's independent clinical research programme and led by Dr

Cristina Díaz de Heredia (Vall d'Hebrón Hospital) and Dr Julián Sevilla (Niño Jesús Hospital), these two projects instantiated our own gene therapy protocol at external sites. This meant that in addition to our Spanish network researchers we could rely on the involvement of research scientists with an outstanding track record in gene therapy clinical trials, such as Dr Adrian Thrasher, Dr Marina Cavazzana and Dr Manfred Schmidt, among others, to prepare a European project in the field of Fanconi anaemia.

In 2012 the project titled *Phase I/II Gene Therapy Trial of Fanconi Anemia Patients with a New Orphan Drug Consisting of a Lentiviral Vector Varrying the FANCA Gene: A Coordinated International Action (EUROFANCOLEN)* – which it is my honour to coordinate – was finally approved by the European Commission as one of only a few projects within the FP7 INNOVATION 1 programme, with the aim of conducting a Europe-wide clinical trial in Fanconi anaemia patients. The trial is now underway and making good progress.

Today, our Innovative Therapies Division at the CIEMAT is not concerned only with gene transfer research in the field of Fanconi anaemia. We are also targeting innovative developments for cell and gene therapy for the anaemia associated with mutations in the erythrocyte pyruvate kinase gene, type 1 leucocyte adhesion deficiency, graft-versus-host disease, and other inflammatory disorders such as rheumatoid arthritis. In order not to extend the length of this paper – and given that the main leadership tasks in the work being done on these disorders rests with other research scientists within our Division – I will eschew any description of the results of these efforts.

Besides the institutions and entities already mentioned, our activities are strongly supported by the Spanish National Research Plan. Moreover, in the wake of our Division's research in gene and cell therapy in partnership with a range of our research peers, in 2012 it was my privilege to coordinate an interesting project funded by the Community of Madrid titled *Una nueva generación de medicamentos celulares más eficaces y seguros (CellCAM)*, which continues and expands upon a project formally coordinated by a good friend of mine, Dr Damián García-Olmo, targeting the therapeutic applications of mesenchymal cells. One of the hallmarks of *CellCAM* is active knowledge transfer among its various teams to develop preclinical and clinical trials involving cells that promise higher therapeutic efficacy.

Finally, thanks to an initiative set in motion by Dr Carmen Ayuso, the head of the Health Research Institute of the Jiménez Díaz Foundation (IIS-FJD), in January 2014 we made an agreement with that entity to create a Hybrid Unit for Cell Therapy involving both the CIEMAT and the IIS-FJD. This venture is a further step in our undertaking to ensure that our Division's research can prove its significance not only in terms of publications in reputable journals but also of transfer to the community, in the form of treatment for patients suffering disorders with poor prognoses.

Conclusions

By way of conclusion I would like to acknowledge the role of all those with whom I have had – and continue to have – the honour to work. I would especially like to mention all my collaborators within the Division, because the greater part of the effort and creativity frequently associated with me in fact has its source in the senior, pre-doctoral, postdoctoral and other research staff among our members. It is thanks to all of them – to their insight, enthusiasm and generosity – that I can say that I lead a laboratory that is exceptional in its scientific and human aspects alike. To be its leader is both a daily challenge and an honour.

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MARIANO ESTEBAN RODRÍGUEZ

A SCIENTIST IN TIERRA DE CAMPOS

03

The People

Beginning to tell one's own story is somewhat of a challenge because the truth is you really do not know where to begin. Logically you have to turn back the clock, and I suppose there is no better way to start telling the story of your life than returning to the time you entered this world. I was born on a summer day, on the festivity of St Anne, 26 July 1944, a difficult time for Spaniards and all people in Europe due to the aftermath of the Spanish Civil War and the battles that had been fought on many fronts during World War II. However, at my place of birth, a town called Villalón de Campos, which then had four thousand inhabitants, on the Castilian plateau in the Tierra de Campos region at the heart of Castile in the province of Valladolid, the sun rose on a peaceful, quiet day. Because it was a mainly agricultural town, most of its people were working in its immense yellow fields, where the sun beats down harshly, requiring protection. I was born at number 23 on Calle Ángel María Llamas in my family home, a typical Castilian manor from the eighteenth century with two floors, many rooms, a courtyard, corral and wine cellar. My parents, Victorino Esteban, the town pharmacist, and my mother, María Victoria Rodríguez, a kindergarten teacher, must have viewed my birth with satisfaction, since I was the fourth of their children to arrive, after my older sister Ana, my second sister María Luz and my brother José Luis, all born in the family home as well, with the help of a midwife. The same took place with the later birth, three years after, of my twin sisters, Rosa María and María Jesús. It was the era when children were given two names, often by adding the "sweet name of Mary". So it was that I grew up in a large family with six children and parents professionally devoted to health care and teaching. Obviously, my parents' upbringing and a good family environment had a great influence over the later development of my calling for science.

My father was originally from Tórtoles de Esgueva, a village in the province of Burgos located at the border of the province of Palencia, near Castrillo de Don Juan. He was the son of Don Mariano and Doña Emilia, my paternal grandparents, who made their living in farming and enjoyed a good economic standing. I never met them, but from what Jesu has told me, my grandfather liked to rub elbows with politicians from Burgos to seek benefits for the town, whereas my grandmother Emilia, a blonde woman with a long braided ponytail, ran the household and the work done by day labourers, who came in from different provinces throughout Spain for the harvest that lasted three months in the summertime. Jesu is one of Esperanza's sisters, and Esperanza is the wife of my father's brother, Clementino, who followed my grandfather's footsteps and worked the fields. Tía Jesu, as we call her, is an extraordinary woman who turned 100 years old in August 2014. She is in perfect physical condition, has an excellent memory, remembers times past and present,

walks without a cane, cooks for my cousins Juan José and Flori and bakes delicious muffins, like the ones she made me a month ago at the house in Tórtoles, as I got the chance to enjoy hearing her tell stories about my paternal grandparents and great-grandparents, all of whom were from the region around Tórtoles and Torresandino, another nearby town. She has never taken any medicines either, and now only uses omeprazole for her stomach. She undoubtedly has a wonderful genome for scientific exploration of genes involving longevity and ageing.

My cousin Juan José is currently the town's mayor, a position he has held for many years with great flair. He was a pioneer in taking advantage of the waters flowing from the Esgueva River and promoted the construction of a reservoir that now gives life to the agricultural economy in Tórtoles. I shared my youthful years with him, because I would spend my summers in Tórtoles as a reward for the good marks I earned in my baccalaureate studies in Palencia, which I will talk about further below. At the age of ten, I learned to live alongside day labourers and share their work. We used to go out to cut the fields very early in the morning. I would help harvest and sow seeds, but what I most enjoyed was mealtime with everyone, all gathered together to seek protection in the shade under a tree or behind a mule-drawn cart. They used to call me "Manolas", because I became famous for singing the song "where the *manolas* live, there is only one Spain..." I believe that this coexistence alongside a wide range of simple people was a lesson that I assimilated at a very young age. It has later helped me deal with the difficulties that life has thrown my way. I learned to ride a horse there so I could take food out to the day labourers on the back of a mare.

As for my mother's side of the family, I met my grandmother Casilda, a very knowledgeable woman, but I never met my grandfather Cándido, who was a member of the merchant marine. Both were from Burgos, but to do business they moved to Los Barrios de Salas, a town declared to be an official historic site near Ponferrada, in the province of León, where my grandfather had a company that produced and sold spirits. My mother was born in Los Barrios, but when my grandfather passed away due to an infection in his appendix (then called the "miserable colic"), my grandmother moved back to Burgos and then to Valladolid, where she raised her five children (Cándido, Casilda, Victoria, Luis and Maruja). Two became doctors, two schoolteachers and the prettiest, Maruja, had beauty and red hair that were enough to ensure her a good marriage, as we used to be told by my mother, who was inseparable from her sister. Grandmother Casilda spent her summers in Villalón and was greatly loved by us all because of her disposition, her discretion about our childhood mischief and the help she gave us in reading proper texts.

I received my earliest teachings from my mother, Doña Victoria, as everyone in town used to call her, since she was the local kindergarten teacher until she retired. As far back as the age of three, I can remember going with her every day to the public school, in a red brick building with unique architecture constructed in the early twentieth century. It is still there today, but it now houses a cheese museum. The building was used to give the girls' classes. There were about sixty of us children in the same classroom, an image that has been handed down through photographs from the era, though this would be unthinkable for an elementary schoolteacher today. With my mother, I learned the first letters, and how to read and write, always with great patience and excellent penmanship. At home,

she taught us how to read properly using the great book by Cervantes, *Don Quixote of La Mancha*. She was a very well-loved teacher, and all her students remember her most affectionately because she was there for them in their earliest education, which is never forgotten. From kindergarten, I went on to the boys' school, in a building identical to the one for the girls, but with teachers of the male gender. With every year that went by, I would move on to another more advanced class, until completing my primary education at the age of ten, though normally it lasted until fourteen. Throughout these years, I learned to interact with other children, all influenced by American action movies, Westerns and war films, and we all formed a group that we considered invincible, at war with other groups of kids from the neighbourhoods of San Juan and San Pedro. This experience in the streets turned out to be quite advantageous in hindsight because those were times in which I learned to coexist with children of my age, to compete in games, to remain loyal to one another and to share the little we had. At that point, I began to stand out as a leader. We all formed a gang and continue to be good friends. I also learned to ride a bicycle, skate and compete in track and field. These were years of total freedom and constant exploration. From the ages of seven to nine, I was selected to be an altar boy at the parish church of San Miguel, a wonderful Romanesque-Mudejar-style building from the fourteenth to the sixteenth century. The minister was very strict about choosing altar boys, so I had to take a Latin exam. What I most enjoyed was ringing the bells in the church belfry, with a different intonation to announce the day's mass, the rosary prayer in the evening, a death or a fire. Climbing to the top of the bell tower and looking off into the distance at the Picos de Europa mountains more than eighty kilometres away was a pleasant view that made you feel larger than life.

The teachings I received in pharmaceuticals came from my father, Don Victorino, as he was called in town. He was a well-loved man because of his open, personable, friendly nature and because he was always willing to help others. The Victorino Esteban Pharmacy was located on the side of our family home, so you could come in and out straight from the house. The back room of the pharmacy was known as a meeting place for long talks with the local doctor, veterinarian, judge, priest and other town authorities. The entrance had a large stack of shelves with many different pharmacy bottles from the seventeenth to the nineteenth century, which are still kept there. Since I was little, I was fascinated by the abundance of flasks, bottles, boxes and other devices that contained products that somehow helped people stay well and improve their health. I did not understand what a medicine was, but I knew that those pills and injections would cure any person who took them. I loved helping make pastes and ointments, which we then put into tubes. These were the master formulas that some doctors continue to prescribe today. My father worked twenty-four hours a day, 365 days a year, because his was the only pharmacy in our town and the surrounding area, so he was always on call. My sister Ana would later take over work at the pharmacy.

School

Right after I reached the age of ten, I began a new stage of life in Palencia, where my parents sent me to study at the Maristas Boarding School. It was a truly rough change. Leaving your home to enter a boarding school with a strict schedule and

harsh discipline required a good deal of self-control. I was at the Maristas school for seven years, from the first year of secondary school to the pre-university preparatory course. These were years that, over time, I have come to remember affectionately, when I learned many things including study methods, competitiveness among colleagues and classmates, how to surmount difficulties, athletics, spiritual getaways at convents (now we would call them "brainstorming", though the objectives are different), and above all camaraderie. Because the school promoted sports, which I am still enthused by, I gradually improved as a football player, a fine left-side defender with a good run on the sideline, since I also ran 100-metre races in track and field. In fact, I was the cadet champion in Palencia at the very first Youth Games as well as a champion in the long jump and 4 x 100-metre relay races. I would later become the champion in the 100-metre races during a competition at the Vallehermoso Stadium in Madrid, as part of the University's Pharmacy Games. One of my greatest satisfactions was going on Sundays to eat at the home of my Uncle Cándido, a skin doctor, and my Aunt Consuelo (we called her Tía Chelo), a pharmacist, who even now at the age of ninety-one remains in perfect physical and mental condition. Having her there in Palencia and being able to rely on her help at any time was my saving grace to get through rough patches, especially when I came down with the Asian flu, and other times when I thought I was suffering from appendicitis. Moreover, they taught me to be responsible and ambitious about the future. It was in my fifth year of the baccalaureate that my curiosity was aroused about science, thanks to the efforts of our teacher, Brother Francisco, to help us understand chemical reactions with great simplicity. Without a doubt, this was a catalyst that, later at the university, helped increase my curiosity about comprehending biological processes. To provide another significant piece of information, I would point out that, of the 120 students who began the first year of the baccalaureate, only twelve of us finished the pre-university preparatory course. In other words, only ten per cent made it to university.

University of Santiago de Compostela and School of Pharmacy Studies

From Palencia I moved to Oviedo to take the *Selectividad*, Spain's university entrance exam, and, once I had passed, I took up residence at the Colegio Mayor San Gregorio, so I would begin my studies in what was known as the *Selectivo*, a set of core subjects common to all science degrees, equivalent to the first year of university studies. At this dormitory, we lived alongside students from different regions, now known as autonomous communities, with a large number coming from wealthy families in the Basque Country. I remember that, shortly after my arrival during a lunch, some older students asked me how many classes I had signed up for that year, and my answer was, "all of them". They went into hysterics, because they considered me a fool. But I got the last laugh at the end of the year, when I passed every subject and they ended up having to repeat the year. My first contact with university was a very pleasant experience. Classes were taught at the School of Chemistry and the professors were quite strict, some of them excellent at conveying their knowledge. The city of Oviedo, with Uria Street, apple cider and some great friendships, made for a wonderful year. By then I had decided to obtain a degree at the School of Pharmacy. In doing so, I felt that I was fulfilling my need for exposure to the subjects that most interested me at that time: medicines and their active ingredients.

My subsequent destination was Santiago de Compostela, with its famous Fonseca Pharmacy School, known by a song that went, "Sad and lonely is left Fonseca, sad and weeping…" Once again I found lodging at a university residence, the Colegio Mayor San Clemente, on the university campus, which was where I ended up living the entire time I completed my degree in Pharmacy, then five years after completing the *Selectivo* course. In other words, it took six years to get your degree, assuming that you passed every single course, as occurred in my case. At that time there were four public Pharmacy Schools (in Madrid, Barcelona, Granada and Santiago) and one private school in Pamplona. Today they have multiplied in number across Spain. My years at the university residence were extraordinary due to the friends I made, with whom I continue to have friendships and frequent get-togethers using any sort of excuse. The residence stood out due to its humanistic ambience, with conferences by guest professors and colloquiums on cutting-edge topics. There was a mural that displayed the contributions by the students most avid to express their thoughts. It provided quite a lesson. At that time the residence was famous for the "hazing" performed on recent arrivals. One night, as we slept, a group of older students came into the room I shared with two fellow students. They were wearing surgeon's uniforms, with white overcoats, caps and masks, and they took us to the toilets, where they had set up an "operating theatre" for the freshmen. There they forced us to strip naked, lie on a table and, using thin paintbrushes and bottles of paint, they began to mark us all up as if we were being operated on, outlining every part of the body until we were covered in paint. The problem was removing this paint afterwards, for which we used a tank of gasoline – a hazardous method that risked turning us into living torches. Furthermore, the residence was distinguished for its athletics in competitions against other university residences, in both football, in which I played left-side, and in handball, in which I was a goalkeeper. We also pulled out all the stops for the residence's parties held once a year. They were famous for the many different hors d'oeuvres we served, and the music and dancing with girls from other university residences whom we invited. At a certain moment, the lights were turned off and we would set the famous Galician drink known as *queimada* aflame. It would light up with its liquor, sugar and coffee beans, and as it burned we would pour it through the air with a ladle, and everybody would burst into Galician songs, ending with the song that is now Galicia's regional anthem. Another entertaining activity during the parties were the "donkey races", a competition consisting of a 100-metre bareback race among those participating. Thanks to my prior experience of riding a horse as a child, I ended up winning several of these.

I began the 1962–63 school year in Pharmacy at the Fonseca School, housed in a magnificent building from the sixteenth century. The number of students per class was about sixty, not including those who took the examination independently. The classes were given in the style of a lecture, with the professor dictating the topic of study and no intervention by students, not because this was forbidden, but rather because we were unaccustomed to asking questions in public. However, Professor Ernesto Viéitez, from plant physiology, who had a long-standing career abroad, opened our eyes to the reality of international research, because he showed slides with his experiments and experiences in the United States. He would inform the most outstanding students among us which courses were the most difficult to pass in our major, such as botany, organic chemistry, galenic studies and

technical studies. It was therefore common for students to attend other universities to pass those courses, which made the Pharmacy School a multi-ethnic place, with students from every region in Spain. This, in turn, helped create bonds that united colleagues. In fact, I still meet with former students from the Pharmacy School living all over Spain.

The subjects I liked most during my pharmacy studies, which would determine my future as a researcher, were biochemistry and microbiology. Biochemistry was taught by a young professor, José Antonio Cabezas, who really knew how to express the most up-to-date knowledge of the times on biochemical reactions and nucleic acids, bearing in mind that Watson and Crick had deciphered DNA just a few years before, as an instrumental part of genetic replication. Professor Cabezas remains active as a numerary scholar at the Royal National Pharmacy Academy. Microbiology was taught by Professor Benito Regueiro, who had worked with Professor Santiago Grisolía in the United States. He was open-minded towards basic and applied research. Since I was fascinated by microbiology, I asked Professor Benito whether he could accept me as a student at his laboratory, and when he witnessed my enthusiasm, he accepted. I became a student with an internship at the microbiology laboratory during the school year of 1965–66, which allowed me to begin working on research. The microbiology laboratory was located on the second floor of the Palacio de Fonseca, with an entrance way and window displaying magnificent architecture. At that time many bacteriological analyses on patient samples were performed for the Hospital Clínico in Santiago, and the person responsible was Professor Ramona Bahamonde, known by everyone as Ramonita. I learned a great deal from her, above all how to begin research on a sample to identify the bacteria present in biological fluids, sputum, urine, faeces, blood and cerebrospinal fluid. With her strict methodology, Ramonita taught us to identify the pathogens found in samples and the tools to be used, all still in use today. Being able to decipher what bacteria are found in a human fluid and determine their sensitivity or resistance to a range of antibiotics aroused a curiosity in me to keep moving forward and attempt to understand the reasons behind it all. Because I had observed a great frequency of *Streptococcus faecalis* in my bacteriological analyses, generally resistant to a broad spectrum of antibiotics, I thought that the research on this bacteria could be of interest in completing my doctoral thesis. When I proposed to Professor Benito that I might complete my doctoral thesis at his laboratory, and I asked him what subject matter he considered to be of interest, he told me that the breakdown of stone by bacterial agents could be a potential topic of study. Without a second thought I got to work, preparing a full set of semisynthetic media, and other synthetic media using different amino acid and salt combinations. For several months I gathered samples from the stones and columns in Santiago, which I later analysed in the lab. As time elapsed and the bacteria were isolated, they were either the usual bacteria one would expect to find or they would not grow in the prepared medium, so I asked Professor Benito whether, instead of the project on the breakdown of stone by micro-organisms, in all likelihood caused by chemical agents, I could change topics and continue with my study of *Streptococcus faecalis*. When he agreed, I focused all my work on this doctoral thesis, the title of which was *Resistance Mechanism of Streptococcus Faecalis to Antibiotics*. Fortunately, the laboratory had just purchased a Beckman L-50 ultracentrifuge, but because nobody had any experience with it, I signed up for an ultracentrifuging course in Munich, which I attended after a long train journey. On the basis of this experience, I introduced the technique of sucrose

gradient fractionation to Santiago, which even included building the device to do it. This allowed me to begin studies on the fractioning of ribosomes and their subunits. In order to determine the interaction of various antibiotics with *Streptococcus faecalis* in greater depth, I got in touch with the most renowned researcher of antibiotic effects in Spain, Dr David Vázquez. With no prior warning, I showed up at his laboratory in the Centre for Biological Research at the Superior Council of Scientific Research (CSIC). In the beginning, David was surprised to receive a visit from me, because he realized it was quite an effort for a student to have travelled by train from Santiago to Madrid, but when I explained the objectives of my thesis work to him, he helped me prepare a bibliography and design experiments. As a result of this meeting, I came into contact with a group of wonderful researchers at David's laboratory in 1968, and later with the group of Dr Eladio Viñuela and Dr Margarita Salas, finally becoming a research professor at the CSIC. This was my foot in the door at this institution. My thesis work allowed me to delve into the topic of genetic information transferred from the DNA in resistant bacteria to sensitive bacteria, classifying the strains biochemically and genetically, isolating new strains infected by phages, and defining the interaction between antibiotics and ribosomes, to name just a few achievements. During the time in which I completed my thesis, I also studied biological sciences, a degree that I had begun in Santiago in 1967 and ended in 1972. I presented my doctoral thesis in June 1970 at the main events hall in the School of Pharmacy in Palacio de Fonseca and received the highest honours. Then I began my postdoctoral stage immediately afterwards.

Postdoctoral period in London

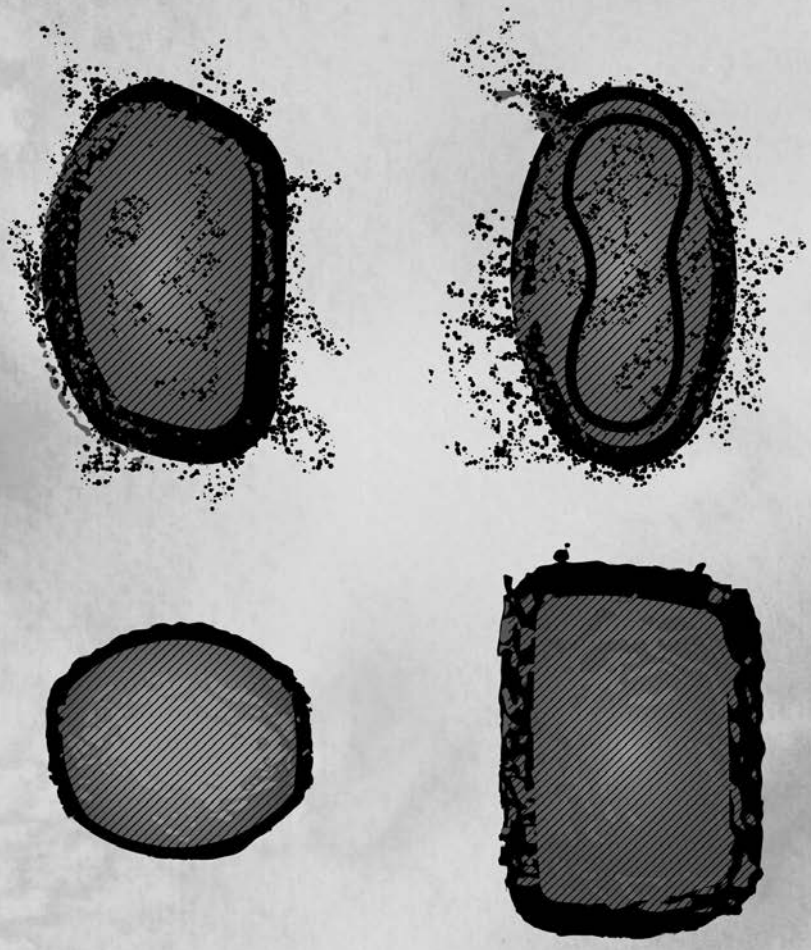
The origin of my postdoctoral work came through several prior contacts made with groups in the United States and the United Kingdom. Although I was accepted at three laboratories, in St Louis (Missouri), Bristol and London, I decided on the laboratory in London. Awarded a fellowship from the British Council, I joined the Virology Department at the National Institute for Medical Research in Mill Hill, London. The Department's director, Dr Elio Pereira, introduced me to the person with whom I would be working, Dr David Metz, at the same laboratory where Alick Isaacs and Jean Lindemann had discovered interferon in 1957. In fact, the desk I was assigned to was the same one used by Alick, which filled me with pride. This was a time when there was a great deal of scepticism about interferon, and whether it was a protein, nucleic acid, or Rnase. Few laboratories were working on the substance. Along with David, we began studies to determine how it worked, using a partially purified extract that contained the active ingredient, interferon. The system we used was based on L929 mouse cell cultures in suspension, treated with interferon and later infected with the vaccinia virus. We demonstrated how interferon inhibited the synthesis of viral proteins at the translational level, preventing initiation, which we published in *Nature* in 1972, followed by several articles that supported these findings. This pioneering work was the foundation for later establishing *in vitro* systems (cell-free systems) that demonstrated viral inhibition (RNA of the encephalomyocarditis virus) due to the effect of interferon, having later identified the proteins induced by interferon, such as the PKR kinase protein and the 2-5 A oligosynthetase enzyme, both responsible for translational control. These were very intense and exciting years, as our group closely collaborated with Dr Ian Kerr's group in biochemistry, which had discovered the two enzymes.

At that time American scientist Robert Friedman was on a sabbatical, and we worked together closely to clarify how interferon affected the synthesis of proteins. During those times, our group in virology and the groups working on biochemistry had the strongest knowledge about interferon in the world, and our work was influential in causing other researchers to join in on the study of this type of antiviral and antitumoural agents. What is gratifying to me as a researcher is that interferon is used today as a clinical medicine to fight various pathologies and can be found in pharmacies located throughout the world.

Our morale was very high, and I was passionate about the research, so I extended my stay, initially for one year, and then to another three with an EMBO fellowship. I was lucky enough to be living in a privileged location, the apartments owned by the Medical Research Council in Hampstead. In fact, I was among the first group of tenants to inhabit the recently constructed building. The ambience in Hampstead was inspiring, with its manorial homes, streets, pubs, gardens and cosmopolitan lifestyle. The local residents included film stars, composers and musicians, who often passed through in Rolls Royce cars, indicating the high social standing held by the community. Also living in Hampstead was the director of the Institute at Mill Hill, Nobel Prize winner Sir Peter Medawar, a great scientist and intellectual. I remember that he used to take in all the postdoctoral foreigners who had recently joined the Institute, putting on a Christmas celebration alongside them with champagne in an open-minded ambience of scientific discussion. At that time the Institute attracted many American postdoctoral researchers. It is a shame that the National Institute for Medical Research will be closing down in 2015–16, when its scientists and supporting staff will be transferred to the new facilities of what will be one of the best research centres in the world, the Francis Crick Institute in London. In London, in 1973, I met the woman who would later become my wife, María Victoria, who everyone calls Chogüi, a dynamic, joyful and very beautiful woman. She was travelling with a group of girlfriends who had gone there to spend the summer working in different fields. Since she was a great singer, I encouraged her to sing at a pub in Hampstead, which is where our relationship began, leading to our wedding in December 1979.

Rutgers University

My time in the United States began with an invitation from Professor Walter Schlesinger, director of the Microbiology Department at the Rutgers School of Medicine in Piscataway, New Jersey. He was a very cultured man of German origin, who I met during his sabbatical at the Virology Department in London. We had been next-door neighbours in Hampstead and struck up a great friendship. He convinced me to go to his Microbiology Department in the United States, and so I did in the summer of 1974. For a few days, I stayed at his home, and, thanks to the help of his charming wife Adeline, I was able to find a nice flat near the medical school. Because I was interested in a research project on DNA replication, I joined the group run by Professor John Holowczak as an instructor, which allowed me to increase my income. Though we were a smaller group, we reached the forefront of research on replication of the vaccinia virus's DNA. We demonstrated the states of initiation, elongation and completion of the DNA of this virus, thereby establishing an *in vitro* replication system. John left on a sabbatical year at



the Scripps Clinic in San Diego, so I took over the laboratory, which allowed me to hire my first intern, Carlos Cabrera, holder of a degree in Biological Sciences from the University of Santiago. He completed his thesis under my direction and presented it in Santiago. Carlos was a brilliant researcher of developmental genetics, with a fine career that was sadly cut short due to his premature death while working at the Marie Curie Institute near London. I will always have fond memories of him and his wife, María.

Ghent

In 1978 I moved so that I could complete a short stay at the Molecular and Cellular Biology Laboratory that was headed by Walter Fiers in Ghent, Belgium, with an EMBO fellowship. This was the era of sequencing the DNA of small micro-organisms, which I was interested in learning, and I did so by sequencing plant viruses. I reached the laboratory at a time when they were celebrating the work they had just published in the journal *Nature* on the full sequencing of the genome (5000 nucleotides) of the SV40 virus. What was then a great achievement seems like a minor detail today, due to the enormous progress made by sequencing teams using computerized processes. By 2014 it had already become possible to sequence the human genome in just a few days at very economical prices. These advancements continue to have a major impact on the understanding of pathological processes, cell organization, tissues and organisms. We are now headed towards personalized medicine. While at the laboratory in Ghent, I received a call from the head of the Biochemistry Department at the SUNY School of Medicine in Brooklyn, New York, Alfred Stracher, offering me a position as an assistant professor, which I accepted days later.

New York

I moved to New York in January 1979 and joined the Biochemistry Department at the Downstate Medical Center of the State University of New York (SUNY), where I began my research activity on two fronts: one consisted in delving further into the mechanism of antiviral and anticellular effects of interferon, and the other was the use of the vaccinia virus as a vaccine candidate against infections by pathogens. Fortunately, at that time the United States government and the National Institutes of Health (NIH) were interested in research on interferon, due to the clinical potential that this antiviral and antitumoural compound held. As a side note, I would mention that this interest by the American government came about, in large part, due to the lobbying carried out by a series of American scientists and business people. In fact, a major impact was made by the organization of a cocktail meeting, to which I was invited, at the home of a researcher for the Memorial Sloan Kettering Center in New York, Dr Mathilde Krim, who was married to the president of United Artists and founder of Orion Pictures. The event was attended by senators and congressmen. Mathilde was famous for the gatherings she put on at her house, acting as a lobbyist during meetings held with Presidents Kennedy and Johnson. One notable historical anecdote is that it was in Mathilde's home that President John Kennedy celebrated his forty-fifth birthday, in the presence of Marilyn Monroe with her famous birthday dedication. After this, interferon (a pipette with a drop of liquid emerging from its tip) would end up featuring on the cover of *Life Magazine*, with the title *Interferon, the Drug Against*

Cancer a report on which I collaborated. With the aid obtained from projects financed by the NIH and foundations, in just a short time the laboratory had been started up, hiring pre-doctoral and postdoctoral students. The inestimable help of Professor Ros Bablanian, a great person and friend, was essential to our swift advancement, in offering me the facilities at his laboratory and staff members as the projects grew stronger. My teaching obligations included biochemistry classes for first-year medical students and students at the Graduate School as well as tutoring. I promoted and participated in directing the teaching of molecular biology as a discipline to the graduate students. Within three years I was promoted to the position of associate professor with tenure (employee level) and after another three I had become a full professor (equivalent to *catedrática* in Spain). I was also named an associate professor of the Microbiology and Immunology Department at the same school.

Along with an excellent group of pre-doctoral and postdoctoral fellows, we attained a series of scientific achievements: for instance, in cells treated with type I interferons (alpha and beta) infected with the vaccinia virus, there was a massive degeneration of viral messenger RNA; the effect of the interferon occurred due to the fact that the PKR kinase protein induced cell death by apoptosis; defining the protein's functional regions; determining that enzyme 2-5 A oligosynthetase produced the breakdown of viral RNA; and establishing that another enzyme induced by type II interferons (gamma), nitric oxide synthase, inhibited viral replication at the DNA level. We determined that in cells treated with interferon and resistant to the vaccinia virus this was due to a process of interference between the virus and the host, as a result of viral genes that counteracted the effect of interferon. We would later identify genes from the vaccinia virus, which block the effect of interferon as well as the immune system, studies that we were to carry out at a later time, when I returned to Spain. We demonstrated which vaccinia genes were the ones that encoded antigens able to produce antibodies in people immunized against smallpox, and we determined the role that some of these proteins play in the virus's entry into the cell.

It was in 1981 that the first cases of infection by an unknown agent started to appear in New York. By 1983 this agent had been identified by a group led by Francois Barré-Sinoussi and Luc Montagnier, who would in 2008 receive the Nobel Prize for their discovery, as the human immunodeficiency virus (HIV) that causes AIDS. What we were interested in was cloning the HIV genes in the vaccinia genome, which is how we got our first article published in *PNAS* on viral vector constructs that were attenuated but replicative as vaccine candidates. We pioneered the understanding of how the virus was distributed in the cell, generating a virus with the luciferase gene, and demonstrating its later localization in mouse tissues infected with the recombinant virus, a work that was also published in *PNAS*.

Through collaborative work with New York University (NYU) and Mount Sinai, we were also forerunners in the creation of protocols for combined vector immunization to induce cell response (T CD8+ lymphocytes), which was correlated with protection against malaria, yet another work published in *PNAS*. These protocols are now in widespread use in vaccination processes in clinical trials. In collaboration with Yale University, we demonstrated the use of recombinants of the vaccinia virus to control infection caused by leishmaniasis.

My two children, Julia and Jorge, were born at the Beth Israel Medical Center in New York. My wife, María Victoria, was already working as a technician responsible for cell cultures at my laboratory in the medical school (SUNY, Downstate Medical Center). Watching my children grow up was a great experience, and Julia would always run when we went out to take a stroll, suddenly stopping at traffic lights, which would scare the people who saw her running. In the meantime, Jorge would be grumpy and say no to everything. They were lucky to learn English with ease at school, while we spoke to them in Spanish at home to keep up their familiarity with the language. They enjoyed Sundays, when we would go out to walk in Central Park, and their favourite place was the statue of Alice in Wonderland and the little duck by the statue of Hans Christian Andersen in the lake, where people would practice sailing small, remote-controlled boats. They also loved visiting the FAO toy store on Fifth Avenue, where they spent the best of times playing with all the dolls, which is why they were surprised to find, on returning to Spain, that they were not allowed to play with the toys when they went into a store. In New York we formed friendships with other Spanish scientists, and the Carnival parties we held at our home on the Upper East Side (333 East 80 Street) were famous.

Along with scientist Ángel Pellicer, for ten years we organized a conference series titled "Science and Scientific Research" at the Casa de España and at the Instituto de España, in which renowned scientists and future promises who would later become well known in the fields of biomedicine, physics and mathematics participated. They were attended by a sizeable group of Spaniards and Americans, including our own Nobel Prize winner, Severo Ochoa, who lived near my home and invited some of us scientists to dinner on more than one occasion. In fact, I remember when he told us that we should not "burn our boats" like Pizarro did on returning to Spain because we had to be sure we could make the return trip. I followed his advice precisely when I decided to come back to Spain, by keeping my laboratory in Brooklyn active for several years. In New York as well, in 1980, along with notable Spanish scientists from many fields of knowledge, we founded the first Association of Spanish Graduates and Doctors in the United States (ALDEEU-Spanish Professionals in America), of which I eventually became the president. Its objective was to spread Spanish culture in America while acting as a meeting point for our colleagues in Spain. This association continues to hold yearly gatherings, alternating between American and Spanish cities.

Returning to Spain

While living in New York, I received a call from José María Mato, then the vice-president of the CSIC, to find out whether I was interested in directing this institution's National Centre for Biotechnology (CNB). Because I had taken part in the early stages of the CNB, I was quite familiar with its origins. I had been working outside Spain and had been named a research professor of the CSIC in 1987, so I thought it might be the time to return. Therefore, I confirmed that I was interested, held interviews with different researchers, government role players and the university, and in 1992 I was named the director of the CNB. After twenty-two years of research at different foreign centres, I moved back to Madrid with my wife and children and took over the centre's management on 1 August 1992, a position I was to

hold for eleven years. Analysing these achievements in retrospect, they were many because, with everyone's help, we managed to start up a centre that earned recognition from the International Scientific Advisory Committee, which evaluated us several times as a world centre of biotechnology excellence (human and animal health, agriculture and environment), all in a very short time span. We created the departments and services. We recruited an excellent staff of researchers. We trained a large number of people who later enriched other research centres, and we were a focal point that attracted young researchers from Spain and abroad. Moreover, we promoted the relationship between the public institution and business by integrating researchers from the centre into companies, creating the Department of Immunology and Oncology, which would later be used as a model by other institutions. When I ceased to be the director in 2003, the number of people working at the CNB had risen to over six hundred. You can read about the CNB's current status here: www.cnb.csic.es.

Vaccines

Along with my administrative responsibilities, I had to create my own research group in Spain, which I achieved through the gradual hiring of postdoctoral researchers who moved from my group in New York to the CNB, while at the same time we recruited pre-doctoral students. My group's main line of research is to study the biology of the vaccinia virus and how to use it to produce vaccines against prevalent diseases. It was initially aimed at HIV/AIDS, malaria and leishmaniasis, and later the hepatitis C virus, chikungunya and prostate cancer. What is of interest about the vaccinia virus in terms of vaccination lies in its use to eradicate smallpox, the only human disease wiped off the face of our planet. In order to avoid safety problems with the current vaccine, we have used weakened strains of the vaccinia virus, the modified virus Ankara (MVA), which was produced after five hundred successive passages through chicken embryo cells, having lost approximately 30 kb of genetic material, and the NYVAC virus, produced by targeted deletion of sequences encoding eighteen specific genes, with the loss of approximately 15 kb. We have demonstrated that both vectors display different immunogenic behaviours, and therefore we are studying the two vectors as different candidates in vaccines. Using the MVA, we have produced vaccine candidates against HIV, subtypes B and C (referred to as MVA-B and MVA-C), which account for more than seventy percent of all the HIV variants throughout the world, and, for the first time ever in Spain, we have carried out clinical trials in phase I using MVA-B as a prophylactic and therapeutic vaccine, demonstrating a positive immune response against HIV antigens. The MVA-C vector is currently undergoing phase I clinical trials in the United Kingdom. These vectors have been patented, and the patent has been approved in Europe and the United States. The planning of a phase II clinical trial is under way. We have also produced MVA vectors against leishmaniasis, malaria, hepatitis C (HCV) and the chikungunya virus (CHIKV). This last virus is transmitted by the bite of the *Aedes albopictus* mosquito and is spreading on every continent, causing terrible joint pain and even death. We have demonstrated that these vaccines induce protection in a murine model in cases of leishmaniasis, malaria and chikungunya. As for HIV, we have also proven that the vaccine based on MVA-SHIV (HIV/SIV hybrid) induces protection in a model with macaques. In the case of MVA-HCV, there is no model with mice to test the protection, though we have demonstrated positive immuno-

logical behaviour and are currently applying its range of effects by selective deletion of viral genes. Due to the interest in controlling infection caused by the Ebola virus, which has a mortality rate range from fifty to ninety per cent, depending on the isolate, we have recently begun an international cooperation project on vaccines with this virus, generating MVA-Ebola vaccines in our laboratory for the virus variants isolated in Sudan and Zaire.

Using the NYVAC vectors, as part of a project financed by the Bill and Melinda Gates Foundation, we have produced vectors that express the antigens of HIV Env and Gag-Pol-Nef for subtype C. They have been tested in a preclinical trial (mice and macaques), and in clinical phase I with healthy humans, thereby demonstrating safety and positive immunogenic behaviour. A phase II clinical trial is currently being prepared in South Africa and will begin in 2016/17. Due to the fact that both the MVA and NYVAC vectors contain genes in their genome that encode for viral proteins that act as immune response inhibitors, our goal has been to ascertain which of these genes or immunomodulators do so, determine their role and remove them from the genome of MVA and NYVAC so as to strengthen their immunogenic ability. We have identified several of the genes that act as signals at different levels, and we have demonstrated that the immunogenic capacity of these vectors is boosted after the elimination of those genes. We are currently carrying out a systematic analysis to identify the relevant viral genes with immunomodulation capacity. As for prostate cancer, we are developing oncolytic vectors with the ability to express tumour antigens so that they may be used to destroy tumour cells selectively, either by direct infection and/or by activation of T-lymphocytes with cytotoxic capabilities.

Our scientific contributions about the way interferons work have been pioneering, promoting a clinical interest in these drugs as antiviral and antitumoural agents. Studies at my laboratory have demonstrated the role that several of the genes induced by interferons play as regulators of programmed cell death (apoptosis). This information may be used to determine more effective therapeutic guidelines for the use of interferons in patients with tumours and in gene therapy. In the field of vaccines, we have developed different vaccine prototypes for use against diseases with a major worldwide presence and high mortality rate, some of which are in clinical trials, while others progress in that direction, which will help control various pathologies.

These scientific contributions amount to more than three hundred works published in international journals, more than 310 presentations given at national and international congresses, ten patents and thirty doctoral theses.

My research in the United States was financed by the National Institutes of Health (NIH) and the National Science Foundation (NSF). Since I returned to Spain, the group's research has been subsidized by the European Union, the NIH, the National Research and Development Plan, the Health Care Research Fund, the Spanish Autonomous Region of Madrid, the Foundation for Research and Prevention of AIDS in Spain (FIPSE) and several companies. In 2005 we signed a collaboration agreement with the Botín Foundation lasting five years to complete research on vaccines against common diseases, and in 2006 my

group was also rewarded with a project by the Bill and Melinda Gates Foundation to create a vaccine against HIV/AIDS, which was extended in 2012.

At my laboratory, students of several nationalities have been trained, and I occasionally receive visiting professors as well. At present, there are fourteen people working at my laboratory, including pre-doctoral and postdoctoral students of different nationalities. I take part in Master's degree academic activities with the Autonomous University of Madrid (UAM), of which I have been named an honorary professor, and with the Complutense University of Madrid (UCM).

I am a member of prestigious international societies (American Society of Microbiology, American Society of Virology, British Society of Microbiology, Spanish Society of Microbiology, Harvey Society, The Society of Sigma Xi, New York Academy of Sciences, American Association for the Advancement of Science). I am on the editorial board and evaluate articles for prestigious scientific journals as well as national and international projects. I have taken part in several European committees (member of the European Action Programme Against AIDS, 1994–97; member of the COST/STD Initiative for a European Vaccine Programme, 1994–97; Member of the European Concerted Action Against Malaria, 1996–98; member of the External Advisory Group [EAG] of the European Commission, key action 2, Control of Infectious Diseases, Fifth Framework Programme, 1998–2002; member of the WHO Advisory Committee on Variola Virus Research, 1998–present; member of the Advisory Group for the Science Foundation of Ireland, 2001, and member of the European Science Foundation [ESF] Group for Research Infrastructures on Biomedical Sciences, 2003; member of the Strategic Advisory Group of Experts [SAGE] for Vaccines and Biologicals, WHO, 2003–07) as well as national committees (ANEP; Large Scientific Installations Committee, 2003–13; member of the EU-CUTHIVAC Scientific Advisory Board, 2013–present, and the Scientific Advisory Board of the CSIC, 2013–present).

I have received awards from the New York Health Council, the State University of New York (SUNY), the Pharmacist of the Year Award, and the Iberdrola Science Award for Visiting Professors.

I am a founder and the president of the first association for Spanish professionals abroad, the Association of Spanish Graduates and Doctors in the United States (ALDEEU-Spanish Professionals in America), and received the ALDEEU medal in 2012; a founder and member of the European Foundation Against AIDS (EuroVacc); a member of the Pharmaceutical Academy of Galicia; an honorary member of the Academy of Armenian Sciences; an honorary member of the Spanish Academy of Histological and Ontological Studies; a numerary member of the National Royal Pharmacy Academy (RANF) of Spain, and the president of the RANF.

I have given a large number of conferences in several countries, organized courses, seminars and international congresses, having been the president of the Eleventh International Poxvirus and Iridovirus Meeting, Toledo, 1996; president of the Fifth European Conference on Experimental AIDS Research (ECEAR-2000), Madrid; co-president of the Second

European Virology Congress (EuroVirology-2004), Madrid, and co-president of the Seventh Vaccine & ISV Congress in Sitges, Spain, 2013.

After this lengthy career, logically I still feel there is much to be done, because science moves forward unstoppably, like life itself. You either remain at the forefront or you get left behind. Therefore, we will continue to develop new vaccines, with the hope and conviction that they may someday reach the people who need them.

Thanks to the Botín Foundation, we have been able to transfer our basic knowledge into clinical practice, while at the same time having protected these discoveries with patents. I would like to express my thanks to everyone who has collaborated with me in the laboratory throughout the years, including pre-doctoral researchers, postdoctoral researchers, technical staff, financial agencies, schools and, most importantly, my wife, María Victoria Jiménez, and my children, Julia and Jorge, for their constant support, allowing me to keep doing what I love most and continue to enjoy: attempting to understand the viruses that cause diseases and how to control them.

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EUGENIO SANTOS

A SCIENTIFIC ROUND TRIP: SALAMANCA-NUTLEY-BETHESDA-SALAMANCA

4

Summary

Eugenio Santos was born in Salamanca. He went to school there and took a degree in Biology at the city's famous university. In 1978 the University of Salamanca awarded him a doctorate in Microbiology and Biochemistry. Santos undertook postdoctoral training at the Roche Institute of Molecular Biology (RIMB, Nutley, New Jersey) between 1979 and 1981 and at the Laboratory of Cellular and Molecular Biology of the National Cancer Institute (LCMB, NCI, NIH, Bethesda, Maryland) from 1981 to 1984. In the period 1985–2000 he was a principal investigator at laboratories on the NIH campus in Bethesda, first at the Laboratory of Molecular Microbiology (LMM, NIAID, NIH) from 1985 to 1990 and later at the Laboratory of Cellular and Molecular Biology (LCMB, NCI) from 1991 to 2000. In late 1999 he rejoined the University of Salamanca as a tenured professor at the Department of Microbiology and Genetics and head of the Cancer Research Centre of Salamanca (CIC-IBMCC), a hybrid institution attached to both the University of Salamanca (USAL) and the Spanish National Research Council (CSIC).

Dr Santos's scientific career has evolved closely in step with the field of molecular oncology. As a young postdoc at the US National Cancer Institute (NCI) in the early 1980s he contributed to cloning and characterizing the first human oncogene (H-*ras*, based on T24 bladder carcinoma cells). Following seminal contributions that inaugurated the field of human oncogenes, his research has focused on analysing the structure, function and regulation of Ras family genes and proteins. In the 1980s Santos continued the pioneering work of isolating the H-*ras* oncogene and proving its oncogenic activation by point mutations. He demonstrated for the first time in humans the presence of an activated K-*ras* oncogene in the tumoural tissue – and its absence in the normal tissue – of the same patient. In the 1990s Dr Santos's laboratory used Ras-dependent proliferation or differentiation models, such as *Xenopus* oocytes or 3T3L1 pre-adipocytes, to achieve further progress in understanding the structure and function of Ras proteins and their involvement in signal transduction pathways that control cell proliferation and differentiation in eukaryotes. Finally, from 2000 onwards, Santos's research has demonstrated the functional specificity of the canonical members of the Ras family (H-*ras*, N-*ras* and K-*ras*) and their specific cellular activators (GEF, guanine nucleotide exchange factors) of the GRF and SOS families, using an experimental approach that involves the genomic and proteomic characterization of knockout mouse strains for several Ras genes and GEFs.

Dr Santos is Spain's national coordinator of the Spanish Cooperative Cancer Research Network (RTICC), sponsored by the Carlos III Health Institute. He sits on several editorial and scientific advisory boards, and has supervised more than fifty postdoctoral and post-

graduate researchers throughout his scientific career. Santos is the recipient of several scientific awards, including the Severo Ochoa Award for Biomedical Research (1996) and the Echevarne Oncology Award (2010). He is a member of the Royal Academy of Medicine (RAMSA, Salamanca, Spain, 2003) and of the European Academy of Cancer Sciences (EACS, Brussels, 2011).

Formative years in Salamanca and early interest in biological research

Eugenio Santos took a precocious interest in biological research while still at school. For several years he was taught biology by José Luis Lozano, and this decisively shaped a vocation in Santos for research in the life sciences. At a time when biology teachers were still termed "natural science masters", Don José Luis, as he was known to his pupils, was ahead of his time: in his lessons he laid stress on all aspects involved in molecular biology (microbiology, biochemistry and genetics). He was an almost obsessive admirer of Severo Ochoa and mentioned him constantly; he would often tell his class about fresh developments in the science surrounding nucleic acids. When Eugenio finished school he was determined to pursue research in molecular biology and follow in the footsteps of his idol, Severo Ochoa. Many years later Dr Santos was delighted to meet Dr Ochoa in person at the Roche Institute of Molecular Biology in Nutley, New Jersey. A friendship was formed.

Eugenio was a member of the sixth class to graduate from Salamanca's Faculty of Biology. This crop of scientists later became known as the *Promoción de Oro* ("golden class") because so many of them later achieved distinguished tenured positions in various biological disciplines both at Salamanca and at other Spanish and foreign universities. In his final year as an undergraduate Eugenio Santos applied to continue his scientific training at the Department of Microbiology, then headed by Dr Julio R. Villanueva. Santos was delighted to be admitted because this department was the most dynamic section of the Biology Faculty. It was open to the new currents and developments in the field, and provided a springboard for him to enter the real world of scientific research. The department head, Don Julio, was well liked and widely respected. Over the years, he shaped and encouraged the research careers of Santos and of many of his cohorts.

Soon after joining the Department of Microbiology Eugenio won a competitive research scholarship granted by the CSIC. Under the supervision of Dr Rafael Sentandreu, he was now able to undertake research projects in support of his graduate dissertation (1975) and his doctoral thesis (1978). Both papers were awarded the highest grades (*sobresaliente cum laude* and *Premio Extraordinario*). Santos's research focused on what was then the new field of biological membranes. His papers made valuable contributions to understanding structural and functional aspects of the plasma membrane of *Saccharomyces cerevisiae* yeast, and provided the starting point for a wealth of publications in microbiology and biochemistry journals, some of which[1] are still widely cited in the scientific literature even today.

First postdoctoral experience: the Roche Institute of Molecular Biology

Having completed his doctorate at the University of Salamanca, Dr Santos found himself at the turning point he had dreamed of for years: to follow the trail of his idol, Ochoa, and train in the United States. He was to undertake his postdoctoral research at one of the leading institutions in America.

He won a competitive postdoctoral scholarship awarded by the Comité Conjunto Hispano-Norteamericano para la Cooperación Científica y Tecnológica (now within the Fulbright Fellowships programme). As a result, in January 1979 he began his first period of postdoctoral training at the Roche Institute of Molecular Biology (RIMB, Nutley, New Jersey) under the supervision of Dr H. R. Kaback. For the young Dr Santos the move to the United States – and, in particular, to RIMB – was an immense experience. It was his first contact with the "Big Science" being done outside Spain. Dr Kaback was a leading scientist widely recognized for his work on the lac carrier and active transport through *Escherichia coli* membranes. Joining his laboratory was a continuation and upgrading of Santos's work at Salamanca with biological membranes. Moreover, RIMB was a top-echelon research institution, a scientific Camelot. The faculty there encompassed a large number of world-class researchers, including Severo Ochoa, Bernie Horecker, Anne Skalka, Aaron Shatkin, Sydney Petska and many others. It was an unforgettable experience for young Santos to present his first seminar (using data drawn from his Salamanca doctoral thesis) in the presence of those august figures after only a few weeks at Nutley.

The research led by Kaback at RIMB was concerned with cloning and characterizing *Escherichia coli* membrane enzyme coding genes involved in generating the electrochemical gradient responsible for active transport processes in that microorganism. This work gave rise to a series of papers[2,3] in this field. They broke ground in so far as they combined traditional bioenergetics with what were then the rising technologies of molecular biology and genetic engineering.

While at RIMB, Santos established a scientific partnership and a true friendship with Dr Severo Ochoa, whose laboratory was one door along from Kaback's. For Santos this relationship carried vast meaning. It was the figure of Ochoa who, years before, while Santos was still at school, so powerfully influenced the decision to study biology and pursue a research career.

In addition, by interacting with members of other RIMB laboratories, Santos widened his knowledge and postdoctoral experience immensely. He became acquainted with the new cloning and sequencing technologies used in Petska's group and the methods for preparing monoclonal antibodies developed by Shatkin's laboratory. This provided support for his own postdoctoral project at Kaback's laboratory. Santos's mastery of molecular biology and genetic engineering techniques was probably one of the reasons why he was offered a second postdoctoral stint at the National Cancer Institute, where those approaches would find use in characterizing new genes related to tumoural processes.

Second postdoctoral experience: the National Cancer Institute. Isolation and characterization of the first human oncogenes

Having successfully completed his first stage of postdoctoral training Santos was presented with the opportunity to change course and focus finally on the research issue he was most interested in: the molecular biology of cancer. In August 1981 he accepted an offer of postdoctoral training at the National Cancer Institute (NCI) in Bethesda, where he would work with Mariano Barbacid at the NCI's Laboratory of Cellular and Molecular Biology (LCMB), then headed by Dr Stuart Aaronson.

The laboratory work in that postdoctoral period was fascinating, undertaken as it was at a frenzied pace in competition with other US-based teams working in the same fields. The first results included molecular cloning based on T24 bladder carcinoma cells of the first identified human oncogene, H-*ras*. After also cloning the normal allele of that oncogene and establishing the relationship of both to the Ras family,[4] in partnership with Prem Reddy the team demonstrated that malignant triggering of these oncogenes was due to point mutations in specific regions of the gene.[5] In 1983, having also identified the chromosomal location of H-*ras*, the activation mechanism was additionally confirmed by the cloning of another Ras oncogene that triggered spontaneously in the laboratory.[6] This groundbreaking work, coupled with the discoveries of the competing laboratories headed by Weinberg (MIT, Boston) and Wigler (Cold Spring Harbor, New York), led to a paradigm shift in research on fundamental cancer mechanisms: proof had been found of the existence of human oncogenes, and one such gene had been specifically isolated. In addition it had been shown that Ras oncogenes were triggered by point mutations.

Having clarified the Ras activation mechanism in cellular lineages, Dr Santos's work turned to analysing activated oncogenes in surgically removed human tumours, particularly lung and colon carcinomas. In 1984 Santos designed biochemical trials based on restriction fragment length polymorphism (RFLP) to distinguish normal Ras genes from transforming Ras genes. This enabled him to prove for the first time in a human subject – a squamous lung carcinoma patient – that K-*ras* was present in tumoural tissue, but absent in normal tissue.[7] This observation was the first of a series of many later observations at other laboratories. It proved the somatic character of Ras mutations and confirmed the specific association of Ras oncogenes with tumour development in humans. In an interview for the Spanish daily newspaper *El País* in 2005, Dr Santos stated that his paper in *Science* in 1984 brought him far greater personal satisfaction than those that had come out in 1982 and 1983, in so far as it finally proved the relationship of oncogenes with the development of real tumours in patients, and removed any earlier doubt he had felt as to whether laboratory advances had anything to do with "real" cancer.

Research on the structure and function of Ras genes in the 1980s at NIAID

After a brief period at the NCI's facilities at Fort Detrick, in Frederick, Maryland, Santos created his own independent team at the Laboratory of Molecular Microbiology attached to the National Institute of Allergy and Infectious Diseases (NIAID) in

Bethesda. From then on, Dr Santos focused on the structural and functional analysis of proteins produced by Ras genes.[8] His laboratory developed a range of new reagents and experimental approaches to analyse the structural and functional aspects of Ras proteins and their involvement in signal transduction in physiological and pathological processes.

Using radiation inactivation[9] techniques and intracellular cross-linking, Dr Santos proved that Ras proteins are organized as homo-oligomers *in vivo*. This oligomeric structure has significant mechanistic implications. It established for the first time that there are clear differences between Ras proteins and the alpha subunits of classical heterometric G proteins. Interestingly, the oligomeric structure of Ras – which for a long time had been disregarded – was recently rediscovered in a series of research papers based on modern techniques using super-high resolution molecular physical/chemical images.[10] In his time Dr Santos had also developed a panel of monoclonal antibodies referenced against the several regions of Ras proteins. This served as a useful tool for structural/functional analysis, immunoaffinity purification and immunohistochemical detection of Ras proteins in clinical samples from various sources.[11]

Dr Santos's work also contributed significantly to clarifying the role of Ras proteins in phosphoinositide-dependent signal transmission pathways. Having proved that cellular transformation mediated by Ras oncogenes brings about specific alterations through these pathways, it was observed that exactly the same alterations are created by transformation through cytoplasmic oncogenes (*mos*, *raf*) and membrane oncogenes (*src*, *met*, *trk* and so on), whereas nuclear oncogenes (*myc*, *fos*) do not cause those alterations.[12] These results ruled out the functional implication of Ras proteins as direct regulators of phospholipase C (PLC) or phospholipase A2 (PLA2), the two main effectors in those pathways. This further indicated that transformation by cytoplasmic or membrane oncogenes involves common biochemical pathways, and the specific metabolic alterations in these pathways are potentially useful as biochemical markers of the malignant transformation process.[13]

The fact that Dr Santos's team was for several years part of the NIH's NIAID structure, which at that time was focusing on the molecular characterization of the then relatively unknown HIV (responsible for AIDS), meant that Santos and his colleagues did work that, as against what had been suggested by the experimental results of other laboratories, clearly differentiated between HIV NEF proteins and the Ras family of proteins.[14]

Functional research on Ras proteins in the 1990s at NCI

In the 1990s Dr Santos's team made significant additional contributions to understanding the function and regulation of Ras proteins using eukaryotic proliferation (meiotic maturation of *Xenopus* oocytes) and differentiation (adipogenesis in 3T3 L1 cells) Ras-dependent models.

Initially, Eugenio's team proved that microinjection of Ras proteins could induce meiotic maturation (progression from G2 to M in the cell cycle) even in the absence of protein synthesis. Later they also observed that in oocytes Ras proteins induced phosphorylation cas-

cades, starting with MAPK and RSK and ending with CDC2K, the universal regulator of entry into phase M of the cell cycle.[15] In related research, Dr Santos's team worked with Dr Vande Woude's team to show that Ras proteins are capable of acting both in phase M and in phase G2 of the cell cycle. These papers laid the foundations for later analysis of the functional interactions of Ras with other proteins involved in the cell cycle. For instance, in an article published in *Science* in 1991 in cooperation with Santos's former colleague Dionisio Martín-Zanca, the team showed, by means of microinjection in oocytes, that the product of the *trk* proto-oncogen is a functional receptor of the NGF nervous growth factor.[16] In other research efforts of the time, Dr Santos's group used oocytes as an experimental model to characterize the functional role of various molecules regulating the cell cycle, such as VHR dual specificity phosphatase, the CDK $p27^{kip1}$ activity regulator and various isolated modular domains (SH2, SH3, PH and so on) in signalling proteins involved in Ras-dependent pathways.[17]

In another line of research Dr Santos used the 3T3 L1 pre-adipocytic line to demonstrate that Ras proteins are essential mediators of insulin-initiated cell signals and to characterize those signalling pathways in mammalian cells. In initial studies Dr Santos's team proved that Ras proteins are both necessary and sufficient in the insulin-induced adipocytic differentiation process of 3T3 L1 cells.[18] Later, they used this biological model to characterize Ras-mediated signalling pathways at the molecular level.[19] First, they proved that Ras activation by insulin implies tyrosine phosphorylation of GAP-associated molecules and activation of nucleotide exchange molecules. In addition, the essential function was characterized of Ras proteins in the insulin-activation of cytosolic kinases in 3T3 L1 cells. Ras proteins are essential mediators in the activation of kinases such as Raf-1, MSK and RSK. However, the activation of Raf-1 is dissociated from the activation of MAPK/RSK. Furthermore, while the activation of MAPK is required for cell growth, it strongly antagonizes cell differentiation.[19] These results indicate that Ras proteins activate cascades of cytosolic kinases differently, depending on whether they are present in a context of cell proliferation or differentiation.

Finally, in the late 1990s, working at an LCMB laboratory in building 37 of the NIH campus, Dr Santos redirected his research focus to the structural and functional analysis of guanine nucleotide exchange factors, "GEFs", and their role as cell regulators responsible for the mechanisms activating Ras proteins, both under physiological conditions and under pathological conditions such as cancer. In several papers published in this field, Dr Santos's team documented the existence of multiple isoforms of the various GEFs, which, besides being functionally different, are expressed differentially in different tissues and different stages of development.[20,21] These observations provided, for the first time, a suitable conceptual framework for an initial understanding of the mechanisms of the spatio-temporal regulation of Ras protein activation that enable Ras proteins to trigger different responses depending on the cellular and functional context in which they appear.

Return to Salamanca: the Cancer Research Centre (CIC)

In late 1999 Dr Santos left his position as a principal investigator at NCI and rejoined the University of Salamanca as a tenured professor at the Department of Microbiology and Genetics. His goal was to help establish, and then lead, the recently set up

Cancer Research Centre of Salamanca (CIC-IBMCC), an interdisciplinary cancer research institution sponsored by both the University of Salamanca (USAL) and the Spanish National Research Council (CSIC) since it was formally incorporated in 1997.

The main scientific reason that persuaded him to move his family and laboratory from Bethesda to Salamanca was the challenge of combining basic and clinical cancer research in his own country, Spain. While at NCI in Bethesda he focused exclusively on basic research on cancer, Dr Santos believed that at Salamanca there was a realistic opportunity for launching a research institution combining basic, clinical and translational cancer research following the model of Comprehensive Cancer Centres in the United States. Once taken, this decision had no turning back. It meant that in future Santos was to add to his laboratory work the burden of management tasks as required to set up and firmly establish the new centre at Salamanca.

At the Salamanca CIC, Dr Santos's laboratory has continued to make significant contributions. His team has demonstrated the functional specificity of the various members of the Ras and GEF families of cell proteins in different signalling pathways and physiological or tumoural processes. The experimental approach used for these projects has involved phenotypic, genomic and proteomic analysis of various strains of knockout mice – bred in Santos's laboratory – that carry inactivating mutations (individually or in combination) of genes that code for Ras proteins and their GEF activators in the SOS and GRF families (specifically, *H-ras*, *N-ras*, *K-ras*, *Sos1*, *Sos2*, *RasGrf1* and *RasGrf2*). The extensive collection of mouse strains bred in the past few years has enabled this team to preserve a robust competitive position for successful research in Spain.

Despite the similarities of structure and of patterns of cellular expression that hold among members of the SOS and GRF families of GEF proteins, the initial characterization of knockout mice lacking each of those proteins bore out, from the outset, the notion of functional specificity of each GEF (Sos1, Sos2, Grf1 and Grf2) in the mechanisms that activate Ras proteins in various tissues and cell lineages.[22–25]

Moreover, an analysis of mice simultaneously lacking H-*ras* and N-*ras* proteins, published in 2001 in an article illustrated by an amusing cartoon that the editor chose to place on the front page, indicated that, while H-*ras* and N-*ras* are dispensable both individually and in combination, K-*ras* alone is both necessary and sufficient for animals to develop to adulthood.[26]

As to the functional specificity of the different members of the Ras family, Dr Santos's laboratory work has demonstrated that the various isoforms of Ras play clearly distinct functional roles in diverse biological contexts.[27] Specifically, his team's research has shown that N-*ras* proteins are clearly involved in cell defence and immunomodulation mechanisms and in apoptotic responses, while H-*ras* has a preferential link with proliferative responses.[28,29] K-*ras* proteins are fundamental to the progression of the cell cycle through the G1/S phase.[30] Other research conducted by the team on a collaborative basis has characterized differential aspects of the involvement of the various Ras isoforms in intracellu-

lar signalling and the specific contribution of H-*ras* to renal physiological processes and to controlling systemic vascular pressure.

In its independent research efforts, Dr Santos's team has analysed the specific functionality of two members of the SOS family of RasGEF activators in mammalian cells. Analysis of knockout mice bred for these loci had previously shown that Sos1 is essential to embryonic development,[22] while Sos2 is entirely dispensable in adult mice and its absence does not cause any obvious phenotype.[23] However, recent work using a mouse strain carrying a conditional Sos1 knockout allele, inducible by tamoxifen, has shown that Sos1/2 double knockout (DKO) mice die rapidly, whereas Sos1 and Sos2 individual knockout adult mice are perfectly viable. This proves a functional redundancy between Sos1 and Sos2 for the homoeostasis and survival of the complete organism and the development and maturation of lymphocytes.[31]

Finally, the analysis of RasGrf1 and RasGrf2 knockout mice conducted by Dr Santos's team has uncovered the different functionalities of these two GEF proteins, which are otherwise closely similar in sequence and structure. RasGrf1 plays an important role in photoreception and ageing processes, while RasGrf2 cooperates with other cell proteins in T-lymphocyte and lymphomagenesis signalling.[32,33] Finally, in recent work involving international consortia to analyse SNP in human populations based on GWAS (genome-wide association study meta-analysis of directly genotyped or imputed human SNPs), the knockout mouse strains developed in Dr Santos's laboratory also served to document the functional involvement of RasGrf1 in the predisposition to sight defects such as myopia[34] and of RasGrf2 in the predisposition to addictive behaviours such as alcoholism.[35]

The importance of mentoring and management in scientific work

The above text describes how, throughout the stages of his career, Dr Santos benefited immensely from the management and direct support of a wide range of teachers, colleagues and mentors, whom he encountered at the institutions where he did his scientific work. In gratitude and acknowledgement of the importance and necessity of support of this kind, at his own laboratory Dr Santos has always laid stress, over the years, on carrying out all scientific management and mentoring activities required to nurture the progression and advancement of the young researchers within his team. It is very satisfying for Dr Santos that more than fifty research scientists – mostly postdoctoral students – have trained at his laboratory, and that many of them now hold distinguished independent positions at institutions in Spain and elsewhere.

Since his return to Salamanca in 2000, Dr Santos's scientific management and mentoring work has increased dramatically in comparison to his previous years at the NIH. Over the past fifteen years he has devoted significant efforts to ensure that the CIC – which employs about 250 people – becomes firmly established as a competitive cancer research venue. He has focused particularly on integrating fundamental research with high-quality clinical trials, following the template of Comprehensive Cancer Centres (CCCs) in the United

States. These organizational and management endeavours have borne fruit. The CIC is a pioneering model that in comparison with its competing research centres in Spain is unique in a number of ways.

In addition to his local contribution at the CIC in Salamanca, Dr Santos has tried to encourage this same philosophy of translational cancer research – the hallmark of CCCs – to the research community in Spain. Hence, since the institution was formed in 2003, Dr Santos has helped lead, manage and support the collective research efforts conducted in the framework of the successful Spanish Cooperative Cancer Research Network (RTICC), which is present throughout Spain and is backed by the Carlos III Health Institute.

In the past few years, Dr Santos has taken part in a large number of scientific assessment and advisory panels and committees for various institutions in Spain (CNIO, IDIBAPS, CIPF, CICYT, Genoma Foundation, AECC and so on) and overseas (ICGC, NSF, ISF, AIRC and so on). He has organized international symposia and served as a reviewer or member of the editorial boards of specialist journals. Dr Santos is the recipient of several scientific awards, including the Severo Ochoa Award for Biomedical Research (1996), the Castilla y León Award for Scientific Research (1996), the *Encomienda* of Spain's Ministry of Health (2003), the Echevarne Oncology Award (2010), and the National FUNDALUCE Award (2012). He has been a member of Spain's Royal Academy of Medicine since 2003 and of the European Academy of Cancer Sciences since 2011.

The ongoing economic crisis has put pressure on public funds available for research in Spain and elsewhere. It is accordingly appropriate to mention that Dr Santos has endeavoured to support research as a member of the scientific boards of several foundations devoted to the development of the Spanish research community, such as the Scientific Foundation attached to the AECC, the Ferrer Foundation and the Rodríguez Pascual Foundation. Dr Santos has consistently acknowledged that it is an honour and a privilege for him to see his name listed in conjunction with the distinguished research scientists now within the faculty of the Botín Foundation. It is hoped that this text will be received as an expression of gratitude for the support received from the Botín Foundation in recent years, and from the mentors and institutions that have enabled Dr Santos to pursue his scientific career to the present day.

A scientific round trip: Salamanca-Nutley-Bethesda-Salamanca

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CARLOS BELMONTE

LOOK BACK WITHOUT ANGER

5

Rilke said that man's true homeland is childhood. Perhaps for that reason it seems necessary to begin this biography, albeit essentially professional, by briefly evoking the experiences and memories from my earliest years. Some people may be able to brag about having built a useful life after surmounting material, cultural or emotional difficulties associated with a rough childhood. This was not the case for me, though. If I had to summarize the first years of my life, I would do so by saying they were completely happy, as I was surrounded by the warmth of a supportive and caring family. From that time on, my only merit has been knowing how to make the most of all the advantages I was offered in that positive environment. This is why I played with the title given by John Osborne to his drama – *Look Back in Anger* – that reflected the mood of many young people of my generation (but certainly not mine) when they thought about the past.

My paternal grandfather, Nicolás Belmonte, was an ophthalmologist of undisputed professional and social prestige in our city of origin, Albacete. Of his five children, three became ophthalmologists, including the second of them, my father, José. Nicolás Belmonte was a kind-hearted, humane, enlightened man and a convinced Republican. His children spent their university years in Madrid at the Residencia de Estudiantes, an experience that, in addition to allowing them to live alongside the most notable intellectuals of twentieth-century Spanish culture and science, forever marked their attitude to life: open-minded about the world, tolerant, and with a remarkable love for cultural and scientific activities. This translated into a constant concern for the education of the next generation, to which I belong.

Among its members, we also forged an emotional camaraderie that arose from endless summers shared at our grandparents' estate, where each family had its own house and we coexisted as if on a cloud filled with cousins, boys and girls of all ages, playing, studying, swimming and reading, in an atmosphere of affection and freedom. On the other side of the family, my maternal grandfather was an honest, right-wing Catalan. He owned the largest, and only, bookshop in the city, which also included a section with musical instruments and religious items. Because of this, it was burned down on two occasions during the Spanish Civil War years. The immense, varied library inside the reconstructed post-war bookshop was, for a voracious reader like I was as a child, a privileged, endless source, first of comics and adventure books, and later of works by Spanish and universal authors, some ideologically suspect. At that time such works were only available in luxury editions.

In 1951, when I was about to begin the baccalaureate, my parents, José and Cecilia, took us, their five children (José, Carlos, Isabel, Cecilia and Nicolás), and moved to Alicante.

Three ophthalmologists were too many for a small and modest provincial capital like Albacete, so the brothers who were doctors drew lots to determine who would be leaving for the city, where there was an increasing numbers of patients. They had always equally split the revenue earned at the family clinic, and so they continued after parting ways, for more than thirty years. They jointly took on our family's initial expenses in Alicante, my father's professional trips to New York with Castroviejo and his brother Nicolás to Switzerland, and even the whole year the latter devoted to studying for his competitive exams for a professorship. This was such a fine example of generosity and mutual support that it has served as a guide for conduct within the family, passed down as an identifying trademark to children and grandchildren. At that time I combined my winters studying in the bright, happy seaside city of Alicante with summers in my beloved Albacete, soon cut short by a month-and-a-half stay at a private school in Germany to learn the language.

From his times as a boarding school student under the physiology professorship of Negrín in Madrid, my father remembered the importance of German in science, so he was adamant about us learning it fluently as of our childhood, with private classes and trips to Germany, where my elder brother José and I would travel each year, soon going alone even though we were just twelve and ten years old respectively. We even got lost on one of the trips and were repatriated from France by the Spanish Consulate, an adventure we loved and spoke about constantly in our circles because at that time nobody, and even less so two children, travelled abroad and learned another language besides the French we were taught in the baccalaureate. My parents had also decided that the education offered by the public secondary school in Alicante was better for us than going to private religious schools, so that is where I completed the baccalaureate, unlike other children of my social class.

This was a wise decision that allowed me to enjoy wonderful teachers, some of whom were survivors of the cultural cataclysm and persecution of intellectuals that took place at the end of the Civil War. I was a good student who leaned towards literature, the natural sciences and chemistry, undoubtedly due to the enthusiasm that my teachers of those subjects managed to pass on to me, above all my natural sciences teacher, Mr Abelardo Rigual. I would write stories, cultivate seeds, make potions with plants and had a few small pets I was allowed to keep in the house. All in all, I was a curious boy prone to intellectual pastimes, something almost natural in a house that hosted doctors like Pedro Laín and Gregorio Marañón, musicians such as Achúcarro and painters like Benjamín Palencia and Pancho Cossío, many of them former residents and friends of my father's, on their visits to Alicante.

Before I had even reached the age of seventeen, I left home to study medicine in Madrid in 1960. I had a difficult time deciding between that major and chemistry. However, due to a hereditary case of otosclerosis, my hearing was rapidly growing worse, to such a degree that I sometimes had to make use of a poorly concealed prosthesis on my eyeglasses. My father, who was aware of my predilection for scientific research, a very appropriate profession were my deafness to continue progressing, recommended that I study medicine, a field that seemed less hostile to him for later pursuing a scientific career, which might prove to be complicated during those times.

Life at the Colegio Mayor Jiménez de Cisneros student residence, right in the middle of the University City, where my brother was already studying, offered unique potential for cultural and personal development during that era. This College tended to be inhabited by the sons of former students from the Residencia de Estudiantes, who had registered in a wide range of university programmes, with very disparate concerns and backgrounds, thereby bestowing some of the values underlying the Residence's operations upon the Colegio Mayor Cisneros. The library, for instance, possessed works by all the authors prohibited at that time (Sartre, Camus, Kierkegaard, and so on). It also had a classical music room. Films by Buñuel and Bergman were shown in the cinema club. The human and the divine were discussed with reasonable openness, and rugby was played passionately by many students.

The School of Medicine at which I began to study, with more than eight hundred students per academic year in the beginning, was a jungle governed by Darwinist rules. I worked hard at my studies, being attracted during my first year mainly to biochemistry and histology, the latter of which was explained by the last disciple of Cajal, Fernando de Castro, a professor short on eloquence in his classes and severe in exams, but whom I soon recognized to be a genuine scientist who examined concepts in true depth. I would have to attend the packed classes standing, pushed up against the professor's desk so that I could hear him. The next year, I went to speak with Professor de Castro to ask if I could work with him as a student intern in his department, and, because I had obtained the highest marks possible, he accepted me immediately. I only saw him in the laboratory a couple of times, though. As I practically self-taught myself histological techniques, I concluded that they required great patience and special manual skills, qualities that I pretty much lacked. Moreover, I had begun to study physiology with José María de Corral, then the temporary department head, and I was enthused by the dynamics of that discipline, compared with the static nature of morphology and structural biochemistry. By the end of the year I had decided to become a physiologist.

My father, who visited me in Madrid as I began my third year at medical school, went with me to see Antonio Gallego, who had just become the chair of the Physiology Department. He and my father had briefly coincided in their studies and as interns in pre-war years at Negrín's laboratory. I wanted to ask if he would accept me to work under his tutelage. He received my father kindly but also warned him with shocking frankness that he did not want anyone just because he was a friend's son. He therefore sent me to the laboratory of Francisco Clement, an ophthalmologist so motivated by science that he personally financed his research on the biochemistry of the vitreous. From there, I soon moved to the laboratory of José María de Corral, who had trained with Houssay in Argentina and studied the physiology of gastric secretion in dogs. Both professors had different but equally kind-hearted personalities, filled with generosity and affection towards me. They devoted their time and patience to teaching me the basics of experimental research. In addition to helping them, I had to prepare practicums and give them to physiology students almost daily, taking records of dogs' and cats' blood pressure, which caused me to miss classes and forced me to study until very late. One Saturday night, I stayed behind alone in the laboratory at the end of a practicum, trying to locate the phrenic nerve in a dog under anaesthe-

sia. Antonio Gallego had gone to the movies with his wife and walked by the medical school building, all its lights out except the one in his Physiology Department window, where I was working. He came in to see who was there. Somewhat intimidated, I explained to him what I was doing, and as he left he said: "Next Monday at nine in the morning, be in my laboratory. You're going to start working with me."

It is in this almost literary manner that the relationship began with a person who would become my most important teacher in so many ways, whom I loved, admired and remained close to until he passed away in 1992. Antonio Gallego's personal laboratory was located inside the Spanish Pharmacology Institute (IFE), of which he was the director. The lab was set up and financed by the Spanish Penicillin and Antibiotics Company (CEPA) on one floor of the medical school, which was connected to the physiology school. At that centre, applied pharmacological research was carried out as well as basic research. Everything there was comparatively modern and functional. It had technicians, a library with many foreign journals for those times, and a free dining hall so that the staff could complete a continuous work day. At Gallego's laboratory, our group initially consisted of just Luis Cros, a second-year medical student, and myself, a fourth-year student. One year later, Margarita Barón joined us, and shortly after that, Antonio de la Fuente, a self-taught electronics engineer who was tremendously valuable, both intellectually and as a person. In large part, it is he who taught us electrophysiology as well as all the other students who gradually joined the group throughout the subsequent years.

Gallego was an acknowledged expert on the functional morphology of the retina. He devoted every hour he could to studying it, in addition to his frenetic, varied professional activity as a university department head, scientific director of the CEPA and IFE and prestigious professor involved in every university controversy at that time. As a physiologist, he wanted us young students at his laboratory to create the electrophysiological set-up for nerve recording, similar to the one that he had used in the United States with Lorente de Nó at Rockefeller University. As we recorded data on the isolated nerve, we would perform other chronic electric stimulus experiments on autonomic nerves in awake animals, taking electrocardiogram records for entire nights on human volunteers to study sinus arrhythmia, and many other experiments based on the ideas that would constantly arise out of "the Boss's" fertile imagination. It was somewhat chaotic work, but it was always packed with enthusiasm and interest, which forced us to read, sharpen our ingenuity and learn the foundations and techniques required to deal with such a wide range of topics.

This included the subject matter that led to my doctoral thesis, on neural regulation of the intraocular pressure, which arose from Gallego's observations, in histological preparations of the retina, of nerve fibres that ended in bulbous ramifications over blood vessels that were morphologically reminiscent of arterial pressoreceptors. Upon seeing them, his friend Fernando de Castro, who often paid visits to the laboratory, exclaimed: "I'm the one who should have discovered this!" As a result of my two favourite professors' enthusiasm, I devoted the next four years of my life to dissecting and taking records of isolated sensory fibres in the ciliary nerves of anaesthetized cats' eyes, attempting to identify baroreceptors that would respond to changes in intraocular pressure or retinal blood pres-

sure. To do so, I used a Tektronix 565 oscilloscope, the signal amplifiers of which were directly connected to the recording electrodes, with no additional filters, inside a Faraday chamber. To perform these heroic experiments, I had to set up a separate laboratory, whose team initially included student interns José Simón, Juan Jordá, Fernando Cerveró and Roberto Gallego. At that lab, in addition to the sensory activity in ciliary nerves, we studied *in vivo* and *in vitro* arterial baroreceptors, forming a junior scientific group dedicated to sensory neurophysiology.

My work at the laboratory took over my entire life. Suffice it to say that, while still a medical student, I would spend the whole day there at least six days a week, missing class and studying for my university courses during random free moments. Therefore, as of the end of my fourth year, the perfect marks with honours that I had been earning in nearly every subject came to a screeching halt. However, despite the fact that this fast-paced life meant making certain sacrifices, I deeply enjoyed my early dedication to teaching and research alongside Antonio Gallego. This was a man with a captivating personality, intellectually brilliant, brave in defending his ideas and capable of convincing and enthusing the young because he was a nonconformist with a quick-witted, critical spirit. His strong personality sometimes intimidated the faint-hearted, but he was glad to accept reasoned disagreement, even if it came from youngsters like his student interns, a quality completely unusual among the tenured professors of that era. In my years with him, he adopted me scientifically and promoted me in every way. While a sixth-year student, he assigned me to a class on the pineal gland, a topic about which I had written a review on my own, to be given to second-year students. He would always take me to lunch or dinner every time he received visits from foreign scientists, and he would take me with him to congresses, meetings and conferences on physiology and medical education. As soon as I finished my university degree, he gradually entrusted me with many of the internal affairs at his laboratory and in his university professorship. He got me a position as an assistant professor and, as soon as it became legally possible, he arranged an official offer – at that time based locally – for a position as a staff professor. Gallego took the time to help me obtain fellowships and personal aid, which complemented an official salary that was barely enough to survive on without my family. When his son Roberto decided to become a physiologist, Gallego put him to work with me in the laboratory. With the hindsight provided through the years, I can objectively state that Antonio Gallego was a teacher in the most noble sense of the term, to whom I owe an unrepayable debt of gratitude and whom I still remember with deep filial fondness.

Antonio Gallego's prestige as a man of forward-thinking, nonconformist approaches began to attract the brightest students to his department, many of whom were ideologically committed to the left. This created more than one conflict with the academic and political authorities of those troubled times. However, it also produced a unique ambience of scientific and educational discussion, commitment and enthusiasm around him. In 1966, this was enhanced by hiring Alberto Oriol Bosch, who had been a researcher in Germany and the United States for ten years, as an assistant professor in the department. His arrival conferred a different style upon the department's scientific operations and teaching, which ended up leaving its mark behind on all of us there. He introduced us to the focus

and work methodologies of the developed world, which included following and reading international publications, learning English and attending seminars for scientific discussion. Despite the difference in age between Alberto and me, we formed a strong friendship with great complicity in terms of ideals and objectives. His personal and scientific honesty, very in tune with young people, his clear ideas and his ability to deal with a huge work load also made him a magnetic role model for many students beginning their scientific careers. In just a few years, he managed to create a broad, strong endocrinology research group around himself. Oriol and Gallego, two men whose personalities were radically different, deeply valued and respected each other, and their mutual fondness, which remained unwavering throughout life, provided strength and coherence during the rapid expansion undergone by the Physiology Department, making it the main core of physiological research in our country in just a few short years.

By early 1971, my academic career was nearly complete in an administrative sense, because I had obtained a position as a staff physiology professor for Madrid's medical school, one of the first created in Spain, through a competitive examination. The natural next step was to earn my place as Physiology Department director, as several of these positions were about to become vacant at various Spanish universities. However, my personal plans were quite different. Because I was acutely aware of the lacks and limits of my scientific training, I hoped to expand upon it by working with one of the researchers who was publishing the scientific articles I admired – I had spent so many hard times reading them that I had learned written English. When I finished my thesis in 1968, I had already begun searching for the way to take that step. Gallego personally knew Stephen Kuffler and asked him whether he would take me in at his Harvard laboratory.

However, Kuffler's response was that he had no open positions at that time, suggesting his disciple Carlos Eyzaguirre, director of the Physiology Department at the University of Utah as an alternative. Eyzaguirre happened to be a good personal friend of Antonio Fernández de Molina, a research professor at the CSIC who knew me, held me in esteem and therefore offered to contact Eyzaguirre. A few months later, in 1970, Carlos Eyzaguirre came to Madrid on a visit and, after a relaxed, cordial interview, he accepted to take me in at his laboratory. I applied for a fellowship from the Juan March Foundation and received it. A couple of months later, I was informed that I had also been granted an International Fellowship form the National Institutes of Health (NIH) in the United States, which I preferred because it offered a few additional advantages. Therefore, as soon as I passed the national exam to become a staff university professor, I requested a leave of absence so I could head for the United States.

On a personal level, my deafness, which had released me from having to serve in the military, thereby giving me a couple years' advantage over my classmates at university, was radically corrected and remains so up to this day, thanks to a providential surgery that had just been developed. During my summer holidays in Alicante, I had met a Parisian girl, Anny, who was studying Spanish. After a couple of summers together and many trips between her city and mine, she came to live in Madrid, and we got married in May 1968. Our first son, Pablo, was born in July 1970, and in September of the following year the three of us set off on our American adventure.

My postdoctoral experience in the United States would leave a permanent imprint on my career in science, as has happened to most of the young Spanish researchers who have gone to receive training in that country since the post-war period. At that time the Physiology Department at the University of Utah was perhaps the best in the country in the field of sensory neurophysiology, with Eyzaguirre, Hunt, Perl, Burgess and Woodbury leading it scientifically. At last, I had the chance to work surrounded by people who knew more than I did, with a modern laboratory, supporting techniques, mechanical and electronic workshops and the library of my dreams. Housed in recently built apartments for medical residents and postdoctoral researchers next to the university hospital in which the Physiology Department was located, our personal life was idyllic and worry-free, while at the same time our scientific life was exciting, alongside colleagues from every generation to discuss science with. Carlos Eyzaguirre was not only a first-rate scientist passionate about his work, but also a man gifted by quite exceptional personal qualities. With an exquisite education and distinction acquired in their native Chile, he, his wife Elena and his children became like the family we had left behind, especially when our second son, Diego, was born there. My work with Carlos Eyzaguirre revolved around studying the efferent control of the chemoreceptors of the carotid body, analysing the mechanisms for modulating the activity of this peripheral receptor, using what was possibly the first functional preparation of a mammalian nerve structure *in vitro* previously developed by Eyzaguirre. Despite my still rudimentary English, Eyzaguirre asked me to give some classes to medical students, naming me a visiting professor, and he gave me the opportunity to write three chapters in the new edition of his popular book *Physiology of the Nervous System*, which he was preparing with his collaborator Sal Fidone, as a co-editor. Fidone was Eyzaguirre's successor and, years later while directing the department, we also formed a strong, long-lasting friendship based on common personal and generational affinity and scientific interests.

During my stay in Utah, Keffer Hartline came to spend a sabbatical in the department. Five years earlier, Keffer had received the Nobel Prize for his description of lateral inhibition in the nervous system of the eye of *Limulus*, an aquatic invertebrate. Eyzaguirre proposed that I work with him, because he came alone and was seeking someone to collaborate with. I was delighted to accept, because I intuitively felt, as was soon demonstrated, that I was being offered a unique opportunity to work side-by-side with one of the most interesting neuroscientists of the era. We experimentally studied the genesis of action potentials in *Limulus* and in the optic nerve of an arthropod, the scorpion. Moreover, and probably more importantly, I spent hours with him in enriching conversations in which, in addition to learning a great deal, I became aware of the importance of following flawless experimental procedure and the need for self-criticism as well as being a witness to the immense curiosity of a seventy-year-old man who had already entered the annals of the history of modern neuroscience.

Despite the constant temptation to remain in the United States and continue a career in research there, surely more productive than in Spain, from the very beginning of my stay in that country I took it for granted that I had the obligation to return and contribute with my personal effort to reducing the secular scientific backwardness in my homeland, combining a romanticism inspired by Cajal's ideas with the desire to help foster a spirit of the

Enlightenment in the still backward Spain of those years. More than forty years later, I still believe that science and education are the strongest foundations upon which human society can be built and, as a result, I still consider the price I paid for that decision, if there even really was one, to have been worth it. Nonetheless, my relationship with the United States continued and even grew stronger after that first unforgettable experience, through several stays at the University of Utah, where I still hold an official position as associate professor, and at Harvard, where I was a visiting professor on two sabbatical stays, the second upon being granted the Severo Ochoa Professorship National Prize awarded by the Spanish government. I still have a large number of scientist friends and personal friends back in the United States, where my son Diego and my grandchildren live. I still cherish a great fondness towards that generous country convinced of the value of science, where I feel completely at home.

In the early 1970s, vacancies started to open up in Spain for several professorships in Physiology and Biochemistry, which we few associate professors that the country had available were given priority to apply for. At no time had I ever considered returning from the United States to any place but Madrid. However, my friend Benito Herreros wrote to tell me that the chair of the University of Valladolid Physiology Department had passed away, and that he had not managed to obtain the associate professor position that the university had created with him in mind. Because of this, he pleaded with me to attempt to obtain this still vacant professorship. Antonio Gallego advised me to consider the possibility seriously. From a distance, returning to Madrid no longer seemed like such an obvious or appealing choice. It appeared evident that it would become difficult for me to remain on the sidelines of the political and university-related wrangling in Madrid without distracting me from my scientific interests and family life.

At the same time, the challenge of forming my own independent research group at a smaller, lesser-known university felt tempting to me. My uncle Nicolás was a tenured ophthalmology professor in Valladolid and, bearing in mind our close family relationship, his presence there added yet another personal enticement to transfer to that university. In the end, I vied for a position as a professor of General Physiology, Biological Chemistry and Special Physiology at the Medical School of Valladolid and I got it. Early in the summer of 1973, I showed up in that city, filled with plans for the future, to discover my new destination at a university with a reputation for being very conservative. At that time I was twenty-nine years old, and I was accompanied on that visit by Roberto Gallego, five years younger than myself. When we headed for a lift marked as "For professors only", the doorman who monitored the entrance to the medical school informed us that students were not authorized to use the lift. He would remind me of that story with respectful embarrassment every time I saw him during the following years.

The department had spacious laboratories, but they were nearly empty. Benito Herreros was the only professor who did research, as an associate professor working exclusively for the school. He was trained in England, where he had very notably published and studied, under some quite precarious conditions, the biophysical mechanisms of membrane transport, with the help of a small group of bright, motivated students. The department

had two other associate professors, competent educators whose professional clinical activity had, however, prevented them from doing research. The number of students per year (biochemistry in the first, physiology in the second and soon general biology as well) approached one thousand, the teaching workload was overwhelming, forcing us to repeat the same class to three sets of students on the same day. In those years, biochemistry was a science undergoing a major scientific boom, but at medical schools it continued to be relegated to the status of "biological chemistry" within a single Department of Physiology. In order to put an end to something I considered to be behind the times in Valladolid, I asked Alberto Sols, probably the most prestigious Spanish biochemist of the day, to put me in contact with some young and worthy biochemists from his centre who might be interested in seeking a career within the university. He recommended Antonio Sillero and María Antonia Günther to me, a married couple of brilliant biochemists, and disciples of Severo Ochoa, who had been hired back at his Institute. They were using *Artemia salina* as a model to study various cell processes involved in development. After some ups and downs, including the attempt to raise funds to modernize laboratories and equipment and create one full professor's position for Benito Herreros and another for Antonio Sillero, I managed to attract them to our team. María Antonia was hired as a visiting researcher of the CSIC. Thanks to these moves, Benito was able to strengthen his group, and Antonio and María Antonia, who had come with a few of their colleagues from Madrid, quickly expanded their work, attracting new students. Being the excellent scientists they were, and even better as individuals, the three of them set up their respective lines of research in biophysics and molecular biology at the international level.

In my own case, I was aware that my personal dedication to the laboratory to instruct my students would be limited by my management obligations in what had now become the Physiology and Biochemistry Department. To make up for this, I sent the student interns to the United States as soon as they graduated and steadily accepted new students to work with me at the laboratory. Five years later, the hiring of Roberto Gallego as an associate professor in Valladolid, after lengthy training in the United States, decisively strengthened our neurophysiological research. As a task associated with managing the Physiology and Biochemistry Department, I was also assigned the organization and set-up of the clinical laboratory at the recently completed University Hospital, with more than one hundred people under my direction. During those years, I attempted to remain up to date on experimental neurophysiology, taking several summer stays in the United States and attending congresses, while at the same time I managed and supervised the work by my students and graduates. During that period, we concentrated on studying the active and passive membrane properties of chemoreceptor and baroreceptor sensory neurons, we described the pain receptors in the cornea, and we defined some of the sensory and autonomic neural components that played a role in the regulation of intraocular pressure.

The experience in Valladolid turned out to be very gratifying and enriching to me, on both a personal level – my daughter Isabel was born there – and professionally. For the first time ever, I got the chance to develop my own ideas and use what I had learned abroad inside Spain, attempting to further scientific research with the ambition of achieving an international outreach, holding meetings and visits with foreign colleagues and carrying

out scientist exchanges. My stay in Valladolid coincided with politically troubled times all over Spain and great student upheaval in that city, which had become very polarized politically. The university acted as a meeting point for those who promoted democracy. Teaching was constantly interrupted by demonstrations and protests. As a result of the conflicts, the University of Valladolid was closed down by the government for an entire academic year. In those still timid beginnings of democracy, during the time in which I held the position of assistant dean at the Medical School, I was forced to reach decisions that forced me to walk a tightrope between my desire to help promote political openness and my conviction that, despite everything, the university had to pursue its objectives and continue moving forward in terms of teaching and research quality. I had mainly accepted the position to fight for the implementation of a *numerus clausus* in the Medical School, and I quickly resigned when the government announced its refusal to back the idea.

When I left Valladolid in the summer of 1980 to move to Alicante, I did so with the private satisfaction of leaving behind a modern department with more than twenty young, bright, well-prepared researchers. Of the many students who were educated in physiology throughout that time, many became university professors of the discipline and, in turn, created fine research groups, including Constancio González, Javier García Sancho, Ana Sánchez, Arcadi Gual, Fernando Giraldez, Emilio Geijo, Miguel Valdeolmillos, Ana Obeso, Ricardo Rigual and Laura Almaraz, just to name a few of the many who did so in biochemistry, from the groups of Antonio Sillero and Benito Herreros. I also forged a strong friendship with many colleagues from the University of Valladolid that remain completely intact thirty-five years later, thanks to the loyalty that characterizes the old Castilians.

Despite having left Alicante at the age of sixteen, my relationship with that city had remained strong. That is where we would spend all our family holidays. I never seriously thought I would end up living there again, though. The possibility arose in an unexpected manner, when the first democratic government decided to increase the number of universities in the country and, to do so, take advantage of university "colleges", where some students complete the first half of certain university degrees. These colleges were run by the main university in the district. In the spring of 1979, I received a phone call from Antonio Gil Olcina, a professor of Geography in Valencia, informing me that, in accordance with that decision, the University of Alicante was being created. He had been named the rector/president from among the members of its management commission and proposed that I become the assistant rector, above all helping him set up the School of Medicine. The offer to set up a university from scratch seemed quite tempting at the age of thirty-six, because, as a full professor from one of the oldest universities in Spain, I had the impression that it was quite difficult to go any further at traditional universities than I had already managed in my seven years in Valladolid. Roberto Gallego and Milagros García Barbero were willing to move to Alicante as well. Moreover, I received encouragement, pressure and offers of help from local friends and social forces in the city. It was certainly a risky decision which, as always in our daily life, my wife and I reached together. In addition to other changes, it would mean a decrease in our family's income by nearly one half. In the end, we thought it would be better to regret a mistaken decision than to wonder for our whole lives what would have happened if we had not dared to take it. And so we moved to Alicante.

Today it is hard to imagine the effort it required of our minute team led by the rector to start up a university by transforming the university college located in the rundown barracks of an abandoned military airfield, with mostly local professors who had no prior experience at a serious university. We started out with the sole economic funding of salaries for the rector and assistant rector paid by the Spanish Ministry of Education. The university began to operate with no offices for the management team, just one secretary lent to us by the college director, low and erratic financing and the lack of any budget of our own for the first year. The work included everything from designing the curriculum, attracting professors and organizing teaching to performing maintenance on the old electricity transformer. This even meant confiscating room heaters from professors during the first winter to keep the electrical fuses from shutting off and having to suspend classes. It is only fair to point out that Alicante society made a huge effort to help us. The School of Medicine also had to be designed within an unusual context, which included a lack of career professors or a university hospital, factors then essential to attracting clinical faculty. I worked hand-in-hand with Alfonso Puchades, a former classmate from the university and a kind friend, till then the director of the University College of Alicante and a tenured anatomy professor whom we immediately named the dean-commissioner of the school. With the small group of career university professors that we recruited, we strove to complete the task of designing a new and modern medical school. We modified the existing curriculum profoundly, adding new scientific knowledge and social topics, such as economics and patient relations. We explicitly defined the objectives in terms of knowledge and the acquisition of attitudes and skills for the whole medical school programme, and we got the students in contact with hospitals and health clinics from the very first year that we offered the degree. We did away with the framework of course subjects, attempting to integrate them into more coherent areas, such as the structure and function of different body systems in health and disease, seeking to eliminate repetition while putting in place a grading system that included everything taught up to the time. One significant innovation was the creation of a Medical Education Unit, intended for the planning, coordination and execution of all the teaching activities involving professors and students. Milagros García Barbero was for years the unit's heart and soul.

For clinical teaching, we recruited all the service directors and doctors from public hospitals and health clinics and asked them to take part in drafting the objectives of all the contents in our clinical teachings. Last of all, we got the provincial government to build a new hospital oriented towards teaching, in San Juan de Alicante. I personally became deeply involved in the task of designing this hospital, along with its extraordinary architect, Alfonso Casares. We based its construction on the criteria that hospitals in the medicine of the future would be oriented more towards the availability of sophisticated diagnostic and therapeutic techniques than to having a large number of hospital beds, and on the idea that medical scientists require personal space for individual study and for research. It took years of incomprehension and political battles to get the hospital finished and instrumentally equipped, a task for which I was also responsible as the result of a personal decision by the Valencian Government Health Director in early 1997, after completing the hospital. The new medical school's curriculum was unorthodox in many ways, and in others of dubious legality, but during the end of the dictatorship, the whole university system was

undergoing a process to review its models and objectives, and that allowed us to act without too many formal limitations so that we could develop these new ideas. The first to finish their degrees at the Alicante School of Medicine began to earn the highest marks on Spain's MIR (Medical Resident Intern) national examination, and the "Alicante Model", as it was known, inspired many of the reforms in university degree plans at Spanish medical schools, put in place by the central government a few years later.

At the time when forming the medical school, we also designed its research strategy, attempting to avoid too wide a range of topics, which would lead at best to testimonial scientific work of limited quality. As a common, high-priority topic of research, we chose the neurosciences, a multidisciplinary biomedical field that was promising and relatively unexplored. We attempted to ensure that future professors, especially those of basic sciences, would be performing experimental work in neurobiology, in addition to attaining a high level of competence in their own particular teaching discipline. Roberto Gallego, leading the team of neurophsyiologists who came in from Valladolid, Jaime Merchán in morphology, Antonio García in pharmacology and José Manuel González-Ros in biochemistry were the main leaders of the groups formed within their respective areas. In the mid 1980s, taking advantage of the University Reform Act, we gave formal standing to their interaction by founding the Institute of Neuroscience (IN), run under the aegis of the University of Alicante. I was its first director.

For me, the years devoted to creating the University of Alicante and its School of Medicine constituted a period of exhilaration as well as a great personal adventure. When the stage of founding the new university had come to an end, with its first management team, I left the position of assistant rector. Attempting to culminate the medical school's consolidation, I ran in the election to become its first dean and won. As time passed, the logical internal discrepancies arose between those of us who defended the positive qualities of our peculiar teaching system and those who criticized it, because it was very demanding of professors and students. Moreover, some of the new professors resented the loss of power and personal protagonism that it led to. After five years leading that model of medical school to a great degree, I felt that my time there had come to an end and that it was up to others to take over the task. I resigned as dean and spent a sabbatical year in the United States in 1986 to jumpstart my research work once again. Of this time, I spent six months at the Retina Foundation and Harvard's Ophthalmology Department, working on the sympathetic regulation of aqueous humour secretion, and another six months in Utah, devoted to studying the modulation of articular nociceptors by hyaluronic acid.

By the time I returned, the University of Alicante, located at the San Vicente campus, had begun to create centres, degree programmes and professorships at a fast pace. The new building for the School of Medicine was being completed in the town of San Juan, alongside the new University Hospital, forming a separate campus together, to which we doctors were moved. Throughout the subsequent years, I combined my personal scientific work, which revolved around peripheral nociception, with a position as director of the Institute of Neuroscience, for which I was re-elected by my colleagues continually until I resigned in 2007. Thanks to the work of all of us researchers together, we got it to grow quickly in

quantity and quality. We were living times of great public controversy, and the university had become noticeably politicized, turning into a battleground for political parties and labour unions. The rapid scientific development, social protagonism and influence of the School of Medicine and Institute of Neuroscience started to be seen with increasing reticence by colleagues from other schools and by the university authorities. Moreover, the pressure by neuroscientists was somewhat irritating to the latter, since they required greater attention and support for their thriving research, in opposition to their propensity for the "one size fits all" approach of the university heads. As a representative of the Institute of Neuroscience on the university's governing board, I became the main voice of discord against that official scientific policy, which to me seemed based more on parochial criteria and personal loyalties than on seeking scientific and educational advancement and the cultivation of excellence. My status as a "founding member" of the university, the public acknowledgements I received across time for my scientific work, including the Jaime I National Prize (and even the naming of a street in my honour in Alicante!), but above all the steadfast support I was given by my colleagues from the Institute and School of Medicine, conferred legitimacy upon me to speak up loud and clear. That, and the envy caused by the Institute's scientific success, caused some to place stumbling blocks before us and perform other petty acts, as has occurred now and again in Spanish university life. Despite everything, Valencia's autonomous regional government (Generalitat Valenciana) approved the conversion of the University Institute of Neuroscience of Alicante into a centre with national standing at last, in 1994. Moreover, after tough negotiations with the president of the National Research Council (CSIC), the Institute became an Associate Unit of that entity, which made it possible for certain scientists from the Cajal Institute of the CSIC to move to the Institute of Neuroscience in Alicante, thereby reinforcing its growth.

The quality of the research performed at the Institute of Neuroscience and its international projection increased quickly in the second half of the 1990s. Simultaneously, it became more and more distanced from those running the University of Alicante, who constantly refused to assign funding to it in the general budgets for equipment, or to provide funds for a building of its own, despite the momentum provided by the expansion plans developed at that time. In 1995, power shifted in the autonomous regional government, and the new president of the Generalitat and the rector, from opposite ends of the political spectrum, directly faced off. The latter had proposed to the prior autonomous regional government the creation of a third campus within the University of Alicante, to be located in Elche, in order to deal with the fast rise in the province's numbers of students. However, the new government decided to found a second university that would be administratively separate from the University of Alicante, the Miguel Hernández University (UMH), the main campus of which would be located in Elche, with others in Orihuela and Altea. It also assigned the campus in San Juan, which belonged to the University of Alicante, including the School of Medicine and the Institute of Neuroscience, to the new university so that this would include two centres of acknowledged academic and scientific prestige from the very outset. The goal was for them to act as an intellectual motor. As was to be expected, this decision was radically opposed by the governing team at the University of Alicante, but it did have the support of the majority of the professors and scientists at the School of Medicine and the Institute of Neuroscience, because the two centres saw the

new university as an opportunity to do away with the blockade to which they had been subjected in recent years by the heads of the University of Alicante. These centres' assignment to the new university caused a political and social firestorm, and even an appeal of unconstitutionality based on violation of the university's independence, which was filed by fifty members of parliament of the Socialist Party. In the end, it was rejected.

I publicly expressed my position in favour of the separation, because it finally created the possibility to turn our Institute into the centre of excellence that we had aspired to build. Because of this, I was the target of furious accusations and criticism by many colleagues at the University of Alicante. Seen in retrospect, these seem to have been based more on a university power struggle, emotions and short-sighted corporativism than on any objective evaluation of the advantages that the university expansion would end up offering in terms of progress in university teaching and research in the province of Alicante, regardless of the administrative model that was chosen to run it. Though I formed part of the first Governing Board of the Miguel Hernández University (UMH) and participated in designing the university's initial stages, I kept a formal distance from its management and placed a priority on completing development of the Institute of Neuroscience by taking advantage of the now more favourable circumstances. In the following years, we turned it into a Mixed Centre belonging to both the UMH and the CSIC, thereby also raising funds to begin construction of its own building.

Shortly after the UMH was created, I was granted a national award, the Severo Ochoa Chair, which financed the award-winner's stay for one year at the domestic or foreign science centre of his or her choosing. In 1997, I decided to use it to go to the Prince of Wales Medical Research Institute and the University of New South Wales, in Australia, as a guest professor for a few months. There I worked in collaboration with E. McLachlan and J. Brock. Immediately after my sabbatical year, I remained at Harvard's Neurobiology Department along with D. Hubel and E. Raviola, and I spent the final three months at my former Physiology Department in Utah, collaborating with S. Fidone. This was a personally delightful time and was very useful on a professional level, to bring my work up to date. While in Utah, I received a call from Albert Aguayo, president of the Nomination Committee of the International Brain Research Organization (IBRO), proposing that I become a candidate for the position of this organization's secretary general. I was selected by its Governing Council in 1998 and began my work in January of 1999.

The IBRO, which was and still is the top neuroscience organization in the world, was falling behind in its adaptation to the profound changes and expansion being produced in worldwide neuroscience. My first act as secretary general was to convene a committee of five neuroscientists from around the world at our offices in Paris. I asked Torsten Wiesel, then president of the Rockefeller University and a Nobel Prize winner, to chair the committee. We designed profound reform of the IBRO's organization, decentralizing its governance structure, defining and limiting the mandates of those governing it and establishing worldwide regions with their own governance and great management independence. The secretary general then held nearly absolute executive power, so I had to set off on a rushed international tour to convince the most significant direc-

tors of the eighty-four neuroscience societies around the world that make up the IBRO that the changes we were proposing would be positive. These included the American Society for Neuroscience, the European Neuroscience Association at that time, and the African Neuroscience Association as well as national associations on every continent. Fortunately, to do so I had the invaluable support of Torsten Wiesel, who was chosen to be president of the IBRO one year later. The position of secretary general was an experience beyond compare for me, because it allowed me to meet the most notable neuroscientists from around the world, form relationships with them and get a privileged view of the diversity and advancements in brain research in very different countries as well as noticing the influence of cultural factors on research. In accordance with the rules that I myself had promoted, I left the position of secretary general, to which one could not be re-elected, after three years, and I once again concentrated on my scientific work and directing the Institute of Neuroscience, then embroiled in the battle to complete the construction and equipment of its new building, to recruit new bright and well-known researchers as group leaders and to attract other younger researchers through the then recently created Ramón y Cajal programme, so that they would become, as in the end occurred, a new generation of Spanish neuroscientists who could clearly live up to international standards.

My research work during that stage and up to today has been supported by collaboration with Juana Gallar, in the field of ocular research, and Félix Viana, in that of sensory neurobiology, along with their collaborators from the following generation. I must point out that my academic and scientific work has been rewarded, both in Spain and abroad, and perhaps in an overly generous manner, with national and international prizes, elections to scientific committees, plenary conferences, membership in academies, an *honoris causa* degree and so on. In 2006, Queen Sofía of Spain finally came to inaugurate our recently opened building at the Institute of Neuroscience. I had been its director uninterruptedly for over two decades, and I took advantage of this milestone to announce my plan to leave the position in the month of October and pass the responsibility on to Juan Lerma, the assistant director for the preceding two years. Looking back, it is of great satisfaction to me to confirm just what a good decision this was, inspired personally by the advice Cajal gave that "you should leave positions before they leave you," and because Juan Lerma also brought new ideas and initiatives to the Institute, expanding it and strengthening it inside and outside Spain, as the top neuroscience research centre in the country.

However, I was not afforded the peace and quiet one would have expected in the new situation after my resignation because in 2007 I was proposed as a candidate and elected president of the IBRO for a further two consecutive three-year terms, which yet again plunged me into the management and promotion of international neuroscience, though with a bit more calm and experience than during my prior mandate. My basic research work continued. Thanks to the support of the Botín Foundation, I opened a line of applied research that culminated with a patent and a spin-off from the Miguel Hernández University. In the past six years, I have also contributed to the founding of an experimental and clinical ocular research centre for vision sciences in Oviedo, in association with the Fernández-Vega Ophthalmological Institute.

I am writing these lines in the final days of the year 2014, having recently acquired the status of professor emeritus at my university, while still keeping my laboratory active as well as my work collaborating with colleagues from inside and outside the Institute. With the perspective given by the passage of time, I still consider scientific research to be the most entertaining and gratifying of the many I have taken part in throughout my life. My contributions may be summarized in just a few lines, after standing the test of time, and I also acknowledge that they were almost always a result of the efforts made by my collaborators more than my own.

In general sensory physiology, I am proud to have taken part in discovering that the properties of primary sensory neurons depend dynamically upon the tissue that they innervate, and that multimodal nociceptors possess a common molecular mechanism for the transduction of heat and chemical stimuli different from that used for mechanical stimuli, an observation confirmed at the molecular level by Julius with the cloning of the TRPV1 ion channel. I am also proud to have been the first to record electrical activity in an *in vivo* mammal sensory nerve ending and define hyaluronic acid's mechanism of action in articular nociceptor modulation. In recent times, we have established that the transduction and encoding of cold by thermal receptors depends upon various potassium channels, in addition to the TRPM8 channel, as well as obtaining evidence that these cold receptors play an important role in regulating moisture in the mucous membranes, especially the ocular membranes.

In the beginning, I mentioned that I did not want to follow the trend in my family of devoting my life to ophthalmology. As a neurophysiologist, I soon defined my interest in studying the mechanisms of transduction and encoding in peripheral somatosensory and visceral sensory receptors, a choice made in those early times based on the evidence that research on complex brain mechanisms would require sophisticated, expensive technology then inaccessible in Spain. Antonio Gallego decided that my doctoral thesis would be on nervous system regulation of intraocular pressure, which led me back to the eye, my family's subject of study, and that is what occurred during my collaboration with Keffer Hartline.

Upon returning from the United States, thinking about exploring whether acetylcholine was a mediator in sensory transduction, as occurred in chemoreceptors, I found out that the cornea, rich with sensory pain endings, was the bodily tissue with the highest acetylcholine content. This suggested that it mediated in nociception. Although it did not confirm this hypothesis, that study on the cornea led me, this time definitively, to the eye as an experimental model. My most significant findings in ocular research can be summarized as having defined for the first time ever the different functional types of sensory receptors in the eye and their responses to different stimuli under normal conditions and in the presence of inflammation and injury; describing the correlation between activity in these receptors and the conscious sensations of an ocular origin in humans, having developed an aesthesiometer with my name on it that is used in the real world; and having demonstrated that basal tear secretion is modulated by cold receptors on the surface of the eye.

In this perhaps too verbose description of my professional career, I have deliberately left out the emotional experiences that occurred at the same time, adding a touch of colour to my life. They include pain, immense and inconsolable, due to the sudden death of my daughter, and the deep, unconditional affection for my wife and children. There was also the great joy that came with each little achievement and the pleasure I got from the affection of the many good friends I have had the fortune of enjoying. To them, and to all the young people who have always surrounded me, teaching me through their excitement and zeal, I must express my thanks for having majorly contributed to making my life better, one which could be summed up by saying that it has never been routine or dull.

JESÚS ÁVILA

MY RELATIONSHIP WITH THE BOTÍN FOUNDATION

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Ten years ago, the Botín Foundation commenced a programme of support for biomedicine and bioengineering that was utterly novel in Spain, and with a certain resemblance to the programme of Howard Hughes in the United States. I recall very well how the project's creator, Pedro García Barreno, came to propose that I should join the programme. I reflected that in my profession there are great days, albeit few, and that the day of his visit could be counted as one of them. I was not wrong. After these ten years, I am proud to consider myself a member of the project and honoured to be considered by my colleagues, recognized by the Foundation, as one of their own.

To commemorate these ten years, Pedro sent us a letter asking for a brief biographical sketch, and he included a couple of examples to show us how to do it. You usually begin a biography saying who you are and where you come from. Not long ago, Javier Marias asked himself this question in one of his articles in the Sunday supplement of *El País* and quoted Teilhard de Chardin, who said, more or less, that we are the sum total of all those who came before us and we are all that has influenced us. Indeed, we are a small part of a society, and our existence usually depends more on society than on our individualism, as we are quite dependent on our forebears, contemporaries and successors and, to a large extent, we are a messenger who receives a torch from someone who preceded us, with the task of relaying it to someone who comes after us, as depicted by a statue in front of the Medical School of the Complutense University of Madrid.

Regarding those who came before us, I will begin with my parents. My father was from Toledo, but he lived in Madrid from the age of seven. On my mother's side, her family was from Madrid – two generations – and before that they lived in Segovia. My parents passed away years ago, and now my closest family is not from Ávila de Grado, with my parents and sisters, but rather Ávila Villanueva, with my wife Nieves and my daughter Marina.

Although I was destined to work in retail, my parents sent me to complete my baccalaureate in the Ramiro de Maeztu secondary school, a good place to learn Latin, philosophy, literature and the natural sciences. In these subjects I had excellent teachers while, in the others, my teachers were not too bad. However, it was very important to play basketball well in my school, but shorties like me did not exactly stand out in this sport. Later, I studied chemistry at the Complutense in Madrid. I had all sorts of professors. Generally, they were good, but distant. Those I liked the most were in organic chemistry and one or another in physical chemistry. After I finished my degree studies, and after a short stay at the Institute of Nuclear Studies, I began my thesis work in an entirely new field for me: molecular biology. My mentors in this new enterprise were Drs Viñuela and Salas. I continue to feel not only great affection for both, but also great respect, even though they

told me, as soon as I first set foot in their laboratory, that I should call them by their given names, Eladio and Margarita. Margarita was the director of my thesis. As a matter of fact, I was her first doctoral student. I owe her much: due to my lack of manual skills for work in experimental science, she showed me how to perform experiments and obtain reliable and reproducible results, even if I had to spend twice the time that my colleagues did – who, by the way, were good people and handier than me. The key was to check once, twice and a third time. Fortunately, the people who later worked with me on their theses or postdoctoral residences had none of my manual shortcomings. Moreover, my limitations helped me develop my memory and sense of order so as to focus my five senses on what I was doing, make notes and know what process I had to carry out.

After spending many hours in lab work, with long stays in the cold chamber at 4°C or in the fermenter room at 30°C – and receiving the occasional electric shock – I began to achieve results. I tried to combine cold, heat, moisture and electrical discharges, and I finally managed to finish my doctoral thesis on the description of the RNA polymerase *Bacillus subtilis* and publish my first paper in the journal *Nature*. In those days I worked very hard – I did later too – and I had the help of a number of colleagues from the Marañon Institute (CSIC), where I carried out my thesis. Several of these colleagues were in Eladio and Margarita's group, but I also met scholarship students from other groups on the fourth floor, from the CIB, or Biological Research Centre (CSIC), where the Institute was based. I learned quite a lot from them, and I am thankful for it.

After a brief postdoctoral stay in Eladio and Margarita's group, I moved on to the NIH in Bethesda, Maryland. There I worked under the tutelage of Dr Robert G. Martin on a virus that infected the eukaryotic cells of monkeys, the SV40, which, as did the bacteriophage Ð29 of Viñuela-Salas, had small-sized DNA, making it a simple model for studying cellular transformation or – if I may exaggerate a bit – oncogenesis. There I learned to cultivate mammals' cells, and after a few publications in journals like the *Journal of Virology* or *Virology*, I began to learn about other topics, as the NIH was the place where one could attend a good seminar at any time of day. Also, the same building where I worked hosted the group led by Dr Gary Felsenfeld, one of the most prominent groups, internationally, in the study of chromatin. Coincidentally, one of the members of the group was Richard Axel, with whom I interacted and from whom I learned many things. Along with Eladio Viñuela, I think he was one of the people whose natural intelligence has most impressed me, and I think he richly deserved the Nobel Prize in Medicine later awarded to him for studies on the sense of smell. In fact, they could have given it to him for any of the projects in which he was involved, as he was brilliant in everything he did.

After my time in the United States, I came back with an interest in researching and learning more about eukaryotic cells, how they proliferate and divide and, more specifically, about mitosis. Upon my return to the Centre of Molecular Biology (CBM) of the CSIC, directed by Eladio Viñuela, I encountered the group led by the brothers José and Marisa Salas, who were working on the cellular cycle, which was the closest thing to mitosis there was. I started to work with them until, owing to Eladio's generosity, I began to create my own group along with Víctor G. Corcés. We set to work on the majority component

of the mitotic spindle, microtubules, polymers made up of tubulin. I can still remember how Eladio bought us colchicine-H^3 to be able to describe the hog brain tubulin. He gave us that brain after obtaining the blood cells for his infection analysis with the African swine fever virus. Fortunately, our first publication as an independent group was also in the journal *Nature*, and that helped to consolidate us.

Subsequently the group grew with the arrival of more people, some of whom were more intelligent than me, although I do not think they outstripped me in enthusiasm and interest. Without them, I could hardly have achieved anything, as they were the ones who discovered and suggested how we should move forward (a table with many of their names can be found at the end). For example, in discussions with some of them, we reached the obvious conclusion that, in view of tubulin's abundance in the brain – about 20% of a soluble brain extract is made up of tubulin – it must play an important role there, and that it would be useful to work with a brain to learn just what the function of tubulin is. In any case, the idea of working with a brain had always appealed to me.

We began to analyse the microtubules taken from the brain of a hog, cow or rat, and once we were in touch with some slaughterhouses in the area – mainly for cow brains, as we usually obtained hog and rat brains from the Institute – we started to work not only with tubulin, but also with the proteins associated with microtubules, like MAP1A, MAP1B, MAP2 and so on, until we came across the protein tau. The latter would give us a lot to chew on. So our work started with a description of the possible function of microtubular proteins in the process of mitosis.[1] Later, and given that tubulin is a fundamental component of microtubules, the structure-function relationship in this protein was subjected to study and we formulated the first ever description of the importance of the carboxyl-terminal region of protein in regulation of microtubule assembly. This region is the site of interaction of proteins associated with microtubules (MAP) that facilitate microtubule assembly.[2] We also observed that the C-terminal region of the tubulin was a good target for post-translational modifications.[3] There we also found a region were inhibitors of the protein assembly would gather, like calcium.[4] We also studied, in collaboration with the group led by J. C. Zabala (University of Cantabria), a few aspects of tubulin folding.[5] In addition, we formulated a description – in collaboration with Dr Ripoll – of a new MAP in vertebrates, with a function in cellular division (mitosis).[6] This protein, called ASP, has subsequently been the subject of much study by other groups owing to its function in the development of the cerebral cortex in humans (the human protein is known as ASPM).

Given that tubulin is the most abundant protein in the brain of vertebrates, we later studied the characteristics of microtubule-associated proteins, tubulin and MAP in the brain. We studied some MAPs, starting basically with MAPIB, and we examined their possible involvement in axonogenesis.[7,8]

This work was completed with the study of the possible function of another MPA known as tau protein, in neuropathological processes, and we supplied the first ever description of tau's capacity to self-assemble, giving rise to polymers that are similar to those found in the brains of patients (paired helical filaments, PHF) suffering from Alzheimer's dis-

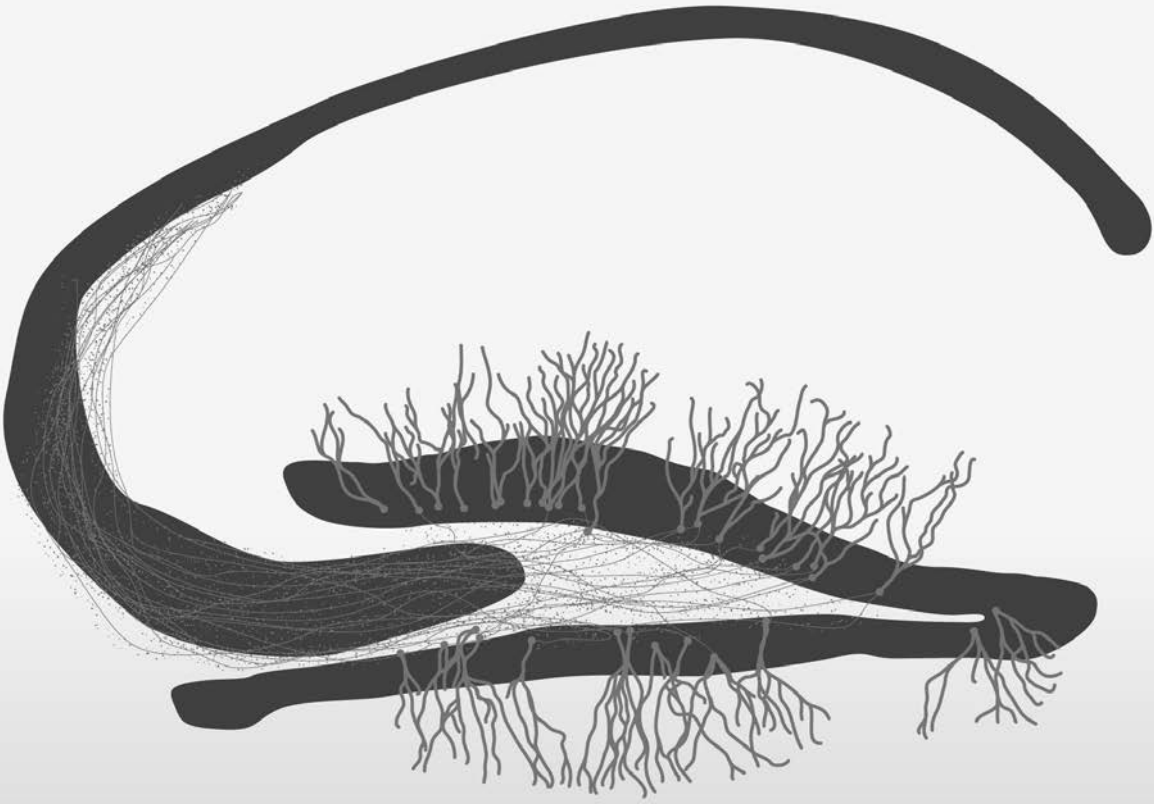
ease,[9,10] Later, we described a novel modification that occurs in this protein, called glycation, which may be involved in the aggregation of PHF in neurofibrillary tangles (NFT).[11] We observed a relationship between phosphorylation and tau aggregation. So our group developed a model of conditional transgenic mouse that overexpresses, under permissive conditions, kinase (GSK3), the protein that phosphorylates the most residues in the tau molecule. With this model, we were able to study some aspects of pathology related to tau dysfunction, based on the fact that such a dysfunction could be related to hyperphosphorylation of tau that occurs in Alzheimer's disease.[12]

More recently, we have also authored other papers that have described conditions (related to oxidative stress[13]) that may explain how phosphorylated tau shows a greater capacity to form the pathological aggregates found not only in Alzheimer's disease, but also in other dementia, known as tauopathies. The importance of phosphorylation was also discussed in another paper.[14]

Further, we have studied not only nerve degeneration, but also axonal regeneration of neurons of the central nervous system, and observed that the transplanting of ensheathing glia cells facilitates such regeneration in injured mice.[15,16] We also obtained immortalized clones of these ensheathing glia cells, and they have been patented for potential use in the future. In 2007, and in relation to the subject of neuronal regeneration in the spinal medulla, I had my first direct contact with the Botín Foundation. As a result of an interview with Pedro García Barreno, who I already had the honour of knowing, he very generously offered me the opportunity to become a scholar of the project he had conceived, for the Botín Foundation. It was a "potion and chips" project, as he had described it in a book published in 2006 by Espasa Calpe. This Botín Foundation group comprised old members of the "Severo Ochoa" Centre of Molecular Biology (CBM) who had been my collaborators (see the list of people in the table at the end). There were also friends from Madrid and elsewhere. A good group.

It was quite easy, after talking with him again, and with Paco Moreno, to be convinced that one of the best things in my entire scientific career was happening to me. I will always be thankful to Pedro García Barreno, Paco Moreno, Rafael Benjumea, Angel Durán, Lala Aguadé and so on, and to the director and president of the Botín Foundation, who treated me like another member of the family and trusted in my work.

At present, our laboratory is focused on the study of tauopathies – the most significant of which is Alzheimer's – and we have also developed new models of transgenic mice that express human tau with some mutations described in a tauopathy, frontotemperal dementia, or that express the protein along with kinases that phosphorylate tau in neurodegenerative processes like Alzheimer's.[17,18] We are also studying how tau pathology expands in Alzheimer's from the hippocampus to the cerebral cortex.[19] As mentioned, our experimental work was supplemented with more general explanations and revisions on aspects related to neurodegeneration, tau, or Alzheimer's disease,[13,20–22] or by collaborating with external consortia through the supply of materials.[23]



One of the most well-known features of Alzheimer's disease is patients' loss of memory. Such loss of memory is related to possible defects in adult neurogenesis that occurs in the dentate gyrus. Recently, therefore, we examined neurogenesis in the dentate gyrus using a mouse model (for Alzheimer's disease). We observed that there are defects in the mouse that resemble those found in Alzheimer's disease.[24,25] These defects can be reversed in young mice, but not in old ones.

Currently, we are still seeking to ascertain what mechanisms, in the molecular realm, can explain the loss of memory found in Alzheimer patients. We have undertaken, jointly with Dr Eduardo Soriano, a study to attempt to find the somatic mutations that may occur in zones of the brain that are damaged in the first stages of Alzheimer's disease. It would seem obvious that, to find genetic or somatic changes in a neurodegenerative disease, one must sequence the DNA of brain cells and not the DNA of blood cells, as has been done until now.

For the most immediate future, we are planning to undertake *in vivo* reprogramming to see if we are able to transform glia cells into neurons with the use of neurodegeneration models in mice. I do not know what I will have time to do before I retire, but I am certain that whatever I am unable to do will, indeed, be done – and better, even if it is difficult for them – by those who are ready to grab the torch. For now, I will continue to work on that silent and devastating disease that Kraepelin dubbed "Alzheimer", after one of his pupils. Lastly, and in keeping with Judeo-Christian tradition, I will say farewell in the way Pedro García Barreno would: *Shalom yom tov*. A literal translation of "Peace and goodwill" that may just be wrong. Even though I had good teachers of letters in the baccalaureate, I regret to say that I was not a very good student.

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JOAN GUINOVART

EMULATING HABAKKUK

7

I was born in Tarragona in 1947. My father died when I was two years old. I was an only child. My mother Josepa (Pepi) Cirera brought me up with great affection, but also with an ironclad determination to make sure that I would become, in her words, "a useful man to society". I grew up in a movie theatre, the Central Cinema, which belonged to my family. I did my homework in the box office with my mother. Only when I had finished would she let me go into the auditorium, so I would only get to see the end of films. I think the first expression I ever understood in English was "The End".

I went to La Salle school, the only religious school for boys in Tarragona and the alternative to what was considered the overly liberal state secondary education school. I was a good student, except in physical education. I remember nearly all the patriotic songs and religious hymns sung at that time and I can still sing the hymn dedicated to the school's founder, though off-key. I used to play football among the archaeological ruins of the Roman Forum, close to my house and completely abandoned at that time – a unique playground by anyone's standards.

Instead of becoming an engineer – which was expected of boys good at mathematics in those years – the influence of my secondary school chemistry teacher, Mr Serafín Sánchez, got me interested in that subject. I started to play with a Cheminova chemistry set and built a chemistry lab at home. I ended up in the School of Sciences at the University of Barcelona (UB). My mother, influenced by her pharmacist uncle's example, pushed me towards pharmacy. We finally agreed that I would study for two degrees. In the end, this proved to be an ideal combination because chemistry gave me a strong grounding in mathematics and physics, while pharmacy added a biological touch that was later incredibly helpful to me.

I found university terribly dull. In general, the lectures were not very stimulating, but the lab classes were fun. These were the years in which the Democratic Students' Union at the UB (SDEUB) was fighting for democracy – a fight that ended with the famous "Caputxinada", a lock-in protest of students and intellectuals at the monastery of the Capuchin Franciscans in Barcelona. We did not manage to complete a single academic year because of the frequent political altercations that led the authorities to shut down the university on more than one occasion. One year, they even threatened to cancel the academic year for all students.

The combination of chemistry and pharmacy naturally made me lean towards biochemistry. My professors in this subject were Vicente Villar Palasí and Manuel (Manolo) Rosell Pérez in the School of Pharmacy and Fernando Calvet in the School of Chemistry. From the

very first biochemistry class, I was captivated by Manolo's magic. He had just returned from a long postdoctoral period in the United States, and you could tell. He was the only professor who recommended we read texts in English, which is how I discovered *Scientific American*. When I finished my Bachelor's degree in 1969, I joined his group. At the same time Vicente Villar Palasí left to become the founding rector of the Autonomous University of Barcelona (UAB). Neither of them lived many years longer. Vicente passed away in 1974 and Manolo in 1977.

I did my thesis with Manolo on glycogen synthase in frogs. I obtained a position as a teaching assistant and received a fellowship of 10,000 pesetas per month. This allowed me to marry Rosa and pay the mortgage on a small flat near the university. My first task as a doctoral student was to travel to the Ebro River delta one weekend to find someone who could supply me with frogs. At that time frogs were considered to be an exception to the general belief that all animals had two forms of glycogen synthase. My results proved that these amphibians were, in fact, just like other animals, with both forms. The laboratory at the School of Pharmacy was quite rudimentary. In order to measure activity, we used ^{14}C-glucose, and the only radioactivity counter was housed in the main building of the university located in the centre of the city. So I had to catch the tram and take the radioactive samples for a ride! As a teaching assistant, I gave countless classes and practicums. By the end of my doctorate, I had given virtually every lesson in the curriculum. That intensive teacher training was critical for my competitive examinations to attain an assistant professorship. Along with Abel Mariné, I was one of the first PNNs (non-tenured professors) admitted to the Governing Board of the School of Pharmacy.

I did my postdoctoral work with Joseph Larner at the University of Virginia (UVA)(1974–75) supported by what was known as a "bases fellowship", administered by the Fulbright Commission and created under the "Program of Cultural Cooperation between the United States of America and Spain". This agreement was a fringe benefit of a more momentous treaty allowing US forces to establish military bases in Spain. At this time these fellowships were among the few funding programmes that allowed Spanish scientists to train abroad. In Charlottesville, I met Carlos Villar Palasí, Vicente's brother, who remained with Larner his whole life. We struck up a great friendship. I worked on the mechanism of action of insulin, seeking the hormone's unknown messenger. Joe had been trained with the Nobel Laureates Gerty and Carl Cori and had witnessed the major discoveries produced by them and their students, including regulation by phosphorylation and cyclic AMP. Joe's hypothesis was that because insulin was an antagonist of adrenaline, the former must produce a metabolite that counteracts the effects of cyclic AMP. It was a shame that such a logical hypothesis could not be corroborated because, as we now know, insulin unleashes a cascade of phosphorylations after binding to its membrane receptor. Joe taught me about the value of hard work and faith in oneself. He died recently, at the age of ninety-three, having worked until just a few weeks before his death. I am happy to have been able to take part in the homage we paid to him in 2011 when he turned ninety.

Arriving in the United States was a cultural and scientific shock. The UVA's Pharmacology Department, despite its modest size, was a hive of activity. Joe, its director, had hired a

series of brilliant young scientists. Two of them, Al Gilman and Ferid Murad, were to go on to win the Nobel Prize. The ambience in the laboratory was very stimulating, and the lights would often never go out at night because somebody was always working. I learned a lot from my colleagues, especially John Lawrence, who died relatively young in 2006, and Peter Roach, with whom I am still bound by a close friendship.

In December 1974, taking advantage of my return home for Christmas, I sat a competitive examination for tenured assistant professorships in the School of Pharmacy that had been announced in early 1973 but was undergoing a huge delay. In theory, I was just testing the waters because I was not considered to be among the favourites. The chairman of the exam tribunal was Professor Ángel Santos. Against all odds, I passed – along with José M. Culebras and Eduard Salsas – in large part thanks to the teaching practice I had during my pre-doctoral training. I was twenty-seven years old, and my life was resolved since this was a permanent position. I therefore had to finish my postdoctoral work and return to Barcelona in late 1975 to take up (formally called "taking possession of") the position, for which I was required to swear loyalty to "the principles of Franco's Movement". Shortly prior to this, the Dictator had died and my daughter Caterina was born. Then Manolo was diagnosed with lymphoma. He left to receive treatment at Johns Hopkins but passed away in January 1977. So at the age of twenty-nine I was put at the helm of the research on glycogen that he had begun. Joan Massagué was my first doctoral student. The biochemistry lab had remained mostly unchanged in my years of absence, but now there was a radioactivity counter in the school! Manolo's demise could have been the end of us, but his "orphans", Fausto García-Hegardt (who was now the boss due to his status as associate professor), Juan Aguilar, Eduard Salsas and I closed ranks and got organized to survive. One of the imaginative formulas we used to raise funds for research was by holding short clinical analysis courses. The students were normally pharmacists with their own pharmacy who wished to expand their horizons by performing such tests. With the money from their tuition, we added to the scant aid of the University Research Funds (FIU), the non-competitive source of funding for universities at that time. Fortunately, I was granted support from the Pedro Pons Foundation, the María Francisca de Roviralta Foundation and the Institute of Catalan Studies, which allowed me to start out with some momentum.

Despite the scarcity of means, the atmosphere in that basement of the School of Pharmacy was exhilarating. We were the only department in which every faculty member had done postdoctoral work abroad, and you could tell in the way we gave lectures (abusing the use of terms in English), the way we treated students (on a first-name basis) and even in the way we dressed (no ties). We were undoubtedly the epitome of change towards modernity occurring all over the country at that time, and we dared to confront the system by giving the first course in Catalan as well. Because we were an appealing department, we systematically managed to recruit the best students and get a large share of the graduate fellowships. We were also the first to obtain competitive funding from the Advisory Committee for Scientific and Technical Research (CAICYT), which had been recently created by the government. Our achievement caused much scandal among the old professors, who could not understand how a bunch of whippersnappers had raised funds that escaped the oversight of the governing board of the School. A generation of scientists were trained in

that department, with a great impact on domestic and international biochemistry. The reason for this was that, despite the hardships, our values were clear and we strove to apply the international standards we had learned about during our postdoctoral training. We knew what had to be done and how to do it. We just lacked the means. During this period I started up projects on the regulation of glycogen synthase by phosphorylation, both *in vivo* and *in vitro*.

In 1983 I transferred to the UAB, on a secondment, to start up the Biochemistry Unit at the new Veterinary School. With me came Carlos Ciudad, Fàtima Bosch and, later on, Joaquín Ariño. Together, we set up a small but decently equipped lab, and we trained future veterinarians. We brought about the innovation of including a pioneering clinical biochemistry course and set up a clinical pathology service for the veterinary hospital. At that time we devoted much effort into studying the effect of different sugars on glycogen metabolism and that of insulin mimetic agents such as lithium and vanadate. While completing his thesis, Joan Enric Rodríguez discovered the insulin-like effects of tungstate – a topic in which I was to work for more than twenty years, collaborating in large part with Ramon Gomis, an "illustrious son of the city of Reus" and the last Renaissance man, who perfectly combines his medical practice with research on diabetes and a writer of literature and theatre pieces.

After several failed attempts to obtain an associate professor position elsewhere, in 1985 I was granted a professorship at the UAB, and in late 1989 I once again competed for a professorship at the UB, this time in the department assigned to the Schools of Chemistry and Biology, where I was warmly welcomed. I yet again set up a new laboratory, this time with the help of Anna María Gómez-Foix. Throughout those years, Roberto Fernández de Caleya called me into the National Agency for Evaluation and Prospective (ANEP) to serve as executive secretary of the Molecular and Cellular Biology Study Section. This opportunity provided a crash course in scientific policy, in which I learned that excellence is the only valid criterion for science. I coincided there with other "captains" who would later play key roles in Spanish science: Ernest Giralt, José López Barneo and Bernat Soria, among others. My successor was Jesús Ávila. One of my duties in the ANEP was to organize a prospecting session that consisted of brainstorming about the future of biochemistry and molecular biology – an event held in Malaga. Participating in the session were Lennart Philipson, director of EMBL, Bernardo Nadal-Ginard, then at the peak of his career, and other European and American figures, including Joan Massagué, whose star was already shining brightly, as well as Àngel Pellicer, my childhood friend and the best scientist ever to come out of Tarragona. One of the conclusions reached in that session was that the borders between disciplines had to be broken down, adopting a cross-disciplinary approach. This is how the concept for a new type of research centre was devised, which Roberto christened "bioleches" (the "bio-whatever" centre). After several attempts, the concept finally took shape many years later as the Institute for Research in Biomedicine (IRB Barcelona).

In 1992, Barcelona's Olympic year, my whole family moved to San Francisco and I took a sabbatical year at the University of California San Francisco (UCSF) with Robert Fletterick, the crystallographer who had published the structure of glycogen phosphorylase and had cloned human glycogen synthase. My project was to determine its structure. This goal

was undoubtedly overly ambitious because the structure of this elusive molecule had to wait another twenty years to be solved. My alternative project consisted of studying the effect of a series of specific mutations on the allosteric properties of phosphorylase. In 2005, in collaboration with Ignacio Fita (IBMB-CSIC) and Joan C. Ferrer (UB), we determined the structure of the glycogen synthase of *Pyrococcus abyssi*, which helped remove the thorn left in my side during my sabbatical year.

Upon my return from San Francisco, I was commissioned with organizing the congress of the Federation of European Biochemical Societies (FEBS), which was to be held in Barcelona in 1996. It was quite a success and, thanks to that, I was appointed meetings counsellor and member of the FEBS Executive Committee – a position that I held for nine years. That same year, I began my term as president of the Spanish Society for Biochemistry and Molecular Biology (SEBBM). With the members of the board, we gave the society great momentum so that it would become the most powerful and influential of Spain's scientific societies. We also built bridges with Latin American societies, particularly those in Argentina and Chile, which remain strong today, and we established ties with those in France and Italy (with joint symposia in Marseille and Alghero). These alliances were made possible thanks to a committee on which Athel Cornish-Bowden and Marilú Cárdenas served. From 1995 to 2001 I was the director of the Biochemistry and Molecular Biology Department of the UB. We were awarded the City of Barcelona Scientific Research Award, a distinction not obtained by any other university department. Throughout this period I channelled great effort into studying the effects of sodium tungstate. In 1995 we patented its use as an antidiabetic agent. That was the UB's first patent to be transferred to a company. Bayer Pharmaceuticals took the compound into phase two for the treatment of obesity. Unfortunately, a lack of funding made it impossible to repeat the clinical trials at higher dosages and over longer periods of time. I continue to be fascinated by the enormous capacity of tungstate to normalize many of the alterations observed in a wide range of disorders.

In 2001, together with Joan Massagué, I began the arduous ordeal of building a research centre that would meet international standards and that would be located in the Barcelona Science Park ("Parc Científic de Barcelona"). It cost us "blood, sweat and tears", and we even fell to "friendly fire"; however, in October 2005, IRB Barcelona was finally founded as an independent legal entity. Thus began the unstoppable take-off that would lead us to become one of the first recipients of the Severo Ochoa Distinction for Excellence (2011), awarded by the Spanish government, as well as of the Narcís Monturiol Award, given by the government of Catalonia one year later. Today, IRB Barcelona is acknowledged as one of the major research centres in Europe. Thanks to its state-of-the-art installations and the help I received from the Botín Foundation, my research group has been able to tackle subjects that I could never have imagined possible. Through the use of genetically modified mice and flies, I have demonstrated that there is "good" glycogen but also "bad" glycogen. Mar García-Rocha, Jorge Domínguez and Jordi Duran have been the cornerstones of my laboratory throughout these years. Thanks to Marco Milán (IRB Barcelona), I have discovered the great potential of the common fruit fly for model human diseases. Also, due to the influence of colleagues in the institute's oncology programme, in particular Roger R. Gomis, I have delved into the metabolism of cancer.

In March 2004 I was elected president of the then recently created Federation of Spanish Scientific Societies (COSCE). The first action I took was to draft the CRECE report, with proposals from the whole of Spain's scientific community to reform the country's research and development system. The document was handed to the vice-president of the Spanish government at the presidential headquarters in the Palacio de la Moncloa. I also started up the project CONOCEROS for politicians and scientists to gain mutual awareness, and the ENCIENDES project, which sought to promote the teaching of science in secondary schools. When I left the presidency in 2011, COSCE had become the recognized voice for the scientific community. From 2010 to 2012, I was the treasurer of the International Union of Biochemistry and Molecular Biology (IUBMB), and I was voted president-elect of the same in 2012. As a result, I will take up the position of president as of 2015.

I am particularly proud to have revamped the old *Boletín SEBBM*, turning it into a journal of prestige and a point of reference on topics in science and society. I am also very satisfied with my activities to promote scientific vocations among young people. Along with Josep Maria Fernández-Novell, in 1997 I organized a series of courses called "I love Biochemistry", for secondary school students, as well as short courses for their teachers. These courses have been given uninterruptedly at the UB ever since. Sadly, our attempts to export the model to other universities did not get beyond the first few editions. We also published a handbook on research projects to be performed at secondary school, which was a pioneering initiative. I have recently launched the programme "Crazy About Biomedicine", which allows secondary school students to spend their Saturdays at IRB Barcelona working under the supervision of our PhD students. The concept has spread to other fields and institutions, and I trust that this type of course will be offered by universities and research centres in most disciplines in the future.

I have received some honours that I probably do not deserve, including the Narcís Monturiol Medal and the Cross of Sant Jordi from the government of Catalonia, and the FEBS "Diplôme d'Honneur". My dual membership in both the Royal National Academy of Pharmacy (Institute of Spain) and the Catalan Academy (the Institute of Catalan Studies) makes me an oddity. Above all else, however, I consider myself privileged for having had the chance to work with excellent students and postdoctoral fellows, without whom I would not have made it this far. They are smart, bright, daring individuals, and I believe my only slight merit has been in giving them opportunities to nurture their talent. For me, it is an honour to be considered a mentor of such brilliant scientists. Recognition of this role is my best reward.

My hobbies include sailing my boat, *Es Sipió*, around the Cap de Creus and hikes through the Toran Valley in the Val d'Aran. I am captivated by southern Chile, "the world at the end of the world", which I got to see thanks to my good friends Ilona Concha, Juan Carlos Slebe and Alejandro Yañez, in Valdivia, and Rafael Vicuña, Vicky Guixé, Jorge Babul, M. Inés Vera, Manuel Krauskopf and many others, in Santiago. In them I have found true "brotherly spirits and bright souls". But above all, I love to wander through the old part of the city of Tarragona, my hometown, which named me "favourite son". I am fascinated by contemplating the statues commonly known as "the apostles" on the portico of the

cathedral. According to tradition, every turn of a century one of them falls off and when none are left the end of the world will be upon us. I feel great fondness for Habakkuk, the second on the left on the Gospel side. He can be identified by the passage "Domine audivi", taken from his book, written in his phylactery. He is the only one of the twelve minor prophets to have been given a statue among a very distinguished set of personages, including the evangelists, major prophets and other high-ranking biblical characters such as Moses, King David and John the Baptist. That is quite a feat! I believe that my position among the beneficiaries of the Botín Foundation resembles that of Habakkuk on the portico of Tarragona's cathedral.

Studying the metabolism of glycogen

Most of my research has been devoted to studying the metabolism of glycogen. The findings in this field were later shown to be of general significance in biology. For instance, the discovery of enzyme regulation by phosphorylation was initially made by studying the control of glycogen phosphorylase. The second protein whose activity increased upon phosphorylation was found to be phosphorylase kinase while the third was glycogen synthase, although in this case it was inactivated when phosphorylated. Glycogen synthase was the first protein in which multiple phosphorylation sites were demonstrated, later leading to the concept of hierarchical phosphorylation. Today phosphorylation is known to be the most commonly used reversible mechanism to control the activity of biological molecules. Glycogen synthase kinase-3 (GSK-3) is another fine example, because it participates in diverse processes, though its name clearly reveals how it was discovered. The concept of the enzyme cascade was also developed by studying the activation of glycogenolysis. Today this concept is a fundamental part of all signal transduction mechanisms. The notion of secondary messengers also arose from research into the effect of glycogenolytic hormones, which led to the discovery of cyclic AMP. G proteins were also identified in a study addressing the effect of adrenaline on adenylate cyclase. Why were so many discoveries of great significance made precisely in the field of glycogen metabolism? The explanation is probably that a series of outstanding scientists coincided in this field, and their work was later acknowledged with the Nobel Prize: Carl and Gerty Cori, Luis F. Leloir, Earl Sutherland, Edwin Krebs and Edmond Fisher, and Alfred Gilman. I am certain that glycogen metabolism still has some pleasant surprises in store for us and that it will be a field that continues to produce important discoveries.

Glycogen accumulation in mammals is a physiological response to an increase in blood glucose concentration after the ingestion of food. The metabolic pathway, which allows the addition of new glucose residues to a growing glycogen chain and involves the successive action of a series of regulatory enzymes and proteins, has been extensively studied. Glycogen synthase (GS), an enzyme discovered by Luis F. Leloir, catalyses the addition of glucose units to the non-reducing end of a nascent glycogen chain through α-1,4-glycosidic bonds using UDP-glucose as a substrate to provide glycosyl residues. This enzyme, which catalyses the key step in glycogen synthesis, has been found to exert the greatest control over the pathway. In fact, GS activity is highly regulated through phosphorylation in many residues and by allosteric effectors, mainly glucose-6-phosphate. However,

recent advancements have shown that the control of glycogen deposition is not performed exclusively by GS and that other factors should be taken into consideration. Moreover, the mechanisms by which glycogen is regulated in the muscle, liver and brain differ.

The structure of liver GS

Determining the structure of GS has been a major challenge. Given the great difficulty in purifying and crystallizing mammal GS, we addressed the crystallographic structure of *Pyrococcus abyssi* GS because it is the smallest known enzyme in the retaining glycosyltransferase family. The quaternary structure revealed that this protein adopts a flat radial trimer form, through the interaction of the N-terminal domains of each monomer. The monomer comprises two domains with Rossmann-type folding common to other glycosyltransferases. It is at the intersection between the two domains where we find the catalytic domain.[1] Thanks to the alignments of protein sequences from the eukaryotic and prokaryotic GS available in databases, we were able to determine that the glutamic residues corresponding to those in positions 510 and 518 in human muscle GS are well preserved throughout the entire evolution of glycosyltransferases and are key residues in catalysis.[2] We also demonstrated that both *Pyrococcus abyssi* and human GS have a binding site for glycogen that differs from the active site and that the former plays a key role in the function of the enzyme.[3]

The activation of hepatic GS

A basic question about liver glycogen metabolism is how an increase in blood glucose triggers the activation of hepatic GS. Preliminary evidence from our laboratory indicated that glucose must be phosphorylated to glucose-6-phosphate in order to be able to induce the activation of liver glycogen synthesis.[4,5] We then discovered that the production of glucose-6-phosphate by glucokinase was necessary for the activation of GS[6] and that, as a result, glucokinase exerts a high level of control over liver glycogen synthesis.[7] Therefore, we proposed that glucose-6-phosphate must, in turn, be considered a precursor and signalling molecule that directs the uptake of glucose to glycogen. This mechanism was not consistent with that proposed by a powerful research group at the Catholic University of Leuven, and it has taken many years for this notion to be accepted. Recently, in collaboration with Kei Sakamoto (Nestlé Institute of Health Sciences, Lausanne), we have demonstrated that, in fact, the activation of hepatic GS by glucose-6-phosphate is the process that most greatly contributes to glycogen deposition *in vivo*.[8]

Intracellular location of enzymes

My laboratory has also described how GS changes its sub-cellular location in response to glucose – an event that constitutes an additional control mechanism. Muscle GS is concentrated in the nucleus when cytoplasmic glycogen breaks down, and when glucose concentration increases, it once again translocates to the cytosol, where it shows a particulate pattern as a result of its binding to the glycogen particles that it generates.[9,10] On the contrary, hepatic GS displays a diffuse distribution in the cytosol in the absence of glucose

and accumulates in the periphery of the hepatocyte when hexose concentration rises.[11] Glucose-6-phosphate is not only responsible for the activation but also for the translocation of GS. The changes in the intracellular distribution of hepatic GS induced by glucose correlate with the stimulation of glycogen synthesis. In hepatocytes, the nascent glycogen particles concentrate near the plasma membrane, and the new glycogen initially synthesizes only at the periphery of the hepatocyte.[12] Afterwards, these glycogen stores grow from the periphery towards the inside of the cell, forming a crown that gradually widens as incubation time with glucose lengthens. Even so, at least while there is net accumulation of glycogen, the synthesis of the polysaccharide remains active near the plasma membrane. The glycogen that has been first synthesized is displaced towards the centre of the cell, and the recently synthesized molecules take its place. The distribution of GS follows the same pattern as glycogen, suggesting that, after the initial movement towards the cell cortex – and as long as glycogen synthesis remains active – the GS remains bound to glycogen.[13] Glycogen breakdown also proceeds in an orderly manner. The orderly deposition and breakdown of this polysaccharide may constitute a functional advantage in the metabolism of this molecule, or may simply allow the liver cell to store large amounts of glycogen or both.

Studies on tungstate

Throughout our research, we proposed that insulin-mimetic compounds might contribute to clarifying the mechanism of action of this hormone in the activation of hepatic glycogen deposition and to discovering new therapeutic targets for the treatment of diabetes. A combination of rational thinking and luck led us to test the effects of tungstate first on cultured hepatocytes and later on diabetic animals. This element is not abundant in nature, although it forms part of some enzymes in anaerobic bacteria. Surprisingly, tungstate displays potent antidiabetic activity without associated toxicity. In this regard, the antidiabetic effects of this compound *in vivo* were described by my laboratory in 1994 in rats made diabetic through a streptozotocin injection (STZ).[14] Oral treatment with tungstate reduced glycaemia and normalized the ingestion of food and water in these animals. It also normalized liver glycogen levels, which are decreased in diabetes. Moreover, this compound had insulin-mimetic effects on glycolytic and gluconeogenic pathways. Experiments involving long-term treatment (eight months) with tungstate showed no reduction in its therapeutic effects. The treatment, in addition to increasing the survival of the diabetic animals, prevented the complications of diabetes, such as vacuolization of the tubular epithelium in the renal cortex and degeneration of the cornea.[15] In healthy rats, tungstate did not display any significant effect on the parameters of hepatic glucose metabolism, and in no case did it induce episodes of hypoglycaemia.

Tungstate is active not only in type 1 diabetes models but also in type 2 models, such as nSTZ rats.[16] The main cause of the moderate hyperglycaemia in these animals is a diminished response of pancreatic β-cells to the glucose stimulus. The administration of tungstate to these animals normalized glycaemia, restored the capacity of β-cells to respond to glucose, and also caused an increase in the insulin content and β-cell mass.[17] In other words, treatment with tungstate regenerated a stable, functional population of pancreatic β-cells, leading to the maintenance of normoglycaemia in these animals.

Models of diabetes induced by STZ only partially mimic the disease in humans. In contrast, ZDF (Zucker-Diabetic-Fatty) rats, a genetic animal model for type 2 diabetes, show a physio-pathology that most closely resembles the disease in humans. In ZDF rats, treatment with tungstate reduced the rise in hyperglycaemia observed in untreated animals, in addition to delaying its appearance by three weeks. At the same time, it drastically decreased hypertriglyceridemia and thus lipotoxicity.[18] Tungstate is therefore also active in genetic models of diabetes.

More in-depth research into the molecular targets of tungstate was required to better understand its mechanism of action and thus advance towards the development of antidiabetic drugs. The working hypothesis was that tungstate either directly or indirectly affected one or more of the components in the insulin-signalling cascade and that these effects drive the antidiabetic action of this compound. Within this context, we observed that tungstate did not change the phosphorylation status of the insulin receptor β subunit, nor did it block or delay its inactivation by dephosphorylation. On the other hand, a clear increase was seen in the phosphorylation of the mitogen-activated kinases ERK1/2, consistent with a temporary activation of these molecules. The phosphorylation of ERK1/2 is needed for tungstate to exert its effect on glycogen accumulation in hepatocytes, as observed in experiments with inhibitors of the phosphorylation of these kinases.[19] Treatment with tungstate affects the proteins involved in glycogen metabolism and the accumulation of this polysaccharide without altering the proliferation of the cells. Therefore, the metabolic effects of ERK1/2 activation in response to tungstate prevail over the mitogenic effects. As for the mechanism through which tungstate promotes glycogen synthesis, we observed that the treatment increases the GSK3β phosphorylation in consensus 9 serine residue. This modification inactivates this kinase and promotes the activation of GS, which explains the stimulation of the synthesis of the polysaccharide. It is important to point out that the phosphorylation of GSK3β by tungstate treatment is not caused by the activation of the PI3K-PDK1 and PKB/Akt cascade, the main pathway responsible for GSK3 phosphorylation in the insulin-signalling mechanism. Moreover, both GS activation and GSK3β phosphorylation induced by tungstate are dependent on the phosphorylation of ERK1/2 triggered by this compound. This observation thus indicates that there is a connection between the phosphorylation of ERK1/2 and GSK3 and the increase in glycogen synthesis in cells treated with this compound.[19]

However, since it was found that ERK1/2 are not the primary targets of tungstate, it was therefore necessary to analyse the proteins located upstream of ERK1/2 in the signalling cascades in order to identify those modified in response to this compound. We found that tungstate triggers Ras activation, and that the phosphorylation of ERK1/2 and GSK3β are dependent upon G proteins. The disruption of the signal through G proteins prevents the activation of the Ras/ERK cascade and the increase in glycogen synthesis caused by tungstate.[20] The most innovative part of this research is probably the description of a mechanism involving G proteins able to decrease glycaemia without activating the insulin receptor. Therefore, these results provide novel data that reveal ERK1/2 and G proteins as potential targets for new antidiabetic drugs.

In other studies performed in collaboration with Ramon Gomis, at the IDIBAPS and UB-Hospital Clinic, we observed that oral tungstate administration reduces obesity in animal models. This compound enhances the expression of UCP1, the most important thermogenic protein in rodents, thereby increasing energy expenditure.[21] Tungstate also induces the expression of genes related to the transport and oxidation of fatty acids in adipose tissue. All of these observations suggested that tungstate could be an effective agent for treating obesity in humans. However, phase 2 clinical trials did not detect a significant effect, probably due to the fact that the dosages tested were too low and the treatments excessively short.

In patients with Alzheimer's disease, GSK3 is abnormally active, which translates into an increase in the phosphorylation of the protein associated with microtubules, known as tau, thus contributing to its aggregation in the form of the so-called neurofibrillary tangles. Along with amyloid deposits, these tangles are a hallmark of this disease. Given that tungstate was observed to induce the inactivation of GSK3, we explored its capacity to prevent hyperphosphorylation of the tau factor, the formation of neurofibrillary tangles and, therefore, the neuronal degeneration associated with Alzheimer's disease. This project was a collaboration with Jesús Ávila at the "Severo Ochoa" Centre of Molecular Biology (CBM-CSIC). First of all, we determined that treatment with tungstate had effects on neurons similar to those observed in other cell types, including the inhibition of GSK3, which was dependent upon the activation of ERK1/2. We then observed that, as a result of the aforementioned GSK3 inhibition, there was a significant decrease in phosphorylation of the tau factor in the residues targeted by this kinase.[22] As a result, as a potential inhibitor of neurofibrillary tangle formation, tungstate may have the capacity to prevent the neuronal death associated with Alzheimer's disease. Tungstate is also effective in muscle, as reported in collaborative studies headed by María Dolores Girón and Rafael Salto, at the University of Granada.

Glycogen and ingestion

Diabetic patients show an impaired capacity to store glucose in the liver, a factor contributing to hyperglycaemia. In studies with diabetic rats, we noted that by restoring the capacity of the liver to synthesize glycogen, the animals showed a decrease not only in glycaemia but also in appetite.[23] We recently demonstrated that healthy mice who have large hepatic glycogen reserves gain less weight even when offered a high-fat diet. By analysing the expression of two neuropeptides associated with appetite control in the hypothalamus, we observed that these mice displayed less expression of neuropeptide Y, an appetite stimulant, whereas they had high levels of the peptide propiomelanocortin, an appetite depressant, thus indicating satiety. There was a perfect correlation between glycogen levels in the liver and levels of satiety molecules in the brain. However, it was necessary to identify the glycogen sensor in the liver that transmits the signal to the brain. We found that the key to the liver-brain connection lies in hepatic levels of ATP, which are decreased in diabetes and obesity but become normalized when glycogen increases.[24] This finding allows us to postulate that strategies to increase liver glycogen production would be effective for improving diabetes and obesity.

Lafora disease: a new unexpected horizon in glycogen research

When it seemed that we knew everything there was to know about the mechanisms regulating GS, we discovered a new one that acts by controlling the degradation of the enzyme. This finding was made possible thanks to the study of a rare condition named Lafora disease, also called Lafora progressive myoclonic epilepsy. This condition is a neurodegenerative disease characterized by the presence of intracellular deposits known as Lafora bodies, made up of aberrant glycogen. It was described by a Spanish doctor, Gonzalo Rodríguez Lafora (1886–1971). The disease is caused by recessive mutations in the gene that codes for a protein with dual phosphatase protein activity capable of interacting with carbohydrates, known as laforin, or the gene that codes for a protein known as malin and that has E3 ubiquitin ligase activity. The molecular mechanisms through which mutations in these genes give rise to the disease had not been established, whereas the physiological role of laforin and malin is still controversial. In collaboration with Santiago Rodríguez de Córdoba (CIB-CSIC), we demonstrated that malin and laforin form a complex that regulates the synthesis and deposition of glycogen in neurons.[25]

We also showed that laforin and malin play a direct role in the formation of Lafora bodies. Therefore, the mice in which we had deleted the malin gene accumulated Lafora bodies (aberrant glycogen), suffered from epilepsy and displayed neurodegeneration, thereby reproducing the natural history of the disease in humans.[26] Moreover, using genetically modified animals unable to synthesize glycogen in the nervous system, we demonstrated that the accumulation of the polysaccharide causes the disease, because when glycogen synthesis is blocked, the disease does not develop.[27] In other animals in which the ability to accumulate glycogen in certain neurons is increased, we observed that neurodegeneration is induced.[28] Likewise, the presence of inclusions known as *corpora amylacea* – similar to Lafora bodies – in the brains of older humans and animals suggests that glycogen accumulation also plays a role in the neurological deterioration associated with age.[29] In such cases, GS becomes a possible therapeutic target, because its inhibition should prevent the formation of Lafora bodies and *corpora amylacea*. The challenge is to find products able to inhibit the enzyme and cross the blood-brain barrier.

One unexpected consequence of these studies has been the demonstration that neurons have the enzymes required for glycogen metabolism. Because neurons do not normally accumulate glycogen, it was taken for granted that the polysaccharide played no role in these cells; however, we have recently demonstrated that neurons have active glycogen metabolism and that it has a relevant function under stress conditions such as hypoxia.[30] This finding forces us to re-examine commonly accepted theories about the energy metabolism of the brain.
This line of research comes with a corollary. Studying a rare disease has led us to the discovery of a new mechanism for the regulation of glycogen metabolism whose existence nobody was able to foresee. And vice versa, knowledge about the cellular mechanisms for glucose storage has shed light on a pathological condition, Lafora disease, which was not suspected to be related to glucose metabolism.

Between 2005 and 2009, my research benefited from funding from the Botín Foundation. Without this support, we would not have been able to generate many of the genetically modified animals used in these projects – models that pave the way to further studies on neurodegenerative diseases. Hence, my most sincere gratitude to the Botín Foundation.

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CARLOS LÓPEZ OTÍN

LIFE AND DISEASE IN THE GENOMIC ERA

18

I was born in the last month of an especially cold year in Sabiñánigo, a small village in the Pyrenees region of Aragon, in the midst of an enthralling natural environment that aroused in me a lifelong curiosity. After the Spanish Civil War, my town grew in a disorderly, haphazard way, clustered around a number of chemical factories that eagerly exploited our most abundant resource: water. In that period one had few opportunities to go to university, but my family always held in high regard values such as hard work, tenacity and strong commitment. Driven onward by these values and by the teachers in what was then called the Labour Institute, I began my studies in chemistry at the University of Zaragoza, with the idea of returning home one day to work in one of those factories, where the workers lived in town and the directors were outsiders. My early mentors' vision seemed clear: to foster social progress through study. On my first day of class in Zaragoza I met a professor whose name appeared to be taken straight out of a novel of manners, Horacio Marco. He was tough and demanding with his students, but he was hugely inspirational. His biology lessons opened my eyes to a way of studying life that was unknown to me. He brushed aside my plan to take my academic pursuits in the direction of medicine, and he urged me to move to Madrid to study biochemistry and molecular biology. The most important decisions in our lives are taken by others, not by us: one day, at the end of the summer of 1978, I boarded a train in Aragon and, after a very long journey, made it to the capital of Spain. There, in the Complutense University, I met extraordinary professors like Margarita Salas and José G. Gavilanes, who decided my calling for science once and for all.

I carried out my doctoral thesis in the Ramón y Cajal Hospital in Madrid under the guidance of Enrique Méndez, and then I worked with Eladio Viñuela in the "Severo Ochoa" Centre of Molecular Biology (CBM) in Madrid. They instilled in me concepts like intellectual curiosity and experimental rigour, which have marked my research work ever since. At different times of my scientific career, I have also worked in the universities of Lund (in Sweden), New York and Harvard, but the majority of my professional work has been carried out in the University of Oviedo, which I joined in 1987 and where I found my place in the world.

Proteolytic systems

In the very place where I am writing these words, we began to very modestly incubate a project that sought to explore the idea that proteolytic systems might play a key role in the progression of cancer. The basis for this decision was laid by the teachings of my mentors, who enlightened me on the foundations of hypothesis-driven science. In our case, the initial hypothesis was to imagine that proteases could open tissue pathways

to allow tumour cells to be not only selfish and immortal, but also travelling entities capable of migrating to multiple, distant sites in the primary tumour and generating the metastases that represent the ugliest face of malignant tumours.

As we further examine the key aspects of the founding hypothesis of our laboratory, we should recall that proteolysis is a process that must be catalysed by a complex group of enzymes called proteases, given the well-known stability of the peptide bond. Initially, these proteins were associated with non-specific reactions of the protein catabolism and they were studied solely from this perspective for several decades. However, the passage of time and biological experimentation broadened our view of the proteolytic universe to an extent that would have been inconceivable to its pioneering explorers. Indeed, we now know that proteases, by means of selective cuts in the relevant substrates, execute irreversible reactions that decisively influence multiple biological processes, such as embryo development, tissue remodelling, angiogenesis, apoptosis, fertilization, blood coagulation, immune system function or the establishment of neural pathways. In view of this multiplicity of functions, it is no surprise to find that many changes in the structure or expression of proteolytic enzymes are associated with numerous pathological processes, including cancer, arthritis, cardiovascular alterations or neurodegenerative diseases. Moreover, many micro-organisms, such as the AIDS virus, use proteases as virulence factors, thus making these enzymes a target for the design of new medications. Hence, there has been a growing interest in recent years in identifying and characterizing the multiple components of proteolytic systems that operate in living beings, from bacteria to humans.

It was precisely this proteolytic diversity that structured the initial work of our group at the University of Oviedo. After formulating the first hypotheses on tumour proteases, we sought to progress in analysing their functional involvement in cancer and other pathologies. Our studies led us to the identification and biochemical characterization of more than sixty new human proteases, which were discovered as a result of their overexpression in different types of tumours or owing to their participation in processes of tissue remodelling that bear a certain resemblance to tumour processes. Having identified these new human proteins, our work centred on broadly characterizing them functionally so as to lay the basis for defining their role in cancer or in other diseases. We also sought to analyse the mechanisms that regulate their appearance in both normal and pathological conditions. Our study of these regulating mechanisms furnished us with new explanations of the etiopathogenesis of neoplastic diseases and other processes, such as arthritis, in which these enzymes are also overexpressed.

The pathological significance of the new human proteases identified in our laboratory largely appeared to arise from alterations in their spatio-temporal patterns of expression during the development of cancer and other pathologies. However, subsequent studies found that congenital deficiencies in these new genes caused different hereditary diseases, including bone and haematological anomalies, or devastating accelerated ageing syndromes. So a leap occurred in the pathological significance of proteins whose functional diversity began to call for new technologies that would enable us to navigate through their growing complexity with a degree of confidence.

Of mice and men

Molecular biology was making advances and, under the aegis of the properties arising from DNA's elegant double-helix structure, new technologies emerged that broadened our way of dealing with biological problems. In about 2000 our group took the initial steps towards studying proteolytic systems based on the generation of animal models that involved overexpression or elimination of protease-coding genes. We thought these approaches might help us achieve a better understanding of the participation of these enzymes in the development of cancer and provide new ideas about their physiological functions. This work, which is still under way, and which will doubtless continue for several generations of students, has indeed allowed us to demonstrate that certain proteases originally discovered in our laboratory due to their alterations in cancer played a decisive role in processes as diverse as iron metabolism, bone formation, the perception of pain or an organism's regulation of ageing.

As an example of this work with animal models, the generation and analysis of mice deficient in the metalloprotease FACE-1/Zmpste24 led us to the discovery of its central role in the formation and stabilization of the nuclear envelope. Experimental work with these animals took on an added dimension with the discovery that mutations in the gene of this metalloprotease or in the lamin A substrate cause a number of accelerated ageing syndromes in humans. Curiously, in recent studies with *Zmpste24*$^{-/-}$ mice, we have observed that the extraordinary premature ageing shown by these animals is associated with the chronic hyperactivation of tumour-suppression pathways, thus lending support to the idea of antagonistic pleiotropy between ageing and cancer. Likewise, we have described the existence of deep alterations in the morphology and regulation of the stem cells of these progeroid mice, which substantiates the hypothesis that ageing arises from dysfunctions in these stem cells. Lastly, we have demonstrated that the dramatic phenotype of the premature ageing of these mice can be alleviated and even completely corrected through strategies of genetic manipulation or through drug treatments aimed at lowering levels of accumulated prelamin A in their cells. These findings have brought about the first therapies for patients with Hutchinson-Gilford Progeria Syndrome, who are now being treated with a combination of statins and biphosphonates designed in our laboratory in collaboration with Dr Nicolas Levy. We strive to alleviate the multiple physiological deficiencies of these patients, improve their quality of life and lengthen their lives as much as possible. This research, coupled with similar work being conducted on a number of progerias, also represents further proof that processes as complex as ageing can be subjected to profound and rigorous analysis that seeks to understand their fundamental molecular properties.

Omic languages and biological regulation

The findings made in these studies in "mice and men" represent clear examples of the need to maintain the enzyme activity of proteases at the right level. In excess, it may further induce tissue destruction such as those that accompany cancer, and a proteolytic deficiency can lead to the development of other pathologies that can also compromise patients' lives. These ideas are the basis of our proposal of global approaches

to the study of proteolytic systems through the introduction of new concepts, such as degradome, to define the array of coding genes of proteases in a given organism. At the same time, we began to implement new methodologies, called degradomics, which gave experimental content to these global concepts that emerged in relation to proteolytic systems.

The application of these degradomic strategies was especially illustrative in the case of cancer. A combination of genetic and biochemical experiments intended to assess the *in vivo* functions of a number of proteolytic enzymes led us to the surprising discovery that some proteases, such as collagenase-2, play a protective role against tumour progression and not the supporting role of cancer that has traditionally been imputed to them. This discovery opened the way towards a definition of new antitumour mechanisms mediated by proteases, fundamentally based on their capacity to regulate the antitumour immune response that accompanies the development of malignant processes. This work also supplied an explanation for the deficient clinical results when patients were treated with broad-spectrum protease inhibitors, as these were unable to distinguish between the pro-tumour and antitumour functions of these enzymes. It therefore became necessary to examine tumour degradome as a whole, to try to define the most suitable tumours in each tumour of each patient as targets of therapeutic intervention. Likewise, the discovery of antitumour proteases made it indispensable to design and use specific inhibitors against pro-tumour proteases.

Immediately afterwards, the confidence gained in the potential of global degradome studies led us to explore, in collaboration with Dr Tony Hunter, the interactions between tumour proteases and kinases, in the first joint study of the two enzyme systems of primary importance in cancer. The finding of multiple evidence of the fertile dialogue between the degradome and kinome contributed to the elaboration of new proposals for the treatment of cancer patients based on coordinated intervention on specific components of both systems. This work also helped to establish the idea that the degradome is a central player in the exquisite and precise network of biological regulation that makes possible every instant of life in each organism.

Accumulated knowledge gained in the field of biological regulation has shown that diverse mechanisms control the expression of the huge amount of information carried by all living beings. Nonetheless, beyond this complexity – which seems as unfathomable as the *Book of Sand* by the great Borges – the studies undertaken by Jacob and Monod, and continued by many authors in subsequent years, pointed to the existence of an essential level of decision: the regulation of transcription. The intricate problem of biological regulation was thus posed in much more accessible terms: to be transcribed or not to be transcribed, that is the main question to be faced by an organism's genes at all times, everywhere. However, as progress was made in finding answers to this question, it became clear that transcriptional control was not enough to explain the regulation of gene expression. Hence, the new levels of transcriptional regulation, including those orchestrated by the numerous microRNA tribes recently discovered on the dark side of the genome, call into question the primacy of all-mighty transcriptional control. Nor is it enough to regulate the production of proteins through the expression of their genes, as they must

also be deactivated or destroyed when their functions are no longer necessary. In addition, many proteins are synthesized as inactive precursor molecules that must be activated at the right place and in the right time. All organisms have developed numerous strategies to regulate the activity of their proteins, once they have been synthesized. Our work in this field, along with that of other groups that have explored similar paths, has definitively cemented the idea that proteolytic regulation is one of the key mechanisms for modulating the life and death of all cells, in all organisms, in health and in illness. This is the only explanation for the surprising fact that our genome has about six hundred different protein-coding genes capable of performing the same biochemical task: the hydrolysis of the peptide bond.

With respect specifically to cancer, we have shown that the diverse components of the degradome exercise regulating functions that impact the practical totality of the "hallmarks of cancer", which have been brilliantly defined by Hanahan and Weinberg as the diverse biochemical characteristics acquired by transformed cells and shared by the majority of tumours. Proteases discovered in our laboratory participate in the acquisition of autonomous mechanisms of proliferation, determine the insensitivity to inhibition signals of cellular growth, favour the generation of strategies of resistance to apoptosis, drive changes in the energy metabolism, cause alterations in the complex inflammatory response associated with cancer and modulate the development of angiogenesis programs that supply the oxygen and nutrients needed for tumour progression. And yes, we ultimately managed to confirm that some proteases are comfortably accommodated in our initial hypotheses and decisively contribute to tumour cells' acquisition of the lethal capacity to invade other bodily territories and generate metastasis.

Cancer genomes

It so happens that, in our study of each gene, each protease and each mouse, time would go by and, after more than two decades examining the complexity of cancer from a minimalist proteolytic perspective, we suspected that the time had come to broaden our view beyond the hypothesis-driven science I had learned from my mentors. The idea arose of presenting our candidacy for a seat on the International Cancer Genome Consortium (ICGC). In late 2008 this project represented a good example of an incipient *agnostic science* based on a massive accumulation of data that could help us formulate working hypotheses when subjected to the expert scrutiny of a human. That year, technology had already beaten back the barriers of the impossible by enabling the sequencing of the complete genome of a tumour in a very short time and at quite a reasonable cost.

Right before our eyes, the ICGC project had every reason to become a major milestone in oncological research, as it was the most ambitious initiative for carefully tackling the molecular study of cancer. In its initial conception, the project sought to determine the complete sequence of nucleotides of at least five hundred tumour genomes of patients with each of the most frequent types of cancer, including patients with the most common haematological neoplasia, chronic lymphatic leukaemia, the study of which was the sub-project awarded to Spain. From 2009 our laboratory, along with the group led by Dr E.

Campo in the Hospital Clinic of Barcelona, undertook the responsibility of coordinating this work, which recently completed its first phase in autumn 2014, with the decoding of the tumour genome of five hundred patients with chronic lymphocytic leukaemia.

Our work in this field has enabled us to identify recurring mutations in several genes, such as *NOTCH1*, *SF3B1*, *POT1* and *CHD2*, which have become the preferred targets of therapeutic intervention in leukaemia and other neoplasias. At the same time, we have examined the genomic biography of other tumours, including lymphomas, melanomas and larynx carcinomas, which led us to the discovery of new tumour-suppressor genes such as *CTNNA2* and *CTNNA3*. Furthermore, our work in collaboration with other groups in the ICGC, and especially with the group led by Dr M. Stratton, has contributed to the identification of new mutagenic mechanisms involved in the genesis of cancer, and the introduction of innovative strategies of personalized medicine for diagnosis, monitoring and treatment. We are entering a new era in oncological research that will define the future clinical handling of a disease that still represents a clear and persistent example of human vulnerability. We are confident that the results of the ICGC, coupled with those from similar or supplementary projects, such as TCGA and PanCancer, will furnish fundamental information in the not-too-distant future on the genetic landscape of cancer, which is not just one disease but rather many different ones. We will therefore be forced to seek out specific therapies for each patient.

Hereditary diseases and the genome

The Cancer Genome Project has brought a series of collateral benefits of great interest to our group and even to those with whom we have had contact personally. Using the technology developed for mutational studies of cancer, including the computer algorithm called *Sidrón*, we have successfully identified the cause of a number of hereditary diseases. Recent works in this field include the discovery of a new form of hereditary premature ageing caused by mutations in the gene *BANF1*, which we have called the Néstor-Guillermo syndrome, in recognition of the exemplary attitude shown by two young patients who came to our laboratory in search of health and information about their disease. This pathology, among the rarest of the rare, consists of a runaway acceleration of the clock of life, as if hours went by in minutes and minutes in seconds. Within a few years, the organism undergoes all the transformations that usually take place over a span of several decades. This work, which was pioneering in Spain and one of the first in the world using this genome approach, was of no use in curing Néstor's advanced illness, but it did bring positive outcomes to his family and to that of Guillermo. A simple mutational analysis of the *BANFI* gene was sufficient to identify healthy carriers of the mutated gene in both families, who can now benefit from the appropriate genetic guidance and avoid transmitting this new form of progeria to their descendants.

Following in the footsteps of Néstor and Guillermo, other patients with diverse pathologies have placed in our hands the responsibility of examining their genomes in search of those minimal changes that transform their lives and those of their families. Quite often, a single mutation in the more than three billion nucleotides making up our genetic mater-

ial is enough to demolish the sophisticated biological plan that governs the development and maintenance of a human being, once again illustrating our fragility and vulnerability. A further example here is the recent study in our laboratory based on the sequencing of the genome of patients with hypertrophic cardiomyopathy, which has led us to discover a new hereditary form of this disease and to identify the gene whose mutations cause it.

Hypertrophic cardiomyopathy is a relatively frequent pathology that represents one of the main causes of sudden death among young adults. In recent years, studies of family cases of this disease have resulted in the discovery of several genes whose mutations cause the development of the disease in approximately fifty per cent of patients. However, it is unknown what genes are causing hypertrophic cardiomyopathy in other patients. This dramatic fact compelled us to study the genome of families with cases of sudden death with the use of new genome analysis techniques designed and implemented in our laboratory during the cancer genome decoding project. Genome studies have allowed us to conclude that mutations in the gene *FLNC*, which encodes the sarcomeric protein filamin C, cause hypertrophic cardiomyopathy in at least eight of the families studied. Having discovered these mutations, our analysis of the mechanisms underlying the development of the disease revealed that these mutations cause the formation of filamin C aggregates in the heart muscle. These aggregates build up over time and prevent the heart from functioning correctly. This discovery has important and immediate clinical applications, as it will allow for providing genetic guidance to families and identifying carriers of mutations in *FLNC*, which are to be subject to continuous clinical monitoring and, if necessary, they may benefit from the implantation of an automatic defibrillator that can prevent the process that triggers sudden death in these patients.

All this work reaffirms the great usefulness of genome sequencing for the study of hereditary diseases, however rare they may be, and it also illustrates the huge potential of these new ways of gaining a deeper knowledge of human biology. Closely connected with this idea, the Botín Foundation, through the work of Marisol Quintero and Francisco Moreno, has backed the creation of the company DREAMgenics, whose main mission is to educate society about genome language and to facilitate interpretation of the coded facts hidden there regarding cancer and any other biological problem written in the universal code of the four nucleotide letters: A, C, G and T.

The keys to ageing

In parallel with these genome and proteolytic studies of cancer and other genetic diseases, our group's work on cellular senescence and progeria syndromes has enabled us to delve into the molecular mechanisms associated with ageing, and analyse their close connections with tumour processes.

In recent years, many scientists have been seeking to find the keys to longevity and ageing in the interior of the cell. The current consensus assumes that ageing arose in the course of evolution because the force of natural selection subsides with age. Hence, the passage of time may manifest in an individual the activity of certain genes that play a harmful role in

the post-reproductive age. A corollary theory points to the necessity to optimally assign biochemical resources between reproductive functions and maintenance and repair, wherein the former clearly predominate. Organisms with a short lifespan favour reproductive functions, while those with greater longevity optimize the mechanisms of repair of molecular damage caused by the passage of time in cells and tissues. Such genetic and epigenetic damage ends up spreading to the proteins coded in altered genes and causes the progressive and generalized functional loss that accompanies ageing, until the organism finally succumbs.

Scientific advances in ageing research from a genome and molecular perspective are astounding. First, the analysis of genetic polymorphisms, and particularly the SNPs, has defined pro- and anti-ageing variants in the human genome, thus providing valuable information about the longevity equation we all carry in our genome. These studies of the genomic landscape of human ageing are only the prologue of others that are now presenting their calling card at the door of knowledge, including analysis of complete individual genomes in different stages of life, or a detailed comparison of the human genome with others that show a longevity that is disproportionate to their closest relatives in the evolutionary tree. Substantial progress has also been made in establishing the molecular connections between processes of cellular senescence and the overall ageing of organs and tissues. Lastly, numerous details have been pinpointed on the pathways and biochemical mechanisms that influence longevity, including autophagy, mitochondrial biology, proteostasis, cellular regeneration, telomere dynamics or alterations that occur in the immune system during ageing.

In this environment of progress towards the scientific comprehension of molecular complexities and the mechanics of ageing, a series of recent works by our group was triggered in a casual manner, with the creation of mice deficient in the metalloprotease FACE-1/Zmpste24. Experimental work in this field of pathological ageing initially led us to the discovery of new genes whose mutations cause accelerated ageing syndromes in human beings, especially the aforementioned Néstor-Guillermo syndrome. Later, we have shown that, in many cases, the functional defects deriving from these mutations also occur in normal ageing. We therefore undertook deeper study of cellular and molecular alterations associated with the passage of time, and this was reflected in a paper entitled "The Hallmarks of Aging" that was published in the journal *Cell*. This paper, authored in collaboration with other researchers of the Botín Foundation, namely Dr M. Serrano and Dr M. Blasco, presents for the first time an integrated mechanistic overview of the complex underlying alterations in the development of a biological process that affects everyone and makes us all equal.

According to our thesis, there are nine common denominators in the ageing process in different organisms, and they can be classified in three categories: primary hallmarks, antagonistic hallmarks and integrative hallmarks. Primary hallmarks are those that set off the process and include genomic instability, telomere attrition, epigenetic alterations and loss of proteostasis. Antagonistic hallmarks constitute responses by the organism aimed at mitigating the damage caused by the primary hallmarks. In principle, these responses are beneficial, but if they are chronic or exacerbated, as is the case at a late ageing, they become deleterious to the organism. This category includes the deregulated

nutrient-sensing, cellular senescence and mitochondrial dysfunction. Lastly, integrative hallmarks are those mainly responsible for the ageing phenotype and include stem-cell depletion and alteration in intercellular communication. A detailed understanding of the molecular mechanisms underlying these nine hallmarks of ageing will enable us to devise future therapeutic strategies for each, thus improving the quality of our lives and, ultimately, extend longevity.

What makes us human?

Finally, and given that "nothing makes sense in biology if not in the light of evolution", we have sought to use our experience in genomic analysis of proteolytic systems and tumour processes to contribute to the genome projects of a number of illustrious passengers on Noah's Ark. Among these adventures in genomic exploration, we have taken part in the sequencing and annotation of the species of great symbolic importance for the study of human evolution or of huge value as models in biomedical research. The gallery of animals whose genomes and degradomes have been subject to preferential attention in our laboratory include the rat, the mouse, the chimpanzee, the orangutan, the platypus and even the legendary bowhead whale, kindly giants of a frozen sea, longevity champions and surprisingly immune to diseases like cancer.

Comparative genomic studies have taught us important lessons about functions that have been acquired, modified or even lost as species evolved. We have found that different genomes have been exposed to significant differential pressures on the genes of reproductive and immunological systems, confirming the idea that these processes constitute crucial mechanisms for governing evolution. We have also shown that hominids possess specific changes in genes related to the lipid metabolism and with strategies of visual perception, thereby opening new pathways in the study of the importance of the two biochemical routes in our own evolutionary experience. Finally, we have found that the genome of other primates has evolved at a slower pace than ours, and that they barely show the tracks of the genomic invasion of certain repeated sequences that have, perhaps, granted us greater evolutionary and functional flexibility by spreading throughout our genome.

In this way, genome by genome, we have attempted to contribute to a series of projects aimed at adding new pieces to the jigsaw puzzle of life. Cautiously, but with perseverance, we are coming closer to understanding some of the essential features of the human condition, those that make us unique and different from all the other living things on a planet that has been overwhelmed by its collision with the human meteorite. In any event, in the genomic era – where we scientists have begun to create life that does not emerge from evolution, but rather from imagination – we are still far from finding specific answers to a vital question: what makes us human?

Looking towards the future

Borrowing a few words from the great Asturian poet Ángel González, I would like to state that for me to be able to write these words "a large space and a long

time were needed". I left an Aragón village while barely a child, yearning to study the fundamental realities of life and disease. After a number of years exploring the boundaries of curiosity in search of knowledge, I landed in Asturias, where I undertook my first projects focused on analysing the mechanisms of cancer progression with the assistance of brilliant students and extraordinary collaborators. The project grew as we went on discovering new protease-coding genes, whose expression was deeply altered in cancer or other diseases. Patiently, we studied the function of the enzymes being identified, and this led us to the decoding of their contribution to tumour progression. And, surprisingly, we found some cases in which proteases had protective functions against carcinogenesis. We also showed that some proteases initially discovered in our laboratory due to their alterations in cancer played a key role in quite diverse processes, including regulation of ageing, which was the starting point for our entry to research on this biological process. In any event, the complexity underlying the proteolytic system was of such magnitude that we were forced to introduce concepts such as the degradome in order to set dimensions and develop procedures that would enable us to carry out a global analysis of the proteolytic universe. In turn, this global view of the genes of the human degradome conveyed us, almost unintentionally, to the world of genome comparison, the analysis of genome evolution and, quite recently, the general study of cancer genomes, in our eagerness to integrate all we have learned over the years.

The detailed analysis of the molecular biography of hundreds of malignant tumours has brought us to conclude that each tumour is absolutely unique in terms of its genomic alterations. For this reason, the future sets out a path in which individual solutions must necessarily be sought for each tumour of each patient. Cancer research is thus entering a new era that promises knowledge, but that does not hide or minimize the long road still ahead. Hence it must be stressed that the decoding of the genome of malignant tumours will not represent a quick and final cure for all types of cancer, but rather the ability to offer oncologists all the biological and molecular information possible about each tumour that will bring about the future establishment of treatments that are more suited to each patient. This global reading of the biography of cancer must be completed with functional studies that will help specify which mutations drive malignant transformation and which ones are merely fellow passengers in the process. The point will be to distinguish between driver and passenger mutations in the tumour cells' voyage towards immortality. Our task will be to understand this path in order to tip the balance towards life.

Similarly, genome research in ageing will result in the discovery of new processes, mechanisms and gene interactions that will help us intervene in the hallmarks that cause the passage of time to "leave us more uncertain, confused, and scatter us to the winds". The voyage of exploration to the tiny nuclear world where genomes live will bring about significant changes in the way we deal with certain diseases, including rare hereditary syndromes that hitherto had fallen under the radar for science but not for patients or their families.

In sum, the extraordinary momentum acquired in only a few years by molecular biology has yielded a rigorous and in-depth analysis of essential questions about the nature of life

and disease, and it has promised to reveal some of its most well-kept secrets, including those that, according to Dostoevsky, we do not dare to tell even ourselves. For nearly three decades, while going around the sun aboard an Asturian laboratory and sailing a sea of genes and genomes, I have sought to help write some of these new chapters in the "book of science". At the same time, I have tried to combine my scientific work with intense teaching activity aimed at showing that hope for the future and the emotion of knowledge are nowhere else felt more intensely than in a laboratory. Today, fifty-five years after arriving on earth on a cold winter morning, I am looking ahead and becoming aware of the extensive *terra incognita* that still remains to be explored in each of the fields of research in which we have worked. For that reason, I hope to have the opportunity for some time still to enjoy my daily encounter with science in this new genomic era through which we are living. Scientific research may not yet guarantee immortality or promise happiness of molecular harmony, but it is still the most valuable instrument conceived by man to improve both the world and our own life.

Acknowledgements

Pedro García Barreno taught me that Isaac Newton was the last of the Babylonians, the last human being to contemplate an ancient world, because everything was different afterwards. With the same premises, Santiago Ramón y Cajal, whose childhood and adolescence were lived in Larrés, a small village barely five kilometres from my hometown, was the last scientist to have earned the right to use the singular on the written page. Today, science is a collective enterprise that must be written in the plural. My thanks go to my teachers and mentors, and to all those who have accompanied me at some point during the last twenty-seven years in our laboratory at the University of Oviedo: G. Velasco, J. M. P. Freije, A. Fueyo, X. S. Puente, V. Quesada, A. Gutiérrez-Fernández, D. Rodríguez, A. Ramsay, A. Kwarziak, F. Rodríguez, P. M. Quirós, C. Garabaya, S. Álvarez, C. Soria, F. G. Osorio, R. Valdés, D. Álvarez, J. M. Fraile, Y. Español, A. F. Fernández, C. Bárcena, A. R. Folgueras, G. Mariño, C. G. Viloria, N. Salvador, S. Cabrera, G. R. Ordóñez, J. Cadiñanos, M. Llamazares, I. Varela, F. M. Lara, J. Uría, Y. L. Boado, M. J. García, R. Cabanillas, P. Bringas, T. Bernal, A. D. Perales, I. Díez, J. Bordallo, R. Heljasvaara, B. Fernández, R. Lorca, A. A. Ferrando, J. Espada, J. R. Peinado, H. Montes, L. M. Sánchez, A. Vázquez, M. Balbín, A. M. Pendás, I. Santamaría, E. Llano, S. Cal, A. Obaya, A. Morán, A. Ugalde, A. Moncada, M. Fanjul, M. Fernández, J. de la Rosa, A. Astudillo, J. Vega, J. Cobo, L. Menéndez, G. M. Albaiceta, A. Aguirre, V. Fanjul, X. Menéndez, K. Watanabe, M. Mittelbrunn, V. Valdespino, S. Freitas, O. Santiago, J. R. Arango and D. Campos. Thank you also to all the institutions that have helped us in all these years, and especially to the Botín Foundation, for decisively helping our laboratory take on a different dimension, thus enabling it to tackle projects that would never have been possible without their support, such as the creation of DREAMgenics.

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MARÍA TERESA MIRAS PORTUGAL

BIOGRAPHICAL NOTES OF A SURVIVOR

9

I was born in February 1948 in Carballino, a town that then had and is still inhabited by 12,000 inhabitants. It is located in the mountains of the province of Orense, at the heart of the inland part of the region of Galicia. Galicia's great writer Emilia Pardo Bazán, who owned a country manor nearby, set her romantic, naturalist novel *El cisne de Vilamorta* (*The Swan of Vilamorta*) in my town. However, nothing could feel further from reality to those of us who were born and grew up there. Our town has always been a sort of lakeside paradise, with a river lined by luxuriant shores and several waterfalls. We were free, and we felt safe in the enormous park, which was more like a forest home to a wide variety of tree species. As children, we were adventurous, and now I need that feeling of freedom, which forms part of me every time I start something new. It is essential to my creative work in science.

My father, Aurelio Miras Azor, was a lawyer with a degree in Philosophy and Letters. The house was full of books and from an early age we were taught to enjoy reading. I can still hear the same phrase ringing in my ears after being repeated hundreds of times: "Put that down and pick up a book". This was no surprise, since I came from a family of teachers who were prone to write, and two even had somewhat successful novels published. My mother, Esperanza Portugal Careaga, was a housewife who could transform any edible item on which she placed her hands into a delicacy. Her *empanadas* and flans were famous. I inherited my optimization and enjoyment of the laboratory through her genes, as well as my love for flowers, and plants in general. We had a lovely estate at the edge of town, where my mother enjoyed taking care of all kinds of flowers, and my father would order new varieties of gladiolus, carnations, lilies and anything else you might imagine one could plant. Even now, when I see certain flowers and trees, I instinctively compare them with those of my childhood. The difference is that I plant them now, especially so that birds can feed off them in the wintertime. My maternal grandparents, with whom I was very close, came from a long line of merchants and worked in the tanning industry. I still perform the reflex action of smelling products made with leather, and the smell of well-tanned leather still stirs up something special in me.

During my adventures and forays into the outskirts of the town, I would collect all types of plants and animals, especially insects. However, my fascination for the unknown arose all of a sudden. I was about eight years old when my father, returning from a trip to Madrid, brought me a treasure, a book titled *The Living Desert*, which years later I discovered was the written version of the first documentary ever filmed about nature. I still have that 1955 edition book. My calling was therefore established: I would become a "discoverer". I still imagine the courageous wasp fighting against the evil tarantula. I still believe that deserts only exist if we dare not look at them in some different way.

I completed my elementary baccalaureate at the school run by the Franciscan nuns of Carballino up to the fourth year. It was right at the beginning of these studies, in 1959, that Ochoa and Kornberg won the Nobel Prize for their research on the mechanisms of biological synthesis of nucleic acids. I had the great luck to be a student of Doña Luz, who gave us literature and history classes. It was then that Yuri Gagarin, on board the spacecraft *Vostok*, took the first journey into space in 1961. Thanks to Doña Luz, we felt as though we played a role in past history and history yet to come, not to mention that our poets had laid down in their verses the feelings that would continue to move readers from the future. From the works we had to read, we were supposed to select the part of the text that had most drawn our attention and describe what we interpreted and imagined in the context, and what we would dream up in different situations. I think this education helped me later to interpret and analyse scientific publications.

When I was fourteen years old, I left to study the higher pre-university baccalaureate in Santiago de Compostela. I was a boarder at the Compañía de María school, a prison that was not easy for me to bear after being accustomed to the nature and freedom in my hometown. I was consoled by the fact that the library was well stocked, and I learned that being free is a matter of your thoughts. On the other hand, I was surprised to find that all the nuns and secular teachers who gave us classes had university degrees, even doctorates. They followed the spirit of the school's founder, Juana de Lestonnac, who was a noblewoman from France, a niece of the famous philosopher Michel de Montaigne and a widow with five children. It was the first Church-approved religious order devoted to educating women. The atmosphere was rational and no-nonsense; the school's historical building, of immense, entirely stone construction, was terribly cold. Our chemistry and mathematics classes were wonderful, and I loved the precise language and knowledge to which they gave access. I will also be forever thankful to and remember Mother Prada, who left her teaching position at a high school to become a nun mathematician. In the meantime, outside those walls in 1962, they would not give the Nobel Prize to Rosalind Franklin along with Watson and Crick for the discovery of the molecular structure of deoxyribonucleic acids; in 1963 the first woman, Valentina Tereshkova, journeyed into space; in 1964, the Nobel Prize was awarded to a woman with a family and children, Dorothy Crowfoot Hodgkin, for clarifying extremely complex biochemical structures using X-ray diffraction, and we must not forget that this was the year when Kennedy was assassinated.

University degree in Pharmacology, between Santiago and Madrid

In October of 1965 I began to study at the Pharmacology School of Santiago de Compostela, where I would complete the first three years of my university degree. At that time its headquarters was located in the Palacio de Fonseca. There I received my university "christening", and was dazzled as soon as I went inside by the beauty of the Plateresque cloister sculpted in grey granite. Two months later, François Jacob, André Lwoff and Jacques Monod won the Nobel Prize. It was the first year in which the selective course was being taught at the Pharmacology School, and the professors were still unfamiliar with the type of student body they would be receiving. They only knew that there would be few of us. It was a very dynamic period, and we were all filled with energy, capable of performing many different academic activities in addition to extracurricular activities and sports.

At the beginning of the academic year, tests were given to student intern candidates. I was lucky and managed to get into inorganic chemistry, the department that was headed by Professor Jaime González Carreró; the truth is that there were not many candidates. The nice part was that I was already donning my lab coat inside the laboratory one week after beginning the school year. Don Jaime had a great sense of humour and explained the course curriculum at a higher level in a way that hooked you, mixing it from time to time with stories about the Venetian silver mirror and other alchemist formulas that left us somewhat perplexed. He had many candidates for doing their doctoral theses because he demanded good work and innovative ideas from those around him. His disciples found positions at good domestic and foreign laboratories, including those for cosmetic products, such as the French firm Vichy. I was not aware of the importance that this held until many years later when, at a dinner with the cultural attaché from the French embassy in Madrid, we began to talk about Santiago de Compostela and he asked me about Don Jaime. I could never have imagined he held so many patents for cosmetics and that he had been one of the pioneers in using silicones in hand creams. I believe that that was the reason why he never let us female students handle the bottles of concentrated hydrochloric acid, because the gases might have damaged our skin. Being a smaller group had the advantage that we did practicums every day for many hours, whether it meant centrifuging by hand or measuring how hard water was with soap. The concepts were clear, and the limitations imposed by scarcity always meant using the imagination.

Professor Luis Josafat Alias was the first-year professor of Geology and Soil Sciences, and he made us dream through the petrographic microscope so we would see the most diverse minerals with polarized light. After we left our theory class, we had two hours of practicums. There was no system for crystallization that we could not crack, including the triclinic system, and no crystal that we did not recognize, because the examination took place in front of a microscope with the crystallization networks. Don Luis soon left Santiago for the University of Murcia, where I found him many years later. To him we owe a study on the soils of Murcia and Andalusia that were later used to optimize the output of soils inside greenhouses.

Professor Ernesto Viéitez was the head professor of Biology and Plant Physio-pathology as well as being the director of the Galicia Biological Mission, originally founded by the former Junta de Ampliacion de Estudios in 1921 before the Spanish Civil War. He devoted a large portion of his scientific activity to studying chestnut tree diseases and to seeking and identifying resistant native chestnuts and new hybrids. He received an award from the United States Department of Agriculture in 1957, and therefore he was nearly mythical in the student body's eyes. During the times when I was a student, his laboratory at the university focused on isolating and identifying the plant hormones associated with the formation of roots and calluses on woody root cuttings. In our practical classes, I saw corn chromosomes for the first time ever, using tinctures that we produced using the tips of germinating seeds. We also suffered for the poor grape leaves whenever we looked at them under the microscope and saw how they were colonized by the hyphae of the fungus *Plasmopara viticola*, producing the feared mildew. He made us feel anguish and sadness for any sick or mistreated plant, as if it were a member of our family.

Professor Pablo Sanz Pedrero, the head physics professor, had an imposing presence and deep voice. We attended his classes with reverential fear. One day, something that I could

never have imagined happened: the young doctoral students in the Chemistry Department, where I was a student intern, planned to carry out a scientific prank. According to them – people who are now directors of hospital pharmacy services at major Spanish hospitals – they were running out of deuterium oxide, D_2O, also known more commonly as heavy water. Because they claimed to be very busy, they asked me to do them the favour of going to the Physics Department and asking for a litre of the aforementioned liquid. Pleased to help and very naive, I set off to do this good deed. I was welcomed by the young assistant professor, Miñones, who very kindly told me that out of respect and proper procedure I should ask the new department head, Don Pablo. With complete self-confidence and in a totally natural way, I proceeded to ask the revered professor for the deuterium oxide. Don Pablo's initial kind smile for me, always a respectful student in his class, immediately turned to an expression of absolute fury. Among other things, he yelled at me that "you students today are not like the ones who came before; we did not have the gall to go to a university and show a total lack of respect for a professor's work". The year was 1965, and I had absolutely no inkling about what was going on. Between the astonished face I must have displayed and Professor Miñones's wide grin, Don Pablo realized he had been sent a real greenhorn. This freshman's prank was on him, too, as he became its second victim. He said "well, well," and once he shut his office door I could hear the two professors' laughter on the other side.

Seven years later, in 1972, while doing my doctoral thesis in Strasbourg, I came across deuterium oxide once again, this time for identifying the presence of a reactive histidine at the catalytic centre of the enzyme that synthesizes the neurotransmitter noradrenaline, dopamine β-hydroxylase, because the ionization kinetics were displaced by 0.5 pH units when performed in heavy water. I have always found that experience is nourished with greater intensity by mistakes and blunders than by successes, and I had the traumatic experience of the heavy water permanently burned into my memory.

Once I had passed the first selective course, the pressure was the same, but we were then familiar with the times and paces. We had some of the same professors as the previous year, but new subject matter enriched the curriculum. What did undergo a profound change was the set of people at the school. Students came from every corner of Spain, including those who were displaced by their various pharmacology schools in search of easier courses. Many others came from Arab countries (Syria, Lebanon, Iraq, Jordan and Palestine, all in perfect harmony). The Latin Americans were quite numerous, and many of those men and women stayed with us permanently. In that multicultural, multi-ethnic, universal environment in Santiago, the political aspects of the dictatorship sounded somewhat old-fashioned to us, while the foreign students did pay it the slightest attention.

Among the new second-year professors, we had the botany professor Matías Mayor, who had recently arrived from the plateau of Castile. He was excited to see the city's glowing green colour and was amazed by the lichen, ferns and flowery plants that grew there, colonizing the walls of buildings as if someone had enjoyed designing a vertical garden. His fascination with Galicia's flora forced us to take constant botanical incursions into every biotope, where we would collect samples for our plant collection, in addition to catching monumental colds from getting wet. With a magnifying glass, clothespins, the proper book

by Gaston Bonnier and an annex on specific flora from Galicia, we would proceed to classify the specimens we gathered. We would spend many hours in the laboratory preparing the herbarium, and they were worth every minute. Even now when I walk out into the country, the memories of their names come back to me, especially those of the poisonous plants.

My third year, and the last in Santiago, brought the novelty of organic chemistry, taught by Professor José María Montañés, who slipped one rainy day and broke his tibia and right fibula. This was no barrier to him leaving his house two hours before class and walking in on crutches, with his leg in a cast, so he could give his course at nine o'clock on the dot, when he started roll-call. He spoke passionately about the "art of organic chemistry". He was working on the synthesis of aromatic ketones with a peculiar smell, derived from biogenic amines performing vascular activities. Along with his disciples, he synthesized numerous compounds with specific effects for the various serotonin receptors, and his school continues to operate very productively today. It originated from the school of Antonio Madinaveitia, who was forced to go into exile in Mexico and founded the Chemistry Institute at the National Autonomous University of Mexico (UNAM). During that period it was common to find exceptional professors who had undergone retaliation by the regime and were sent to the University of Santiago, where they contributed high quality and open-mindedness. The other innovation in third year were the microbiology classes taught by Professor Benito Regueiro, also known as San Benito because of his benevolence when grading, and assistant professor Ramona Vaamonde, known as Ramonita. I will always remember their practicums: the description of pathogenic bacteria, including *Mycobacterium tuberculosis*, was performed using materials brought from the hospital. Today no laboratory would allow this way of working, but in the 1967–68 academic year and in Galicia, we future professionals were hardened on every front. Some kinder practicums included the production of yogurts and other fermented dairy products, which people were not used to eating back then. For the first time in my life I tried yogurt. Some of my colleagues later set up factories to produce them.

During those first three years of my university degree in Santiago, because I was tall, I was signed up for the Fonseca handball team. I soon realized that competitive sports were not my cup of tea. I had better luck with the university theatre group. I even acted in a Eugene O'Neill play titled *The Dreamy Kid*, in the part of Irene, and another by Anton Chekhov, in which I played young Natasha in *The Marriage Proposal*. We filled the house and received a huge round of applause. The reason for our success lay in the fact that we had an enthusiastic, involved audience made up of our complaisant classmates. My time in Santiago was about to come to an end. My lifelong boyfriend, Fernando Varela, had earned a chair in Mathematics by competitive examination and was completing his doctoral thesis at the Complutense University. We decided to get married at the end of summer and to begin the new school year together in Madrid.

The academic year of 1968–69, now at the Complutense University in Madrid, was the complete opposite to Santiago. I was awestruck by the classes given in enormous amphitheatres. They were multitudinous and gave a sensation of distance and depersonalization of students. Practicums were less common and very inflexible in design and methods. The faculty was much older and you could almost breathe in a sort of conformist dogmatism.

The possibility of combining my studies with other cultural activities was very difficult if I hoped to get a good record. You could say that, while in Madrid, my nurturing of personal concerns became more limited. On the other hand, I discovered the importance of biochemistry, and I remember the very well-organized and precise class by Professor Ángel Santos Ruiz. Without a doubt, biochemistry offered a new perspective for understanding the essential processes in living beings. It sufficed to look at the areas of work by Nobel Prize winners to realize how much future potential they held. I had reached the decision to devote my work to researching biochemistry, and Don Ángel very generously accepted me at his laboratory. At that time it was a mixed council-university centre, the Centro Alfonso X el Sabio, with an excellent library, the best scientific equipment of that period, and many researchers who were just as hopeful as I was myself. I completed my degree in Pharmacology and received a special award for my university studies in June 1970 as well as the National University Final Project Award. These distinctions allowed me to apply successfully for fellowships to do my doctoral thesis. However, that is another chapter.

The doctoral years

Having completed my university degree, the next academic year, 1970–71, I began experiments on the enzyme glutamine synthetase, of major importance in the brain's metabolism. This first contact furnished me with the possibility of becoming familiar with the generosity and knowledge of researcher Pilar González, who guided my first steps, and the brilliant and kind Professor Ángel Giménez Solves, an exceptional educator. That first year, I successfully registered for all the doctoral courses that should normally be taken in two years, but which I absolutely had to finish in one. The reason was that my husband had been accepted at the Advanced Mathematics Research Institute of Strasbourg, the famous IRMA, which had earned so many Field Medals throughout the years. I had to manage to get myself to Strasbourg and a good research centre there. I wrote to several of them, and their response was affirmative in every case, possibly due to my academic record. Some of the directors were surprised to find my file included marks in subjects like religion and "formation of national spirit". As a huge favour, I asked the person responsible for the secretariat at the Pharmacology School in Madrid to expunge these notoriously controversial subjects from my academic record. I studied the publications by the centres that had accepted me, and I finally chose the Neurochemistry Centre for two reasons: first of all, because I was attracted to neuroscience, and secondly, because of the exceptional scientists who were working there.

I got to Strasbourg in October 1971, when my son Fernando was just a few months old. Searching for a day care centre was a true odyssey worthy of an entire book in and of itself. However, I will leave that for another occasion. I had been granted a fellowship by the French government, which entitled me to medical care and other benefits, though the economic amount was quite low. This fellowship was actually given to the laboratory that took me in, because it was to receive a considerable amount to cover my research expenses. A few months later, when I was least expecting it, I was awarded a fellowship by Spain's Ministry of Education to complete my doctoral thesis abroad. It took them nearly one year to make effective payment, and I had to go and collect it in Paris. My last academic year in Strasbourg, 1974–75, I was fortunate to be awarded a fellowship from the Juan March Foundation that was truly generous for those times.

The director of the Neurochemistry Centre at that time was the prestigious Professor Paul Mandel. Years earlier, he had discovered that many nuclear RNAs have a tail of polyA added to them and the advantages that this created for protein synthesis. Another line of work that he co-directed with Noelle Virmaux was on the protein composition and presence of metabolites in different ocular structures, having been the first to discover that GMPc was abundant in the retina and that its levels depended upon light stimulation. The study of the different types of hereditary epilepsy, and the animal models used to generate the *status epilepticus*, held special connotations for family-related reasons, and the tests with different drugs were performed in collaboration with the Sandoz Laboratories of Basel, having tested the first benzodiazepines and valproate. Though there were many researchers working on the topic, his son Jean-Louis Mandel, who later became a professor at the Collège de France, like his father, was one of the most active and created the great world reference laboratory in the field. Among his disciples working on the synthesis of nucleic acids and controlling their expression was Professor Pierre Chambon, whose earliest articles on RNA polymerase date back to 1961. They collaborated until 1970. When I arrived, Pierre Chambon had an impressive, separate laboratory, and he was very demanding of those who collaborated with him. The centre's seminars given by Chambon's group were followed with special interest. In 1970, they had just discovered that alpha-amanitin was a specific inhibitor for one of the two RNA polymerases dependent upon DNA known at that time in eukaryotes. At present, we know them as RNA polymerase II, which synthesizes messenger RNA, microRNA and others. Its three-dimensional structure led the Nobel Prize in Chemistry to be awarded to Roger Kornberg in 2006. His group's first seminar, which I attended in early 1972, presented evidence that there were at least three RNA polymerases dependent upon DNA in the eukaryotic nucleus, which was published in subsequent years. Perhaps what most impacted me were the first electronic micrographs, in 1974, showing the hybridization of messenger RNA with large DNA fragments from the oviduct of a hen. These studies of what we now refer to as exons, introns and gene expression control were published much later, even after I had already returned to Spain. I have always thought it was such a privilege to be able to witness a historical event and the birth of a very powerful new science.

I began my doctoral thesis in Gerard Mack's group. He specialized in adrenergic neurotransmission and the problems associated with various models of epilepsy and behaviour. I was soon assigned a work topic, studying the enzyme that synthesizes noradrenaline on the basis of dopamine, dopamine-β-hydroxylase, or DβH, in widespread use to distinguish dopaminergic neurons from noradrenergic neurons in maps of the brain. This enzyme and its connotations were to accompany me for a long time. I began by fine-tuning the most diverse techniques for dealing with the enzyme from every perspective. The beginnings were difficult. In addition, because I wanted to complete my thesis, I had to stand the litmus test of remaining completely alone. After a few months, when they saw that I performed well and had developed and fine-tuned techniques, including an affinity column for purifying the enzyme, thanks to my knowledge of organic chemistry and my "daringness," I was already considered to be worthy of collaboration and exchanges. That is how I learned to fight to be a survivor. From those times, I still keep some friendships and collaborators, including great scientists such as Jean Zwiller, Max Recassens, Eliane Kempf, Christo Goridis, Giorgio Gombos, Jean-Pierre Zanetta and many others, because the Neurochemistry Centre was one of the most in-

ternational in France, where researchers from around the world went on sabbatical stays. In addition the centre's seminars, the group meetings, were held on Tuesdays at six in the evening at Professor Mandel's home. It is nighttime at that hour in Strasbourg from October through March, and the meetings lasted about two hours. On the table, there were always dates from Israel and from time to time some Belgian chocolates. The results were explained in a context of prior knowledge, highlighting what was innovative and what direction we were hoping to take. You could almost always hear the muffled sound of a piano coming from upstairs. Everyone in the family was an accomplished musician, and he was a violinist.

The most productive collaboration took place with Dominique Aunis. During my stay, we published fourteen articles in very good journals, in addition to continuing to collaborate and publish once in Spain. One of the first articles was about the presence of histidine residues in the catalytic centre of the enzyme, making use of the deuterium isotope in the effect of pH on enzyme activity. That is when I remember Don Pablo and the heavy water. We were later able to purify the enzyme stored in secretion granules in just one step using affinity with lectins, because it was a glycoprotein with a terminal mannose. The world of lectins and glycobiology were another of the surprises that allowed me to analyse the modifications in the enzyme dopamine-β-hydroxylase, DβH, once released from the secretion granules and present in the blood plasma, a lead that I would take to its end much later when I was at the University of Murcia. My work with this enzyme allowed me to present my doctoral thesis in 1975. I discovered that the levels of the enzyme in human plasma are more abundant than in any other mammal, which made it possible to measure the enzyme activity in all the Mack group's research and try to imagine an interpretation. Professor Mandel, who had a great eye for business, managed to convince those responsible for the supersonic Concorde aeroplane to study its effects on human stress. He suggested that these effects had to be measured using sympathetic activity parameters. The suggested parameters were none other than the levels of noradrenaline and its enzyme for synthesis in the blood plasma, DβH. Said and done: we soon had to fine-tune the technique to measure the enzyme in the blood of pigs subjected to the ultrasound of the supersonic plane. The benefits for the laboratory were substantial, it appears, because the Belgian chocolates become even more plentiful at our weekly meetings.

In May 1973 the third international symposium on catecholamines was held in Strasbourg, having been organized by Paul Mandel, and the book of synopses and articles was published under the title of *Frontiers in Catecholamine Research*. It was my first congress and my first poster. We were fascinated with the Swedes, because they had brought slides developed in blue, which was quite a novelty. Who remembers that nowadays? We attended presentations of the brain maps showing the distribution of catecholamines: dopaminergic, so well collected and precise, noradrenergic and serotoninergic, with a much broader distribution. Their authors had been Annica Dahlström, Tomas Hökfelt and Kjell Fuxe, whose presentations were one of the congress's highlights and continue to be in current textbooks. The important guests at the congress were Ulf von Euler and Julius Axelrod, Nobel Prize winners in 1970 for describing the storage, release and deactivation of catecholamines; Roger Guillemin, who worked on neuropeptides and their secretion, in addition to being French and curiously receiving the Nobel three years later, in 1976; Solomon Snyder, who discussed the mechanisms of the action of psychoactive drugs and would later discover opioid recep-

tors; and finally, among many others, Arvid Carlsson and Paul Greengard, who would be awarded the Nobel Prize in the year 2000 for contributing to understanding the molecular and cellular foundations of Parkinson's disease and its treatment.

Top-level scientific meetings were often organized with a very small number of participants. I remember those in Mont Sainte-Odile, in the heart of Alsace, where you could contact the best in each speciality and see how far the limits of knowledge had reached. There I was able to speak at length with Rita Levi-Montalcini, Viktor Hamburger and many others. They were all delighted to discover how many young people were excited about science. The mathematicians' meetings that my husband attended were completely different. They were more family oriented and we would take our children. They were usually held in the typical mountain chalets for school holidays in the snow: extremely beautiful but very austere places. The meetings were organized by the IRMA, whose director was Professor Georges Reeb, famous for founding the topological theory of foliation. He was a generous Alsatian who took us in as members of his family. The meetings were generally held in May and would always include the greats in the field. There you could find Alexander Grothendieck, Jean Dieudonné and Réné Thon, creator of catastrophe theory. We would take long hikes through the Vosges, and at the end of the evening there was always music. The contacts made by mathematicians on both sides of the Rhine were very fluid. The difference with Germany was that theirs were financed by the Volkswagen company, so they were low on austerity.

Science moved forward, as did my thesis, and life followed its course. In February 1973 my second son, Alberto, was born. My husband's PhD thesis, at the highest French level known as *Thèse d'État*, had reached a very advanced stage, and it would open up all sorts of doors to positions as a professor or researcher. In the end, he was hired as a staff researcher at the National Scientific Research Centre (CNRS), within the Advanced Mathematical Research Institute (IRMA) in Strasbourg. In July 1975 I defended my doctoral thesis at the University of Strasbourg. It was titled *La dopamine-β-hydroxilase (EC 1.14.17.1) du serum humain* and earned *magna cum laude* honours and congratulations from the jury. That jury was international and, in addition to my thesis directors, Professor Paul Mandel and Professor Gerard Mack, two other members had attended. One was a professor of Pharmacology from the Paris Descartes University, Jean-Charles Schwartz, a member of the Academy of Sciences of the Institute of France, known worldwide for his discoveries on serotonin receptors in pharmacology. The other jury member was Professor Merton Sandler, of the University of London, who was a pioneer in the development of biological psychiatry and psychopharmacology, who had related catecholamines and serotonin with depression and migraines. Among his scientific books, perhaps the most greatly distributed is *Design of Enzyme Inhibitors as Drugs*, published jointly with H. John Smith. It allowed for a better understanding of many drugs, their development and improvements, having been republished on several occasions. It can be considered a book with a cult following that I have used extensively in my teaching work.

In 1983 Dominique Aunis, with whom I had collaborated throughout my entire stay in Strasbourg, was named research director, publishing many articles on the mechanisms of exocytosis and the regulation thereof by G proteins and cytoskeleton. In 1991 he created his own research unit at the National Institute of Health and Medical Research (INSERM),

known as "Cell Communication Biology", and he opened up new fields of research in drug addiction, neuroimmunity and pathologies associated with prions. He later became the director of the Neurosciences Institute of Strasbourg and president of the Neurosciences Society of France. We would continue collaborating after I returned to Spain, with the help of bilateral and European projects.

In September 1975 I reached a tough decision along with my husband, that of returning to Spain. Our friends from mathematics and neurosciences did not understand how we could leave our positions to return to a country that was, according to everyone, fraught with uncertainty. Professor Mandel and Professor Reeb offered us the chance to return at any time if we thought any danger existed. They were right: the 27 September shootings – just twenty days after returning – left us truly horrified.

Returning to Spain: from the UCM Pharmacology School to the UAM School of Medicine

I returned to Madrid, headed for the Biochemistry Department at the Pharmacology School with a contract from the Spanish National Research Council (CSIC), which had been achieved through great effort by Professor Ángel Santos. The contract term was one year, the academic year of 1975–76. The following two years, I got a position as an assistant professor in practical classes. My husband was a professor of Differential Topology for the School of Sciences at the Autonomous University of Madrid. I continued publishing with the group from Strasbourg, and I also had a small but exceptional group of young people who did their undergraduate theses with me, including Antonio Jordá, José Sánchez Prieto and Francisco Vara. I published with them as of the very first year when I returned to the country. In the department, the vast majority of researchers had gone on stays abroad or were about to leave, and the scientific discussions and weekly seminars were very enriching. Few departments in Spain enjoyed that vitality, and many of my colleagues were and still are professors and researchers, including José Miguel Ortiz Melón, Sebastián Cerdán and Manuel López Pérez.

At that time the system for remaining within the university required successfully passing an open examination, and in the field of biochemistry it was highly competitive. The explanation of one's record as a researcher and educator was a key point, but you also had to have a well-designed programme and know how to explain each of the topics as if you were giving a master class. Ten numbered balls representing different subject matters were drawn at random, and the exam tribunal would choose one of them, indicating the topic that the examinee had to explain. We all knew the programme very well, which required even greater effort and dedication. This unavoidably meant putting research onto the back burner, no matter how cutting-edge and excellent it might be. In these competitive examinations, I coincided with exceptional educators and researchers, including Joan Guinovart, José María Medina, Juan Carmelo Gómez and Natalia López Moratalla.

I earned a position as an associate professor in 1978 and was lucky to be able to vie for a spot in the Biochemistry Department at the Autonomous University of Madrid's School of Medicine,

directed by Alberto Sols, where I remained for four academic years, from 1978–79 to 1981–82. The students were of a very high level, given the selection process and requirements. It was a new school at the university, and a great staff of researchers from the Biological Research Centre (CIB) had been hired. The CIB was devoted mainly to enzymology. There you could find the relevant scientists Gertrudis de la Fuente, Carlos Gancedo, Juana María Sempere, Juan Emilio Feliu and Lisardo Boscá, among many more, all dedicated to studying glucidic metabolism. This was the period of searching for isoenzymes, their tissue relationship with pathological statuses and how to differentiate them clearly for their use in testing. With different lines of research one could find, among others, Antonio Sillero, María Antonia Günther-Sillero and Roberto Parrilla, with whom I would later collaborate. The centre's spirit much resembled the spirit in Strasbourg: the seminars were excellent, the guest professors were many, and there were always new people collaborating. It was a centre open to all schools of thought, a permeable distributor of the knowledge that entered its doors. One of the guest researchers who most impressed me due to his extensive knowledge and culture, as well as his refined sense of irony, was Professor Luis Leloir. He had been awarded the Nobel Prize in Chemistry in 1971 for the active forms of sugars, with UDP-glucose being the first discovered. I remembered him thirty years later, in 2001, when UDP-glucose turned out to be the physiological agonist of the P2Y14 receptor, the last P2Y receptor for nucleotides discovered to date.

The Biochemistry Department was indebted to Professor Sols, who had earned his stripes at many international forums and was aspiring to make Spanish science cross new borders. He was the creator and promoter of the Spanish Society for Biochemistry (SEB) and led our memberships in the Federation of European Biochemical Societies (FEBS) and the International Union of Biochemistry and Molecular Biology (IUBMB). His spirit was universal, and his ability to break down complex problems into simple questions that could be dealt with experimentally was admirable. I felt especially honoured when the Spanish Society for Biochemistry and Molecular Biology (SEBBM), many years later, granted me the highest distinction, the Alberto Sols Medal, in the year 2005. I learned a great deal from his way of analysing things and his sense of humour, which denoted an exceptional intelligence. In 1954, he published the specificity of substrates in cerebral hexokinase, including 2-deoxyglucose, which, once phosphorylated, could not continue its metabolism and was built up in cells. Almost thirty years later, 2-deoxyglucose and [18F]-2-deoxy-2-fluoro-D-glucose were used for the first *in vitro* studies of metabolism and brain function using positron emission tomography (PET). Interestingly, the *coup d'état* on 23 February 1981 took place on the same dates as the publication of the first articles on PET and the hypothetic potential that the technique held. It is a good thing that the first failed and that the second has become a powerful study and diagnostic tool.

In that environment, I completely changed the direction of my research. Catecholamines were a highly competitive subject matter, and I needed to find my own road to travel down in a field that was much less congested. After a few articles on glucidic metabolism in neurons, resulting from the atmosphere of collaboration with Juan Emilio Feliu, Rosa Sagarra and Ana Millaruelo, who ended up writing her thesis on the topic, I decided to devote my studies to other components in neurosecretory vesicles. Among these components were the nucleotides ATP and ADP. Releasing the contents by exocytosis required neces-

sary recovery, and I began to study that of adenine and adenosine in neural cells. Pedro Rotllán and I described enzymes, new inhibitors and capturing in living cells, beginning the study of transporters. Since then, extracellular nucleotides, their receptors, their signalling cascades, their molecular and cellular biology, with the physio-pathological implications that they entail, have been my battlefield, with a few surprising discoveries and a few disasters, but it has always been original and has belonged to me.

At the same time as defining the field of research for my future, I applied to take the competitive examination for a full professorship at the university, which at that time were referred to as "Professor Agregado". They allowed for direct access to a full professorship position later on. Once again, this meant exhaustive subject lists, full-time dedication to the organization of topics and research suffering from delays. In 1981 I earned the full professorship position at the Medical School of Oviedo, where Professor Santiago Gascón was the head of the department. They very generously granted me a secondment, and I stayed in Madrid as professor during the academic year of 1981–82. That very same year, my husband earned a full professorship and department direction in Geometry and Differential Topology at the University of Murcia, to which I managed to get myself transferred.

The University of Murcia and returning to Madrid

I went to Murcia in September 1982. José Antonio Lozano, at that time the rector and full professor of Biochemistry at the University of Murcia, had achieved the creation of a well-equipped multi-school department with an excellent central library. It was he who facilitated my arrival at the Medical School and later a professorship in the School of Biology. It meant starting over again in a new place, but I admit it was very enriching. It forced me to think in a new way and see possibilities where there was uncertainty. I was in Murcia for four academic years, from 1982–83 to 1985–86. The students were brilliant and hard-working. Hearing one sentence was enough to get them to start work on any undertaking. The city was bright, and I enjoyed this life in a fun environment that smelled of orange blossoms.

I was given my first projects as a main researcher and began collaborations and exchanges with groups within the university and abroad. It was a very productive period, with articles in *Cancer Research*, *Diabetes USA*, *Journal of Biological Chemistry*, *Journal of Neurochemistry* and so on. We had fine-tuned original techniques for transporting metabolites to cells using silicone gradients, with the cooperation of Roberto Parrilla, of the CIB; exocytotic parameters in diabetic rat models with the Department of Physiology and Hospital de la Arrixaca, with Andrés Muñoz and Tomás Quesada; we synthesized the first radioactive compounds for quantifying adenosine transporters in collaboration with Pedro Molina of the Organic Chemistry Department, and we developed all sorts of cell cultivation techniques, above all for chromaffin cells. Last of all, the doctoral theses by Andrés Muñoz, Magdalena Torres Molina, Pedro Rotllán and Esmerilda García Delicado were ready for presentation. It was a frenzied time, and I also got the chance to collaborate closely with the person who would become my successor in the professorship, Francisco García Carmona, undoubtedly the best enzyme kinetics analysis specialist in this country and one of the best in Europe in enzyme kinetics in biological and synthetic membranes. Once in Madrid, we would

give shape to some of the data that were difficult to understand by interpreting the kinetics of membrane transporters, with many surprises in store.

However, Murcia was very far from the family we had in Galicia, and we wanted to return to Madrid. In the meantime, as a sort of joke by the government, the rules of the game had been changed, and associate professors had become full professors without having to shift out of their respective positions in order to gain access to professorship. Since there was no other choice, in early 1986 I took the competitive exam for the professorship once again, in biochemistry and molecular biology at the Veterinary School of the Complutense University.

I began my stay in Madrid in the academic year of 1986–87, and I have remained there ever since. When I entered the facilities of the Biochemistry Department in September 1986, I was left speechless. The laboratory did not even have water of the proper quality for making solutions. This would mean starting from scratch. I survived thanks to my collaborators and some begging. The good side of the problem was that there were some great people there, like Amando Garrido and Milagrosa Gallego, who were willing to work hard, and we sought financing by applying for new projects of all types and origins. We therefore quickly changed the appearance of those laboratories. The flip side was that some educators had perfectly adapted to this state of decadence and had grown complaisant. With too much free time, they enjoyed a very "creative idleness," making life impossible for any living beings who actually dared to do some work. They loved to hold all types of meetings and assemblies, a way of working that has been a true deadweight on the advancement of research at Spanish universities, above all those considered to be "historical". The teaching load was huge, with nearly seven hundred students in second-year biochemistry and over five hundred in first-year chemistry. This number did not decrease until a cap was placed on the number of students who could be accepted into the major of veterinary medicine. The establishment of the Complutense University's Biochemistry and Molecular Biology Department IV was important to the proper teaching that we managed to achieve in the following academic year, 1987–88, and I was its first director. Coinciding with its creation, the year of 1987 also led to the granting of positions to stabilize the status of many young people in the university, and competitive exams were held the next year. Many members of my veterinary medicine group earned positions as associated professors at the university, including Magdalena Torres, Esmerilda García Delicado and María Dolores Fideu, as well as recovering José Sánchez-Prieto, who had done his undergraduate thesis with me and continued as an assistant at the School of Pharmacology.

The beginnings of research at the Veterinary School of Madrid

We began our research by finishing off the work on adenosine and glucose membrane transporters that we had begun in Murcia, with some good science produced. Unexpectedly, the equilibrative adenosine transporter ENT, SLC29 had a wonderful surprise in store for us, because it displayed mnemonic kinetic behaviour and was the first of its kind to be described with such features. We published about this in 1993. To support the finding, the kinetic analysis performed by Professor García Carmona, of the University of Murcia, was indispensable. Years later, in 1996 and 1997, we would find similar behaviour

in the vesicular transporters of nucleotides VNUT, SLC17A9. I performed this work with Javier Gualix as part of his doctoral thesis.

In 1987 a great discovery was made in the laboratory that caused us to reorient our research on nucleotides. A brilliant researcher was hired to complete a postdoctoral stay, Dr Antonio Rodríguez del Castillo, who had been trained with my disciple, Professor Pedro Rotllán, at the University of La Laguna. He was an expert on high-performance liquid chromatography (HPLC), and we thought it would be of interest to quantify the different types of nucleotides in the neuroendocrine-secreting granules in the adrenal medulla. The problem was that we did not have the HPLC equipment, so we asked Professor Roberto Parrilla for help. At that time he had just returned to the CIB. It was with that borrowed equipment that we discovered the presence of diadenosine polyphosphates (ApnA) in the chromaffin granules. They are dinucleotides whose presence was not predicted, which is why they were important, because up to then they were considered similar to by-products created due to secondary reactions in the activation of the tRNA. Now we know that ApnA are by-products of nearly all kinases reactions. Studying the molecular and cellular biology of diadenosine polyphosphates has been a constant in our research, with the first article published in 1988. Being pioneers provided us with a platform in the purinergic field and a large number of references. In 1989, at the International Neurochemistry Congress, I met Professor Burnstock, considered the father of purinergic neurotransmission. I began to attend the purinergic meetings and organize international meetings and courses in this field. In 1990 a group of us researchers founded the Purinergic Club, which has never ceased to grow because the actions mediated by nucleotides are ubiquitous in all living beings, and possibly the most philogenetically primitive signals. A young researcher of my group, Jesús Pintor, began to do his thesis on diadenosine polyphosphates, first studying the ectoenzymes that broke them down and later searching for specific receptors in neural and endocrine tissues. In 1995 we identified a specific ionotropic receptor for diadenosine polyphosphates in the presynaptic terminals of rat brain. In 1997 another young researcher, Javier Gualix, synthesized the diinosine polyphosphates, very powerful inhibitors of the polyphosphate receptor, as well as P2X1 and P2X3, which are all ionotropic receptors. These compounds were used to establish very productive exchanges and collaborations with Professor Burnstock's group in London, Professor Zimmermann's in Frankfurt and with other important researchers, financed by a BIOMED project of the European Union. We completed the electrophysiology studies thanks to an INTAS collaboration project between the European Union and former countries of the Soviet Union, with Professor Oleg Krishtal of Kiev and Professor Levon Piotrovsky of St Petersburg. The most significant result of this collaboration was discovering a post-synaptic excitation current mediated by nucleotides, thereby confirming the neurotransmission function in the hippocampus. Participation in international projects was essential to our group, because it allowed us to form an international network of collaborators that facilitated the circulation of researchers and ideas.

Thanks to our original results, we would receive invitations to take part in numerous congresses and meetings by entities including the European Neurochemistry Society, of which I was chosen to be a member of the ESN Council by way of a vote in 1992, and the Society of Molecular and Cellular Biology of Chromaffin Cells, having been selected as a member of its Advisory Board in 1991. A few years later, in 1995, I became an elected coun-

cil member of the International Society for Neurochemistry (ISN), and I held the positions of international relations director and electoral coordination director, which allowed me to make contact with important neuroscientists from around the world. Moreover, I was a member of the editorial board of the *Journal of Neurochemistry*, which is the official publication of the scientific society. My last task at the ISN was to chair the International Scientific Committee to organize the scientific programme of the Twenty-First Joint International Congress of the International and American Societies (ISN-ASN) that was held in Cancun in August 2007. These were coupled with the congresses and meetings of the Purinergic Club and the organization of many different symposia and courses in this field, most notably the international events financed by the Ramón Areces Foundation. I have always considered the work of spreading science and facilitating contact among scientists to be very gratifying, and yet another facet of my commitment as an educator.

New research projects and other functions

Major developments in the field of purinergics forced us to constantly re-examine our objectives and the need to update our methodology appropriately, which became even more apparent as of the year 2000, when we organized the World Purinergic Congress in Madrid. We had to perfect the most cutting-edge techniques in video microscopics and microfluorimetry, thanks to a notable researcher, Enrique Castro, and we managed to record the response in terms of calcium in cells and even individual synaptic terminals. The functional studies were followed by identification using the immunohistochemistry of the type of receptors on each of the cells or synaptic terminals. This identification of the terminal type, parallel to the responses at each type of receptor, allowed us to understand the potential of the purinergic signal in various nervous system pathologies. The equipment necessary for this work is very costly, and it was paid for through national and European projects as well as through private foundations. Among the private foundations, the Ramón Areces Foundation was the first to award us a project, which meant an enormous amount of generous support to foster more ambitious objectives. The project awarded by the La Caixa Foundation allowed us to gather the first evidence of the involvement of nucleotide receptors in neurodegenerative diseases.

Thanks to the great project by the Botín Foundation, granted in 2006, whose director general was Rafael Benjumea, with Pedro García Barreno as president of its Scientific Committee, we were able to become fully involved in studying the physiology and physio-pathology of nucleotide receptors in the nervous system and collaborate with excellent groups from Spain and abroad. One of the major milestones was determining a physiological function defined for P2X7, which we had described as very plentiful in the mature brain's synaptic terminals. In 2008 we discovered the presence of functional P2X7 ionotropic receptor at the axonal growth cones, which occurred in collaboration with Dr Juan José Garrido, Dr Miguel Díaz-Hernández and Rosa Gómez-Villafuertes, members of my research groups. It was completely unexpected to find that the receptor's activation, with the entry of calcium, put a stop to axonal growth. No less surprising was discovering that *in vivo* the axons for growth must destroy ATP and other agonists using a wide range of enzymes called ectonucleotidases, which are expressed by colocalizing with the receptor. We had studied these enzymes since our first research work in Madrid, at least twenty years earlier, and we brought them

back once again. P2X7 proved to be a perfect target for controlling axonal growth. A member of my group, Dr Miguel Díaz-Hernández, got the chance to confirm the discovery *in vivo* in a model of epilepsy, in collaboration with Dr Tobias Engel of the College of Surgeons of Ireland. In effect, in epileptic lesions with destruction of hippocampus neurons, the new neurons form axons that branch out and abundantly express the P2X7 receptor at their terminals. Moreover, excessive activity of the receptor produced *status epilepticus*. The administration of antagonists led to an attenuation of epileptic seizures, confirming their importance in different forms of epilepsy, above all those refractory to the drugs in current usage.

The behaviour of receptor P2X7 in neurodegenerative diseases varies greatly depending on the type of disease. In terms of Huntington's disease, in collaboration with Professor José J. Lucas, we used genetically modified mice as models for this disease. In these models the axon terminals originating from the motor cortex that reached the GABAergic medium-sized spiny neurons in the *substantia nigra* overexpressed the P2X7 receptor. Thus, the excess of calcium internalized by these receptors favoured the terminals' destruction. As for animal models of family-related Alzheimer's disease (J20 animals), the P2X7 receptors do not appear to be increased, and are neither the expression of the messenger nor that of the protein, though their activity is. According to our results, the P2X7 receptor activates the GSK3 enzyme through a signalling cascade, which leads to a decrease in the activity of α-secretase, which is the enzyme for non-amyloidogenic processing of the amyloid precursor protein, APP. One poorly understood aspect is the presence of the functional receptor P2X7 in astrocytes, discovered by Esmerilda García Delicado, with the help of Professor Antonio Rodríguez Artalejo, a full professor of Pharmacology, with whom we closely collaborated. He has electrophysiologically described all the ionotropic P2X receptors on which we have worked, as well as the processes that require biophysical parameters.

There are many aspects of purinergic signalling that we are researching at the present time. One of them has meant returning with a new methodology to the central actions of diadenosine polyphosphates, because their levels in perfusion medium *in vivo* are sufficient to activate very diverse nucleotide receptors, and to the role played by enzymes that specifically destroy these compounds in neural differentiation.

In any case, I have completely left out our work on metabotropic P2Y receptors, and these are the ones that have taken on most importance after the discovery of specific antagonists on P2Y12, which prevent the formation of clots and are used to prevent strokes. Many other P2Y receptors have notable vascular effects, and compounds already exist that act upon P2Y2, P2Y4. They are used to treat dry eye and glaucoma. In my group, Raquel Pérez-Sen and Felipe Ortega have made some very original contributions, at the basic science level in principle, but with great therapeutic potential looking towards the future, because they protect the neurons from cytotoxic stress and radiation. These studies allowed us to take one step further in understanding the signalling cascades of the P2X and P2Y receptors, for the first time involving P2Y13 receptors in the regulation of dual phosphates, DUSP, which reverse the signalling cascades. In this same direction, in collaboration with Antonio Cuadrado, we have verified the importance of the P2Y13 receptor on oxidative stress, involving hemooxygenase. We have also studied the effects of the P2Y1 and P2Y13 receptors

on glycine transporters, with the connotation of pain control at the central level, in collaboration with Carmen Aragón and Cecilio Giménez's group. The constant presence of the P2Y13 receptor in all these processes is of the utmost interest, and we will eventually see the apogee of its pharmacology in the upcoming years.

However, I would not want to end this section without mentoning another very innovative aspect, the result of recent collaboration with Professor Lisardo Boscá. Studying the effects of nucleotides in macrophages, he discovered that the effect of prostaglandins on the response of P2Y receptors activated by uridine nucleotides (UTP and UDP) is not due to the interaction of their receptors, but rather to the direct action of the prostaglandins on an atypical PKC that is found in the signalling cascade. This mechanism may possibly be widespread in many inflammation processes. The surprises and innovations in this field are constant, and they are just sitting there waiting to be discovered. As so often happens when you become deeply involved in something and you feel passionate about it, like the research we were doing, and for the first time in my life with no economic tribulations, higher forces can arise that distract us from the main goal. On these occasions, it is a good idea to read the classics and remember what Baltasar Gracián advised: "There are odd concerns that are like moths eating away at our precious time". I would not regard some of the activities that I have performed during these recent years as separated from my teaching and research work, but you may think of them as an extension going beyond the realm of the university. The truth is that they used up a large part of my time, which I was lacking on many occasions. Some of these activities increased my visibility and allowed me to design and introduce changes into rigid structures. I was even able to express my opinion aloud. The best part is that, on this journey, when you try to do something, you come across people who are as idealistic and naive as yourself. In general, they are very stubborn people convinced that it is worthwhile attempting to do things they way they should be done. The problem is always the system's inertia.

The higher forces arose in January 2001 when I read my speech upon admission as a Numerary Academy Member of the Royal National Academy of Pharmacology, having been introduced by Professor Ángel Santos Ruiz. In December 2006 I was elected president of the Royal National Academy of Pharmacology. Becoming the first female president of a Royal Academy of the Institute of Spain was apparently quite a revolution. The reality was that the situation lived up to the old Spanish saying "in the sin lies the penance," because the learning process was intense, and the topics to which I had to pay attention were very distant from my work as an educator and researcher. I had the great luck to be given help by all the academy members, who were very generous with my initial awkwardness. The advice of Mr Juan Manuel Reol Tejada, the prior president, always wise and fitting, was essential to surviving my first months in the position. I was later elected a Numerary Academy Member of the Royal Academy of Veterinary Sciences, the Royal Academy of Pharmacology of Catalonia, the National Academy of Pharmacology and Biochemistry of Argentina and the European Academy of Arts, Sciences and Humanities (EAASH)/ Académie Européenne des Sciences, des Arts et des Lettres (AESAL). Especially enjoyable for me was the conference that I gave at the headquarters of the French Academy, where I presented my research work as an Academy Member of the National Pharmacology Acad-

emy of France in May of 2012. It was very moving for me, because I have always held great respect for French science and culture, to which I consider myself indebted. In 2011 my English and German colleagues, with whom I had collaborated for many years, proposed my candidacy as a member of the European Academy, in Section C3, Physiology and Medicine. I have been a member of that academy since October 2011. One great, unexpected honour was being named Doctor Honoris Causa by the Rey Juan Carlos University of the Autonomous Region of Madrid, with an award ceremony of major academic character on the date of 28 January 2013. I will always be grateful to them for having thought of me.

I must say that the national and international evaluation committees and the invitations to form part of prestigious juries do help one become more familiar with the creative undercurrents and hard-working, valuable people in our country, who not only give their all, but should also be considered a true example to everybody. I never considered these meetings to be just routine work, and it is of deep satisfaction to me to find that there are people of enormous worth in building science. Last of all, my naming as president of the Ministerial Committee for the Reform and Improvement of Quality and Efficiency in the Spanish University System in 2012 took shape in 2013 with a document delivered to the Minister of Education, José Ignacio Wert. It was drafted through the efforts of a top-level committee concerned only about the suitability of Spanish universities in line with the present times. We hope that somebody actually heeds its words someday.

One last factor, which comes with many years of work and age, is that you are given an award from time to time. In addition to others, I received the award of the Confederation of Employers and Industries of Spain (CEOE) for Biomedical Sciences in 2004, the Research Award of the Autonomous Government of Galicia in 2008, and the Research Award for a Career in Science from the Autonomous Government of Madrid in 2011. They all indicate that I have reached an age at which I have become deserving, and so I am twice as thankful for them. However, the award that moved me the most was the first prize for research that was awarded in my home town, the Arenteira Science Award. It is a bronze sculpture of a beautiful oak leaf, named for the River Arenteiro, a treasure of nature.

Final remarks

I have always thought that science is a collective labour performed by normal people with great effort and dedication, with a few peaks offering original ideas that are actually rather scarce. In order to be developed, science requires a society that is convinced that science is needed and respected. As an educator, I believe that research is essential to the development of the university itself, because therein lies the stimulus for the creative strength of future scientists, and it is where the most original ideas are conceived because the brains at work are younger.

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JOSÉ LÓPEZ BARNEO

MEMORIES AND REFLECTIONS ON MY SCIENTIFIC CAREER

10

The first thought that occurred to me as I embarked on this autobiographical account of my scientific career was that, even though it has had its disappointments and a few failures, I feel fortunate to have studied and worked in the field that I dreamed of as a child. Furthermore, I had the good fortune to live in a special time in Spanish history, during which a great deal of development occurred after a period of stagnation. It was a time in which scientists' work, in addition to advancing knowledge, also contributed to the progress and modernization of the country. During these years we created a National Science and Technology System, which, although it has numerous limitations and defects, is unique in Spanish history. Spain has joined the international community, which has diluted the "patriotic" component that doing science had in our country. From a more global perspective, recent decades have seen a major transformation in biomedical research. The explosion of molecular biology and subsequent advances in genetics have erased the barriers between the disciplines and transferred results from fundamental research to clinical medicine and biotechnological applications. In this changing scenario, electrophysiology and neuroscience, my areas of specialization, were among the most affected.

In 2008 I had the honour of being named a "favourite son" of Torredonjimeno (Jaén), my hometown (1952), where I spent my childhood and adolescence. The event was held in the "Instituto Santo Reino", where I went to school until I was fifteen years old; as a memento, the director of the centre gave me a copy of my enrolment card that I had filled out when I was ten years old, and in which I stated my desire to become a doctor – and that football was my favourite sport. When, after many years, you think about the path you have taken, you find key moments that could have played out differently and completely altered your destiny. The different, sometimes complex circumstances that are often difficult to understand and appreciate in real time are determining factors in people's fates. However, I also believe that teaching us to generate an "internal personal motor" that motivates us to persevere in the achievement of our dreams is an essential task of families and schools, especially in the early years.

My father, Amando López Rísquez, was a truck driver for a transport company, and my mother, Emiliana Barneo Carpio, cared for us at home. Both their educations consisted of only a few years of grammar school, but from a very young age they stimulated my natural desire to learn and advance in my studies. Even though there were no university graduates in my family, I received a solid education thanks to my teacher Mr Rafael Fernández, when I was between seven and ten years old, and afterwards from the teachers at what was then called the "Labour Institute" (from ten to fifteen years). At this school they combined the classical fields of study with workshops on mechanics, carpentry and electricity. It was a form of teaching that focused on giving the better working-class students a

basic cultural level, so they could later learn a skilled profession in Schools of Industrial Apprenticeship. In my case, I did not follow that path, but all that contact with machines and tools served me well when, years later, as an electrophysiologist, I used ¡nstrumentation in research laboratories. I received my secondary education thanks to a scholarship from the "Patronage for Equal Opportunity", which required that recipients pass all their classes in the final exams held at the end of June each year. Those who wished to go on to high school had to receive a grade of 7 or higher. This is the environment in which my medical vocation was born. It was influenced, I believe, by my frequent conversations with my mother and by reading the biographies of Fleming, Pasteur and other "heroes" in the field of biomedical research.

On finishing my secondary education, I received a scholarship to study for a technical baccalaureate. I was "sent" to the Alcalá de Henares "Labour University". I studied for two years at this centre, which had just opened and had excellent chemistry, physics, mathematics and electronics teachers, as well as talented teachers in other fields, all of whom left an indelible mark on me. The technical baccalaureate required a "major", which in my case was electronics. We had electrical engineering, radio and television rooms, where we spent at least three hours every day with top-flight equipment made available to students. In 1967, during the height of the Franco dictatorship, we had a television to work on, when in the neighbourhood where my parents lived there was only one family that owned such a luxurious piece of technology. The Alcalá Labour University gave me everything necessary to progress intellectually and as a person. Everyone who was there – from different regions of Spain – had gone through a very strict selection, for which reason we were all hard workers, bright and from working-class families. In this environment it was only natural that we would feel proud of our origins and conscious of the opportunities that studying opened up for us. My education in science and technology was also a very valuable complement to my spontaneous medical vocation. I had a good memory, and ever since I was a child it had enabled me to easily master the most encyclopaedic aspects of my studies. The workshops obliged me to approach knowledge in a different way. In addition to memory, this form of knowledge required practical reasoning and an acceptable level of motor skills. In June 1969 I took the university entrance exam at the Complutense University of Madrid – actually in the main hall of the Medical School – and a few weeks later I received a letter granting me a scholarship to study medicine at the university of my choice. I transferred my transcript to the University of Seville, where I started my studies in October 1969.

Compared to the Labour University, the Medical School in Seville seemed poor, lacking in infrastructure and with a teaching faculty that was in many cases worse than what I had previously experienced. Nevertheless, I owe a great deal to this university. I had some brilliant professors, who not only strengthened my desire to study biomedicine, but also showed me the path to become a researcher. All this occurred in a complex, but very attractive period in Spanish universities, in which a significant percentage of the professors and students were at the forefront of the anti-Franco movement. Elizabeth Pintado Sanjuán, a fellow student on the course, with whom I have been married now for over forty years, introduced me to the "student movement" and, as a result, in the last three years of my studies (between 1973 and 1975), in addition to my academic work as an intern

in the Department of Physiology, I was also the union delegate of my class. I studied for my clinical courses with great interest because I liked them, and also because I knew that my grades were not only important in terms of graduating from the course, but also for securing a scholarship to write a doctoral thesis. However, I understood early on that it would be difficult to combine a medical practice with basic research, a dilemma that has remained a constant throughout my career.

Between 1975 and 1978, as a teaching assistant and doctoral student, I worked as a physician for six months at a military camp and in a village (Peñarroya-Pueblonuevo, Córdoba), where I believe I carried out my duties competently. I still bump into former patients who remember that almost forty years ago I treated them for arterial hypertension, kidney stones, or some other illness. The salary of a doctor was much higher than that of a doctoral student, which allowed me to save money so that I could live decently with my family in Paris during my first postdoctoral fellowship. The decision to become a physiologist arose from my general and special physiology coursework with Diego Mir Jordano, a professor who had just arrived from Yale University where he had worked for several years with José Manuel R. Delgado. Delgado was a controversial Spanish psychiatrist; a US resident who, in the 1970s, had some very original ideas related to the control of cerebral functions through electrical stimulation, but who, in the general opinion of his peers, was unable to make solid advances in his experimental work. The excellent classes given by Diego Mir and the intrinsic beauty of the physiological mechanisms that he explained, often using data from recent discoveries at the molecular level, attracted me immediately to this field. As a result I ruled out other options and requested an internship in the Physiology Department.

My first foray into experimental research involved a study on the brains of anaesthetized rats into which we introduced fine tungsten electrodes that we used to stimulate different parts of the brain or to record electrical activity (a sort of deep electroencephalogram). I wrote my doctoral thesis on the neurophysiology of the amygdala, and, even though we did not really know what we wanted to discover, my colleague, Juan Ribas, and I worked in the lab, eleven or twelve hours a day, from Monday to Saturday. From very early on, I learned what it meant to do experimental research in Seville in 1975. Our department comprised empty rooms and hallways with no equipment or personnel, very similar to what Santiago Ramón y Cajal described in his works as the normal situation in Spanish institutions at the end of the nineteenth century. Diego Mir made a huge effort to teach and organize coursework that was acceptable for the over 1500 students in the department and to obtain resources to put together a research laboratory. Although in this period Spanish science had started to make significant inroads in fields such as biochemistry, neurophysiology was practically non-existent, with just a few nascent groups, although better equipped than ours, in Madrid, Valladolid and Santiago de Compostela. I learned from Diego Mir how to distinguish between good and bad scientific work (and teachers) and it was he who showed me the path to becoming a scientist. With the assistance of Juan Ribas and José María Delgado García, who received his doctorate before us and was at that time a postdoc at New York University, I was able to obtain some recordings of electrical activity from neurons in the brainstem that regulate eye movements. This became my thesis topic. During this period (in 1977) I gave my first scientific presentation at a physiology

conference held at the Autonomous University of Barcelona. I think my presentation was well received, although it was rather fast paced since I had to explain nearly thirty slides in fifteen minutes. Antonio Fernández de Molina was in the audience (which I imagine was shocked by the spectacle), and many years later told me that I gave the impression of a "runaway horse". Fully aware of the limitations of my surroundings, I requested a grant from the European Brain and Behaviour Society to spend nine months in Paris to progress as a researcher. This grant had a major impact on the direction my life took, since if I had not received it I would have pursued a medical specialty. Apart from this turn of luck that had such a profound influence on my life path, I have never understood why I was so "loyal" to neurophysiology, and why I did not reorient my career towards another discipline like biochemistry, which I liked. Furthermore, this discipline already had its first groups and institutions in Spain and these already had standing on an international level.

In January 1978 I moved to Paris, with my wife and two-and-a-half-year-old twin daughters, to embark on my first postdoctoral stay. Elizabeth, who spoke French very well and was studying for her doctorate in the recently created Biochemistry Department, was able, after overcoming some difficulties, to come with me and work at the Collège de France with Jean Girard. I worked with Alain Berthoz in the CNRS Laboratory of Sensorial Neurophysiology, located near the Collège, together with the École Normal Supérieure, in the heart of the Latin Quarter. Berthoz, one of the leading French neurophysiologists of the time, was a collaborator of Robert (Bob) Baker, who José M. Delgado García worked with in New York. I joined a project that had recently started and was being developed by Christian Darlot, one of Berthoz's doctoral students. The objective was to study the neurons in the brainstem that regulate eye movements. In that period, the oculomotor system was one of the most developed systems for the study of motor functions and neuronal plasticity. One of the hot research areas involved the identification of the place where the electrophysiological signals were generated that, acting on the motoneurons of the ocular muscles, produced movement and maintained the position of the eyes. Together with Christian, Alain and Bob, I carried out a study using alert cats in which we analysed the electrical activity of nearly two hundred neurons in the *prepositus hypoglossi* nucleus, a zone located in the brainstem between the abducens and the hypoglossal nuclei. Many of these cells had an action potential firing frequency that encoded, in an extraordinarily precise manner, the velocity and/or the position of the eyes in the horizontal or vertical planes. Based on these experiments and those we carried out the following year, we wrote an article[1] in the *Journal of Neurophysiology* that went on to become a classic in the physiology of the oculomotor system. The publication of this article, due to its scope and implications, was my debut on the international physiology scene, and though I later abandoned this research field, I felt from this moment in time that "I could do things" and that "I had a scientific story to tell". Despite the difficulties and uncertainties inherent to embarking on a professional career, my memories of this time in Paris are unforgettable. Alain Berthoz and his group took me in with respect and generosity and during my few months with them I really came to understand the academic environment in scientifically advanced countries.

During my sojourn in Paris there was another fortunate coincidence that had a decisive impact on my professional career. I unexpectedly met Rodolfo Llinás, a neurophysiolo-

gist originally from Colombia, who was the director of the Department of Physiology and Biophysics at the New York University Medical School (a post he held until just a few years ago). At the time Rodolfo Llinás was already one of the world's top neuroscientists. Together with John Eccles (Nobel Prize in Physiology and Medicine in 1963) he carried out an excellent study on cerebellar neuronal circuits and had just discovered that neuronal dendrites were not passive elements, as was previously thought, and that they could actively generate calcium action potentials. For reasons I never discovered – perhaps because he had always had a great appreciation for Spain and Spaniards – Rodolfo invited me to dinner on one of the few days he spent in Paris. During dinner, he gave me a thorough exam on physiology, medicine, and even philosophy, making me see that to become a "true" electrophysiologist I would have to spend three or four years in the United States to receive the proper training. I would first have to work with a group of biophysicists focused on membranes, and later in his laboratory working on the intrinsic electrical properties of central neurons. I still remember returning home that night, totally blown away by the conversation with Rodolfo, a highly intelligent person with tremendous powers of persuasion and, without doubt, one of the most important physiologists of the twentieth century. Rodolfo advised me to write to Francisco (Pancho) Bezanilla (University of California, Los Angeles) or Clay Armstrong (University of Pennsylvania) in order to work with them on the squid giant axon. At the time these physiologists were the best in the field following Alan Hodgkin and Andrew Huxley (Nobel Prize in Physiology and Medicine in 1963), since several years earlier they had discovered *gating currents*, capacitive currents that are produced by the movement of electrical charges inside the thickness of the membrane and that are due to the movement of the gates that open and close the membrane ion channels. The measurements of these currents – a technological milestone at that time – were of great conceptual importance, given that they pointed towards the existence of the ion channels regulated by membrane potential proposed by Hodgkin and Huxley.

After returning from Paris (1978–79 academic course), I was appointed interim associate professor in the Department of Physiology at the Medical School in Seville, which resulted in a notable improvement in my salary and in the quality of life for my family. With colleagues, we put together an excellent experimental set-up to study the electrophysiology of the ocular motor system in alert cats. I designed and carried out, for the first time in my life, experiments with a precise scientific focus and concrete objective. This resulted in several papers being published in international journals, something I still feel quite proud about. This was a transition period, and at the same time I started looking for a postdoctoral position in the United States. Pancho Bezanilla's laboratory was ruled out for at least a year since he had no space. However, Clay Armstrong offered me an NIH funded postdoctoral position, so that I could join his group as soon as possible. From April to September, Armstrong's laboratory relocated to the Woods Hole Marine Biology Laboratory (Massachusetts) to work on the squid giant axon, a model that was practically the only one at the time in which biophysical methods could be used to study transmembrane ionic currents. The laboratory did not usually have experimental activity going on during the winter months, so Armstrong proposed that I do a project on the electrophysiology of endocrine cells. At the time the study of endocrine cells was appealing to biophysicists because some of these cells – such as those of the pituitary and the pancreas – had voltage-gated ion channels

similar to those present in the so-called "excitable cells" – neurons and muscle – and were therefore able to generate action potentials.

I went to the United States at the end of January 1980, leaving my family in Seville, since we thought that our daughters should finish the school year in Spain. Elizabeth had been accepted into a postdoctoral programme at the University of Pennsylvania Biochemistry Department, which she started a few months later. During the flight to America, I decided to work on the parathyroid gland cells, since their electrophysiology was unknown and their secretory activity was activated through the lowering of extracellular calcium, something that was unusual, since secretory systems normally require the entry of calcium from external media to induce exocytosis. Initially, I was surprised by Armstrong's laboratory. When you are in the country that is the global scientific leader, you expect modern facilities and the highest quality equipment. Instead I found a room full of parts and broken equipment. The only thing that worked was a computer, which was open on all sides, and which Armstrong used for doing his calculations and models. However, before going to Woods Hole that year, we were able to put together a perfectly functioning setup and generated the first data on the electrophysiology of parathyroid cells. Armstrong showed me – with great patience – how to use the operational amplifiers and other microchips, and in a few weeks we had built a recording amplifier for high-resistance microelectrodes, stimulators, heating systems for the solutions and other equipment. The study, completed in subsequent years, showed that the parathyroid cell membrane potential is regulated, in a very precise manner, by extracellular calcium and other divalent cations. We proposed, for the first time, the existence of a calcium receptor in the membrane and, using the cryofracture technique, observed this receptor as transmembrane particles with a large extracellular domain.[2] Inspired by our work, a few years later Edward Brown's group at Harvard University cloned the extracellular calcium sensor or receptor using cow parathyroid cells. This consisted of a protein with seven transmembrane helixes and a very large extracellular domain rich in dicarboxylic amino acids, which can bind calcium ions. In addition to the parathyroid glands, the extracellular calcium receptor can be found in numerous tissues – brain and kidney, among others – although its functions in these cases are not fully understood.

During the summers in Woods Hole, the research project involved measuring the ionic currents in small "patches" of squid giant axon membrane, with the idea that current flow through a single ion channel could be recorded. At the beginning of 1980 there was some debate as to whether ion channels functioned as discrete entities that permitted the flow of a "package" of ions through the membrane as long as the channel pore remained open. Inspired by a recent work by Erwin Neher (Max-Plack Institute, Göttingen), where, using the patch-clamp technique, he had been able to record currents through single K^+ channels, we tried to do the same for Na^+ channels. For several months I worked intensely at Woods Hole, but with few results. Neher came to give a seminar and showed us very high-quality measurements of single Na^+ channel currents in myotubes using a new technique (gigaseal) that he had just discovered. Years later, in 1991, Erwin Neher, together with Bert Sakmann, received the Nobel Prize for Physiology and Medicine for their discovery of the patch-clamp techniques. In the squid axon we obtained nice recordings of

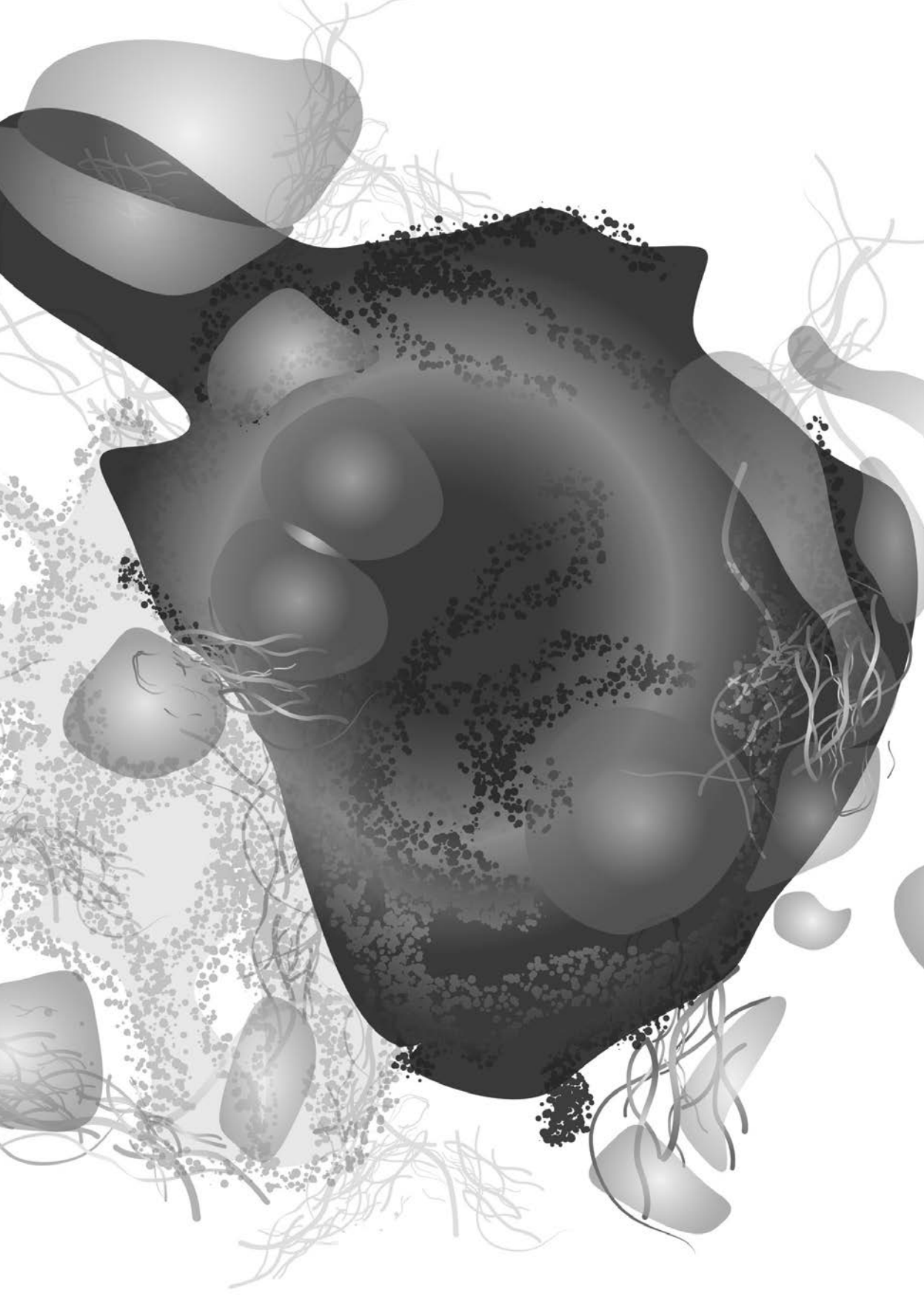
between fifteen and twenty Na^+ channels and with this data we gave a presentation at the Biophysical Society congress held in Denver on 23 February 1981 – the same day that the Spanish Parliament was occupied at gunpoint by anti-constitutional civil guards. That was my first oral presentation in English before a large audience and I think it went well. In September 1982 the entire family returned to Spain after I was offered a position as an assistant professor in the Physiology Department at the Medical School in Seville. While the laboratory was being built, I returned alone to New York (between February and May of 1983) to complete my postdoctoral fellowship, working in the laboratory of Rodolfo Llinás. The exams for the appointment as an assistant professor had been held several months earlier in Alicante, and the president of the tribunal was Carlos Belmonte, who treated me in a manner that I will never forget and for which I will never be able to thank him enough. Ever since then, Carlos has been my mentor and friend. In New York, I coincided with Antonio Fernández de Molina, who was one of the most influential physiologists in Spain. The friendship we forged in that period was later of great help when I had to get established as an independent researcher in Seville.

Even though my postdoctorate in the United States did not result in many published articles, I always thought that it had met my desired objectives. Clay Armstrong and Rodolfo Llinás were two excellent scientists, although very different from one another, but I was treated extremely well and gained complementary knowledge from both of them. Clay was very nice and had a solid conceptual mind and a rigorous experimental approach. He taught me electronics, how to build the recording apparatuses, and the fundamentals of membrane biophysics. This gave me a great deal of confidence and the knowledge and independence necessary to put together my own laboratory in Spain in a field that, though it changed very quickly, was only considered appropriate for "experts". In Clay Armstrong's laboratory, I saw the beginnings of cloning and the molecular identification of ion channels, one of the holy grails of modern biology. Between 1980 and 1990, genes encoding for ligand- and voltage-gated ion channels were identified. I remember the seminars at Woods Hole, where the first projects on recombinant channels were described, showing that many of the ideas proposed by biophysicists in previous decades were being confirmed at the molecular level: hydrophilic pores formed by transmembrane alpha helixes, charged domains in the membrane acting as "voltage sensors", structures gating the opening and closing of ion channels, and so on. This revolution made it clear that ion channels are real proteins and not imaginary entities, and that these proteins participate in cellular signalling just like receptors, enzymes and transcription factors. Electrophysiology, which at the time was greatly simplified by patch-clamp techniques, ceased being an exclusive science limited to those who enjoyed working with wires, as it had been before. Many laboratories started to use it in combination with molecular or cell biology. In 1999 the Lasker Prize was awarded to Bertil Hille, Clay Armstrong and Roderick MacKinnon, three representatives of the generation of biophysicists who brought the field of ion channels from "idea to reality".[3] It was speculated that this same trio would win the Nobel Prize for Physiology and Medicine, but it was not meant to be. Several years later, MacKinnon received the Nobel Prize in Chemistry for his studies on the three-dimensional structure of potassium channels. He shared the prize with Peter Agre, who discovered the water or aquaporin channels.

Rodolfo Llinás had a very attractive personality, but on a personal level he was usually somewhat distant. With me he was always attentive and sometimes kind, perhaps because we shared the same language and many cultural traits. In his laboratory, each postdoc worked on a different brain structure, but everyone studied the intrinsic electrical properties of neurons. This idea, generated to a large degree by Llinás,[4] sought to show that the neurons in the different structures in the nervous system had different electrical properties due to the variations in the type and distribution of the ion channels in their membranes. Therefore, each cellular type integrates the information it receives in a different way. Up to then, neurons had been considered totally passive elements in which the output signal in each case depended mainly on the morphology of the somatodentritic tree. The ideas of Llinás and his laboratory added a new level of complexity to neuronal integration and helped explain phenomena such as the generation of neuronal rhythms that underlie sleep and wakefulness. After having worked for three years with Clay Armstrong, joining Llinás's laboratory did not pose any problem and in the few months that I was there, working between twelve and fourteen hours every day, I learned to look at neuronal physiology in a new light. Although I have always considered myself a neurophysiologist, I developed a great respect for the brain and decided – for better or for worse – to investigate more specific topics, on the cellular or molecular level, and to leave the challenge of understanding the organ to future generations. After this stay in New York, which came to an end in May 1983, my postdoctoral training, which I had started in 1978, was over. I remember that I was in a hurry in those days, not just to rejoin my wife and daughters who had stayed in Seville, but to create my own group that would gain international recognition and, in this manner, contribute to the scientific and social development of my country. That period was a critical moment in Spanish history, and most young professionals were very hopeful and positive about the future.

On returning to Seville in the autumn of 1983, I started the task of creating, practically from nothing, the Cell Biophysics Laboratory, since the equipment we had acquired before I left for the United States was being used in the Neurophysiology Laboratory. After more than a year of waiting, during which time I gained experience as a teacher, giving physiology classes to medical students, and applying for grants from different bodies, I received my first grants as the principal investigator from the Advisory Committee for Scientific and Technical Research (CAICYT) of the Ministry of Universities and the Ramón Areces Foundation. The first doctoral students (Lucía Tabares and Guillermo Álvarez de Toledo) started in the laboratory, and they were joined shortly afterwards by Juan Ureña and Antonio Castellano. Our scientific objective was to take advantage of modern electrophysiological techniques (patch clamp and intracellular recording in brain slices) to identify ion channels in neurons and in endocrine cells that carried out functions of physiological relevance. We were especially interested in studying how different stimuli applied to secretory cells are translated into electrical signals that trigger the release of hormones or other transmitter agents.

Among the different projects during this period, I would highlight, for their impact on my research career, the work we started on the carotid body in collaboration with Constancio González at the University of Valladolid. He had completed his doctorate with Carlos Bel-



monte and subsequently did a postdoc with Carlos Eyzaguirre at the University of Utah. The carotid body is a small sensory organ next to the bifurcation of the carotid artery whose physiological function is to detect changes in the level of oxygen and other parameters in blood – CO_2, pH and glucose, among others – and to activate adaptive cardiorespiratory reflexes. In conditions of hypoxemia, for example, the activation of the carotid body leads to hyperventilation and sympathetic activation, which increases oxygen in the blood and its delivery to tissues via the circulatory system. We owe our knowledge of the sensory role of the carotid body to the pioneering work of Fernando de Castro and Cornelius Heymans. The latter won the Nobel Prize for Physiology and Medicine in 1938, but he did not receive it until 1945, when the Second World War ended. Subsequently, the carotid body attracted numerous researchers, among whom Carlos Eyzaguirre was one of the most notable. He demonstrated that carotid body sensory cells, called glomus cells, form chemosensory synapses with afferent nerve fibres that terminate in the brainstem respiratory centre. Despite these significant advances, in the mid 1980s the physiology of glomus cells was practically unknown, due to, among other reasons, the fact that they are very small (10 to 12 microns in diameter) and easily damaged by the microelectrodes used for intracellular recording. For this reason we thought they would be ideal cells for applying patch-clamp techniques. In 1987 José Ramón López-López, a doctoral student of Constancio González, came to my laboratory, and in the following months we inaugurated a new chapter in sensory physiology that has had a major international impact. We published an article in *Science*[5] in which we showed that the glomus cells are neuronal elements that produce action potentials and have potassium channels regulated by oxygen. In subsequent work, we were able to demonstrate that glomus cells are presynaptic-like elements that, in response to hypoxia, release transmitters that activate afferent sensory fibres. This model of chemotransduction was confirmed by other research groups and shown to work for other chemoreceptor organs. In addition, the regulation of ion channels by oxygen proved to be a more general phenomenon that participates in other functions, such as the control of vascular tone or the contraction of the *ductus arteriosus* after childbirth.[6]

Research on the carotid body has occupied a large part of the activity of my group during the last thirty years. It was once again an unexpected circumstance that led me to become interested in this organ and not another. Scientists normally "search" for problems to study, but on some occasions the problem "finds" the scientist; sometimes they are even involuntarily "captured" by a topic. In the initial phases, I worked on the carotid body thanks to the huge unexplored field that we opened up as a result of the first experimental study, and because our laboratory, more than any other, had gathered together the human and technological resources needed to perform electrophysiological studies on small glomus cells. Afterwards, due to the need to respond to the new, continually arising questions, I ended up, without even realizing it, spending three decades working in this area. In fact, it was only recently that we were able to respond with experimental data to some of the basic questions that arose in 1988. Sometimes I ask myself what the evolution of my group would have been if we had not been "captured" by the carotid body.

The 1980s were not only about scientific progress, they were also about personal and professional maturity. A few years after returning to Seville, I received an offer from Carlos

Belmonte to go to Alicante, to the newly created Medical School. Although I seriously considered it, the plan did not materialize because at Diego Mir's request the university created a chair in Physiology to entice me to stay in Seville. A few months after I became the chair (January 1986) an extraordinary person came to see me in Seville, Roberto Fernández de Caleya, who I had never met before. Roberto had been appointed director of the National Agency for Evaluation and Prospective (ANEP) and had been tasked with creating an internationally accredited peer review system for the research projects presented to the National Plan for Scientific and Technical Research. His idea was to appoint different scientists (called "coordinators") who would oversee different areas, select the anonymous evaluators and make a proposal regarding whether or not to finance the project. Although I resisted a little because I did not want to be distracted from my scientific research, which was going well, Roberto convinced me because he promised to install a telefax in my office (an invention that I had not heard of), so that I would not need to travel to Madrid frequently. The promised telefax was never installed and over the next three years I had to travel to Madrid several times a month, staying overnight. However, contributing to the creation and development of the ANEP and working with Roberto and all my colleagues (scientists from other fields, evaluators, managers and administrative staff) has perhaps been one of the most enriching professional experiences I have had. The discussions about the evaluators and projects, the meetings and the presentations, the trips abroad to evaluate bilateral integrated actions, my stays in the CSIC Student Residence and many other activities were always characterized by high technical quality and camaraderie. Since then I have always had vivid memories of that period. In addition to my work in the ANEP, I had the privilege to participate in numerous committees that contributed to the modernization of the Spanish biomedical research system, in particular the Fund for Health Research (FIS). In this environment, management activities were almost as creative as scientific ones, and as a result of hard, focused work, the research community came to view the ANEP as the most prestigious institution in the Spanish National Science and Technology System. It is a pity that the same people who backed this project were unable to carry out a university reform in accordance with what Spanish society needs in order to modernize the R&D structures in our country. The creation of a National R&D Plan and the ANEP's initiative multiplied the number of researchers in Spain, which had the effect of creating new scientific societies that had never before existed in Spain. I played an active role, which I believe was important, in the creation of the Spanish Biophysical Society, where I was on the management committee. I was also involved with the Spanish Society of Neuroscience, where I was one of the founding partners, the vice-president and later, the president. My research group grew and matured, with the presentation of the first doctoral theses, the incorporation of new doctoral students, and the postdoc studies abroad of my first graduate students. With the change in the university legislation, the classic chairs were phased out and new departments were created. I was elected director of my department in 1987, which, under the new design, was called the Department of Medical Physiology and Biophysics, the name it still bears today. During that period we approved agreements, such as the need for new professors to have worked at foreign research centres, which were fundamental for ensuring that the department attained the quality it has today, one of the highest in the University of Seville.

A collateral advantage of my work with the ANEP was that, after the statutory period, I was "awarded" with a grant to spend a sabbatical year at Stanford University in Palo Alto, California. Between September 1991 and August 1992 I worked in Richard Aldrich's lab in the Molecular and Cellular Physiology Department in the Medical School. The department was located in the recently created Beckman Center, an elegant building with magnificent facilities where top-flight biomedical Stanford researchers did their work. Rick Aldrich, who I had met when I was a postdoc, directed one of the most active groups, which was studying the functional-structural relationships of potassium channels. I wanted to learn about the molecular biology of the ion channels so that when I returned to Spain I could work on the identification of the molecular mechanisms responsible for the regulation of the potassium channels by oxygen, a phenomenon that would help complete the work we had done earlier on the carotid body. My studies of molecular biology proved to be fruitful and pleasant, thanks to Rick and the members of his lab. I started from zero, purifying DNA, performing site-directed mutagenesis and sequencing. To apply what I had learned, I started a project related to the molecular basis of inactivation of the potassium channels, a process that allows the channels, once open, to stop conducting ions. Ion channel inactivation has a very important functional value, since it determines the duration of the refractory period and the frequency of the action potentials of excitable cells. Curiously, the speed with which this process occurs may vary by up to almost one thousand times between different types of potassium channels. Some channels go inactive in 10 to 20 milliseconds, other take several seconds to do so. Comparing the sequences of amino acids of the already cloned channels, I saw that even though they were very conserved, there was an amino acid in the external mouth of the conduction pore that changed from one molecule into another. We showed that by mutating this amino acid we could generate channels with changes in the inactivation kinetics similar to that of native channels, which represents a fundamental discovery. In the final weeks of my sabbatical – while I was suffering from withdrawal symptoms after having given up smoking – I quickly wrote an article that I sent to the journal *Neuron*, which was rejected for reasons I still do not understand. This same text was accepted by a less "fancy" journal,[7] and over the years has become a classic in the field. Despite the fact that the journal is no longer published, this paper continues to be one of the most cited in my professional career (five hundred citations) and I continue to receive requests for PDF copies of the study. Obviously, peer review has its faults, and in this case the editors of *Neuron* committed a clear error. Like the majority of scientists, I have had various articles rejected, some unjustly, in high profile journals. However, it is difficult to find a system that works better than international scientific evaluation. My confidence in this system has only increased over the years. Nevertheless, I think that, in a similar manner to how researchers are evaluated based on the quality of their publications, scientific journals should be subjected to an independent evaluation of their technical quality that obliges them to revise and refine the procedures that they follow in their decision-making processes.

My sabbatical year in Stanford was not only fruitful on the professional level. My entire family enjoyed highly enriching experiences that we still remember with nostalgia. Elizabeth worked on the genetic basis of autism, which allowed her to subsequently conduct research and genetic diagnoses in her laboratory of the Fragile X Syndrome, and my daughters

completed a full year at high school, experiencing all aspects of the American way of life. I had to travel twice to Seville from San Francisco in order to participate in meetings with the regional authorities regarding the creation of an Andalusian Biology Laboratory (LAB). This project was designed by Enrique Cerdá, the chair of Genetics at the University of Seville. As a promising young local researcher, I was invited to join, together with three other colleagues who worked in foreign labs: Miguel Beato and José Campos Ortega in Germany and Manuel Perucho in the United States. The objective was to create a biological research centre of excellence in Seville, governed by guidelines similar to those prestigious European centres such as the EMBO lab in Germany, or the Medical Research Council centres in the UK. It was a very exciting environment, where I learned a great deal. We had several meetings, and secured financing to construct a building in what would be the Pablo de Olavide University, which was designed to be a more modern and cutting-edge university than what had existed up to then. However, although we never received formal explanations, the project was cancelled by the authorities, and the building remained empty for several years. Later, the building, which had been built and equipped thanks to our efforts – especially those of Enrique Cerdá – housed the Andalusian Centre of Developmental Biology (CABD), where a group of young researchers is now doing excellent work. Miguel Beato was able to complete his project in Barcelona, where, with the assistance of the Catalan authorities, he created the Centre for Genomic Regulation (CRG), now one of the world's most prestigious institutions in this field.

For my group, the 1990s was a decade of international endorsement and recognition. With the help of Antonio Castellano and María Dolores Chiara, we developed molecular biology techniques and expanded the scope of the group's scientific objectives. I had numerous invitations to attend workshops, write reviews, and to join the editorial boards of international journals, in addition to receiving several prizes and accolades for my work. Some of my first doctoral students had returned from their postdoctoral studies abroad and were creating their own research groups, which indicated that the seeds of experimental research had taken root. During this period we expanded our work on the physiology of glomus cells, showing their dopaminergic nature[8] and revealing the existence of calcium channels regulated by oxygen in arterial smooth muscle cells.[9] We cloned the gene of a new regulatory alpha-subunit of the voltage-gated potassium channels[10] and we carried out intense work on oxygen regulation of the recombinant channels with the aim of identifying the molecular domains responsible for the sensitivity to oxygen. This effort did not result in robust and replicable results. The massive amount of experimental data obtained was only published in the form of congress abstracts and after several years of work Patricia Ortega Sáenz, the doctoral student involved in the project, had to start a new project for her doctoral thesis.

In the middle of 1997 an extraordinary meeting took place that proved to be crucial for the future of my research group. Juan Negrín, a neurosurgeon from New York and son of the last president of the Second Spanish Republic, came to Seville to meet me because he was interested in our work on the carotid body. He and other surgeons had tested the removal of this organ in the treatment of asthma attacks. During our conversation we discussed surgical treatments for Parkinson's disease and the possibility of using the carotid body in cellular therapy as a donor organ for dopamine. After this interview, we launched a

preclinical research project involving the intrastriatal transplantation of carotid body cells in parkinsonian rats. The results, which were very satisfactory, were published in *Neuron*[11] and made a major impact in the international scientific community – as well as in the media – due to the fact that Parkinson's is a very common illness with no effective treatment. A few months later we completed a similar study on monkeys, with good results, carried out in collaboration with researchers from the University Clinic of Pamplona. As a result, research into Parkinson's became, along with studies into the cellular responses to hypoxia, one of the main lines of work for my research group, and remains so today.

In the same period in which we started the study on Parkinson's, I was invited by Gabriel Pérez Cobo, then the director of Virgen del Rocío University Hospital (HUVR) in Seville, to move my group to that centre, far from the place where I had worked since I was a medical student, to start up a scientific development project at the hospital. This was a centre that had prestige in Seville, due to its high level of healthcare, but with limited academic tradition up to that point. I decided to commit to this project due to my conviction that Spain needed to have institutions where biomedical research was undertaken in a hospital environment, which favoured the transfer of knowledge to the clinical activity and vice versa, and allowing the most important medical problems to be investigated in the lab with animal and cellular models. To move our research activity to the hospital, the Laboratory for Biomedical Research (LIB) was created, which comprised a group that grew to forty members and included colleagues from the department, such as Juan Ureña, Juan José Toledo, Antonio Castellano and Miriam Echevarría. In addition to directing LIB, I was also appointed the general research coordinator of HUVR. Fired with enthusiasm, with a great deal of effort we started a scientific programme (seminars, scientific meetings and collaborations with clinical groups) and also secured external resources that in just a few years transformed the hospital's profile. New postdoctoral researchers joined the team, in particular José I. Piruat and Alberto Pascual. In addition we entered into a collaboration with Óscar Pintado, director of the Centro de Investigación y Experimentación Animal (Animal Research Centre) of the University of Seville. As a result of these developments, the scientific objectives of my group expanded into molecular biology and the generation of genetically modified animals, which allowed us to make some important advances in cellular physiology. In parallel, we started to carry out clinical trials on parkinsonian patients thanks, among other contributions, to a research grant of 150 million pesetas (~ € 0.9 million) from the Juan March Foundation. At the time this was one of the largest grants ever given to a research group in Spain. Between 2002 and 2005 we carried out two clinical trials, with six patients in each one. The trial involved extracting the carotid body and, in the same surgical procedure, transplanting this organ, in minced form, into the basal nuclei of the brain (caudate and putamen) through a cannula implanted using stereotactic surgery. We carried out the selection of the patients, the implants and the clinical follow-up together with Ventura Arjona and Adolfo Mínguez, eminent neurosurgeon and neurologist, respectively, at the Virgen de las Nieves University Hospital in Granada. The neuroimaging studies (positron emission tomograghy) were done in London with David Brooks's group at Hammersmith Hospital. This collaboration with the Granada group, which was very fruitful from a scientific standpoint and pleasant at a personal level, started in a casual, simple way. In a meeting on Andalusian scientific policy, I sat next to

someone I did not know, who ended up being Ventura Arjona. Arjona asked me what sort of work I did. I briefly told him what I was working on and said I needed to find a place to conduct trials on anti-parkinsonian cell therapies on humans. He answered: you just found it. The clinical trials once again brought me into contact with patients, and, on seeing the disease first hand, once again reaffirmed my belief in the need for top-level centres focused on medical research and technological development at large hospitals. The results of the two human trials were satisfactory,[12] but less promising than what we had seen in preclinical trials with rats and even monkeys. We found that age and the amount of tissue transplanted were among the prognostic factors that affected the clinical result most. For this reason we decided to interrupt the trial on patients and start a basic project with the aim of expanding the carotid body *in vitro* before the transplant, in order to increase the amount of tissue available.

One of the most interesting properties of the adult carotid body is that, despite being an organ of neural origin, it grows in size in conditions of chronic hypoxia to increase the afferent signals that stimulate the respiratory centre and favour acclimatization (adaptation) to the situation in which there is a lack of oxygen (hypoxia). The plasticity of the carotid body has been known for decades, but the mechanisms on which it depends have not been revealed until recently. Since necessity spurs ingenuity, it occurred to us that maybe the growth of the carotid body depended on the existence of stem cells or progenitors that remained active in adult life. If that was the case, then these cells could be used to obtain a greater quantity of tissue and improve the efficiency of the transplants. At the end of 2001, before the unfortunate and unnecessary situation arose in which stem cells became the subject of heated debate in Spanish and Andalusian political circles, Ricardo Pardal, a doctoral student who had just defended his thesis, went to work in the laboratory of Sean Morrison, in Ann Arbor (Michigan), to do postdoctoral work on stem cells in the peripheral nervous system. Ricardo did excellent work in the United States and, on his return to my laboratory as researcher in the Ramón y Cajal programme, we started a project where, together with other colleagues, we discovered a population of stem cells in the carotid body, the first ever identified in the peripheral nervous system. These cells are responsible for the growth of the carotid body in conditions of hypoxia.[13]

During the first decade of the twenty-first century, coinciding with our incorporation into HUVR, I have become increasingly involved in committees and initiatives aimed at developing biomedical research in the Spanish National Health System. In 2002, together with Federico Mayor Meléndez and under the auspices of the Spanish Foundation for Science and Technology (FECYT), I organized a very high-quality and well-attended forum at the University of Seville to discuss the concept and development of the university hospital. This event concluded with the drafting of a manifesto that highlighted the need to promote and foster the university hospital as a basic institution in translational research. For various years during a period in which several ministers from different political parties held his post, I was a member of the Advisory Council of the Health ministry directed by Juan Rodés. As members of the council, we could apply significant pressure on the Ministry of Health to support the development of the health research institutes at university hospitals, an idea that served as a model for the Institute of Biomedicine of Seville (IBiS), where I am currently the director.

When the grant from the Juan March Foundation ended, with the sad news that the programme, which had only been extended to two other researchers, had been cancelled, I received a visit from Pedro García Barreno, chair of the Surgery Department in the Complutense University of Madrid, who came in the name of the Botín Foundation. He offered economic support for my research group, with what seemed to me to be a proposal of stratospheric proportions. This gave me the opportunity to become a part of a programme where, in addition to economic assistance, I would have the support of personnel specialized in the protection of intellectual property and the transfer of knowledge and technology to the business realm. This was, without doubt, one of the most critical moments in my professional life. Never before had I received such special treatment; never before had I been offered such a wealth of resources to advance in my research without being asked for anything in exchange, just that I continue working on what I liked, just as I always had. At the end of this grant, which lasted four years, I had the privilege of being named a "Botín investigator" until 2017, which is why I have been invited to write this autobiographical text. During these years, the Botín assistance has been essential for my group and thanks to this support we have been able to advance in the development of new models of genetically modified mice, to sustain technological transfer projects, and to reach scientific goals that would not have been possible otherwise. The technological transfer project into which we put so much effort initially was the expansion of the carotid body *in vitro*, with the aim of carrying out a new clinical trial with parkinsonian patients. From the scientific perspective, the project was satisfactory, which allowed us to publish highly relevant results regarding the growth mechanism of the carotid body in response to hypoxia.[14] In recent months, this project has culminated with the identification of the oxygen sensor mechanism used by the arterial chemoreceptors – something we have been looking for over twenty-five years! However, from the technological perspective, it has not progressed at the rate that we might have desired because, for unknown reasons, the growth and dopaminergic differentiation of the stem cells obtained from human tissue does not occur with the required intensity. In parallel with this project we developed another one based on previous work where we demonstrated that the GDNF trophic factor is absolutely necessary to maintain the nigrostriatal pathway in adults – the neurons that are destroyed in people with Parkinson's disease.[15] The technological objective of this project is to develop methods (pharmacological and/or electrophysiological) that allow for the activation of endogenous GDNF production; this would increase the protection of the neurons of the *substantia nigra*, and eventually slow down the advance of the disease.

During the last two decades our group has developed various patents but, except for those related to the creation of genetically modified animals, none has resulted in a product of technological interest. Although in recent years the academic environment has come to understand the need to "transfer" research results into the medical practice or the business sphere, in general it is forgotten that this process is, on a global level, highly inefficient and that the majority of the well-focused, soundly designed projects meet with failure. I have seen up close several multi-million-euro corporate projects aimed at developing new therapies for neurodegenerative diseases that, despite having been based on solid preclinical data, failed in the clinical transfer phase. Naturally the correct response to this situation is what occurs in other countries: patiently invest in research, conduct

more and better research, and promote high-quality management and transfer. In Spain we already do science that is qualitatively comparable to other developed countries, and therefore there is no reason to think that we will not be successful in technology transfer. If political corruption and opportunistic separatist movements do not sink our country, we will be able to take another step towards its modernization, and in a few years we will have an elite standing in this field just as we do in other scientific fields and other facets of cultural production.

As I approach the end of this biographical summary, I would like to refer to the institutional project that I have coordinated over the last several years. My academic life has been closely linked to the generation of institutions that allow research to be carried out in this country, perhaps because, like other members of my generation, our objective was not just to do science and make important discoveries, but to contribute to the transformation and progress of the society where we were born and where we grew up. During the seven years that I was the director of the Department of Medical Physiology and Biophysics, I put all my effort into modernizing it and transforming it into an international research centre. Subsequently, I worked on creating LAB (today the Andalusian Centre of Developmental Biology, CABD) and the construction of the building that currently houses CABIMER (Andalusian Molecular Biology and Regenerative Medicine Centre). Lastly, four years ago I could see how we were able to set up the Institute of Biomedicine of Seville (IBiS), the project that was my reason for moving to HUVR and which I have had the privilege to coordinate and direct over the last ten years. IBiS was conceived as a multidisciplinary and multi-institutional biomedical research centre, located at a large university hospital. Its aim is to develop translational research programmes related to the most prevalent pathologies affecting the population. After I joined HUVR, I coordinated the research group with which I started to take the necessary steps to start up the project. In just a few years we created IBiS (2006) and became the second centre to be accredited by the Carlos III Health Institute (2008). In addition, we constructed an excellent building, furnished with the most modern infrastructure, which has functioned since 2011 with over 250 permanent researchers on staff. Over the last several months, having entered a phase in which I can contemplate the institutional work that has been completed, I have often wondered whether all the effort – which was intense, demanding and not always in consonance with the demagogic attitude of certain authorities in our society – was worth it. Naturally, I feel a deep satisfaction that I was able to contribute to the creation of a space where top-level research can be carried out in Seville and in the HUVR campus. However, I am disappointed by the fact that it will not be possible for IBiS – or any other Spanish research centre – to reach and maintain a real level of international competitiveness unless the institutions in our country are modernized, which would entail the implementation of a professional and depoliticized governance regime similar to what exists in equivalent institutes in developed countries. Our healthcare and research centres must be dynamic and independent, and ruled by the principles of merit, efficiency and accountability. Spain must undergo a new modernization that adapts our rules of coexistence to the changes that have occurred in recent decades. In the specific field of scientific research, the fact that we have accomplished a great deal should drive us to work harder to make sure that everything that remains to be done becomes a reality.

Now that this scientific biography is complete, I think it is clear why I consider myself to be fortunate. My efforts, which have taken their toll, have been compensated many times over by a stimulating, independent and creative professional career, and above all by the appreciation of alumni, colleagues and Spanish and Andalusian society (including people from my hometown). My education and scientific activity were made possible thanks to the support of my family and my mentors. My wife and daughters have accompanied me in my professional career and our lives have been enriched by sharing our dreams and individual projects with each other. Honestly, I believe that we have shown that with effort and generosity – especially on their part – it is absolutely possible to reconcile professional and family life. My scientific vocation has always been accompanied by a dedication to teaching in universities (especially of medical students) and the training of doctoral students and postdoctoral researchers. For reasons of practicality, only some of them have been cited in this autobiography, though all of them have been equally important. I have spent many hours of my life with doctoral students and postdoctoral researchers, and this is reflected in our publications, the most prized results of my professional work. My teaching work and research has been undertaken in academic environments, especially in the University of Seville, my "alma mater", which has always supported me and to which I have the privilege of belonging.

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LAURA M. LECHUGA GÓMEZ

DIAGNOSIS WITH LIGHT: A LIFELONG GOAL

11

Synopsis

The relationship between science and technology has always been one of the main foundations for progress in human knowledge, with many examples existing in our society and everyday lives. In much the same way that the world of conventional electronics underwent a revolution with the advent of the first field-effect transistor (FET) in 1947, sensoristic sciences have moved forward with the help of microelectronics, microtechnologies and, more recently, nanotechnology, making it possible to produce sensor devices of a very small size with excellent features. It is a field still undergoing expansion, but in the near future we will have access to nanosensors on demand that will be connected to our mobiles, giving an instant diagnostic of our health status, the presence of contaminants in our drinks or food, or any environmental pollution in a matter of seconds. It is precisely on the technological development of biosensor devices that I have focused the nearly twenty-eight years of my scientific activity, at no time forgetting to include their social applications while targeting their development in that direction.

Biosensors are devices able to detect all types of chemicals and biological substances in real time, with no need for fluorescent or radioactive markers, and with a high level of sensitivity and selectivity. A biosensor is made up of a biological receptor (antibodies, enzymes, DNA probes, cells and so on) specifically designed to detect a substance and a physical transducer able to detect the biomolecular recognition reaction at the time it occurs, translating it into a quantifiable signal. The usual techniques for analysis are laborious, time-consuming and expensive, and usually require specialized technicians. In comparison, biosensor devices offer the possibility of carrying out tests with reduced samples of body fluids, in a specific way and in a very short time, with a high level of accuracy and sensitivity.

There are different types of biosensors, but my work has focused mainly on nanophotonic biosensors (plasmonic silicon-based integrated optics biosensors) and optonanomechanical biosensors, due to their high-performance features when making use of the particular way in which light is transmitted within optical circuits. Sensors are preferably produced with microelectronic technology, which makes it possible to manufacture dozens, or even thousands, of devices for the simultaneous detection of substances. One of the main objectives of my work has been to create high-sensitivity and miniaturized devices for integration into lab-on-a-chip platforms with original and proper designs, as well as the technology transfer of the results, with a special interest in scientific and technological developments that can be transformed into products of interest to our socio-economic environment.

As a result of my activities throughout these years, I believe I have contributed with a pioneering work in both Spain and at the international level involving the implementation of innovative nanophotonic biosensor technology, with an emphasis on demonstrating its use in decentralized diagnostics.

My work in science has been, is and will always be linked to achieving greater technological development, with "technology" understood as being the side of "science" whose main goal is to achieve results whose medium and long-term application will lead to widespread societal benefits.

Early life

I was born in Seville on one rainy night during the Christmas season of 1962, shortly before midnight. My father was a member of the Spanish Navy and my mother a dressmaker. My first years of life were spent in this bright city, before moving to Cadiz, a southern city surrounded by the sea, with incredible beaches and a laid-back way of life. All my upbringing, from the age of five to my entrance into university, was spent at two Catholic girls' schools, something not very typical during my teen years, when most of my friends were already going to different sorts of public schools and institutions. The education and discipline at the school were quite strict, but the school had a very active and highly motivated secular faculty. My most important memory from school is learning about the important values of hard work, effort and advanced study as the main goals to achieve. Some of my enthusiastic baccalaureate teachers vanished on one of the regular days of class and became the first Socialist parliament members in Spain's recently restored democracy. The education at this religious school contrasted to a great extent with the free and open Andalusian way of life.

In that period, living in one of the smallest, most economically depressed cities in the country also meant that the choices offered at the small university that was then the option in Cadiz were quite few. Not being able to gain access to other more important universities because of economic limitations meant I had little choice about the major I would be studying, because the only viable ones were chemistry or medicine. I opted for chemistry. And my arrival at university from a Catholic school then meant a significant life change, at a time when the female presence in science majors was still quite uncommon. The School of Chemistry had recently been created, and both the faculty and laboratories where we held our practicums had yet to be "premiered". I therefore have no special memories of any of my professors as great educators or inspiring figures, with the exception of one technical chemistry professor, Dr Manolo Galán, who became one of the pioneers in very successfully transferring knowledge from university to business. I have continued to maintain a close relationship with him ever since.

During those years at university, I actively participated in its political life, becoming a student representative in all its governing bodies, first in the School of Chemistry and then in the university. These were years of change, in which students were for the first time ever given the chance to take part in university governing bodies and elect the rector.

With some very active militants, we managed to unify all the votes cast by the University of Cadiz students to achieve massive support for the rector who had made the most promises of change to us. Very shortly afterwards, once I had begun my doctoral thesis, I left my political militancy for good when I discovered how unappreciated that sort of work is, despite the effort and dedication. This was due to political interests, and above all the low intellectual level in the surrounding environment, which could have contributed to making a better university if it had been less deficient at that time.

My beginnings in research

In the final years of my university studies, I entered the Inorganic Department at the School of Chemistry with a collaborating student scholarship and began my first foray into the world of research. While there, my conviction about completing a doctoral thesis formed, as a necessary and indispensable stage of my education before deciding whether I would devote my career to scientific research or work in a company. My tasks there focused on searching for new antitumoural drugs using stereoisomer coordination compounds similar to the antitumoural drug cisplatin (cis-diamminedichloroplatinum). This type of compound has comprised one of the most commonly used families of drugs in chemotherapy for treating solid tumours, with a high level of effectiveness, but at the same time showing significant and devastating side effects. At present, more than 700,000 patients recently diagnosed with cancer continue to receive this treatment in Europe. The working mechanism of these complexes is based on their bonding to cellular DNA, causing apoptosis. My work consisted of synthesizing, characterizing and separating into its isomers the complex ion bis (meso-2,3-butylendiamine) cobalt oxalate (III) to later select the structure with the most notable neoplastic activity and potential for fewer side effects. We relied on laboratories for chemical synthesis and stabulary for *in vivo* evaluation of the prepared compounds. During this time, I became closely familiar with the intense work entailed by scientific research and the many hours in the laboratory necessary for chemical synthesis and the characterization of the materials. However, the repetitive work similar to that which I had already been performing did not seem especially stimulating or creative to me from a scientific perspective, which motivated me to seek out other subject areas and horizons.

Through one of the most active professors at the School of Chemistry, who belonged to my department, Dr Rafael García Roja, I was offered the chance to get an interview at a very recently created research centre in Madrid, the National Microelectronics Centre (CNM) of the Spanish National Research Council (CSIC). The CNM was a pioneering initiative by the Spanish government in 1984 to improve the fact that Spain was so far behind in the field of microelectronics, and it was given significant investment for the time (nearly three billion pesetas). At that time microelectronics was considered to be one of the priority sectors of development and, in supporting it, they were attempting to contribute to the modernization of Spanish science. Designing integrated circuits and the technology for manufacturing chips, and studying new semiconductor materials were just a few of this new centre's scientific objectives. In 1988, the year in which I signed up to begin my doctoral thesis, there were more than a hundred people working at the CNM, whose headquarters was divided between Barcelona and Madrid. They were managed by Professors

Francisco Serra, Emilio Lora-Tamayo and Fernando Briones. The centre's goal was to create prototypes and collaborate with industry. Though the greatest possible miniaturization of electronic circuits in the field of microelectronics is achieved, there are many other specific uses in which microelectronics are necessary but do not require integrated circuits. Among these applications were sensors, which at that time were not even manufactured in Spain.

Chemical gas sensors and biosensors with III-V semiconductor technology (1988–92)

With my doctoral thesis, a new line of research was opened up at the CNM on sensor and biosensor devices, starting a pioneering research line at the National level in that period. With the support of our proper designs and production processes that could be carried out at our laboratories, we designed and produced innovative gas sensors based on Schottky barrier diodes produced by combining catalyst metals (Pd, Pt and Ir at the nanometric scale) with gallium arsenide semiconductors. It was necessary that the semiconductor structure had a low level of doping and excellent structural quality, so it was required to be produce by using a sophisticated ultra-high vacuum molecular beam epitaxy (MBE) technique. The devices detect the gases absorbed through the variation in their electrical characteristics at the interface between the nanostructured metal and the semiconductor with low doping, providing a rapid, and precise analysis of the gas concentration, able to detect even few parts per million (ppm). The completion of this work constituted a great intellectual and professional challenge because the project mainly had a component of material science and microelectronics engineering, and obviously my training on those subject matters was scarce, or perhaps even non-existent. I had to fine-tune the methods for producing devices, including vacuum techniques such as the deposition of metals assisted by electron beam or sublimation, in addition to mastering all the techniques for the structural and electrical definition of the various steps in the production and assembly of my first prototype, a small reactor for the evaluation of sensors in a controlled gaseous atmosphere, connected to a sophisticated gas dilution and control panel.

While completing my thesis, I managed to create a set of innovative H_2 and NH_3 sensors, as well as completing a thorough characterization of them as devices to define their interval of applicability and mechanism of interaction. The sensors achieved were extremely sensitive, operated in a broad range of temperatures and remained operative even at room temperature. Moreover, they were fully reversible and had a long life span.

I later developed what constituted my first microelectric biosensor device based on the prior sensors. It was the first device of its type to be produced in Spain. In an enzymatic reaction, the specific substrate of the enzyme breaks down and, during that breakdown reaction, gases are given off which can be detected using our sensors, thereby acting as a quantitative and selective measure of the enzymatic substrate used. Therefore, the goal was to develop a urea biosensor by combining an enzymatic layer of urease with the NH_3 sensor, detecting the ammonia produced in the catalysed reaction of the urea, compound of clinical interest. The urea was determined within the interval of 0.4 to 10 mM, which is the usual range in clinical tests.

This thesis work gave rise to a large number of international publications that had a great repercussion in their field. Even today, twenty-two years later, some of them are still cited in the specialized literature. However, perhaps the most notable part of this work, besides the international publications, was that the dissemination of these results in the mainstream scientific magazine *Química e industria* drew the immediate attention of the Tabacalera company, one of the largest companies in the country at that time, because of the difficulties that they were encountering in analysing the NH_3 content of tobacco exhalants. If the NH_3 content in the smoke was not the appropriate, this could indicate a poor adjustment of the tobacco's composition, which could give rise to major toxicity for consumers. A few weeks after this publication, I began to work closely with Tabacalera, S.A. The year was 1991, and that became my first foray into the world of research and development in the private sector. It is worth mentioning that I have never smoked in my life, so the visits to the R&D laboratories at Tabacalera, with their smoking "machines" and odours, was true hell for me. The company regularly sent the samples to the laboratory in balloons that gave off a terrible pestilence, and on one occasion one of the balloons exploded, making it necessary to evacuate and ventilate the entire institute so as to prevent any major harm.

It should also be mentioned that, in that period, none of us involved in the research (my thesis director, the Institute's director or myself) ever brought up the possibility of patenting those sensors, even though they were unique in terms of both their design and their excellent operation, and of obvious industrial interest. This is an excellent example of the non-existent culture of technology transfer and the scarce importance that was placed on that concept in 1992, even at a centre like this one, which had been created for the purpose of industrial collaboration.

Dutch tulips (1992–94)

After the work I completed with gas sensors and biosensors, whose working principle was based on electrical changes, my scientific interest turned towards optical sensors and integrated optics because I believed that this technology and these types of sensors were really advantageous as compared with electrical sensors. At that time they were emerging as one of the technologies with the greatest future impact, as has later been demonstrated over the years. To work in the field, I completed a postdoctoral stay at the MESA+ Research Institute, located at the University of Twente in the Netherlands. This was a pioneering centre in the development of microdevices and optoelectronic devices, and it had one of the best microelectronic production facilities (known as a clean room) at that time. During my postdoctoral stay, I worked with the group led by Professor Rob Kooyman, one of the forerunners and visionaries in multidisciplinary scientific work, who developed the first plasmonic and interferometric biosensors with immunological applications, masterfully combining a photonic sensor with a single layer of receptor antibodies. At that time our knowledge of the biosensor field was quite limited, and we knew little of covalent immobilization techniques or methods for orienting antibodies to increase capture effectiveness. But, even so, the group performed notable work towards achieving biosensor devices that proved to be those with the greatest sensitivity published

to date, with no need for fluorescent labelling. During my postdoctoral years one of the PhD students in the group set up a spin-off using part of the knowledge produced there, and another of my postdoctoral colleagues, Dr René Heideman, latterly founded the spin-off company Lionix BV in 2001, of which he is currently the scientific director.

During that time, I learned to work in a clean room with special facilities for microelectronic production and a highly controlled environment in terms of the presence of dust, oxygen content and temperature. There I learned the skills of handling sophisticated equipment and manufacturing micrometric-sized chips, following strict optoelectric production guidelines while dressed in special suits so as not to contaminate the chips, making it difficult to move. In doing this, I instructed myself about the design and production of innovative optical sensors based on microelectronic silicon technology, using optical waveguides. My main achievement was the creation of a device known as the planar Mach-Zehnder interferometer with an optimized flow system (my group had not achieved the implementation of microfluidic systems until my arrival), also showing a set of measures as an immunosensor (specific antigen-antibody interaction) with the lowest detection limit (10^{-11} M or 10^{-2} nm in biolayer thickness) achieved for this type of device in a direct measurement (that is, with no need for fluorescent labels as in the traditional methods) at that time.

Once again, completing this work constituted an important professional challenge because it required knowledge of optics, optoelectronic technology, microfluids and biology, and my training on all those subjects was quite limited.

My first research group (1997)

After some productive training during my postdoctoral stay, on my return to the CSIC National Microelectronics Centre in Madrid I started up a new line of research in 1997, once I had obtained a position as a tenured scientist of the CSIC. Setting up a new group was not a simple task because I was given no initial budget for hiring PhD students or acquiring consumables or the minimum equipment required. These were very complicated years in which the unconditional support of collaborators such as Ana Calle, Carlos Domínguez and José Ramón Sendra, of the CNM, were essential.

In my new group, our research was oriented in the development of evanescent field bio-optical sensors based on optoelectronic devices. They were produced with microelectronics technology, using all the knowledge I had learned during my postdoctoral stay and extending it to include new technologies. These sensors are based on the principle of evanescent field modulation and can be used to detect a biospecific molecular recognition event, such as an antigen-antibody interaction or DNA-DNA hybridization. They are devices with exceptional features because they allow for this type of detection both directly (without labels) and fast (from seconds to minutes); no other method in biotechnology provides both of these features. Within this line of research on optoelectronic biosensors, two types of sensors were developed at the same time: a surface plasmon resonance (SPR) sensor and a Mach-Zehnder integrated sensor (MZI). The research carried out had the particular feature that it was both technological and multidisciplinary because a wide range

of areas were involved, including optics, chemistry, biotechnology and microelectronics. And they were always oriented towards end applications.

In the case of the integrated interferometric sensors, for several years we performed intensive scientific and technological work to create technologically advanced biosensor devices in which the strict specifications required were made clear: optical waveguides had to be single mode (only one mode of light may travel through it), with a high surface sensitivity towards biomolecular interactions, which, in practice, meant fabricating a device of reduce dimensions (waveguide measuring just 3 μm in width, 100 nm in thickness and just 4 nm in height). Therefore, their design, production, handling and evaluation were extremely laborious. These devices also included the channels for separating and recombining light in submicronic photon channels. It must be pointed out that this was the first integrated interferometric nanodevice based on conventional waveguides with which an immunological interaction was taken directly and in real time, which allowed us to demonstrate the generation of devices at a micro/nanometric scale with the highest surface sensitivity (10^{-8} effective refraction index) known in the specific literature.

At the same time, and in the case of the SPR – a sensor based on the high reflectivity of a nanometric layer of gold that is sensitive to minute changes in the refraction index of the medium in contact with the sensor's surface – we managed to produce complete SPR biosensor prototypes, including the sensor, the excitation and coupling optics, the microfluidics and the flow control system, in addition to the electronics and the software and hardware necessary to handle them, reaching a detection limit in the order of a 10^{-6} refraction index. Moreover, we developed surface chemical modification methods and anchoring by a covalent bond of the specific biological receptors for each specific application.

For the first time ever, we demonstrated that it was possible to directly detect toxic contaminants, such as the organic pesticides present in trace concentrations in natural waters, through the use of a portable immunosensor based on this technology. The analysis was carried out through an immunotest in an inhibition format in which the analyte was covalently immobilized on the surface of the sensor and the contaminant was recognized by a specific antibody. To do this, the biological receptor's functionalization protocol was optimized, which made it possible to reuse the sensor device for more than two hundred times. The achieved detection limit was just 1.38 $\mu g.L^{-1}$ (ppt, parts per trillion), thereby meeting the requirements of European legislation regarding pesticide residue detection. The analysis was completed in just twenty minutes. All these values indicated a great competitive advantage compared with the standard techniques normally used to study contamination, which require prior conditioning of the sample and the use of specialist technical staff.

As a result of this research, I signed my first contract with a company located in the Basque Country, devoted to environmental control but linked to the Spanish Defence Ministry. The work was carried out quite normally until the company attempted to appropriate itself of our knowledge after completely changing the contract in force, which by the way was of low economic income. That was a symptomatic sign of the nearly non-existent relationships between academia and private companies, the mutual lack of

knowledge in those years and the low level of preparation for research and development by business owners in that period.

At the same time, we began some collaborations works with the Boeing company (United States), and together we organized several workshops with the sponsorship of NATO to explore the applicability of biosensor technology to control chemical and bacteriological warfare, a topic that had aroused great interest at that time.

In our group, SPR devices had been used throughout all those years very successfully in the detection of the main families of pesticides, food contaminants, specific mutations in DNA (indicating genetic defects), evaluating biomaterials for implants in humans, early cancer detection by seeking protein biomarkers and microRNA, evaluating doping hormones, evaluating viral particles for the detection and treatment of HIV-1 and, more recently, for the analysis of allergies in patients' serum as well as for the early detection of colon cancer.

The first business adventure: Sensia (2004)

The excellent results in the environmental evaluation achieved in the laboratory directly using real samples to detect the presence of organochlorine, organophosphorated and carbamate pesticides, all in widespread use in agriculture, led us to take the leap towards creating an academic spin-off. So, in the year of 2004, Sensia was created, an innovative technology-based company devoted to marketing surface plasmon resonance biosensors, an analytical technique based on optical principles that allows for the analysis of many chemical and biological substances with a high level of sensitivity and selectivity, in real time, with no need for fluorescent or radioactive labels, and requiring a small sample amount for the analysis.

Using our first laboratory prototype and the excellent results verified in the realm of environmental control, we decided to begin this business adventure along with the biotechnology company Genetrix, thereby combining scientific knowledge and technological innovation with business management. Essential to this process was the pre-existing relationship of productive scientific collaboration with the group headed by Professor Carlos Martínez-Alonso and Dr Cristina Garmendia, president of the Genetrix group.

The company's main objective when it was created was to offer robust analytical technology with high added-value products and services, creating the first company in Spain to develop and market the SPR technology based on our own developments. Potential fields for application of this technology included centralized and decentralized clinical testing, environmental control and food and veterinary controls, as well as many others, providing a powerful tool not only for simplifying tests, but also for performing basic studies on the kinetics of biomolecular interactions.

That same year, Sensia received the first prize in the Second Contest of Researchers' Spin-Off Ideas, Madrid+d, in the category of business plans, awarded by the Autonomous Regional Government of Madrid. In 2006 Sensia completed the development of the first prototype of

the SPR biosensor and placed it on the market, just two years after it was created. In 2007, after determining there was a need to include an industrial partner for the full industrialization of a totally automated unit, as well as to introduce the product on the market as a robust and reliable unit, several cooperatives in the Mondragón Group decided to form part of the company. In July 2009 those Mondragón Group companies jointly became the majority shareholders of Sensia. In 2012, with the new totally automated SPR unit placed on the market, a unit that was easy for users to handle and completely autonomous, Mondragón acquired the rest of the company and Sensia was taken over by it in its entirety.

My participation in the Botín Foundation Programme (2007–12)

One of the major challenges to be dealt with by medicine this century is finding new diagnostic methods that are faster, more effective and more specific and that, in addition to providing early diagnoses, reduce the costs involved to the greatest extent possible. Early identification would allow for a fast response time and immediate application of the specific treatment, thereby offering greater potential for patient recovery. (Nano) biosensor devices may provide a proper response to such a need. That is why, as of the year 2007, my main research line shifted towards the field of nanomedicine and my scientific activity focused on the development of micro/nano biosensor systems and their integration into portable clinical testing platforms.

The changes that have been undergone by technologies in recent years are at this time allowing me to include developments that were created in the field of nanotechnology. This has led to a notable improvement of the features offered by our devices. The main goal was creating nanobiosensor devices and integrating them into lab-on-a-chip- platforms that can be used in real situations, such as early clinical testing and genetic, environmental or bioclinical monitoring. Using these sensors, it will become possible to directly evaluate ultra-small concentrations of proteins or variations of one single base in DNA in just a few minutes, requiring sample volumes of the order of just a few microlitres and, on some occasions, the samples to be analysed (urine, blood serum) will not even require prior treatment. A lab-on-a-chip platform that integrates various bionanosensors could offer a complete diagnostic test using a drop of blood by identifying molecular changes that are otherwise not perceptible.

In 2007 I joined the Botín Foundation *Technology Transfer Programme*. In accordance with my own goals, the Botín Foundation Programme provided me with the tools and vision necessary to demonstrate that it is possible to perform technological development of devices based on prior high-quality science and, later on, their technology transfer. It also allowed me to more clearly and precisely visualize the need for multidisciplinary work within one single research team in order to successfully tackle the competitive development of diagnostic biodevices on an international scale.

My years of participation in the Botín Programme were one of the most productive periods for my research group, with pioneering work at the international level in the development of new types of biosensor devices, such as the biosensor based on bimodal optical

waveguides, the magneto-optic surface plasmon resonance biosensor and the wavelength modulated sensors. Moreover, it promoted the search for real clinical application of our technologies and important collaborative work was set up with other participants in the Botín Programme and with the most important hospitals in the country.

Throughout this time, research was performed on new systems to increase the sensitivity of the SPR device, which took shape in the form of two pioneering world developments: the magneto-optical surface plasmon resonance (MOSPR) biosensor and the introduction of a new technique for increasing sensitivity based on wavelength modulation (ML-SPR). A new line was also started up to evaluate other alternatives based on the use of nanoparticles and plasmonic nanostructures. These nanostructures may contribute to a higher sensitivity level as compared with conventional SPR. Using these systems, theoretical and experimental studies were carried out aimed at optimizing and increasing the sensitivity of nanostructures based on their shape and size. A demonstration of the biosensor capacity of innovative nanostructured nanoplasmonic platforms based on gold nanodisks (even at the level of one single nanostructure) was carried out by evaluating the hybridization of DNA probes, which opens up the possibility of multiplexed detection of hundreds of biomolecular interactions in real time and with no need for labelling, using platforms with sizes of less than just a few centimetres.

At that time we also carried out pioneering work, proposing a new nanointerferometric device based on biomodal nanometric waveguides and mountable diffraction couplers for coupling light in the sensors. These nanophotonic biosensors fabricated with integrated silicon photonics at the same time combine a high-sensitivity level (several orders of magnitude greater than for nanoplasmonics), mechanical stability, miniaturization and large-scale production capability. The main works completed included the theoretical design and modelling of new optical structures based on waveguides, the production of devices in clean room facilities, the design and production of micro/macro flow cells with the later implementation of flow systems, the development of modulation and optical scanning systems, the development of electronics, data acquisition and control software, the analytical characterization of the devices and, last of all, use in clinical testing. This new nanophotonic technology work has been done intensively in recent years with the integration of lab-on-a-chip microsystems, which include on the same platform the micro/nanosensors, lasers, microfluidics, light detectors and the software/hardware necessary for their full integration, and on creating portable analysers for decentralized testing sometime in the future.

Close collaboration with the Botín Foundation has allowed for active evaluation of the degree of innovation and inventiveness of the new ideas that were generated in my laboratory. During this time, several patents were created, and one of them, awarded in all the national phases throughout recent years, was licensed in April 2014 to the company PROMAX Electrónica, which marks a huge step towards marketing this technology. Without the help received by the Programme, not only in terms of financial support, but also in the proper way of focusing my research group's objectives and priorities, this highly competitive development would not have been possible.

Also in 2007 my group was selected to become one of the founders of the Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), one of the nine CIBER consortia in the country, the creation of which was headed by the Carlos III Health Institute (ISCIII) to promote research of excellence and attain a critical mass of researchers in the field of biomedicine and health sciences. CIBER-BBN was officially established on 4 December 2006. CIBER-BBN's research programmes included three large subject areas: bioengineering and biomedical imaging, biomaterials and tissue engineering, and nanomedicine. And the research was oriented towards both the development of prevention, testing and tracking systems, and technologies related with specific therapies, such as regenerative medicine and nanotherapies. My activity within CIBER-BBN formed part of the nanomedicine area and, more specifically, in the line of bioanalytical molecular testing platforms for the identification of biomarkers and mass screening studies, both to achieve early diagnoses and to track and predict diseases.

Some main objectives of CIBER-BBN include offering high-quality research whose results contribute to improving health care offered to patients and creating wealth in the country by transferring research results to industry, as well as promoting its outreach within its field of activity and its participation in competitive development programmes at the international level. In line with these objectives, as of our entry into this new centre, our research has focused more and more on real clinical applications, in accordance with the goals set by the Botín Foundation Programme.

In 2008 I began a new stage of my scientific career at the recently created Catalan Institute of Nanoscience and Nanotechnology (ICN2), a joint centre of the CSIC and the Autonomous Regional Government of Catalonia, forming part of the CERCA network of research centres in Catalonia, awarded with the Severo Ochoa seal of excellence in 2014. My group's addition to this new centre entails a notable change in the model for doing our research because it has gained access to a more flexible system in which excellence and quality research hold precedence, in a more competitive environment. Coinciding with our participation in the Botín Foundation Programme has meant taking a huge quantitative and qualitative step in my research group's everyday tasks.

Applications in clinical testing

One of the main facets of my work has been performing real demonstrations of the applicability of the testing systems that we have been developing throughout these years in the clinical field. I would like to highlight some of these results so as to show the value of our technologies:

- The possibility of analysing the presence of pesticide metabolites in patients' urine has been demonstrated, which opens up the possibility of testing for potentially toxic substances that build up in the human body.

- The direct detection (without labels) of specific mutations in DNA sequences corresponding to the BRCA1 gene has been demonstrated; they indicate a predisposi-

tion to develop inherited breast cancer in women. Our technique makes it possible to test for four relevant mutations simultaneously. The test is carried out in real time and with a minimal blood sample, which is a noticeable improvement compared with conventional genomic techniques.

• Our testing systems have been used for the detection of growth and fertility hormones at physiological levels, as well as hormones related with the thyroid in urine and human serum samples. A methodology has been developed that, unlike conventional clinical analyses, makes it possible to perform an assessment with no need for prior treatment of the sample, in real time, with no fluorescent labels or enzymatic amplification, and with minute amounts of sample. The results have clearly demonstrated that the technology is useful for clinical testing of human samples, which opens the way for decentralized clinical testing.

• Biosensor technology has proved to be useful for the real-time evaluation of interactions between the GPCR cell receptors (receptors with seven transmembrane domains coupled to G proteins) of chemokines and their specific ligands. These receptors are involved in a wide range of physio-pathological processes, including neuron transmission, cardiac function and cellular movement, and they are fundamental in many physiological and pathological processes (asthma, rheumatoid arthritis and so on). They are considered optimal therapeutic targets and are the subject of great interest by pharmaceutical companies. However, most studies run up against the high complexity of their isolation from the cell membrane without losing their functionality. In our work, viral particles were used as agents containing the receptors, which is a pioneering pathway for obtaining cell membrane receptors while preserving their functionality. The use of biosensor microsystems has made it possible to analyse the interactions between receptors and their specific ligands in real time, thereby demonstrating the reliable measurement of the interaction's kinetics, in addition to providing a quick screening method that is easy to use on many samples, like those which are required to search for antagonists.

• Biosensor detection with high sensitivity to messenger RNA (mRNA) originating from cell extracts of pathogenic bacteria. RNA is involved in many biological processes and is of interest in fields such as epigenetics, disease detection based on the alteration of its levels of expression in cells or to identify infectious agents. In our work, innovative nucleic receptors with a tail-clamp structure were used that, by forming triplex-type structures, achieve a high capacity for capturing highly structured RNA sequences. This strategy is an innovation in the field of RNA detection using biosensor devices with no need for labelling, in real time and with excellent detection levels (pM).

• Determining alternative RNA splicing isoforms with biosensor technology, which adds great simplicity compared with conventional techniques. Splicing is a process through which, in the RNA taken from the transcription of a gene, the introns are eliminated and the exons are bonded again. In an alternative splicing process, different protein versions may be generated with sometimes even antagonistic func-

tions on the basis of one single gene. This process may cause major disorders in cell metabolism and even cancer, but the ultimate mechanisms that produce it are not known. Most of the methods currently used for this type of analysis are extremely complex and very expensive. In our work, the usefulness of biosensor technology in the evaluation of alternative splicing in the FAS gene, which plays a role in regulating the process of apoptosis, or programmed cell death, crucial to preventing the development of cancer, has been demonstrated. Our methodology based on biosensors has become a different option of interest when compared with conventional methods because it allows for detection in just a few minutes, in real time and with no need for labels or amplification.

• Application of the biosensor methodology for allergy testing in patients through the use of dendrimers as a bioreceptor. The tests were performed directly on just a few microlitres of patient blood serum samples, previously evaluated at a hospital using conventional techniques. Our results are very similar to those obtained at the hospital, which indicates that our device has great potential as an allergy testing system that creates minimal disturbances for patients.

As a whole, my research has consisted of pioneering work in the field of biosensor devices, a field that has become a priority line of activity today at many research institutes and centres as well as biotechnology, pharmaceutical and electronic engineering companies in our country and around the world. The work performed has combined science, technology and transnational research with a demonstration of industrial technology transfer, having reached the market thanks to a combination of basic research, innovative devices and technology transfer with a clear future vision The early detection of diseases, their immediate customized treatment and the later follow-up will become possible in the upcoming years thanks to the use of these new nanotechology tools. I hope that my work in the field of nanodiagnostics will make it possible in the near future. I would like to contribute to achieving full testing of each patient, allowing us to determine their genetic and immunological profile, resistance to antibiotics, and the bacteria and viruses present in their bodies. Thanks to all this, prevention plans could be personalized and habits modified so that we can all enjoy the highest possible quality of life.

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MANUEL SERRANO

DISCOVERY OF P16 AND ITS IMPORTANCE IN MEDICINE

12

Introduction

This essay is about the discovery of CDKN2A/B, a gene with wide implications in medicine. The story of this discovery is closely linked to my own research career, and I shall mention my involvement throughout. I apologize in advance for the fact that this approach enlarges my own role to the detriment of many highly accomplished scientists. CDKN2A/B has the "privilege" of being the only gene that is involved simultaneously in a large number of human diseases, including multiple forms of cancer, cardiovascular disease (atherosclerosis, myocardial infarction, ictus), type II diabetes and Alzheimer's among others. The involvement of a single gene in so many disorders is startling, and continues to be the subject of debate and further research. I shall present a unified model of how this gene functions that may explain why it is so significant in so many diseases. In the final section, I list my team's main scientific achievements with respect to CDKN2A/B and related issues in the field of tumour suppression, undertaken with the sponsorship of the Botín Foundation, to which I express my heartfelt gratitude.

Background

In the 1970s three research scientists working independently on model organisms that at first sight appeared to be very distant from medicine (yeasts and sea urchins) – Paul Nurse, Lee Hartwell and Tim Hunt – discovered that the process of cell multiplication (technically called the "cell cycle") was driven by a number of proteins that were later dubbed CDK. The name of these kinases reflects the fact that in order to be active they must be associated to another protein called cyclin, hence the name "cyclin-dependent kinases". This finding eventually allowed them to understand the molecular basis of cell multiplication and, due to its importance, the three scientists were awarded the Nobel Prize in 2001. A decade after the discovery of the CDK genes in yeasts and sea urchins, in 1987, Paul Nurse and another research scientist, David Beach (with whom I was to work later), independently discovered the human counterpart CDK genes. This aroused the interest of many laboratories, which took up the challenge of deciphering the molecular machinery of the cell cycle in humans. The following years brought forth new findings at a dizzying rate.

Discovery of p16

In October 1992 I joined the team of David Beach, as a postdoctoral fellow, at Cold Spring Harbor Laboratory in Long Island. This research centre was already legendary because of the famous scientists who had worked there. It had been headed for fifty

years by James Watson, who, together with Francis Crick, had discovered the structure of DNA. I chose to work at David Beach's lab because I wanted to take part in and witness some major scientific advances, and Beach's team was at the leading edge of our understanding of the cell cycle in humans.

Only three years earlier Stan Fields had developed the "two-hybrid screening" technique, which was a way to screen the entire genome to find proteins associated to a given protein of interest. This relatively easy and accessible method was adopted by many laboratories, including David Beach's. In our case we wanted to find out which other proteins associate to human CDKs. By the time I arrived at David Beach's lab, it was already known that many different CDKs existed and I was lucky to be assigned to work on the CDK dubbed "CDK4". The number had no further significance other than the fact that this was the fourth CDK to have been identified. Just three months after joining Cold Spring Harbor Laboratory, I completed a two-hybrid screening. All the proteins I found to be associated to CDK4 turned out to be the same one, a protein about which we knew nothing except that it had a molecular weight of 16 kDa: we therefore called it p16.

We then followed the logical steps that would give us an insight into the function of this new protein. We found that CDK4 and p16 really associate in human cells; in addition, we observed that as a result of this association CDK4 (or more accurately the CDK4/cyclin D active complex) is deactivated. In the light of its inhibiting effect on CDK4, we chose to call it "INK4" ("inhibitor of CDK4"). The discovery of p16 was published in *Nature* in December 1993, about a year after my arrival in New York.[1] This protein was the first known inhibitor of any CDK – whether in humans, yeasts or any other organism – and, because the protein works in opposition to cyclins, we proposed that: "The biochemical properties of $p16^{INK4}$ suggest that it could act as a negative regulator of the proliferation of normal cells".

One of the major cancer-protective genes

Soon after the discovery of p16, a research team employed by the pharmaceutical company Myriad Genetics made a startling new find. These researchers had already spent some time looking for a potential cancer-protective gene. They expected it to be in chromosome 9 and more specifically in the band 21 of its short arm, that is, chromosomal region 9p21. This region had aroused interest for a long time because this chromosomal region was often lost in many types of human cancer, thereby suggesting that 9p21 contained one or several genes that antagonized the development of cancer. This is the defining characteristic of genes that protect against cancer, which are technically termed "tumour-suppressor genes". Myriad Genetics had identified a small region within 9p21 where, they believed, there existed the key gene for cancer protection. The problem was to find out which gene, out of all the many genes in that region, held the key to cancer protection. While this was going on at Myriad Genetics, we published our paper announcing the discovery of p16 and suggesting that it might work against proliferation. The scientists at Myriad Genetics immediately realized that p16 was one of the genes at the critical region of 9p21. Only a few further tests were needed to confirm that p16 was the key gene they were looking for. Not only was it eliminated in many cancers, but in those

cases where it was not, it was frequent to identify mutations (sequence aberrations) that had inactivated the gene. This was incontrovertible proof that p16 was a tumour-suppressor gene. Given that in many different types of cancers the p16 gene was either absent or mutated, it was dubbed "MTS1" ("multiple tumour suppressor 1"). Myriad Genetics published its findings in *Science* in April 1994. Since then p16 (INK4 or MTS1) became one of the most important genes involved in our understanding of cancer and, of course, in the development of potential therapies.

Since 1994 a huge volume of research literature has been produced on the role of p16 in human cancer. Today it has been firmly established that p16 is a member of a triad of very special genes: the only three genes that protect against practically any form of cancer. Listed by order of discovery, the general cancer-protective genes are p53, p16 and PTEN. There are many other tumour-suppressor genes. However, as a rule, their effects are circumscribed to only one form of cancer or a small cluster of cancers. The p53, p16 and PTEN triad are clearly distinct from the rest in so far as they are involved in a large majority of cancers, regardless of their specific form. All this has been amply confirmed by the International Cancer Genome Consortium (ICGC) – an international cancer-sequencing project – which, time and again, has verified the special significance of these three cancer-protective genes.

Genes linked to p16

Thanks to the work done by Myriad Genetics, which had sequenced the p16 gene and its neighbouring regions, and following later work by David Beach and Chuck Sherr among others, it was discovered that the p16 gene is in close physical proximity to another two genes that are also tumour suppressors. In other words, p16 was part of a triad of adjacent genes, which frequently behave in unison, all being tumour suppressors: p16, p15 and ARF. Biology is always far more complex than we would like.

The p16 (INK4/MTS1) RNA messenger is formed by three exons, two of which are shared with the ARF RNA messenger. But these shared exons are read in different frames and therefore proteins p16 and ARF have nothing in common. The p16 gene was officially named "CDKN2A", and came to be treated as a "double" gene formed by CDKN2A(p16) and CDKN2A(ARF). In addition, CDKN2A is adjacent to another gene called CDKN2B, which codes for a protein called p15. Protein p15 closely resembles p16. Both share the biochemical property of inhibiting CDK4. To summarize, CDKN2A/B contains three genes: CDKN2A(p16), CDKN2A(ARF) and CDKN2B(p15).

So in the space of only a few years what had initially appeared simple became quite a puzzle. Questions arose about the three genes (p16, p15 and ARF). Were all three equally important in cancer? Was one of them more important than the others? Or did the answer depend on the form of cancer? As at today, the answer appears to be that all three of these propositions are true. The commonest situation in cancer is for the three genes to be simultaneously inactivated (through loss of region 9p21 or methylation of CDKN2A/B). However, in a small but by no means negligible number of cancers, p16 is the only one of the three genes that is altered (whether by mutation or by methylation of its promoter

DNA element). It is an interesting fact, for instance, that in congenital melanoma and in pancreatic cancer p16 is the only altered gene. Finally, in a minority of cancers only p15 or ARF are altered.

Mechanisms

Realizing that a given gene is significant in cancer is important but of little help in itself if we do not know exactly how it acts, how it fits into the network of molecular interactions within cells, how it responds and how it is regulated. The p16 gene played a major role in this, too, because it was on the basis of p16 and, later, of ARF, that two molecular pathways were identified that are immensely significant in cancer. Both when I was at David Beach's lab and later, at the Centre of Molecular Biology (CBM) in Madrid (which I joined in January 1997), it was my privilege to help clarify these two cancer-protective biochemical pathways, which are now described in biology textbooks (see, for instance, the chapter on cancer in the popular undergraduate textbook *Molecular Biology of the Cell*, Alberts et al.). These pathways have the attractive feature of providing a simple nexus among several tumour suppressors and oncogenes. In this context the simile is often invoked of the cell as a car, the speed of which is determined by a balance between the brakes (tumour suppressors) and oncogenes (accelerators). In essence, proteins p16 and p15 (tumour suppressors) deactivate the oncogene CDK4/cyclin D, and this in turn deactivates the "RB" or retinoblastoma tumour-suppressor gene.[2,3] For its part, ARF deactivates the oncogene MDM2, which in turn deactivates the p53 gene. The RB and p53 proteins are in charge of switching off the cell-cycle machine at multiple levels. Recent data emerging from cancer genome sequencing is confirming incontrovertibly that these two pathways are altered in the immense majority of human cancers. For instance, as outlined earlier, many cancers show a loss of the p16 gene, and those that do not show such loss display hyperactivity in oncogene CDK4/D, or have lost the RB gene. In whichever way it might be, this pathway is always profoundly affected. The same can be said of the ARF/MDM2/p53 pathway.

The largest contribution of my own in this field was achieved in partnership with David Beach and Scott Lowe, when we discovered that CDKN2A/B is a sensor of oncogenic signals. We found that the presence of oncogenes (genes that promote an abnormally high cell proliferation) strongly fired the activity of the entire CDKN2A/B gene, produced p16, p15 and ARF proteins, and thus switched off the molecular engines of the cell cycle, both through RB in the case of p16 and p15 and through p53 in the case of ARF.[4–6] For the first time, then, the activity of CDKN2A/B was linked to an overabundance of proliferative signals. As we shall see, this later became a recurring theme. From now on I shall refer to this mechanism as the "mitogenic hyperstimulation mechanism".

Cellular senescence

As described above, the activation of CDKN2A/B by mitogenic hyperstimulation switches off the cell-cycle machinery through the RB and p53 genes, but this switching off is special. Often, when faced with minor damage, cells can temporarily block the

cell cycle, repair the harm and restart their proliferation. However, it was soon observed that the activation of CDKN2A/B triggers a "deep switch off" of the cell cycle from which the cell generally does not recover. The permanent blockage of proliferation was already known to cell biologists as "cellular senescence". The study of cellular senescence had a long history but only one mechanism was known to be responsible for it, namely, loss of the ends of DNA chromosomes, or "telomeres". Our work added a second one, mitogenic hyperstimulation led to the activation of CDKN2A/B and the simultaneous production of p16, p15 and ARF resulted in cellular senescence.

Against this background, rapid progress was made in our understanding of cellular senescence up until very recently. Some of the milestones in this field were achieved at my laboratory, beginning in 2005 with the finding that cellular senescence is not circumscribed to *in vitro* cells but really does occur within live organisms in response to oncogenic stimuli.[7] And recently, in 2013, we discovered that the ultimate purpose of cellular senescence is to initiate tissue renewal even during embryonic development.[8]

Our work, and the work of so many other scientists who have explored this fascinating phenomenon, has uncovered a far richer and more complex story surrounding cellular senescence. When tissues are damaged, cells are generally eliminated by means of two processes: cell suicide, technically known as "apoptosis", whereby damaged cells self-digest and become small inert vesicles; and also by the process that I have dubbed assisted suicide, or "cellular senescence". In this latter scenario damaged cells stop proliferating, generate alarm signals (inflammation), and are finally eliminated by specialized inflammatory cells called macrophages (in a process called phagocytosis). Therefore, the outcome of cellular senescence is tissue repair.

The involvement of cellular senescence in many human diseases is now widely documented. Prima facie both senescence and apoptosis are beneficial processes that eliminate damaged cells and – in the case of senescence – support repair. However, in the case of chronic damage – which is highly frequent in ageing processes – excess senescence and excess apoptosis may become a problem on their own. For instance, in pathological scenarios, senescent cells may fail to be eliminated by macrophages and so build up and eventually contribute to disease.

From cancer to the rest of the major human disorders

The decipherment of the human genome (completed between 2000 and 2003) identified millions of genome loci having common variations that make each individual human unique. This in turn made it possible, for the first time, to analyse large numbers of people suffering from the same given disease to identify the genes involved. This research approach is termed "genome-wide association studies" (GWAS). To my astonishment, every major paper published on the human diseases (type II diabetes, atherosclerosis, myocardial infarction, stroke, aorta aneurysm, Alzheimer's, glaucoma, endometriosis and, of course, many forms of cancer) identified CDKN2A/B as one of the genes involved. Ever since, research has gone over the same ground and extended to a range of

human populations. Today, the evidence is incontrovertible that CDKN2A/B is involved in practically all widely prevalent human disorders. A recent paper analysing all GWASs published up until 2013 concluded that the CDKN2A/B gene is the only gene in the genome involved in a large number of human diseases (other than the multiple histocompatibility complex, which is involved in autoimmune and inflammatory disorders).

GWAS are highly effective in determining which genes are involved in a particular disease. However, the downside is that they do not ascertain in what way specifically those genes take part in the disorder. In other words, small changes in CDKN2A/B affect many human disorders, but it remains to be determined whether a more active CDKN2A/B variant is beneficial or detrimental (or whether a less active CDKN2A/B variant is beneficial or detrimental). In the case of cancer it is clear that a decrease in the activity of CDKN2A/B is harmful in so far as it weakens protection against cancer. But what about the other diseases mentioned earlier? It might be the case that, as with cancer, the other diseases are aggravated when CDKN2A/B decreases, or that only some of them do, or even that they behave in the opposite way to cancer, such that a decrease of CDKN2A/B is in fact beneficial.

The answer to this question is key to understanding the function of CNDK2A/B in human disease. No final answer has yet been arrived at. There are, all the same, a few clues that I should comment on here. My laboratory bred the first genetically modified mice carrying a modest rise in the activity of CDKN2A/B. To our surprise, not only were these mice protected against cancer, but, independently of cancer, they were longer-lived than their counterparts having normal levels of activity of CDKN2A/B. In addition, they were better protected against diabetes and atherosclerosis.[9–13] All this suggested that a modest rise in CDKN2A/B, far from being harmful, protects against multiple diseases (at least cancer, diabetes and atherosclerosis). This notion has been substantially reinforced in the case of atherosclerosis by other researchers. It can now be asserted that a slight rise in CDKN2A/B protects against atherosclerosis in both mice and humans. Based on the current evidence, it appears that CDKN2A/B is a gene that intrinsically protects against multiple diseases, including cancer, atherosclerosis and diabetes, and possibly even Alzheimer's, glaucoma, endometriosis and other disorders. So CDKN2A/B seems to be an entirely exceptional gene that protects in general against many disorders.

A common mechanism for many disorders

Curiously, although much is known about the function of CDKN2A/B in cancer, its role in the other diseases to which it has been linked is far less clear. Several different interpretations have been offered, and the data is still insufficient for any reliable judgment to be made as to which is correct. According to one hypothesis, CDKN2A/B plays a different role in each disorder, that is, in each disease the mechanism is different. And yet it is also possible that CDKN2A/B operates through a single mechanism in all disorders – the known mechanism whereby CDKN2A/B halts cell proliferation in response to mitogenic stimulation. Personally, I believe that there are increasingly robust arguments to assert that CDKN2A/B acts by a common mechanism in all disorders.

Specifically, in many of the diseases mentioned earlier the common factor is a process of chronic damage associated with a proliferative response. This is the case, for instance, of Alzheimer's, which is usually associated with gliosis or glial scarring as a result of excess mitogenic signals (or neuro-inflammation) triggered in order to repair the neuronal damage. It is still debated whether neuro-inflammation and gliosis are a cause of Alzheimer's or a consequence. A similar situation arises in many cardiovascular diseases, where chronic endothelial damage generates an overabundance of mitogenic signals that contribute to the formation of atheromatous plaques. In both these examples, Alzheimer's and atherosclerosis, we can expect that slowing down the cellular proliferation involved in these diseases will slow down their progression. So the CDKN2A/B gene may be acting in these diseases in the same way as it does in cancer: once triggered by mitogenic hyperstimulation, it induces senescence and so facilitates repair while limiting the potential for excessive, non-functional cell multiplication. It may be that a similar situation takes place in the rest of diseases in which the CDKN2A/B gene has been found to be involved.

The first drugs emulating CDKN2A/B

In the field of biomedicine the process of converting new scientific knowledge into therapies is unpredictable, difficult, slow and expensive. Several independent studies have estimated that the average time elapsed from a biomedical scientific discovery to its clinical application is seventeen years. This is also the case of CDKN2A/B. It was identified in 1993, but it is only now that its medical applications will finally come into their own. Several pharmaceutical companies have developed experimental drugs that deactivate CDK4/D (and hence emulate p16 and p15) or MDM2 (thus emulating ARF). The CDK4/D inhibitor known as Palbociclib (developed by Pfizer) has already been approved by the FDA for metastatic breast cancer. Clinical trials of Palbociclib are delivering promising results for gliomas, sarcomas and other forms of cancer.

In the light of what we know about CDKN2A/B, I am confident that these new drugs will also prove beneficial in many other human disorders.

Final thoughts

Over twenty years have elapsed since the discovery of p16 in 1993. Since then our understanding of its role has moved forward immensely. The history of CDKN2A/B exemplifies the scientific process particularly well. For the sake of brevity I have not addressed the scientific debates and controversies that, as could not be otherwise, emerged along the way. The vicissitudes of this journey were an immense experience for me: I witnessed at first hand – and sometimes played a central role in – the collective telling of the scientific story. I also became aware of the immense difficulty involved in converting laboratory discoveries into medical applications. Nonetheless, the main thing is that the applications finally arrive. This leads me to an obvious point that is sometimes forgotten: if there are no laboratory discoveries, there is nothing to be turned into medical applications. What is more, every step forward casts up new questions that nobody expected, driving us into uncharted waters. There is no room for boredom in a research career.

PROJECTS SPONSORED BY THE BOTÍN FOUNDATION, AND THEIR RESULTS (2007–14)

Anti-ageing effect of tumour suppressors

The tumour-suppressor genes ARF and p53 have anti-ageing properties

We have made a major effort in generating new mouse strains in which we can study tumour suppression and its interrelationship with ageing. We had super-p53 mice (carrying three copies of the p53 gene) and super-ARF mice (carrying three copies of the ARF gene). It is known that these two genes, ARF and p53, work together, and constitute one of the main cancer-protection mechanisms in mammals. Super-p53 and super-ARF mice display significant protection against cancer when compared to unmodified mice. We combined these two transgenes in a single mouse strain.[10] Our most meaningful and unexpected finding, however, was that these mice not only show a lower rate of cancer but also age more slowly. Our cellular and molecular analysis prompted us to suggest a model in which the ARF/P53 duo detects both the endogenous, chronic, low-intensity damage that is characteristic of ageing, as well as the acute, high-intensity damage responsible for cancer. We found evidence that p53, in response to the endogenous damage responsible for ageing, trans-activates the genes involved in antioxidative protection.

Anti-ageing effect of the CDKN2A/B locus

The Ink4/ARF (or CDKN2A/B) codes for a triad of tumour suppressors, p15INK4b, ARF and p16INK4a, which together make up one of the main cancer-protective mechanisms in mammalian cells, being equal in importance to the tumour-suppressor genes p53 or PTEN. We have examined the fertility, cancer susceptibility, ageing and longevity of mice that have been genetically modified to carry one or two additional intact copies of locus Ink4/ARF.[11] We showed, first, that an increase in the Ink4/ARF dose prevents the production of male germ cells. We also observed a lower incidence of cancer associated with ageing in proportion to the gene dosage of Ink4/ARF. We determined that an increase in the gene dosage of Ink4/ARF involves a decrease in ageing markers and a lengthening of average longevity. We saw higher survival in cancer-free mice, indicating that cancer protection and slower ageing are separable effects of locus Ink4/ARF. Mice carrying one or two additional copies of p53 display normal longevity despite enjoying increased protection against cancer. We concluded that locus Ink4/ARF has an overall anti-ageing effect, probably because it encourages quiescence and prevents unnecessary proliferation.

Regulation of the CDKN2A/B locus

Chromatin remodelling in the CDKN2A/B locus

We have an ongoing interest in understanding the regulation of locus Ink/ARF (or CDKN2A/B). For this reason, we focused on a non-coding DNA component, which is conserved in all mammals, termed the "regulatory domain" (RD). We found that it is possible to ma-

nipulate the chromatin state of this locus simply by aiming small interfering RNA (siRNA) at the RD element.[14] The remodelling of chromatin using siRNA is a poorly characterized phenomenon in mammals, and we made use of this experimental approach to explore the mechanisms involved. We found that siRNA-mediated chromatin remodelling shares a range of characteristics with siRNA-mediated disruption of mRNA. In particular, siRNA-mediated chromatin remodelling requires the Argonaut proteins that are carried by siRNA and recognize transcripts from nascent RNA. Hence one condition for siRNA-mediated chromatin remodelling to occur is the existence of an overlapping transcription throughout the DNA being targeted. We observed that in fact locus Ink4/ARF as a whole, including the RD element, is transcribed in an antisense direction, and that most of the transcription arises from a later gene called MTAP that codes for a protein involved in nucleotide metabolism.

Function of CDKN2A/B in nuclear reprogramming

The CDKN2A/B locus is a barrier against iPS cell reprogramming

Locus Ink4/ARF (or CDKN2A/B) codes for three powerful tumour suppressors, p15Ink4b, p16Ink4a and p19ARF, which have their basal expression in differentiated cells and are overexpressed in response to aberrant mitogenic signals. We proved that this locus is completely silenced in iPS cells and in embryonic stem cells, while retaining the capacity to be reactivated after differentiation.[15] We found that cell culture conditions during reprogramming increase the expression of locus Ink4/ARF, hence the importance of silencing the locus to enable reprogramming and proliferation. The silencing of this locus is a limiting factor for the efficiency of reprogramming, and temporary inhibition may significantly improve the generation of iPS cells.

In vivo effect of reprogramming factors

In vivo *cell reprogramming*

Until we began our research, cell reprogramming was achieved only under highly controlled *in vitro* culture conditions, while the *in vivo* tissue micro-environment was in principle destined for cellular differentiation and would oppose reprogramming. Even in the face of this, we decided to attempt cell reprogramming within an organism. We showed that temporary induction in mice of the so-called four reprogramming factors (Oct4, Sox2, Klf4 and c-Myc) entails rapid de-differentiation in multiple tissues.[16] This de-differentiation arose in variable magnitudes, including loss of the expression of keratin and acquisition of expression of certain pluripotency markers indicating reprogramming. Over longer periods the mice developed multiple teratomas. Our "reprogrammable" mice also had iPS cells in the bloodstream and, at the transcriptome level, these *in vivo*-generated iPS cells are closer to embryonic stem cells than IPS cells generated *in vitro* in the standard way. We concluded that cell reprogramming within an organism is feasible, and confers on the *in vivo* reprogrammed cells a number of totipotency features that are lacking in standard iPS cells and embryonic stem cells.

Cancer and pluripotency

A link between p27 and Sox2

It is very frequent for tumoural cells to undergo a loss of differentiation and acquire generic features. In this context it is important to understand how pluripotency genes are repressed in normal differentiated cells and how that repression is lost in cancerous cells. We discovered an unprecedented mechanistic link – having relevance to cancer – between the tumour-suppressor p27 and the transcription factor Sox2: p27 binds to and represses the expression of Sox2.[17] The clue enabling us to link p27 to Sox2 came from our study of the reprogramming of cells lacking the p27 gene, where we observed that iPS cells could be reprogrammed without need of the ectopic expression of Sox2. This prompted us to explore the question of whether there is a link between two previously unrelated proteins: p27 and Sox2. We proved that p27 contributes to the transcriptional repression of Sox2. Absence of p27 leads to defective repression of Sox2 in different tissue types, and to incomplete and late silencing of Sox2 during the differentiation of pluripotent cells. In the absence of p27, pituitary tissue expresses high levels of Sox2, and this is the basis for the development of pituitary tumours.

NANOG fluctuations are epigenetically controlled

Pluripotent stem cells are distinctive for keeping a delicate balance between their dedication to self-renewal or to cellular differentiation. This balance is associated with certain levels in the expression of NANOG. NANOG levels in pluripotent stem cells are not constant; rather, they fluctuate asynchronically in each individual cell. Although it was known that Oct4 and Sox2 activate NANOG transcription, little was known about the negative regulation of its expression. We identified enzyme Ezh2, a methyltransferase that is responsible for the tri-methylation of histone 3 in lysine 27 residue (H3K27me3) as an important negative regulator of NANOG.[18] We found that the NANOG promoter coexists in two epigenetic configurations: one carrying the positive H3K4me3 epigenetic marker and another carrying the negative H3K27me3 epigenetic marker. iPS cells lacking Ezh2 have the capacity to differentiate and an expanded subpopulation of cells having a high expression of NANOG in comparison to iPS cells that do carry Ezh2. While in some forms of cancer (prostate) Ezh2 is oncogenic, in others (lymphomas) it is a suppressor. The mutational inactivation of Ezh2 (observed in some lymphomas) may contribute to raising the expression of NANOG levels, so increasing the self-renewal capacity of cancerous cells.

NANOG is linked to tumours derived from stratified epithelia

We found that NANOG is selectively expressed in stratified epithelia (skin epidermis, oesophagus lining and the epidermis of external mucous membranes such as the tongue).[19] We saw that *in vivo* overexpression of NANOG is restricted to stratified epithelia, where we noticed an increase in cellular proliferation and in hyperplasia. NANOG specifically regulates the cellular proliferation of these tissues by binding to and activating the Aurka gene promoter, which codes for the Aurora A kinase mitotic factor. We showed that

overexpression of Aurka recapitulates the same effects as NANOG in the oesophagus. The inactivation of NANOG in cells derived from oesophagus carcinomas and squamous cell carcinomas in the head and neck entails decreased proliferation, and the expression of NANOG and Aurka is positively correlated in squamous cell carcinomas (SCCs).

NANOG favours squamous cell carcinomas

No direct link had yet been established between NANOG and SCCs. We observed that the inducible overexpression of NANOG in mouse skin epithelia encourages malignant conversion of skin tumours induced by chemical carcinogenesis, leading to an increased formation of SCCs. Our analysis of gene expression in premalignant skin indicated that NANOG induces genes associated with the epithelial-mesenchymal transition (EMT). In primary keratinocytes, endogenous NANOG is linked to the promoters of these genes and induces features of EMT. These results provide direct *in vivo* evidence of the oncogenic function of NANOG in stratified epithelia.

Reprogramming effect of NANOGP8, a member of the NANOG family widely expressed in cancer

Human cells carry eleven paralogues of NANOG, of which only three (NANOG1, NANOG2 and NANOGP8) code for full-length proteins. We have found that NANOGP8 is expressed in many cell lineages of human cancers, and is as active as NANOG1 in promoting reprogramming to pluripotency.[20] So NANOGP8 may contribute to cancer, possibly by promoting cellular de-differentiation and/or plasticity.

Oncogenicity of the developmental transcription factor Sox9

The transcription factor Sox9 plays key roles in embryogenesis and is required for the development, differentiation and establishment of lineages in various tissues, including the intestinal epithelium. We collected clinical and functional data that disclosed that Sox9 has a very wide functionality in tumourigenesis.[21] Sox9 is overexpressed in a wide range of human cancers, in which its degree of expression correlates with malignity and progression. A rise in the number of copies of Sox9 is detectable in some primary colorectal cancers. Sox9 displays several pro-oncogenic properties, including its ability to promote differentiation, inhibit senescence and cooperate with other oncogenes in neoplasm transformation. In the primary fibroblasts of mouse embryos and colorectal cancer cells, the expression of Sox9 facilitated tumour growth and progression, while its inactivation reduced tumourigenicity. We have ascertained that Sox9 is directly and actively linked to the BMI1 promoter, the overactivation of which represses the locus of the tumour-suppressor Ink4a/ARF. Human colorectal cancers show a positive correlation between the expression levels of Sox9 and BMI1, and a native correlation between Sox9 and ARF in clinical samples. Taken as a whole, our results provide direct mechanistic evidence of the involvement of Sox9 in the pathobiology of neoplasms, particularly in that of colorectal cancer.

Cellular senescence

Programmed cellular senescence in embryonic development

Cellular senescence is increasingly being viewed as a key aspect of tissue remodelling. Senescence had so far been associated with accidental rather than programmed tissue damage, this being the case in certain pathological situations such as cancer. We have discovered that senescence also happens, in a programmed way, in the course of the embryonic development of mammals, and that it plays an active role in multiple processes of tissue remodelling.[8] We focused on the mesonephros and the endolymphatic sac of the inner ear. Senescence in these two structures depends strictly on p21, and is independent of damage to DNA, p53, or other cell-cycle inhibitors. We also show that the TGFβ/SMAD and PI3K/FOXO pathways regulate senescence during embryonic development. Embryonic senescence is followed by a process of macrophage infiltration and elimination of senescent cells, so completing the cycle of tissue remodelling. In the absence of p21, the loss of senescence is partially compensated by apoptosis, which entails detectable development anomalies. Human embryos also display senescence markers in the mesonephros and in the endolymphatic sac. We proposed that senescence emerged in the course of evolution as a tissue remodelling process and later adapted to post-damage tissue repair.

Cellular senescence induction by nutlin

By analysing the effects of nutlin we identified senescence induced by this drug.[22] Nutlin is a selective activator of p53. In approximately fifty per cent of human cancers, the functionality of p53 is lost, but – and this is relevant to nutlin – in the remaining fifty per cent p53 remains functional. Nutlin activation of p53 in certain cancer cells triggers cell suicide or apoptosis. Not all cells, however, initiate apoptosis in response to activation of p53: this is the case of fibroblasts and the cancer cells deriving from them. We found that after treatment with nutlin fibroblasts and fibrosarcomas did not undergo apoptosis; rather, they went through permanent proliferative blockage, known as cellular senescence. These effects are entirely dependent on the presence of functional p53. Cells that are deficient in p53 are insensitive even to high doses of nutlin. Our findings lent additional support to the potential use of nutlin as a therapeutic agent to block the growth of cancer cells by means of functional p53.

Function of DNA damage in senescence induced by oncogenes and in p53-dependent tumour suppression

It has been proposed that oncogenic signalling damages DNA and this in turn prompts p53-dependent cellular senescence. We tested this hypothesis using a range of experimental murine systems,[23] and concluded that in mice oncogene-induced senescence can arise in the absence of any response to DNA damage. Our data suggest that, in mice, DNA damage plays a minor role in oncogene-induced senescence and in p53-dependent tumour suppression, and that its tumour-suppressor activity is restricted to maintaining genome stability.

Regulation of the transcription activity of p53

MSK2 inhibits the activity of p53 in the absence of stress

MSK2 is a negative regulator of p53. We examined the molecular mechanisms that underpin p53 inhibition by MSK2.[24] We found that in the absence of stress stimuli MSK2 selectively suppresses the expression of a subset of p53 target genes. Basal inhibition of p53 by MSK2 is independent of its kinase activity and of the signalling of kinase proteins activated by mitogens in sections preceding MSK2. We found that apoptotic stimuli promoted the degradation of MSK2, so lessening its inhibition of p53 and enabling efficient p53-dependent activation of the Noxa promoter. We thus identified a new mechanism that regulates transcription activity in response to stress.

Ribosomal biogenesis and ribosomal stress in cancer and pluripotency

Depletion of the L37 ribosomal protein induces p53

The activity of p53 is primarily regulated by the MDM2 proto-oncoprotein. Inhibition of the function of MDM2 is a universal requirement for the activation of p53, which is an important regulator of DNA damage pathways and protein synthesis. Coupling these two processes may be significant in the prevention of oncogenesis. Disruption of ribosomal biogenesis is the third relevant p53 activation pathway. The depletion of certain riboproteins is followed by the binding of RPL11 to MDM2, inhibition of MDM2 and activation of p53. We observed that depletion of RP L37 halted the L11 and p53-dependent cell cycle.[25] We found that genotoxic aggressions lead to stabilization of L11-dependent p53. Our work showed that DNA damage can be detected through disruptions in ribosomal biogenesis, linking cell growth and division to genotoxic stress via p53.

Analysis of ribosomal stress in pluripotent cells

Ribosomal biogenesis is the process requiring the most energy input among those that occur in proliferative cells and is coming to be viewed as a critical sensor of cellular homoeostasis. If ribosomal biogenesis is disrupted, certain free ribosomal proteins – particularly the L11 protein – bind to and inhibit MDM2 (a negative regulator of the tumour-suppressor p53), thus triggering activation of p53. This pathway had been characterized in somatic and cancerous cells alike, but its function in embryonic pluripotent cells was still unexplored. Our team discovered that treatment with a low dose of actinomycin D or depletion of the L37 ribosomal protein – two firmly established ribosomal stress inductors – activate, in an L11-dependent manner, p53 in embryonic stem cells in mice and induced pluripotent stem cells.[26] The activation of p53 entails the transcription induction of p53 targets. Finally, ribosomal stress sets off an apoptotic response, dependent on L11 and p53, in the cell types mentioned earlier. Our results extend the functionality of the ribosomal stress pathway to pluripotent cells. This enables us to speculate that this may be a significant point of cellular control during early embryogenesis.

Ribosomal stress and non-genotoxic activation of p53

Interest is high in identifying chemotherapeutic agents that activate p53 in a non-genotoxic way. Nucleolus disruption is a non-genotoxic mechanism that causes the appearance of free RPL11, which binds to and inhibits MDM2, thus activating p53. In partnership with the CNIO's *Experimental Therapeutics Programme*, we identified a group of acridines that trigger an efficient nucleolus disruption and activate p53 in the absence of DNA damage.[27] These acridines inhibit the transcription of ribosomal RNA genes in a process that includes selective degradation of the RPA194 subunit of RNA polymerase I. Our findings have provided the basis for non-genotoxic chemotherapeutic approaches that attack the nucleolus in a selective way.

In vivo function of the Par-4 tumour suppressor

Identification of Par-4 as a new human tumour-suppressor gene

We wanted to expand the scope of my laboratory's interests to the inflammatory component that is known to be the initiator and/or promoter of several human cancers, such as colon and prostate cancer. We focused on "prostate apoptosis response 4" (Par-4), a pro-apoptotic protein that is a candidate to be a tumour suppressor. Our earlier research had established that Par-4 is an inhibitor of atypical protein kinase C (PKC), and thus attenuates two of the major cell survival pathways, NFkB and Akt. We bred mice lacking Par-4. We observed that they spontaneously developed tumours within the spectrum of interest, mainly affecting the prostate of male mice and the endometrium of female mice. Guided by these observations, we examined the status of Par-4 in human endometrial cancers, and discovered that Par-4 certainly behaves as a tumour suppressor in that cancer, with a high rate of inactivation (~30%) through hypermethylation of the promoter.

The tumour-suppressor Par-4 in lung cancer

Our hypothesis was that, in the absence of Par-4, cells would acquire unusually high levels of survival signals that would encourage tumoural progression. We proved that Par-4 is highly expressed in human lung tissue, and that that expression is lessened in human lung cancer samples. Using a mouse model that develops lung tumours induced by the K-Ras oncogene, we observed that the absence of Par-4 drastically raises the number and aggressiveness of the lung carcinomas formed,[28] so demonstrating genetically that Par-4 is an important barrier to lung cancer.

Simultaneous in vivo *inactivation of Par-4 and PTEN leads to synergetic activation of NF-kB and invasive prostate cancer*

The tumour-suppressor Par-4 is highly expressed in the prostate. We have shown that the expression of Par-4 is lost in a high percentage of human prostate carcinomas, and that this arises in association with loss of PTEN. We determined that mice lacking Par-4, much like mice that are heterozygous for PTEN, develop only benign prostate lesions, whereas,

in mice, concomitant Par-4 ablation and heterozygosity for PTEN lead to invasive prostate carcinoma.[29] Our results established that cooperation between Par-4 and PTEN is relevant to the development of prostate cancer, and involves the NF-kB pathway as a key event in prostate tumourigenesis.

Function in the pancreas of the Sei1 cell-cycle regulator

Mice lacking the Sei1 cycle regulator display normal proliferation and tumourigenesis but impaired pancreatic function

Sei1 is a positive regulator of proliferation that promotes the assembly of CDK4-cyclin D complexes and raises the transcription activity of E2f1. It is overexpressed in several forms of human cancer. We bred a mouse strain that is deficient in Sei1.[30] It is known that insulin-producing pancreatic cells are particularly dependent on the activities of CDK4, cyclin D and E2f1. We observed that Sei1 is highly expressed in pancreatic islets in comparison to other tissues. Mice lacking Sei1 presented a smaller number of pancreatic islets, a decrease in the area of β cells, impaired insulin secretion, and glucose intolerance. We concluded that Sei1 plays an important role in pancreatic β cells. This points to the existence of a functional link between Sei1 and the basic cell-cycle regulators specifically in the context of the pancreas.

Sirt1 in cancer, metabolism and ageing

Sirt1 protects against the metabolic damage induced by a high-fat diet

Our hypothesis is that the genes that protect us against metabolic damage may also have a positive protective effect against cancer and the deterioration associated with ageing. We focused on Sirt1 in the light of indirect evidence that this histone deacetylase might be key in protection of the organism against harmful metabolic effects. A compound that activates Sirt1 can, in mice, confer protection against a range of diseases arising from a chronic high-fat diet. To address this issue on a genetic basis, we bred transgenic mice that overexpressed Sirt1 to a moderate extent under the control of its own promoter and following physiological patterns of expression.[31] We found that our Sirt1 transgenic mice are protected against most of the harmful effects of a high-fat diet (HFD). These mice exhibit less fat-induced inflammation, are protected against the diabetic effects of a high-fat diet and, completely protected against hepatic steatosis.

Sirt1 improves healthy ageing and protects against cancer associated with metabolic syndrome

At the molecular level, the protection against the physiological damage caused by an HFD observed in our Sirt1 transgenic mice reflects the activity of Sirt1 as a negative regulator of NF-κB, and as a positive effector of PGC1α and FoxO1. Chronic exposure to an HFD leads to so-called "metabolic syndrome", which may cause liver cancer and heart failure. We observed that our Sirt1 transgenic mice displayed lesser DNA damage, a decrease in the expression of the p16Ink4a gene associated with ageing, better overall health and a smaller

number of spontaneous carcinomas and sarcomas.[32] These effects are not powerful enough, however, to affect longevity. Nevertheless, Sirt1 does have a positive effect on the health of elderly mice, as shown by the fact that Sirt1 transgenic mice are less intolerant of glucose, less affected by osteoporosis and display a lower incidence of cancer. As to liver cancer associated with obesity, the Sirt1 transgenic mice exhibited a drastic rise in resistance to this disorder. Taken as a whole, our data demonstrate the tumour-suppressant activity of Sirt1, which is particularly powerful in the case of liver cancer associated with obesity.

Sirt1 promotes thyroid cancer

Although genetic evidence in mice pointed to an intense tumour-suppressant activity of Sirt1 in a range of cancer models, there had so far been no certainty as to its *in vivo* oncogenic activity. We have now proved that the transgenic expression of Sirt1 is oncogenic in prostate and thyroid carcinogenesis initiated by PTEN deficiency.[33] We observed that Sirt1 increases transcription programmes and, in thyroid cancer in Sirt1 transgenic mice, c-Myc levels are higher. Similarly, Sirt1 is overexpressed in human thyroid cancer and correlates positively with c-Myc protein levels. We also produced evidence in cultivated thyroid cancer cells that Sirt1 stabilizes c-Myc. Our results as a whole implicate Sirt1 as a new candidate target for the treatment of thyroid cancer.

Function of the PTEN tumour suppressor in metabolism and ageing

The tumour-suppressor PTEN positively regulates brown adipose function, energy expenditure and longevity

Tumour suppressors are known for their capacity to confer protection against cancer. At a deeper level, they seem to protect cells against many forms of damage that need not be cancer-related. Evidence already existed of simultaneous protection against cancer and ageing by the tumour-suppressor genes p53, Ink4a and ARF. We then examined this paradigm in the tumour-suppressor PTEN. The pre-eminent function of PTEN is to counteract the activity of class I phosphatidylinositide 3-kinases (P13K), which mediate the signals triggered by insulin, insulin-type growth factors and other molecules usually involved in cell growth, metabolism, survival and proliferation. We bred transgenic mouse strains carrying additional genomic copies of PTEN, known as PTEN-Tg mice.[34] Our PTEN-Tg mice displayed protection against cancer and exhibited a significant lengthening of their lifetimes independently of their lower incidence of cancer. PTEN-Tg mice have increased energy expenditure and enjoy protection against metabolic disorders. In these mice brown adipose tissue is hyperactive and contains high levels of the Ucp1 decoupling protein, which we have shown is a target for the Foxo1 transcription factor. We also found that a synthetic PI3K inhibitor also raises energy expenditure and hyperactivates brown adipose tissue. These effects can be recapitulated in isolated brown adipocytes. Combined with other observations we made, this research revealed the involvement of PTEN in promoting energy expenditure, so decreasing the storage of nutrients and the damage associated with this process.

Therapeutic strategies for non-small cell lung carcinoma (NSCLC)

A new therapeutic strategy for NSCLC

We deciphered one of the molecular pathways that lies behind lung cancer. We used this knowledge to identify an experimental drug that, in mice, blocks lung cancer growth. Deregulation of the Notch cellular signalling pathway is involved in numerous human diseases, including cancer. Depending on the cell type, it can be oncogenic or tumour suppressant. In the case of NSCLC, high activity in the Notch pathway has been observed in up to fifty per cent of cancers, and this is correlated with a poor prognosis. Function-gain mutations have also been described in a small percentage of patients. We used a murine model in which oncogenic K-Ras is induced experimentally in adult mice to produce NSCLC. We proved that a loss of function in the Notch pathway that is simultaneous with the activation of the K-Ras oncogene completely prevents the generation of NSCLC.[35] Lung carcinogenesis directed by oncogenic K-Ras is therefore strictly dependent on the presence of a functional Notch pathway. It was known that Ð-secretase inhibitors (GSIs) inhibit the Notch pathway. We made the relevant discovery that after fifteen days of treatment with a GSI – provided by Eli Lilly – NSCLCs promoted by K-Ras stopped growing. For these trials we conducted diagnosis and follow-up of lung carcinomas in mice using TEP/TC, replicating the usual practice with human patients.

The genetics of human longevity

The APOB gene contributes to familial exceptional longevity

Exceptional longevity (EL) is a rare phenotype that runs in families. In partnership with the National Genotyping Centre (CEGEN) of the National Cancer Research Centre (CNIO) and the Ageing and Frailty Cooperative Research Network (RETICEF), we sequenced a total of seven exomes drawn from exceptionally long-lived individuals (older than a hundred years) from three unrelated families, each having at least two centenarian siblings.[36] We focused on rare functional variants (RFVs), and observed that a single gene, APOB, carried RFVs in all members of the three families. APOB codes for a component of the lipoproteins that carry cholesterol and triglycerides in conjunction with the APOE gene, and variations in these two genes have been associated previously with human longevity. We also identified candidate longevity genes that were shared across two families or within an individual family. Our research provides an initial catalogue of genes that may be contributors to familial exceptional longevity.

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Patents managed by the Botín Foundation

Inventors: M. Serrano Marugán, J. Pastor Fernández, S. Martínez González, A. Ortega-Molina, J. R. Bischoff and J. Oyarzabal Santamarina (2011).

Owner: Fundación Centro Nacional de Investigaciones Oncológicas Carlos III.

Invention: new use of PI3K inhibitors.

Number: PCT/GB2011/051030.

LUIS SERRANO

CHANGING, ALWAYS CHANGING

13

What Pedro García Barreno has asked of us is not easy. Writing a scientific autobiography is a delicate task. You have to take care not only to select the successes from your life, but also to include the doubts and failures. Furthermore, you should not spend too much time examining personal matters. This is made more difficult because we can neither write an entire book nor limit ourselves to a one-page summary. Having said this, I will place my trust in inspiration and do the best I can. It may be true that our childhood and adolescence bear a great influence on what we do later in life, along with a dash of genetics perhaps. In both branches of my family tree there are doctors and pharmacists, some of them well known, at least in certain provinces of Spain, such as Dr Rodríguez, of whom there is a bust on display in La Coruña. As may be true for many members of my generation, my forebears came from the two sides that faced off in the Spanish Civil War. My father's family, made up of landowners from Guadalajara, escaped to the Nationalist side walking for several days across the country to avoid being shot by the Republicans. As for my mother's side, they belonged to the intelligentsia of La Coruña, and my grandfather, the city hall's secretary, was shot at gunpoint by the Nationalist troops. I was born in Madrid and spent all my summers at an estate in Guadalajara, near Cuenca, with another eleven cousins. It was a manorial home without running water. The water we had was brought in by the watchman on a mule from a nearby water source. There was no refrigerator or television either, so we would spend the month of August playing outdoors, going on excursions or bathing in the river. I got the opportunity to live in the country, and I was delighted by nature. However, despite my great love for the forests and animals, I was not drawn to being a naturalist.

I have to admit that I did not stand out much in school. I would usually fail three or four classes each month, but I was lucky because I would always pass the course in the end. There was just one year when I failed English, so I spent the whole summer studying with a teacher and my father. I was only a good student in the last three years of my studies. Perhaps this was because of my biology teachers, or because I took chemistry, physics and biology at all, subjects that I loved. Whatever the reason, I went from being among the worst students to becoming one of the best, and the last year of school I was the valedictorian. This might demonstrate the importance of having good teachers and studying subjects that arouse students' interests.

In my final months at school, I was not sure what to do with my life, but there were two university majors that attracted me: biology and medicine. I was undecided, so my father managed to get me into an operating theatre to find out whether I could handle seeing blood. In any case, when the time came to choose a major, I still could not reach a decision, so I tossed a coin and ended up with biology. That is how I began to study biology at the Complutense University in Madrid.

I must say that the first year was not very exciting. More than anything else, all we did was review what I had already studied during my last year at secondary school. If it had not been for making a good friend, Alfonso Valencia – who is currently an important figure in biocomputing – I would have gone mad. Things improved a bit in the second year, but it never ceased to be a typical Spanish university, with lots of lectures, few practical classes and a great deal of memorization. I studied and received good marks, but, in all sincerity, I was not enthused by the university. Luckily – and here I must once again highlight the role of good teachers – I had an excellent genetics professor in the third or fourth year (I cannot remember which) whom we called "Nano". There were two ways to pass a class with him. The first was by the typical exams, on which you could receive up to an A, while the second involved completing a research project, for which you could received up to an A+.

Creating a team, Alfonso and I chose to do the research project. Suddenly, we found ourselves in the library of the old Centre for Biological Research (CIB) poring over indexes and searching for a topic of study. It was fascinating. Reading real articles and thinking about the work opened my eyes, so I concluded that I must like research. It now seems funny to think about that project; it had to do with the evolution of a virus and how a bit of DNA can encode two overlapping proteins, and at present my team is working with microbacteria that also display overlapping genes.

Alfonso and I got through five years of university together. We met our future wives during those times and also did our military service together. We both went to a military academy with the university militias and reached the rank of second lieutenant, though I was demoted and spent three months more in a disciplinary battalion. All this worked out so that we would not miss a single year at university and were able to end on time. When I look back, it is interesting to realize how ignorant we were. At university, nobody had explained to us what a scientific career meant. Nor did we know how important it was to do our doctorate at a good laboratory or study abroad. Just by chance, we got into the Genetics Department at the Complutense University with Dr Lola Ochando to complete a Master's programme on this topic.

This was very interesting because, for the first time ever, I was researching, and Alfonso and I formed a team within the project. Our topic of research had to do with the sexual vigour of natural populations of *Drosophila melanogaster*. We travelled to Almería to hunt down flies and then studied their sex life in the presence of males and females from other populations. We also learned to observe the chromosomes in their salivary glands and did a study on inversions and duplications. I am still proud of the Master's thesis I wrote at the end of the programme. Completely a work of my own, it consisted of putting flies on a Petri dish connected to another so as to then study the effect that lack of food or sexual activity had on migration, and to what degree the tendency to migrate could be hereditary. After several entertaining episodes, like the time when Alfonso and I flooded a room on the ground floor where they kept preparations made by Ramón y Cajal himself, we finished our Master's theses and decided to do our doctorate.

Once again, we were completely lost for ideas, and luck took the form of a friend, Gerardo Pisabarro, who came into our laboratory and mentioned that the Centre of Molecular Biol-

ogy (CBM) was a good place to do a doctorate. Alfonso and I headed for the CBM and got interviews with different people there. At that time we also had the opportunity to become assistant professors in the Genetics Department at the Complutense University. Since my record was better than Alfonso's and I could get a fellowship, the two of us decided that I would go into the CBM while Alfonso remained at the university. That is how I was accepted as a doctoral student on Jesús Ávila's team. I still remember my first day, when he showed me an SDS gel and asked for my opinion on it. Of course I had none to offer him, and he said that it had to be the last time I was unable to respond.

My doctoral thesis revolved around analysing different isoforms of tubulin using an isoelectric focus. The beginnings were difficult. Since we did not have a great deal of money, I remember that I would go along with other colleagues from the laboratory, including Francisco Wandosell and Esteban Montejo, to steal Eppendorf tubes and pipette tips from Margarita Salas's laboratory, or we would reuse tips that we already had. However, Spain's economic situation was improving, and after a year we had a reasonable amount of money for research. It was truly exciting and we had an excellent team, including María Ángeles, Paco and Esteban, and secret projects that we would only tell Jesús about when they worked out right. In fact, of the twelve articles I published from my thesis, only one came directly from the initial project. Then Ricardo Maccioni, a Chilean researcher, showed up at the laboratory. He was interested in observing the structural domains of tubulin using limited proteolysis. I came to form part of that project and began to break down tubulin using trypsin and chymotrypsin, in the presence of drugs, among others, with polymerized tubulin. One day we decided to try another protease: subtilisin. I discovered that, shortly after starting to break down, the buffering solution became slightly cloudy, and, when using a gel, I discovered that a bit of tubulin was missing. Under the electron microscope, we discovered that, upon removing the last aa of tubulin, this protein spontaneously polymerized, forming microtubules with no associated proteins (MAP). We had discovered that C-terminal tubulin was involved in the regulation of tubulin's polymerization, and we later verified that many MAPs had to do with that part of the protein. It was a true revelation that helped us greatly to understand how the formation of microtubules was regulated. During that period I attended a doctoral course on proteins, taught by my thesis director, and as a research project I proposed designing a molecule that would change in structure upon fixating calcium. I read several articles on the structure of proteins and liked the idea of designing them.

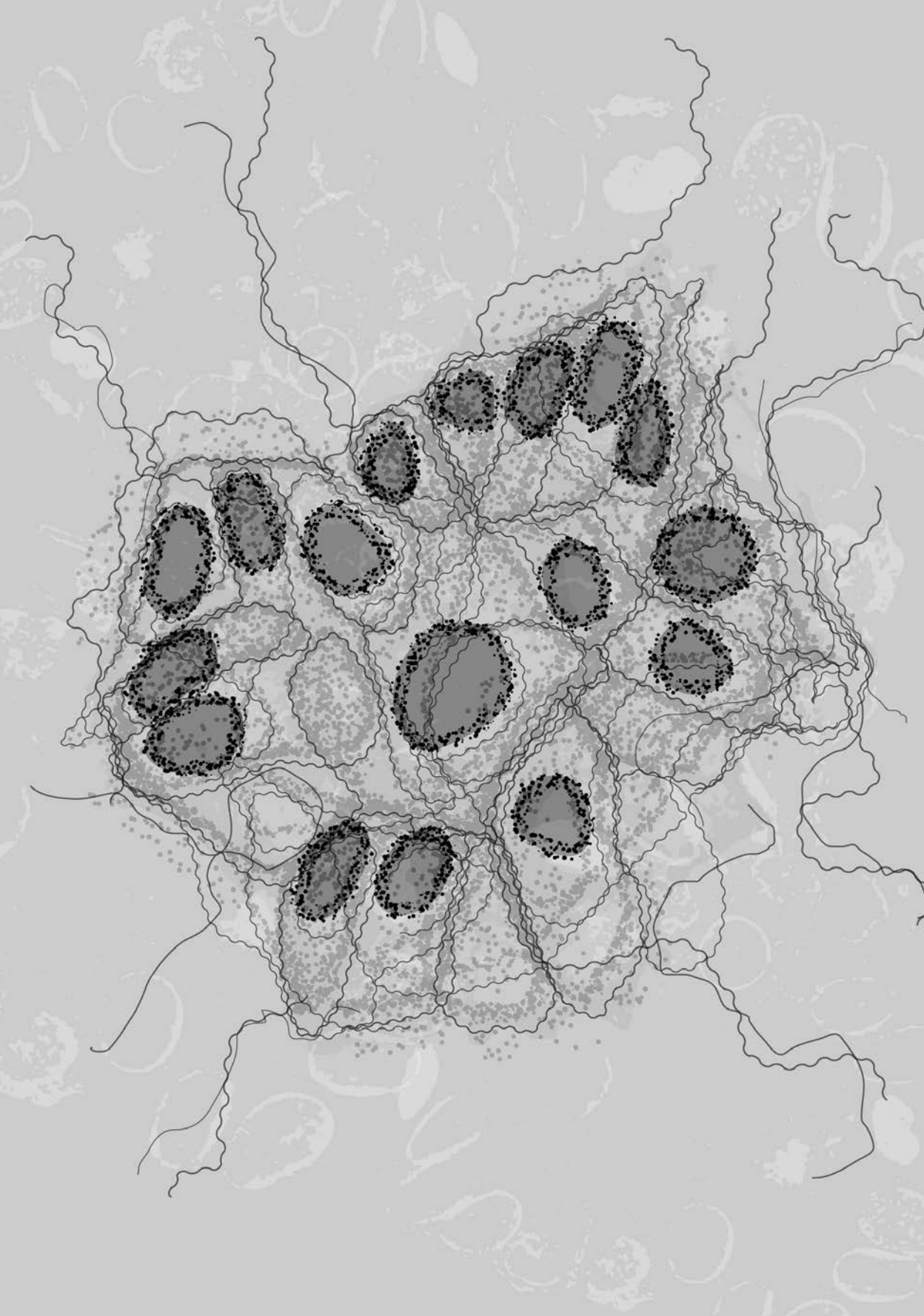
In the meantime, I applied for a postdoctoral fellowship at David Baltimore's American laboratory to study transgenic mice with various C-terminal mutations. I received a Fulbright fellowship and was willing to leave when I realized that my wife Isabelle Vernos, who was still doing her thesis with Roberto Marco, still had two years left to finish. So I cancelled the fellowship and decided to accept a quite beneficial one-year postdoctoral fellowship with Jesús. To avoid travelling too far away, I decided to do postdoctoral work in the United Kingdom, studying the then new field of protein design. Since I had no idea which laboratories were good in that subject area, I consulted the editorial advice in *Protein Engineering Design and Selection* and *Protein Science* and addressed all the members who were residing in the United Kingdom. To my surprise, they all accepted me, and when I

was ready to join a team that was working on antibodies a former lab colleague of Jesús's spoke to us about Alan Fersht, who was working on protein folding at Imperial College. I changed my mind, and in late 1987 I accepted Alan's offer. I therefore found myself on my way to the United Kingdom with a fellowship from the European Molecular Biology Organization (EMBO).

When I reached London, it was mid February, and I had the luck that Cayetano González and Salud Llamazares, who were at the CBM and had arrived before me, welcomed me at their home for a few days until I could find my own accommodation. It was a gloomy time: it rained all day long, my future boss was ill, and my colleagues at the laboratory had made it clear that they had their own areas of research on which I was not supposed to trespass. In that rainy London, I lived in a room with no bathroom, and my only company was a teapot. In addition to this personal depression, those first months were hard because I did not know much about molecular biology, and my project meant designing mutants on the basis of barnase, Alan's favourite protein. That was before the polymerase chain reaction (PCR), and we were creating mutations of the M13 phage using oligonucleotides, so as then to search for the mutant on Petri dishes using a P32 oligonucleotide and performing washes at various temperatures. It took me some time to learn the technique, as well as to understand my boss's English and his jokes about Spaniards.

Eventually I found my niche and made transcendental discoveries about the structure of proteins, including the role of the N-terminal and C-terminal of helixes in stability, or how to quantify the contribution of hydrophobic groups to thermal stability. However, my main achievement occurred when I formed a team with Andreas Matouschek, a German doctoral student. Together, we discovered the protein folding system for the first time, a work that was published in six articles all in the same issue of the *Journal of Molecular Biology* (*J Mol Biol*). It was a fascinating time when the possibility of changing the amino acids in a protein at will allowed us to play with those molecules and attempt to understand in what way the linear sequence of a polypeptide bore its three-dimensional structure encoded within. In a certain way, something similar is now taking place in synthetic biology with the ability to design genomes. During that period I applied for and was given a position at the CSIC, but I thought it was still too soon to return to Spain.

In all, I stayed in the United Kingdom for four years, one in London and three in Cambridge. I had the option of becoming a researcher with the Medical Research Council (MRC), and my wife Isabelle had also applied for a job at Cambridge. When nobody was expecting it, Ramón Serrano, who was at the European Molecular Biology Laboratory (EMBL) in Heidelberg, contacted me. I knew Ramón from my times in the doctorate. Since both of us had the same surname, I would sometimes receive his mail, and he would receive mine. When he contacted me, Ramón was leaving EMBL and wanted another Spanish group leader to be placed there. At that time I was not familiar with EMBL, and the idea of going to Germany was not very appealing to us. Therefore we decided that if Isabelle got the job in Cambridge we would stay in the United Kingdom, and, if not, we would leave for EMBL. Isabelle got an excellent score, but budget decreases kept her from being hired, so we moved to Heidelberg. I became a group leader in the



structural and computational biology programme, and Isabelle began to work as a postdoctoral candidate with Erik Karsenty.

It was a stimulating period because I was going to be running my own team for the first time ever. I began with one doctoral student, Víctor Muñoz, who had done his Master's thesis with me at Cambridge; with Jesús Prieto, a postdoctoral candidate, and also a friend from the university; and with our technician, Deborah. Being at EMBL was like a dream come true: I had money to pay salaries, buy equipment and do research, in addition to having excellent facilities and a wonderful atmosphere. People talked about science in the lunch room. We would hold parties in the Operon Foyer. On Fridays we would meet up for beer. There were seminars... In those times, Philipson was the director general, and the moment was stimulating because EMBL was still a young institution. I decided to continue working on protein folding, though I also began to design proteins, something I had always dreamed of.

Because there were no good protein design programs in those years, people just dived into this exciting and innovative field without looking. I therefore switched barnase for the chemotactic CheY protein, which is structurally related to p21 ras. I intended to introduce a bonding site for GTP in CheY, as well as to study how it folded. At that time I met Manuel Rico and his team at a meeting in Madrid, and I discovered the world of nuclear magnetic resonance (NMR). Manuel's team was a pioneer in using NMR to study protein fragments in solutions. I was quite lucky because Francisco Blanco, who had completed his thesis with Manuel, joined my team with the status of a postdoctoral researcher. Relying on him, Víctor and Mexican student Marina Ramírez, we began to observe the peptides that folded on their own until forming beta or alpha helix strands. We discovered the first peptide that folded by forming such a strand. Also with Víctor, we created the AGADIR computer program, which is still used by many scientists. It could predict the tendency of amino acid sequences to fold into alpha helixes. The discovery that short peptide sequences would partially fold without the rest of the protein created the foundation for the development of very productive protein design programs, like the one by David Baker's team. We also discovered that we could design thermally stable proteins, simply by stabilizing their secondary structure. A few years later, the doctoral student Emmanuel Lacroix joined my team to work on developing a computer-based design program that we called PERLA. It was the basis of the protein design algorithm named FoldX, which is currently being used by many different teams. We now know that whenever we have proteins with good structures, we can provide them with great stability and a high capacity for fixation, and this is made possible thanks to the discoveries made by my team and by others in that period.

In 1998 I was promoted to senior group leader at EMBL with an open-ended contract. My team continued working on protein folding and design, but I was drawn to a new discipline that was just starting to take its first steps at that time, but did not yet have a name. Later on, after some controversy, it would become known by the name of "systems biology". The reason for which I decided to delve into this field was an opinion article published in *Nature*, in which the possibility was brought up of combining biology and computer models to understand biological systems quantitatively. At EMBL there was another team,

Eric Karsenti's. He was also interested in that idea and had invited Stan Leibler to the centre, a French physicist who was working in the United States. To tell you the truth, back then I had no idea what systems biology entailed, nor did I know anything about computer models, but I was fascinated by the prospect of understanding an organism to such an extent that I could reproduce it. In a certain way, it was like designing a protein, but with an animal. At that point, I had the luck of meeting Attila Becskei, a Hungarian physicist who studied medicine, during EMBL's process for selecting doctoral students. He joined my team and began the first projects related with systems biology and synthetic biology. We published our first article in *Nature* about the analysis of a very simple genetic circuit, a negative feedback loop. That text and another by Leibler's team were the first on systems biology, and on synthetic biology.

Shortly afterwards, as a result of the unfortunate death of Matti Saraste, the programme's coordinator, in 2001 I was nominated to hold that position along with Peer Bork. Therefore I was no longer just responsible for my team but was also in charge of a programme involving twelve research groups. Peer and I led the programme towards the integration of the computer with the structuralists, also developing electron tomography at EMBL in Heidelberg. We had great fun together and managed to collaborate and have the same view about the integration of structural biology and systems biology. Sometime around 2005, when Isabelle's nine years as a group leader at EMBL were drawing to an end, I had to leave the centre. We considered several possibilities, but we both knew that she did not feel very much like working at a German university. At that time in Munich, the Max Planck Institute got in touch with me to ask me to apply for a director's position at one of its centres. Back then the proposal was quite stimulating because it meant starting up my own systems biology institute from scratch. I went to the interviews and had an offer on the table, but sadly for Isabelle the only possibility was becoming a group leader at my centre or to go as a researcher to the German university.

That is when Miguel Beato got in touch with me, after a conversation I had with Cayetano González in a Heidelberg grocery store, and he offered me the chance to return to Spain, to the recently created Centre for Genomic Regulation (CRG). They also wanted to create a cellular biology programme that Isabelle could fit into. I remember speaking with Iain Mattaj, the new director of EMBL, and he said to me: "Luis, you have done stupendous work at EMBL. Whether you stay here or become the director of a Max Planck centre, you will follow the same path. However, if you accept Miguel's offer, you will have the opportunity to create a cutting-edge institution in Spain for the first time ever." The decision was agonizing to reach but, in the end, the challenge of doing something stimulating in my own country was what convinced me to accept Miguel's offer. At that time the decision was risky: the CRG lacked basic financing and was a centre that had just begun, but there were two former members of EMBL there, Fátima Gebauer and Juan Valcárcel, who also did their best to convince us. Later on, during my transitional period from EMBL to the CRG (2006–07), I rejected two other offers from the Max Planck Institutes in Dortmund and Marburg.

So in 2007 I started work at the CRG as the Systems Biology Programme coordinator. It was an exciting time, and because Miguel had raised financing, we were able to offer a

combined welcoming package to the new group leaders. We were advertising abroad, so the first foreign group leaders started to arrive. We were also starting up an international doctoral programme and, among other things, a programme for technology transfer. New programmes arose, like the one in cellular and development biology, coordinated by Alfonso Martínez Arias, for a brief time thereafter by Isabelle Vernos, and later by Vivek Malhotra.

The year before our move to Barcelona, my team began to collaborate with Peer Bork and Anne-Claude Gavin on one of the smallest bacteria that can be cultivated in the laboratory, *Mycoplasma pneumoniae*. The goal of the project was to attain full understanding of a living being in an absolutely quantitative manner so that it could inhabit a computer. It was truly an ambitious undertaking, and to achieve it we would need solid economic support. That is when, one day while I was in my office, Dr Pedro García Barreno showed up and told me that he was representing the Botín Foundation. He wanted to offer me considerable financing for a five-year period and told me I could use the money as I saw fit. It was as if heaven had answered my prayers. More or less at that time, too, I applied for advanced research aid given by the recently created European Research Council (ERC). The project I submitted focused on modifying *Mycoplasma pneumoniae* to turn it into a pill that could be used to treat human diseases. I was lucky and was granted the aid. Thanks to this, with solid economic backing, I was able to take on the project that, besides producing many other articles, has taken shape in three texts published in the same issue of the journal *Science*. We also began to collaborate with Sanofi to manipulate the bacteria in order to treat human diseases, something which could lead to some very promising results. As we worked in that field, half of the team dealt with the task of quantitatively analysing the signalling of human cells, focusing on the MAPK-ERK pathway, which is frequently related with cancer.

In 2007 I was also named assistant director of the CRG, and in 2011, when Miguel retired, I successfully applied to become the centre's director. Since I obtained the aid from the Botín Foundation, I have adhered to its initiatives for promoting Spanish science and, more importantly, for training Spanish scientists about technology transfer. The Foundation has done excellent work hiring cutting-edge Spanish scientists, providing them with training and support in their initiatives, who intend to repay to society part of what society has given to them.

In my position as the director of the CRG, I believe that our priority is scientific excellence, but we must also place an emphasis on technology transfer, as well as on education and communicating with society. I hope that in the upcoming years, with the cooperation of the Botín Foundation, we can achieve these three objectives. As for my team, what I hope for is to combine my position as the director of an international institute of excellence made up of 450 people with the running of my own laboratory. Up to now, I have managed to do so, thanks to the excellent members of my group, and to having Christina Kiel and Maria Lluch on my staff, two scientists who take care of everyday management. I have not left science, and I am still excited about discovering new things with the team and having new revelations. However, I have changed, in the sense that I am no longer as excited over

getting an article published in an important journal. I am more interested by things that help people or that have a true impact on science. As for the CRG, my goal is to have one of the best institutes in the world and contribute to the education of excellent scientists who can go anywhere. I do not know whether I will end my career here, however. I like to do new things and take on new personal and professional challenges. In a certain way, I like the comparison between life and a watercolour painting made by Toquinho in one of his songs: "Imagine that your future is a watercolour painting, and that your life is a canvas to fill with colour".

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JUAN VALCÁRCEL

SPLICING LOOSE ENDS TOGETHER

14

Wrong number?

"Are you sure you haven't got the wrong number?" That was my first reaction – fortunately, restrained – when, one morning in September 2007, Pedro García Barreno phoned me at the time of the weekly meeting of my research group to tell me that he would like to visit our laboratory at the Centre for Genomic Regulation of Barcelona (CRG). I had met Pedro a few weeks earlier at a conference at the School of Molecular Biology at the Menéndez Pelayo University organized by Margarita Salas and Carlos López Otín in honour of Eladio Viñuela, one of the most pleasurable scientific meetings I know, due to its stimulating atmosphere of learning and discussion, the motivation and participation of students and the extraordinary natural setting of the Palace of La Magdalena, host to the courses in Santander. And also, of course, out of respect for Margarita, Eladio and Carlos, both for their pioneering contributions to establishing and developing molecular biology in Spain and for their example and influence on my own career.

I found Pedro's talk to be prescient for his common sense in diagnosing the causes of Spain's relative lag in science and technology compared to other countries of a similar economic level. And he spoke not only of the causes, but also of the possible solutions. Indeed, that talk prepared me, unwittingly, for the conversation we had when he visited my laboratory. He started by explaining the philosophy behind the Botín Foundation's *Technology Transfer Programme* and he offered me the opportunity to join it. He must have read my mind because he then added: "And we don't want you to change anything you're doing; you just have to talk to us once a month to discuss your science". My first reaction – "Just think what we could do in the laboratory with the plentiful funding that would come with joining the Programme!" – was followed by dejection that I could hardly conceal. I had never filed a patent application, never thought of setting up a company, and even though I was aware of the possible applications of my research group's work I assumed they would later be developed by others with greater acumen for technology and for business. My only professional goal up to that time had been to carry out work of the highest possible scientific quality in the field that had absorbed me since my doctoral thesis, and to which I will return below. My perception was that any distraction from this goal would end up undermining the quality or the impact – or both – of my group's work. "We must each do what we know how to do best," was the quote I had read from Howard Temin, the discoverer of one of the pillars for the development of biotechnology, the reverse transcriptase enzyme. However, he had shown no interest in exploiting the results of his revolutionary work.

At that moment I thought that the honest thing to do would be to express profuse gratitude for the invitation but to find some courteous way of declining. However, I could only

manage to do the former, and stammer that "the fact is that I have no experience in technology transfer," which Pedro brushed aside, saying that that was precisely the profile they were seeking for their mission of transformation. Being part of an experiment is the best way to convince someone with a scientific background, so I gladly accepted the proposal and assumed that, at the very worst, I could be useful as some sort of negative control of the experiment.

Odd syntax

I grew up in Lugo, a city founded in the year 25 BC by the Roman magistrate Paullus Fabius Maximus in honour of the emperor Augustus, on top of a pre-existing Celtic settlement. I was lucky to have wonderful teachers and a family that taught me to enjoy learning about the workings of nature along with drawing, languages and literature. My best memories are of reading books about DNA, the life of stars or the behaviour of bees or chimpanzees, and the novels of Gabriel García Márquez and Günter Grass in the forests and rivers that surround the city of Lugo, or in the cliffs on the coast near Ribadeo. I studied biology and chemistry in the University School of Lugo and continued in the Department of Biochemistry and Molecular Biology at the Autonomous University of Madrid. I had the fortune to find work, at the suggestion of Juan Fernández Santarén, in the laboratory of Juan Ortín, in the "Severo Ochoa" Centre of Molecular Biology (CBMSO). Laboratory work centred on studying the replication mechanisms of the flu virus, and Juan offered me a doctoral thesis project the aim of which was to identify the proteins involved in the generation of the genetic diversity that makes it so difficult to devise effective vaccines against this infection. Working on a subject of such importance in an institution like the CBMSO – home to so many great Spanish scientists, and a pioneer in molecular biology in Spain – was a dream come true. This, combined with the great human and professional quality of Juan Ortín, who was not only a great supervisor, but also, and continues to be, a great friend. But the first experiments in the laboratory would lead us down quite a different path.

A few years earlier, researchers at the Massachusetts Institute of Technology, Cambridge (USA), and the Cold Spring Harbor Laboratory on Long Island had discovered that one of the fundamental principles of molecular biology, gene-protein collinearity, had exceptions in certain animal viruses. This principle holds that the nucleotide sequence (A, T, G, C) of DNA, which constitutes the genetic inheritance of species and organisms present in each of our cells, determines the sequence of amino acids making up the structure and functions of proteins. These are the molecules that build our organisms, coordinate our metabolism and enable replication of our genetic material and reproduction.

The monumental work of many groups, including that of Severo Ochoa in New York, contributed towards deciphering the genetic code by which combinations of three nucleotides specify the twenty-one amino acids that make up proteins. But the new discoveries in viruses indicated that genetic text is sometimes interrupted by meaningless letters. Using as an example the famous initial phrase of Marquez's *One Hundred Years of Solitude* – "Many years later, as he faced the firing squad, Colonel Aureliano Buendía was to remember that

distant afternoon when his father took him to discover ice" – in our genome, that piece of information would be written in a quite odd form. Something like: "Manyyearsdadfdlaterdkeaswowkhefefaceddevhjtheknbfdsjjkfefirfiringsdfkdfsquaddfkfdlfkddkaidjfkadjfakdf". A most unusual syntax that shatters the universality of the principle set forth by François Jacob: "What is true for the intestinal bacteria *Escherichia coli* is also true for the elephant," revealing a fundamental difference between the mechanisms of gene expression of prokaryotic organisms – whose genetic messages are continuous – and eukaryotic organisms, which have a nucleus in their cells, and most of whose messages are discontinuous.

Within a short time, the conclusion emerged that this unexpected organization of genetic messages was not a rarity of certain viruses, but that the immense majority of genes in multicellular organisms are written in this odd syntax. What is more, at the time I was beginning my thesis, it was becoming ever more evident that organisms use this broken syntax to increase the number of genetic messages generated from the same region of DNA. By discarding certain parts with meaning, or combining them in many ways, phrases can be generated in which Colonel Aureliano Buendía did not remember that distant afternoon, or in which it was not his father, but rather his mother, who took him to discover ice, or that it was not ice but fire that the Colonel discovered. The possibilities that were opening up with this surprising organization of genes fascinated me. I imagined the cell the way the reader of a novel may not only read the chapters in a different order – the way Cortázar tried to make his readers participate in the creative process – but also generate, based on each sentence, different meanings. And this could be done with every sentence from the very beginning of the novel all the way to the end. Immense possibilities of combination for an infinity of novels that are related to one another, but different! Now we know that at least ninety per cent of human genes use this method of combination to multiply the informational content of their genes, and that some – such as the gene Dscam in *Drosophila* – are capable of generating several tens of thousands of different messages, an amount that is greater than the total number of genes in the organism.[1] Using the metaphor of Borges's *Book of Sand*, our gene resembles a book that contains another book between one page and the next.[2]

From the flu to cancer (with a glance at fruit flies)

In addition to the project on the variability of the flu virus, Juan Ortín proposed that I carry out experiments to study the expression of the seventh gene – the virus has only eight genes – that can produce two types of messenger RNA and proteins, depending on whether a region of the precursor RNA is eliminated or not. The further we advanced in studying this phenomenon, I became ever more fascinated by the machinery the cell uses to eliminate certain RNA regions, which cuts the molecule with surgical precision and splices loose ends together to generate sentences that can be read in a continuous manner. In our literary example, the cellular machinery would start with the text "Manyyearsdadfdlaterdkewowkefefe...", cut and discard the meaningless texts (the technical term for these is intron) and would join "Manyyears" with "later" (these individual pieces are known as exons) to generate the legible content of the messenger RNA "Many-

yearslater...". Throughout my thesis, we observed that the efficiency of this process in gene 7 increases during a viral infection[3] and is altered in the absence of other viral products.[4] This means that the activity of the splicing machinery can be modulated within the cell itself as a result of changes induced by the progression of the viral cycle. This malleability, which can also be seen in other viruses, would point to the existence of dynamic cellular mechanisms through which the process can be modulated. If we could learn what those mechanisms are, we could dream of influencing them to control gene expression and choose what version of the novel we want to read. I immediately decided to devote all my attention to the study of the splicing machinery ("splicing" is sailor jargon for joining two ends of rope). My fascination for this machinery, its effects and regulation has only grown since then and, as I hope the reader will gather from what follows, I am sure that it will continue to do so in the future. Juan Ortín was very generous in allowing my work to digress from the general interest of the laboratory to follow this line of research, and I will always be thankful to him for it.

One privilege of a science career is having the opportunity to explore new areas of research, once your doctoral thesis is complete, which is generally associated with living somewhere else in the world for a time. To understand the regulation of splicing, the biological system in which the factors regulating the process were best defined was the fruit fly, *Drosophila melanogaster*. Genetic data indicated that the Sex-lethal protein, which is expressed solely in females, coordinates the process of sexual determination by controlling the splicing of several target genes. I was fortunate that Michael R. Green, one of the world's leading experts in the splicing process, accepted me as a member of his laboratory at the University of Massachusetts, for which the granting of a postdoctoral scholarship by the European Molecular Biology Organization (EMBO) was instrumental. Although the laboratory did not work in *Drosophila*, we set about researching how Sex-lethal regulates splicing and we found that in some target genes it does this by binding to the RNA and preventing certain intron sequences from being recognized by the splicing machinery.[5] This mechanism also allowed us to understand how a protein involved in the recognition of the 3' end of the intron (U2AF) performs its function, by showing that the fusion of a region rich in arginine and serine amino acids of this protein is sufficient for Sex-lethal to shift from being a suppressor to being an activator of the splicing process.[4,6] Once again, the idea sprang to mind of modulating the splicing process in a predictable and relatively simple manner. The years in Massachusetts were enormously stimulating for me and for my wife, Fátima Gebauer, who was doing her postdoctoral thesis with Joel Richter, because of the intellectual stimulation of working with real pioneers and the opportunity to learn from the dynamism, ambition and rigour of science and technology in the United States. Personally speaking, we both enjoyed the multicultural environment, our little house near Lake Quinsigamond and the splendid natural environment of North America.

In 1996 I had the immense fortune of being able to set up my research group at the European Molecular Biology Laboratory (EMBL) in Heidelberg. I was able to do so thanks to the unconditional support of Juan Ortín and Michael Green, and to the new director Fotis Kafatos's policy of hiring scientific personnel from southern European countries. In the following years, I was able to link my research into the mechanisms regulating splicing to

the control of cell division and programmed cell death, both of which are of crucial importance for the creation and progression of tumours.

From Odenwald to the Mediterranean

Located on the outskirts of Heidelberg, in the forests at the edge of the Odenwald natural park, EMBL is a unique research centre. The majority of its groups are led by young scientists eager to establish novel lines of research. The maximum period of residence at the centre is nine years, which results in a constant turnover in its personnel and a non-stop flow of new ideas. Alongside its tradition in the development of new technologies, its interdisciplinary partnerships and its mission to educate young people at all levels of their scientific career, EMBL has played a critical role in the development of molecular biology in Europe.

Jointly with a small group of highly dedicated and talented collaborators, we continued to study the mechanisms through which Sex-lethal regulates other targets in the process of sexual determination in *Drosophila*. What I had envisioned to be an initial, short-term project to set up laboratory techniques soon became a six-year project in which the study of these mechanisms unearthed significant information on the molecular basis of the identification of intronic sequences and the regulation of their use in different stages of the splicing process.[7–14] These results revealed the great plasticity of the splicing machinery and, in connection with it, a variety of regulation mechanisms: the idea was taking root that the machinery for reading the genome is malleable and, ultimately, controllable.

These studies had the added value that some of the regulatory factors we identified were proteins previously involved in other cellullar processes, which led us to wonder if these functions might be explained on the basis of their activities as splicing regulators. For instance, we found that the functions of the proteins TIA-1 and RBM5 in the regulation of the process of programmed cell death (also known as apoptosis) were related to the activities of these factors in the control of alternative splicing of the gene Fas, which encodes a key receptor in the sensing of signals of cell suicide.[15–17] In this case, alternative splicing generates two variants of the receptor: one integrated in the cell membrane and the other secreted from the cell, with opposing functions: while the former fosters apoptosis, the latter inhibits it, demonstrating the importance of the control of alternative splicing in the reading of genes. Collectively, these results, along with many other publications by other groups, cemented the idea that control of splicing may play a key role in many aspects of tumour progression.[18] To my fascination for the broken syntax of genomic messages and for the RNA processing machinery, the perspective of their relevance in human diseases, including cancer was added.

The years in Heidelberg were an excellent experience both professionally and personally, owing to the international environment of EMBL, the cultural opportunities in the heart of Europe and, especially, the birth of our daughter Flora, who grew up among the beech trees of Odenwald and the kindergarten of EMBL, an exemplary crèche for any research institute.

In 2002, Fátima and I accepted offers from Miguel Beato to join the recently created Centre for Genomic Regulation (CRG) of Barcelona. It was a very difficult decision because we both had very competitive offers from other institutions in Europe and the United States, while the CRG was a huge question mark at that time. But the idea of taking part in the creation of a new centre committed to achieving international excellence, inspired by EMBL, coupled with the optimism and scientific weight of Miguel Beato and the strong support of the Catalan government minister Andreu Mas-Colell, convinced us that the future perspectives of the challenge made up for the risk. In the last twelve years, the CRG has consolidated itself as a competitive and international research institute, with seventy-five per cent of its scientific personnel coming from other countries. The centre's location facing the Barcelona seaside, along with the investment in the latest technologies and the atmosphere of open collaboration and discussion, also helped us attract excellent young scientists ready to set their own seal of talent, scientific quality and innovation at a worldwide level. As in the case of EMBL, the majority of the group leaders have contracts that are limited in time, thus allowing for a renovation of ideas, fields of study and new opportunities of collaboration. The first generations of alumni have attained positions as senior researchers in prestigious institutions, which attests both to the talent of these researchers and to the success of the CRG in recruiting them and providing them with the right environment to become international leaders in their fields of research.

The centre receives external resources that double the institutional investment, and we are confident that in the near future the wealth it generates in terms of technology transfer will contribute substantially to the functioning and scientific expansion of the centre, as institutes of a similar size and scientific calibre in other countries have done. A contribution here will surely be made by the work carried out for the last seven years in our centre by the Botín Foundation, through the support it has provided to the groups of Luis Serrano, current director of the CRG, and to mine. The Foundation's meticulous and highly professional work, which is exquisitely respectful of researchers' freedom, ends up impregnating the modus operandi of the centres with respect to technology transfer. In our case, very direct support has arisen from the fact that Pablo Cironi, one of the managers of the Foundation, has taken over the reins of the Technology Transfer Department of the CRG.

Three transformations in Barcelona

The group's work in Barcelona has undergone profound transformations at three levels. First, technological and conceptual advances have enabled us to study the process of splicing and its regulation both in its mechanical details and in terms of the global programmes that coordinate its functioning in cells. This makes it possible to describe the dynamics of hundreds of proteins involved in eliminating each intron and to study the effect of these factors, throughout the genome. These technologies and study methods allow us to condense, to a single experiment, analyses that would have required decades of work by many researchers using traditional methods. The bioinformatic analysis of data and computational modelling of the systems have become indispensable tools that make the quality difference in results and publications. Indeed, there has been a qualitative leap in the capacity of analysing biological systems that will, unquestionably,

cause a true revolution in biomedicine. To take just one example: in a decade, we have gone from sequencing the human genome in ten years at a cost of 3 billion dollars to doing it in one afternoon at a cost that is a million times lower. Paradoxically, this explosion in analytical capacity coincides, in recent years, with a contraction in the public resources devoted to biomedical research, both at a global level and, particularly, in Spain. Certainly, this fact does not speak well of the foresight of centres of economic decision-making, and it highlights the value of the efforts made by the government of Catalonia to protect the funding of outstanding research institutions in the face of the severe effects of the economic and financial crisis.

This technological transition was possible in my group through the incorporation of young people of great talent, who are capable of establishing a fluid dialogue between experimentation with large-scale technologies and the concepts and tools of systems biology. It has also been crucial to establish strategic partnerships, both on the part of the CRG with groups such as those of Roderic Guigó or Ben Lehner, or with international consortia financed by the European Union or the Human Frontier Science Program Organization (HFSPO). Of particular importance was the European Alternative Splicing Network of Excellence (EURASNET), a consortium of forty-two European laboratories in which our group played an important role, along with its coordinator, Reinhard Lührmann, from the Max Planck Institute for Biophysical Chemistry of Göttingen, who is also one of my most esteemed colleagues.

A second profound transformation in the group's work was its new focus on biomedical problems. This was facilitated by the scientific and medical environment of the Barcelona Biomedical Research Park (PRBB), whose campus houses the CRG, and the integration of molecular and clinical research groups in the RNAREG consortium, which I have been privileged to coordinate, with the financial support of the Consolider-Ingenio Programme. Dialogue and collaboration between groups, joint publications, technological development and its applications show the added value of such programmes, which the Ministry of Economy and Competitiveness has, unfortunately, decided to discontinue.

The group's third transformation was catalysed by its partnership with the Botín Foundation. The persistent and eloquent work of its professionals throughout these years, namely that of Pepa Limeres, Pablo Cironi and Daniela Piazzolla, has made the group aware of the opportunities being opened up by our research to find industrial applications. Without straying a single inch from our primary objective of understanding the molecular logic of splicing, the group has achieved a positive dynamic around innovation. This ranges from attention to detail in recording results, to open and ongoing discussion of possible applications, and the establishment of lines of work with eminently practical goals, or the registration of our first patent application.

Choosing from among different versions of the genome

Our studies of alternative splicing of the gene Fas, which, as I have mentioned, produces messenger RNA that either foster or inhibit cell suicide, have enabled us to identify a variety of mechanisms and regulatory factors.[17–20] Even finding that the spliceosome,

the molecular complex that performs the process of discarding introns, is not only one of the most complex machineries of our cells, but also exceptionally dynamic and flexible, allowing its regulation in basically each of the steps of its assembly and molecular function.[20,21]

One of the regulating factors, the protein PTB, fosters the elimination of the alternative exon Fas. By dissecting the parts of the protein responsible for this effect, we managed to identify a small sequence of amino acids (a peptide) that can foster elimination of the exon when it fuses with a small nucleic acid (oligonucleotide) that is supplementary to exon sequences. This work, which we are carrying on in collaboration with the group led by Enrique Pedroso at the University of Barcelona, immediately pointed to the possibility of using such reagents to induce elimination of any exon by simply fusing the peptide to a supplementing oligonucleotide. This would open up a wide range of possibilities for modulating the splicing of any gene of biomedical and biotechnological interest, thus yielding at-will readings of the genome. Although we have yet to find a way of universalizing this application to any gene, our group has learned much in this project about the requirements – and difficulties – of achieving applications from basic observations with no apparent practical use.

Our development of high-performance technologies to detect alternative splicing profiles enabled their application to biological samples from tumours.[22–25] It also allowed us to systematically identify the target genes of splicing regulators whose alterations influence tumour progression, such as the protein of the Ewing sarcoma and tumour suppressors WT1, SPF45, RBM5 and RBM10.[26–29] One overall conclusion of these studies was that processes of tumour transformation and progression are deeply influenced by the expression of different forms of splicing. In some cases we were even able to prove that the effects on cell proliferation of alternations in splicing regulators can be blocked by reversing the splicing patterns of certain genes. For example, the greater cell proliferation associated with mutations in the protein RBM10, which are quite frequent in lung cancers, can be inhibited by reversing the splicing pattern of one of its target genes. This target gene encodes for the protein NUMB[29] an important regulator for the Notch signalling pathway, the inhibition of which has therapeutic effects in models of mice of lung adenocarcinomas, as shown by the research group of Manuel Serrano at the Spanish National Cancer Research Centre (CNIO). These results reveal the potential therapeutic value of the modulation of alternative splicing.

The connections between the splicing process and cancer have taken on a further dimension with the identification of products of bacterial fermentation that possess antitumour properties and that act on the components of the splicing machinery.[18] A number of these compounds bind to the protein SF3B1, which is considered essential for the general intron splicing process. Adding further interest to this connection, the group led by Carlos López Otín at the University of Oviedo discovered that SF3B1 is one of the most frequently mutated genes in patients with chronic lymphocyte leukaemia. How can compounds that act on molecules essential in the splicing process have antitumour effects without being toxic for normal cells? Our group found that, even though they act on a general component, when

used in concentrations that inhibit cell proliferation, these compounds modulate alternative splicing of genes involved in the control of cell division.[30] The mechanism through which these compounds act is related to molecular proofreading processes that facilitate precise recognition and verification of splicing sequences, an area of great interest for my group.[31–33] Our team is actively investigating the detailed mechanism of action of these compounds and has developed high-performance methods to identify the molecular targets of these drugs.[20,21]

Visions of therapeutic windows

Is it realistic to think that modulating a process as basic for gene expression as splicing can eventually offer therapeutic possibilities? In other words: can we conceive of designing procedures specific enough to affect only certain molecular events in specific genes without substantially altering the processing of a multitude of other RNAs, with the likely negative consequences this would have for the cell? Two main paths have appeared on the horizon.

The first is the use of modified oligonucleotides capable of preventing recognition of sequences in messenger RNA precursors by the splicing machinery or by regulatory complexes. Therapies based on the use of nucleic acids complementary to the mRNA to be inhibited (that is, antisense therapies) were all the rage among investors and the biotechnology industry in the 1980s and 1990s, but the results failed to meet those expectations. One possible reason lies in the mechanism of action of classic antisense therapies, which aims to block the translation of messenger RNA into proteins. This process is carried out by the complex machinery of the ribosome, which contains numerous enzyme activities designed to eliminate just those double-strand structures that tend to naturally form between different regions of messenger RNA. In the case of splicing modulation, however, these oligonucleotides appear to be more effective, probably because they "divert" their function towards an alternative processing sequence rather than launch a frontal attack on the splicing machinery. These molecules have been very successfully used in animal models to correct genetic defects associated with spinal muscular atrophy, one of the most prevalent genetic diseases. The introduction of stabilizing chemical modifications has yielded oligonucleotides with a notable activity and average lifetime in tissues, even weeks after intravenous injection. Such pioneering compounds are now in the advanced stages of clinical trials.

The second model is based on the identification of small molecules that modulate recognition of alternative splicing sequences. We have already discussed the antitumour effects that microorganism fermentation products have on the protein SF3B1 and their consequences on the expression of genes regulating cell division. Another recent example is the identification of compounds that can modulate the alternative splicing of the gene SMN2 to correct gene defects that cause spinal muscular atrophy. Although their underlying mechanisms remain unknown, these compounds open up the possibility of generating therapeutic "magic bullets" based on the specific alteration of the splicing process in the relevant genes of a pathological process.[34]

Either of these strategies, or an intelligent combination of both, can offer realistic methods for specific modulation of the splicing process and, hence, contribute to the creation of new therapies and pathways for exploring gene function. Making the most of these therapeutic windows will require a detailed understanding of the molecular interactions that underlie the spliceosome function, our reader of messages from the genome. In this direction, our group has developed a combination of computational and experimental methods that can determine the network of functional interactions of the spliceosome and it can use these tools to identify molecular mechanisms of regulation, including targets of drugs and compounds that modulate alternative splicing.[20,21]

When I was a doctoral student, I had the privilege of hearing a lecture by Arthur Kornberg on the occasion of the tenth anniversary of the Centre of Molecular Biology. Kornberg, who had received the Nobel Prize along with Severo Ochoa for their enzymatic synthesis of nucleic acids, ended his talk by saying that it was a great time to do science and, in particular, to study biological processes. I believe Arthur Kornberg would agree that the opportunities arising today for the study of living beings and the alterations associated with their pathologies are even broader than what was conceivable at that time. Let us hope that we can all keep splicing those loose ends together.

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MODESTO OROZCO

THE IMPERCEPTIBLE CERTAINTY OF DOUBT

15

I have always been fascinated by paradoxes. One of the most troubling was proposed by Austrian physicist Erwin Schrödinger in 1936. His paradox forced us to imagine an opaque box containing a radioactive source, a Geiger counter, a hammer and a flask full of poisonous gas. Once the box is ready, we introduce an unfortunate cat into it, seal the box shut and wait for a certain time until the radioactive source has a fifty per cent chance of having decayed. The classical mechanics of the story teach us that, if this decay occurs at any time during the experiment, the Geiger counter will detect it, activating the hammer that will break the flask, release the poisonous gas and kill the cat. Otherwise, once the experiment is over, the cat will be bored but alive. This classical way of telling the story implies that, when the box is opened, there is a fifty per cent chance that the cat will be alive and another fifty per cent that it is dead. As Schrödinger pointed out, quantum mechanics provides us with another viewpoint, in which the cat is defined on the basis of a wave function resulting from the combination of two different states existing in equal measure: one corresponding to the bored but living cat, and another corresponding to the dead cat. As a result, the quantum cat is both alive and dead at the same time. Only upon opening the box will these two equally frequent states collapse into one alone, corresponding to either a dead cat or a living cat.

When I look back, I often think about Schrödinger's cat, and I try to imagine alternative lives of a Modesto Orozco who might exist if the universe forgot about me, and my wave function was defined in accordance by a series of completely different states. I encourage you to put yourself in that situation and to discover different and exciting parallel universes.

I was born in Barcelona in October 1962. My parents had just barely studied, and my mother, despite being one of the most intelligent people I have ever known, was practically illiterate. I need not mention that neither culture nor money were frequent visitors to our home. My mother and my father both worked twelve hours a day, with little payment in return and no acknowledgement from others whatsoever. Already as a child, I had reached the conclusion that the probability of being successful in life – and at that time I was not really sure what that even meant – could be expressed as follows:

$$Ps = \alpha \cdot \beta \cdot \gamma$$

in which α represents your own innate abilities (such as physical strength or intelligence), β stands for training (in other words, level of education) and γ is a random parameter (yes, "luck"). All the variables in equation 1 are standardized. In other words, they range from 0 to 1. It is worthwhile to point out that, if any of them has a value of zero, you are better off waiting until you reincarnate.

It did not take me long to realize that equation 1 was too simple to reproduce the true likelihood of success. It was not only a matter of luck, innate abilities and training. There was also an overlap between abilities and the definition of success itself:

$$Ps = \alpha s \cdot \beta s \cdot \gamma$$

In which $\alpha_S = \alpha \cos(\delta)$ and $\beta_S = \beta \cos(\delta)$; δ represents the angle existing between the definition of success and abilities. Perverse, right? In order to be "successful", you not only need to have luck, innate abilities and training, but also your own objectives (which is what defines success for each individual) must be aligned with your abilities and training.

Let us go back to Schrödinger's cat and think about Modesto Orozco in the parallel world, who might also exist if nobody (including myself) were paying attention. To make it simpler, let us just think about the lives in which a reasonable level of success is reached, and assume that my random "luck" coefficient is equal in them all. The requirement of achieving "reasonable success" eliminates a significant number of possibilities. For example, I surely would not be a famous singer, artist or football player, and I probably would not be a hair stylist or fashion expert either. However, I think I could be, to provide some examples, a reasonably good chef, not too bad a writer or a passable secondary school teacher. So, why did I turn out to be a scientist in the end?

Curiosity had a great deal to do with me heading towards science, there is no doubt about that. As a teenager, I had already begun to carry out chemistry experiments in the kitchen, most related with improving gunpowder – fortunately they were not very successful. After a few accidents that forced me to explore other fields, I became fascinated by astronomy. I spent my nights looking for stars – not easy when you live in downtown Barcelona – and I even conceived of an ingenious method for calibrating the distance to the stars, a feat for which I have never received proper recognition, since it was already used back in ancient Egypt. During my rough adolescent years, my interests leapt endlessly from biology (for instance, I was fascinated by viruses) to physics (*Investigación y Ciencia* magazine was to blame) and chemistry (I will always be indebted to Isaac Asimov).

My formal education was also important in nudging me towards science. I completed my primary and secondary school studies inside Franco's education system. It was a dark period. Classrooms were packed, and the information you got was blatantly biased. Physical punishments were not uncommon and no effort was made to help children with learning problems. However, that system did have one big advantage: it was practically free, and if a boy or girl from a poor family, like me, had the ability and proper interest he could survive and progress in it. Since I was not a bad student, I managed to move forward in the educational system and pass my university entrance exams. Once I had resolved my doubts between physics, chemistry, biology and medicine, I decided to study chemistry at the Autonomous University of Barcelona (UAB). In those times, Spanish universities were living a veritable revolution because democratic bodies were being created and young, motivated teachers were entering departments, many of them educated abroad and with the clear mission of "changing everything".

In order to become a chemist in Spain at that time you had to spend five whole years in class, and normally one more year doing experimental work (for the *Tesina*, or undergraduate thesis). The first three years were devoted to general chemistry and the last two to the "speciality". When I registered in chemistry, it was clear to me that I wanted to specialize in theoretical biochemistry. However, when the last year of general courses drew to an end, I was filled with doubt once again. The culprit was Juan Bertrán, probably the best professor I have had in my entire life. Professor Bertrán's classes on quantum chemistry were unbelievably fun. At the end of each, you were never sure just what you had understood about the principle of uncertainty, Hückel's model or the tunnel effect, but you were convinced that quantum chemistry was the most exciting subject in the world. It was very difficult for me to follow my initial plans and choose biochemistry as my field of specialization. Nonetheless, when I analyse my scientific career, the influence of Professor Bertrán's classes stands out glaringly.

I must admit that I did not enjoy the two years of my field specialization. At that time studies in biochemistry were mainly about memorization, and the largest challenges lay in remembering an endless list of enzymes, cycles and metabolites, completely lacking any logic or meaning. The curriculum revolved around the "what", and not the "why" or "how". In other words, it was completely outside my realm of interest. I spent two years thinking that I had made a poor choice, and when I finished my university degree I had little doubt that I would attempt to do my undergraduate thesis on topics related with theoretical (bio)chemistry, distancing myself as much as possible from what seemed to me like dull description. In hindsight, little doubt can remain that my scientific orientation was greatly marked by my formal education, the academic curriculum and the teaching abilities of the professors.

The first dose of science

In 1985 few teams were applying theoretical chemistry to the study of biological systems, and my colleague Javier Luque – now a full professor of Physical Chemistry at the University of Barcelona – and I had the great luck to join one of those teams, directed by Ferran Sanz, at what was then called the Municipal Institute of Medical Research (IMIM). At that time Ferran was creating the group and holding down more than one job involving different topics, so for a certain time I was a bit lost. However, the experience was wonderful. I completed my *Tesina* and when I left the group to finish my military service I was convinced that I should attempt to do a doctorate in Theoretical Chemistry/Biology.

I completed the last stage of my military service on Majorca. The ambience was laid-back, the island was marvellous and I had a decent salary with a lot of free time, which I used to write my first articles, based on the work I had done at IMIM. They were not masterful scientific works, and the scientific community paid no attention to them, but they were important for helping to reinforce my thoughts about becoming a scientist. As explained stupendously by writer Carlos Ruiz Zafón, when you see your name printed on paper the sweet poison of vanity enters your blood. Whether we like it or not, similarly to what moves politicians is power and bankers money, what moves scientists is vanity – evidently we are not so different from pop stars and artists in this sense. Locate a scientist who says

he is not vain and you will have found a liar. Only after painstaking training and years of experience can you learn to conceal vanity, and its first cousin arrogance, but if you scratch a bit below the surface of the scientist's soul, you will find a thick layer of it. I am afraid so: vanity is one of the main reasons why I dedicated my life to science.

The thesis

My doctoral thesis was directed by Professor Rafael Franco at the Biochemistry Department of the University of Barcelona. Rafa's laboratory wished to study the behaviour of an enzyme, adenosine deaminase, which is a catalyst for the conversion of adenosine into inosine. My doctoral years were wonderful. I had a very good relationship with my director. The members of his laboratory were young and active. My collaboration with Javier Luque was productive, and I took advantage of the pleasant theoretical chemistry environment in the chemistry building. Seen from today's perspective, those were exciting years because the darkness of the present did not keep us from dreaming about a bright future. Don't be mistaken, though: life for a computational chemist was quite difficult in the late 1980s. Programs were neither reliable nor easy to use, and you really had to know the methodological basis well, which forced me to return to classes at the UAB to learn more quantum chemistry. Not only were the programs poor, but the computers were like oversized turtles, so slow that any modern-day laptop is much faster than the mainframe computer at the University of Barcelona back at that time, and that was the computer providing service to hundreds of groups. The software and hardware limitations drastically reduced the number of calculations that could be performed in those years, but the sad situation had three obvious advantages: you had to plan calculations very carefully, understand the margin of application of the methods you used very well, and all this encouraged you to grab a pencil and paper and to develop methodology, something that did not require any computational muscle.

Since my fellowship allowed me to spend three months abroad, I used them to visit Professor Stephen Neidle's laboratory at the Institute for Cancer Research (ICR) in Sutton, UK. Those three months were decisive in my career because they opened my eyes up to the things happening outside Barcelona. Stephen, a crystallographer who had discovered the power of computerized methods, had purchased a large computer and hired Charlie Laughton, an intelligent Oxford-educated postdoctoral fellow, to carry out simulations using DNA. Charlie, who is still one of my best friends, took care of me at the ICR and, showing infinite patience, introduced me to the world of DNA and the use of classical simulation techniques. I returned to Barcelona with a fascination for the potential offered by these new techniques in pushing biochemistry into the realm of the physical sciences. However, in the final stages of my doctorate, I was overcome with doubts once again: should I stick to the path of academic research or join the industry? Should I apply for some position abroad? Or should I just forget everything and prepare for competitive exams to become a secondary school teacher?

Professor Joan Guinovart's arrival in the department was crucial for my career – yes, there you have that luck coefficient yet again. He was and still remains a very enthusiastic, very

empathetic person talented at taking part in down-to-earth discussion. Joan convinced me that I should remain within the world of science and that getting a permanent position in the Biochemistry Department was a good step in that direction. At that time achieving such a thing at a Spanish university was not as difficult as it is now. With an acceptable CV (not necessarily outstanding), a bit of teaching experience and some talent for communication you could get a permanent position. Honestly, it is not at all clear whether that was good for Spanish universities, but I passed my competitive exam in November in 1989 anyway, and by 1990 I was the youngest associate professor in the Biochemistry Department at the University of Barcelona. Spain had yet another civil servant.

At the age of twenty-eight, I was guaranteed a salary for life, something undoubtedly positive, but there was no room for doing research in the department, or money to start up projects, or computer resources. All I had to help me was a Master's degree student, Carlos Alemán, who earned a living by giving private classes and organizing the students' computer room (Carlos, still a good friend, later did his doctorate at the Polytechnic University of Catalonia (UPC), where he is now a full professor). The worst thing, though, was that my way of viewing science was very narrow-minded when I was twenty-eight. To sum it up, the possibility of having a good scientific career by staying in Barcelona was practically none (in the real world, thank God, probabilities cannot fall below zero!). I was not unaware that I had to come into contact with a more active research environment before trying to create my own research group. Once again, I was lucky and received a Fulbright scholarship to work in the United States, where a true revolution was taking place at the frontier between physics, theoretical chemistry and biology. I will always be indebted to the Fulbright Commission.

Time in the United States

In 1991 my wife Mari Luz and I packed all our belongings into three suitcases and moved to New Haven, Connecticut. At that time I began my postdoctoral period, officially a sabbatical, in the Chemistry Department at Yale University, a university designed to impress while convincing you of its grandeur and your own smallness. At Yale, the weight of history is omnipresent, and you can feel the excellence not only in its laboratories and offices, but also in the hallways, at the cafeteria and on the tennis court. Yale, like other emblematic universities, creates a strong sensation of belonging, making people proud of working there and contributing to its future reputation. Some day, when our universities inspire this same feeling, we will finally be able to consider ours to be a mature society.

My mentor at Yale was Professor William Jorgensen, a pioneer in the use of computerized focuses to study complex chemical and biochemical systems. Before transferring to Yale, Bill had already developed the famous TIP3P water model (to date, the article in the *Journal of Chemical Physics*[1] in which he presented that model has been cited on more than 15,000 occasions), as well as the first version of his OPLS force field for proteins, which cleared the way towards the fascinating possibility of simulating protein dynamics. I began to work on two high-risk projects, one on the mechanism for recognition and catalysis of a

rotamase (FK506BP) and another based on the chemical unfolding of proteins. Both were groundbreaking, entailing enormous technical and theoretical problems and huge computations. In fact, one of my projects on the development of barnase, induced by urea, ended two years after I left Yale and required three full years of constant CPU-use. Bill was and still is an imposing person. He aroused respect, admiration and a bit of fear in everyone. Whenever you spoke with him, you got the feeling that he was examining you, and there were only two possible outcomes of such an examination: either he placed you in a large box where almost everyone ended up, or in a tiny box where there was only room for those whom he considered "brilliant". Bill is very intelligent, a solid, visionary and extremely meticulous scientist. What he was most afraid of then, and I think still now, was publishing any data based on a programming or calculation error, or due to a mistaken interpretation of results. Despite the fact that we only spoke in person once a year, I learned many things from Bill, such as the need to link calculations to experiments, the importance of detecting biological problems of interest before beginning simulations and the risks entailed by adapting problems to methodology instead of methodology to problems. The side effect of all this was my sickly obsession with the reliability of calculations.

Bill's team, which was huge, was well financed and brimming with postdoctoral researchers who were well educated. Some of them were "hawks" striving to get ahead academically. As a result, the scientific ambience was highly advantageous to a restless youth like me. It was a laboratory that offered unbelievable potential to anyone who wanted to learn from a wide range of people and in many different specializations. My main source of knowledge was Julián Tirado Rives, Bill's main collaborator, and still a great friend of mine. From Julián I learned a large part of what I know about molecular simulation, but the most important, after many coffees in his office, where he kindly picked apart my "brilliant" ideas, was that I learned to tone down my inborn arrogance, to listen attentively to others and to appreciate those colleagues who, though very intelligent, do not attempt to show it off constantly. Vanity is a constant pitfall for scientists, but with Julián I learned to control it, speak less and listen more.

When my Fulbright scholarship was reaching its end, doubts stalked me in New Haven. My wife had a permanent position at Yale's Chemistry Department as the manager of an organic chemistry teaching laboratory, and we were delighted to be in the United States. It was obvious that possibilities existed to gain a potentially permanent position in the US, whereas in Barcelona a young theoretical chemist who worked in biology could not expect too much. I still have not fully understood why we returned to Spain. It was probably a mixture of personal reasons (in Catalan, we say "*roda el món i torna al born*", in other words, "travel the world and return home"): there was Javier Luque's presence at the Pharmacology School in Barcelona, my phobia about changing plans and the rather important topic of my permanent position at the University of Barcelona, a factor that ensured stability. When I think how close I came to spending my scientific life in the United States, it seems impossible for me to understand the enthusiasm with which our science system powers American or European research teams, already wonderful on their own, giving them the gift of our best postdoctoral researchers, whom we then refuse to take back. Do we expect the best to wait for years until they finally get a short-term contract with salaries that

mean earning half what they would make abroad, with no money for projects, no stability and no future prospects, all within a terribly depressed scientific ecosystem?

First steps as a leader of my own team

The experience at Yale was excellent to get a vital view of science, to convince myself that I could compete with the discipline's elite, to establish contacts that I still have today and to earn the respect of the computational biophysicist community. The worst part was returning to Barcelona, where everything was frozen in time like a still photo taken two years earlier. Space and resources were limited, and in the department the fact that a new team was asking for its fair share of both inspired no great enthusiasm. After many arguments, I was given ten square metres of space in a dark room located at the end of a hall. Ten square metres do not sound like much, but a lot of enthusiasm fits inside them. Thanks to an excellent generation of students (from Carles, Cristóbal, Bego, Elena, Xavi to Robert, to whom I will always be grateful), the first scholarship I received from the European Union and my close cooperation with Javier, I began my own career, working in two different fields: the development of simplified models to represent the effects of solvent and the study of macromolecular recognition.

In the late 1980s and early 1990s, computational chemists understood that the solvent was not just a mere spectator, but rather played a key role in the modulation of chemical properties and reactivity. The transcendental work by Jorgensen, Karplus, McCammon, Levitt, Warshel, Kollman and others (Karplus, Levitt and Warshel – KLW – received the Nobel Prize for Chemistry in 2013) had demonstrated that a large part of the reactivity which we know about today cannot be explained without bearing in mind the effect of water and that, as a result, it was necessary to include solvent effects in the calculations of real chemical-biochemical systems. Unfortunately, this is no trivial matter because a solution is defined on the basis of a moving set of solvent molecules, the number of particles to be considered and their mobility, meaning that they cannot be represented by quantum mechanic (QM) methods, precise but too computationally expensive. At that time another possibility was considered, after being suggested by Jorgensen: setting aside the quantum nature of the solute and representing it all within the realm of molecular mechanics (MM). Another possibility, proposed initially by KLW and called the QM/MM method, represented the solute at the QM level and the solvent in the form of classical mobile particles. The QM/MM focus was extremely slow, and the combination of the quantum and classical parts of the system was not trivial. On the other hand, the use of plain MM calculations, while slightly more efficient, suffered from the limitations derived from the neglect of the quantum nature of solutes. Javier and I decided to test an alternative approach proposed earlier by Professor Jacopo Tomasi in Pisa. In the 1980s Jacopo, an outstanding quantum chemist, developed a method to explain the electrostatic response of solvents in the presence of a diluted solute based on the quantum version of the self-consistent reaction field (SCRF) for continuum solvents. Jacopo's ideas had been published in a modest quantum science journal and few people had paid much attention, but we believed that they could be taken advantage of to develop a new, fast and accurate dilution method. Our intention was to more precisely define the border between solute and solvent, incorporating non-electrostatic terms, which

were parameterized on the basis of atomic simulations and experimental data. These were exciting times in which Javier and I relied on Jacopo's support and the help of many other colleagues, among whom the most influential was perhaps Jiali Gao, a former student of Jorgensen's and a postdoctoral associated with Karplus (Jiali, also a good friend of mine, is now a full professor at the University of Minnesota). The model[2] (known as MST in honour of Miertus, Scrocco and Tomasi) provided free energies of solvation with chemical precision for the first time ever (this explains the more than 450 citations of our *Chemical Reviews* paper[2]). This made it possible to study in what way solvents modulate the chemistry of a process. If I had to choose just one contribution made by the use of MST, it would be the discovery that the pairing of bases by Watson-Crick in DNA is nothing more than a side effect of an aqueous solution, because water disturbs the natural tautomeric preferences of keto-enol and amine-imine in the nitrogenous bases.[3]

For over a decade we dedicated great effort to our model of the continuum solvent, developing versions able to work with systems of an enormous size, and others adapted to the environment of drug design. However, at a certain time it became clear that the model had already reached maturity, and it made no sense to devote Herculean efforts to achieve marginal improvements. It is not easy to give up a productive line of research, but neither Javier nor I wanted to tie down our entire scientific career to continuous solvation methods. We therefore left the field little by little to focus our energy on others. Nonetheless, in my heart there will always be a spot tucked away for the beauty of the equations used to represent solutions within the theory of SCRF. As a friend once told me, an article can live on in its readers' memories for one, five or ten years, but an equation lasts forever.

The SCRF's methods were wonderful for studying the properties of molecules in condensed homogeneous stages, but when I returned from Yale it was very clear to me that I not only wanted to study small solutes in condensed phases, but also real biological macromolecules in physiological environments. Unfortunately, in Barcelona the computer resources were so bad that for a few years the only chance I got to perform simulations of any importance was during brief visits to Yale; otherwise, the only thing I could do was carefully study the basic interactions that take place in protein and nucleic acid protein systems (hydrogen bond, stacking cation-π interactions and so on). If I had to choose one article that represents this period and its studies, it would be the one published in *Proceedings of the National Academy of Sciences*,[4] cited on more than two hundred occasions to date. In it we demonstrated the impact of polarization on cation-π interactions for the first time. I am especially proud of that article for many reasons: sixteen years later it is still considered "the reference" in its field. It was completed using quantum methods created on our own and contributed to the discovery of new interactions (anion-π interactions) and – here comes vanity once again – I wrote practically the entire article during a long flight from California. The technical knowledge that we were provided by those studies has been of incalculable value to my career. When I look back, I must acknowledge that we may have been quite lucky to work with such pitiful resources at the time. Poverty forced us to stay more imaginative, seek out unexplored scenarios and, because we had no computer "muscle", use our brains more.

Hope for change in Barcelona's research ecosystem

In the late 1990s, my team had increased in size and somewhat in visibility. I was related with the image of the "young wolf". We published in good journals, and I was often invited to speak at international congresses, while the laboratory began to receive funds on a steady basis. We bought some computers, and Josep Lluís Gelpí joined our group. He was an enzymologist with great programming knowledge, which expanded the range of projects on which we could work. We obtained some financial aid from Europe, which we used to increase our computing power, allowing us to carry out our first dynamic study on biomacromolecules. At that time the laboratory received the first researcher on a sabbatical year, George Shield, a former postdoctoral student of Steitz (another Nobel Prize winner) at Yale (where he solved the CAP-AND complex) and at that time a professor at Lake Forest College. George had thought he would spend his sabbatical year in Barcelona enjoying the city and shifting from crystallography to simulation. I had wanted to work with DNA since my pre-doctoral times in the United Kingdom, but to do that I needed powerful computer resources. This and the need to solve many methodological problems made it very difficult to take that step and, as a result, I had avoided DNA for many years. The presence of George, a very well-educated person, not in a rush to present a thesis and delighted to spend a year of work collecting results, was the excuse I needed to leap into the world of DNA. The system we chose to set off on our adventure with nucleic acids was triple DNA, a molecule subject to long-standing debate between X-ray crystallography and RMN regarding the nature of its structure (of type A according to X-ray experts, of type B for experts in RMN). The basic idea of our simulation was quite simple: carrying out two unbiased DM simulations of triple DNA in water, with the helix originally defined in one of its canonical compositions (A and B). In an ideal world, the MD simulations should drive the structure to the most stable form and the convergence therein of the two simulations. The idea was simple, but the potential for success practically none, because the force field was not very precise and our sampling capacity was quite limited (we could not perform more than a million integrations of Newton's motion equation per year; in other words, we could muster up about 1 ns/CPU per year). We were very lucky because the A→←B happened on the timescale of the subnanosecond, and George observed a magnificent, spontaneous transition from A→B, ending the debate on the structure of triple DNA in a solution (backing the models of the RMN). The results, published in the *Journal of the American Chemical Society*,[5,6] gave us great visibility in that field, soon led to a large number of citations and convinced me that the simulations with nucleic acids were high risk but potentially very productive. George returned to the United States, and since then the simulation of nucleic acids has been one of my team's main fields of research, and perhaps the one that has attracted the most international visibility for us.

In the late 1990s things were going reasonably well: the team was made up of about ten people, and we had several computers (including a set of robust SGI Origin servers, probably the best workstations in history). However, despite this positive improvement, we were still confined to the same dark room, with no hope whatsoever of any change. The heat produced by both people and computers, and our privileged location on the top floor of the chemistry building put us at constant temperature of 39 to 42°C all summer long.

When the first of us started to faint, we convinced the university to install air conditioning, but since there was not enough power to keep the computers and air running at the same time, the CPUs would regularly switch off. In the midst of this chaotic situation, I received a few offers from the United States, from both academic institutions and pharmaceutical companies, and I was plagued yet again by doubts. Should I keep trying to run a team under these conditions or give up and leave for the United States, taking advantage of the fact that my first son, Mode, was still little and, therefore, easy to move? In the end, I decided to stay in Barcelona, and one of the main parties responsible was Professor Màrius Rubiralta. At that time Màrius was the vice-rector of Research at the University of Barcelona, in addition to continuing research on topics involving organic chemistry (by then, we had already published a few articles together). He understood that the university system was not optimal for doing research and that it was truly bad to move from research to industry. He conceived new research centres known as "scientific parks", which would be organized under a more flexible and competitive paradigm than universities, improving cooperation with industry. Màrius was a leader who had a wonderful knack for selling his ideas, and thanks to the favourable economic times it became possible to lay the foundations for the Barcelona Science Park (PCB) and create the general core concept from which all elite Spanish institutions arose. From a personal perspective, the possibility of moving to a new research centre governed by the paradigms followed in the United Kingdom and United States was just the excuse I needed to continue my relationship with Barcelona.

The new century began with a great deal of hope and excitement. The birth of my second son, Sergi, brought a considerable increase in household chores for me. The ideas for the Science Park started to take shape, and we had experimental colleagues around us, leaving behind the apprehension towards theoretical work. This offered us the chance to back up our models and undertake more ambitious projects that would combine theory and experimentation. Encouraged by the collaboration with experimentalists, we returned to the world of simulation with proteins, which, due to the huge computer-related limitations, I had previously had to give up when I returned to Barcelona. The collaboration with medical chemistry teams ended with the development of very powerful drugs that reached clinical stages[7] and our cooperation with crystallographers was crucial to understanding the reaction mechanisms of various enzymes.[8] The joint work with Ramón Eritja, a synthetic chemist with the CSIC, drove our DNA research, because the possibility of verifying the results of our simulations encouraged us, for instance, to design modified nucleotides capable of stabilizing concrete forms of DNA or to examine how DNA and drugs interact. I am very proud of the creativity and the impact our work had at that time;[9–14] some of the articles on DNA published then are considered "classics" in the world of simulation with DNA.

A new ecosystem for research in Barcelona

The presence of Professor Andreu Mas-Colell in the Catalan government was crucial to creating new research centres and, in general, setting up a map for research in Catalonia. These new institutions began to take shape in the first decade of this century. It culminated in the construction of the first stage of the Barcelona Science Park and, almost at the same time as receiving my tenured professorship at the university, we moved

in to the new space. For me, the move did not mean a change in salary or more funds to do research, or new computer equipment; it meant setting up a brand-new laboratory measuring 150 square metres that had been specifically prepared for us. The most important part of the move was that I was surrounded by highly motivated researchers who came from different institutions, at a centre at which the organization of staff and the quality of the laboratories promised to come closer to those at cutting-edge international centres. In northern Europe, things are usually planned with great care, and the plans are stuck to until the end, which means that projects are normally carried out in a laminar flow regime. In other words, they are dull to journalists, but effective. In southern Europe, people are stupendous at making plans – we can easily make up to thirty a day – but they are not usually based on consensus and their half-life is just a few days. As a result, our projects are always carried out under a shadow of confusion. It was clear that the Barcelona Science Park was a southern project that, from the very beginning, had to survive in the turbulent regime – I must say with a very high Reynolds number. During the first few years of the new century, for quite a prolonged period, the Science Park's management was impossible to understand, and its long-term policies only remained in force until the following weekend. However, little by little, the PCB was turned into a receptacle for research centres with scientific and legal independence. This new scenario is the one in which, thanks to the work of Joan Guinovart and the support of Andreu Mas-Colell, the Institute for Research in Biomedicine (IRB Barcelona) officially came to life in 2005. In less than ten years, the IRB has achieved maturity, becoming one of the best biomedical research centres in Europe. To a great extent, its success can be attributed to Joan. From here I send my admiration, respect and gratitude for his efforts throughout these years.

As the genesis of the IRB was occurring, Mateo Valero and some other colleagues from the Polytechnic University of Catalonia (UPC) were trying to convince IBM that the European headquarters of its new supercomputer should be in Barcelona. Against all logic, they were successful and so the *Marenostrum*, the fourth-largest supercomputer in the world and the largest in Europe at the time, came to the city, being installed at a new and dashing institute: the Barcelona Supercomputing Centre (BSC). Barcelona very quickly became a reference in large-scale computing, mainly thanks to the work of a small group led by Mateo and by Francesc Subirada, a person from IBM who was crucial to the "boom" at this centre. I am proud to have contributed to the BSC's success from my discreet position as director of the Life Sciences Department, a department that, over time, turned into a joint BSC-IRB research programme on computational biology, to which the Centre for Genomic Regulation (CRG) has recently been added. I am very proud of the unbelievable development that has been seen at the BSC over the last ten years and the influence it is having on biological research in Europe. However, most of the centre's success must be attributed to Mateo and Francesc. It has been an honour and a privilege to work with them.

It might seem like I spent that whole period devoted to scientific policy, but, to be sincere, the effort was made by others, while I devoted a large part of my time to research, in which important changes took place. The most decisive probably had to do with the addition to the team of Xavier de la Cruz, a Ramón y Cajal researcher and later a research professor at the Catalan Institution for Research and Advanced Studies (ICREA). He had

come from Cambridge and had a solid background in bioinformatics. Xavier, a very calm, solid scientist, convinced me that I should use focuses based on quantitative data to deal with problems that could not be dealt with using our physical models. In short, Xavier brought bioinformatics to my team, which opened up new and unexplored horizons. The main result of Xavier's stay with the team was possibly PMut, one of the most commonly used methods for predicting pathological mutations. The server has thousands of access every year, and the two articles in which we explain the method behind it[15,16] have been cited nearly five hundred times to date.

A senior scientist

It is surprising, but one fine day you attend a meeting and you realize that people no longer see you as a young, aggressive, promising researcher, or as a former collaborator of Jorgensen's. They just see you as a senior scientist. That is what happened to me in the second half of the last decade, and frankly it was not pleasant. I still feel more at ease considering myself a young scientist, but indeed, I must acknowledge that by the end of the first decade of the 2000s, I was a senior scientist taking on clear responsibilities in research management. As I have mentioned, the most gratifying of these administrative tasks was that which led to the creation of the Life Sciences Department at the BSC and the Joint Research Programme on Computational Biology signed by the IRB and the BSC. Because the economy stoked the fires of science, for the first time we had a real chance to hire foreigners with talent. It was a dream-like period for the programme, in which we hired highly talented young scientists (Patrick Aloy and David Torrents, of EMBL; Juan Fernández-Recio and Xavier Salvatella, of Cambridge University; and Victor Guallar, of the University of Washington). In just a short time I was surrounded by smart, enthusiastic young people, which provided an unbelievable opportunity to learn from them, as well as making it possible to create a small research platform that has swiftly achieved international visibility.

From a purely scientific perspective, gaining access to the BSC's facilities and the Botín Foundation's support allowed us to play in the first division of molecular simulations. For example, we were able to create the first dynamic map of protein flexibility, allowing us to think about the possibility of systematically exploring the correlations between functional movements and the intrinsic flexibility of proteins. With this same objective in mind, and the encouragement of adding several physicists to the team, we began to develop various coarse-grain models. After careful parameterization, they proved to be quite capable of detecting protein flexibility at a low computing cost (these methods were recently reviewed in *Chemical Society Reviews*[17]). In the field of DNA, our most visible product throughout that period was the development of a new force field for DM simulations in DNA. After more than three years of great effort, Alberto Pérez, a highly talented student on the team, parameterized the parmbsco force field, considered the "gold standard" in simulation with DNA.[18] In less than seven years, the two articles with which the force field was presented and validated[19] have been cited on more than nine hundred occasions. Access to parmbsco opened the door, for example, to studying never-explored processes, such as the folding and unfolding of nucleic acids,[20,21] facilitating the development of

coarse-grain flexibility models for DNA. Those models were so efficient from a computational perspective that we were able to use them to analyse the physical properties of DNA at the genome level. We combined our knowledge of those properties with the experience acquired in the field of bioinformatics to create ProStar, a program developed by Ramón Goñi, another excellent graduate student. Using ProStar, Ramón predicted the presence of human promoters six years before they were demonstrated experimentally.[22,23] These results aimed at the existence of a correlation between physical and regulating properties of DNA. Many of our current projects with DNA and chromatin arose from this discovery.

Recent years

Physics is not amenable to lacks of continuity because they break the rules and make predictions unreliable. Moreover, physics does not tolerate changes in direction in temporal or entropy arrows. Unfortunately, neither politics nor economics are bothered much by going against the principles of physics. They enjoy a lack of continuity and have no problem going twenty or thirty years back in time. Personally, I cannot complain. Both the BSC and the IRB are secure environments that have managed to overcome the economic crisis with good financing from the European Union. However, it is difficult not to identify with the good scientific teams that have vanished throughout this period, and impossible not to notice the terrible effects these dark years have had on the research ecosystem in Spain.

As it became harder to find resources to do research, my administrative tasks increased. The goal ceased to be expansion by hiring new group managers that would create a research environment equivalent to that of the finest centres in Europe and became limited to pure and simple survival. All our efforts revolved around keeping our own group leaders from leaving for other centres in Europe and the United States. Attacking is much more creative than remaining on the defence, and these years trying to sail against the current have been exhausting. The positive part is that, when writing this text, I can proudly say that our micro-environment is weathering the crisis, the reigning pessimism and the risk of structural collapse quite well. Because four of our group leaders receive grants from the European Research Council (ERC), and because the Joint Research Programme was recently expanded to include the CRG (the other large focal point of research on computational biology in Barcelona), I am convinced that, when the crisis is over, we will be ready to grow again.

In our own research, the most exciting change happening in these recent years has been that related with the start-up of an experimental laboratory. In late 2014 there was little doubt that theory had already sunk into the mainstream of biological research. Clearly, the border between theory and experimentation was blurry, and there were no longer major differences between experimentalists and theorists. As a result, the paradigm that divided laboratories into "dry" and "wet" no longer exists, and laboratories entered a "misty" environment. I would love to be able to say that I was a visionary when, years ago, I convinced Joan Guinovart that a laboratory had to be set up as part of the Joint Research Programme (of incalculable value in achieving that goal and many others was the support of Francesc Subirada). Sadly, I must acknowledge that I merely adopted other people's ideas, above all

those of Jorgensen, who organized an experimental lab ten years ago while at the peak of the theoretical world, and Patrick, who reached the IRB with a clear idea about the need to validate theoretical models experimentally. The EBL (Experimental Bioinformatics Laboratory), which has been operating for more than five years, is now a consolidated platform and a model that is being copied by other institutes and similar programmes.

The influence of experimentation on our latest contributions cannot be doubted, not only because our team gathered evidence to support our theoretical studies, thereby increasing our confidence in theoretical models, but also because many experimentalists suddenly trusted our simulations and wanted us to take part in their projects, or they were so kind as to collaborate in ours. Before they were even published, we had the pleasure of seeing our calculations directly backed by experimental measures. For example, our prediction of the dielectric constant of DNA was validated at the same time by an AFM study;[24] the dynamics of DNA in anhydrous environments[25,26] was verified by other colleagues who worked with ESI-MS, whereas the structure and behaviour of unusual DNA deratives,[27,28] or those of common DNA in unusual solvents,[29] were confirmed by NMR data. The close collaboration with teams that use X-ray crystallography allowed us to analyse DNA's enzymatic susceptibility to a level of precision that was impossible when using simple theoretical calculations.[30] The inclusion of experimental data was also crucial to better understanding the great structural transitions undergone by proteins and how the transfer of information is disturbed in them (for example, the NMR data presented in *PNAS*,[31] or the X-ray data in *Nature Commun*[32] or in *PNAS*[33]).

This exercise of looking back has been very interesting. I do not trust fate, and I am still convinced that, if I fell asleep as the universe forgot about me, I could wake up in a completely different life and use my abilities in whatever new environment I found, perhaps one orthogonal to science. I am a scientist because my skills fit in well with those required to do such work, because I like the feeling of knowing something that nobody else knows (a reformulation of vanity) and because I am curious by nature, but also due to stochastic conditions, situations and people who combined in my life, driving me towards this point, at which I do something that I truly like.

Because it appears that the retirement age is being delayed in Spain, I will probably continue at the front lines for a few aeons more. How do I see the future? Well, Niels Bohr is attributed the following phrase, though a friend told me that it is actually just an old Danish proverb: "It is very difficult to make predictions, especially about the future". Regardless of the fact that the author of this reflection received the Nobel Prize, I completely agree with it. Researching is like telling a story; in the beginning the characters in the book do what you had planned, but after a while they start to take on lives of their own and reach their own decisions in the direction set by the story. It is useful to design research plans for the following three, five or ten years, but every scientist knows that those plans are not adhered to. Nonetheless, despite all the uncertainty involved in predicting future interests, I know I will devote the rest of my scientific career to taking magical thought out of biology and trying to improve the description of living beings on the basis of simple physical and chemical rules.

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ÁNGEL CARRACEDO

NETTING GENES HERE, THERE AND EVERYWHERE

16

I never found out where it had come from, but in the library in my house, which at the time was without doubt the biggest private library in the small town of Santa Comba, there was a copy of *Biología*, by Salustio Alvarado. I was fascinated by the part of the book that dealt with genetics. I did not understand all of it, but I did understand that it held the key to why a pine cone produced a pine tree, whereas a pea produced another type of plant entirely. I was already obsessed with the topic at that point. I was twelve years old, and during those endless Xallas winters, reading books and competing with my brothers at memorizing long passages, or at "It Pays to Enrich Your Word Power!" (a feature in *Reader's Digest*, which my parents subscribed to) were part of our daily life. The exams themselves were not at all easy. Some of the questions were about topics that we had never studied either at home or at the academy, and the results that my brothers and I got were not always good. However, I remember that in addition to my parents' taking extra special care of us during the exam period, my mother always told me that it was not the results that mattered, but the fact that I had truly made an effort, and it was clear to her that I had.

Late in the course of my schooling, my parents decided that their children should switch to the Manuel Peleteiro School in Santiago de Compostela. While moving to the city was not easy for a kid from Galicia's rural environs, adapting to the school itself certainly was. Within a year or two I was at the top of my class and, more importantly, I had made some very good friends. As my son Guille, an excellent student himself, once said to me. "The hard part isn't getting good grades, it's keeping good grades from ruining your reputation". To me, this is clearly indicative of the negative socio-educational dynamics that often reign in schools.

I do not know why I chose to study medicine. I guess I did not have to think much about it, for several reasons: the field of genetics interested me a great deal; a biology professor at my high school, who also happened to be a doctor, was one of my favourite teachers; and my maternal grandfather was also a doctor. Of course, I did not hear a single word about genetics at any point during my studies; incredibly, even now there is not a single core course in genetics or genomic medicine for those who major in medicine in our Department of Medicine.

Our graduating class was part of the first IMR (Internal Medicine Residency), but to my surprise there was no specialty in genetics, in spite of the fact that such a specialty existed everywhere else. We would have to wait a good long while to get one; even now, such a specialty has only just been approved, and will not actually be an option for students for another two years. After passing my exams, I did not start looking for a job straight away. In fact, I did not know what to do. I thought of becoming a professional fisherman – I was good at it then, and still am – or becoming a lighthouse keeper. This latter idea was origin-

ally that of a close friend from back then, and was eventually realized by my brothers, who are currently lighthouse keepers on the islands of Ons and Sálvora. I went to speak with Luis Concheiro, a professor of Forensic Medicine. He was the professor who had infected me with his enthusiasm for his work. He was also one of the top forensic pathologists in Europe, and certainly the best in our country. During our talk he explained to me that for him, forensic medicine did not exist as a separate specialty; that he was in fact a pathologist; that he had just hired a specialist in chemistry, and was looking for someone to develop the genetics subfield, which at the time was known as forensic biology. He eventually convinced me to join him there in the Department of Forensic Medicine; I applied for an FPI (Personal Research Training) grant, and began working in the department as soon as it was granted. This was in October of 1978.

Thus began my medico-legal period. Though I was not particularly interested in them, I attended the autopsies that Professor Concheiro so masterfully performed; I learned histopathology from him, and above all I enjoyed the company of those who came to visit him, especially a professor of Penal Law named Agustín Fernández-Albor, and a psychiatry professor named Antonio López. It seemed to me that I needed to begin studying law, so I did, and was particularly interested in my Roman Law course. As it happens I dropped the course for reasons that I will soon explain, and also because I was convinced that I was only truly interested in penal law, which I could study on my own, reviewing my doubts and questions with people who knew a great deal about the field, people to whom I already had access, including Fernández-Albor himself.

However, my main dream was to develop the field of forensic genetics, and that is what I set out to do. Forensic genetics proposes to use the study of human variation to help solve legal problems, including family relation conflicts (such as proof of parentage); it also entails the analysis of biological samples of interest in criminal cases, including blood, sperm, saliva or hair. Back then, these samples were dealt with in terms of blood groups, an approach which, while it could be helpful with simple paternity cases, was generally of very limited use in criminal cases, as it required the obtention of a very large, very fresh genetic sample – two conditions that rarely occur in most forensic cases.

At that point I had never seen laboratory work first hand before; my early development was helped along greatly by a clinical analyst named Teresa Mora, and a haematologist named Juan de la Cámara. In addition to learning about the HLA (human leukocyte antigen) system that had recently been discovered, I read all the books and magazines at my disposal – pretty much everything was at my disposal because Professor Concheiro had, and still has, an obsession with being up to date in the literature – and in them I learned that everyone had begun using polymorphisms in erythrocyte enzymes and serum proteins that had been separated via electrophoresis, a biochemical separation technique that I was familiar with only in theory. I believe that I first met José Luis Blázquez when I went to visit the Biology Department to ask how the electrophoresis process worked. It turned out that he and I were there for exactly the same reasons. He was about to begin his thesis over in the Anthropology Department, and the aforementioned type of polymorphism presented the possibility of a new approach for analysing the dynamics of human populations.

We decided to join forces and avoid duplicating our efforts. One of us would do a thesis on serum proteins, and the other – that would be me – would work on erythrocyte and leukocyte enzymes. We would use a common thesis design, and share our respective sampling data with one another. It was an extremely fun time in our lives, as we travelled together all over Galicia gathering blood samples in schools, at residents associations, even in bars, not to mention in university departments where the students – med students especially, among whom were the two women who eventually became my wife and my sister-in-law, respectively – were willing to donate in massive quantities. And that is where another story began, one that I am not going to tell here, but it is the story of the happiest part of my life.

Blázquez and I made our own electrophoresis units, and distilled our own water with a burner and a still; that and a power source built by an electrician friend constituted all the infrastructure we had at our disposal. I was dividing my time in all kinds of ways. I taught classes in which I was also just another student, and I attended autopsies; I studied law and read a great deal. That said, I also had time to indulge my hobbies.

However, electrophoretic techniques soon underwent a revolution thanks to the appearance of isoelectric focusing, which allowed us to separate the protein isoforms much more precisely using isoelectric points, and made it possible to substantially increase the number of polymorphic markers. As mentioned, I did manage to put a unit together on my own, but then, while reading *Electrophoresis* magazine and reflecting on the fact that I needed a stronger theoretical foundation in physical chemistry and genetics, I saw an advertisement for a Master's degree in Biochemical and Genetic Separation Techniques at the BMC (Biomedical Centre) of Uppsala University. In addition to the three openings they had for Scandinavian students, there were also three openings for students from the rest of the world, and I was lucky enough to be awarded one of them. It was a dream come true: this is where Tiselius invented electrophoresis, where Svedberg discovered differential centrifugation, where Svensson invented isoelectric focusing. But the dream did not come cheap. The tuition and residence together cost more than what my FPI grant could cover.

That was when it occurred to me to write to Joaquín Arias, who was at that time the director of both Banco Pastor and the Barrié Foundation. My father was then – and remained until his retirement – an employee of Banco Pastor's branch in Santa Comba. In fact he had spent his entire professional life there and, as the children of bank employees, we had received financial aid from the Barrié Foundation to help with our studies. I wrote a letter to Mr Arias explaining my situation and my dream, and he answered me immediately, saying that he had opened up a bank account in my name with funds sufficient to ensure that I would be able to fulfil my dream without difficulty. My impression is that when he realized there were likely to be others in my same situation, he decided to utilize the Barrié Foundation to launch the grant programme that has done so much good for research in Galicia. In fact the Barrié Foundation – on whose Board of Trustees I now serve – has always felt like home to me.

Two foundations tied to banks have been of great importance to my life as a scientist. As mentioned, the first, the Barrié Foundation, has given me personal and financial sup-

port. The second, the Botín Foundation, was fundamental in changing my understanding of the importance of transferring research results into active practice, and for helping me to see the importance of intellectual property rights.

As soon as I finished the research aspect of my thesis, I left for Uppsala. Everything was new and exciting. The difference between the humble laboratory in our Forensic Medicine department and that of Uppsala's BMC was like the difference between night and day. I learned several new biochemical separation techniques, and a great deal of physical chemistry theory. I was very impressed by Tiselius's first electrophoresis system, and by how well the rights relevant to the historical discoveries that had been made there at the centre were protected. Not long before my arrival, Sanger had invented his new sequencing technique, and I was able to perform my first DNA sequence. I was especially thrilled to help develop the theory behind capillary electrophoresis. In fact, I remember every experiment of which I was a part, both inside and outside the centre. I spent my free time analysing the data from my thesis research, and sending handwritten pages to Professor Concheiro, who dictated them to his secretary Adelaida, who then typed them up. My brother Jesús produced the necessary imagery, and at long last my thesis was ready. That was in June 1982.

That same year I published four articles from my thesis in the finest forensic medicine journals to be found.[1–4] Unlike other disciplines, forensic medicine in Spain lacked the tradition of publishing in international journals, so these were the first publications born of Spanish forensic medicine to be published in English. We began publishing on a regular basis, and a couple of innovative techniques – Cajal's silver staining method for dying proteins after separating them out via isoelectric focusing,[5] and a similar technique for analysing keratins (hairs being notoriously difficult to identify)[6] – provided us with something of an international reputation. As a result, for the first time ever foreign forensic experts sought out our lab from abroad; the cases involved illegal animal trafficking in the United States.

We were just beginning to become quite well known internationally, and thus to compete well on the world stage, when our field underwent a sudden change. Alec Jeffreys and his team discovered the key to the process of genetic fingerprinting using hypervariable polymorphisms in DNA.[7] This would lead to a genuine revolution, a radical shift in both paradigm and methodology. Fortunately, our Physiology Department faculty included Fernando Domínguez, who had just returned home from the United States. He knew how to work with DNA, and how to use the relevant probes. It was not difficult to implement the new concepts and adapt ourselves to them. The first case utilizing DNA testing done in Spain was brought before the Provincial Court of A Coruña in 1989. It allowed a person who had been wrongly accused of rape to go free. This was shortly after the world's first such cases had been handled by Peter Gill, a scientist with whom I have always enjoyed a warm friendship.

Kary Mullis's discovery of the PCR (Polymerase Chain Reaction),[8] and, shortly afterwards, the discovery of microsatellites[9], created yet another revolution in our field. These were the years when the group's research really began to take off, with the discovery and forensic validation of many of the microsatellites that are now being used in forensic laborato-

ries all over the world. The job of refining these new methodologies went on non-stop, and we acquired the first automated sequencer in all Spain – I do not know how I dared to buy it when we did not actually have the money for it, but I did. It took me five or six years to pay off, but it put us in the vanguard in terms of the ability to analyse mitochondrial DNA, which is absolutely essential for degraded samples and hair. The first two cases in which the results of mtDNA analysis were taken before a court of law were, firstly, that of P.W. Ware, in the United States in June 1996, a case handled by the FBI and involving a rape and subsequent murder in the state of Tennessee, and, secondly and simultaneously, a criminal case that was solved by our laboratory, known as Brief 2/95, Court of First Instance and Inquiry of Puente Genil, in Cordoba, Santiago Institute of Legal Medicine report 23/1996.

Mitochondrial DNA also caused a revolution in the field of genetic studies of populations, allowing scientists to use phylogeography to better understand the movements of human populations. Forensic genetics is in a certain sense an applied branch of the genetics of human populations, and research in this field is a natural ally to our own, as we recognized by incorporating Antonio Salas, soon to be a towering figure in the field, into our research group.

On a parallel track, these were also years of major developments in the field's organizational structures. My part in this was helping to create within the International Society for Forensic Genetics (ISFG) a working group in Spanish and Portuguese known as GHEP-ISFG. Shortly afterwards I became the director in charge of all the ISFG working groups, and, in the end, I was named president of the society itself. I focused on promoting standardization (through the creation of the DNA Commission) and quality control (the control standards of the GHEP are exemplary), and worked decisively to encourage the society's internationalization. Today, very nearly all experts in forensic genetics worldwide are members of the society.

I later led the Institute of Forensic Medicine, which had only recently been created, but was nonetheless already undergoing an institutional crisis. Its scientific output justified its ranking as the finest such institute in the world, a position it held for a decade, from 2001–11. The group has already begun to solidify, and was fortunate to include as a member María Victoria Lareu, one of my first disciples and currently a full professor in the Forensic Medicine department.

We soon began to receive important international cases, ones with strong repercussions in the media, including the triple murder case of the Alcàsser Girls, and the Olot pharmaceutical case, both from here in Spain, as well as the Baneheia case from Norway. The introduction of new techniques developed by our group – including SNPs (single nucleotide polymorphisms) and INDELs (Insertion/Deletions) for degraded material,[10] SNPs in areas protected by nucleosomes,[11,12] and, with the same goal, the AIMs (ancestry-informative markers) that can predict the geographic origin of samples[13] or use new markers to predict physical characteristics – all contributed to raising demand for our services. Courts and police forces from many countries sought our help. We eventually handled cases of profound public impact, including the 11-M (March 2004) train bombings in Madrid (the most significant terrorist attack in the history of our country); the identification of victims of the 2004

Indian Ocean tsunami; the parentage case of Emmanuel (son of Clara Rojas, a member of the Colombian congress who was kidnapped by FARC); the Minstead operation in the United Kingdom (the most dramatic serial rape case in history); and the Bretón case in Cordoba, among many others.

In 2012 I left the reins of the Institute in the hands of María Victoria Lareu, who has carried on with great enthusiasm the adventure of maintaining the Institute's position as the world leader in forensic research. I began to limit my hours in the field, and at the moment, in addition to helping with research, I hold several posts of international responsibility, including that of co-director (alongside my friend Peter Schneider, professor of Forensic Medicine of the University of Cologne) of a network dedicated to European excellence in the field known as Euroforgen (the European Virtual Centre of Forensic Genetic Research). I am also a member of DNA commissions, and hold the directorship of the magazine *Forensic Science International: Genetics*, which I founded, and which is currently the most influential magazine in the world in the field of forensics.

Several years ago I began another adventure, one that takes up most of my energy at present. The tipping point was a series of offers to lead forensic medicine institutes here in Europe, offers that I did not accept. In some cases they carried a heightened media profile, which involves a kind of pressure that I find unbearable, and which is incompatible with my dream of promoting a wider understanding of genetics.

Since the beginning, I have been most passionate of all about clinical genetics – in particular for using classic genetic polymorphisms to search for the genetic components of complex diseases – and that has not changed. However, since the moment we were first able to sequence DNA, we have been getting more and more requests for assistance from hospitals. With recent genomic advances, and the by-products of the Human Genome Project, the number of identifiable genetic diseases has been growing rapidly. It was no longer merely a question of chromosomopathies and cytogenetics, but, more and more, of molecular analysis.

Every hospital in Galicia wanted to make use of the latest genomic advances, and they all called us to help them do so. It was important to make sure that all such development in the field occurred in an organized way, and it was my idea to create a centralized structure from which to provide the relevant services to everyone who needed them. Such a field as ours – in which vertiginous changes, progressively more complex and expensive infrastructures, and great diversity in terms of interdisciplinary knowledge – could not be allowed to scatter itself across a landscape of separate hospitals and service providers, as was happening everywhere else in the country.

Fernando Domínguez has been helping me to develop this idea since the beginning, and we also have the support of one of the most intelligent people I have ever known, Professor Manolo Salorio. Though by profession he was an ophthalmologist, he gave our idea a home at the public foundation that he directed, the Galician Institute of Ophthalmology, which later changed its name to the Galician Institute of Ophthalmology and Molecular Medicine, only to return to its exclusively ophthalmological origins a few years later when the Galician Public

Foundation of Genomic Medicine was founded. Clinical genetics had leapt forward into the world of genomic medicine; now it was a matter of being able to analyse the genetic components of complex diseases, not just the elements that affected the germinal line directly.

The development of this new centralized structure was now unstoppable. We were joined by several experts in the areas of forensic medicine and physiology. Francisco Barros (who was one of my first graduate assistants, and my right-hand man as regards clinical genetics), Lourdes Loidi, Ana Vega, Clara Ruiz-Ponte and Celsa Quinteiro formed the original nucleus out of which developed a group of more than fifty professionals undertaking an enormous amount of work in the genomics clinic. Subsequently we also welcomed Luz Míguez and Manuela Ariza to work in the field of cytogenetics, and Teresa González as a specialist in oncohematology.

It has always been my opinion that it is impossible to have clinical excellence without excellence in research alongside. In conjunction with our clinical work, we began to do research in the field of cancer, particularly genetic research regarding two rapidly growing healthcare fields, breast cancer and colorectal cancer. We also did research on rare diseases, but little by little we began to specialize in finding the genetic components of complex diseases. One decisive landmark in this process was the creation of CEGEN (the National Genotyping Centre). For some time I had been working together with my friend Xavier Estivill on the genetics of psychiatric diseases, and with Javier Benítez on cancer genetics; together we entered and won a competition for the right to create CEGEN, and we then put our plan into action. I am currently the coordinator for the centre, which has two nodes: one within the CNIO (the Spanish National Cancer Research Centre), and another that is part of our group (with two platforms coordinated by María Torres and Inés Quintela, respectively), where we provide support and analytical services to groups and consortia who require SNP analysis, normally on a fairly large scale. In particular, the type of SNP analysis known as a genome-wide association study (GWAS) has revolutionized our understanding of the genetic component of common diseases.

It soon became clear that it was necessary to form large clinical consortia with immense sample banks in order to get a handle on the genetic component of all these diseases, thereby opening new routes towards understanding them. Our analytical and genotyping capabilities were complemented by the addition of a large number of excellent researchers to our group. In a very short time, in addition to the cancer genetics groups directed by Ana Vega and Clara Ruiz, we also had a neurogenetics group run by María Jesús Sobrido; a group dedicated to the genetics of psychiatric diseases, helmed by Xabi Costas; a cardiovascular disease group run by María Brión; a group addressing the genetics of parasites, directed by Xulio Maside; and a group studying the genetic epidemiology of cancer, run by Manuela Gago. Meanwhile, Francisco Barros was in charge of coordinating all the research activity in the area of pharmacogenomics, which is extremely important to us given its direct application to our clinical activities.

The creation of a cutting-edge sequencing service coordinated by Beatriz Sobrino, a bioinformatics service headed by Jorge Amigo, and a statistics service helmed by Raquel Cruz, has given a great deal of added value and support to both our research efforts and our health care. On the research side of the equation, we have begun to publish articles of much higher

impact than ever before. Among the most noteworthy articles have been those detailing the discovery of new genetic components of SIDS,[14] the discovery (made within an international consortium) of new genes implicated in cases of schizophrenia[15] and autism,[16] and advances made in the genetics of colorectal cancer.[17] These discoveries and advances were made possible by another consortium, the EPICOLON network, where Toni Castells is producing exemplary work, above all in the area of pharmacogenetics, applied to colorectal cancer (where it is allowing us to provide more personalized treatment for each case),[18] as well as to other diseases for which we are likewise discovering new biomarkers as the key to treatments[19] that will doubtless have an immense impact on clinical practice.

I have lately been paying particular attention to the field of pharmacogenetics, which, it seems to me, is an area in which we must be particularly careful as regards how we translate theory into practice. This implies a great deal of work still to be done, not just in terms of research, but also regarding the field's educational and training elements, not to mention its regulatory and organizational aspects. And while I am on the topic, I should note that it was while serving as the Spanish delegate to the EMA (European Medicines Agency) that I learned what a regulatory organization truly does.

I have been an active supporter and promoter – the help of my friend Montse Baiget has been essential for both of these roles – of SEFF (the Spanish Society of Pharmacogenetics and Pharmacogenomics). I am currently working, once again with the support of the Botín Foundation, on a related structural project involving personalized medicine in the hospital environment. It was the Botín Foundation that opened my eyes to the need for the proper transfer of research results into practice and, as a result, in addition to several other spin-off projects, we have been at work on the Innopharma platform, a joint venture between our group and the Biopharma group directed admirably by Mabel Loza. The goal of this venture is to hasten the discovery of medicines that can serve as genomic tools.

At present I am especially interested in the genetics of psychiatric diseases. Genetic components are of extreme importance in this field – much more so even than for any kind of cancer – and yet we know so little about them. Finding the genes related to specific illnesses is like going fishing, another of my favourite hobbies. In the case of cancer, there are a few fish still left to catch, but the ones that remain are extremely elusive. In the case of psychiatric illnesses, on the other hand, there are countless fish, but we do not yet know how to catch them, or even which fish are the exact ones we are looking for: it is supremely difficult even to properly define a psychiatric illness. For any fisherman worth his salt, it is an extremely interesting challenge.

That said, psychological and psychiatric illnesses have an even bigger problem: the social stigma that they carry. I am currently dedicating particular attention to ensuring that the children and adolescents who suffer from these diseases receive better medical attention than they have in the past. I want to dedicate my highest efforts towards research that will help us understand the genetic foundations of such diseases, including obsessive compulsive disorder and autism.

Over the years, I have realized that dissemination is as significant as investigation. Doing science is important, but it is just as important, or even more so, to share what we know, and to talk about the many, many mysteries that we have not yet been able to unravel. This is essential not only so that people value the role that science plays in our lives, but also because it is the only way in which others can come to understand the world that surrounds us, and to think critically about what they are told: the only way to become more free. With that as my goal, I try to dedicate my highest efforts to spreading the word about our research. Every other week, I either visit a school or institute to talk about what we do, or I welcome visitors to our centre for the same purpose. I have to admit, I love talking to these kids – maybe I should have been a teacher all along.

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FRANCESC POSAS

A LOOK BACK AT THE PAST WITH A VIEW TO THE FUTURE

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It is no easy task to reflect on those factors that have left a decisive mark on your scientific career and the events that have drawn you towards research. The framework of the Botín Foundation memoir provides us with an excellent excuse to write an overview of those past experiences and how they may have impacted the development of our research groups, as well as our way of viewing science and the knowledge transfer that it produces. It is in this commitment to the future and in transferring the results of research to society that the Botín Foundation has played and is still playing a key role, which I will describe further below, because in the beginning I too had a very particular view of what it meant to become a scientist. Now I know that our responsibility as scientists goes beyond just creating knowledge.

In the beginning, it was all fireworks

I could not say precisely what within my family environment marked my youth and education as a scientist, but I feel it is clear that it did have a great impact. I was born in a town not far from Barcelona, where my parents ran a family business that included a livestock farm. From a very young age, I worked with them on the weekends and during every "summer holiday". Not that it made me particularly happy; I just assumed that was how it had to be. Dedication to work and the effort required to keep the family business going marked my ability to handle both my studies and research. I have always felt with great clarity that if someone wants to achieve something they have to be willing to fight for it, work hard and strive until they succeed. I believe that this idea has influenced the rest of my career as a scientist. I must also say that my parents have always been very generous with me and have supported me in everything I have set my mind on doing, even if they did not understand it. This included my decision to leave the family business so that I could devote myself to studies that they had no certainty would be of any use to me.

When I was a child, I had the idea of becoming either a veterinarian or of working in economics so that I could help improve my parents' business and their farm. However, what I failed to bear in mind in these plans was what was going to happen later on, when a professor opened my eyes to the fascinating world of biology. In any case, that occurred somewhat later. During my primary school years, I was lucky that my town's school had an enthusiastic group of teachers, and from that moment on studying and understanding things began to interest me and take hold.

When I was eleven or twelve years old, a series of events took place that perfectly illustrate some of the early contours of my investigative mind, as well as my devotion to learning and researching the topics that fascinate me. Like any boy, I was fascinated by rockets and

firecrackers, so I decided that I had to build rockets capable of reaching great heights. This led me to start researching what gunpowder was and how to make it with a classmate. The next step consisted in experimenting, which is when we began to produce our own gunpowder, at first using low-cost materials and later with more pure ingredients purchased at the pharmacy, not without a bit of suspicion on the pharmacist's part. Already back then we would use a notebook to write down the speed at which each mixture would be consumed, how fast it caught fire, the intensity of the flame and, of course, all the improvements and additions we would add to the rockets when launching them. Everything moved forward brilliantly and the rockets reached increasing heights, which forced us to change their outer casings and the pressure at which we would compress their fuel, until one day, when one of them blew up while we were packaging it and I almost lost my hand. Luckily, it turned out that all I had were a few burns, as well as having to have a few stitches and spending a couple of weeks with diminished hearing. This was a lesser evil, if you bear in mind that we were handling the rockets next to a bunch of butane tanks. For me, the bad part was that I was forbidden to use any flammable materials after that and, even worse, the notebook containing the results of all our "experiments" vanished into thin air. This is how my potential career as a rocket engineer went up in smoke as well. In any case, this experience helped me learn to research the things that interested me and to be organized about the way I studied them. From then on I have always placed great value on laboratory notebooks.

An interest in biology

As has surely happened to many people, I had teachers who helped mark my path. The possible university studies rolling around in my head clearly changed during my high-school studies. In those years the first genetically manipulated mouse and plant were created, the first vaccine that used genetic engineering, the polymerase chain reaction technique (PCR) and the proposal had been made to sequence the human genome. The professor of Natural Sciences (biology and other subjects) that I had for two years was able to transmit not only an interest in knowledge, but also his fascination for biology, introducing contents that were cutting-edge. He would comment on news that had just come out, information that had not yet appeared in books. Thanks to that enthusiasm and his passion for knowledge, it became very clear to me from that moment what I wanted to do: investigate how cells function.

That is how I ended up at the School of Biology in the Autonomous University of Barcelona (UAB). I chose UAB and not the University of Barcelona (UB) for one practical reason. At the end of the high school I sat in on a few classes at UB as an observer, and when I saw that in first-year biology there was an infinite number of students, I decided that I would rather go to a university with a much smaller student body and a more open campus. At that time I did not realize just how right my decision was, but for reasons that I could not even have imagined then.

In the first year the courses were very basic and perhaps somewhat unappealing. Even so, along with a few other students, I applied to be a volunteer at a company that designed large-scale bioreactors for waste treatment. There I began to carry out my earliest chem-

ical and bacteriological tests. What fascinated me most was the biological complexity of the bioreactors and how difficult it was to keep them active. The relationships between the different bacteria populations and their evolution is a topic that has always enthused me. During my second year, due to my prior experience, I applied to work in the microbiology laboratory at the recently founded UAB Veterinary School. I still do not understand very well how its director, Dr M. A. Calvo, dared place her trust in a second-year student, but the truth is that the whole group gave me a fantastic welcome. There I learned how to make bacteria and fungi grow, to classify them and to evaluate their sensitivity to antibiotics. This opened my eyes up to research in a laboratory and to the field of microbiology as such.

However, my greatest opportunity arose while finishing my second year, when the Department of Biochemistry at the School of Veterinary Medicine was looking for students to collaborate in a laboratory. At that time the Biochemistry Unit was enjoying great momentum and standards beyond the norm, with researchers who had recently arrived from the United States. It was led by Dr Joan J. Guinovart, who is currently the director of the Institute for Research in Biomedicine in Barcelona (IRB). It was then that Dr Joaquín Ariño returned from his postdoctoral stay in the United States, where he identified new protein phosphatases using molecular biology techniques, something absolutely innovative at that time. That is when Joaquín came to implement state-of-the-art molecular biology techniques in a department that was studying cell metabolism. I learned to sequence DNA with him, as well as the basic techniques of molecular biology, and we even got as far as obtaining the first PCRs and oligonucleotide synthesis machines. When I completed that academic year, I already had a clear idea of what I wanted to study: I would devote my career to biochemistry and molecular biology. Throughout those years, I combined my studies with work at the laboratory. I would spend every summer in the lab as well. People treated me wonderfully. Some of my best colleagues are from those times, and it was not only useful to help me learn and begin my training as a researcher, but also to get extra motivation to attend classes in the undergraduate degree programme, since they were not always as stimulating as they should have been. When I finished that year of my degree, I had already decided that I was going to continue in the world of research, so I put my full efforts behind that goal.

Phosphatases and cell metabolism

During the 1960s the mechanisms of phospho-dephosphorylation of proteins were discovered and defined, and shortly afterwards these mechanisms were identified as key factors in controlling enzyme activities. Protein phosphorylation consists of introducing a phosphate group onto a specific amino acid, normally serine, threonine or tyrosine, thereby changing its structure and chemical charge. In 1992 Dr Edmond H. Fischer and Dr Edwin G. Krebs received the Nobel Prize in Physiology or Medicine for their description of the mechanisms of phosphorylation and dephosphorylation linked with the regulation of enzyme activities. Whereas the study of many kinases, the enzymes responsible for protein phosphorylation, were very advanced and were being constantly identified in the 1980s and early 1990s, the role of protein phosphatases, the enzymes that

reverse the action of kinases, and their importance in controlling cell metabolism, was just emerging.[1] The protein phosphatases were the poor siblings in the whole business. However, that was what we were betting on: we proposed to identify new phosphatases using molecular biology techniques and to define their function, above all in cell metabolism.

The model organism chosen to identify these new phosphatases was yeast. The idea was that once identified we could define their biological role much more easily than in mice. At that time yeast was already a point of reference in terms of its genetic potential, and very important discoveries were being made in the field of cellular biology and, obviously, in intracellular signalling. This posed a challenge. We had to learn to work with this organism using molecular biology techniques, and many different laboratories, including that of Dr C. Gancedo, for instance, helped us to do so. After two research stays, one in Germany at the laboratory of Dr F. K. Zimmermann, and another in Belgium at the laboratory of Dr M. Bollen and Dr W. Stalmans, we were finally able to identify new phosphatase proteins, such as PPZ and PPG,[2] as well as studying the role of other phosphatases in the metabolism identified at other laboratories. These studies allowed me to see the huge potential held by yeast as a model organism. Even today, it still plays a prominent role in the studies we do at our laboratory.

At the same time the field of cell signalling using phospho-dephosphorylation mechanisms underwent an incredible heyday. It must be pointed out that in the early 1990s the identification of MAPK (mitogen-activated protein kinases) pathways was achieved in both yeast and mammals. The MAP kinase signalling pathways consist of a cascade of kinases that are activated sequentially and are essential to efficiently transducing information on the presence of growth factors and other stimuli from outside the cell to the inside. The MAPK Fus3 in yeast was the first described by the laboratory of Dr G. Fink, and afterwards its counterpart in mammals, ERK,[3,4] was identified, as well as the kinases MEK1 and Ste7.[5,6] In fact, it was at a congress in Italy that I had the pleasure of hearing a wonderful talk about the phosphatases that regulate a MAP kinase pathway, known as HOG. This signalling pathway had just been identified in yeast and was important for cell survival upon osmotic stress.[7,8] The genetic study presented by Dr Haruo Saito greatly impressed me, and I soon took notice of all the works published by his laboratory in Boston. That is when kinases similar to Hog1 were also identified in mammalian cells p38 and JNK.[9–11]

This was what convinced me that I had to travel to the United States and start work in the field of cell signalling and MAP kinases. For obvious reasons Boston was my choice, with the advantage that the huge concentration of laboratories existing there would allow both me and my wife Gemma to find the laboratory we were looking for. Gemma did her thesis in the same Biochemistry Department, working with mice, after obtaining her degree in Veterinary Medicine. In Boston she focused on studying the proteins that decouple mitochondrial activity. Obviously, the fact that both of us were researchers and the mutual support that we have given each other at all times have been essential for developing a career devoted to research.

The pre-doctoral stage is an educational period that, beyond just what one learns, is fundamental to the development of any scientist. It is when scientists begin to understand

what science is and how to manage it. I must say that the atmosphere and environment in which this took place for me were of great importance in being able to get prepared and start the next stage of my education with great enthusiasm. I did this at the laboratory of Dr Haruo Saito in Boston.

Boston, a window on knowledge in a privileged environment

My arrival in Boston was not easy. It was 3 January and the airport was shut down due to a snowstorm. We were forced to spend that night in New York. The first week, half a metre of snow fell, and the laboratory that I was going to join had just published an article in *Science*[12] in which it described how the HOG signalling pathway on which I was going to work was activated. Not a very encouraging start. However, in environments in which science works at a vertiginous pace everything can change overnight: a genetic observation by the laboratory made me think that the HOG pathway was controlled in a manner similar to bacterial sensor systems. The combination of these genetic data with a set of biochemical experiments allowed us to understand how the sensor worked when subjected to stress, being the first two-component system that was described in eukaryotic cells.[13] This experience helped me to understand how important it is to combine different experimental approaches for finding answers to complex topics.

I must admit that if I learned one thing at Dr Haruo Saito's laboratory it was the potential held by genetics to understand biology and the concept of work that Asian people have. Haruo is an amazing geneticist and has an ability beyond the norm to deal with extremely complex topics using great simplicity. Whenever we would discuss possible hypotheses and experiments in his office, I ended up with one more screening system in my collection. However, it was the combination of those genetics with biochemical testing that gave us clues as to how the pathway's activation mechanisms worked, as well as how to identify and characterize new key elements in the transmission of information from that pathway. Throughout this time we were also able to demonstrate, in conjunction with other laboratories, the great conservation of this pathway in the p38 pathway in mammals, thanks to experiments for complementing and identifying the corresponding kinases in mammals.[14,15]

Identifying Ste11 as an element in the HOG pathway was something completely unexpected.[16] Ste11 was an essential element in another signalling pathway, but we were able to demonstrate that even though Ste11 was present in different pathways they could maintain trustworthy signalling with no crosstalk or disturbances. This was important for understanding the transmission of signals in these signalling channels a bit better in yeast and mammal cells.

In those years we held joint meetings with other groups from the same department (Harvard University). There, Dr P. Silver's group was one of the pioneers in the use of GFP (green fluorescent protein) to visualize proteins and their cellular locations. The fusion of Hog1 to GFP allowed us to visualize the movement of Hog1 to the cells' nuclei, seconds after its activation in response to stress.[17] This observation suggested that MAPK played

a very important role in the nucleus. This discovery stimulated the research that I began later, once back in Barcelona, to understand the role of Hog1 in controlling transcription and the cellular cycle.

There are two more elements that I would like to highlight about my stay in the United States, which go beyond just the realm of the laboratory and the specific research carried out there. I believe that these two aspects are required for understanding why science is so highly acknowledged in that country and the reason why biotechnology and the development of new technologies in general are so deeply rooted in the American model. First of all, I must mention that the impact I experienced when I saw that they would highlight the discoveries that had just been published in important science journals on the news each week. This was news aimed at the general public, and I had never seen it in my own country. Now this is changing. For a few years now, there have been good reporters and media that pass on the news about scientific events with rigour. This aspect, which scientists themselves must look after, is key to getting society to look positively upon using a part of our taxes on research and, of course, to increasing awareness about the need to promote research if we hope to have an economy based on something more than just the construction sector. The second surprising thing for me was to see the huge cluster of biotechnological and pharmaceutical companies with headquarters in the area around Boston. There the researchers took part in the companies, many of which were spin-offs, or companies derived from the universities themselves. They recruited researchers in the different departments and worked on topics closely related to what we were working on. Inevitably, knowledge and results were transferred through contracts with pharmaceutical companies, such as Sandoz, which analysed the development of different lines of research at the Dana-Farber Cancer Institute. At that time I did not have a very clear idea as to whether that was the right way to go about things or not, but intuition told me there was a constant siphoning off of basic research to those companies, which could make products that had a positive effect on society. This meant there was a partnership beyond just the work in the laboratory that I had never thought about as a scientist; I was always more interested in answering basic questions on how cells worked.

Returning and the chance to form a research group at the UPF

A series of re-hiring scholarships is what brought my wife and I back to Spain. In my case, I went to the UAB, not without doubts about what was going to happen there, but with the hope of managing to set up a laboratory where I would be able to develop something of great interest to me in order to better understand intracellular signalling. The idea was to attempt to identify and characterize the functions of MAPKs in the cell nucleus. The opportunity arose at the perfect time. The Pompeu Fabra University (UPF) was looking for professors for a new degree in Human Biology in Barcelona. This initiative, led by Dr J. Camí, with the involvement of Dr M. Beato, who later became the director of the Centre for Genomic Regulation (CRG), and a group of fantastic scientists who really wanted to do something different, was just getting under way. In late 1999 they proposed that I form my own group in a set of still vacant spaces, which did not take long to fill with researchers from different specialities. It was a time of some uncertainty but great excitement about being able to take part in a new and revolutionary project.

In the beginning, forming a new group and starting to teach systematically was no easy task, but I got a lot of help from both inside and out. Those of us in the group included Dr Eulàlia de Nadal and myself. She signed on as a postdoctoral researcher upon completing her thesis at the UAB, and she also believed in the project. Eulàlia has been a key person in the research group's development since its very beginning and is now leading the group with me. Our funding was not impressive, but it was sufficient and constant from the outset, and that made it possible to begin developing all our ideas. For example, in 2000 the European Molecular Biology Organization (EMBO) offered me the opportunity to take part in the young researchers' programme and that, together with a project from the ministry and a project by the government of Catalonia, allowed us to start off with some momentum. From then on, a large number of training and postdoctoral researchers have passed through our group and have made exceptional contributions towards crystallizing those initial ideas and others that had yet to come.

In addition, I must mention at this point that EMBO not only provided me with economic funding, but also provided us with a mentorship and training programme for young group leaders. The way I see it, this training is essential for any young person who wishes to create his own research group. In fact, during the different stages of training in general, we received instruction as scientists for researching, but not for managing research teams. That is why I suggested to the Barcelona Biomedical Research Park (PRBB), the scientific complex in which our research group was located, to set up these training courses for the researchers in our field. Under the umbrella of the Intervals programme, these courses are given on a scheduled basis at our centres and, without a doubt, this is an essential part of any researcher's training.

The impact of stress on cell biology, from yeast to human cells

Our initial efforts consisted in seeking possible targets of MAPK so as to define new biological functions for these MAPKs. At that time people already knew that MAPKs regulated transcription, but the mechanisms through which they acted were not well defined. We found that Hog1 played a very important role in gene regulation thanks to one of the first arrays, or gene expression matrices, existing for yeast. It was in the laboratory of Dr J. Ariño, when I returned from Boston, that we were able to perform these experiments.[18] Once at the UPF, we began to work on identifying and better defining the molecular mechanisms through which MAPK was able to modulate gene expression. This is a task that we continue to perform today, and it has allowed us to understand basic aspects of gene regulation unknown until now.

Initially, these studies were completed using the basic techniques of gene expression, biochemical techniques and tracking by double hybrid. This led to results that made it possible to identify new transcription factors under the control of MAPK.[19,20] However, one truly interesting aspect was to discover that MAPK associated with DNA through transcription factors.[21] This association with DNA serves to create a direct contact between the MAPK and RNA polymerase II[22] and occurs in both promoters and in the coding regions of stress genes.[23] This discovery has been essential to understanding how many kinases involved in cellular signalling are able to regulate transcription.

The qualitative leap from these studies took place when Dr De Nadal was at the Federal Institute of Technology (ETH) in Zurich, Switzerland, and completed one of the first screenings with the entire collection of mutations in yeast through the use of robots. Thanks to this, we were able to identify a large number of genes fundamental to allowing proper expression of stress genes. We discovered that different activities such as SAGA, Mediator and, much more interestingly, the histone deacetylase complex RPD3 were important for stimulating transcription. This was important because up to that time histone deacetylases were only considered elements that repressed gene expression.[24] It was later, but also in connection with that initial screening, that we were able to define how important the status of chromatin is and how it is remodelled in response to stress, through the RSC complex in the transcriptional response. These studies, initially carried out with a restricted number of genes, were analysed at the genomic scale thanks to new hybridization and sequencing technologies, resulting in a better understanding of the full spectrum of regulated genes and the role of chromatin.[25] These studies also allowed us to discover a non-coding RNA set that was expressed in response to stress.[26] The role of non-coding RNA is still a mystery that is being researched in many laboratories. In conclusion, our studies, along with those completed by other laboratories, have allowed us to establish that this MAPK is able to regulate practically all the steps in the biogenesis of RNA, from its origin to the generation of proteins, thereby showing the regulating potential of one single kinase in a key aspect of cell biology.[27]

Another of the aspects that has always interested us was understanding whether an MAPK was able to regulate progression in a cellular cycle. This attempted to answer the basic question of whether cells needed to stop their progression in stress situations so as to be able to adapt without undergoing damage during cellular cycle phases, such as replication and mitosis. Different laboratories tried to respond to this question by analysing the cycle in the presence of stress. The results were disappointing; however, it was the use of hyperactive mutants from the pathway that allowed us to clearly define at what points in the cycle MAPK was acting. This opened up a whole new field of study that has allowed us to establish that Hog1 is able to regulate basic parts of the cellular cycle machinery. We initially studied how Hog1 regulated the transition between phases G1 and S (replication) of the cycle, and we observed that the phosphorylation of a kinase inhibitor that controls the cycle made it possible to inhibit its activity and block the cycle.[28] This encouraged us to continue exploring further stages in the cellular cycle, and we found that G2-M was also being actively regulated by Hog1. The idea that arises from these studies is that because the cells cannot choose when they are going to undergo stress they must be prepared to respond to environmental changes at any time. With this idea in mind, we started to research what happened during the S phase of the cellular cycle. This is the phase in which DNA is replicated and therefore it is essential to genomic integrity. At this point, we verified that MAPK was able to coordinate the replication and transcription processes to avoid collision between the two cellular machineries and prevent genomic instability. This discovery is of great relevance because it allowed us to determine a new checkpoint unknown up to that time.[29] The data obtained from yeast, due to its relevance in controlling the cellular cycle, as well as other aspects of cell biology, have led us to study these aspects in human cells as well.

The use of yeast as a model organism has always been of great help to us in understanding the basic stress response mechanisms and how they impact cell biology. However, our main goal has always been to achieve an understanding of these processes in human cells. As I have mentioned before, evolutionarily these pathways are highly preserved both structurally and functionally, so they are found in both yeast and mammals. This high level of preservation made us assume that this could also be true with the targets and mechanisms that regulate them. Using mammal cells, we were able to verify that both the transcriptional regulation and control of the cellular cycle are regulated by very similar mechanisms.[30] This opens up new prospects that make us think that much more advanced studies on yeast may have an impact beyond just the understanding of its biology.

We expect the studies on yeast and mammals to determine much more precise knowledge of how signalling pathways transmit the information in response to what happens around them, and how cells adapt to those changes through the regulation of basic processes. What we have learned up to now not only reveals a part of this process, but also allows us to understand new biological regulation processes that could, in some cases, be susceptible to new therapeutic targets in a not-too-distant future.

I would like to add that the studies performed at our laboratory to understand cell response to stress would not have been possible without the cooperation of a large number of laboratories. This collaboration has not only allowed us to perform experiments and use techniques beyond our experience, but also to explore new fields and integrate new viewpoints into our work.

The interface with theoretical biology and the implementation of biological computation

In 2005, as a result of our studies on the cellular cycle, we realized that simple data analysis was not sufficient to predict how the process was being regulated by the MAPK. This was due to the complexity and the number of the mechanisms regulated. For some time the mathematical modelling of biological systems proved useful in many cases, such as studying the cellular cycle.[31] This is where an active adventure with physicists, mathematicians and engineers began that still lasts today. The road was not an easy one: the differences in language and logic used in these worlds mean that sharing projects is no trivial affair. However, after some time, the effort made has demonstrated that the results that can be achieved in joint approaches are incredible. In addition to modelling aspects of the cellular cycle with Dr E. Klipp's group in Germany,[32] we also allied with the complex systems group of Dr Ricard Solé of the UPF. Initially, this served to study aspects related to signalling in the HOG pathway,[33] and in one of Ricard's fantastic presentations we saw that we could complement our experience in biology with his theoretical ideas. That is how the challenge of implementing living systems able to compute stimuli in a programmed manner came about. Using our preferred tool, yeast, we obtained a European project and, later, a grant from the European Research Council (ERC) to pursue this objective. Its first results were already published in 2011,[34] presenting a new way to make simple what even today is viewed as a very complex process. It is the combination of knowledge and experience that allows projects beyond the imagination to work in reality. This is the field of synthetic

biology, in which cells are developed with additional capabilities, in which our imagination appears to have no limits. We have no idea where it might take us.

From knowledge to technology transfer

Throughout my research career, and while developing the research group, discovery and curiosity have always been the driving forces that inspired my work. However, in recent years, I have also been pairing that vision with the potential for finding possible applications of the results of our basic research. This has been stimulated especially by the Botín Foundation, thanks to the possibility of participating in the *Technology Transfer Programme* within the Science Area. The Programme's basic goal is to promote the conveyance of knowledge from laboratory to society, pursuing a profound change in mentality and a commitment by researchers and the institutions in which they work to reach that goal. This has not meant a change in our way of researching or in what we are interested in researching, but it has led to serious thought about the possible short- or long-term application of the results we obtain. Through ongoing education and professional tracking of the group, we have identified those aspects that have potential for producing future applications. This process has been neither easy nor obvious, but from the initial stages of scepticism about how those results from basic science could be applied, we have shifted to a stage of hope and very different approaches that have led us to propose the initial screening of compounds with potential pharmacological use or with biotechnological applications, using cells generated in the laboratory. We do not know with certainty where these studies will lead us, but what is quite clear is that this cultural change will forever mark the way our research group operates and acts.

This notion that our responsibility as scientists goes beyond just producing knowledge is what has driven me, now as a manager, initially as a director of the Department of Experimental and Health Sciences (DCEXS) at the UPF, and later as vice-rector of Science Policy at the University, to attempt to provide our centre with the stimuli, training, elements and structures that can facilitate the transfer process by researchers. This return of training and also research to society that is carried out at our universities is undoubtedly essential in the effort to be made to obtain support from society for our work.

A message of thanks

I would like to end by expressing my thanks for the work and effort made by all the students and postdoctoral researchers who have completed research at the laboratory, as well as all those people who have taken part in my training, who have collaborated with the laboratory or who have simply supported me during my scientific career and whom I was not able to name in this article. Without them, this would not have been possible. I would also like to express my thanks for all the sources of funding that have made our work possible at the laboratory, including the Government of Catalonia, the Spanish Ministry of Economy and Competitiveness, TV3's Fundació La Marató, the Sant Joan de Déu Research Foundation (CIDI), the EU (FP6 and FP7), EMBO, the ESF, the ERC, the Botín Foundation and ICREA (ICREA Acadèmia).

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MANEL ESTELLER

A PERSONAL ODYSSEY OF EPIGENETIC PROPORTIONS

18

I suppose I should start at the beginning. My journey began on a glorious 6 September 1968 in Sant Boi de Llobregat, near Barcelona in Catalonia. My mother, Rosa Badosa Vallas, was very much a local girl, while my father, Manel Esteller Agustina, originally hailed from Valencia but had come to Catalonia in search of a better future. On my mother's side, my grandfather Pepet had been a chess champion, while my great grandfather, Joan Badosa Briquets, had been a Republican. He had been the town's local reporter and chronicler, head of the Unió de Rabassaires (association of vine growers) and had felt the full wrath of Franco's regime. He died when I was just six, but he remained a hugely important figure within the family due to his achievements: he learned to read and write when he was forty, and yet went on to become chronicler for the town of Sant Boi. He also built the house where my family still lives today. My brother Joan, who is three years my junior, was my best friend when we were growing up. We used to spend endless hours playing with our toys and going on imaginary adventures together. I have always believed my imagination to be one of my few true virtues. In fact a teacher once told me off for having too much. I also have a sister, who was born fifteen years after me, and who I therefore look upon more as a daughter than a sibling. She belongs to another generation, allowing me to relive my youth through her. I was a home-loving lad, and ventured out relatively little, preferring to spend most of my time reading comic books or gawping at the television crashed out on the floor. This routine only changed in summertime, when we would visit my father's small village in Castellón, which was already a yearly highlight for me. Lately I have begun to realize that perhaps my having too much free time for fun and games (surely above average for a child of my age) has made me more mentally dextrous and flexible at resolving human illness and disease.

I had few friends at junior school (Escola Joan Bardina), where I completed my basic general education, although I was relatively well liked and stayed out of mischief. I used to sit next to a boy called Gerard in class, and he was my best friend at that time, partly because he was also prodigiously creative. I had a mixed bag of teachers. In those days television was still in black and white, though by the time I finished fifth grade, colour TV was already available. At that time I remember my father signed me up for after-school English classes, something I will always be grateful for because having a good grasp of English opened up the world to me. Very little Catalan was taught in those days and only behind closed doors, though a number of brave teachers, such as Mr Obiols and Mr García – lest we forget that in those days teachers never had a first name –, used to throw some into their classes. "That child just is not the studying kind", one of the teachers once told my parents. How right she was! I quickly decided I wanted to be a doctor and finished the sixth grade of my general basic education. Despite being somewhat scared of what illness and disease can do to people, I felt that curing them was a virtuous pursuit.

I was lucky enough to gain a place at what I believe to be an excellent institute, namely the Escola Esportiva Llor de Sant Boi de Llobregat. A number of world-class sportsmen are ex-alumni, including Pau and Marc Gasol, both brothers currently playing in the NBA. My Catalan teacher, Maite, was a supremely good influence on me. She once gave me a full dressing down while I was still a rebellious teenager, saying all the potential I had would come to nothing if I did not buckle down. My chemistry and physics teacher, Jordi Carvajal, aroused my interest in research work and thanks to him I began doing what I liked most: scientific work and writing short articles, including my first laboratory experiment. I view education as the most powerful weapon in changing the world. Parents and teachers alike are directly responsible for the future of each child. That explains the importance of role models: we tend to become what we observe. There were two things that really shaped my future during my time at the institute: firstly, my best friend was a truly brilliant student, called Chilla, and recommended a number of good books to read; secondly, I walked away with the award of the Interdepartmental Committee for Research and Technological Innovation in both 1986 and 1987. Winning these awards back to back, when I was seventeen or eighteen, really got me going. After the institute, I pulled out all the stops to achieve what I wanted more than anything at that time: securing a place at the Faculty of Medicine. Obtaining top marks on the University Orientation Course (which all students must complete to get into university) let me off the enrolment fee, which certainly eased the financial burden on my family. The country should hang its head low if any boy or girl with potential talent slips through the net because they cannot afford a place.

My reasons for studying medicine were not your typical ones. I pursued my career because I wanted to become a doctor, but, as I mentioned previously, not to treat sick people but to investigate the underlying causes of disease and explore new treatments. The faculty also had its fair share of brilliant and dreadful teachers. I have always tried to emulate the former and I have retained every single thing I learned from them. The bad teachers are long lost to me, unless their names happen to come up when chatting with old classmates. There were two professors at the faculty who left a profound impression on me: Dr Ferran Climent, who had a gift for making people understand such a hugely complicated subject as biochemistry, and Dr Cristóbal Mezquita, who just loved to teach. He was, for me, the best teacher I have ever known. I realized from the outset that biochemistry and molecular biology were my true passions and I spent every minute I could in the department from the day I started university to the day I left. Dr Jesús Ureña taught me proper laboratory procedure and the need to combine hours of flat-out work with good ideas (remember, success is 5% inspiration, 95% perspiration). Yet the more clinical course subjects began to be a drag. Thankfully, the afternoons I spent in the laboratory and the first meetings of young researchers provided a welcome tonic. It was at one of these events that I met, albeit only briefly, Nobel Prize winner Severo Ochoa, who was already a very old man, and his disciple Margarita Salas. Then, one day in 1992, with Barcelona in full Olympic fever and with Barça celebrating their league victory following Real Madrid's shock defeat in Tenerife, I walked away from the faculty with my university degree tucked firmly under my arm.

I had hoped to carry on my research activity close to patients, but in those times research centres were not like they are now. Research work was limited to university departments

or small laboratories within hospitals. In the end, I settled on biochemistry and molecular biology, which I started investigating within hospitals and other healthcare centres. Yet luck was not on my side in the early days. One of the centres I handed my CV to never even got back to me, while at another I needed just one day to see that nobody had the faintest clue about what they were doing, so I calmly walked out the door. After these failed experiences, I finally managed to track down a medical investigator from the Vall d'Hebron Hospital and cornered him in a lift, offering him little chance of escape. And my tactic worked because he accepted me into his group. At my new home, I started working on my own line of research into the molecular genetics of cancer, and studied the DNA mutations associated with cancer of the body of the uterus. During this fruitful period of my life, I worked closely with surgeons and pathologists and we rose to become one of the world's leading authorities on these kinds of tumour. Subsequent training with our Scottish brethren at the University of St Andrews allowed me to study hereditary breast cancer while immersing myself in a whole new culture for the first time in my life. My work took me deeper and deeper into the genetics of tumours, yet despite the progress we made one question always eluded me: why did some tumours not suffer mutations? There must have been alterative mechanisms for getting the better of the genes.

I am not sure how, but one day I found myself reading an article recommended by the first public sector oncologist in Spain to have acquired in-depth knowledge of molecular biology, a certain Rafael Rosell. The article suggested that there could be mutations beyond the genes responsible for the cancer. The study was headed by investigators from Johns Hopkins University and Oncology Centre in Baltimore. I had to go. So, in early 1997 I finally arrived at my new North American laboratory. Interestingly enough, the day before I was set to leave and with my bags already packed, I received a call from a very prestigious hospital in Barcelona offering me a much coveted position. These things in life are sent to test us! But in my eyes I was still enthralled with the idea of living and working in the United States and so it ended up being an easy decision. I buckled right down in Baltimore, putting in twelve to fourteen-hour shifts. We did not feel it because we were all intoxicated with the enormity of what we were discovering. Between 1998 and 2001 we published the fundamental discoveries of our work, which lent further credence to the existing observation that there are anti-cancer genes that become silent in tumours due to a chemical signal called DNA methylation. And all this without a single mutation! The genes remained intact, but were what we might call asleep. These were happy times, and on top of our professional success my personal life also became more stable and I became more comfortable financially. That said, I did not yet have the added responsibilities of having a team under me.

The United States is a prime example of how things should be done, as one's worth is based solely on one's merits, without nationality, wealth, beliefs or religion being in any way relevant. Had it not been for the investigative freedom given to me by Dr James Herman and Dr Stephen Baylin, my career would have taken a completely different path. I also got to share a laboratory with Dr Bert Vogelstein, one of the fathers of modern molecular genetics of cancer. It was a golden period full of scientific breakthroughs and wonderful anecdotes, such as the call our young Maryland-native secretary picked up from the Spanish royal family, or the publication of our article in the *New England Journal of*

Medicine, which also made television news, concerning a molecular trial we had developed to predict patient response to chemotherapy.[1]

And in 2001 I had to give a conference in a seaside city on the East Coast. As scientists we are used to such events, as they provide us with an opportunity to present the results of our work while giving a valuable insight into what our peers are up to. At one of these congresses, in 2001 to be precise, I finally got to meet Mariano Barbacid, who had discovered the first mutation in an oncogene and who was one of the doctors I admired the most as a young man. As a young boy, I had a poster of Marilyn Monroe up in my bedroom, but had it been a scientist instead, it would surely have been Barbacid. Mariano told me that he was setting up a large centre in Madrid, the Spanish National Cancer Research Centre, or CNIO for short, and he offered me my own epigenetic research team. Naturally I was thrilled with the compliment, but it suddenly crossed my mind that it might well mean having to live in Madrid. Shortly afterwards I received another offer, this time from Barcelona, but I could not realistically let the opportunity of working with someone I admired, and who I still admire, pass me by. So I talked it over with my wife and in October 2001 I arrived at the CNIO as team leader of the Cancer Epigenetics Laboratory. I had surely chosen the right time to return to Spain. It was in September 2001, 11 September to be precise, when the world witnessed the terrorist attacks on the Twin Towers in New York. The United States has since taken a decade to rediscover part of that creative innocence for which it had always been known.

I settled in well in Madrid and I have only good things to say about the city and its people. Barbacid and I are friends because we are practical, seeking merit regardless of colour. My group was the first to be set up at the CNIO. We worked in coats and scarves when we started because the central heating had yet to be installed. I recall a visit from Spanish minister Celia Villalobos and she was shocked to see us like that. But it was a fresh challenge. It was my first position as head of a laboratory and once again it turned out to be a period full of valuable findings, changing the way we look at tumour cells. I was by then skilled enough, or perhaps lucky is the word, to find a good starting team for my laboratory, including Esteban Ballestar and Mario Fraga. This was absolutely key. There are many people involved in a discovery, and you are only as good as your team. Messi could not score all his goals without built-up play. During my wonderful time at the CNIO I cannot help but remember our first studies, in which we demonstrated that two genetically identical individuals (monozygotic twins) are epigenetically different and, as such, can have different susceptibilities to disease. This discovery made the front cover of the *New York Times* and was the leading news story on both NBC and the BBC. We also found that the way DNA is packaged, creating a kind of fibre two metres in length in each and every cell, is completely altered by cancer. Our work opened the eyes of many researchers working in a wide range of different fields.

In 2008, with a small son in tow and my parents getting on in age, I thought that after twelve years of globetrotting the time had come to return home. In October, I accepted the position of director of the Cancer Epigenetics and Biology Programme at Bellvitge Biomedical Research Institute (IDIBELL), where all research at the Bellvitge campus is conducted,

including its general hospital, the Duran i Reynals cancer hospital and part of the University of Barcelona. I also started lecturing at the Catalan Institution for Research and Advanced Studies (ICREA), which had been set up by the irrepressible Dr Andreu Mas-Colell to recruit non-staff investigators seeking to work in Catalonia. I also returned to the Medicine Faculty, this time as professor of Genetics, knowing deep down that if I could inspire just one of my students to become a researcher, my time there would have been worth it. These last six years have been tough because of the cutbacks: conducting top-quality research under testing economic conditions, from having my own team to working in a department with various group leaders and lead investigators, and so on. Yet, despite the difficulties, it has all been worth it because the discoveries have continued to flow. Uncoding the alterations in the so-called dark genome, or non-coding DNA, in tumours; extending the scope of our research to embrace Alzheimer's following the death of my grandmother Paquita; or developing the first epigenetic drugs now being used to treat leukaemias and lymphomas, to name but a few. And when the director of the world's leading cancer treatment centre, the MD Anderson in Houston, remarks "if it is related to epigenetics, best ask that investigator who works in Barcelona", you really begin to appreciate just how highly your work is valued, and by extension the work of your team and the work being carried out in little old Spain.

I would now like to take you on another journey, this time going into greater detail as we narrow in on my work: from cancer genetics to the epigenetics of diseases. A journey inside the human body and its pathologies to explain to you just how we in the laboratories have helped broaden human knowledge and awareness of these issues. Some may see my description as overly short and believe I am somehow making light of their own experience in these areas. Others, on the other hand, might think I have gone too far and perhaps overdone it with the technical jargon.

Human beings are made up of cells. Some organisms have only one, such as *Escherichia coli*, which can send us running to the bathroom, while others, such as we human beings, have millions. There are two types of cell: some more primitive we might say, called prokaryotes, and others more advanced, the so-called eukaryotes. The bacteria *Escherichia coli* is a prokaryote, while our cells are eukaryotes and will be the subject of the rest of my discussion. The cells of the human body have three parts: a membrane that encloses them, made up largely of fats (lipids); a viscous liquid substance inside the membrane, which is made up of water and other dissolved substances; and lastly the nucleus right in the centre. This cellular nucleus contains the deoxyribonucleic acid (DNA), our genetic building blocks and which, for better or worse, comes from our parents. DNA is a linear jigsaw made up of just four kinds of piece (nucleotides) called A, C, T and G. The multiple possible combinations of these explain why no two people are the same. There are fragments of DNA and, therefore, sections of the jigsaw comprising thousands of A, C, T and Gs. These are our genes, the functional units of DNA. From these a molecule called ribonucleic acid (RNA) is born, which is then tasked with originating other molecules, namely the proteins. DNA gives instructions on how the organism functions so that the cells can manufacture the proteins. You might say it is in charge. The genes are the parts of the DNA that actually contain these instructions, while proteins carry out the dirty work,

meaning they carry out the specific functions prescribed, such as transporting oxygen around the body (haemoglobin), controlling blood sugar levels (insulin) and allowing you to see the outside world through your retina (rhodopsin). That is the presentations out of the way. Now let's start the show.

Cancer is a disease of the cells. These bodily units, which were initially obedient and only too happy to help, become self-centred and only interested in personal gain, without caring who or what might get caught up. Now, a number of genes from the cancerous cells, called oncogenes, activate, triggering an uncontrolled proliferation of these cancerous cells, while other cells that should protect us (the tumour-suppressor genes) are silenced. One of the first oncogenes to be found was the K-ras gene, while important tumour-suppressor genes include the likes of p53 and Rb. These genes are more likely to become altered as we get older and also when we expose ourselves to carcinogens, such as the components of tobacco and radiation.

From the discovery of the first oncogenes (at the start of the 1980s) through to the mid 1990s the only gene alteration in cancer to have been described was mutation. But then we came along, as did other research groups, to break this blinkered view of reality. Although from 1992 to 1996 I was able to unearth new mutations in the DNA of the kind of tumour I was studying, I later discovered something far more interesting. A guardian gene called hMLH1, involved in hereditary cancer, which accounts for ten per cent of all tumours, was never found to be mutated. How could that be? Well, precisely because the gene was "methylated".[2] It was not a typical mutation, rather an alteration, a chemical marker that causes them to become confused. And now I really should stop to explain this clearly. As you will remember, DNA is what orders the cells around. While all our cells contain the same genes, a neuron and a heart cell carry out very different functions. The former creates neurotransmitters, while the latter is there to beat. How has evolution resolved the matter? By introducing "chemical switches" to turn the genes of our DNA on or off. Using the same example, a gene expresses itself (or is active) in a neuron, but not in the heart because there it has no function. Unfortunately cancer causes these switches to malfunction. The human body is equipped with genes to protect against cancer, such as the hMLH1 we were studying. This gene stops protecting us against the disease due to a chemical signal that stops and blocks gene expression. It is then that the cell changes from normal to tumorous. This chemical alteration is known as methylation and it is partly responsible for the appearance of tumours.

We can now confirm that the development of hereditary cancer does not occur solely because an altered gene has been inherited, but also due to the simultaneous arrival of other cellular alterations, such as methylation, which silences our anti-cancer genes. We made our first discoveries on the methylation-triggered inactivation of these guardian or tumour-suppressor genes towards the end of the 1990s. It was then we discovered the first altered genes. This in turn opened up further study and led to a boom in this kind of research. There are currently hundreds of anti-cancer genes we know do not function and are turned off due to methylation.

The value of certain breakthroughs can sometimes be felt for decades and some are even now leading to new discoveries. For example, in 1999 we found that the inactivation of the DNA repair gene known as MGMT occurred in human tumours through the chemical process of methylation.[3] In 2000 it was shown that tumours presenting this alteration died more quickly when their DNA was damaged by chemotherapy.[1] Nowadays, determining the methylation of the MGMT repair gene is one of the tests conducted on patients with brain tumours (gliomas) to decide on what therapy to apply. And just recently, in 2013, we discovered that this test can also be carried out for cancer of the colon.

Without wishing to be a pain, allow me to give you another example. The year 2000 also witnessed the discovery of the inactivation through methylation of the breast cancer gene BRCA1.[4] This finding has enabled us to predict those mammary tumours that will be more sensitive to drugs such as PARP inhibitors[5] and platinum-based drugs.[6] We have even found that a first cousin of the BRCA1 gene (the SRBC gene) becomes inactive in colorectal tumours, predicting the response to other chemotherapy agents that also act at DNA level.[7] By looking at the dates of these discoveries one can clearly see that there are findings with a ten-year gap between the first clues emerging and their eventual clinical application. This sometimes makes explaining the importance of science to impatient officials and politicians a frustrating exercise, as they refuse to look beyond their own terms of office.

What has all this got to do with epigenetics? Let us summarize. Epigenetics embraces everything that lies beyond and around genetics, which regulates our genes, our genome, our DNA. And it has a hugely important application in certain human diseases, including cancer. Cancer is a product of modifications in the genetic material, making normal cells malignant. We have known for some time now that there is not always a mutation, and by this we mean a modification in the sequence of letters making up our DNA (A, C, T and G) that in turn triggers gene function to change, giving rise to a tumour. Chemical modifications can also do the trick. These alterations, which affect the function of a gene (activating or silencing it) without actually modifying the DNA sequence, are what we call epigenetic changes. Epigenetics can explain things beyond the grasp of genetics. We all have DNA, which we might call the alphabet as it makes up our cells and arranges them in proper order. But we also need a spell and grammar check to place the accents on words, add capital letters and brackets where necessary, and so forth, and which ultimately ensures the alphabet makes sense when arranged. This is epigenetics. If we think of a computer, the machinery, or hardware, would be the genetics, while all the programming, or software (which makes it work), would be the epigenetics. The DNA methylation I was discussing a little earlier is an epigenetic signal: it changes gene activity without mutating the gene. All the tissue and organs found in the human body have the same genetics (DNA), but different epigenetics, meaning different chemical signals or switches that regulate the DNA and let genes express themselves in the right place and at the right time, whether in the eye, the skin, or the heart.

Two clear examples come to mind of the power of epigenetics deriving from the study of genetically identical beings. The first concerns a discovery made by Dr Rudolf Jaenisch based on mouse cloning. He showed that if we clone a mouse, and by that we mean replicating

its DNA, the cloned mouse will not be exactly the same as the original one. Although his mice had the same DNA (the same genes), their epigenetics (how their genes are regulated) were different. Their DNA had been successfully copied, but not their epigenetics, and therefore their chemical markers were different. This example clearly illustrates that the cloning process is not perfect and mints imperfect copies. The second of my examples concerns a finding made in my own laboratory, after years of head-scratching, first in Baltimore and later in Madrid. The subject here is monozygotic twins, who can have the same DNA and who we might consider clones produced naturally. If the DNA were the only thing that mattered, these people should be photocopies, or spitting images of each other, and yet they are not. They can have different anthropomorphic features, personalities and diseases: one of them may develop cancer while the other may not, and so on. So, based on this premise, we demonstrated in 2005 that monozygotic twins, despite having the same genetics, have different epigenetics, which manifest with different levels of DNA methylation affecting hundreds of genes.[8] They may during their life develop chemical markers in their DNA that mark them apart and have different elements of their genetic make-up turned on or off. Gene regulation acquired during life means a disease may or may not be expressed. There is no such thing, therefore, as genetic determinism. This discovery was published in the journal *PNAS*, of the National Academy of Sciences of the United States,[8] and is one of the most cited articles in the journal's long and prestigious history. Recently we have demonstrated that when a twin falls ill with diabetes or cancer, their epigenetics change even when their genetics have not. We have even seen that many differences between non-twins are rooted in epigenetics, which would explain the different tolerances to food, drugs and infections.[9]

If you think things have been easy so far, allow me to shake things up: DNA methylation is only one of the dozens of epigenetic changes our cells can undergo and moreover genes only account for ten per cent of our genome. It turns out that in addition to this DNA methylation, genetic material can also be controlled by "squeezing" or "relaxing" it. As I mentioned previously, DNA is a two-metre thread that fits not only inside the cell, but within the walls of the cell nucleus. Yet it is only able to squeeze into such a tiny space because it is very tightly packed, and the way DNA is coiled up in an intestine cell is very different from how it is coiled up in a white blood cell. It is all highly regulated. In charge of this packaging process are the histones. Looking something like a pearl necklace, these proteins roll up the DNA and allow it to fit inside the nucleus. And here is the thing: just how hard the histones squeeze or relax their grip on the DNA, and in doing so either activate or turn off a gene, depends on chemical modifications (the chemical methylation of the histones, and also others such as acetylation, phosphorylation, ubiquitylation and sumoylation), which respond to epigenetic changes. They are effectively epigenetic markers. Many of these alterations were discovered in yeasts and worms and will be up for a Nobel Prize in the coming years. Dr David Allis is one of several whose work in the field is certainly worthy of the award.

Our laboratory took the knowledge acquired from this work and applied it to human cells and, subsequently, to cancer. From there we discovered that histone acetylation and methylation were both found to be altered in human tumours. It was the first time this

phenomenon had been witnessed and the findings were published in the leading genetics journal in 2005.[10] It also provided the platform for many subsequent discoveries, including the development of epigenetic cancer drugs, such as DNA methylation and histone deacetylase inhibitors, which attempt to restore a normal epigenetic structure. These drugs reprogramme the tumour cells, much like a computer antivirus, and eliminate the wayward chemical markers present in the cancerous cell.

I would also like to explain my statement that genes only account for ten per cent of our DNA. So where does the remaining ninety per cent come from? Around half is leftover evolutionary baggage, akin to archaeological remains from when we were other species, or molecular parasites that have tagged along for the ride. Yet the other half of this dark DNA (or non-coded DNA) has a role to play in living beings: it contains the switches that turn the switches. It produces small RNA molecules responsible for activating or deactivating genes, but does not generate proteins. It regulates the rest of the DNA; another law enforcement body if you like. Ever since the first clues emerged as to the possible existence of this "dark genome", we committed ourselves to the task, and in the period since 2007 we have been able to demonstrate that changes in dark matter molecules are related to tumour formation.[11–16] They suffer the same epigenetic changes in cancer as the conventional genome does. More knowledge, more tools and more targets to aim at as we strive to beat the disease.

Laboratory investigation is ongoing. After having obtained the first human epigenomes in Europe,[17] providing the keys to understanding how epigenetics contributes to ageing,[18] we have since 2001 been conducting valuable research into Rett syndrome alongside the affected families.[19] Rett syndrome is a rare disorder in which an epigenetic switch fails. We are also continuing our investigation into other syndromes, such as Sotos and Rubinstein-Taybi syndromes, and we have opened new lines of research to study the altered epigenetics associated with other human diseases, such as Alzheimer's. In 2013 we showed the first epigenetic lesions in the cerebral cortex of Alzheimer's patients.[20] Neurodegenerative diseases are one of the major challenges facing us in the twenty-first century. We will be ready and our research will not cease.

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JUAN ORTÍN

MY JOURNEY FROM CHEMISTRY TO VIROLOGY

19

There are times in life that are decisive to each person's professional and personal future. In these pages I would like to reflect upon my memories about a lot of those times, the people involved in them and their effects on my professional life.

My earliest education

I was born in Madrid in the spring of 1946, during hard times in Spain's post-war period. My mother had been born in Valencia, where my father met her during the Spanish Civil War. My father was born in Madrid and was a cadet at the Military Academy when war broke out. Although he was stationed at Cuartel de la Montaña – where you can visit the Egyptian Temple of Debod today – at that time he was on leave in Murcia, visiting his father's family. For that reason, he was saved from the Cuartel de la Montaña massacre and was able to serve in the Republican army as a transmissions regiment officer in Spain's south-west. Like many others who fought on the Republican side, when the war came to an end he was judged but fortunately found innocent. He did lose his status as a soldier though. From that moment my father had to rebuild his life, but his great intelligence, his strength and his ability to adapt made it possible for him to start out as a simple draughtsman and then finish his professional life as one of the directors of Otis Elevators in Spain.

For many years my family lived in the neighbourhood of Cuatro Caminos, and I completed my primary school and baccalaureate studies at the Colegio del Buen Consejo, run by the Augustine fathers at the far end of Avenida de la Reina Victoria, next to the old Metropolitano Stadium. There I received a serious education based on Christian morals, but much more liberally and less politically doctrinarian than at other schools and institutions of the period. Because the school was private and my family's economic situation precarious, my parents could not afford my education without a scholarship that exempted them from paying tuition. Therefore, before beginning the baccalaureate, I had to pass an exam to gain access to the free tuition scholarship and then keep up the high marks that this required throughout my studies.

From those times I would like to remember two of my professors. First of all, there was my English teacher, an old Augustine priest in love with teaching, who spent his summers in England and made strenuous efforts to get us to pronounce correctly and speak English in class. What he taught us was enough so that shortly after finishing the baccalaureate I was able to spend a summer abroad working at a restaurant in England and survive the whole endeavour. More significant to my professional life was my fourth-year baccalaureate chemistry professor. His passion for the subject and the teaching style he used in his

classes led me to lean towards this field of specialization when the time came to choose my later university studies.

In the world of chemistry

During the following five years I was a student in the School of Chemical Sciences at the Complutense University of Madrid. As commonly occurs, the first year was a shock to me: a new environment, lecture classes with three hundred students, almost all strangers, and complete independence for students, with a lack of control by teachers. My father's teachings about how to organize work and deal with studies were decisive at that time. Despite this, I only managed to pass the first year by the skin of my teeth, which was also quite commendable because the first year was "selective", or in other words it had to be passed in full to continue studying that major at the university.

From my university studies, I remember physics with particular delight, both the subject in general and mechanics, which we were taught by Professor Luis Bru, one of the most prestigious Spanish physicists of the twentieth century. Chemistry, physics, spectrometry, quantum mechanics and, above all, organic chemistry as well. In the end, I decided to choose the last of these as my specialization, which included the subject of biochemistry (at that time there was no major on this subject matter). From the last two years, I most affectionately remember the long days of practicums in the organic chemistry labs with a group of colleagues, who are still friends today, and the discussion sessions on chemistry problems that we frequently organized. From that period, I would like to highlight the work by one of my organic chemistry professors, Ángel Alberola. Professor Alberola was a serious person and, in a certain way shy, as he had a significant speech impediment. However, he managed to surmount that limitation and taught his classes in organic reaction mechanisms with enviable clarity, enthusiasm and depth. At a time when tenured professors were generally inaccessible individuals, Professor Alberola made his classes feel so personal that discussions were often held with students, and there were nearly a hundred of us. We were also surprised by his humble nature and intellectual honesty. On more than one occasion, when a student asked a question, he would answer, "I cannot answer you at this time, but I will study the topic". And he would devote the next class to analysing and solving the problem brought up. Such was his prestige that, at the beginning of the school year, a new student showed up in class. His name was Friedemann and he had come all the way from Germany. He soon joined our work group, and when we asked him how a German came to study his fifth year of chemistry in Madrid, he answered that Professor Alberola was the best European specialist on his topic.

The School of Chemical Sciences required both the university degree programme examination and the completion of an undergraduate thesis in order to achieve the degree of "*licenciado*". I completed my thesis in the Department of Biochemistry, which was then directed by Ángel Martín Municio, one of the key figures in biochemistry in Spain. With the average marks obtained during the degree, the degree examination and the thesis, the school awarded the Extraordinary Degree Award to the valedictorian of each year, but in my year it turned out that two of us students obtained exactly the same average grade.

Therefore, the school called us in for an ad hoc test to determine who the winner would be. Since the other student was from Physical Chemistry, the exam included questions from both majors, and for me it was a joy to be awarded the aforementioned prize.

A biology apprentice

Upon completing my university degree, I had decided I would do my doctoral thesis, and the easiest way was to remain at the Biochemistry Department where I had done my undergraduate thesis. Though this was a very reasonable possibility, I was not fully convinced because the work I saw around me seemed too descriptive. That was one of those key moments in terms of my professional future, and it was decided by pure chance. One day, when I was returning home from class on the bus, I ran into Enrique Méndez, who I am sad to say has recently passed away. Enrique was from the year above mine, but I knew him because his brother had been a colleague in the hardships of the university militias. Enrique told me that he had just begun his thesis at the Biological Research Centre (CIB) in the group that had just been set up by Eladio Viñuela and Margarita Salas, both recently returned from Severo Ochoa's laboratory in New York, and that they had begun some very interesting work on molecular biology. In fact, he went on to say that Eladio was giving a conference on the topic at the school and that I could get more information there. And so I went to listen to Eladio and became convinced that that was a world apart in science. When I met Eladio and Margarita, and they accepted me to do my doctoral thesis with them, I reached a fundamental decision in my career, which meant switching from chemistry to molecular biology and getting the chance to learn what it meant to work in that field at the cradle of this subject matter in Spain. Throughout those years, I was lucky to share a laboratory with students of the stature of Enrique Méndez, Jesús Ávila, Antonio Talavera, Víctor Rubio, Galo Ramírez and José Miguel Hermoso (Eladio and Margarita's first generation), and later with José L. Carrascosa and Fernando Jiménez. Working in that environment was tough, but very stimulating and formative. At the bibliographic seminars, Eladio would teach us to eviscerate papers, and to understand experimental strategies and the reasons behind controls (and above all what controls were lacking), to reach the less obvious conclusions and to propose verifiable hypotheses in the future. There I learned that scientific work never ends, that hypotheses cannot be proven but only refuted and that a hypothesis that cannot be experimentally analysed cannot be used to move forward.

The subject of our study was phage F29 ("a small, morphologically complex phage, but with small-sized DNA", as stated in the introductions to the articles in that period), for which we dealt with structural, biophysical and genetic aspects, as well as its interaction with the host. My thesis project within this context was to study the general structure of the phage's genome, a DNA molecule, and though formally my thesis was directed by Margarita, in work discussions both she and Eladio would critique the data and suggest new experiments. For quite some time, my work was frustrating because I was unable to find any of the typical characteristics of other phages' genomes in the DNA of F29, but in the end I did find something very novel: the viral DNA was covalently associated with a protein, forming complexes that I could recognize under an electron microscope and

by sedimentation. To do so, I was lucky to receive help from César Vásquez, an Argentinian professor who spent a few months at the lab and taught me to make DNA preparations for observation under an electron microscope. With this support, we were able to demonstrate that F29's genome adopted circular structures that could be linearized using protease treatments.[1] Later, similar situations were found in the genomes of adenovirus (DNA) and poliovirus (RNA), and the door was opened to discovering a new mechanism to begin the replication of nucleic acids, which has turned out to be quite widespread.[2]

At the end of my thesis and throughout the postdoctoral year in which I remained in Eladio and Margarita's group, I had the pleasure of sharing the laboratory with José Antonio Melero, who was starting his thesis on proteins with an affinity for DNA in cells transformed under cultivation. His experiments on control of the cellular cycle, along with those of Manolo Perucho, opened up the prospects for me of shifting to animal viruses during my postdoctoral period.

The outside world

As soon as I finished my thesis, it was clear to me that my next step would be to spend a postdoctoral stay abroad and that I had to move into the world of animal viruses. This meant an important technical change, coming from the world of phages, but it drew me closer to biological problems that had an effect on real life. In those times, studying so-called oncogenic viruses was very in vogue (SV40, adenovirus and so on), and that was an appealing, though very competitive, field for my postdoctoral period. When the time came to choose my professional future, I was also given the decisive support of Eladio and Margarita. They mentioned to me that Professor Walter Doerfler was going to open up his laboratory at the Institute for Genetics of the University of Cologne and recommended that I write to him. Walter was a professor at Rockefeller University and had worked at Stanford University before that, using adenovirus as a biological system. When I contacted him, he was a visiting professor at the University of Uppsala, in Lennart Philipson's group, because his new laboratory in Cologne was still under construction. Thanks, as well, to the support of César Vásquez, I was accepted by Walter, applied for an EMBO fellowship and, with that financing, moved to Cologne with my family. During that time my wife Maleles, who is also a doctor of Chemistry, reached the difficult decision to put her career on hold until we returned home and devoted those years to taking care of our children, a decision for which they and I will always be grateful.

My postdoctoral stay with Walter was a very happy, productive and scientifically interesting time that was very educational as well, both professionally and personally. From a technical perspective, I had to deal with the world of mammalian cells and with that of RNA, as well as taking the first steps in genetic engineering techniques (these were times in which we had to purify restriction enzymes on our own, using bacterial strains that we got from colleagues, mainly Rich Roberts, who would later win the Nobel Prize). Scientifically, I dealt with transcription control problems in the genome of adenovirus 12, one of the oncogenic serotypes.[3] However, I not only learned a lot about scientific and technical matters, but those years were also extremely formative in other essential aspects as

well, including relations between science and the world, handling a work group and other non-science topics. All this was thanks to the extraordinary personality of Walter Doerfler. Walter is a rigorous scientist with a great vision and huge work capacity, but he is also a person of exceptional human qualities, great culture and a cosmopolitan personality. These qualities were faithfully reflected in his work group, a colourful mixture of students, postdoctoral researchers and technicians from a wide range of countries, including the United States, Indonesia, Holland and Spain. The official language used was English, something quite uncommon in the Germany of that time. In fact, I do not remember a single scientific conversation with Walter in German, and I was only able to learn the language when I asked the German students and Monika Westphal, Walter's technician and a great friend, to speak to me only in German outside the laboratory. I have wonderful memories of some of my colleagues and friends from back then, including Ellen Fanning (Vanderbilt University, who sadly passed away last year), Harold Burger (Wadsworth Center), Ernst-Ludwig Winnacker (who was president of the DFG), Karl-Heinz Scheidtmann (University of Bonn), Dennis T. Brown (University of North Carolina) and Sian Tjia (University of Cologne).

Walter's ability to do work was phenomenal. He never stopped for lunch and always returned to the laboratory after dinner. At that time I had to do a lot of kinetic experiments that required staying in the laboratory late into the night, and on many occasions Walter and I would take advantage of those quiet moments to discuss the widest range of scientific and worldly topics. Years later, when Walter and his wife Helli visited us in Spain, or on my last visit to his family's home in Bavaria, we continued to enjoy long and pleasant talks until the late hours of the night, discussing every topic imaginable. Walter was and still is a greatly respected person around the world, and this could be seen in the fact that the most important scientists from across the globe would pass through Cologne to give seminars and visit our group. Walter made us participate actively in those discussions, and in the meetings at his home afterwards, which is how I got to know personages such as Ernest Winocour (Weizmann Institute), Lennart Philipson (who would later become the director of EMBL), Bernard Roizman (University of Chicago), Dick Compans (Emory University) and Hans D. Klenk (University of Marburg), as well as many others. I would like to make a special mention of two of them, Max Delbrück and Daniel Nathans, both Nobel Prize winners, because they impressed me due to their modesty and openness.

Back home

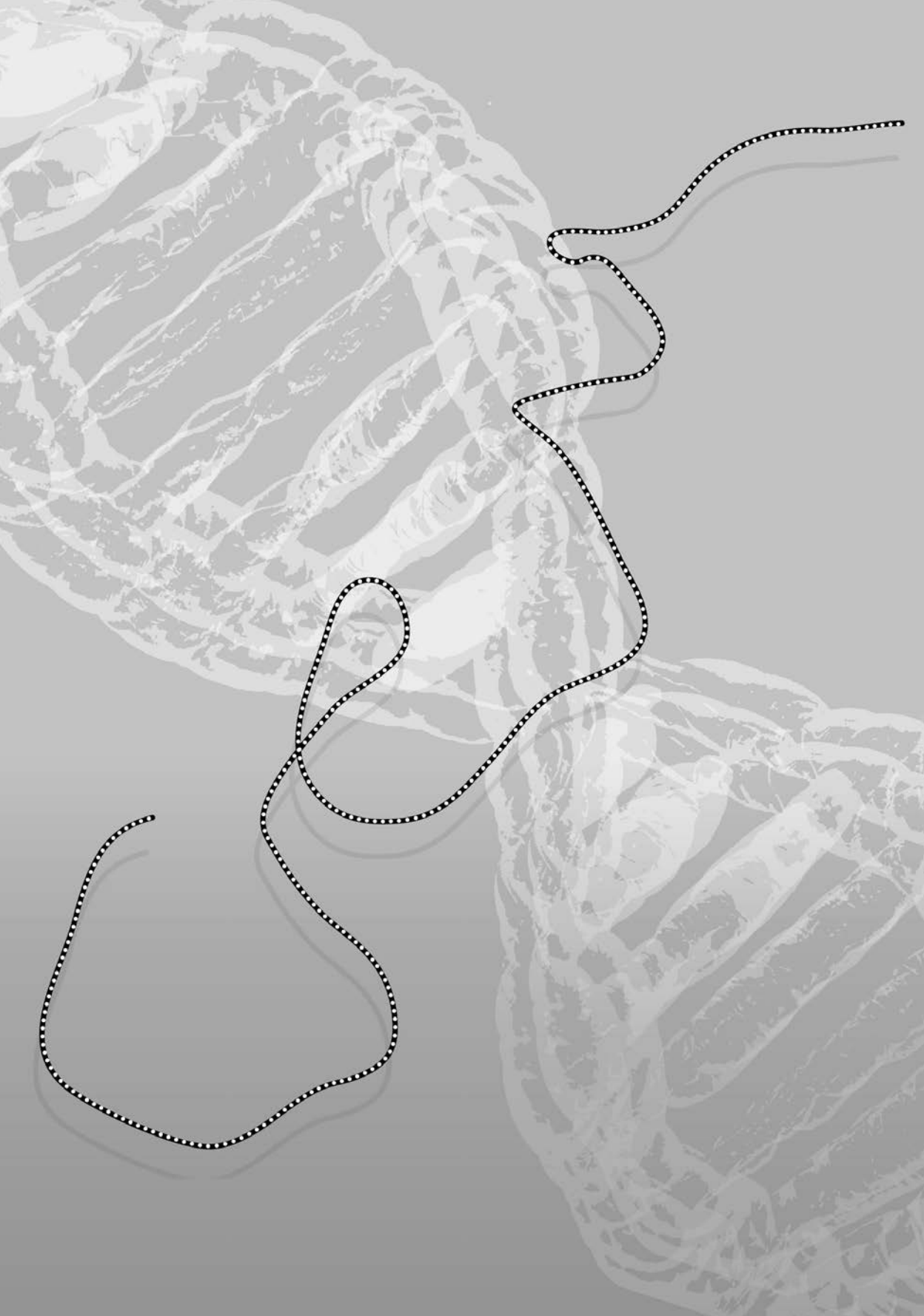
During my stay in Germany, Eladio began a new research project to study the biology of the African swine plague virus (VPPA), the causal agent of a devastating disease in the domestic pig that had become endemic on the Iberian Peninsula and was causing major economic damage. Given my recent experience with adenovirus, I asked him if I could join his group, thinking that I could contribute new aspects to his study. In Eladio's group, I briefly coincided with Luis Enjuanes, who had laid down the foundations for the study of VPPA, since he had adapted it to multiplying in cultured cells and not just in macrophages. He had established a virus biological assay and had purified the viral particles. My contribution was focused on the viral genome, a large-sized DNA molecule. I

was able to demonstrate that its two chains were covalently linked, in a manner similar to poxviruses.[4] However, VPPA requires the presence of the cell nucleus in order to multiply, despite replicating in the cytoplasm, a property that distinguishes it from poxviruses.[5] These observations were later verified and extended throughout Eladio's group.

During those years, Eladio was very much involved in the creation and establishment of the new Centre of Molecular Biology (CBM), one of the milestones in the specialization's history in Spain, and I had the luck to be given responsibility for everyday management of the group. By doing so, and with the increase in my experience, my scientific aspirations grew and I proposed taking responsibility for one of the many aspects that could be dealt with in research on VPPA as well. In light of this proposal, Eladio advised me that I should find my own ecological niche myself and propose a completely new and independent research project. In addition to giving me this wise advice, he suggested that I join forces with Esteban Domingo, who at that time was working at Margarita's laboratory and also wanted to form his own group. Esteban had just published a key article for understanding the evolutionary dynamics of viruses, and together we decided to propose a project that would study the genetic variation of viruses that contain an RNA genome, from its most mechanistic aspects to those most closely related with population genetics. To do so, we suggested using two different complementary biological systems, the virus of foot-and-mouth disease (picornavirus with an RNA genome of positive polarity) and the influenza virus (orthomyxovirus with an RNA genome of negative polarity), which are also of great importance in animal and human health. For over ten years, Esteban and I shared laboratory 201 at the CBM, along with our students, postdoctoral researchers and technicians. Despite the very high population density, these were years of peaceful coexistence, sharing seminars, new experiences and very lively and productive scientific discussions. It was a pleasure to work with Mercedes Dávila and Conchi Martínez, lab technicians who started out with us; with the members of Esteban's group, Francisco Sobrino, Juan Carlos de la Torre, Nieves Villanueva, Encarna Martínez Salas, Mauricio García Mateu and Juana Díez; and with my students, Lucía del Río, Agustín Portela, Susana de la Luna, José Ángel López de Turiso, Juan Valcárcel, Paula Suárez and Puri Fortes.

During this time, my group evolved towards studying the structural, biochemical and genetic foundations of influenza virus replication so as to be able to analyse the mechanisms through which the virus varied genetically. In the first stage, we laid down the foundations of the study problem by analysing the genetic variation of natural influenza virus strains.[6] At the same time we established the experimental procedures for analysing the activity of viral RNA polymerase, the subject of our interest in the long term,[7] and we analysed the genetic bases of the virus's variation.[8]

We then enjoyed close cooperation with José Antonio Melero, who, first with Laboratorios Abelló and then with the Carlos III Health Institute, studied the antigenicity of the influenza virus. Here I would like to make a special mention of Amelia Nieto, a scientific collaborator of the CSIC, who joined our group in the late 1980s and with whom we have worked ever since. In the early years, Amelia was a member of our group, and she later created her own, but this has been no problem in terms of continuing our constant collaboration,



enjoying seminars together and receiving her scientific support and personal advice on all the decisions I have reached for decades.

In the late 1980s, the new National Biotechnology Centre (CNB) was created, and its then director and assistant director, Michael Parkhouse and Víctor Rubio, asked me to become a candidate for the position of one of its departments' directors. When they named me head of the Molecular and Cellular Biology Department I became a participant in and witness to the CNB's scientific organization, first under the direction of Michael and Víctor, and later under the direction of José L. Carrascosa and Mariano Esteban. Since we moved to the CNB, I have enjoyed working at a cutting-edge centre in Spain, with a stimulating, easy-going atmosphere.

Three decades with the influenza virus

In order to be able to study the influenza virus's replication machinery, we began our efforts from a very early time to clone and express in mammalian cells the viral genes involved in the process, the three subunits of polymerase PB1, PB2 and PA, the nucleoprotein NP, as well as the regulating protein NS1.[9,10] At the same time we developed gene transfer systems and, in collaboration with Antonio Jiménez's group, new selectable markers for mammalian cells. In fact, the marker for resistance to puromycin[11] is still one of the most widely used around the world today.

With this experience and technological ability at hand, we tackled the *in vivo* reconstitution of recombinant systems for replication of the influenza virus that allowed us to dissect the viral mechanisms involved. For instance, in collaboration with Agustín Portela, who had created his own group at the Carlos III Health Institute, we described strategies to reproduce the functional machinery of replication in uninfected cells. These instruments allowed us to work on structure-function studies of the polymerase's and nucleoprotein's subunits,[12] and to analyse the architecture of the polymerase heterotrimeric complex, as well as locating the domains of the polymerase that interact with the genomic RNA.[13] To do so, it was essential to produce collections of specific monoclonal antibodies for the subunits of the polymerase and nucleoprotein.

Having reached this point, it became clear that in order to deepen our knowledge about the viral replication process it was indispensable that we obtain structural data on the responsible machinery that could help us propose new hypotheses and test them directly. Given the existence of one of the most powerful electron microscope and three-dimensional reconstruction units at the CNB, led by José L. Carrascosa, José M. Valpuesta and José M. Carazo, it was obvious to us that we had to establish close collaboration with them to analyse the structure of the viral polymerase and the viral ribonucleoprotein (RNP) ensemble, the biologically relevant entity in replication and transcription. In the late 1990s one of my students, Joaquín Ortega, and one of the postdoctoral researchers of José L. Carrascosa, Jaime Martín-Benito, started a collaboration that has continued ever since, allowing us to become leaders on this topic worldwide. Because viral RNPs are 2-6 MDa-sized structures of great complexity, Joaquín created collections of mini-RNPs that were smaller but

fully functional *in vivo* and *in vitro*, to analyse their structure using an electron microscope, which is how they obtained the first detailed images of the viral machinery.[14]

The use of three-dimensional reconstruction techniques based on these data gave rise to the first structure of a biologically active RNP, including the polymerase complex, though with a very limited resolution level,[15] and the polymerase structure within the RNP was dissected by analysing monoclonal RNP-antibody complexes.[16] The structure of the free polymerase or polymerase bound to the viral genome RNA was determined using similar experimental strategies, in collaboration with Óscar Llorca,[17,18] as well as the structure of a domain of subunit PB2[19] and, last of all, the structure of the mini-RNP was determined with cryo-electro microscopy at a resolution of 12 to 18 Å.[20] Until recently, this information has been the most accurate available for any viral RNP and has made it possible to visualize the connections between the monomers of nucleoprotein and the polymerase complex.

Although recombinant mini-RNP is biologically active, its structure is different from that of the complete RNPs present in the viral particle. More recently, in collaboration with Jaime Martín-Benito, we determined the structure of these native RNPs by cryo-electron microscopy and cryo-electron tomography, which has made it possible to establish that these are left-handed helices with two antiparallel strands of nucleoprotein monomers associated with genomic RNA. Associated at one of the ends of this double helix is a polymerase complex, whereas the other contains a link that allows for a change in the strand's polarity. This structural model helps to interpret a series of biological processes involving viral transcription and replication, the generation of defective particles and the orderly packaging of the eight viral RNPs inside the viral particle, and it provides a foundation for proposing new hypotheses about the way in which this molecular machine works.

The gathering of structural, genetic and biochemical data[19,21] throughout these years made it possible for us to pose specific questions on the mechanisms that RNPs use to transcribe and replicate the genomic RNA that they contain. For instance, we asked whether the polymerase present in RNP is responsible for the synthesis of viral mRNA during transcription or for progeny RNP during replication. Using *in vivo* experiments with transcription and/or replication recombinant systems, we were able to propose new models that postulate a transcription process in *cis*, whereas the replication would be carried out in *trans* for a polymerase other than the one residing in the paternal RNP.[22,23] Moreover, the assembly of progeny RNP would be initiated by a third polymerase, different from the replicative polymerase and that associated with the template RNP, which would become a resident polymerase of the new RNP. New data published by other groups support these models, at least in part.[24]

Interaction of viral machinery with the infected cell

Another of the aspects that has most interested me throughout my study of the influenza virus is the interaction of the transcription machinery with the infected cell. To deal with these topics, our group successively used the different strategies that were set up for this type of analysis. For instance, in a first stage we used the double hybrid technique in yeasts to identify cellular factors that interact with the polymerase sub-

units of and with protein NS1. This protein was included in these studies because we had gathered clear evidence that it interferes with systems of cell splicing[25,26] and viral splicing,[27] affects viral translation and replication,[28] and interacts with the virus's transcription-replication machinery. Scanning a human gene library using NS1 as bait allowed us to identify the protein hStaufen,[29,30] the homologous human protein to the Staufen protein in *Drosophila melanogaster*. With it, we had the opportunity to study both the cell function of this new protein and its role during viral infection. The hStaufen protein turned out to be essential in the formation of mRNA transport granules,[31] for the specific recognition of a collection of cellular mRNAs[32] and for the proper dendritic branching of human neuroblasts *in vitro*.[33] Contrary to what we expected, the hStaufen protein is not involved in viral transcription-replication processes but, instead, in one of the late stages of infection, probably the encapsidation of the different RNPs in the viral particle, in accordance with prior genetic data produced by our group.

Initially, we scanned a human gene bank using the subunits of the polymerase as bait and identified the protein hCLE, of which there was practically no information at that time. Its study, as well as that of the CHD proteins also identified, has been a topic of work for Amelia Nieto's group in recent years and has made it possible to link the viral transcription-replication machinery with chromatin remodelling and cellular epigenetics processes. More recently, we identified cellular factors involved in viral transcription-replication by purifying intracellular complexes of recombinant polymerase or its subunits using affinity chromatography and proteomic analysis of cell components. In this way, nuclear factors were found that are involved in various processes as well as cytoplasmic factors that were stably associated with viral polymerase.[34] Most notable among them are importin alpha isoforms, which are differentially associated with diverse influenza viruses and are important for the activity of polymerase [34] and the SFPQ splicing factor, which does not alter viral splicing but is essential so that polymerase can synthesize the poly-A tail of viral mRNA.[35]

Last of all, we also carried out a more general analysis of the virus's interaction with the innate cell response to infection. Initial data from Adolfo García-Sastre's group indicated that protein NS1 is also a factor that blocks the innate cell response, and these results were later verified by us and many other groups.[36] As a result, lately we have carried out genetic examination using deep-sequencing techniques to analyse the relevance of various NS1 protein domains and those of other viral genes to counteract the innate immune response of the cell.[37] These studies have made it possible to conclude that the influenza virus adapts its entire genome to the existence of the innate cell response and undergoes an overall drift towards new optimal sequences when it is released from that selective pressure.

The Botín Foundation, a new way of understanding scientific work

A few years ago I had the pleasant surprise of receiving a call from Pedro García Barreno to ask me when he could visit me at the laboratory. I knew Pedro from several meetings, and because he is the brother of Blanca García Barreno, of the Carlos III Health Institute, with whom I have had the pleasure of collaborating on several occasions.

However, the surprise to me was much greater when Pedro paid his visit and began asking me to forgive him for not having visited me sooner. He continued by informing me that the Botín Foundation wanted to finance my research. It was the first time in my life that an institution was offering me financing without even having requested it, and I would like to express my profound gratitude to the Botín Foundation for this opportunity.

During the pleasant conversation that ensued with Pedro, and afterwards with Francisco Moreno, I was informed about the Botín Foundation, what its general objectives are and, in particular, the goals of its *Technology Transfer Programme* in which I was being invited to take part. From then on, I was able to tackle new goals in my work and above all to do so with a new mindset because the Botín Foundation's help was not just economic but also fundamentally allowed me to think about experiments in a different way. Throughout my career, I have many times come across discussions about the (apparent) dichotomy between "basic science" and "applied science" (also called "translational research" in biomedicine). I have always thought that what is truly important is to carry out innovative, high-quality research and that any potential applications would simply occur as a result of the aforementioned. However, it is true that if basic scientists are not on the lookout for possible uses of their findings those findings can be passed over and opportunities lost. The Botín Foundation knocked on my door precisely to avoid those problems, to help build bridges between basic results and their potential use to create new products and processes. At this point, the fundamental figure in the programme appeared, the project manager (in my case, Michael Tadros, who I express my profound thanks to for his work with us). He regularly met with our group to learn about our advancements, advise when or when not to divulge results and help us propose opportunities for collaboration with potentially interested companies, always backing our initiative in terms of the topics to be studied and the experimental focuses to be used.

In summary, the Foundation has worked with us on knowledge transfer topics in a proactive instead of a reactive way. By doing so, we have been freed of concerns in this sense, which has allowed us to devote ourselves to what we truly know and like to do, without losing technology transfer opportunities that might not have been obvious to a scientist. This has been a new way of working in science, which seems ideal to me and should be adopted by all those institutions that do scientific research in Spain.

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MARÍA DOMÍNGUEZ

MY LIFE AS A FLY SCIENTIST

20

One of the key challenges of science is that our discipline constantly revises and broadens original hypotheses by offering new concepts and discoveries that rule out the initial idea. Looking back over my career and what I have achieved, I believe I have helped to undermine a "dogma" or two. I have also suggested new hypotheses. I hope these have served as a springboard for others to formulate broader ideas and fresh concepts – or to show how I went wrong. I have focused on growth and form (morphogenesis). Anyone who loves art appreciates the aesthetics of proportion and symmetry. However, the way in which proportion and symmetry in human beings and other animals develop from an ordered and precise sequence of biological events still seems to me a deep mystery. The goal of my research is to help illuminate the factors that determine the proper growth, proportion and form of the various parts and organs of the living body. I believe that the main determinant is genetics. Yet I cannot deny the involvement of environmental influences. For instance, nutrition plays a role in determining size. Form, however, is set by genes. What better testimony to my thesis can there be than the resemblance of children to their parents. Some features – usually the least attractive – are inexorably inherited from parents to children and grandchildren by way of a family "hallmark". An understanding of the genetic basis of this determinant also helps us comprehend, diagnose and treat diseases that affect both normal growth and cancer.

I was the fourth-born of five siblings. Among them, I was the only one to take up science. My father is a retired judge though he continues to be active in the law with his scholarly writings and legal commentary. My mother, a highly intelligent woman who devoted herself to bringing up her children, played a major role both in my father's career and in our education. I am certain that my aptitudes for mathematics and drawing are inherited from her. I was born in 1965 in Alcántara, in the province of Cáceres. This was the home of my parents, my grandparents and my great-grandparents. When I was four, my family moved to Valencia. Later, we were posted to Rota, a coastal village in the province of Cadiz. Then we moved to Seville. I took a degree in Biology. My family continued to move in response to my father's various transfers and promotions. I believe these shifts explain the fact that my spoken voice does not betray any regional accent; nor do I feel rooted to any physical place in particular. I moved to Madrid to do a doctoral thesis at the Autonomous University of Madrid (UAM) – the "Severo Ochoa" Centre of Molecular Biology (CBMSO) specifically – obtaining my PhD in April 1993. Then I undertook postdoctoral training: at Zurich from 1993 to 1996, and at Cambridge from 1997 to 2000. In January 2000 I returned to Spain, and obtained a position as "*Cientifico Titular*" from the Spanish Research Council (CSIC) at the Institute of Neurosciences (IN) in Alicante, where I still work today. My key influences have been Professors Juan Modolell, Antonio García-Bellido, Peter A. Lawrence and Ernst Hafen. I am indebted to them for their insightful critiques and unstinting support.

From the outset I took an interest in chemistry. In the latter years of my biology degree at Seville, from 1986 to 1988, I worked at the Biochemistry Department, then headed by Professor Manuel Losada. However, the biochemistry of photosynthesis failed to fire my imagination. I did not particularly stand out in my work, either. And yet those years enabled me to absorb basic knowledge and concepts about scientific exploration and encouraged me to undertake a research career, albeit in other fields. In my final year, 1987–88, I took a "Developmental Biology" module taught by Professor Enrique Cerdá Olmedo. Cerdá was the first Professor of Genetics to be appointed at the University of Seville, and in 1969 had founded the Department of Genetics. He pioneered the use of microbial genetics to decipher complex biological mechanisms. He made a wealth of contributions to our understanding of the biology of the *Escherichia* and *Salmonella* bacteria and the *Phycomyces*, *Blakeslea*, *Fusarium* and *Saccharomyces* fungi, and their biotechnological applications. His work has formed the basis for hundreds of research projects involving proteins and complex biological processes. Enrique Cerdá was and continues to be an outstanding public speaker. He was cultivated and intelligent and – why not say so – a bit abrasive. Some of his students did not appreciate this trait as perhaps they should have. He never ceased to challenge us. In me, he awakened a genuine interest in developmental biology, to which I have remained loyal throughout the rest of my career.

I took finals in June and was awarded a prize by the Compañía Eléctrica Sevillana in 1988. Then I undertook doctoral study in Madrid. I had earlier worked as an intern at several laboratories, but I must say that my research career really began at the CBMSO in the laboratory headed by Juan Modolell, under the immediate supervision of Sonsoles Campuzano. I have worked on *Drosophila* ever since, and am likely to continue to do so until I retire: I am a devout "fly" scientist. I believe that this experimental animal model offers unparalleled advantages for finding answers to certain questions. A wealth of research has shown that *Drosophila* is an exceptionally good organism for researching the mechanisms of the determinants of growth. However, despite extensive research and the clinical significance of these determinants, we are still at a great distance from understanding how the cells of an organ, the organ itself and the entire organism measure, adjust and maintain their dimensions. We have studied these problems for years, applying genetics, molecular biology, biochemistry, cell biology and a few hunches.

In the 1980s Juan Modolell's laboratory published pioneering papers involving pro-neural genes – particularly involving a gene complex called "achaete-scute", which is responsible for the majority of the fly's sensory organs called bristles. Their highly specific patterns are modified by certain mutations in the achaete-scute complex that were very hard to explain. Antonio García-Bellido, among others, had already studied the genetics of these mutations. But Juan Modolell was the first to apply molecular biology to clone and sequence these genes. My doctoral thesis analysed the fourth and least-known gene of the achaete-scute complex, a gene known as asense. Our research uncovered significant differences between this gene and the other pro-neural genes, including differences of regulation and function. The greater part of my thesis was published in 1993 in an article in *EMBO Journal*. Sonsoles and I were mentioned as the only authors. This scant but apparently distinguished scientific output won me a scholarship from the Ramón

Areces Foundation and, soon afterwards, a highly prestigious bursary from the Human Frontier Science Program (HFSP).

My years in Madrid and my experience of genetics are inextricably linked to the team headed by Antonio García-Bellido, which then comprised Marcos González Gaitán and Fernando Díaz Benjumea – both, like me, from Seville – and José Félix de Celis and Marco Milán, who are all distinguished scientists today. Antonio García-Bellido chaired the examining board for my thesis, and ever since my relationship with him has been outstandingly good.

I moved to Zurich in September 1993. I began postdoctoral research at the laboratory headed by Professor Ernst Hafen, of the Department of Zoology, then led by Dr Rüdiger Wehner, who was well known for his work on the navigation system of desert ants. I have few good memories if any of my stay at Ernst Hafen's laboratory, although, perhaps paradoxically, this was one of the happiest periods of my life. I fell in love with Zurich, and have stayed in close touch with many colleagues and friends of that time. During those years I worked tirelessly and for the most part alone, and for that reason many of my papers of that time carry my name as leading author. Most of this research was published in reputable journals. One particular article that appeared in *Science* in 1996 is still my most cited paper to this day. These findings later took on special relevance when it was discovered that they had a direct impact on processes relating to human health, such as cancer. At this time I first met Professor Walter Gehring in Basel, where Ernst Hafen had defended his thesis. Later, Gehring invited me to several meetings he was hosting on retina development in *Drosophila* flies. This enabled me to contact other researchers working on Pax6 genes, which Gehring had discovered in the 1990s. Walter Gehring and his team discovered the genetic network associated with this factor, which is responsible for specifying the retina. They found that the process was conserved to an amazing extent throughout the animal scale, despite the morphological and ontogenic differences of the different types of eyes. Further analysis in flies turned up a pair of related Pax6-like genes, on which I worked intensely during my first stage at Alicante. These findings on the genetics of eyes prompted a frequent crossing of paths with Gehring. In 2014, just before a research convention (on flies) in Crete, Gehring was killed in a car crash. This seems to me to have been the end of a chapter in the research on the genetics of eye development.

In the summer of 1996, at a meeting of the European Molecular Biology Organization (EMBO) in Heidelberg, I met Peter Lawrence. He was impressed with the work I was involved in and offered to support me in continued efforts on an independent basis at his laboratory in Cambridge. I moved to Cambridge in January 1997 and stayed for more than three years of postdoctoral work at the Medical Research Council-Laboratory of Molecular Biology (MRC-LMB). At Cambridge I continued my research on the role of the Hedgehog pathway in the early development of the retina. It was there that I developed most of the tools and modified flies that later enabled me to establish my own team at Alicante. To work in Cambridge and at the MRC-LMB specifically was a real privilege that I did not fully appreciate until I moved to Alicante.

I worked both on my own and in cooperation with other Cambridge-based researchers. A highlight was my collaboration with Jonathan Wasserman, a student supervised by Matthew Freeman, on the regulation and function of the EGF-R/Ras oncogenic pathway in retina proliferation, survival and differentiation. Our paper was published in 1998 in *Current Biology*. This article has had a great impact and is still widely cited. It was also at this time that I started to research the Notch pathway in relation to eye development in *Drosophila*. This has been the focus of my work for the past few years, and has laid the foundations of present lines of research on cancer mechanisms in connection with the Notch pathway. My first paper on Notch, in collaboration with José Félix de Celis, was published in *Nature* in 1998. Two later papers, one of them in cooperation with the student Florencia Cavodeassi and Sonsoles Campuzano, my former thesis supervisor, provided new evidence underpinning the Notch-pathway-based growth model we had proposed, which is widely accepted to this day. Later research – conducted when I was already in Alicante, and published for instance in *Nature Genetics* in 2004, *EMBO Reports* in 2009 and *EMBO Journal* in 2011 – has corroborated and extended the original hypothesis.

In 2000 I returned to Spain. I had obtained tenure at the CSIC (the Spanish National Research Council), and was posted to the Institute of Neurosciences (IN) attached to the Miguel Hernández University (UMH) in Elche, Alicante province. The IN had just signed an agreement with the CSIC. Carlos Belmonte, who founded and for many years was head of the IN, had recruited the Madrid-born neurobiologist Fernando Jiménez, on the basis of his findings on the nervous system in *Drosophila* flies. Sadly, soon after joining the IN, Fernando died of a heart attack. His "disciples" and I inherited his equipment and laboratory space. Physically, the IN was then located in the "departments building" of the UMH on the Sant Joan campus. Though well equipped, the laboratories were scattered and isolated from one another, which limited group interaction and cooperation. In 2004 the IN moved to its own building. Today, it employs twenty-six researchers, who work on neuronal plasticity, neuro-protection and neuro-repair, molecular systems neurobiology, neuron migration, glial cells, memory and learning and so on. The IN is now a dynamic research centre that enjoys an international reputation.

In 2001, soon after taking up my new job, I was selected for the EMBO Young Investigator Programme. This accolade was more about recognition than funding, but it nonetheless enabled me to establish networks and partnerships in Spain and Europe involving other young research scientists. In 2005 I was promoted to the status of "Investigator" by CSIC, followed by a promotion to full professor in 2007. I was also elected a member of EMBO. During those years I undertook management duties (deputy director from 2002 to June 2005) and sat on the CSIC Biology and Biomedicine Committee (from December 2004 to 2008). These commitments distracted me from my true vocation – research – but also afforded me a broader view of the Spanish research landscape and of the intersections between science and policymaking.

Our beginnings at Alicante were tough. However, with the support of other established scientists and the director, Carlos Belmonte, I was able to continue my research and keep up a high standard of scientific achievement. First I published a paper (co-authored with

another researcher) in *Nature* in 2001. Three years later, I published an article in *Nature Genetics*. This was my first paper produced entirely at Alicante. It was reviewed in *News & Views* – featured on the front page – and selected by *Faculty of 1000*. Following a riskier line of research than we had pursued so far, we created modified or "humanized" flies into which we inserted a human gene to rule out an initial idea about an oncogenic mechanism that had been suggested years before but hitherto analysed only in cell culture. The sequencing of the fly and human genomes had found that over sixty per cent of genes involved in human disease and seventy per cent of genes determining the cell cycle and cell death (these mechanisms being closely linked to cancer) were present in flies. As a rule the fly genome is a simplified version of the human genome. In this case, however, the fly has four versions of the Pax6 gene (two canonical Pax6 genes and two Pax6-like genes), while the human genome has only one. Yet that single human PAX6 gene codes for several different forms of the protein, although only the canonical version had been considered and studied. In 2004 our research on non-canonical Pax6 genes identified them as the oncogenic forms, in addition to enabling us to describe the early development of eyes in flies in a new way. This work using human genes in flies gave us an insight into the high degree of conservation that supports the extrapolation of basic and general principles using genetic models in flies. This in turn also placed us in a position to propose novel cancer mechanisms. Four years earlier, fly-based research on the source of cancer had been circumscribed to only a few laboratories in the world, although many development-related investigations in flies had in fact served as the basis for extrapolating discoveries to the understanding of cancer mechanisms in humans. Our first study of 2004 prompted us to investigate more thoroughly the process of cancer using flies.

How could an insect equipped with only four chromosomes and destined for a lifetime of just eleven days help anyone to understand a complex disease like cancer, which sometimes develops over a period of years? Significant pioneering work had already been done on cancer in flies. In the 1960s, for instance, Elisabeth Gateff and others had described malign tumours of genetic origin in flies. However, for many cancer scientists this research went unnoticed. These pioneering enquiries proved the existence of tumour-suppressor genes and the importance of the antitumoural function of the immune system. Our work at Alicante has shown, for instance, that causes of cancer that had so far been regarded as independent are in fact connected. By 2004 cancer was already viewed as a disease the source of which was to be found not only in genetic anomalies but also in epigenetic alterations of gene expression. The potential for reversing epigenetic changes led to an explosion of possible epigenetic therapies for treating cancer. In normal cells the epigenetic transmission is essential to preserve cell memory, and to help maintain cell identity and the pluripotentiality and plasticity of stem cells. It is therefore unsurprising that many epigenetic factors are altered in different types of invasive carcinoma, lymphoma and leukaemia. However, owing to the importance of these factors in healthy cells, a therapy targeting them must be based on our best understanding of how cancer springs from them, and of why those epigenetic alterations turn healthy cells into cancer precursors. One of our first research projects on cancer conducted in the early years at Alicante, and which led to a paper published in *Nature* in 2006, discovered an unexpected cooperation between the Notch pathway and epigenetic silencing pathways in tumour initiation

and progression. This work was followed by related research and enabled us to establish collaborations with other scientists in the field of the Notch pathway and epigenetics. The finding that discoveries in flies were conserved in tumoural processes in humans enabled us to firmly establish the *Drosophila* model. Our research in 2006 had a wide scientific impact, even beyond the *Drosophila* field. It was mentioned in numerous reviews and recognized by *Faculty of 1000* as "exceptional". Our paper spent several weeks among the top ten life science articles in the world (fourth place) at *Faculty of 1000*, and at the top of articles on development biology. The tools and screening method to interrogate the fly genome with which we detected the connection between the Notch pathway, epigenetics and other cancer mechanisms in *Drosophila* flies are now used by many laboratories around the world. This work raised our international visibility and helped us to attract Spanish, foreign and private funding. In particular, it led to our agreement with the Botín Foundation.

In January 2007, in collaboration with the team headed by Dr Adolfo Ferrando of the Institute for Cancer Genetics attached to Columbia University in New York, we published a paper explaining the connection between the Notch pathway and the PI3K/AKT oncogenic pathway in the formation of leukaemias in humans and invasive tumours in *Drosophila* flies. At present there is no treatment for this type of leukaemia, which is particularly aggressive when it develops in adults. The new "-omics" technologies, combined with genetic analysis in intact animals, may turn out to be a highly useful and effective preclinical pharmacological strategy to develop new therapeutic tools for cancer. Four years ago, largely funded by the Botín Foundation and a European programme, we implemented this strategy in the laboratory, focusing on metastasis and Notch-Akt tumours – a so far incurable type. "-omics" technologies are expensive and the development and screening of compounds is a protracted process, even in short-lived animals such as flies. This strategy has been set in motion contemporaneously with ongoing decision making and interaction with Botín Foundation officers. This has enhanced our effectiveness and, we hope, will aid a rational use of the knowledge we have produced.

The Botín Foundation made us understand the importance of protecting any progress that might have practical applications. At our laboratory we work with several cancer models, and we have used various models for systematic (high-yield and extensive) screening using flies. Systematic screening is the riskiest phase in the discovery of new compounds and drugs, and implementation and proper use of flies may constitute a valuable preclinical alternative.

In 2008 our fundamental cancer research was awarded the Francisco Cobos Prize in Biomedical Research. We were powerfully motivated by this accolade. Genomic analysis of the effects and consequences of cancer in flies using "-omics" technologies led us to further unexpected discoveries, such as insulin/relaxin-like peptide 8 (ILP8), published in a paper in *Science* in 2012. For years we had placed excessive emphasis on the autonomous determination of organ growth, and cancer research had been based on cell culture. But neither organ growth nor cancer occurs in isolation (in a Petri dish); rather, it happens in the context of a complete organism. Every organ and every body-part must grow harmonically and in proportion to the other parts. This means that signal-mediated coordination

and communication exists to an extent that had previously been unsuspected. These signals also appear to play a role in oncogenic processes. In 2012 the discovery of ILP8 in my laboratory turned out to be key to understanding how developing organs are involved in this communication. Flies that are deficient in this peptide of the insulin/relaxin family hormone are unable to maintain control of size and are more varied in their final size than their wild type, normal flies. The lack of this hormone also produces animals that are bilaterally asymmetrical. Various chronic diseases and environmental issues may delay growth and development in children and adolescents, leading to adults who are short in stature and display facial and body asymmetries. In many cases a spontaneous "catch-up" growth takes place, such that the affected children reach an adult stature close to the normal range and malformations are repaired and unnoticed in the adult. In our research we found that, in flies, numerous growth-altering circumstances trigger activation of the ILP8 gene, which is then responsible for catch-up growth and development, allowing a harmonious growth control as a whole. Our work opened up new avenues of research into the biological foundations of certain problems of juvenile growth and, beyond that, prompted a paradigm shift in the field, having shown how, through inter-organ communication and brain-driven mechanisms, juvenile organisms are capable of recovering and producing adults of a normal size even when faced with alterations, injuries and adverse environmental challenges. New findings on the receptor of this type of insulin (the data are still to be published) may explain how these processes are guided throughout the development of the bodily pattern of the fly and other more complex animals.

So my research focus has been on the genetic determination of growth and for many of the resulting discoveries and recognition, I am greatly indebted to the Botín Foundation. I thank the Botín Foundation for its genuine interest in the specific needs of research scientists and their work and, above all, for the Botín Foundation's desire that the partnership should move forward throughout these five years.

I am also indebted to my family, mainly my husband. Throughout the many years during which I have researched *Drosophila* flies, he has patiently given up many weekends and nights while I was in the lab or writing manuscripts or grant applications. He has forgone his career to encourage and support my own.

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JOSEP SAMITIER

MEMORIES

21

The use of nanotechnology in the field of biomedicine did not truly emerge until late in the year 2000, when the president of the United States, Bill Clinton, began an intense campaign to promote the development of research on nanotechnology through the well-known National Nanotechnology Initiative (NNI). At that time advances in nanotechnology were proposed from two different focuses: the top-down approach, or how to miniaturize complex systems, and the bottom-up approach, or how to build materials or devices based on the individual control of thousands of atoms or molecules. That is how my personal and professional advancement has worked as well; sometimes from the top down and sometimes from the bottom up.

In 2000, when the Clinton administration started up the NNI with the goal of promoting the discovery, development and deployment of science, engineering and technology at the nanoscale, research underwent a natural progression that shifted from studying microsystems to nanosystems. For example, electronics evolved to include more functions in smaller circuits, moving towards sizes at the nanoscale.

For about fifteen years I worked on developing microsystems based on silicon, with the goal of adding different physical and chemical functions "on-a-chip", such as pressure sensors, accelerometers, micropumps and different types of integrated chemical sensors, which, along with microelectronics, could help us obtain relevant information from the environment or from our own bodies. Therefore, this advancement in research towards a decrease in size, the ability to include more functions and a reduction in the size of circuits, sensors and all the other parts that work in systems allowed my career to move from the microscale towards the exploration of new possibilities in functional engineering systems at the nanoscale. This was the top-down focus.

Almost at the same time the bottom-up approach revolved around the basic principles of physics and chemistry. From this perspective, the objective is to control and modify the properties of the materials that are used at the nanoscale with the intention of building new complex systems. Using the same biomolecule assembly principles that we see in biological systems, we can develop surfaces or 3D structures with molecules and atoms organized in the same way, using powerful microscope systems, such as the atomic force or scanning microscope, to detect new structures. That is how my professional career progressed. In actual fact, it had begun outside basic physics, in the field of electronics, from a bottom-up focus.

Around the year 2000 these two focuses merged, which made it easier to apply nanotechnology to different realms, because if you want to use nanotechnology for applications in

the real world, you have to integrate them along with macro and micro devices as part of a system, such as a mobile telephone or medical device. For me, it was a natural move forward to integrate nanotechnology into the development of microsystems using the basic principles of physics.

Just like Clinton's initiative, both originated in the United States. The bottom-up approach was an initiative by American chemists and physicists who were already working in that field and who were immediately followed by the Europeans, beginning to integrate nanotechnology in order to develop their own systems.

The difference lay in the fact that in Europe a feeling of mistrust arose within society, just the opposite to what happened in the United States when nanotechnology began to emerge, and nanotechnology was not well received. At that time the European public was very sensitive to everything related to genetic manipulation, which was perceived as a threat that jeopardized health.

Here, a great uproar has been caused by several books that exaggerated the potential threats of nanotechnology, such as the novel *Prey* by Michael Crichton, published in 2002. Although books like *Prey* had come from the United States, nanotechnology did not arouse the same feeling of mistrust there, or, if it did, it was not as widespread a sentiment as it became in Europe. Perhaps this is because the public's doubts and fears had already been dealt with. In Europe, on the other hand, there were certain minority groups that made terrifying claims about the potential dangers, and they explained that very small particles produced in large amounts could get released into the atmosphere and enter our bodies and cells. The fact that they were small (invisible) but intelligent particles (capable of following pre-established instructions) seemed to be what most concerned people.

In fact, in the first few months after my laboratory moved from the facilities at the University of Barcelona to the Barcelona Science Park (PCB) in 2003, we were already aware of the negative sentiment. Graffiti appeared in the streets nearby with messages like "Danger! They are working on nanotechnology here!" Of course, it was our responsibility to respond and calm people down, so we organized informational activities to explain what our research consisted of, above all so that people would know that there was no danger because we scientists were working with a great sense of responsibility to minimize the risks.

We worked hard to explain to society that nanotechnology did not always involve working with invisible and seemingly free nanoparticles; it is, rather, a way to control and manipulate matter at the nano level with great precision. Nanoparticles are simply a sub-element of nanotechnology, and most of the time when nanoparticles are used, they consist of solutions inside a device, not in the air.

Along with the PCB we also promoted the new Nanotechnology Platform that we had there – it is now the responsibility of the organization that I direct today, the Institute for Bioengineering of Catalonia (IBEC) – and explained the different initiatives to which it contributes and in what way nanotechnology could be beneficial to people's health and society,

as well as what the real risks were. However, it was clear that something had to be done in coordination with other laboratories to inform people and help them become more familiar with what we were doing and how nanotechnology was advancing.

In 2003 the European Commission approved Nano2Life, the first European network of excellence in nanobiotechnology for biomedical applications, as part of the Sixth Framework Programme. Its main goal was to coordinate different groups and people who were working in nanotechnology into one single collaborative network, but it was also meant to demonstrate how useful nanotechnology is in many fields and how important it could become in medical advancements. Nano2Life was the first major European initiative, and shortly afterwards the European Science Foundation also approved a document on nanomedicine – a new term at that time – focusing on nanotechnology for the administration of drugs. These were perhaps the two most important decisions leading to the coordination and promotion of all the activities related to the nanotechnology field in the realm of biomedicine in Europe, and they truly foretold real change in the general public's perception.

Today, the way in which society perceives nanotechnology is more positive. All the projects and initiatives involving nanotechnology and its advantages, which clarify the true scope of its risks, have been productive, and therefore people have a better understanding about how the benefits more than outweigh any potential risks. Used in medicine, they could clearly see that this was a new and effective focus in developing new testing systems and therapies.

My relationship with Nano2Life goes back to its creation, when we were the only group in Spain involved in the excellence network. At that time my nanobioengineering laboratory was located at the PCB and was in part a precursor to IBEC. Nano2Life also involved people and groups from countries such as France, Germany, Israel, Sweden and Switzerland, so it became a powerful pan-European network. The advancement of Nano2Life led to the creation of the European Nanomedicine Technology Platform in 2005.

We were selected by our European colleagues as the representative of Spain in Nano2Life for various reasons, despite the fact that, at that time, there were several groups in the Barcelona area and many others in the rest of the country who were already working on nanotechnology. We played a decisive role in creating the Nanotechnology Platform at the PCB, with the objective of offering state-of-the-art equipment to produce and characterize microdevices and nanodevices and structures for biomedicine with the desire to seek public-private collaboration. Moreover, I was very involved in the founding and management of what would later become IBEC, a research organization that would be dedicated to this new concept of nanomedicine, as well as to bioengineering. At that time we had also signed an agreement with the European Science Foundation (ESF) to set up summer school courses on nanomedicine throughout Catalonia. Together with the Carlos III Health Institute, the ESF formed part of the commission to create a national virtual centre dedicated to bioengineering, biomedicine and nanomedicine, which later became the Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine, the CIBER-BBN.

In 2004, just four years after nanotechnology truly began to emerge as a prospect in biomedical research, Barcelona appeared in several European reports as one of the five most important European groups in nanobioengineering. It was astonishing how fast it was welcomed and progress was made in this field, with the cooperation of many colleagues, and the speed with which it has been able to produce concrete results that may be turned into new testing systems or therapies. Professionally, these have been highly satisfying years in which I have focused on nanobioengineering as a field of research. I have always felt attracted to topics and fields of research that might lead to rapid growth, progress and change, as is the case with microelectronics and the many applications in which it results. I have based my entire career on the use of basic concepts, or on basic research with a strong focus on applications within the real world, in fields in which advancement was taking place very rapidly, such as microelectronics, microsystems and nanomedicine.

This fascination began when I was still quite young. I do not come from a scientific or academic family; in fact, I was the first in my family to go to university. My parents came from a very small town in Catalonia, located approximately a hundred kilometres south of Barcelona in the Penedés region, where my grandparents were farmers. After my parents got married in the late 1950s, they moved to the city because the economic boom that had begun by then in Spain after the Spanish Civil War – in which my maternal grandfather died in a labour camp, leaving my mother an orphan – offered many more opportunities.

I began primary school at the same time as my father found work in the construction sector. We then lived in the doorman's flat of a building whose owner was responsible for some of the most important construction projects in Barcelona. When I started school, that man's son began studying engineering, and from time to time he would come over to tell me stories about life at university and all the opportunities it created, including some "foot races" between students and the police. It sounded like a fascinating world, and I could not avoid being taken over by his enthusiasm. That is why, at the age of just eight, I had already told my father I wanted to go to university when the time came. Another thing that also influenced me was the moon landing in 1969. I watched it on television with my parents, and though I was still very young at the time, I remember it most vividly. I was already starting to become interested in topics involving this subject, the universe and the rules of physics.

In that period it was very difficult to go to university in Spain, not just because of the cost, but also because society was very class-oriented and the university was reserved for a select few. Sociologically, I form part of the generation whose parents were workers in the 1960s who managed to put their children through school only through great effort, as a way to improve their professional skills. When I started secondary school, I proved to be very good at sciences, which always appealed to me more than the humanities, and I was one of the most notable students at school, I believe in part due to the luck of having very good teachers in mathematics and physics, both excellent women teachers who stimulated my curiosity. At that age, having the right teacher can make a major difference.

At the age of fourteen, I wanted to study something related to the sciences, mathematics or perhaps even medicine. In the end, I opted for physics. To me it seemed like a good way

to remain close to the field of mathematics, while at the same time applying the knowledge I acquired to solving real problems. At that time I was already certain that I wanted to follow a path that would allow me not only to study theory, but also to get a focus on applications in the real world.

My mother was a bit surprised when I decided to study physics; I remember that she insistently asked me what I would work in afterwards because she saw no relationship between majoring in physics and any profession of the future. From that time, I also remember that my earliest days at university coincided with the premiere of the movie *Star Wars*, by George Lucas, and because of that concepts more or less related with physics started to become very popular.

I finished my undergraduate studies in 1982, right at the time when work was beginning on IBM's first personal computer. During the last year of my undergraduate studies at university, I used a Commodore with diskettes and 128 kB of memory, and I learned the programming languages popular at that time such as Basic and Fortran. When I got my first 5 MB hard drive, it was like a miracle: five whole megabytes! I remember that I thought it would be impossible to fill.

Although I wanted to understand the principles of physics, I also had the aspiration of attempting to relate them to real applications. That is why, instead of specializing in theoretical physics, I began to apply physics to materials. I have used physics to be able to understand the properties and characteristics of materials and matter and to create better devices. When I left to do my doctorate, I spent some time from 1984 to 1985 as a visiting researcher at the Philips Electronics Laboratory in Paris. At that time I was already involved in studying semiconductors made of elements in groups III and V of the periodic table, such as gallium arsenide, gallium aluminium-arsenide or gallium phosphorus-arsenide, which have constituted the foundation for developing laser and LED semiconductor devices. The first article I got published discussed this topic, with red, green and white LEDs. In this sense, I was satisfied that the work by Isamu Akasaki, Hiroshi Amano and Shuji Nakamura, creators of the blue LED, was acknowledged with the Nobel Prize in Physics in the year of 2014.

This is an example of what was being done at that time which would later come to form part of the field of nanotechnology. Back then, we did not call it "nano", but it was clear that the semiconductor industry offered a potential application of this new focus, due to the properties that arise upon creating nanostructures using epitaxial processes that make quantifications at the nanoscale possible. For example, in order to develop a laser using electronic semiconductors, you need to produce nanostructures to stack materials with different properties in the energy states that an electron can hold, to be able to complete a population inversion (electrons occupy higher energy levels, leaving the lower energy levels free) in order to achieve the creation of the coherent beam of photons characteristic of lasers.

We were therefore applying quantum mechanics to the development of real devices. It was the first opportunity I got to use the general principles of physics with a specific objective and use in mind.

Starting in the 1980s, studying semiconductor materials used in electronics evolved at a spectacular speed, and my field of interest quickly shifted from compound semiconductors to the development of microsystems. I concentrated on attempting to integrate mechanical functions, such as pressure sensors or accelerometers, on the same chip as the microelectronics. Just at that time I got involved in a European project with Robert Bosch GmbH, Fraunhofer and SEAT to develop 3D accelerometers for airbags, which allowed me to obtain my first patents. This project made a significant contribution to turning airbags into a standard feature during the production of automobiles in Europe. After this came a considerable number of European projects devoted to a wide range of microsystem developments based on the control and use of the surface or structural properties of materials. Shortly after that, when nanotechnology began to emerge, it was obvious that its advancement would occur very quickly. We already had the ability to control materials and develop new and more precise technologies, so the potential for developing new applications and integrating them into real-world devices would become very immediate. It was also clear that, in the field of medicine in particular, the use and control of materials in the same range as cells, bacteria, viruses or biomolecules would allow the rapid development of new applications that had not been possible in the past.

One very important mentor of mine was Joan Ramón Morante. When I completed my doctorate, he was the director of my thesis on the characterization of semiconductor materials, and after the thesis we continued working together in that field for some time. During those years, the National Microelectronics Centre (CNM) was created, where the applied research on microelectronics has been carried out in our country under the direction of Professors Francesc Serra Mestres and Emilio Lora Tamayo (current president of the CSIC). However, I must acknowledge that, beyond the many instances of collaboration and individual influences, first by my professors and tutors, and later by my colleagues in joint collaboration projects, I have always been stimulated by the possibility of creating structures that would make it possible to deal with scientific and technological challenges that reach beyond just my own individual effort. Talent is personal, but a good scientific institution can serve to attract that talent and take on challenges that it would not be feasible to deal with if there were not a platform for mutual assistance.

In this sense, the learning and knowledge at centres such as the LEP in Paris, the CNM, the LAAS-CNRS in Toulouse, the CEA-Minatec in Grenoble, the Fraunhofer Institute, the Max Planck Institute, MIT and the Star Alliance in Singapore have helped me to attempt to implement the best strategies for performing cooperative research.

When I obtained a permanent position at the University of Barcelona, after my stay in Paris and an exchange period at the CNRS in Toulouse, I began to progress in work on microsystems. At that time I was starting a new research group and had my first doctoral student. We began to work on the use of microsystems for biomedical purposes. That was the beginning of my shift towards biomedical engineering.

The first step was to attempt to develop microsystems that use dielectrophoresis. The concept was based on using microelectrodes in a microfluidic system to apply electrical fields

that could trap or move cells in a specific way, to control cells within the microsystem or distinguish them from one another, depending upon their characteristics. First we did this with nanoparticles and later with the cells themselves.

Another application of this new "nano" concept was based on controlling the surface of materials using nanotechnology to control the behaviour of cell cultures on them. We wanted to know how we could define or modify the proliferation, adherence or differentiation of the cells by modifying superficial characteristics such as topography or the chemical bonds on a surface. In fact, this has been one of my main research topics up to today. In that moment, we were partners in an important European project, the largest project integrated within the nanotechnology priority forming part of the European Commission's Sixth Framework Programme, coordinated by the Fraunhofer Institute for Biomedical Engineering in Germany. The project, which was called *Cellular Programming of Devices at the Nanoscale* (CellPROM), had the objective of controlling the differentiation of cells in a precise, non-invasive manner. The chance to work with the director of the Fraunhofer Institute, the biophysicist, professor and doctor Günter R. Fuhr, was also a fundamental influence on my research activity.

At that time the management and direction of projects formed an increasingly important part of the work in my career. My laboratory was already involved in developing new technologies, such as soft lithography and nanoprinting, processes and systems that could be used to manage the surface or structures of polymers or other materials. This was the stage I had reached prior to the founding of IBEC in 2005, when several groups at the University of Barcelona and the Polytechnic University of Catalonia met to form a research organization dedicated to bioengineering and nanomedicine.

First of all, we created a mixed research unit within the University of Barcelona dedicated to bioelectronics and nanobioengineering, which Antonio Juárez, Daniel Navajas, Fausto Sanz and I formed part of. In conjunction with this, we began similar collaborative work with the Polytechnic University of Catalonia, and more specifically with Josep A. Planell (who would become the first director of IBEC), Pere Caminal, Raimon Jané and Alícia Casals.

I was the youngest main researcher who formed part of the joint research unit at the University of Barcelona, but even so they decided I should become its director. My involvement in the PCB's management provided an opportunity to physically move the joint unit there, and because the collaboration alliance with the Polytechnic University of Catalonia also remained in force, we were able to combine the two entities at the new physics site.

As a result of the decision to unite both organizations, Professor Josep A. Planell, who was the director of the equivalent research unit within the Polytechnic University of Catalonia, and I worked jointly to ask the Catalan government to create the Bioengineering Reference Centre of Catalonia (CREBEC), the precursor of IBEC, as a stable collaboration network.

The joint work at the CREBEC led us to further the idea of creating a stronger research structure in the form of an institute with its own legal status as a private non-profit foun-

dation dedicated to interdisciplinary research on bioengineering and nanomedicine. Together, we prepared the documentation and began the negotiations with the Catalan government and the two universities, which later became IBEC's founding entities. When we were involved in this process, the University of Barcelona offered me the position of vice-rector of International Policy, and I accepted due to my institutional commitment and my friendship with the rector, Màrius Rubiralta, who had been the promoter and director of the PCB. From Màrius Rubiralta, who is a great visionary of research administration, I have learned the importance that the management of scientific policy can have on the promotion and improvement of research organizations. When, in April 2008, Rubiralta was named State Secretary for Universities, I replaced him as the acting rector of the University of Barcelona.

It was, therefore, impossible for me to continue working as a full-time researcher and at the same time remain involved in these management positions, but when my commitment to the university ended in 2008, after losing the elections to become rector of the University of Barcelona by a handful of votes, I got the opportunity to focus once again on doing my research at IBEC, and I then became involved in promoting that institution as an associate director. For me, the experience in management at the University of Barcelona, though short, was very illustrative in terms of understanding the problems that universities in our country are experiencing, to see how they have changed in recent years and to understand the need for introducing reforms in the way they are organized if we hope to get them to reach the level found in other cutting-edge European countries. Focusing on my work at IBEC was very gratifying because it was a small, flexible research organization dedicated to my areas of interest, and consequently I could feel how important it was to be managing the organization's results much more closely, while at the same time taking part in that organization.

Returning to research was not easy. I had spent more than three years juggling it with management tasks, and therefore as a main researcher I had to submit new projects, design new research strategies and get new doctoral students and postdoctoral researchers into the group, as well as building relationships for collaboration with other researchers.

It was in this period that I was presented with the wonderful opportunity to collaborate with the Botín Foundation. In December 2008 I was paid a visit by the Foundation, which offered me the chance to take part in a new programme intended for promoting research in applied fields of biomedical engineering. The idea was that the programme would not only finance some research projects, but would also help to increase our awareness and understanding of how to evaluate the knowledge produced in a research laboratory to transfer it into the form of patents or technology, or to translate ideas and inventions into a new product or service that benefits society. Within this context I began to collaborate with the Botín Foundation, which made excellent managers of technology transfer topics available to me, so as to analyse the new ideas and results obtained at my IBEC nanobioengineering laboratory. That was a very interesting and productive process and, though our formal collaboration officially ended in late 2013, we continue cooperating with the Foundation even today.

Although my plan was to focus on continuing to build and develop my research group to the greatest extent possible, something unexpected changed things in 2013: Professor Planell announced his decision to leave IBEC's administration to become the rector of the Open University of Catalonia (UOC). It was the right time to accept the opportunity being offered to me to become the new director of IBEC.

My current plan, at least for the next eight to ten years, is to continue guiding IBEC's growth and advancement. For a young research institute like ours, I believe this new era will make it possible for us to establish and build on our institutional activities, as well as achieve the proper development and progress in our lines of research so that they may become strong assets for the organization.

I continue working at the University of Barcelona as a part-time professor. I have remained involved by developing and running some of its undergraduate and Master's degree programmes, including the recent graduation of the first class of students in the Biomedical Engineering degree programme, of which Professor Navajas and I were the promoters. That was a very special moment for me. I like and want to continue taking part in teaching and educating the next generation of scientists, as well as playing a role in the organization and management of research, coordinating programmes and promoting international collaboration.

However, when I look back at the professional path I have taken up to now, what I am most proud of is having attempted to open up new lines of research, whether at the university or at IBEC. If I had to choose one achievement, patent, invention, discovery or award that has made me feel most proud or satisfied with myself – quite a difficult feat, since the most recent result is always the one that seems most interesting to you and is the freshest in your memory – I believe I would choose three different moments.

One memorable time came at the beginning of my career as a researcher, when I published my works in collaboration with Philips on the behaviour of semiconductor materials for optical applications. I felt this was a very important step because it was the first opportunity I got to show myself that I was able to carry out real research and have solid results published. When I began this research, Spain did not form part of the European Union, so it was difficult for a Spanish researcher to compete at the same level as people from the United Kingdom, Germany or France, especially in applied science. Therefore, those first publications form part of the time when I truly thought, "Yes, I can do this!"

The second took place when I received my first patent, which was for automotive applications. This was proof that the research I was doing actually had potential to help deal with problems in the real world and that, at some future time, they might even have some impact on society.

The third came much more recently. In fact, it is still happening right now. We are doing a lot of interesting things in the laboratory, such as developing "organs-on-a-chip" as platforms for studying pathologies or improving drug designs, having made great advances

in 2014 with the first "splenon-on-a-chip" developed with the collaboration of researchers from the Barcelona Centre for International Health Research (CRESIB). This type of research makes it possible to combine microfluidics, functionalized biomaterials, biosensors and cell cultures in 3D to mimic an organ's physiology. As a result, I am integrating the knowledge gathered in my lines of research over the last few years.

More than two hundred years ago, a Catalan surgeon and physicist, Antoni Cibat i Arnautó, highly influenced by the ideas of the Enlightenment and the advancements by Galvani and Volta in electrophysiology, wrote: "Without knowledge of physics, chemistry and botany (pharmacopoeia), nobody can consider himself fully knowledgeable about medicine and surgery". Today, with the introduction of nanotechnology into biomedicine and the advancements in bioengineering, this statement is more valid than ever.

As I look towards the future, one thing that I would truly like to be able to say at the end of my career is that I helped develop an early diagnosis system that is useful for some relevant pathology, such as cancer or neurodegenerative diseases. However, my most important contribution might perhaps be that my activity as a researcher and trainer of researchers helped a series of young people who have worked with me to develop their potential as scientists so that they can transform our country into a cutting-edge force in biomedical engineering and nanomedicine.

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MARÍA A. BLASCO

TELOMERES AND THE ORIGINS OF DISEASE

22

I have summarized my career in science in the following twenty-eight sections.

Thesis

1. After finishing my Biology degree in 1989 at the Autonomous University of Madrid (UAM) I undertook doctoral study. I could have worked on a topic that interested me greatly – cancer and ageing – but instead chose to take up a place at a reputable research laboratory. Under the supervision of Margarita Salas, at the Centre of Molecular Biology (CBM) (CSIC-UAM) in Madrid, I researched the problem of how the ends of a linear virus genome replicate. Without being aware of it, I was working on prokaryotic "telomeres".

Postdoctoral stage

2. When considering the question of what to do at the postdoctoral stage of my career, I knew nothing about telomeres, which I had not been taught about as an undergraduate – in addition, this was an "unpopular" field. At the time, the "hot" topics were tumour suppressors (p53 in cancer), the cell cycle and developmental biology.

3. However, the fact was that telomeres are the mechanism by which eukaryotic organisms resolve the so-called "terminal replication" problem (how the ends of the chromosome replicate), and in 1990 it had been postulated that telomeres might play a key role in ageing and cancer.

4. A colleague at the CBM, Crisanto Gutiérrez, told me about Carol Greider, a spirited young scientist who, with Elizabeth Blackburn, had discovered telomerase activity in the *Tetrahymena* model organism, and was now studying telomeres. I chose to join Greider's team rather than Blackburn's because Carol was working at Cold Spring Harbor Laboratory (CSHL) in New York, and Margarita Salas, who had taken a course there, told me it was the leading molecular biology institution in the world. Moreover, the director of CSHL was Jim Watson, the co-discoverer of the double-helix structure of DNA and a science visionary.

5. When I joined her team in 1993, Carol was still in her early thirties. She had been given a young team of researchers to lead at CSHL straight after successfully completing her doctoral thesis: this was because Jim Watson was a believer in the significance of telomeres in disease, while Barbara McClintock, who had first discovered telomeres (in corn) in the 1940s, had spent most of her career at CSHL (where she worked until her death).

6. To fund my stay at Carol's laboratory, I applied to the European Molecular Biology Organization (EMBO) for one of its postdoctoral fellowships, which were among the best-funded

and most prestigious scholarships in the field. One of the steps in the selection process was an interview with a European scientist. I was quizzed by a German researcher who was working on fungi. She told me I had made an unwise choice. Telomerase, she said, was still only an incipient field; Carol's research team was still undeveloped, and did not even have the cloned genes available. This meant – in her view – that my project was too risky. I should have picked a topic that was at a more mature stage, something more like my work with Margarita Salas. It need hardly be said I found her attitude mistaken and short-sighted. So I did not get the EMBO fellowship. But this setback did nothing to blow me off course in my determination to work on telomerase with Carol. Years later, the EMBO awarded me its Gold Medal. At the prize-giving ceremony I took the opportunity to tell the audience about this anecdote.

7. A biography of Elizabeth Blackburn records that she complained that the scientific community had ignored her discovery of telomerase activity. Everybody was interested in the cell cycle and cancer-protective genes as the keys to cancer. It was only in 1997 that some of the big names in cancer – David Beach, Robert Weinberg and Ronald DePinho – took an interest in telomerase and telomeres.

8. I applied for a postdoctoral scholarship from the government ministry then in charge of this matter. A colleague told me that my application had been discussed along the same lines as the EMBO fellowship. Luckily, he had intervened to make clear that the host research team was a leading group working in a spearhead discipline with a great future ahead of it. So in the end I was granted support to go to CSHL.

9. At CSHL, my previous background in molecular biology turned out to be a real asset since Carol had less experience in cloning and recombinant DNA techniques. I was the first member of her laboratory to buy a restriction enzyme. My research project was highly ambitious. I had to clone a mouse telomerase gene (only the *Tetrahymena* counterpart had been cloned so far) and engineer a telomerase-deficient mouse. As I had no idea of how to breed knockout mice, the first thing I did was to take a mouse embryology course, where I learned how to create genetically modified mice.

10. Cloning the mouse telomerase gene turned out to be very tough – it bore no resemblance to the *Tetrahymena* counterpart. This was 1994. Working in parallel with the company Geron, we finally isolated an RNA that was a candidate to be the telomerase gene. That same year, Geron published research showing that telomerase activity is increased in cancerous cells. Using the isolated sequence for the putative telomerase RNA, we searched for the mouse gene and analysed its levels of expression in normal and cancerous cells. When it was observed that it was highly expressed in tumoural cells but not in normal cells,[1] I got a hunch that we had cloned the correct gene. In addition, I detected that the telomerase gene is inhibited in adult tissue, as is its activity. Everything seemed to be indicating that we were on the right track, and that we had at last got our hands on the first mammalian telomerase gene. We dubbed this gene "TERC" (telomerase RNA component).[2] I immediately began to design a strategy to generate the knockout mouse. We were in a hurry because, if Carol's team did not get the mouse soon, Geron would generate it too.

11. I generated the vector to delete the gene in the germ line in record time; but we needed the right embryo microinjection equipment to breed the mice themselves. CSHL did not have the necessary infrastructure. It then happened that a postdoc researcher who was working at the Albert Einstein College of Medicine with Ronald DePinho, and who was a friend of Manuel Serrano's, persuaded Manuel to join Ron in breeding a knockout mouse for one of the genes that Manuel had isolated at David Beach's laboratory at CSHL, the p16 gene. Manuel had generated the p16 knockout mouse in cooperation with Han-Woong Lee, a Korean thesis student who was working with Ron.

12. I then persuaded Carol to enter into a partnership with DePinho: this would be the quickest way to get our knockout mouse now that Geron was so hot on our heels. We spent a day at the Albert Einstein College of Medicine to convince DePinho – who did not know what telomerase was – to work with us to obtain the knockout mice for the candidate telomerase gene.

13. Han-Woong Lee and I generated a mouse strain from which the gene had been knocked out. The most exciting day was the one on which I first observed that fibroblasts derived from embryos lacking this gene had no telomerase activity. Our next step was to reintroduce the gene we had isolated in those cells lacking telomerase activity. We found that telomerase activity returned. Finally, we had a mouse strain that was going to help us understand the role of telomerase in cancer and ageing. This research was conducted in 1995 and 1996.

14. Carol was excited to reveal these results to Titia de Lange, and the two of them made a bet. Titia de Lange was a colleague at Rockefeller University who had just identified the first protein that specifically binds to telomere repeats, so-called "TRF1" (telomere repeat binding factor 1); this was the first component to be isolated within a complex of six proteins now known as shelterin. Titia thought that telomerase was unnecessary to fertility – "not for life, nor for sex" – while Carol believed it might be essential to life. They wagered a case of beer. Titia won – at least at first. We found that telomerase knockout mice were viable and did not display major disorders. At a seminar I gave at CSHL to present our findings about the knockout mouse strain, Jim Watson exclaimed "But this is a disaster!", meaning that his hunch that telomeres were important had turned out to be wrong. But time proved otherwise. To an extent, the viability of the mice was to be expected because, as long as the telomeres were sufficiently long, telomerase was not perhaps necessary to sustain life itself. To force shorter telomeres, we created succeeding generations of telomerase knockout mice. It was only in the sixth generation that we observed ageing phenotypes in very young mice.[3] In my team, we observed that if we introduced deletion in a genetic background of shorter telomeres, then in the course of only three generations we could reach the critical telomere length, and ageing phenotypes began to manifest.[4] We now know that even in the first generation of mice lacking telomerase, accelerated ageing occurs, leading to shorter-than-normal average and maximum lifespans.

My own research team

15. In 1997 I returned to Spain and set up my own research team in Madrid at the National Biotechnology Centre (CNB), a division of the CSIC (the Spanish National

Research Council). Having settled in there, my first task was to handle the points that were still outstanding for me to be able to publish a description of telomerase knockout mice. One crucial issue was to determine whether or not telomeres became shorter and, if so, if such shortening led to chromosomal aberrations. This was key to showing that telomerase was the enzyme that maintained telomeres in mammals (this had so far been shown only in single-cell organisms), and to demonstrating that the shortening of telomeres caused chromosomal aberrations. I began to work with Peter Lansdorp, who had developed a telomere measurement technique based on FISH (fluorescence *in situ* hybridization), specifically "quantitative telomere FISH" or "Q-FISH", to carry out these analysis tasks. The results were truly spectacular. For the first time we proved that telomeres shorten at a faster rate in telomerase knockout mice; therefore, telomerase is the enzymatic activity responsible for maintaining telomeres in mammals. Carol agreed to include our data in the paper. This was my first publication from Spain, in *Cell*.[5]

TERT

16. In 1997 the teams headed by Bob Weinberg and Tom Cech published a paper on the first ever isolation of the gene for the human telomerase protein component, TERT, which encoded for a reverse transcriptase. In 1998, from Madrid, we published a paper on the isolation of the mouse telomerase protein component gene and first studying its regulation during normal development.

17. In 2000 we published highly illuminating research on the role of telomeres in cancer.[6] We showed for the first time that short telomeres behave as powerful cancer-suppressor mechanisms: non-telomerase and short-telomerase mice exhibited a lower cancer rate. This was the first validation in an animal model of the notion that telomerase might be a good target for designing cancer drugs.

18. In 2001 we published a paper on the generation of the first transgenic mouse strain for TERT. This mouse model enabled us to show that telomerase expression in adult mice might favour the emergence of cancer in step with ageing: this would explain why telomerase is repressed in adult tissue after birth.

19. Also in 2001, in parallel with Carol's group, we published a seminal paper[7] on what has later become one of the most distinctive topics of my research team. We showed, through genetic activation of telomerase, that the rescue of short telomeres in a telomerase-deficient mouse that was otherwise set to display ageing-related disorders prevented the development of those disorders (infertility, bone marrow failure, intestinal atrophy or anaemia) and, in addition, conferred a normal survival duration.

The biology of telomeres

20. In later years my team diversified to embrace research on various aspects of the biology of telomeres, including their interaction with the machinery of DNA repair. We showed that short telomeres interfere with the proper repair of DNA breaks,

so raising radio-sensitivity. We also demonstrated that some proteins involved in repair – Ku and DNAPK, among others – play a significant role in protecting telomeres. It is now widely accepted that short or unprotected telomeres are equivalent to a DNA break that is incapable of repair – that is, persistent damage.

21. My team also pioneered the characterization of mammalian telomeric chromatin and, specifically, its heterochromatic nature; we also led the field in identifying the key chromatin modification activities for establishing telomeric chromatin. Among others, we identified HMTases, DNMTases and DICER as important for telomere chromatin.[8–12] These papers form the basis of a model whereby telomeres, when they lose their heterochromatic nature, lengthen more easily, whether by the action of telomerase or alternative recombination mechanisms.

22. Our understanding of the nature and regulation of telomere chromatin led us to an unexpected discovery. We observed that telomeres are transcribed, thus generating long, non-coding RNA strands that, in addition, bind to the telomeres of practically all chromosomes.[13] More recently, we identified the proteins that bind to these RNA strands, called TelRNA and TERRA,[14] and this in turn enabled us to enrich these findings and detect, using mass sequencing techniques, their site of origin.[15] Identification of the TERRA locus will finally let us elucidate, through the use of genetically modified mice, the function of that protein in normal development and various disorders.

Telomere binding proteins and their importance in cancer and ageing

23. Telomeres bind to a complex known as shelterin, which comprises six proteins playing several roles in telomere protection and telomerase regulation. In recent years my team has set out a range of mouse models for conditional function gain or loss in respect of all shelterin components so as to examine their significance in cancer and ageing.

My group began by generating the first transgenic mouse strain having high levels of shelterin TRF2[16] and demonstrated that this telomeric protein plays a significant role in cancer and ageing, and that there is a genetic interaction between TRF2 and the NER (nucleotide excision repair) pathway.[17] Later, my team generated murine models for conditional deletion of shelterin TRF1, TRF2, RAP1, TPP1 and TIN2.

Our functional studies of models carrying altered expressions of shelterin TRF1[18] and TPP1[19] enabled us to discover that these two shelterin components are responsible for premature ageing and increased cancer risk. This constituted the first proof that a telomeric protein can act simultaneously as a tumour-suppressor and as an anti-ageing agent.

More recently, quite unexpectedly, we found that shelterin RAP1, in addition to binding to telomere repeats, also binds to extra-telomeric sites and regulates gene expression.[20] This provides a new connection between telomeres and gene expression programmes. Moreover, we discovered that RAP1 plays a highly significant role in metabolism: it regulates

expression of the PGC1α/PPARa axis, and thus protects against metabolic syndrome and obesity through its extra-telomeric role in the regulation of gene expression.[21]

In a paper published last year we identified POT1 as the first telomeric protein to mutate in human cancer so as to favour the acquisition of the malignant features of chronic lymphocyte leukaemia cells,[22] in the form of greater telomeric length and higher chromosomal instability.

Finally, the creation of murine models for conditional deletion of shelterins also provided us with models for so-called "telomeric syndromes", which are human diseases characterized by serious telomeric defects owing to mutations in telomerase or one of the shelterins, including aplastic anaemia[23] and idiopathic lung fibrosis. In particular we generated a murine model of aplastic anaemia through specific deletion of TRF1 in bone marrow. Mice whose bone marrow lacks TRF1 develop aplastic anaemia over the course of a few weeks, thus faithfully following the human disease, including extreme telomeric shortening.[23] This model will be of great use in preclinical studies of telomerase activation strategies. Finally, TRF1 models for conditional and specific tissue function loss prompted us to design drugs against TRF1 that are effective to slow the growth of lung cancers. This was the first time that small molecules were developed targeting telomeric proteins: so far, only potential drugs against telomerase had been addressed.

Consequences of short telomeres

24. One of my team's main goals continues to be to understand how short telomeres trigger ageing-related disorders, so as to delay ageing and thus disease itself. We pioneered exploration of the question of whether short telomeres affect the function of so-called adult stem cells, which regenerate tissue to maintain the organism in good condition. Although we worked with several teams on several stem cell compartments in the context of short telomeres, the most illuminating research was that in which we discovered that the presence of short telomeres in the stem-cell compartments of the skin decrease the ability of stem cells to respond to regenerative stimuli.[24] This defect anticipated the fact that short telomeres involve faster ageing and a lower rate of cancer. We further observed that p53 was an essential protein in preventing short-telomere cells from activating to regenerate tissue: this is undoubtedly a mechanism to ensure that regenerated tissue is damage free and hence less prone to developing cancer. We then clearly perceived what we had already suspected in 2001 in our work on telomerase reactivation and prevention of short-term telomere disorders in mice:[7] if we could lengthen the telomeres in these stem cell compartments, then we would be able to lengthen disease-free lifetime and hence longevity.

Development of telomere measurement techniques: when technology is needed to drive knowledge forward

25. Since 1997, when we worked with Peter Lansdorp's team to use his quantitative FISH technology to observe and quantify changes in the length of non-telomerase mouse telomeres, one of my team's goals has been to adapt this highly laborious

and complex technology – which involves meta phases, and hence *in vitro* cell culture – to mass, rapid and accurate use in circulating blood samples and tissue sections without need of cell culture. These are the biological samples that are in fact usually available in blood and tissue banks. We first developed what we called "high-throughput QFISH" in blood samples,[25] allowing for the rapid determination of an abundance of short telomeres in many samples simultaneously. This technology was licensed in 2010 to a start-up, which had emerged from the Spanish National Cancer Research Centre (CNIO), called Life Length. The formation of Life Length was made possible by the support of the Botín Foundation *Technology Transfer Programme*. The Foundation believed in my projects and in their market potential. Since 2010 the Botín Foundation has walked hand-in-hand with me in the task of accelerating the process from fundamental research to marketable products and potential applications in disease diagnosis and treatment. By 2014 Life Length was operating in over thirty countries around the world, marketing this technology as a technique to diagnose the presence of shorter-than-normal telomeres. This may be of use in preventive medicine and early diagnosis of cancer and cardiovascular disease. Our team continues to examine the importance of telomere length as a biomarker having prognostic value in a range of disorders. For instance we are working alongside the National Cardiovascular Research Centre (CNIC) in the context of the PESA study.

We later adapted the concept of detecting individual telomeres within each cell to detection in each cell of a tissue, thus creating telomere length maps. This technology, which we called "telomapping",[26] is protected by a United States patent. Telomapping enabled us to discover that stem-cell compartments have longer telomeres than differentiated cell compartments. This in turn led us, in partnership with other research teams, to identify new adult stem cells that were so far unknown.

The telomapping technique also allows for biopsy analysis in normal or pathological tissue (for example, tumours). This may be highly useful in ascertaining the effect of various treatments on telomere length.

Telomerase and pluripotency

26. One of the recent milestones achieved in biomedicine was Shinya Yamanaka's reprogramming of differentiated cells into pluripotent cells – which are capable of forming any cell type or even an entire organism, and normally exist only for a short time at the early stages of embryonic development, when, in addition, telomerase levels are very high. This prompted us to explore what happens to telomeres and telomerase during the reprogramming process. Would they lengthen "magically" in record time? If so, might this show us new ways of lengthening telomeres and so achieve younger and more long-lived cells and tissues? Could it reveal to us which genes and activities are necessary for the telomere rejuvenation process? We found that telomeres lengthen spectacularly when a differentiated adult cell becomes a pluripotent cell,[27] and that this lengthening is necessary for the resulting pluripotent cells to be of high quality. The process is executed by telomerase,[28] and requires the necessary proteins for telomerase to access the telomeres, such as TTP1 protein.[19] Interestingly, the longest telomeres in pluripotent

cells continue to grow as they divide until becoming what we call "hyper-long" telomeres. We observed that this is because the telomere chromatin in pluripotent cells enables the telomerase to manufacture increasing telomeres until achieving exceptionally long ones. The "hyper-long" telomere phenomenon also occurs during *in vitro* cultivation of natural pluripotent cells derived from the blastocyst. We are using this insight to try to breed mice carrying "hyper-long" telomeres, which may be able to live longer than those having normal-length telomeres.

We recently saw that one of the proteins that protect telomeres – shelterins – called TRF1, is induced by the Oct4 reprogramming factor at very high expression levels in pluripotent cells, and in fact constitutes an excellent marker to select the highest quality iPS cells.[29]

Telomerase activation and longevity

27. One of the contributions that I believe to be among the most important ever achieved by my team after the breeding and analysis of telomerase knockout mice is the demonstration that telomerase is sufficient to lengthen youth, delay the emergence of disease and increase longevity. First, our team generated transgenic mice having increased resistance to cancer (carrying extra copies of the tumour-suppressor genes p53, p16 and p19ARF) and overexpressed TERT, the protein component of telomerase. These trebly transgenic mice displayed better general health, systemic delay in ageing and extended longevity.[30] This research proved for the first time that telomerase has anti-ageing effects. Later research conducted by my team highlighted the potential of telomerase-based anti-ageing therapies and cast light on the involvement of telomere shortening in biological ageing.

We found, for instance, that dietary administration in mice of a natural telomerase-activating compound lengthens short telomeres and improves the health span of elderly mice without raising the incidence of cancer. We also showed that gene therapy with telomerase in adult and elderly mice achieved markedly beneficial effects on health and state of form, including glucose sensitivity, osteoporosis, neuromuscular coordination and other ageing biomarkers, with no observed increase in cancer incidence.[31] Using the gene therapy strategy, we could also show that mice treated with telomerase lived significantly longer. These benefits were not observed, however, when therapy used catalytically inactive TERT: this shows that telomerase-mediated extension of telomeres is necessary. The results obtained with our telomerase-based gene therapy prove the principle that treatment of adult mice with telomerase is sufficient to delay ageing-related disorders and extend longevity without incurring any increase in cancer incidence.

In humans and mice suffering from a defective telomere maintenance system, aberrantly short telomeres entail decreased longevity. Inter-individual variation in telomere length in humans and mice is wide, but it was not known whether this was associated with longevity potential. To find out, our team conducted a lifelong telomere length study in wild and transgenic mice. We found that mouse telomeres shorten a hundred times faster than human telomeres. It is the rate of increase in the percentage of short telomeres, rath-

er than the monthly rate of telomere shortening, which significantly predicts lifetime duration in both cohorts of mice. The individuals displaying a higher rate of increase in the number of short telomeres were also shorter lived. These findings demonstrated that short telomeres have a direct impact on mammalian longevity.

To improve our understanding of ageing mechanisms we determined the serum metabolomic profile of a range of mice having different genetic backgrounds and ages. We defined a robust metabolomic signature and a metabolomic scoring system that reliably and accurately predicts the age of wild mice. In the case of telomerase-defective mice – which have a shorter lifespan – metabolomic scoring predicts a greater age than expected. In mice that overexpress telomerase, metabolomic scoring predicts younger ages than expected. Late reactivation in adulthood through telomerase-based gene therapy significantly converted the metabolomic profile of elderly mice into profiles that would be characteristic of younger mice, again confirming the anti-ageing role of telomerase. The metabolomic signature associated with natural ageing in mice predicts the ageing caused by telomere shortening, which suggests that natural ageing in mice is partly caused by the presence of short telomeres.

I recently took part in drafting a proposal as to the question of which are the hallmarks representing the common denominator of ageing in different organisms.[32] In this paper, it was assumed that, for a given characteristic/activity to constitute a hallmark of ageing, it must satisfy the following three tests: it arises during the normal ageing process; its experimental worsening should accelerate ageing; and its experimental improvement should delay ageing, and hence increase healthy (disease-free) lifespan. The gradual shortening of telomeres with increasing age is one of the nine hallmarks of ageing. Telomere attrition with age satisfies the three requirements for it to be regarded as a hallmark: it occurs during physiological ageing in mammals; pathological telomere dysfunction accelerates ageing in mice and humans; and experimental stimulation of telomerase delays ageing. My team contributed decisively to proving each of these three requirements. As a result, telomere shortening is one of the molecular pathways of ageing that has been most fully characterized, and there is a wide base of genetic evidence that this phenomenon modulates "health span", or disease-free lifespan.

Telomerase activation and disease

28. Telomere shortening associated with ageing is one of the main risk factors for cardiovascular disease (CVD) in humans and mice alike. Mice carrying unusually short telomeres owing to telomerase deficiency develop cardiomyopathy, characterized by an increase in the death of cardio-myocytes and cellular hypertrophy, which is concomitant with ventricular dilation, thinning of the cardiac wall and cardiac dysfunction. Myocardial infarction induces far-reaching alterations in ventricular architecture, with formation of scar tissue, ventricular dilation and hypertrophy of the non-infarcted myocardium. CVD associated with ageing and myocardial infarction (MI) entail a few shared cardiac alterations. The regenerative capacity of the heart is lost with age. The heart of a newborn mouse can undergo complete regeneration after partial surgical removal or in-

duced MI. This potential for complete regeneration is lost, however, after the first week of life. In parallel to the loss of capacity for complete regeneration, the expression of the essential telomerase genes TERC and TERT is also lost during the first week of postnatal life.

These facts encouraged me to speculate whether the re-expression of telomerase in the adult heart might aid its regeneration after MI. Telomerase activation through overexpression or through telomerase-based gene therapy as a strategy to lengthen telomeres and delay ageing and its associated diseases had already been applied successfully by our team in the past. Since it had been found that the transgenic expression of TERT leads to an increase in heart hypertrophy and an increase in the proliferation *in vivo* of cardio-myocytes, we decided to use our recently developed telomerase-based gene therapy to investigate the possible therapeutic effects of telomerase expression after MI.[33] To do so, we used an MI murine model in which the event is induced by fusing the coronary artery and viral vectors for specific expression of TERT in the heart. We found that telomerase activation in the heart of mice following MI improves heart functionality and morphology metrics, lengthens telomeres, induces the activation of several pathways associated with cardiac protection and regeneration, and significantly reduces the post-MI heart failure death rate. Our findings are a "proof of concept" for the development of innovative strategies based on telomerase activation to treat chronic and acute heart failure.

Our group is also interested in using telomerase activation strategies to treat diseases other than CVD. So we are now exploring the therapeutic use of telomerase activation for the treatment of telomeric syndromes such as aplastic anaemia and familial idiopathic pulmonary fibrosis. As I have said, telomeric syndromes are human genetic diseases associated with mutations in telomerase and the shelterin proteins. The mutations lead to unusually short telomeres and premature loss of tissue regenerative capacity.

Aplastic anaemia is a potentially fatal disease characterized by hypocellular bone marrow and a lowered number of blood cells. We have bred a murine model to recapitulate the phenotype of aplastic anaemia patients;[23] we shall use it to try out our telomerase-based gene therapy. We expect that telomerase reactivation will lengthen the short telomeres in stem cells and haematopoietic system precursor cells and hence remove or delay the bone marrow failure.

Idiopathic pulmonary fibrosis (IPF) is a fatal disease caused by chronic damage to the lungs. Its aetiology is still not well understood. Telomere shortening triggered by telomerase mutations is linked to cases of familial IPF. Telomerase-deficient mice do not develop IPF owing to their premature death caused by other diseases. The breeding of a murine model to recapitulate IPF is yet to be achieved. Given that TRF1 depletion leads to rapid telomere dysfunction and cell death independently of telomere length, my hypothesis is that TRF1 depletion lung epithelia may lead to IPF in mice. We are now in the process of developing that animal model. If it fulfils expectations, we shall use it as a preclinical model to develop therapeutic strategies based on telomerase activation.

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RICARD SOLÉ

AUTOBIOGRAPHICAL NOTES BETWEEN ORDER AND CHAOS

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When does a career in science in begin? At what moment does that uncontrollable curiosity awaken from which you are never set free? How can we human beings become so obsessed with finding a solution to a problem? I do not have the precise answers to these questions. However, I do suspect the seed that grew into the scientist I became formed in a remote time of childhood of which I can unfortunately remember almost nothing. As scientists say, we must forget early childhood in order for our minds to become fully developed. So, it is an exercise in futility. If we look back at the footprints left behind by our existence, they are erased almost in the moment when our shoe prints are still small and our footsteps uncertain. Among my memories of what Manuel Vicent called "the light days of childhood", I see myself sitting on the ground next to my mother, observing ants in a tiny park by the Sagrada Familia. From time to time, when I would lift my eyes, I could see other creatures inside the huge structure being raised towards the heavens. The dream of an architect and engineer inspired by nature was coming true thanks to the inspiration of the genius Gaudí.

As far back as my memory stretches, I have always felt an enormous curiosity about everything around me. That curiosity and the sense of adventure associated with science have a great deal to do with my parents, their life experience and their conviction that knowledge is the most valuable thing we possess, provided we use it the right way. They came from a tragic childhood and adolescence marked by the Spanish Civil War. My father came to Barcelona from Aragon while still a child, and I had the luck of going to the school run by the Catalan government, or *Generalitat*, located inside the Ciutadella Park. The school was run by physicist Josep Estalella Graells, who was not only a pioneer in the science of his times, but also a revolutionary in his way of viewing education. The school, an heir to the Free Teaching Institution, was very advanced, with a secular, co-ed model and a strong experimental component based on a scientific view of the world. Far removed from the method of teaching by memorization alone, girls and boys were taught to think through a strict but entertaining implementation of the scientific method. Estalella considered it important to use teaching methods that brought science closer to future citizens, and the school's vision and educational method were summarized in its slogan, written on one of the school's walls in 1933: "We are first and foremost determined to make good men; if they are strong, that is good, and if they are wise, too, all the better". My father always remembered those years, in which experiments and nature outings into the country formed part of the everyday learning process. Many of those experiments are described in a small book my father kept, and which I now possess: *Course on Physics and Chemistry*, by Dr Estalella. Most of those experiments are no longer done at our grade schools and high schools today. They were used to pose questions that served as a source for mental exploration. Many years later, my father still remembered those happy times with great nos-

talgia. My mother was a Republican as well, very curious and restless, a limitless lover of reading – something that I have inherited – and of her own dreams, like that of becoming an archaeologist. She could never make her dream come true because economic hardships to come would keep her from continuing her studies. Even now, when she recalls the day her parents told her she would never be able to study again, she cannot keep the tears from welling up in her eyes.

Estalella's dream of a society of "wise men" ended when the war began. My father had the bad luck of being old enough to get sent to the front while still too much of a child to understand what that meant. When just seventeen years old, he formed part of the sadly famous *Quinta del Biberón*, or so-called "Baby-Bottle Draft", and fought in the Battle of the Ebro, from which he managed to return, but not his friends. The war ended, ushering in a forced silence that failed to snuff a certain sense of rebellion that also effected my personality. For all scientists, questioning higher authority is a basic principle: there is no room for authority or tradition in our rational view of the world. And though we did not have very much money – we never owned a car or house of our own –, books were never lacking. My parents would buy new books in abundance, above all second-hand books, and the library formed part of my years discovering literature, with Jules Verne and Edgar Allan Poe as the great protagonists. The old-fashioned bookshops on Calle Aribau, now almost all gone, were a sort of second homeland to me, where I could spend hours searching through miles of bookshelves. Like the explorers in stories, an unexpected treasure would occasionally pop up in the least expected corner. Some of the bookshop owners got to know me well because my visits lasted for many years, until the bookshops finally shut down. Our home library was also a hidden corner of freedom, which I delved into to find works like *Frankenstein* and *The Three Musketeers*.

My father was a merchant marine and spent half of his time away travelling. Partly for this reason, and despite not being a believer, my siblings and I ended up attending the school of the Marist Brothers, which was located just one minute from our house. I imagine he was expecting a certain discipline from that education which it was unable to provide. My memory of those eight years of required secondary schooling in Franco's Spain are quite poor, if not to say a complete waste. The teachers were incredibly dull, and the physical violence, especially by certain priests, was a constant element. My lack of interest was usually accompanied by a somewhat defiant attitude and questioning of authority. At that school, so distant from the one my parents had known, it was very difficult for me not to get punished for one reason or another. During those years, I received a microscope and two chemistry sets as gifts, and my grandmother, who could not imagine they would cause an explosion and had never experienced the slightest fire, lent me a room in her house so I could set up my first laboratory. On returning from his trips, my father used to bring back exotic animals, which would end up getting sold in the end, including an iguana and a crocodile. The iguana often escaped from his box, and one time I found him under my bed, fleeing from that strange world that had so little do with the lost world to which he would never return. The crocodile spent his week in transit lying in our bathtub, which undoubtedly helped improve our relationship with reptiles while notably diminishing our family's level of hygiene.

With time, my interest in nature was pushed aside by smaller things, and at the same time by an interest in our planet's past. I most affectionately remember a small book of fossils that illustrated Darwin's theory of evolution and the connection between the different forms of life, which harked back to a past that had to be measured not in terms of thousands of years, but rather millions. The impact it had on me to discover this was an invisible – but very powerful – process that seemed to explain the origin of the enormous complexity of the living world. In his day, Darwin had also run up against traditional religion, which mounted a veritable crusade against his theory. Although the Catholic Church remained at odds with this viewpoint, rejecting the idea that human beings formed part of that chain, I understood that Darwin was the winner, and so it is not surprising that he, along with Galileo, would become one of my intellectual heroes. The theory of evolution's elegance was overwhelming, while religion's logical impoverishment as a way to explain the world seemed obvious to me even then. This led to an inevitable confrontation. Precisely at that time my siblings had a private language and literature teacher who had recommended "problematic" readings to them. I began adolescence overhearing those conversations, in which some guy named Nietzsche was mentioned. He had proclaimed the death of God no less in one book! Much earlier than I should have, I read the book that has made the greatest impression on me ever. I have read it over and over again throughout the years: *The Plague*, by Albert Camus. All that reading, which I discussed with my classmates, caused the school's directors to write to my parents and warn them that I would be expelled if I continued down this path. I finished eighth grade and left that school forever. To be fair, though, I will also be grateful for those black-cassocked priests, with thoughts no less dark than their clothing, for their help in distancing me from all dogmatic authority.

At the Jaume Balmes School, I found myself in a radically different intellectual and human environment. In addition to a very welcome lack of forced religion, I came across some teachers who truly changed my life's path. They were politically troubled times, and my later interest in unstable systems may have incubated during those days of demonstrations, assemblies, running from the authorities and societal uncertainty. Because I have always increased in mental activity as the day transpires, I decided to attend night classes. My classmates were students who worked and studied, and I still have very lively memories of those colleagues in the trenches with whom I was able to share political, philosophical and scientific concerns. Back then, I had already discovered what would become my passion in a book published by Alianza Editorial titled *Towards a Theoretical Biology*, edited by someone named Conrad H. Waddington. In that thick tome, I discovered several authors with whom I would not only end up collaborating, but with whom I also became friends. The different chapters of the book examined the problem of defining a new discipline, theoretical biology, which was to build bridges between descriptive biology and mathematical theories, drawing it closer to other more quantitative disciplines like physics. It included topics such as the brain, genetic networks, the problem of hierarchy in biology and the stability of systems. The book suggested the possibility of redefining biology, using a systems-based vision in complete opposition to the dominant reductionist viewpoint that understanding the details of a system's components was the only thing necessary – as we were told and taught at university – to understand the system as a whole. That book

already outlined the idea that there are global properties that cannot be reduced to just the components in the system, no matter how well we might come to understand them. In the same collection, I greatly enjoyed two wonderful biology books: *The Brain* and *Molecular Biology: Structural Focus*, both by C. U. M. Smith. The second especially guided me towards the molecular foundations of biology, to a great extent replacing my naturalist interests. The complexity of cells, my understanding of which has never ceased to grow throughout these years, has never stopped surprising me. And with those books also came my first computer, a ZX81 with just 1 kB of memory. I could not have imagined then that cells and computers might share something in common beyond just a few interesting metaphors.

Without a doubt, my best memory from those years at Balmes are of my physics professor, Professor Carreras, who was also giving classes at the University of Barcelona School of Physics. I had never regarded physics as something relevant or interesting, though a high school colleague and I built a rather large telescope together, and I spent many nights gazing at the stars, when it was still possible to do so through Barcelona's sky. However, astronomy and physics seemed like completely unrelated disciplines to me. I suppose that until then I had not had a good physics teacher, and it was very clear to me that I would be studying biology at university. However, my first class that year changed everything. I still remember the day when Carreras showed up looking like a young Einstein. The first topic he covered was "field vector theory". It was more of a mathematics class than I would have imagined, and his explanation was so elegant that I grew intrigued. Where was the physics? As we moved ahead, however, I discovered that the formulas that others had forced me to memorize came up time and again in a natural way. And the foundations we had laid in the beginning were used to raise a theoretical edifice of enormous power. Problems that were very different – at least in appearance – could be solved using the same equations. Hiding behind just a few mathematical expressions was a universe of possible solutions. As has happened to nearly everyone who discovers the beauty of formal science, the search for elegance and simplicity in my later work was undoubtedly marked by this experience. I will never be thankful enough to Professor Carreras for his wonderful classes and for opening a window through which I could observe nature with the mind of a theorist.

When the academic year ended, I had understood I must complete my degree in Physics, though without giving up my initial plan to study biology. It was not a very common decision. What I had most often heard about trying to do two undergraduate degrees at the same time usually involved mixing physics and chemistry or physics and mathematics. As if things were not hard enough, they became more complicated when I had to leave home due to disagreements with my father. I could not stay there, but that also meant I had to find a new place to live and some way to earn a living. For some time I took on any sort of job, such as giving private classes in physics and mathematics to first-year students from different schools. After some time I had earned a bit of a reputation as a teacher and was able to do reasonably well. This forced independence brought with it the companionship of a group of travel mates with whom I would share several years of my life living together in different student flats. Our complete freedom allowed us to wander as we pleased along the borderlines. Although we lived a somewhat scattered life, we also managed to put

together an amazing library, had our own collection of personal computers and had walls constantly covered with chalkboards full of formulas and mathematical theories. We would spontaneously organize discussions about logical problems, artificial intelligence, philosophy, genetics, neuroscience or history. This bohemian lifestyle melded with a zeal for knowledge that seemed limitless. We lived with such intensity that I believe I was always aware it would come to an end someday. And that critical spirit with which we analysed the world and the way in which science approaches it did not allow for any concessions. That was a great opportunity to seal my education in place, coming full force into the terrain of borderlines between disciplines. Those travel mates became friends for life.

During my years of university study, I had to surmount considerable difficulties to attend class. It was simply impossible to get to them all because they would constantly overlap, and because my own private classes took up such a significant number of useful hours. Fortunately, the Schools of Biology and Physics at the University of Barcelona are right next to each other, separated by just one street. Therefore, after a class in molecular genetics, I could run over to the other building to take a course in quantum physics, and then return to the other building once again to attend a class on evolution. I should point out that I was not a "good" student. With some exceptions, I rarely followed the subject matter defined in the curriculum but instead devoted myself body and soul to studying those parts of the curriculum that seemed interesting to me, often leaving out a large part of the syllabus. The result was that I received low marks in most classes. I want to highlight this point to remind people how different obtaining good marks is from developing a scientific mind. At the School of Biology I had some extraordinary professors, such as Jaume Baguñà, Mariano Marzo, Montse Aguadé and Roser González. At the School of Physics – whose course materials were mostly anchored down to the knowledge from one hundred years before – I took a course in "thermodynamics of irreversible processes" taught by Jorge Wagensberg. That class was incredibly stimulating to me, and it was the closest thing I experienced to a melding of physics and biology. For the first time ever, the word "complexity" took on its full meaning in every dimension. It was also an initiation into the concept of self-organization: nature was free from all the bonds of reductionism, and everything turned out to be more than just the sum of its parts. However, the impossibility of reducing global behaviour to the properties of components did not mean the system was beyond our comprehension. Quite the contrary, because the emergence of order on the basis of molecular chaos was perfectly explicable through the prism of theory. Wagensberg's classes also introduced a very important component that now plays a central role in most fields of knowledge: the concept of information. Beyond just matter and energy, information is in many ways the true key to our understanding of living beings. Life has triumphed to a great extent because of its ability to grow and adapt by properly using information. Even today, it is hard to provide a proper description of how information is processed and integrated into living systems and how it has played an active role in the evolution of complexity. What we are certain of is that each major innovation that has taken place (the advent of life, cells, multicellular organisms, sex, cooperation and language) has come with a new sort of information or a new way to store it and transmit it. Among the innovations that Wagensberg spoke to us about were the books in the collection *Metatemas*, written by thinkers, mathematicians and scientists such Jacques Monod, Benoît Mandelbrot,

Ilya Prigogine and Douglas Hofstadter. That year, the last of these had just published the Spanish edition of the classic *Gödel, Escher, Bach: An Eternal Golden Braid*. In class, Wagensberg explained that the book dealt with the connections between Bach's music, the surprising engravings made by the ingenious Maurits Escher and Gödel's theorem, as well as other problems related to recursivity. For a reader as obsessive as I am, it seemed like an exceptional book that I should get a copy of as soon as possible. That afternoon, I went to browse through the book at a bookshop in downtown Barcelona. It was truly extraordinary, but I was in economic ruin at the time, so I could not buy it. For days I returned to the bookshop to read the huge tome until I could finally get a copy. Hofstadter's book, to which I have returned time and again throughout my career, proposed surprising connections, and in its pages he endlessly intertwined the genetic code, computer programs, ants, the mind and viruses. In it I discovered unexplored territories that I was determined to examine more closely, if I got the chance.

As I completed my undergraduate degrees, a complicated dilemma arose for me. I wanted to continue my career as a scientist and make the inevitable move to my doctoral thesis, but yet again I felt unable to reach a decision. Therefore, I ended up starting two theses at the same time, one at the School of Biology and another at the School of Physics. The former, under the direction of Jaume Baguñà, was about certain problems in development, while the latter with Jorge Wagensberg was on the theory of information and ecology. As part of my training, I was given a scholarship from the Catalan government (with Jaume's support) for short research stays. The initial idea was to work for about three months with Robert Ransom, a pioneer in biological development simulation models at the Open University in the United Kingdom. The excitement of working with an institution abroad was coupled with the fact that I would be taking my first trip outside Spain at the age of twenty-six. One of my concerns was how I would understand English when speaking with English people. My foreign language skills were limited to the French I had studied in school and a set of technical words I used to get by when reading scientific articles. I decided I could somewhat correct the problem by watching films in the original language version. If I paid close attention, I could learn to say a few coherent phrases that would put me a step ahead of the Apaches I saw in the movies. I went to see *House of Games*, a very good film about fraudsters planning their crimes, and I loved it. I ended up watching it four times. The end result was that the sentences I could remember tended to resemble "Don't trust anyone!" or "I'll kill you, you damn bastard", not at all useful when looking for a grocery store or talking about the weather with normal people, as I would verify soon after.

When I got to the university, somewhere in the English countryside, I encountered a bleak scenario: Ransom had decided to leave the university to work for a private company. That was one of the many effects of Margaret Thatcher's ultraconservative government, which did terrible damage to the science world in the United Kingdom. I therefore had to look for some alternative. Next door was Brian Goodwin's office. He was a professor in the Department of Biology and was well known for being a radical scientist. I was very familiar with his name because he was one of the authors of *Towards a Theoretical Biology*, which I had already re-read in school, and he had been a student of Waddington's. According to some, Goodwin denied the existence of genes and rejected Darwinian evolution. I mentioned to

Ransom that I intended to speak to Goodwin about the possibility of working with him, and he responded something like: "Go ahead and try, but bear in mind he's crazy". I believe Jaume Baguñà had a similar opinion, but even so he encouraged me to try. With such recommendations, I was not expecting much from our interview. However, when I entered Brian's office, I came across a charming individual who was very willing to argue his ideas. We talked for an hour, almost beginning a discussion on the nature of biological evolution and how to understand the origin of complexity. While I did share some of the ideas that Brian expounded upon during our meeting, my education as a biologist was very biased towards the importance of genes as the key to answering basic questions. Goodwin's viewpoint, however, was that natural forms and the rules of embryonic development followed laws of a mathematical sort. Due to these universal laws, the full set of possible forms was quite limited, and the role of natural selection was less relevant as a mechanism for producing complexity among organisms. Though Goodwin did not deny the role of genes, he thought they could not explain the origin of living beings. The conversation continued, but Brian understood my attitude was one of great scepticism and that he was far from convincing me, so in the end he said to me: "Think about it. How many things do genes explain, and how many do they not? How does the organism and its development fit into the evolutionary scheme?" When I left, I walked to the university library, thinking that this patient and kind man must be mad. Even so, the truth is that those questions were still there, with no answer. And after all these years, I have never forgotten them. Despite strong criticism, Brian always held onto his views, many of which have ended up becoming a part of the way we now understand biology.

That same year, I returned to Goodwin's laboratory to study a problem involving the formation of structures in *Drosophila* embryos. This is the famous fruit fly used to build the foundation of a large part of modern genetics. My work was theoretical and consisted of determining certain potential signals of spatial organization based on experimental data gathered at different times in the embryo's development. The work was tedious and, for someone like me, it was not very stimulating. Therefore, I decided to ask Brian whether he had any other topics to research. It was a risky wager. When you have someone working on a project, the possibility that efforts might be diverted in some other direction is not the optimal scenario, but Brian was generous, and after thinking about it, he pulled a folder out of a filing cabinet and gave me a copy of an article that they had sent to a journal shortly before. The topic was fascinating: how to represent an ant colony in terms of something like a simple, artificial network of nerves in which the social interactions among artificial ants (computer-simulated) could be described as interactions among neurons. Each ant would be an ensemble of neurons, and these interactions would make the system able to reach decisions on how to distribute tasks throughout time. This problem was of great interest because it was known that, in ant species in which individuals are morphologically identical (and, therefore, there is no association between morphology and tasks), the colony is able to redirect efforts towards different tasks based on what is needed at each given moment. In some way, the interactions between individuals make it possible for the system to remain aware of its internal status and needs, and to adapt to them. I walked to the library, extremely excited. This was just the type of problem I was searching for, so I sat down to read books on social insects, brains and computers

until the library was closed. In addition to others, I once again enjoyed *Gödel, Escher, Bach* and Hofstadter's discussion of ants, brain and logic. Somewhat later, I developed a theoretical model of ant colonies that I called "fluid neuron networks", which allowed me to explain a strange phenomenon that was observed in the colonies of certain species. In these colonies, individuals' activities do not remain constant over time. When we observe them, at certain times we see very low or no activity, without any movement. When an ant becomes active, which happens at random, it begins to move about, and when it comes into contact with another ant, that ant is activated. The result is that a wave of activation propagates, reaching the entire colony. Ants perform several roles while they are active, but the wave ends up weakening little by little, and the system becomes inactive again. If we observe how the system's activity changes across time, we discover a signal that is reminiscent of the brain, which also fluctuates across time in the form of partially regular waves. I discovered that the phenomenon can be explained, so I designed several related experiments using the hypothesis that ants acted like a set of mobile neurons that could move, thereby making contact with the others. This could cause their activity to be triggered in a manner similar to what is observed in the cerebral cortex. However, the system's status would be similar to a sort of liquid that gradually changes over time. The phenomenon was inexplicable when viewing individuals because they clearly lacked an internal clock that made them move in waves. The interaction among the elements was what created the overall order, and that was my first successful model.

A second component would end up changing the course of my destiny as a scientist for good: chaos theory. Close to the end of my studies, I read that certain extremely simple mathematical systems could display extraordinarily complicated behaviours. Using one single equation, it was possible to create series of data so complex that they appeared to be random. Everything indicated that many real systems (from heartbeats to the solar system) follow the laws of chaos. However, contrary to common sense, the world's complexity could be encapsulated in a very simple model, which we could use to reach a perfect understanding of what we see, though also remaining unable to predict what will happen in the long term. At the end of an article in *Scientific American* titled "Chaos", the authors – with whom I would eventually do research – pointed out that perhaps even the brain might obey this sort of dynamics. When I finished reading the last paragraph, I closed that scientific journal and decided I would stop work on the two theses I had begun in order to devote my studies to chaos and, if possible, I would do my thesis on that subject. It was a risky decision because I had already used up several years of work and was not very sure how this change in direction might play out.

One especially appealing aspect of the potential in this theory had to do with the fluctuations in natural populations across time. It seemed clear that certain measured booms and busts in populations might have occurred due to chaotic processes. However, population drops created somewhat of a paradox: a population that is reaching small numbers faces extinction: shouldn't we expect evolution to select parameters that would distance these species from chaos? At that time I had begun to collaborate with Quim Valls, a disciple of Jorge Wagensberg's who had developing ecosystem simulation programs. Quim agreed to accept me as a doctoral student at the Polytechnic University of Catalonia (UPC),

and we soon discovered that models, if properly adapted to certain scenarios, displayed chaotic behaviours. My future doctoral thesis was gradually oriented towards the dynamics of populations and their instability. However, my greatest discovery had to do with space and solving the paradox of extinction. Most theoretical models of ecosystems, and nearly anything you could find in textbooks, assumed that natural populations can be seen as mixtures of individuals of different species. This assumption gave rise to certain paradoxes. What would happen if we considered something as simple as the fact that populations grow and spread throughout the habitat they occupy? To study this problem, I used a type of mathematical model known as "coupled map lattices", developed by Japanese physicist Kunihiko Kaneko. This would make it possible to study population models over a discrete space, in something resembling a chess board. The basic element was a simple system of equations that described the interaction between predators and prey. The system had been analysed before, but not considering what could happen if the population also spread out over an area – something so obvious that nobody seemed concerned. What happened was both surprising and revealing all at once. On the one hand, populations continued to increase or to fall towards extinction, but that all took place at the same point on the board. The total population, adding up the sum of every square on the board, was much more stable, even constant. Because of space, local chaos turned into stability within the system. Moreover, populations moved around the space, spreading in an orderly fashion, forming spiral waves, for instance. Chaos and order coexisted and the paradox ceased to exist. Without knowing it at the time, I was taking part in the birth of what would be named "spatial ecology" along with other researchers. Its influence has been enormous, and it has changed many of our initial views on the behaviour of ecosystems. Another important aspect of my thesis would be the so-called "self-organized criticality", a fascinating proposal made by Danish physicist Per Bak. Bak had proposed the idea that many natural systems spontaneously tend to form what in physics – using a somewhat lax definition – are called "critical points". They separate orderly behaviour from disorderly behaviour. At these critical points, very complex behaviours occur, fractal structures are created, and systems can react swiftly and coherently to external signals. As I discovered along with Susanna Manrubia, one of my first doctoral students, tropical jungles and even large-scale evolution could play a part in these critical dynamics. We both collaborated with Per Bak, who sadly died when he had reached the high point in his theory. Though sometimes he was a bit too talkative for his audiences – he loved to stir people up –, Per Bak was charming and had an endless curiosity about all kinds of topics. Although his theory received aggressive criticism, I think it is fair to say that the key idea was extraordinarily elegant, and in recent years it has gained acceptance and much supporting experimental evidence, particularly in biology.

Shortly before completing my doctoral thesis, I decided to give five seminars on chaos theory at the UPC Computer Science School, where I was an associate professor. The seminars included mainly mathematics, but also many examples from ecology, physiology, evolution, physical systems and brushstrokes of what was referred to as quantum theory. Many students and a few professors attended those improvised lectures. Some of those students were greatly surprised and became frequent visitors to my office. I must say that both then and in later years, working on chaos and complexity was somewhat frowned upon.

Some colleagues in the Department of Physics even suggested I should work on "something more serious". Even so, before completing my own thesis, I had already agreed to direct the doctoral theses of four of those students. The first of them was Jordi Bascompte, who would end up becoming an international figure in theoretical ecology years later, though while doing his thesis he was unable to get any financing whatsoever. Therefore, following a fait accompli policy, all five of us set up operations in the same office, and so began what would become the Complex Systems Group. With hardly any financing, all four theses came to a positive end. As for myself, I continued visiting Brian Goodwin, and on one of those visits I met the legendary Stuart Kauffman. Once again his name was familiar to me because of Waddington's book, though I had already read some of his classic works. Several decades before, Kauffman had seen the genome as a complex system that should possess emerging properties that could not be reduced to the properties of genes alone. In Kauffman's vision, the complexity of organisms was a result of interactions among genes, which he already imagined as a network. Inspired by ideas in cybernetics, the models known today as Kauffman Models became one of the classic systems in the field of complex systems. As a result of that visit, the chance arose to visit the Santa Fe Institute, which was beginning to shine as a unique place, located on a hill near the city of Santa Fe, in the middle of the American Southwest. Shortly afterwards, I read how the Institute was founded in the book *Complexity* by Mitchell Waldrop. It was created by a group of extraordinary scientists (including some mythical Nobel Prize winners like Murray Gell-Mann and Phil Anderson), who were willing to explore the frontiers of complexity, giving up the reductionist view of systems, with their sights placed not only on biology, but also on society, history and economics. I decided that no matter what happened I had to see that place, and in the end I was able to spend a month at what was then considered the world's main centre of reference on the topic of complexity. Kauffman received me in his office and, from the very first moment, we began to discuss all sorts of problems. A friendship arose that has lasted until today. At the Institute, I discovered that what seemed impossible was possible: for researchers from very different fields to sit down and talk in such a way that ideas could flow without barriers and without using each field's specific jargon. That was something we had already seen in Barcelona, where my small group, lacking financing and credibility, had already achieved that goal, but we had received no acknowledgement at all in the field of complexity.

My visits to the Santa Fe Institute continued, and during the year after the first while walking home – it was actually quite far – a car stopped at my side. The woman driving, whom I had seen at the Institute on several occasions, asked me whether I wanted a ride. I got in and we started to chat. She asked me what I was doing at the Institute, and I explained the projects that were starting to show results. When I finished, I asked her what she was doing at the Institute, and she answered, "I'm the president". As I contemplated the possibility of jumping out of her moving vehicle, Ellen Goldberg laughed at the sudden pallor on my face, and then simply continued asking me details about what I did. That situation would surely have had a very different outcome in my country of origin. The next year, president Goldberg came to my office and told me I should consider the possibility of becoming an Institute member. Not much later, I became an external professor, then forming part of one of the most creative communities I have ever known. Yet again,

something happened that had little to do with the way you were supposed to progress according to the academic world from which I had come. In any case, the ambience at the Institute promoted intellectual ambition, and I ended up posing my own questions, as well as pursuing a research programme in which my main ambition was, and still is, to discover the basic laws that shape biological complexity (if there are any). At that time the architecture of this complexity (how the elements in a system interact to form a network) was not yet well known, but that situation was about to undergo drastic change.

During the early years, I managed to develop some ideas and models that demonstrated the theory's ability to understand complex phenomena. I came up with a very simple approach to the organization of high-biodiversity ecosystems based on an approach in which each species made up of a set of individuals would be represented by a series of balls with a specific colour. They were mixed in an urn along with balls of other colours that represented all other species. In the simplest case, the changes in these populations would take place in the form of random removals and replacements, following the rules of "the Pólya model" formulated by economist Brian Arthur, who was at the Institute at that time. The idea is quite simple: a ball is removed at random, it is taken out and put back in, and then another ball of the same colour is added. From time to time, an error is made, and one of the balls put into the urn is of a different colour (this is equivalent to a mutation or immigration by an individual from some other place). A few years before, Arthur had used the Pólya model to study and demonstrate the existence of many solutions to the economic systems dynamic. In those studies, an infinite urn was assumed with a finite set of species (colours). When I studied the problem using an urn with a fixed number of balls and an undetermined number of colours, I discovered that the system tended to move towards a status in which most of the colours were represented by just a few balls, while some, very few, became completely dominant. The statistical law could be determined using a very simple mathematical model, and the most interesting part is that it matched the observations in abundant species within ecosystems. With such simple rules, which could be generalized to bear in mind the type of interactions among elements (competition, predation or mutual aid), it became possible to obtain the vast majority of properties observed in real ecosystems, even though, in many ways, there are a large number of details not even considered within the model. Though I did not know it when I began to work with this model, American ecologist Stephen Hubbell was finalizing a similar theory (the neutral theory of ecosystems) that ended up becoming a classic reference. This theory evidently supported the idea that complexity in the world can, to a great extent, be understood by simple models. In some way, there must be a "physics" of biology that we are just starting to comprehend.

During the following years, I found myself involved with several problems related to instabilities in viruses and cancer. In a collaborative effort with colleagues from Yale University, we published one of the first articles that demonstrated (using experimental data and models) that tumours display a wide range of mutations in space; in other words, different areas in the tumour display different mutations. This result contradicted the dominant viewpoint of tumours as "clone" systems in which the most advantageous mutations are dominant in the tumour, making it especially homogeneous. Our study demonstrated

that tumours in fact resemble diverse ecosystems in which – as we had already demonstrated a decade earlier – space plays a fundamental role by sustaining great diversity. Similarly, along with my colleague Thomas Deisboeck, of Harvard, I proposed the idea that cancer evolves towards a state of disorder that has a critical limit of instability beyond which the tumour is unable to advance. For now, this idea has been demonstrated only indirectly, but it seems to be supported by the evidence obtained from *in vitro* cell systems, studies on the evolution of genomes and clinical data. If correct, it would become possible to design tumour fighting strategies based on making the cancer's instability rise above this critical point, above which the anomalous population would be unable to survive. This problem could be formulated theoretically using mathematical models that were developed in a study on virus populations, once more demonstrating the potential of models able to transcend the field in which they are used.

With the turn of the century, a revolution took place in the field of complex systems. In part, it has come to dominate everything that has happened since. Two studies appearing in *Science* and *Nature* demonstrated that the structure of the networks which define the interactions between elements in a real system are far from simple. For instance, when the individuals in a social network, the computers on the Internet or the nodes in an electrical system were used, it was verified that these systems define a "small world" in which two elements chosen at random are separated by a very small number of leaps within the network. At the same time the networks studied, in general, displayed great heterogeneity: most of the elements were connected with just one, two or a few components, whereas a very small number of them were hyperconnected. Yet again, it was possible to demonstrate that these properties are related to very fundamental rules on growth and the connection of items in a network. These ideas came years after my complex systems laboratory had been studying models of genetic, ecological and artificial networks. For years we had raised many questions about the origin of complexity, and all of a sudden we had an incipient theoretical framework to support our work. Within the space of barely one year, we completed pioneering work to study ecological, linguistic, technological and cellular networks. These studies had a major impact that even reached the pages of the *New York Times* and other media. I remember that time as extremely intense and exciting. Plus, I was able to share it with an exceptional group of students who included Sergi Valverde, Jose Montoya and Ramon Ferrer i Cancho, as well as some no less brilliant postdoctoral researchers, in particular Romualdo Pastor Satorras and Eric Smith. We had to define the networks of human languages – non-existent up to then – and demonstrate that their architecture might perhaps explain some of their strange properties (such as their ambiguity and enormous efficiency). Our analyses of ecological networks revealed, on the one hand, great stability in the random loss of species, but major fragility in the extinction of key species. Our analysis of technological networks, in particular, which define the interactions between parts of a computer program, demonstrated that, despite being objects made by human design, they were in many ways similar to the networks of interactions between proteins in cells. This observation had a profound interpretation that I discovered during a stay in Santa Fe. Attempting to understand the way in which evolution can produce genetic networks of increasing complexity, I had developed a complicated model in which different genes produced proteins that activated or repressed other

genes. The model included an error process based on random duplication of one gene (and its interactions), making it possible to study the effect of growth. In those days, some of the properties of protein interaction networks were becoming better known. They seemed to indicate the existence of a small cellular world, while also displaying great heterogeneity. My plan was to verify to what extent the functions of genes were altered by this process, and whether maintaining stability or homeostasis bore any relationship to the network's architecture. However, the model was slow and difficult to study. At a certain point, I decided to make a clean cut and forget about genes, functions and proteins. Let us imagine – thinking like a physicist – that we have a set of balls connected by wires. We choose one at random and "duplicate" it: we add a new ball that is a copy of the chosen ball and also copies its connections. We know based on the genome study that many of these connections, now redundant, are quickly lost, and also that new ones are created from time to time. When using these simple rules, which lack a connection with the functionality of cellular networks, something surprising happened: it was possible to obtain most of the structural properties observed in the real world. The simulated proteome had a small world configuration and the same heterogeneous structure we already knew about. And it required nothing more than the mathematics associated with the aforementioned rules. That seemed to support an idea formulated by French biologist François Jacob, who very appropriately indicated that biological evolution is produced to a great extent in a makeshift fashion. Unlike an engineer, who can in principle do without all of the above, evolution must use what it has available and then reuse it over and over again. The process of duplication and reconnection is one clear example, and my results resoundingly indicated that a large part of the observed structural order was, as Kauffman would say, "order for free". In fact, however, what was observed went even further because it indicated that desirable properties of stability compared with disturbance or mutations had no reason to be strongly selected by evolution. They were just an inevitable result of the makeshift process, and the structures produced could then be used to perform the proper functions and do so in a very efficient way. The existence of an important part of biological complexity and technological complexity as well, might simply be unavoidable. The theory of complex networks gradually took shape over a span of years, and it is currently one of the theories with the greatest impact, to such a degree that it has made inroads into all sorts of disciplines, from the pure sciences to the humanities. Personally, it was an essential part of searching for fundamental laws, and throughout those years we used it as a theoretical framework to keep seeking answers to difficult questions, including the origin of human language and its nature, or the similarities and differences between natural and artificial evolution.

My latest adventure was ushered in by synthetic biology and biological computation. The twenty-first century began with the beginnings of a new way to manipulate cellular systems that comprised a qualitative leap in terms of traditional genetic engineering. So-called "synthetic biology" arose as a nearly engineering-style approach to biology. The underlying idea is that we can redesign the networks of molecular interactions on the inside cells so that they can carry out totally new functions. The scope of this field has yet to be seen, but it has undoubtedly changed our perception of what can be done in terms of modifying life. My laboratory got involved in several projects related with this field,

including an international project that unsuccessfully attempted to create an artificial cell and a later successful project in which we developed a new way to build something resembling "biological computers". The possibility of getting sets of genetically modified cells to perform pre-programmed detection and response processes is of great importance: we can create systems that detect and eliminate tumour cells, or even make cells with tissues other than "natural" tissues carry out necessary functions when damage is caused to the original tissues. Many other biomedical applications are being considered at present. One problem with this approach is that the design of these systems has substantial comparative differences. An example is designing electronic circuits. In a conventional circuit, each component (resistors, diodes, transistors and so on) is connected to others using identical wires. Inside a cell, the situation is quite different. A gene gives rise to a protein that, by acting on another gene or genes, plays the role of a "wire". Wires cannot be identical, though, because, if they are, communication between genes would be useless, given that any gene would influence any other indistinctly (we could literally say that they "get their wires crossed"). Different proteins make it possible to avoid this ambiguity, but this means that molecular wires possess an identity. If we hope to design a complex circuit that detects signals and reaches decisions, we must also use or design different wires. The problem is that the difficulty in such a design rapidly increases, and the more different connections we add, the more likely it becomes that we will modify other parts of the cellular circuit. In summary, beyond just one connection type, problems multiply in general. At the same time synthetic biology was originally designed to be a biological equivalent of circuit design, the traditional version of which meant we could build anything with simplicity by combining existing parts as if they were Legos. However, the vast majority of biological systems designed to perform a task (whether synthesizing a drug, detecting a chemical signal or responding to external stimuli) are only useful to perform the function for which they were created and cannot be reused. Can we escape the limitations imposed by biology? The answer is yes. By developing a theoretical concept that I had explored years before in collaboration with a colleague of mine, physicist Javier Macía, using yeast cells genetically modified in the laboratory of researchers Francesc Posas and Laia de Nadal, we were able to create cell libraries that formed basic modules in our circuits. We were able to demonstrate that, by breaking certain basic rules of standard design, multicellular circuits could be designed that would carry out complex tasks while allowing for enormous combinatory flexibility. To a certain extent, we were imitating other forms of computing that exist in nature, such as that of social insects. Ants in particular process information in a distributed manner, and our system for manipulating cells allowed us to come close to that type of computation. This new type of engineering opened the doors to a large number of biomedical applications, and it also allowed us to look at fundamental questions once again. We can now contemplate scenarios that seemed like science fiction just a few years ago, like redesigning parts of a failing organ by "relocating" the absent functions in other types of cells completely unrelated to those of the original organ.

During the beginnings of that collaboration, an unexpected turn of events took place. The Botín Foundation chose me as a researcher, within the framework of its commitment to science and research. This initiative, completely unique in our country, supports research groups considered to be cutting-edge at the forefront of biomedicine. Someone informed

me that a representative of the Foundation would come and visit me in one week, and what I had heard up to then was amazing enough to keep me awake for nights. For five years, the Foundation would support any research carried out by the group by making a hefty investment, providing full freedom and the additional incentive that, should the time come, it would help transfer the result of the research to the applied world. The day arrived and a man appeared in my office with a briefcase. He represented the Botín Foundation and formally asked me if we could speak for a while. We sat down and I immediately attempted to explain to him – as I had mentally rehearsed on the preceding days – the work we were doing at my laboratory. I was concerned that the eminently theoretical path taken by my laboratory might constitute an impediment that would keep us from being selected. In one minute, my visitor interrupted: "You don't need to explain yourself. We already know who you are." He very kindly asked me to let him explain: "I have come to make a proposal to you". The proposal was essentially what I had heard about. My visitor introduced himself as a doctor with experience in the world of research and a clear concern for the state of science in our country. His proposal was unique in our world. He trusted researchers and the fact that they would put forth their best efforts and talent to carry out research without interference by their benefactors. There were only advantages, and as Dr Pedro García Barreno explained those implausible benefits, I asked myself whether this could truly be possible. When he finished, in the same courteous manner as at the beginning, he said to me: "It would be an honour if you would accept our proposal". I did not know what to say, other than an incredulous "yes", from which I have not yet fully recovered. This would be the opportunity to do what was not otherwise possible: to create an experimental laboratory where we could develop our ideas on synthetic biological systems in the real world, beyond just our chalkboards with equations. We therefore created our synthetic biology laboratory, in which we are currently developing completely new lines of research through which we hope to understand biology, while at the same time exploring other alternatives. You could actually say that a dream has come true.

Synthetic biology offers us a unique opportunity to approach the problem of the origins. Instead of just observing reality or building models, we now have the chance to modify living organisms themselves and create new forms of interaction among cells, change their ageing process, print organs and tissues, and even connect computers and cells to each other. In addition to other possibilities (and these are just some of the ones we are currently studying at my laboratory), we can recreate some of the great inventions in evolution, including multicellularity, symbiosis, cooperation and even, perhaps, certain types of intelligence or complex communication. The questions and challenges are increasing in number: can we convert simple cells into microrobots capable of communicating and acting like ants? Could we use our synthetic ants to explore the inside of an organism and reconstruct damaged parts? Will we be able to design micro-organisms capable of integrating into our bodies to perform new functions? Can we communicate with the microbiome to make it respond to signals that allow us to take action against pathologies? Will the resulting synthetic systems be similar to those that developed during the evolution of our biosphere? Or, on the contrary, will we observe alternatives that never arose on their own, or perhaps which we alone can create? The answers to these questions may shed light on the nature of living systems and their evolution. They may show us alternative

worlds that never came into being on our planet. Perhaps they will lead us to reconsider our ability to modify our own bodies and find new ways to deal with diseases that could not be treated up to now. I believe we must even bear in mind a possibility not yet contemplated in our relationship with the planet we inhabit, which we are going to lose if we do not do something. Perhaps this is the best time to consider our chances to redesign the biosphere by using the tools synthetic biology has to offer. If we scientists have a privileged viewpoint of the cosmos, this is also because we are able to project our theories and models into the future. In just a few human generations, the world as we know it may change drastically, undergoing one of the critical transitions that distinguish an inhabitable biosphere from another that is completely unpredictable. In some way, it may lie within our reach not only to cure our diseases, but also, in the words of James Lovelock, to cure our own planet.

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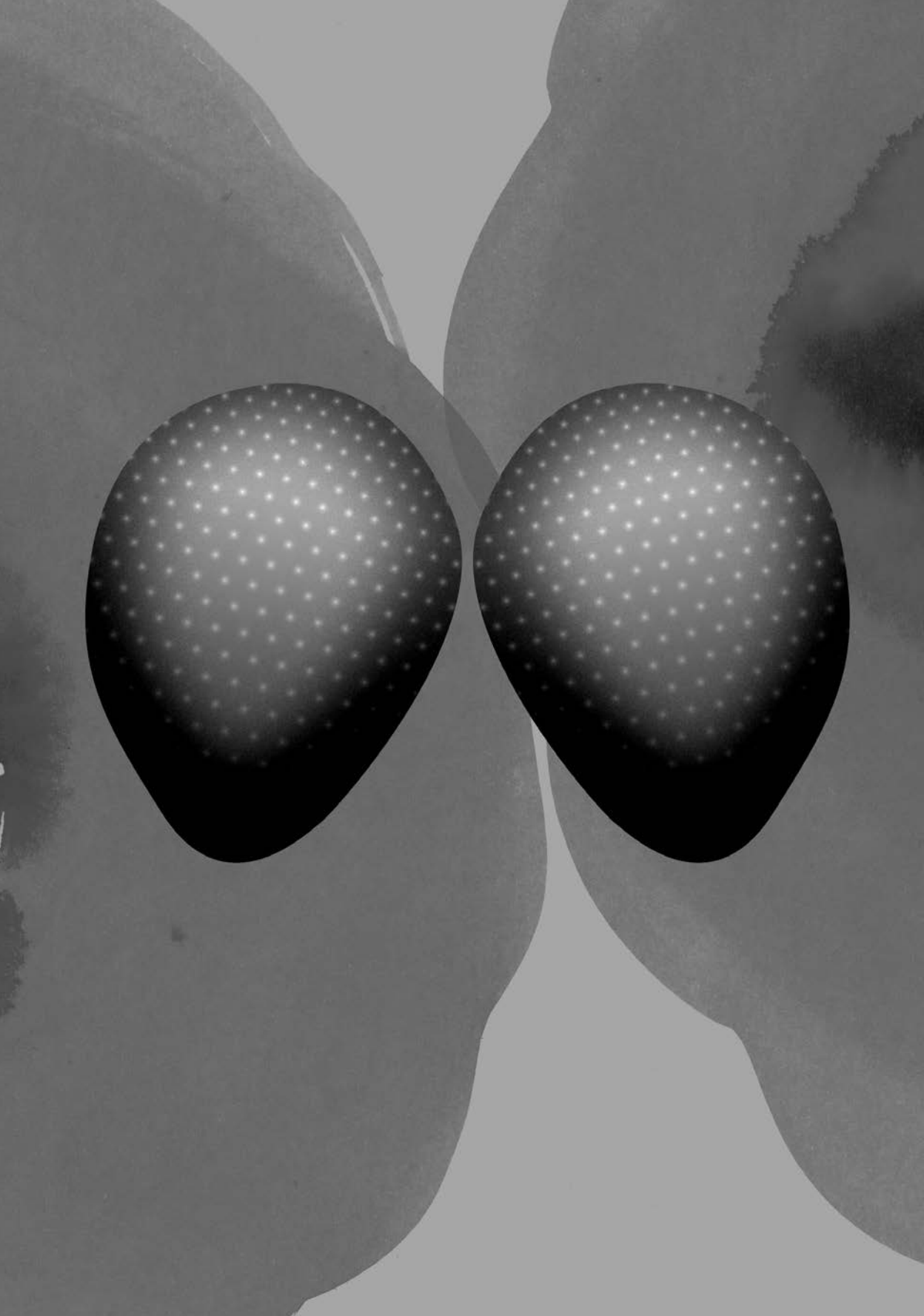

GUSTAVO GUINEA

JACK OF ALL TRADES, MASTER OF NONE

24

My haughty Fair a sonnet bids me make...* A disclaimer

I feel quite uncomfortable writing about myself. I am not old enough to offer any tasty nuggets of wisdom from my past experience, and my life history has not been particularly notorious or noteworthy. Writing has never been a hobby of mine, let alone a job, and I have never kept a diary aside from the jottings in my laboratory notebooks. I thus wish to warn you that you may risk becoming bored or irritated – or both – before you have finished making your way through these pages. For readers who have absolutely made up their minds to get to the end, I recommend that they speed-read their way through; I will do my best to encourage such a strategy by making my paragraphs as concise as my memory and writing skills will allow.

To those who might ask why I have gone ahead and written the following lines in spite of everything I said in the previous paragraph, I would answer that I have done so in compliance with one of the principles that has guided my life, or at least one that I have always taken seriously: obedience. Several months ago, Professor Pedro García Barreno wrote me a very kind letter requesting an autobiographical essay – this very one – to form part of the institutional memoir that was being published to help celebrate the tenth anniversary of the founding of the Botín Foundation Science Programme. As I am always inclined to follow orders, particularly when the person giving them is someone I respect and admire, as is the case here, I have knuckled down and got to work. If you are reading this now, you will soon be in a position to decide whether your obstinacy is strong enough to overcome your boredom.

However, one by one, I have ek'd out four... The early years

I was born in Madrid in 1962; fortunately for me and my future, my family gave priority to my education. My father had studied at university, and had built a library of nearly a thousand volumes that he taught me to treat with reverence. Not until my adolescence had nearly begun was I allowed to touch any of the books without first proving irrefutably that I had just washed my hands. I am an only child, and my parents were already quite old when my mother gave birth to me. Extremely old, in fact, by the standards of the time, and even the standards of today – my father was turning sixty when I was born. I believe that the two elements that were primarily responsible for the path my life later took both personally and scientifically were the fact of being an only child, and the vast number of hours I spent in and around my father's books (which, due to lack of space elsewhere, also lined the walls of my bedroom).

The special attention that I received from my parents and other close relatives was such that I was reading and calculating skilfully at a very young age. I still remember the frus-

tration I felt during my first day of primary school at Colegio Salesiano San Juan Bautista de Madrid, where, in accordance with the rules of the time, I was put into a classroom full of students my age. I already knew a good deal more than they did, and the result was an extremely boring first year – the first great disappointment of my academic life.

In spite of such an awkward beginning, my memories of school are happy ones. I do not recall ever feeling particularly overworked, even when the courses were academically demanding. Teachers back then tended to give a good deal of homework, and I took mine very seriously. As I have implied, I always prefer to follow the rules, and I have never had trouble respecting authority or giving credit where due; as a result, all my teachers thought I was a good student, but would never have accused me of being a swot or a toady. To use a more modern expression, you could say that I always made the classroom's Top Ten.

In terms of athletics, I never stood out, but neither was I a complete oaf. The fact that as of age nine I already had to wear glasses to correct a hereditary myopia – my mother and father would both lose their sight to this disease – tempered my interest in contact sports, and led me instead towards mountain climbing and rock climbing, two activities I still enjoy.

From a very young age I felt a special affinity for maths and applied geometry because of their astounding (or so it seemed to me) ability to solve common problems and predict the behaviour of our physical surroundings. I have always been rather gifted mechanically, and as a child I loved to think up and solve mechanical problems. One of the youthful accomplishments of which I am most proud – I must have been eleven or twelve years old at the time – was predicting the geometry of the nozzle of a *churro* maker in a snack food factory near my house. I found myself wondering how they went about making closed-loop *churros* out of a steadily moving rectilinear bar of dough interrupted only by the guillotine-like movement of the operator's finger. I finally got to see the *churro* maker in action up close: obtaining the experimental proof of the accuracy of my prediction was without doubt my first great scientific satisfaction.

During my school years I was very fortunate in that I had a number of excellent maths, physics and chemistry professors who taught me a good deal more than just the rules and procedures of their respective subjects. Both the friendly demeanour and the intellectual rigour of Professor Pascual, Professor Luis and Professor Bartolomé had a great deal to do with my enthusiasm for physics and my subsequent professional development in that field.

These rhymes, said I, I never shall complete... The baccalaureate years

When I began studying for my baccalaureate – once again at Colegio Salesiano, once again with the same excellent teachers from previous years – my affinity for maths and above all for physics crystallized to form the vocation that has since determined the course of my life. It was in these years that I acquired the mathematical maturity necessary to deal with increasingly complex problems. Discovering infinitesimal calculus, integral calculus and vector algebra opened my mind to a universe of physical models that I found absolutely delight-

ful. In this sense, I think that earlier educational programmes were superior to those of today. We may well have advanced on other fronts, but it is both painful and disappointing to me each time I am reminded that so much of the physics, maths and chemistry that I learned for my baccalaureate are now taught either incompletely or not at all.

I especially enjoyed looking through my father's technical books, some of them quite old, and contrasting them with what I had learned in modern textbooks. The archaic language, the strange units of measurement, the out-of-date theories and the grim black-and-white dip pen illustrations seduced and hypnotized me. I learned a great deal trying to translate concepts and theories from one epoch to another, as if the different time periods were different languages. This theoretical apprenticeship, begun when I was just a child, led me to experiment fearlessly and without regret on all types of objects. In addition to my own toys, my materials included – much to the annoyance and chagrin of my progenitors – my father's watches, the miniature steam-powered machines he collected, and our entire collection of household appliances. Perhaps the biggest problems occurred during my adolescence, when I was almost never able to completely rebuild the things I had taken apart; I would say my reconstruction projects generally stalled at around seventy per cent. Since then, I have held the designers of toys, watches and other such mechanisms in the highest esteem.

In addition to my fondness for mechanisms of all sort, during my adolescence I developed a genuine passion for books, one that led me to read a significant portion of my father's library, and a great many other tomes besides. Looking back, I do not remember another period of my life when I read so fruitfully, not even now, when my academic tasks require that I be up to speed on all the latest articles in my field. I have to admit that more than once, swept along by my fervour for literature, I took funds that my parents had intended me to use for schoolbooks, and, instead, used them to buy books that, while excellent, were not strictly speaking necessary. Such was the case with the non-purchase of a maths book for the second year of my baccalaureate; in its place appeared the three volumes of the physics text by Alonso and Finn,[1] purchased at a cost four or five times higher than my parents had authorized. I am proud to say that I still have all three volumes, and still consult them relatively frequently.

My interest in philosophy and metaphysics also dates back to this period, perhaps, once again, because I first explored these territories in the company of a professor who offered rigorous explanations of the basic concepts on which more complicated theories had then been built. It goes without saying that as a dutiful child of my era – the 1970s – I read a good deal of modern and contemporary philosophy. It was not until I was well into my thirties that I turned my gaze towards classical and medieval philosophy, finding it much more natural and human than work from later centuries. Physics and metaphysics are my two great intellectual passions; I consider them the two sides of a single coin.

And found the second Stanza half way done! The university years

The high grades that I obtained in my baccalaureate, particularly in the more technical subjects (maths, physics, chemistry, drafting), made it clear to me what

path my university schooling should take. They made it even clearer for my parents, who wanted more than anything else to see my baccalaureate success used as a launch pad for a university education in engineering. However, I much preferred physics.

Once again, the conflict was resolved by applying the principle of authority. As an obedient son, I agreed to study engineering. Forced to choose more precisely, I went with telecommunications engineering, which, with its notions of waves and electrons, seemed closer to physics than the other options. I received high marks on the university entrance exam, and chose the School of Telecommunications Engineering at UPM (the highly prestigious Technical University of Madrid). Two boxes for back-up choices remained to be filled; I picked Aeronautical Engineering and Civil Engineering, at the same university, and in that order.

Here I should pause briefly to note that, looking back, it was at this very point in my life that I first noticed the existence of a deeper ordering principle, a unifying thread guiding the decisions which, regardless of whether or not I was fully aware of it at the time, marked out the route that my life was to take. I no longer believe that there is such a thing as coincidence.

In spite of the high marks I had received on the entrance exams, and in complete disregard for the specific entrance requirements of the fields I had chosen, fate or administrative laziness (or both) resulted in me gaining admission to only one school: Civil Engineering, my third-place choice. My efforts to appeal the decision were fruitless, not because I was wrong but because in the early 1980s first-year students in all the different engineering fields took the same courses, at least on paper. I had no choice but to agree to study civil engineering, with the intention of transferring to telecommunications the following year, assuming I kept my grades up. Such was my second big dust-up with academia.

At the beginning of the summer before my university courses began, I took the exams one had to pass in order to become a technician for the national telephone company, Telefónica. Money was always tight at home, and for the most part the job would only require an understanding of the sort of maths and physics I had learned for my baccalaureate. Moreover, the job would help prepare me for the brilliant career I envisioned for myself as a telecommunications engineer.

Unfortunately, this plan did not work out either. I passed Telefónica's technical exams, and was the one and only applicant to fail the eye exam. It turned out that while the myopia I had inherited from my parents had not posed a problem during the exam, the deuteranopsia my own genes had added to the mix meant that it was difficult for me to distinguish certain shades of red and green. Stoplights would never give me any trouble, but the Ishihara Colour Vision Test was pitiless. I did not get a single answer right. But I am a hard nut to crack, and it was not long before Telefónica posted the dates for the exams required to become a repairman. I signed up once again, and this time I had some invaluable help: a friend provided me with a complete copy of the Ishihara Colour Vision Test book. Once again I passed the technical exam, and I walked confidently into my eye exam, my memory stuffed with the numbers that I would be asked to identify on each page. But there was a new ophthalmologist this time. And he had a new book of tests. And once again my failure was complete.

I have always been one to seek opportunity precisely where others see only disadvantage. My weak vision (and my acknowledgement thereof) decisively influenced my next decision regarding my future: I concluded (mistakenly, I now believe) that if I went on to study telecommunications, I would end up failing the rigorous medical exams given by businesses in that sector. So I decided it would be a good idea to carry on with my civil engineering studies, particularly given how well I had done the first year. I finished the six-year undergraduate degree programme in 1986, and two years later I obtained my licentiate degree in Physical Science from UCM (the Complutense University of Madrid), having started that programme while in my third year of civil engineering at UPM.

In spite of the ups and downs of my early years at university, the truth is that I really enjoyed studying civil engineering, I was quite the star in my class, and to this day I consider it my true profession, even though I may only take part in the field as a university professor.

On the first Triplet thus I enter bold...
The doctoral degree and early years of research

My relationship with UPM's Department of Materials Science (my second home in every sense – in fact it might even be my first home, given how much time I have spent there) began in the summer of 1983, just after I finished my third year in civil engineering. I wanted to find a summer internship in a laboratory, not some company, so I headed over to the Physics Laboratory. The professors there were surprised to see me. It was very uncommon for engineering students to be at all interested in research. My life would surely have turned out very differently if my request had been turned down, but instead it was accepted. The department head, Manuel Elices – my mentor and teacher from that day forward – welcomed me with open arms. After working with him for several summers, I decided to get my PhD and dedicate myself to research. I made this decision in spite of a new-found passion for bridge design in my original field of study. I was very sorry to be unable to accept Professor Javier Manterola's offer of employment in the Project Management Office of Carlos Fernández Casado SL, a world-class engineering firm. It was not an easy decision. My parents and my girlfriend – now my wife – never understood why I chose the path I did. But my life's unifying thread remained unbroken, and would not let me simply drift away.

I have a powerful curiosity regarding the ways of the natural world – the supernatural world too, though this is not the moment to talk about that – and few things escape my curious eye. I had received a scholarship to do my PhD at UPM's Department of Materials Science and shortly after I arrived I blew out the head gasket on the old Renault 5 that I had inherited from a family member and was using to get around. Wasting no time, I took advantage of the abundant surgical-mechanical materials available in the department's workshop, and early one morning I began the operation. Hours later, I had all the motor's parts laid out in careful order on the floor. Valves, seals, rocker arms, cams, washers, nuts and all the other pieces waiting nervously to be put back in their original positions. My daring collaboration with David Culebras, the laboratory technician, had made short work of the first half of the job.

Many of my classmates offered us encouraging words, albeit tinged with tones of sympathy and scepticism. For some of them it was quite a surprise to hear the motor come to life. We had successfully put the motor back together, and not a single part had been forgotten! What is more, the engine now idled much more smoothly, in spite of the fact that it had been adjusted with tools that could hardly be considered specialized. That day I won the respect of many people there in the laboratory, including several who were reluctant to tell me so until years later.

My doctoral thesis, which I finished in 1990, was entitled "Measuring the Fracture Energy of Concrete", and was co-directed by Manuel Elices Calafat and Jaime Planas Rosselló. I do not have the words to express how much I learned while working alongside these two people. At the risk of being reprimanded by one or both when they read these lines – they are both deeply modest, as befits their great magnanimity – I will say only that in my entire life I have only met one other person who can compare to them in terms of intelligence, intellectual brilliance and personal warmth. It was a privilege to work with them. I greatly enjoyed it then, and I enjoy it now.

In the course of the years I spent becoming a research scientist, I specialized in Materials Science, delving into the relationships that exist between the composition and structure of materials on the one hand and their mechanical behaviour on the other. I was particularly interested in fracture mechanics. I spent time as a visiting scholar at the Joint Research Centre that the European Union created in Ispra, Italy, and also at the International Centre for Theoretical Physics in Trieste (Italy), working on both theoretical and practical aspects of the fracture of materials. My thesis focused on civil engineering's archetypal material: concrete. I know that many people consider concrete to be a second-rate building material, but that is not at all the case. The scientific quality of a material resides less in the material itself than in the intelligence we bring to bear when studying it. And Manuel Elices and his group have studied concrete with a high degree of intelligence indeed. Extremely high, I would say. The contributions they have made in the field of nonlinear fracture mechanics, especially as regards analytical, numerical and experimental models of cohesive fracture, are the irrefutable proof of my assertion.

And, as it seems, my speed I still may hold... Academic initiatives

It will come as no surprise to those who know him personally to hear that Manuel Elices is an extremely energetic person in all facets of his life, including the academic. After establishing himself in Spain as a pioneer of fracture mechanics studies – in 1975 he personally developed the course by that name that became a permanent part of the civil engineering curriculum, and in 1984 he founded the GEF (Spanish Fracture Group) – he later accepted the challenge of creating Spain's first official university degree in Materials Engineering, which he founded at UPM in 1995.

This project required a tremendous amount of effort and forbearance on his part, all of which I witnessed from up close. By his side almost from the start, I did what I could to help

as a contributor and coordinating secretary for the degree. In 2009, following the steps outlined in the Bologna Process, an undergraduate degree programme in Materials Engineering was created at UPM, and in 2013 a Master's programme in the field was added as well.

There are currently more than four hundred students studying Materials Engineering at UPM, and that number has grown steadily each year. Such are the results of the coordinated efforts of many professors from many different departments (Aeronautical Engineering, Civil Engineering, Industrial Engineering, Mining Engineering, Naval Engineering, Telecommunications Engineering and so on) who have shown great generosity and altruism in lending their abilities and energies to the task of educating our students in this field. I have observed all this first hand over the past several years, as I stepped in for Manuel Elices when he retired in 2008.

As for my own career trajectory here at the university, in 1991 I was awarded a post as associate professor; I was placed in charge of the Fracture Mechanics course, a position I hold to this day. In 2002 I took on the additional task of transforming the GEF into an official scientific association known as the Spanish Structural Integrity Society; I served as the society's first president from 2002 to 2011.

My experience in academia has confirmed that research and teaching should ideally go hand-in-hand, with the latter serving as the engine that powers the former. It is during one's university years that one's research vocation is most often forged – my own life serves as an example of that. Moreover, keeping close contact with the teaching process helps make researchers more rigorously analytical, more organized, more critical, more attentive, more open to innovation and the exchange of new ideas. Finally, there are the many benefits that students derive from being in contact with active researchers, which are so obvious as to hardly bear mentioning.

Teaching and research form a binomial in which the former holds the superior position, as do all activities that prioritize human contact. The possibility of becoming better human beings is always present in the course of such activities. We should take this very seriously indeed.

Since this Foundation is so fairly laid... Biological materials

Towards the end of the 1990s Manuel Elices began researching structural biological materials (those whose most salient property is their physical strength) from the perspective of materials science: he sought to understand the relationships that link composition, structure and mechanical behaviour. As the epitome of his studies, he chose spider silk, which is to this day unequalled in terms of its mechanical properties.

I joined his research group near the beginning, after being awarded the chair in Materials Science in 2001. Together with my colleagues José Pérez Rigueiro and Gustavo Plaza Baonza, I have been researching silk intensively since then. I can say with satisfaction that our research group has well and truly earned its place among the few elite organizations who

have made significant contributions to the scientific understanding of the microstructure and property control of silk.

That said, our interest in biological materials is not limited to silk alone. We have also researched mollusc shells and their extraordinary structures, and, more recently, collagenous tissues, which are fundamental to the make-up of ligaments, tendons and many of the human organism's other most resistant structures. Similarly, together with my colleagues José Miguel Atienza and Francisco Javier Rojo, I have been investigating the strengths and biomechanical behaviours of human blood vessels and the tissues used to create prosthetic heart valves.

Now for the Second. – And so well dispos'd... The Centre for Biomedical Technology and the Botín Foundation boost

In 2008 UPM's previous rector, Javier Uceda, gave the order to create the CTB (the Centre for Biomedical Technology) with the goal of galvanizing biomedical research at our university, which at the time had no organization dedicated specifically to work in that field. The CTB opened its doors on the Montegancedo campus in Pozuelo de Alarcón (Madrid) in the spring of 2011, following years of extraordinary efforts – so extraordinary, in fact, that there are not words enough to acknowledge them – by its current director and driving force, Professor Francisco del Pozo Guerrero. The centre is profoundly interdisciplinary; from the beginning it has welcomed researchers from both inside and outside academia. Our research group, which is dedicated to investigating both biological materials and biomaterials, has its home in the laboratory on the ground floor.

The goals of our research group can be grouped into three well-defined lines of inquiry: the study and development of fibres and biomaterials based on silk fibre proteins; the study of the mechanical behaviour of collagenous tissue and its use in bioprosthetics; and the study of the mechanical interaction between cells and tissues. In each case, but above all in the first-mentioned area, the group had a good deal of experience to begin with, and had already developed the basic characterization techniques necessary. All the same, we lacked a great many things, especially in terms of personnel, and it was thus necessary to find a stable source of funding that would allow us to meet the challenges of the research we were undertaking in the recently opened centre.

I do not remember the exact date – I believe it was in the autumn of 2009 – when Pedro García Barreno invited me to join the Botín Foundation's Science Programme. However, I do remember how perplexed I felt at receiving such a generous and seemingly nonsensical offer. I say that because, like all true Spanish researchers, I had had plenty of experience in the ever more chaotic process of writing and justifying requests to fund specific projects, and the idea that someone might be able to offer us medium-term economic support so easily seemed like something straight out of a fairy tale. But Pedro García Barreno is not some otherworldly prince in shining armour, let alone a fairy godmother. After wavering in disbelief at first, there was nothing for me to do but come to terms with the fact that his offer was as real as it was generous.

It is now four years later, and I can confirm that my first impression was correct: the assistance the Botín Foundation has given us has been extremely generous in both human terms and material ones. Their help allowed us to start off on the right foot; since our doors first opened, their funding has provided stability to the CTB's Biological Materials and Biomaterials Laboratory, and underwritten a large portion of our personnel expenses.

It would be difficult to quantify the exact degree of in/dispensability to assign to the help they have given us, or to calculate the percentage of my group's research that would have been impossible without their funding. Any given number – 100%, 0%, anything in between – would be wrong in the sense that rather than simply paying for a given amount (difficult to quantify or otherwise) of our research, the Botín Foundation funding has, instead, created a paradigm shift (to abuse the term coined by Kuhn[2]) within our research group. That is, in addition to providing resources to help us along our scientific path, the Foundation has fostered a new way of both "seeing" and "doing" science and technology, encouraging us to explore more creatively, to seek ever stronger connections between applied technology, its dissemination throughout society and its socio-cultural commitments.

For all these reasons I am and will always be profoundly grateful to the Botín Foundation, to its late president Emilio Botín, to its directors (Rafael Benjumea in the early years, and Íñigo Sáenz de Miera now), and in particular to Pedro García Barreno and Francisco Moreno, the most intellectually engaged and most visible members of the Science Programme. I am also thankful to all those who have worked so closely with us over the years, regardless of their place in the Foundation's chain of command. Finally, special thanks go to Pablo Cironi, Michael Tadros and Lala Aguadé for their support, availability and approachability.

My Muse appears, that Thirteen lines are clos'd... The challenges that remain

What have I accomplished? What do I want to accomplish in the future? These are hard questions to answer, but it is imperative that we ask them. Having reached a certain age, one is forced to recognize that little in this life can be achieved on one's own, particularly in the world of science. To paraphrase Newton, we are able to see farther than others only because we are standing on the shoulders of giants. All the same, it is essential to take stock of where we have been, and to form a personal plan for the future.

I turn my gaze towards the authorities – a word I use in its most positive sense, is there another? This technique has never failed me. And it has always seemed to me a good idea for large research centres and consortia to surround themselves with those famed for their wisdom; to ask them to help find answers to the first of the questions above, and suggest future paths for the second. I humbly attempt to do the same, surrounding myself with those who have earned my highest respect. And it is not any sort of unthinking or unreflective adherence. The paragraphs that follow have for the most part been distilled from conversations with people I consider to be flawlessly trustworthy, both as scientists and as human beings.

It is not without a certain pride that I look back at what I have accomplished in academia, perhaps because, as I have already mentioned, I believe that working in direct contact with others and contributing to their education are the shortest paths to personal and institutional improvement. For me, my signature achievement in this field was working together with Manuel Elices to get the Materials Engineering degree programme up and running. But I am also proud of the classes I have taught, the hours of mentoring, and the good company of so many students who have chosen to share my path.

Another source of personal satisfaction is the contributions I have been able to make in the field of nonlinear fracture mechanics, particularly in terms of the cohesive fracture model, which has proven so useful for the study of cracks in materials as seemingly disparate as concrete and polymethyl methacrylate. The work done by Manuel Elices and Jaime Planas in the 1990s established a foundation for the cohesive zone model and opened up new routes both for its numerical implementation and for measuring material parameters.[3–8]

The bulk of my scientific work has without a doubt been devoted to biological materials. And my contributions in the field have been, I believe, outstanding. Among them, I am proudest of those related to the recovery and property control of the dragline thread (also known as major ampullate, or MA, silk) of web-spinning spiders, as well as their flagelliform (or capture-spiral silk) thread.[9–12] Also of note was our experimental proof that the ability to recover from deformation – a quality found nowhere in the natural world except spider thread – can be brought forth in fibres composed of other proteins, such as the fibre produced by silkworms, via a proper processing.[13] I believe that these discoveries will have substantial repercussions in the not-so-distant future as regards the production of biomimetic fibres.

The expansion of our knowledge of the microstructure of silk through the use of atomic force microscopy also bears mentioning,[14–16] as do the Raman microscopy projects directed by Dr Fernando Agulló at the ICMM (Materials Science Institute of Madrid), and the work on X-ray microdiffraction done in collaboration with Dr Christian Riekel at the ESRF (European Synchrotron Radiation Facility) in Grenoble, France.[17–19] And together with Dr Todd Blackledge at the University of Akron in Ohio, and Dr Cheryl Hayashi at the University of California at Riverside, both of the United States, we have followed the philogenetic pathways of the most salient properties of spider silks.[20–22]

Our results in other areas have been equally important for applied science and technology, if perhaps less significant for basic scientific theory. The properties of arterial blood vessels play a deciding role in the development of both the pathologies that affect them and the strategies used to treat them.[23–26] We have not yet invented a foolproof procedure for selecting collagenous membranes that can guarantee the durability of the flaps on the valvular bioprosthesis. However, we have made modest contributions in both of these areas, experimenting with new trial methods and procedures for both the characterization of arterial tissue and the selection of collagenous membranes.[27–29]

Of course, there remains a great deal to be done; in fact, I believe that the most interesting work is still before us. We began producing biomimetic fibres some time ago, taking advan-

tage of the priceless knowledge we have acquired regarding silk thread. While the composition of the thread produced is of course important, we firmly believe that finding the proper production process will be the key to final success. The poor results achieved thus far by everyone in the field (our team included) with artificial proteins produced via genetic and biotechnological engineering is proof that our current silk-spinnining techniques are insufficient to allow for the subtle manipulations at the microstructural level that such thread requires. That is why we have been working together with Professor Alfonso Gañán of the University of Seville to create a new method, currently in the patent-writing phase. The preliminary results are highly encouraging, in terms of both the quantity and the quality of the thread produced, and I hope that we will soon have a new process to offer the scientific community.

Something similar has occurred with the use of silk proteins as scaffolding on which to engineer new tissues. Having demonstrated the regenerative qualities of silk, we are building the foundations that will allow us to use such proteins to build a molecular and cellular capsule for said tissues to keep them from dissolving prematurely, and to better control the release of chemical elements into the surrounding environment. Meshes made of nano- and microfibres are extremely useful for this process, as are thin films and gels. And the early results from tests on mice have shown promise: encapsulated stem cells taken from bone marrow have survived transplantation to areas of the brain affected by stroke. The preliminary experiments, done in cooperation with the research group led by Dr Daniel González Nieto of the CTB, will be published shortly.

Finally, I must say that I am extremely optimistic – perhaps overly so – regarding the consolidation and development of a research line dedicated to the study of cellular mechanics and of the biological response to mechanical stimuli, both individually (at the cellular level) and when grouped together (at the tissue level). We have begun a study on the ageing process in mice, observing the changes that occur in T cells, which are particularly sensitive to the age of their host. We are working on this project together with a world expert on the subject of ageing, Professor Mónica de la Fuente from the Complutense University of Madrid. Once again the early results – currently on their way to being published – have shown that atomic force microscopy is a powerful tool for analysing cellular mechanics, given its ability to register the changes caused in T cell rigidity.

The simultaneous and complementary use of more traditional techniques (including micro-aspiration and traction of cells embedded in gel) allows us to compare and calibrate the more direct measurements made via atomic force microscopy. All these techniques are in use in our laboratory, and we are currently developing new trial techniques to be used on skin cells and myocardiocytes.

Now count the whole fourteen! The sonnet's made. A sort of conclusion

If I have anything at all to offer the world of biology and medicine, it is on one hand my training in materials science and mechanics, and on the other my engineering mentality, which seeks always to understand and solve applied problems. I am proud

to be following in the wake, so to speak, of other civil engineers who did not simply content themselves with the slow, steady development of a career in engineering alone, but went on to explore as far afield as teleprocessing (Torres Quevedo), aeronautics (La Cierva) and even literature (as in the case of our Nobel laureate José Echegaray). While any merits and abilities I may possess certainly cannot compare to those of the aforementioned engineers, I believe that I have the same sort of restless, enterprising spirit that they did.

Arm-in-arm with my mentor and friend Manuel Elices, filled with humility and awe, I have drawn near the frontiers of the biological world, working far from the realm of engineering as such until quite recently. Biological systems (those at all levels, from the biomolecular to the organic) hold without doubt the great challenge of the century in which we find ourselves. Seen from this far side of my life's watershed, I firmly believe that my work and the knowledge I have acquired are of a certain value – minimal, perhaps, but value nonetheless – to the course of human development.

One last note. It may be that the few readers who have made it this far are surprised by the influence that the principle of authority has had on my life. The ability to trust is as necessary for life as the ability to breathe. Individualism and relativism have undone the framework of our society, and we are now paying the consequences. I know in whom I have placed my trust, and this has calmed me, has brought me peace in the most difficult moments of my life. I hope that all of you have found – or one day will find – someone to trust as well.

12 October 2014, Our Lady of the Pillar, National Day of Spain

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EDUARD BATLLE

EXPLORING THE FRONTIER BETWEEN STEM CELLS AND CANCER

25

The research that I have carried out throughout my scientific career has attempted to clarify the relationship between stem cells and cancer. All the tissues in our body, and particularly the skin, the blood and the digestive tract, are subjected to constant wear and tear due to their own functions (transporting oxygen, absorbing nutrients and so on) as well as to external aggression (ultraviolet rays, ingestion of certain foods, infections and so on). In order to avoid breakdown and premature ageing caused by this wear and tear, tissues continually replace damaged or aged cells with new cells. This mechanism, which allows our organs to maintain their functions for years, is made possible thanks to a small number of cells that have extraordinary properties: they live indefinitely and have an unlimited ability to regenerate tissue. This population of special cells, known as stem cells, is present in all our body's tissues. There is no field in today's biomedicine that offers more promise and hope for improving our well-being than research on stem cells. In fact, stem cells have been used in medicine to heal our bodies for many years; the existence of these cells is the reason why skin grafts on burn victims or bone barrow transplants for patients with leukaemia are effective therapies for regenerating these tissues. In the future, we hope to be able to perform similar interventions to regenerate other organs, such as the pancreas, liver or kidneys.

My team investigates to understand how stem cells work at the wall of the intestine, the organ with the highest cellular renewal rate in our body: more than one billion cells on the inner layer of the intestine (the equivalent of 5 grams) are regenerated every day by intestinal stem cells. In particular, our research is aimed at discovering how changes in the homeostasis of intestinal stem cells may lead to colorectal cancer, one of the most common malignancies in our society. In the following pages, readers will find a historical summary of the path I have travelled since my earliest days as a university student, until reaching this field of research. Just looking back twenty years, the scientific and technological advancements in the field of cancer and stem cells have made enormous strides. It inspires a certain amount of pride, as well as nostalgia, to recall here my contribution to the development of new concepts that are now fully accepted by the scientific community and that, in certain cases, led to the creation of new fields of research in which hundreds of laboratories are now working.

Academic background

I was born in 1970, into a working family from the neighbourhood of Santa Eulalia, in the city of L'Hospitalet de Llobregat near Barcelona. My parents owned two local bakeries, and between the two of them they managed to keep the family business running. My sister and I spent many hours at the bakery. Occasionally, when my scientific tasks are going through particularly arduous and demanding times, I remember the work and sacri-

fices my parents made at the bakery, and I thank them profoundly for never having forced us to continue with the family tradition. Quite the contrary, they always encouraged us to follow other paths. My father would repeat two adages to us: "Never take over the family business" and "Whatever you do, always strive to do your best". Because my parents worked during shop opening times, I spent many hours at home alone and very bored, at weekends as well, which somehow must have sharpened my creative spirit. As a child, I remember experimenting with coffee makers, putting all types of materials into the coffee holder to see what would happen in each instance. Later I played around with my first personal computers, and I self-taught myself how to program at weekends while my parents looked after the bakeries. Perhaps the first influences that marked my future as a scientist were my classes in biology, physics and chemistry, which were taught by wonderful baccalaureate professors at the PROA school. Though I was initially fascinated by physics more than anything, the fact that mathematics was never my strong point led my later interests to lean towards biology.

I studied biology at the University of Barcelona, where I obtained high marks that helped me afford my university studies. In the third year, my natural interest in research, together with the existing competitiveness to obtain a pre-doctoral position once undergraduate studies were completed, motivated me to work as a research student in my spare time, of course without earning a penny. Dr Rafael Romero was kind enough to take me in when I applied for a job in the Genetics Department. His group, along with that of Jaume Bagunyà, the department's head professor, was studying regenerative processes in the *Dugesia (Girardia) tigrina*, a small flatworm with an amazing ability to regenerate. Back in those days I was fascinated by topics related to developmental biology. We were looking for factors that allowed a flatworm cross-sectioned at the pharynx to regenerate this entire organ in all its complexity. Whether it involved the anterior or the posterior fragment, the flatworm had the wondrous ability to regenerate its entire body thanks to a group of multipowered stem cells known as neoblasts. This implied the re-establishment of positional information in the adult that allowed the proper regeneration of the organs regardless of where the site of the cut was located. My contributions as a research student consisted in the characterization of monoclonal antibodies developed in the laboratory, which marked different cell populations throughout the regeneration process.

During this period I realized that work in the laboratory was tremendously exciting and, moreover, that I was good at it. My relationships with Dr Rafael Romero and Dr Jaume Bagunyà provided a very positive influence during the final years of my undergraduate studies at university. The field of research that has always interested me lies at the frontier between developmental biology and cancer, and I have no doubt that this is a reflection of the influences I received during this period of my education. It seems like an odd coincidence, a nod to my career, that years later some of my most relevant contributions have been precisely in the field of stem cells.

Doctoral studies

My cousin Estanislao Navarro, an extraordinary molecular biologist and the only person in my close family to have studied at university before I did, was working

for the Municipal Institute of Medical Research (IMIM) in Barcelona at that time. At a Christmas dinner he told me that IMIM was searching for doctoral candidates. The flatworms were interesting, yet studying cancer was more appealing to me. It was 1992, and though I still had two years left before finishing my degree in Biology, I had an interview with Dr Francisco X. Real, head of the Immunology Department. Soon afterwards I began to work at IMIM under the supervision of Dr Myriam Fabre and later under the direction of Dr Antonio García de Herreros, who was my thesis director and, in the end, has become my mentor and personal friend. This was my first contact with studying cancer, and more specifically colorectal cancer, the field of research to which I have dedicated my scientific career. During this early stage, I explored the mechanisms involved in the destruction of the junctions existing between epithelial tumour cells, a phenomenon associated with the acquisition of mobility during invasion and metastasis. Colorectal tumours progress because of the successive accumulation of genetic alterations in a limited number of oncogenes and tumour suppressors. Though these genetic alterations represent the engine driving the tumour's malignancy, it had been proposed that epigenetic factors could influence this progression. In the case of colorectal cancer, it had been reasoned that biliary acids and fatty acids from the diet might act as tumour promoters through direct activation of protein kinase C (PKC). We demonstrated that activation of the PKC alpha isoenzyme in colorectal cancer cells that displayed a differentiated phenotype exacerbated their malignant behaviour, increasing their ability to migrate and invade adjacent tissues. These data were published in *The Journal of Biological Chemistry*.[1]

Following the lead found in our prior research, we made an extremely important finding for cancer biology. Most epithelial tumours develop mechanisms to increase their capacity for migration and dissemination. As a central part of this process, many types of tumours silence the cell adhesion protein E-cadherin when they become malignant. Although this phenomenon was being intensively researched at many laboratories, including that of Dr Walter Birchmeier in Berlin, Germany, as well as others, the mechanism responsible for silencing E-cadherin was unknown. In our prior work, we had described how cells with high levels of PKC-alpha activity displayed poor cell-to-cell adhesion due precisely to a decrease in E-cadherin levels. A mechanistic analysis of this phenomenon led us to identify Snail, a transcriptional repressor that blocks the expression of E-cadherin in tumour cells of an epithelial origin.[2] This phenomenon also occurs physiologically during embryonic development in order to transform epithelial tissues, such as the ectoderm, in mesenchymal tissues such as the mesoderm, and is known as epithelial-mesenchymal transition or EMT. Identifying Snail transcription factor represented a fundamental contribution to cancer biology and has been essential for understanding this key stage in the progression of many types of tumours. Our work, along with a second article with identical conclusions completed by the teams of Ángela Nieto and Amparo Cano,[3] which was published jointly in *Nature Cell Biology*, led to the founding of a new field of research: that of epithelial-mesenchymal transition in cancer. Since then, our findings have represented the conceptual framework for the research by hundreds of laboratories all over the world. The fundamental role of the Snail gene in the malignancy of tumours has been demonstrated for many types of cancer, including breast, colon and skin cancer. Without a doubt, Snail was one of the most important discoveries in my career, but it was not the only one made

during this period. While I completed my doctoral thesis, I also met Elena Sancho, who would become my professional partner and lifelong companion. With her I have shared all the contributions in my scientific career, including the discovery of Snail.

Postdoctoral period

With my mind set on my future postdoctoral training, in 1999 I attended a congress in Vienna, where for the first time I heard Professor Hans Clevers give a conference. Research on colorectal cancer was going through exciting times right then. In 1997 the publication of three consecutive articles in the journal *Science*, two from the laboratories of Clevers himself, along with Kinzler and Vogelstein, and a third from the laboratory of Paul Polakis, described a fundamental discovery for colorectal tumorigenesis.[4–6] Virtually all colorectal tumours display activating mutations in components of the WNT signalling pathway, mainly in the APC tumour-suppression gene or in the beta-catenin oncogene. These genetic alterations are the only ones present in early premalignant lesions of the intestine, and therefore they constitute the first transforming event in colorectal cancer. However, the mechanism through which changes in APC or beta-catenin lead to colorectal cancer was unknown. The scientific community correctly hypothesized that this mechanism should be the philosopher's stone that would make it possible to understand the beginning of tumorigenesis in the intestine. For the first time, these three articles described the interaction between beta-catenin and the TCF4 transcription factor, giving rise to a transcription activating complex, which represented the final executive component in the WNT signalling pathway. All three articles related the genetic mutations in components of the WNT pathway to the constitutive activation of the beta-catenin/TCF4 complex in early colorectal cancer and in melanoma lesions.

Ever since its publication, I was drawn to this discovery. I remember many passionate discussions about these articles with Antonio García de Herreros and Paco Real in the hallways of IMIM. I had decided to get in touch with Hans Clevers to find out about the possibility of working at his laboratory, when I crossed paths with an opportunity that was hard to reject. Another great scientist, Miguel Beato, was planning to create a new institute in Barcelona, the seed of what eventually became the Centre for Genomic Regulation (CRG), and he was looking for postdoctoral researchers who, after a stay at his laboratory in Germany, would join the team at the new research centre in Barcelona. The offer was very appealing, and Elena Sancho and I agreed to join his laboratory in Marburg to research signalling by hormones in breast cancer. Nevertheless, events sped forward quickly, and Miguel Beato's return to Barcelona was moved ahead, to a point that it became unfeasible to complete a productive postdoctoral stay. It seemed risky to me to end this period with no publications to accredit my professional advancement and allow me to nurture my leadership as a scientist. Miguel Beato understood perfectly, so I retrieved the idea of writing to Hans Clevers to ask for a job interview.

Hans Clevers received Elena and me in Utrecht in late January 2000 to give a seminar and hold an interview with both him and the rest of the members of his laboratory. We crossed a snow-covered Germany to reach his laboratory at the University Medical Centre of Ut-

recht (UMC). The seminars were still being given with physical slides, and I vividly remember the scene: I, a doctoral student who had recently graduated, stood in front of Hans Clevers' whole laboratory, introducing the slides into a carrousel one by one, without shaking, even though this was the first seminar I ever gave in English. Hans was quite impressed with the work on Snail, and the laboratory's members had no problem accepting us both as future colleagues. This helped us surmount Hans Clevers' initial apprehension towards having a couple as postdoctoral fellows working in his laboratory. Three weeks after the interview, we received an e-mail from Hans that said: "The decision was a hard nut to crack, but after having spoken to the people in the lab, I would be happy to have both of you in it". That difficult nut to crack changed our lives, and the stay at his laboratory left a definitive mark on the rest of my scientific career.

We arrived in Utrecht on a Thursday in August, and on Friday we had already begun working. After discovering the beta-catenin/TCF complex as a fundamental component of WNT signalling during development and in cancer, nearly all the members of Hans's group focused their projects on this aspect. The working hypothesis was that the genes controlled by beta-catenin/TCF in the intestinal epithelium were promoting the transition towards benign adenomas or polyps. Marc van de Wetering, one of the senior researchers at the laboratory, had generated tools to analyse this hypothesis. Due to the mutations that are normally found in this type of tumour, colorectal cancer cell lines displayed constitutive activity by the beta-catenin/TCF complex. Van de Wetering generated inducible clones in these cell lines, which expressed a dominant negative version of TCF4 that blocked the complex's transcriptional activity. It was 2000, and transcriptomics had just come into existence, though at that time the technology was of restricted use. Hans contacted Pat Brown, of the Biochemistry Department at Stanford University (United States), one of the pioneering laboratories in the development of microarrays, and set up collaborative work with his team to compare the genetic programme of colon cancer cells when the beta-catenin/TCF complex was active or inactive due to the negative dominant expression of TCF. A Dutch colleague at Pat Brown's laboratory, Cor Verweij, carried out the experiment, and by the time we reached Hans's laboratory, the list of target genes had been torn apart, with each member of the laboratory choosing one favourite target gene to pursue his/her postdoctoral research. The Friday we arrived, Hans was away on a trip, so we asked Van de Wetering whether he could let us see the list of candidate genes, or at least what was left of it.

I spent that first weekend in Utrecht at the apartment we had rented studying the function of each of the genes appearing on that list, until something drew my attention. Among the genes activated by the complex, there were two tyrosine kinase receptors of the EphB family, whereas among the genes that were induced in an indirect manner upon blocking the complex's activity, I encountered ephrinB1, precisely one of the ligands of EphB receptors. I read articles describing the role of this family of receptors and ligands during embryonic development, and I learned how their function consisted in compartmentalizing cellular territories. Expression of the receptor in one cell population and that of its ligand in another, such as the rhombomeres in the brain, gives rise to the formation of adjacent zones, distinguished by the expression of one molecule or the other, and delimited by a physical frontier that is created by the interaction of the Eph receptors with their ligand

ephrin. The epithelium in the large intestine is arranged into invaginations in the form of a sack, which are known as crypts of Lieberkühn, in which two compartments can also be distinguished. Found at the bottom of the crypts are the progenitor cells undergoing constant proliferation, whereas the upper half is occupied by differentiated non-proliferating cells. The differentiated cells are continually replenished by the progenitors as they migrate towards the surface. During that first weekend, the hypothesis that EphB receptors and ephrins may be involved in separating the proliferative compartment and the differentiated compartment took shape in my head. Somehow, this mechanism fitted in well with the organization of the normal intestinal mucosa. However, we had identified the EphB receptors and the ephrins in tumour cells under the control of the WNT pathway and this piece of the puzzle, which did not fit in well with the whole picture in the beginning, turned out to be decisive in connecting for the first time the activity of intestinal stem cells to colorectal cancer, as I describe in further detail below.

When Hans returned to the laboratory on Monday, I explained to him my thoughts from the weekend, while also asking him to let me purchase antibodies to study the expression of receptors and ligands in the intestinal crypt. Hans agreed incredulously, and I remember him saying: "It would be nice if you discovered something during your first week..." A few days later, and greatly excited, I found myself looking through the microscope to discover a complementary staining between the EphB3 receptor and ephrinB1. I also used immunohistochemistry to analyse many of the genes activated by beta-catenin/TCF and genes induced after blockage of the complex's activity in colon cancer cells. I was surprised to observe that they all followed the same complementary pattern as the EphB receptors and ephrins. In other words, the set of genes regulated by the beta-catenin/TCF complex in the initial colorectal cancer lesions was also being expressed by normal progenitor cells in the intestinal crypts, whereas those genes inhibited by beta-catenin/TCF in the tumours were being expressed in differentiated cells of the intestine. These results, coupled to revealing the presence of nuclear beta-catenin at the bottom of the crypts, which Elena Sancho achieved not without technical difficulties, demonstrated the presence of physiological activity by the WNT signalling pathway in the intestinal epithelium. I realized that WNT's activity controlled the proliferation/differentiation switch at the crypts' base, the place where intestinal stem cells reside.

These observations built the foundation for the two articles published in the journal *Cell* as the result of our stay at Hans Clevers' laboratory.[7,8] We demonstrated that, at the beginning of colorectal tumorigenesis, the acquisition of mutations in components of the WNT pathway imposes a progenitor cell phenotype on mutant cells and makes them independent from the physiological signals that would force them to become differentiated.[8] At that time this hypothesis was very innovative and it meant that tumour cells acquired a stem-cell phenotype at the origin of intestinal tumorigenesis. The second article resulting from my stay at Hans's laboratory elaborated on the function of the EphB tyrosine kinase receptor family in the intestinal epithelium. In this work, we described how a fundamental part of the genetic programme directed by beta-catenin/TCF regulates the position of intestinal cells along the crypt axis.[7] This represented another basic discovery for understanding the organization of adult tissues and the loss of their architecture during

carcinogenesis. It has served as a conceptual foundation for research at numerous laboratories, including those of David Wilkinson (MRC National Institute for Medical Research, London, United Kingdom) and Elena Pasquale (Sanford-Burnham Medical Research Institute, United States).

Given the importance of the discoveries described above, our two articles received many commentaries in top-level scientific publications (*Nature*, *Nature Medicine* and *Nature Reviews Cancer*). Over time, they have become classics and have been cited more than two thousand times to date. They both also represent central focuses of the research that I have carried out afterwards as an independent scientist.

Returning to Barcelona: ICREA and the IRB

As of the publication of these works, I began to explore the possibility of beginning to direct my own research group. Although being the primary author of the works mentioned above ensured me some good job offers anywhere in the world, the option of returning to Barcelona began to gain momentum. To many, this idea meant little more than committing scientific suicide, or at least being willing to endure great suffering until I was able to set up a competitive laboratory. However, at that time the situation in Barcelona had begun to change. The head of the University and Research Department of the Autonomous Regional Government of Catalonia, Andreu Mas-Colell, had started up a series of instruments and initiatives to attract talent and promote scientific excellence. This ambitious plan, maintained by the region's various governments throughout the past fourteen years, has had a huge impact, to such an extent that it has turned the science done in Catalonia into a reference for quality in Europe. One of the key tools created was the Catalan Institution for Research and Advanced Studies (ICREA), a foundation aimed at hiring researchers from around the world who wish to work at Catalan institutions. The only prerequisite is that their research must be cutting-edge and excellent. The second tool was the creation of new research centres equipped with a budget that would make them competitive, along with modern and independent organization. When I was still working at Hans Clevers' laboratory, I was given a contract as an ICREA professor, which put me in the best possible position for negotiating working conditions with research centres. Throughout 2003 I underwent several interviews with Joan Guinovart and Joan Massagué at the Institute for Research in Biomedicine (IRB) in Barcelona, with Josep Baselga at the Institute of Oncology of the Vall d'Hebron Hospital (VHIO) and at the Bellvitge Biomedical Research Institute (IDIBELL) with Jaume Borrás and Gabriel Capellá. I received offers from IRB Barcelona and the VHIO, both exceptional centres, which made my decision very difficult. In the end, I accepted IRB Barcelona's proposal after Josep Baselga recommended I do so, and I returned to Barcelona in mid 2004. The opportunities and support that I have been offered by IRB Barcelona since 2004 have been extraordinary and equivalent to those given to researchers working at the most cutting-edge centres on the planet. I have never regretted returning to Barcelona instead of accepting the offers I received from abroad. In large part, these privileged working conditions are the result of the effort and resolve of Joan Guinovart and Joan Massagué to build an institution that could compete with the world's elite.

The research I have performed at my laboratory at IRB Barcelona revolves around three main fields: one lies in further understanding the relationship that exists between stem cells and cancer; the second on the role of EphB receptors in cellular positioning; and, more recently, we have devoted a great deal of our efforts to understanding the mechanisms involved in metastasis, the most deadly stage in cancer's progression. I have also had the opportunity and privilege of contributing to the organization and growth of IRB Barcelona. Since 2007 I have combined my work as a main researcher with that of Oncology Programme coordinator.

A new mechanism for tumour suppression: signalling mediated by EphB receptors controls the compartmentalization of normal and tumoural stem cells

The earliest suspicion that EphB receptors could act as suppressors of colorectal cancer progression arose upon analysing a collection of tumours with different states of malignancy at the University Medical Centre of Amsterdam. In the articles published in *Cell*, we had just described how each of the genes expressed under the control of beta-catenin/TCF in the progenitor cells of the crypts was expressed in the initial neoplastic lesions (adenomas and dysplastic crypts) as a result of the constitutive activation of the WNT pathway at the beginning of tumorigenesis. The EphB2 and EphB3 receptors were a clear example of this. However, we observed that the expression of the two receptors was silenced around the adenoma-carcinoma transition in nearly all the patients analysed. This finding contrasted with the fact that the mutations that activated the WNT pathway were maintained throughout the entire carcinogenetic sequence, from initial lesions to metastasized tumours. The level of silencing in the EphB genes correlated strongly with the loss of differentiation in the tumour, which suggested that it had occurred concomitantly with the acquisition of malignancy and independently from the signalling by WNT. The question at that time was obvious: did the loss of expression of the EphB receptors provide some advantage to colorectal cancer cells? Our genetic experiments in animals were unequivocal. We used mice deficient in EphB2 and EphB3 receptors obtained from Tony Pawson (Samuel Lunenfeld Research Institute, Toronto, Canada). We used these models to create a strain of mice with a mutation in the APC gene, in a genetic background with low EphB2/B3 activity. Under normal conditions, mice that only had mutations in APC spontaneously developed benign neoplastic lesions in the intestine. However, in the absence of EphB activity, this process sped up drastically and led to the formation of aggressive adenocarcinomas.[9] Our work in the journal *Nature* described these results and demonstrated the role of EphB receptors in suppressing tumours for the first time.

The continuation of this research, carried out fully through my laboratory in Barcelona, provided evidence for the tumour-suppression mechanism mediated by EphB in colorectal cancer: EphB receptors impose borders on the growth of cancerous cells through a mechanism dependent upon adhesion mediated by E-cadherin. In our work in *Nature Genetics*,[10] we demonstrated both *in vivo* and *in vitro* that the compartmentalization mediated by EphB restricts the ability of cancer cells to grow within positive ephrinB1 territories during the beginning of intestinal tumorigenesis. Mutant APC cells that begin tumours are

surrounded by normal cells that express ephrinB1 ligands that confine their growth and expansion within the intestinal crypts. Our results suggested that colorectal cancer cells silence the expression of EphB receptors to avoid repulsion interactions imposed by the ephrinB1+ tissue that surrounds them.[10] My laboratory's latest contribution to this field has been to clarify how one mechanism of cellular compartmentalization mediated by EphB receptors involves the destruction of adherent junctions by the proteolytic shedding of E-cadherin ectodomain by ADAM10 metaloprotease.[11] This latest work proposes that the combination of differential adhesion and repulsion controls the position of cells within the intestinal epithelium.

Linking colorectal cancer with stem cells in the intestine

Ever since I discovered the role of EphB receptors in cellular positioning, I thought I could use their expression in the membrane to isolate the population of intestinal stem cells from the crypt. Hans Clevers was following up on the same idea, but using classical genetic tools and, in fact, he was the first to locate stem cells in the small intestine of mice using the marker Lgr5.[12] Far from becoming discouraged, we applied our idea to human samples because no methods existed for purifying patients' colon stem cells. It took great effort by several members of my laboratory, including mainly Mar Iglesias, Anna Merlos Suárez and Peter Jung, until we succeeded, first of all, in purifying human colon stem cells and stem cells from the mouse's small intestine and, secondly, in developing experimental conditions that allowed for expanding them in the laboratory.[13] This dual technology has meant an extraordinary advancement in research with adult stem cells because it opens up a whole range of possibilities for their use in regenerative medicine applied to inflammatory diseases of the intestine such as Crohn's disease and ulcerative colitis. At present, in collaboration with the Hospital Clinic in Barcelona, we are exploring the therapeutic potential offered by stem cells from the colon.

The CCR is organized hierarchically, similar to the normal intestinal epithelium, and cancer stem cells are identified at the head of that hierarchy

Studying stem cells promises not only to revolutionize regenerative medicine, but also to help understand the causes of many diseases related to ageing. Among these, cancer has most benefited from research on stem cells. In particular, the discoveries made in the field of stem cells have promoted two key aspects. Firstly, we now understand that most cancers come about due to the accumulation of misfortunes over the years as a result of our body's ageing. It has been demonstrated that the origin of the errors that cause cancer lie in defects in the way stem cells function in adult tissues. Secondly, studying stem cells has fostered the notion that, in a similar manner to the healthy tissues in our body, many cancers contain a population of tumour stem cells responsible for regenerating the tumour. The regeneration of cancer after therapy is a frequent phenomenon associated with high mortality rates. One of my laboratory's main objectives is to understand the role played by tumour stem cells in the regeneration of colorectal cancers.

Recent advancements made by my laboratory demonstrate that most colorectal tumours are made up of two cell populations with phenotypes similar to either stem cells or to differentiated cells from the normal intestinal epithelium.[14] Only those tumour cells with the stem-cell phenotype display the ability to self-renew in the long term and have the ability to propagate the disease. The methodology developed to purify normal stem cells from the intestinal epithelium has allowed us to isolate tumour stem cells using samples from patients and to decode their genetic programming. This information has been instrumental in demonstrating that patients with colorectal tumours that display high expression of genes characteristic of stem cells have a higher risk that the tumour will reproduce after curative surgery.[14] Globally, these data identify the stem cells of colorectal cancer as the origin of disease relapse after therapy.

Since we made this discovery, a large part of our research has revolved around answering two questions: what genes control the expansion of tumour stem cells? And how can these contribute to the susceptibility towards developing cancer? This research recently led us to identify a transcriptional circuit directed by the GATA6 transcription factor, which regulates the self-renewal of stem cells in adenomas and intestinal polyps.[15] These lesions are benign but constitute a serious danger to health because they are the substrate upon which cancer originates. The transcriptional circuit controlled by GATA6 controls the expression of morphogenetic BMP4, which acts as a powerful limiter of intestinal tumorigenesis. This work creates the foundation for future studies on the varying susceptibility of the population to suffering colorectal cancer because the regions regulated by GATA6 at the BMP4 locus contain various polymorphisms that are very significantly associated with a higher risk of developing the disease.

A key discovery for the clinical management of colorectal cancer: the colonization of target organs during metastasis depends upon molecular reprogramming induced by TGF-beta in the tumour's micro-environment

My laboratory's third line of work revolves around researching the main cause of death due to colorectal cancer: metastasis. From forty to fifty per cent of all patients with colorectal cancer develop metastasis, which may either be present at the time when they are diagnosed or appear after months or years, after the curative therapy has been completed. The metastatic dissemination of colorectal cancer has a very poor prognosis, with a five-year survival rate of just eight per cent. At present, there is no effective therapy for eradicated colorectal cancer that has spread, or any way to predict which patients will develop metastasis. This is due in large part to a lack of knowledge about the determining factors that confer the metastatic capacity upon colorectal cancer cells. In this sense, it must be pointed out that there are no genetic mutations associated with this process. In my laboratory, we have associated the biology of stem cells in the intestine with the regenerative capacity of tumour cells. At the same time, we have discovered that metastasis depends to a great extent on the expression of a genetic programme induced by the hormone TGF-beta in the micro-environment of tumour cells.[16,17] Signalling by TGF-beta instructs the cells in the tumour stroma to secrete pro-

survival and prometastasis factors during the stage of nesting and colonization of the distant organ, usually the liver or lungs. We have demonstrated that the use of TGF-beta inhibitors at this stage blocks the metastatic growth of colorectal cancer cells.[16,17]

One fundamental aspect of this research lies in the fact that those patients who have tumours with low expression of the genetic programme directed by TGF-beta are at practically no risk of developing metastasis. This information could contribute to considerable improvement in the predictive power of the current patient status classification system drafted by the American Joint Committee on Cancer (AJCC), used at all hospitals in the world to reach clinical decisions on what treatment to follow. The results found at our laboratory may therefore have a great repercussion on patients with colorectal cancer because, on the one hand, they demonstrate that TGF-beta inhibitors prevent metastasis and, on the other, they are the foundation for the development of a test (Colostage) that predicts the risk of a relapse in the disease after therapy.

It is at this time that I have established a relationship with the Botín Foundation through two of its initiatives to create incentives for technology transfer. I would like to thank the Foundation for the generous contribution received through the *Mind the Gap* programme, which has allowed us to embark on the development of the test that I have just mentioned. This predictive test will make it possible to improve decisions when clinically dealing with patients and may also be used by pharmaceutical companies to identify patients with poor prognosis who may respond to their therapies in clinical trials. Undoubtedly, participation in the *Mind the Gap* programme has been absolutely essential for us to be able to begin transferring the results obtained at my laboratory in relation to the role of TGF-beta in metastasis. At the same time, since 2013 the Botín Foundation has provided support to my laboratory within the framework of the collaboration established as a researcher in its *Technology Transfer Programme*. Perhaps it is too premature to state anything concrete in this text, but I am convinced that the great experience and professionalism of the Botín Foundation's team will be essential in facilitating, at some time in the near future, the transfer of several technologies developed in my laboratory to important companies in the pharmaceutical and biotechnological sector.

Having reached this point, I would like to thank all the people who have collaborated on the research that I have taken part throughout these years, from my mentors to the members of my laboratory. Each of them has played a notable role, and without their contributions many of these discoveries would not have been possible. I would especially like to express my thanks for the contributions and help of Elena Sancho, who has undoubtedly been the most important factor in all the successes achieved throughout these years. I feel privileged to have been able to devote each day to research. Passion makes the world go round, and most of mankind's great advancements have been made thanks to people who are passionate about what they did or believed in. Without a doubt, a passion for this work is what has fuelled my career. I sincerely hope and wish that my laboratory's work will be useful in providing solutions to some of the biomedical challenges that I have described above.

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RAÚL MÉNDEZ

DECODING THE TRANSLATOR

26

"There are no such things as applied sciences, only applications of science"
LOUIS PASTEUR

This phrase above and its numerous later versions – "there is no applied science if there is no science to apply" (Bernardo Houssay) –, to quote just two Nobel Prize winners, is as obvious as it is often ignored, to the point that we scientists tend to pigeonhole ourselves into one of the two categories, albeit subconsciously. However, recent history is littered with examples of applications of sweeping economic and social impact that emerged from research that had seemingly been driven by sheer curiosity and human beings' inherent need to understand ourselves and the world in which we live. Modern medicine would not be conceivable without the work that established the structure of DNA; the first cure for the flu came from the determination of the structure of neuraminidase, the enzyme that uses the virus to infect the cell that, like the structure of transthyretin, allowed for the development of drugs against Alzheimer's. Whether it is the Internet, the laser or other things, the list is endless, but perhaps the best example is the discovery of one of the most effective antitumour drugs, cisplatin, the origin of which lies in the experiments of Barnett Rosenberg,[1] a biophysicist who was studying the effect of an electromagnetic field on bacteria division. Rosenberg saw that, under the influence of an electrical field, the bacteria stopped dividing, but not for the reasons that initially led him to undertake the experiment, but due to a chemical reaction between the ammonium chloride and the platin of the electrodes: he had discovered *cis*-diamminedichloroplatinum (cisplatin) and its properties for inhibiting cell proliferation. Years later, these properties would be applied to arrest the uncontrolled proliferation that characterizes tumours, thus increasing the cure rate from ten to eighty-five per cent for testicular cancer and to more than fifty per cent for cervical cancer.[2–4] At present, cisplatin is used in the treatment of a wide variety of solid tumours and it has been the subject of more than 56,000 research projects. Although the impact (application) of these discoveries was not evident when the work was being carried out, one need look no further than California or Massachusetts to realize the huge projection that basic research can have in a knowledge-based technology industry.

In early 2014, when I received the phone call from Pedro García Barreno, I had already heard a bit about the philosophy of the *Technology Transfer Programme* of the Botín Foundation from friends and colleagues I admire and who were part of the initiative. It was an idea that, due to its sheer simplicity, was innovative in a country where the huge potential in academic research has not translated into related technological applications. It is not only obvious but true that basic research leads to applications, but how often is it the same group who traverses the entire path from one to the other? How could we, working at the base of the base, transfer something if we did not even speak the same language as companies? Where would

we start? A few days later, Pedro stopped by to see us at the Institute for Research in Biomedicine (IRB) of Barcelona and I began to understand the true value of the project. It was not only about supporting our research, it was also a matter of using his experience to make our way through a realm that was unknown to us. Maybe he had come at the right time?

Everything is in books

I was born and grew up in pre-constitutional Madrid in a family that lived from and for books: publishers, translators and authors. Despite the huge influence of literature, it soon became clear that I was more awed by the Guadarrama mountain range than the Aqueduct of Segovia and by the gorillas in the Uganda rainforest than Plato's cave. I could not, however, completely immunize myself to family influence, so the adventures of Richard Francis Burton and Charles Darwin were to be my own personal synthesis of literature and biology. What could have been better than to sail on the *Beagle*? Childhood is childhood, and Jack London is the other side of the coin of Darwin; it was quite easy for me to combine zoology books with life in the southern seas or the epic sleigh journeys down the Klondike. And I was not far off target because years later I would hear an eminent scientist say that after being a pirate the riskiest profession was that of a scientist, and I am sure that Jack London would have written more than one magnificent novel on the review process of many scientific works. In a moderate version – I am certain that my mother would have liked it to be even more moderate – of *My Family and Other Animals* by Gerald Durrell, I arrived at a secondary school where "Don Carlos", the professor of Biology, helped me understand that the Krebs cycle was every bit as exciting as the sources of the Nile and that the voyage in a cell had the same, if not more, potential as that of the BEAGLE. So I gave up Durrell, Burton and Darwin for Lehninger (who wrote the bible of biochemistry), Asimov and Luria (whose book *36 Lectures in Biology*, a gift from a good friend, was my bedtime reading for many years). London also gave way to Hemingway, Gabriel García Márquez, Upton Sinclair and many others who would come later. At the same time I discovered mountains and climbing, but that is another story. With these arms, the *Atlas de microscopia* by J. Bernis Mateu and a field microscope my mother had given me, I arrived at the Autonomous University of Madrid (UAM), where I had the good fortune to encounter wonderful professors. Just to mention a couple, and in full knowledge that I am committing an injustice of omission, there was Federico Mayor Zaragoza. From him I not only learned enzymology, but also that communication is a part of science. And Ginés Morata, who revealed to us both developmental biology and the fact that what we do not know is much more interesting that what we do.

Starting with the mechanisms

Following a brief stay at the Cytogenetics Department of the UAM in the fourth year of my studies, I then discovered what would become my passion: the translational regulation. In spite of the homonymy, this is quite different from the translation my parents and grandparents did, and that kept a roof over my head during my childhood. Or maybe it was not really that different, and I actually did keep up the family tradition? In biology, translation refers to the decoding of information stored in the genome, in a

four-letter code, to the proteins, functional components of cells made up of more than twenty amino acids. The genome is made of deoxyribonucleic acid, a four-base polymer, structured like a double helix (with two antiparallel chains) compacted in chromosomes and stored in the nucleus. This organization is optimal for exact copying and transmission of information from a cell to its progeny. However, DNA cannot be directly translated to proteins. It needs an intermediate molecule called a messenger ribonucleic acid (mRNA). mRNA is made up of a modification of the same four bases but of a single chain, which can be translated. The mRNA also contains summarized genome information, including only those sequences that codify for the amino acids of the proteins flanked by untranslated regions (UTR) that contain regulation signals. Once they are synthesized (transcribed) in the nucleus, mRNAs are carried to the cytoplasm, which contains the machinery responsible for translating them into proteins (the translation factors and the ribosomes).

In long family table talk, I tried to explain to my parents, who were experts in Romanic philology, what I was doing for all those hours in the laboratory. And, although I am not a fan of analogies, the following example helped me explain the profession to which I was intending to devote the rest of my professional life. The genome would be a collection of all the music ever composed, and related material. This "music" would be in a powerful computer (the nucleus) that would store it in a binary code (DNA). This computer could easily copy all the music (to transmit it to our children), but could not play it. At a certain moment, we would like to make a selection of songs to listen to, so we would copy to a CD these pieces while still in binary code (mRNA) although now we could take these copies into the living room (cytoplasm) to listen to them. However, this CD does not produce music, but only a sequence of zeros and ones. To transform it into music, we need a CD player to convert the information of these two components into the multiple notes that make up the music (proteins). The CD player would be the ribosome, and the form in which the player deciphers the code to play music would be the translation. The order in which the songs are played, their volume and equalizing would constitute regulation of the translation. Obviously, if the reading is not done correctly or if the regulation is not right, we would have an unbearable cacophony instead of a pleasant evening of music. I was going to devote – so far – some twenty-seven years of my life to studying the regulation of translation initiation and the pathologies that occur when the reading is not done correctly.

In the fifth and final course of my degree studies, I wrote to Dr Severo Ochoa to inquire about the possibility of joining his laboratory. So I joined the group led by Dr César de Haro at the "Severo Ochoa" Centre of Molecular Biology (CBMSO). Dr Haro would end up directing my thesis and becoming a great friend. These were the biochemistry-times of the translation initiation factors, in which advances were directly measured by the hours we spent in the cold chamber purifying the basic machinery that carried ribosomes to the mRNA, in order to reproduce the process in a test tube. As in any initiation, that of translation also has its dogmas and, for a biochemist, the "bible" had been written by Arthur Kornberg in the "Ten Commandments of Enzymology".[5] For my doctoral thesis, the most important "commandment" was the fourth one – *Do not waste clean thinking on dirty enzymes* – and it took me more than five years to obtain something reasonably clean. With molecular biology, this commandment began to diminish in importance, but not the third one – *Do*

not believe something just because you can explain it. Kornberg reached this conclusion when studying DNA and, blinded by what he expected to find, he ignored the evidence that would have led him to the discovery of mRNA.

Considering the fact that for the ribosome to recognize mRNA and decode the information stored in the genome required more than 300 polypeptides, purifying each of them and reproducing the process in a test tube was a Herculean task in which Merrick and Hershey were the masters. I decided to follow in the footsteps of one of the most famous tandems in the golden age of translation biochemistry, Tim Hunt and Richard Jackson (Tim Hunt would later win the Nobel Prize in the field of cell cycle due to the discovery of cyclins). I spent five years purifying and describing a kinase that phosphorylated one of the translation initiation factors, the eIF2, which is responsible for carrying to the smaller ribosome subunit the transfer RNA that includes the first amino acid of the protein that will be synthesized.[6,7] This kinase, which has been given many names (HCI, HRI and HCR), couples globin synthesis to the available levels of iron in precursors of red globules, optimizing haemoglobin production. This, along with the struggle between viruses and cells to control the machinery of cell translation, was one of the few known cases of gene expression regulation in translation at a time when the focus of gene expression regulation was centred almost exclusively on transcription.

Diabetes, by accident

In the early 1990s, in congresses in the field of translation, two groups of "intruders" began to emerge: developmental biologists and cancer cell biologists. For me, it was a revolution. The point was not only to see how the machinery that translated mRNA into proteins worked, but how their regulation, or deregulation, was essential in basic processes such as embryo development, or pathological ones such as cancer. These spatial and temporal processes of translational regulation defined cell polarity and differentiation: from a single cell there emerged an organism with different specialized organs. The key to this process was that these transcriptions not only used the general translation machinery, but also contained specific sequences in their untranslated regions (UTR) that were recognized by factors that conferred a regulation that was independent from other mRNAs. At the same time the group led by Dr Nahum Sonenberg showed that regulation of the levels and activities of the general factors that control the translation of all mRNAs, such as eIF4E (the initiation factor that recognizes mRNA so as to "introduce" it to the ribosome), could act as oncogenes fostering tumour formation.[8] The key to this discovery was that general factors of translation initiation could also act as specific regulators of transcripts that encoded for proteins that promote cell transformation. The new perspective underlying this discovery is that RNAs have intrinsically different efficiencies and that tumours make use of them to elude the barriers to cell proliferation, and thus grow in an uncontrolled manner.

This new perspective unleashed huge potential for post-transcription mechanisms of gene expression regulation in essential biological processes, including cell division and differentiation, and pathological processes such as cancer. Due to their significance in human

pathologies, I decided to undertake postdoctoral studies on the role of eIF4E in cancer with the group led by Dr Robert E. Rhoads at the LSU-Medical Centre (LSUMC), in Shreveport, Louisiana in the United States.

My arrival at the new laboratory could not have been more accident-plagued. First, I discovered that the "deep South" was deeper than I had thought. Then I found out that the project on eIF4E as a tumour booster already "had an owner". And, lastly, I broke two ligaments in my knee in a volleyball game at my welcome party, which prevented me from starting experimental work right away. There is always a silver lining to any cloud, so those weeks of inactivity gave me time to read publications and design a new project. To do this, we set up a collaboration with the group led by Dr Morris F. White at the Joslin Diabetes Centre, in Harvard, Boston. This also had the added incentive that it allowed me to spend a few weeks a year in Boston, with which I had a closer cultural affinity than with Shreveport. Some pioneering publications in this field arose out of this collaboration, in which we dissected the signal transduction pathway through which insulin regulated translation through eIF4E, including one of my most oft-quoted papers.[9] At LSUMC-Shreveport I met Mercedes Fernández, a postdoctoral researcher who had come from Barcelona to work in the laboratory of Dr Matt B. Grisham, and we jointly decided to move to the University of Massachusetts Medical Centre (UMASSMED), one of the fastest-growing centres in the United States, to which we had been referred by Juan Valcárcel and Fátima Gebauer. Juan and Fátima were old colleagues of the doctoral programme at the CBMSO with whom I would continue to cross paths all the way to the Botín Foundation.

Developmental biology, in the "toad with claws" (*Xenopus laevis*)

At UMASSMED I joined the group led by Dr Joel D. Richter, who was studying how the translation of maternal RNA is regulated during meiosis (the cell division in which eggs are generated) and embryo development. At UMASSMED we had the opportunity to soak up an environment in which true pioneers of RNA biology were working, and a way of doing science that was unafraid and fired with absolute enthusiasm and dedication. These were fantastic years in which we could do science and enjoy hospitable New England without having to concern ourselves with anything else.

Gametes – eggs and sperm – are unique cells in so far as they contain only half the genetic information; when fertilization occurs, a complete copy is reconstituted of the genes necessary to form an organism. Further, each gamete contains a unique combination of genes that is the result of "blending" genes inherited from the maternal and paternal lines in a process that is known as "meiotic recombination". To obtain these cells, two cell divisions must occur, without replicating the DNA. These two divisions and the first cell divisions in the embryo (embryo divisions) are carefully orchestrated so that the gene information is correctly distributed among all the cells that will make up the adult organism. At the same time, each cell gradually acquires the functions they will perform in the adult organism. This process of cell differentiation is what enables a single cell to generate different organs. Obviously, the fact that we have two legs, two arms, a head, a heart and so

on requires that each cell express a specific protein combination at defined moments and at specific locations. However, during meiotic and embryo divisions, the information contained in the DNA is not accessible. To overcome this limitation, the cells store silenced mRNAs that code for all the proteins needed in the mitotic divisions and in the first stages of the embryo. The existence of these mRNAs, which are called maternal mRNA because they are inherited through the egg, was known since the mid twentieth century, but it was not until the end of the century when we began to understand how they were regulated by the release of the information they contained at the right time to generate the embryo. In 1990 the groups of Joel D. Richter and Marvin P. Wickens identified a specific sequence, cytoplasmic polydenylation element (CPE), in the region that does not encode for proteins (3'-UTR) of these maternal RNAs and, in 1994, the group led by Joel identified the protein that recognized this sequence (called CPEB). The identification of these elements controlling the translation of the maternal RNAs allowed for studying the molecular mechanisms that guide the formation of oocytes of *Xenopus laevis*, one of those living fossils that got stuck at a halfway point of evolution before frogs and toads took different paths (that was paradise for biochemist turned cell biologist). *Xenopus laevis* is a model of vertebrate that is reasonably close to humans to which any extrapolation is immediate, and its oocytes are the only system in which *in vivo* work in biochemistry can be done in a single cell. With CPEB recently discovered and a world of unknowns before me, I decided to apply the knowledge I had gained in the group of Bob Rhoads and study how stimulation by progesterone regulated CPEB, and how the latter, in turn, controlled cell division in embryo development. From a strictly scientific viewpoint, this was probably the most gratifying period of my career. An "almost virgin" field, a scientifically fascinating question, a brilliant mentor and fabulous laboratory colleagues. Also, as a postdoctoral fellow, I was free from any sort of administrative burdens, and from the need to obtain financing, while Joel gave me absolute freedom to take my line of experiments in any direction I saw fit. In this environment we carried out some pioneering work on the action mechanism of CPEBs that had a major impact on the translational impact of mRNAs controlling cell division.[10–12]

Starting in the subsoil

The millennium was closing in and it was time to bring this postdoctoral period to an end and start making plans to set up our own laboratories. Everything seemed to indicate that we would stay in the United States – swapping the east coast for the west, and a bit further to the north – when we saw an ad in *Nature* about a new institute that was being founded in Barcelona. I wrote to them and a few days later found myself in Barcelona being interviewed by Dr Miguel Beato, the director and, at the time, the sole group leader of the Centre for Genomic Regulation (CRG) of Barcelona. Miguel has never been short of enthusiasm. The CRG had no physical home: a new building was to be built on the shore but they had only got as far as digging a hole for the foundations, which was regularly awash with seawater. And yet Miguel's clout, and the idea of creating a centre that would be "different", resembling EMBL, with strong support from the Catalan government (and in particular the backing of the *conseller* Andreu Mas-Colell), persuaded me that the CRG had a bright future ahead of it and that the gamble would be worth it. In late

2011 I joined the CRG, even though it was not until the summer of 2002 that – after doing more work with architects than with scientists – we had laboratories in which to work. By that time, the CRG had managed to recruit a good number of excellent groups, among them those of Juan and Fátima, with whom I had crossed paths during the thesis and in UMASSMED. In this new setting the first group I would have the opportunity of directing was established, in addition to a small number of collaborators with the necessary dedication to embark on an adventure that had more of a future than a present and sufficient talent to keep it afloat. As is always the case in science, the "environment" shaped our initial forays, leading us to a more global perspective, and we decided to start off with an apparently simple question: how is it decided which of those maternal mRNAs are activated at any given time? And how is it specified where synthesis of the proteins for which they coded will occur?

These questions did not prove to be as simple as they seemed, and it took us more than five years to answer them. But the answer bore a new code, like a bar code, in which RNAs include in their non-coding regions (those that do not codify for a protein) the information necessary to know when and where the proteins would be synthesized, and how a cell is capable of reading that "bar code".[13–16] This enabled us to not only "read" mRNAs and predict their behaviour, but to generate artificial mRNAs that would behave in a certain way. It also included a surprise: the targets of this mechanism regulating gene expression went far beyond the few maternal mRNAs that controlled early embryo development and included up to twenty per cent of the genome, thus involving a multitude of functions of the adult organism.[17] But no less important for us, in those early years in Barcelona in which both Mercedes and I were creating our own research teams at the August Pi i Sunyer Biomedical Research Institute (IDIBAPS)/Hospital Clinic and the CRG, was the birth of our children, Pablo and Ana. Since then, they have been the engine driving our research forward, giving us the "what for" perspective of the work we do.

In parallel fashion, the large sequencing projects enabled us to see that the CPEB is not only a protein, but a family of four members (CPEB1, CPEB2, CPEB3 and CPEB4). The first evidence of a function of CPEBs in an adult organism came from the group of Joel D. Richter, the discoverer of these proteins, who showed that CPEB1 was involved in the establishment of long-term memory. From a molecular point of view, memory is established through the marking of connections between neurons (synapse), so they will "know" if they have previously been stimulated. In 2000 Dr Eric Kandel won the Nobel Prize for proving that this "mark" is based on a local synaptic protein synthesis in response to stimulation. Richter's laboratory showed that the mechanism controlling this localized translation was the same that regulated maternal mRNAs during egg formuation.[18] Recently, the same laboratory has shown that the cognitive delay caused by Fragile X syndrome, which is second in frequency to Down's syndrome, can be reversed by eliminating CPEB1.[19]

For our group, the leap to biomedical applications arose from a coincidence that illustrates the importance of a multidisciplinary focus in research in an environment that facilitates interaction. Deciphering the code in which mRNA carries information on their spatio-temporal regulation allowed us to "read them" and seek out possible targets. Lots

of time was killed reading sequences that we thought could be relevant, or that were sent to us by colleagues to ascertain if they should explore this mechanism in their favourite genes. One of these came to us from Pilar Navarro and Paco Real, who were at the Hospital del Mar Medical Research Institute (IMIM), with whom we shared the building. Pilar and Paco were working on pancreas tumours in which they had identified a protein (TPA) that overexpressed itself in tumours, allowing them to grow. To their surprise, when they measured the levels of mRNA that coded for that protein in healthy tissue and in patient samples, they saw that there was no direct correlation, but the opposite, between protein levels and mRNA levels. This is a clear indication of translational regulation, so they contacted us and asked us to "read" the mRNA sequence that coded for TPA. Five years later we finished the job, demonstrating that in tumours – but not in healthy pancreases – CPEB4 is ectopically expressed, reactivating a pattern of gene expression that is inherent to embryonic cells, allowing the tumour to grow and invade adjacent tissues. In fact, eliminating CPEB4 reduced the tumour growth by more than eighty per cent and prevented it from vascularizing, keeping the tumour from receiving the nutrients and oxygen it needed to grow.[20] Soon after, we would identify another CPEB, called CPEB1, as a factor that mediated the reprogramming of gene expression in tumour cells deriving from Hodgkin's lymphoma.[21] These observations, and the experience of combining our mechanical knowledge with other groups working closer to the realm of clinical research, encouraged us to explore the possible role of CPEB in pathologies that involved the formation of new blood vessels. This time the collaboration arose from an even closer source. Mercedes was working on chronic hepatic diseases, such as cirrhosis, and the formation of new blood vessels associated with the course of the disease. Four years later, we had shown that the CPEB deregulation contributed to the development of the disease.

Currently, we have under way some half dozen collaborations that are studying the possible involvement of CPEBs in pathological processes.

However fantastic the system of *Xenpus* oocytes for defining the molecular mechanisms of CPEB activity and regulation may be, this organism has two intractable problems when it comes to genetic methods to enable us to systematically define the functions of these proteins in an adult organism and its possible pathological implications. First, it is a tetraploid organism. That is, it has four copies of each gene, which is the reason that it has not become part of the experimental models in which their genome has been sequenced. Second, it is quite a long-lived organism, and it takes about five years to attain sexual maturity, so producing genetically modified individuals would take decades. So we decided to change systems and start to generate mice models and modify each of the four CPEBs.

Around the world to travel six kilometres

At this point in our research we were about to reach the eight-year mark that was seen as the standard limit for a junior group leader at the CRG, so we started to think about changing research centres. In addition, the new directions opened up with the new functions of CPEBs in the adult organism and their possible involvement in a number of pathologies could benefit from an environment in which other groups were working on

mouse models and cancer. All researchers have a nomad within, so any change starts by taking out a map of the world and the phone. A family conclave ensued: should we return to the US, or go to Madrid? Should we try our luck in Switzerland, or Germany (two of the countries that dedicate the most work and investment to research)? In Sweden the winter is never-ending and Australia is really too far away, so they were both ruled out. In the end, the answer was much closer to home. The Catalan Institution for Research and Advanced Studies (ICREA) and the Institute for Research in Biomedicine (IRB) of Barcelona, another two projects born from the vision of Andreu Mas-Colell. It took no more than two meetings with Joan Guinovart, director of the IRB, and Joan Massagué, then vice-director of the IRB and now director of the Memorial Sloan Kettering Cancer Centre, to see that what we needed was not a map of the world but one of Barcelona's metro system. The IRB had a vision of the future, and the enthusiasm and atmosphere of collaboration we were looking for. Furthermore, at the IRB I would have access to cutting-edge technologies and the environment of scientific excellence necessary to develop the mouse models and collaborations that are crucial to achieving an in-depth understanding of the physiological and pathological role of CPEBs. Also, by staying in Barcelona, we could continue with the collaborative work we had previously undertaken in the CRG, allowing us to keep up the most basic research work into the action mechanisms of CPEBs. The IRB's philosophy also hugely boosted technology transfer, thus perfectly complementing the spirit of the Programme of the Botín Foundation.

For the three years we have been working at the IRB, we have continued our work studying the mechanisms through which CPEBs regulate gene expression, how CPEBs are regulated in response to the signals received by the cell; we have established the structure of CPEBs in collaboration with Dr Frederic Allain at the ETH of Zurich;[22] we have described some of the physiological functions of CPEBs in the adult organism; and, using the mice models in which we have, one by one, deactivated the four CPEBs, we have begun to define the pathological consequences of incorrect regulation of these proteins. The results of this research have revealed the huge clinical potential of specific drugs that act on CPEBs and we are working on a system that will enable us to systematically identify these compounds. However, at the end of the day, you are just a basic researcher, a biochemist, and the leap towards any application of that knowledge makes you feel a little vertiginous, even more so when research during those three years has been hit by a severe economic crisis. At a moment of doubt as to whether we would be capable of making that leap to technology, the phone rang and it was Pedro.

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ÓSCAR FERNÁNDEZ-CAPETILLO

FIGHTING AGAINST CANCER AND AGEING

27

In his book *Asimov's New Guide to Science*, one of the best popular science writers of our times, Isaac Asimov provides the best definition of science that I have ever found. This formidable book's first chapter begins by defining science itself: "What is Science? Almost in the beginning was curiosity". And it is precisely into that definition that I fit. Although it is my profession, I do not consider myself a biochemist, or a geneticist, or a cancer researcher. I just consider myself a person with a frustrating curiosity that is trying to understand the world around us.

I was born in Bilbao on 4 August 1974, exactly one year after my parents got married. As will become obvious in this document – and I honestly believe it is the truth, though most frequently biographies tend to be embellished – my life has not followed a coherent direction but, instead, has been moulded by chance on the basis of a small set of specific events that has caused my path to turn suddenly changing directions. I, for one, am a believer in the theory of "punctuated equilibrium" as a guide to explain how our lives move along, similar to the one proposed by Niles Eldredge and Stephen Jay Gould within the context of evolution. Shortly before I was born, Niles and Steve proposed that evolution is not constant, but rather remains relatively slow across very long periods of time, and it is in very short intervals of time that an unlikely event (a catastrophe, a drastic change in temperature and so on) may cause a massive expansion and selection of life forms, from which a new set of beings emerges. As you will see, it is easy to understand why I have the impression that our lives also follow this model of "punctuated equilibrium". Periods of stability that end abruptly due to more or less fortuitous events and decisions.

Although unusual for someone coming from Bilbao, I was born into a humble family. My mother, Margarita Ruiz, had worked as a stenographer but, as often happened in those times, left her profession as soon as I was born. My father, Juan José Fernández-Capetillo, then worked in customs for a company named Babcock and Wilcox which, like so many others, eventually became famous for forcing early retirement of its employees or laying them off when Spanish labour ceased to be among the cheapest in the world. We lived in a small flat in Astrabudua, one of the poorest areas at the outskirts of Bilbao, along the Nervión River, in the years when the water in this channel was somewhat of an emulsion of petroleum and heavy metals. My mother tells me that back then we had no money for heating and had to get by with blankets and thick clothing, but I always thought she was just trying to make her old stories sound more exciting. In any case, it was during those times that my life took the first very sudden turn that I am aware of: my parents won the Christmas lottery.

I guess everyone wonders who – besides politician Carlos Fabra – has won the lottery at some time. Well, ecce homo. Luck smiled on my parents with a winning Christmas lottery ticket. Back in 1978 this was enough for us to move to a new house, buy a new car and, for what concerned me, change schools. We moved to Ibarrekolanda, a neighbourhood near the School of Economics and Business at the University of the Basque Country (UPV), though the only thing that ever seemed appealing to me about that campus were its gardens. As for my school, I was sent to Askartza Claret, a relatively new *ikastola*, or Basque language school, run by the Claretian order for "upper-class" kids. My parents thought that some of their influence might stick and that going there would keep me busy until six in the evening, since I had to come home by bus. They were right on both accounts.

Already back in my grade school days, it was obvious that I was a science boy. I had a practically constant 7.5-point average grade throughout my entire pre-university education. This figure is easy to explain: 10 in all my science courses and 5 in everything else. Science was fun for me; it was like a game, and everything else bored me to tears. I have never understood the educational philosophy of turning students into walking libraries. This non-participatory teaching style is one of the reasons why medical doctors in Spain, unlike in the United States, focus mainly on applying already known cures rather than looking for new ones. We are not educated to change and improve the world we live in, but instead to remember what others have already done before us. Memorizing without understanding is absurd, and is not of much use unless you are trying to impress others during a meal or while playing trivia, neither of which interests me. My opinions on our educational system aside, this is how things went for me until I took the *Selectividad* university entrance exam, on which I also received a perfect 7.5. My regularity was worthy of an award.

My arrival into the university years caught me without having a very clear idea about what I wanted to study. I knew it had to be something science-related, but did not know exactly what. I leaned towards physics because one of my favourite books – which still sits by my side in my office at the Spanish National Cancer Research Centre (CNIO) – was *Cosmos*, by Carl Sagan. Although I have devoted myself professionally to biomedicine, that does not change the fact that I still think the truly important questions have nothing to do with knowing how we human beings work, but rather with the "big" question(s) of why matter, energy and the fundamental laws that regulate their interactions exist. In other words, what is this whole universe thing, in which we have stuck our heads out by chance for just a brief space of time, all about? And no, the answer "God" does not convince me. An idea is not valid just because it cannot be refuted. Some Vikings thought that the shell of a giant turtle held up the world. Obviously, no Viking could demonstrate otherwise. I can live acknowledging that I do not have the answer to every question without having to create myself an arbitrary interpretation to make me feel more at ease.

Getting back to the times of the *Selectividad* exam, I was relatively convinced about studying physics or, as an alternative, mathematics. I thought that it would not require too much of an effort and, besides, it was the major my Uncle Carlos had studied. My mother's brother, he has been one of the key role models in my life. Carlos was a bit older than me,

and I spent a large part of my childhood and youth with him. He got his undergraduate degree in Mathematics with extraordinary marks. However, once he finished that degree, he reflected on his future and realized that mathematics did not fulfil him. He had a calling to help others. So he decided to study psychology and help children with educational problems, and he devoted his life to that. "One in a million", as the Americans say. And I can attest that he was. That, coupled with our sharing of many personality traits, and the fact that he constantly drilled me with logic puzzles, made Carlos a key reference for my own life. As I was trying to make up my mind between physics and mathematics, another catastrophic moment arrived that would cause my boat to change course. During those months, I lost two very close relatives to cancer. I witnessed the whole process at first hand, watching how they agonized. I had been in the trenches of cancer twice and seen the enemy's face. All this was rather traumatic and made me change direction once again. I decided to study biology to cure cancer, and this is where I stand today. However, I did not make it to this point without a few more extra turns.

At the UPV, which was about two hundred metres away from my school, things changed in a somewhat predictable manner. My marks of 7.5 rapidly rose to an average of 10, and I obtained an A+ in every single class for the first three years at university. In practical terms, this translated into me attending the University for free, which is something I valued since, from as soon as I could, I have always tried to help my parents financially. In addition to studying for free I tried to earn income in a wide range of ways – from playing handball in the national league to harvesting potatoes. Since then, I never had to ask for help to cover my own expenses. At the university, these were times of upheaval. My first class was with a chemistry professor who introduced himself by mentioning that, as a Basque, he did not recognize the Spanish civil service, and therefore he could not make sure that his marks would be officially valid. Worthy of Woody Allen, but true. I will restrain myself from saying what I think about these issues. I believe that sometime around 1936, in the events hall at the University of Salamanca, Unamuno gave a historical lesson on "isms" that ended with his dismissal as rector. In any case, even today, the simple fact that I am from Bilbao and speak Basque forces me to define my position on miscellaneous nationalisms in too many places. Since I have always been a bit sharp-tongued, I decided to spell my name with the letter "K" in my signature, a practice that endures today. I do not know whether I am more amused by those who immediately label me a Nationalist for signing with a "K" or those who assume I belong to the Opus Dei because I have four children. Clearly I am a master at disguise.

At the university, once I completed my first three years, I decided to opt for the field of biochemistry because it seemed somewhat closer to my interests than general biology. If I started from square one again, knowing what I know now, I would have become an organic chemist. A golden age in the development of new molecules with biomedical properties awaits us, and it has caught me off guard. At the UPV, I established a great friendship with another student, Gontzal Cebas, an enlightened anarchist and great conversationalist, who made the tedium of lecture classes more bearable for me. Sadly, I also lost Gontzal, in the fifth year at university, when he took his own life after suffering from bipolar depression. It was then that I faced the nature of mental illness for the first time. Nothing

is more frustrating than seeing someone you love lose his bearing without being able to do anything for him. I will never make the mistake of trivializing the origin of these diseases. With nothing else worthy of mention from those years, and having started my fifth year at university, it was clear to me that the time had come to explore other worlds.

That year, I had started working as an undergrad at Ana María Zubiaga's laboratory. Ana had recently returned from doing postdoctoral work at Harvard and talked about things like mutant mice, the molecular biology of RNA and growing lymphocytes on Petri dishes, which back then sounded like heavenly music at UPV. Therefore, a brief conversation sufficed for Ana to convince me to work there. However, I actually wanted to explore other worlds, and Ana had already taught me enough to know that the truly good groups were not at my university, but rather in centres located abroad, and that I should seek them out in the best-known (printed) journals – back then, the Internet was not what it is today. With Ana I share the fact that we love all science beyond any field of specialization, and she passed on to me a love for reading *Nature* and *Science* on almost a weekly basis to get a panoramic view of the science going on around us. Therefore, my first strategy for finding a place to do my PhD was to select articles from those journals whose themes seemed interesting to me and to write directly to their authors asking for shelter. This tactic worked reasonably well and led me to visit several places, including David Tollervey's group in Scotland. He was working on the molecular biology of RNA and ended up offering me a fellowship. The visit I paid to Tollervey in Edinburgh was my first flight outside Spain. Though David was very kind, he also had a bit of a stutter. The experience of my first conversation ever in English outside Bilbao with a Scottish stutterer caused me to ask myself whether the language I had learned at school was really English. In the exchange that ensued from the airport to the hotel, I was only able to identify a few random words, which I combined to imagine all possible meanings: "We will go to the laboratory and then have dinner at the hotel". "The people at the laboratory are expecting you at the hotel for dinner". "Today we won't have dinner, and I want to show you the laboratory on our way to the hotel". Regardless of what he said, I felt like an immigrant who had just landed at Ellis Island. I now recall this episode with a smile.

At the same time, and after the most competitive selection process I have ever gone through in my life, I also gained the chance to do my doctorate at the European Molecular Biology Laboratory (EMBL) in Heidelberg, the jewel of the European crown in molecular biology. However, at EMBL I was not going to work on molecular biology, but on developing instruments instead! This love for electronics and devices I owe to my grandfather, who, with no training whatsoever, was able to fix any electronic device he got his hands on – a feat that fascinated me. At EMBL, I was going to get my PhD under the mentoring of Wilhelm Ansorge, which shortly afterwards I learned would have been a very bad decision. Luck smiled upon me again in an unexpected way.

In those years, I was a rather wild person outside the university. Without entering into much detail, suffice to say I was not on the best path if it were not for another accident that changed my life. Ana Zubiaga's first graduate student was Matilde Murga, a rather prim and proper student a few years older than me who had come from the University

of Navarre. Though Matilde and I came from completely opposed worlds, I immediately found her devastatingly attractive. Luckily, the attraction also worked in the opposite direction, and soon we began to go out together. However, I had just finished my studies at university that same year, and was getting ready to leave for EMBL to do my PhD in building instruments. Once again, something unexpected happened. That August I travelled to Bilbao to enjoy a few days of our "Semana Grande", the city's week of festivities. Upon arriving home, a huge surprise was waiting in the form of a letter from the Basque government, notifying me that I had been awarded a PhD fellowship at the UPV. This caught me completely by surprise since I had not applied for such a fellowship. When I spoke with Mati, the mystery was solved: she had applied for the fellowship under my name. At that time her family's situation was rather complicated, which made it impossible for her to leave Bilbao. She proposed a deal: if we were both to stay at the UPV for our PhD, she would subsequently follow me wherever I went to do my postdoctoral work. I hesitated for a few hundredths of a second. Instruments and Germany could wait. I was in love with her to the very core of my being.

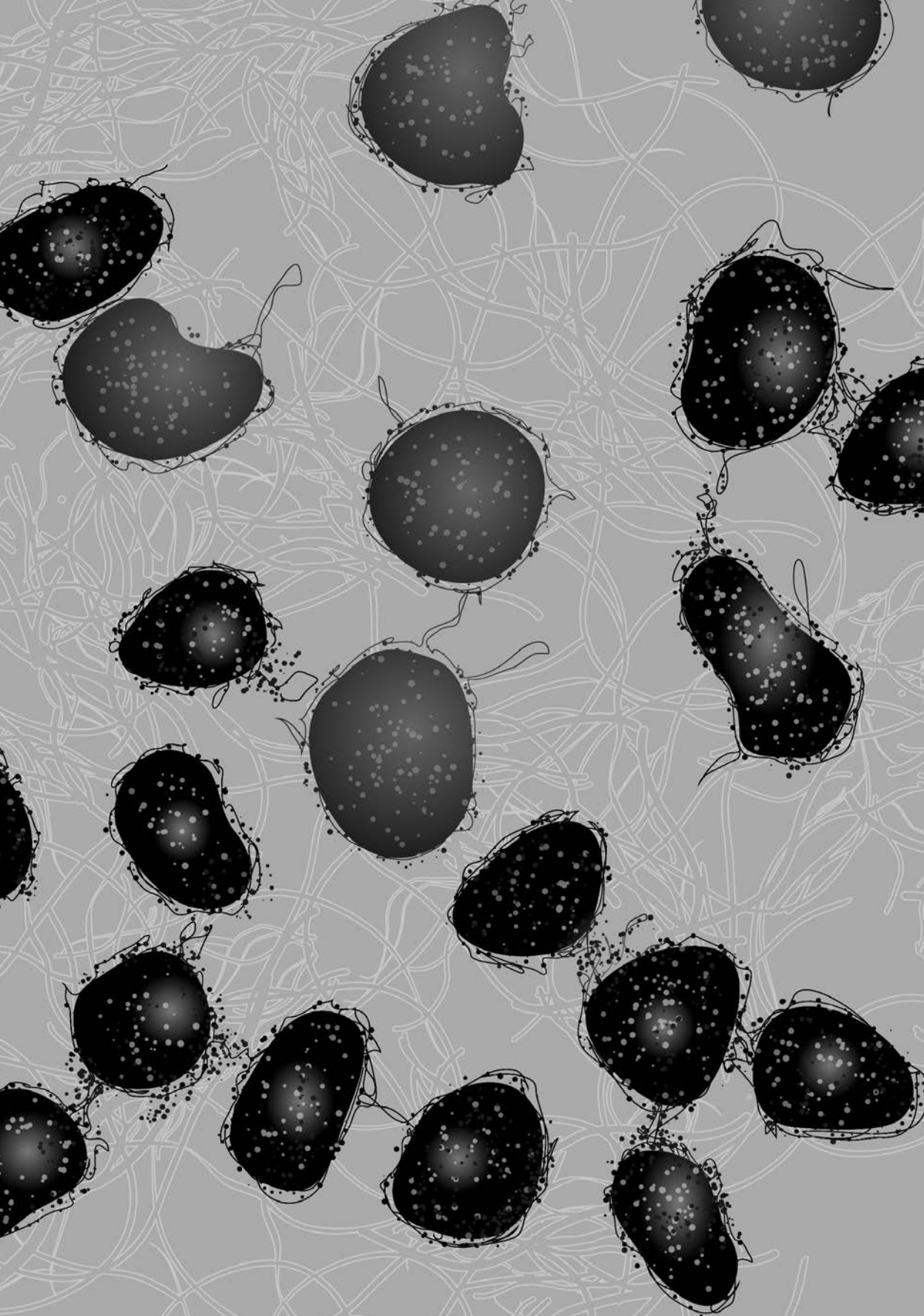
At the UPV, we literally started from scratch. Ana brought in a lot of energy and passion for science, which she was good at passing on to me. Much of the scientist I am today I owe to those years. We started out in an empty laboratory and devoted part of the first years to just putting a laboratory in place. Some techniques, such as cell culturing, had to be done in another of the university's buildings. And for other techniques, we had to travel as far as Santander or Madrid. The trips to Madrid were particularly heroic. We would wake up at four in the morning to go up to the UPV and grab a few mice. We would put them in a camouflaged box and head down to Bilbao to get on the first bus leaving for Madrid – the box had to be camouflaged because animals were not allowed. On arriving in Madrid, we would finally take a bus and a commuter train to reach the National Biotechnology Centre (CNB), the leading biomedicine centre in late-twentieth-century Spain. There we would sacrifice the animals and run from one lab to another to analyse their immune systems with every instrument we could. We would finish around eleven in the evening and literally run to catch the last bus to Bilbao at one in the morning. My visits to the CNB left a collateral effect. The closeness to the young groups of Manuel Serrano and María Blasco, as well as others that were more consolidated, like that of recently arrived Mariano Barbacid, had impressed me. Although we would only cross paths years later, those days were decisive for me. I had decided what type of scientist I wanted to be.

My PhD began with a focus on analysing the stability of messenger RNA (mRNA), a topic on which Ana Zubiaga had worked at Harvard. Although it was frustrating because this was a very methodical project that left little room for exploration, it helped me cut my teeth on molecular biology before the age of "kits" came along. This equipped me with the knowledge necessary to know how to refine a protocol when things do not work out, something that is being lost among PhD students today. As my students say, I am still the king of cloning. The origin of this facet of mine lies in those years. However, the RNA project was not especially fulfilling to me, and I began to collaborate with Mati on another one that was much more entertaining: the characterization of mice that were mutant for a set of genes involved in the biology of the cell cycle. Plus, this work was potentially

related with cancer, the field I was hoping to head towards. Our research ended with the discovery of one of the first autoimmune suppression genes, and revealed the existence of transcription factors in the E2F family that worked as repressors. Years later, groups from Boston rediscovered this phenomenon of E2F repressors and published it in *Nature* as an invention of their own. Well, these are the some of the dark spots in our business. It is difficult for an article based on the results of two young students from Leioa with their adventures on the bus to have the same repercussion as an equivalent one developed by the giant Bostonian pressure machine. Nevertheless, the work was published in the journal *Immunity*, the main immunology journal at the time. Bearing in mind the conditions at the UPV in which we started, I am still amazed about how we achieved this. There have been many more articles since, and I hope there will be many more, but it will be hard for any ever to have the same value that this one holds for me.

Despite that project's success, those years at the UPV had worn me down quite a bit. The RNA project was particularly frustrating and raised many doubts about whether I truly wanted to work in science. My unsettled nature made me explore other alternatives. On the one hand, I had always liked computers, so without too much thought I reached the conclusion that I wanted to understand how they worked and comprehend the process through which a machine is able to process an order as simple as "two plus two". Therefore, I signed up to study technical engineering in systems computing at the National Distance Education University (UNED). This kept me entertained many nights, and the truth is that I enjoyed it quite a bit. It revitalized me. I learned about digital electronics, programming and many other things that I had always had a weakness for. However, the magic of computers ended with my second course in computer architecture. Once I understood the secret of how the magic box worked, the rest of computing sciences bored me more than biomedicine. Nevertheless, this incursion into the world of computers was essential for keeping my curiosity alive during those years. This activity led me in my own thesis to program a software for the analysis of expression microarrays, which were back then being done using nylon membranes. The source code of that program was attached to my thesis, and curiously it was the focus of my doctoral thesis defence. For the members of my thesis tribunal, a biochemist who knew how to program computers was like a monkey with guns, a rare bird. This was not the last time that knowing how to program has helped me in my work, though.

In addition to computers, during those years I also wandered through the worlds of the stock market. The period from 1995 to 2000 was that of the first Internet newsgroups, and curiosity led me to spend my nights reading what was then the only discussion group on the stock market in Spain. In the beginning, the stock market seemed like a fun pastime. There was a program called MetaStock into which, with a bit of skill, you could enter historical series of stock market values. The software was filled with statistical tools that, if you had the knack, you could take advantage of. These were the early days of Internet trading, and it was not hard to use the information available to earn money. I started to invest my savings, and the manna began to flow. These were good times, and the truth is that things were going really well for me. I had designed a fairly refined system that allowed me to complete intra-day transactions with securities and come out smelling like



a rose. When things began to go really well, I created a pseudonym and began to act as a guru within the Spanish newsgroup. They must have thought I was a fifty-something economist with vast experience managing portfolios, and not just a mere biochemistry student hooked up to a Pentium amid experiments and sandwiches. Things went so well that I was hired as an adviser by a firm in Zaragoza, at which point I earned more through this activity than through my own fellowship – not a difficult feat in those days. I also remember that, for a few months, I was even investing the savings of some of the professors in our department. It was madness; things were completely out of control. It was fun, though. However, as happened to me with computers, I suddenly grew bored of the stock market as well. It was simply a game, a pastime. Making money for the sake of making money did not fulfil me at all.

Amid autoimmune mice, computers and stock market values, the time came to make my next move and take a decision. Mine had more or less been made, and it was not directly related to research. However, this time it was Mati who pushed me ahead. She was determined to do postdoctoral work in the United States and dragged me along. She asked me to give one last chance to science. If it were not to work out, I should give up on it. Having recently married, I had no other choice but to trust that she was right. She had selected a group in Boston, but, before joining them, their group moved to the National Institutes of Health (NIH) in Bethesda, near Washington, DC. So, my task was to search for potential groups that I might like to work with at the NIH. Not knowing exactly how to choose a group, I went back to my original strategy. I chose NIH groups that published articles that seemed creative to me, and I wrote to them all. I was surprised to have made a good impression on some of them, so I organized a trip to "America" to hold interviews with some of them. One of the interviews was with Andre Nussenzweig at the National Cancer Institute (NCI), who led a small, recently created group working on genomic instability. They had developed a technique for visualizing broken chromosomes. Fascinating. Moreover, Andre was working in cancer that, at last, was what I had always wanted to do. In any case, personal chemistry was decisive – as it always has been in my decisions. Andre seemed like a smart, nice guy, and even if I was not exactly sure why, it felt like working with him was going to be good for me. I was right.

My years in the United States (2001–04) were decisive for my future as a scientist. Andre was educated as a physicist – including his PhD and postdoctoral work –, and he had an amazing ability to be able to identify the truly important questions and set aside superfluous details. That helped me focus on interesting projects and, without a doubt, is the most important thing I have learned from him. Furthermore, for the first time ever, I had plentiful funds and instruments! I could therefore spend as much time as I wanted in the laboratory, doing all the experiments I felt like. And sure enough this is what I did. In those years, my research revolved around understanding the role played by histones, proteins that package DNA, in detecting and signalling chromosomal damage. Bearing in mind that DNA damage is involved in the development of tumours, this was a field directly related with my original interests. I hunkered down and managed to complete a large number of articles for prestigious journals during that time period. I was invited to speak at several conferences, where I started to become known. The wind began blowing in my

sails. I was enjoying research, and for the first time I started to believe my future might be linked to biomedicine.

My postdoctoral research at the NCI focused mainly on understanding the role that a histone, H2AX, plays in maintaining the genome's stability. Shortly before I arrived, Bill Bonner, a neighbour at the NCI, had discovered that this histone was phosphorylated in response to agents that damage DNA. How Bill Bonner and his brother managed to demonstrate that the phosphorylation of H2AX took place exactly at the broken ends of a chromosome is worthy of a tale on its own. As far as I was concerned, at first glance it seemed of interest to me to attempt to understand how a protein supposedly involved in packaging DNA could also be involved in its repair. Among other things, I discovered that H2AX regulated a new signalling pathway in response to radiation, the pairing of sexual chromosomes during gametogenesis and the coordination of telomeric movements. Moreover, I discovered a new phosphorylation of another histone, H2B, in response to damage in DNA, which proved the idea that histones are actively involved in protecting the genome. All this work made it clear that there was an active relationship between chromatin and genome's stability, which is still a very active field of research today. As for me, I was happy. I felt that I was able to do competitive science, and I wanted more. However, research with mice has long waiting times, and the genetic studies felt eternal to me. I made up my mind: I wanted to do another postdoc to learn about genetic experimentation in yeast. There is no faster model for genetic studies. As you can by now imagine, Mati came up with a more sensible option once again.

Though they now seem distant, good times were afoot in Spain in 2002. Spanish postdoctoral students abroad were excited about the creation of a new type of contract called "Ramón y Cajal", which promised to bring the Anglo-Saxon tenure track model to Spain. Dreamers. Moreover, this was also a time when every Spanish region was building its own research centre, not because there was any special desire to do research, but rather because it was just important to build things. This was, in any case, welcome. This being the situation and having recently become parents, Mati wisely decided it was the right time to come back to Spain. We applied for the "Ramón y Cajal" and began to explore our options for returning. The doors were opened for us in several places, including the CNB, but the researchers whom I admired at the CNB were leaving to go to a brand-new institution: the Spanish National Cancer Research Centre (CNIO), which was going to be directed by none other than Mariano Barbacid. Fortune smiled upon me once again in an e-mail from Manuel (Manolo) Serrano, inviting me to apply for a programme to recruit young groups at the CNIO. Manolo knew me because I had spent a brief stay at his laboratory while doing my thesis. What I could not imagine – now that I know him better I can – is that he had followed my trail at the NCI. He mentioned that María Blasco was going to head a new research programme at the CNIO in which some of Spain's top players in biomedical research were going to be working, including him, Mariano Barbacid and Ángel Nebreda. I felt overwhelmed, truth be told. It was too good to be true. After a selection process that I remember experiencing in a state of great excitement, I obtained a position as a junior group leader in the Molecular Oncology Programme led by María. However, we were not going to make it to the CNIO without manoeuvring a few more hairpin bends.

At the CNIO, the opportunity to lead a group was only offered to me, not to Mati. Therefore, despite being flattered, we said "no" to the CNIO and decided to seek other options (I vividly remember how hard it was for me to hit the "send" button on that e-mail). However, *noblesse oblige*. Bilbao had also built its own centre, the CIC bioGUNE, which would obviously have been the easiest option for us, but, after the interview with its director, it was not completely clear to me whether our science would really fit in there. The other opportunity opened up for us in Valencia. They were building a lovely centre next to the City of Arts and Sciences, known as the Prince Felipe Research Centre (CIPF). Valencia was a coastal city with a good climate, and my office was going to overlook a dolphin aquarium. Mati would also be leading a group on stem cells. Could we ask for more? Not until we met its director, Rubén Moreno, who had been named by the Valencian government to run the CIPF for reasons that were not strictly scientific. In our interactions with him, rough edges began to appear in his discourse, and it was obvious that he was clueless about managing a competitive centre. Non-scientific politics were marking the path taken by the CIPF. And so our Valencian adventure ended one morning when we woke up in the United States and saw Matilde's name on the front page of the online edition of *El Diario de Levante*. They had used her name to explain why they were not bringing Bernat Soria to the CIPF, because they were bringing Matilde, a world expert on stem cells, instead. Well, this was a quagmire we did not want to have any part of. That very same day, Mati told me that, given what she had seen in Valencia and Bilbao, we had to leave for the CNIO. The other centres we had explored did not inspire much confidence in us either, whereas the CNIO seemed destined for success. I will never be thankful enough for this move from Mati. She was voluntarily giving up her own chance to start up a group so that I could start my own at the CNIO. For the first time, I was the one who would be deciding where we would work.

In November 2004 we started at the CNIO to create the junior Genomic Instability group. I had just turned thirty, so the word "junior" was a good match. I remember those early years at the CNIO laboratory as one of the best times I have ever experienced in science. As had already happened to us during our PhD at the UPV, we had to begin setting up a laboratory from square one, the difference being that this time we knew the routine. Having Mati in the group was also essential to start working quickly. She had always been very talented with experiments and helped me instruct the first scholarship recipients and postdoctoral candidates. Strictly speaking, as far as experimentation went, she is the one who mainly trained them. This allowed me to focus on thinking up interesting projects, travel, meet with groups similar to ours and attempt to obtain financing wherever I could. Thanks to the fact that we had a flexible position financed by the CNIO, we were able to use it as a landing pad for our first students. Once the students got their own scholarships, we would get the CNIO position back and repeat the process. Therefore, in little less than one year, there were six of us. As for the selection of personnel for my laboratory, I always attempted to make sure they meet two basic conditions: that they like science beyond just biomedicine and that they are nice, good people. I have always managed to make my laboratory a pleasant work environment. I am a steadfast believer that happy people are more productive. I cannot understand laboratories managed like production lines. Truly important discoveries are not made by brute force. In fact, my philosophy in life is similar to my philosophy in the laboratory. I like to be surrounded by optimistic,

pleasant people, while at the same time trying to avoid egos and divas that are unable to make a constructive comment to a student who is just starting out. Unfortunately, this latter class of people has some notorious members in our academic world. Though I have fun fighting with them and making it clear that their emperor's clothes are invisible, in general I just try to avoid them. It is amazing how many Newtons surround us!

In my early years at the laboratory, we began to focus our research on very elementary questions in our field: how does DNA compaction influence the recognition of genomic damage? Could we design a trick to activate the DNA damage-response machinery, but without such damage existing? Was there any communication between the different DNA damage signalling pathways? We gradually published these and other contributions in solid journals, and our way of thinking and doing research began to receive acknowledgement from our colleagues. In these early years, I also attempted to remain very active by participating in national and international research networks, explaining our work in every possible forum. However, it was one of Mati's projects that truly opened the way to the most productive lines of research we have followed up to now. Her first project focused on creating a mouse model of a human disease known as Seckel's syndrome. Penny Jeggo's group in Sussex (UK) had described the mutation in patients, and we thought we could use that information to create an animal model of the disease. The syndrome's molecular basis lay in a mutation that decreased levels of the protein ATR. This protein is the main activator of the cellular response against a type of damage to DNA known as replicative stress (RS). Therefore, both the patients and the mice that we produced had low levels of ATR and suffered from high levels of RS. Mati's Seckel's mice reproduced all the disease's clinical traits and, more importantly, they were resistant to cancer. This made us begin to believe that inhibiting ATR's activity may be especially toxic to cancer cells, though at the time we never remotely dreamed that from our small research group we would be able to develop such ATR-inhibiting molecules for chemotherapy. However, we were given an opportunity and we took advantage of it.

The CNIO had created a department for developing new therapies that included medical chemists and libraries of chemical compounds for developing new drugs. At the same time a student from my laboratory, Luis Toledo, had developed a system to activate ATR in cultured cells. I then realized that, if the therapy programme could provide us with compounds and decided to collaborate with us, the possibility could exist of becoming competitive at developing ATR inhibitors. We knew that there was competition from big pharmaceutical companies like AstraZeneca, but I was convinced that our screening strategy could give us a substantial advantage. After some initial doubts, the therapy programme decided to help us, and we managed to develop ATR-inhibiting compounds that displayed antitumoural properties in cultured cells. This was 2011. Over the next two years, we concentrated on improving those compounds to such a degree that they would become molecules that could be administered orally and be capable of curing some tumours in mice. Once this work was done, it was time to knock on the doors of big pharmaceutical companies. The CNIO could not afford the costs of the clinical development of ATR inhibitors, and if we wanted to get those compounds out to patients we had no other option than attracting the interest of a company willing to invest in the project and get it into clinical use. In De-

cember 2013 the compounds were finally licensed to the German company Merck, in one of the largest antitumoural compound licencing deals ever made in Spain in the academic world. As of today, our group continues to collaborate with Merck actively so as to take part in the clinical development of these compounds and to define which patients could benefit from them. Though this part of our research does not require the greatest part of my creativity, I must say it is the part that feels most fulfilling to me. Throughout my years at the CNIO, I have also lost my father and my Uncle Carlos, both to cancer, and both too young. All this adds a certain sense of duty to the work we do. Many years ago I decided to get an education to try to find ways to cure cancer, and though I got side tracked several times, from now on I will not be steered off course by one single degree.

Although I have focused our work on replicative stress over the last five years and that is where we have achieved recognition, I am not a person who seeks the shelter of success. As happened to me with computers and the stock market, once I understand something, I tend to grow bored. Therefore, my nature causes me to use my nights for studying organic chemistry, and little by little we are turning towards other areas of research at the laboratory: cancer, ageing, pluripotency, haploid cells, mechanisms of radioresistance, drug development and so on. This is just a brief list of the activities that we are dealing with today. Luckily, we are doing so with one of the most competent and pleasant teams of people I have yet directed. Everything good that has happened to me I owe it to the people who have gone through my laboratory: to their efforts and fine work. Nothing fulfils me more than to feel the gratitude of those who worked with me, and nothing frustrates me more than not being able to help someone succeed. Though we are moving away from the hot topic of replicative stress, I am confident that a future filled with interesting discoveries lies ahead of us. "Don't rush, but never stop", said Saramago, in one of my favourite quotes. And that is what we are working at.

In these nearly ten years since we returned to Spain, a lot of things have happened in my life outside the CNIO, but none as worthy of mention as the birth of my three younger children: Emma (nine), Luca (seven) and little Marco (two). Along with Ian (eleven), they form an army of affection that has surely kept me from falling into some of the depressions typical of people my age. It is fascinating to see that, though we have brought all of them up similarly, they are completely different. However, I do see a common denominator: they are good people, which is good enough for me. I hope I manage to pass on to them the curiosity that moves me and the value of attempting to improve the world around us. And, while it is too soon to know where the currents will take them, and their lives will surely also be shaped by unknown twists of fate, one thing I can say: they love *Cosmos*.

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ISABEL FARIÑAS

MY PERSONAL VOYAGE IN NEUROSCIENCE

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As is often the case with us scientists, I found my calling as a researcher at an early age, but it did not completely solidify once and for all until the combination of a biology course and a motivated, dynamic professor showed me exactly what I wanted to do with my life. Even though I could not explain precisely what my profession entailed, my family never questioned my somewhat precocious decision. For many parents of the generation that grew up in post-war Spain, higher education had been impossible to come by; as far as they were concerned, it did not matter what your job was, as long as you performed it with dedication and honesty. My parents, both natives of Galicia, emigrated separately to Barcelona, where they met and formed a not-quite-Catalan family; they always believed that their children's education would be their own greatest legacy, and that seeing us well trained would make all their efforts worthwhile.

However, I had not yet made it to university when my family suffered a major blow in the form of the crippling illness that befell my father, and I thought that perhaps I should quit studying to work and help out at home. That summer was a difficult one; I spent it trying fruitlessly to find a part-time job that would somehow allow me to afford university tuition. I will never forget the day that one of my classmates at the preparatory institute mentioned that having graduated high school with top honours meant that I was exempt from having to pay the first year's tuition at university. That exemption, together with unconditional family support even during the hardest times and my occasional jobs over the course of the following years, made it possible for me to obtain my undergraduate degree in Biology at UAB (the Autonomous University of Barcelona) in 1985 – the first such degree in my family's history.

For a true understanding of my training and vocation in those early years, two aspects of my university life are particularly illuminative. One of those was my interaction with three classmates who shared my major and were likewise passionate about their scientific calling. Together, we prepared for each class; together, we discovered and were amazed by the library of what was then known as the Institute of Fundamental Biology at UAB, where we learned what scientific journals were and what original research consisted of, and where there was nothing we did not want to read. I shared with them the search for a laboratory where we could work and do the final project we had to go through to graduate with honours, and the seminars and conferences we had to attend, and the class projects on which, among laughter and arguments, we outdid ourselves out of pure passion. Of that group, all four of us became professional scientists, and one, Ignacio Marín, became the life partner with whom I am united to this day.

The other aspect worth mentioning is that my desire to do research led me to request that I be allowed to do an internship in the Department of Cellular Biology and Physiology, in

a research group that was working in the field of neuroscience. That request was granted, and there I met young researchers who were in the process of doing their doctoral theses – people who went into the field to hunt lizards for use in their experiments, who travelled (taking me with them) to scientific congresses, who could happily spend all day every day in the laboratory. Their enthusiasm was infectious, and I interwove my part-time jobs, my classes and my study sessions together with my laboratory experiments, many of which I performed at noon while everyone else was eating lunch, in between my morning classes on scientific theory and my evening lab sessions. As time passed, I learned more and more about neurobiology and electron microscopy.

During my fourth year at the university, the fiftieth anniversary of the passing of Santiago Ramón y Cajal was celebrated with an international symposium on neuroscience in Madrid, and several of us from the laboratory attended. A number of scientists who had authored papers I had read were also in attendance, and I will never forget the impression some of them made on me. We were also joined there by young scientists from all over Spain, and from abroad as well, helping me to realize how vast and international the scientific community really was. Maybe it was because of these youthful impressions that I never left the field of neuroscience. Or perhaps it was the fact that, as someone once pointed out to me, scientifically speaking I am Cajal's great-great-granddaughter.

It was at that same congress that Alfonso Fairén, who was at the time a researcher at the Cajal Institute of the CSIC (Spanish National Research Council) in Madrid and a leading expert on the cerebral cortex, and is to this day a dear and respected friend, suggested that I move to Madrid to do my doctoral thesis with Javier de Felipe, who had just returned from a postdoc stay in the United States. The following year I finished my undergraduate studies, defended my degree final project on depictions of the olfactory bulbs of lizards created using electron microscopy, and began to make preparations for the move to Madrid that had been made possible by a pre-doctoral grant from the Ministry of Science and Education; I had decided to make the move even though it meant briefly abandoning Ignacio.

My thesis director was and is an eminent specialist in the cerebral cortex. A straight forward and profoundly good-natured person, he is also a multifaceted scientist, with many talents above and beyond his vast knowledge of the cortex's microcircuitry, including the ability to create artistic representations of cerebral imagery, and a deep critical reading of Cajal's work. (Javier would be considered Cajal's scientific great-grandson, as his thesis was directed by José Rodrigo, whose thesis was in turn directed by Fernando de Castro, a direct disciple of Cajal himself.)

Javier suggested that I attempt to determine whether pyramidal neurons from the cat visual cortex that differ from one another in terms of the specific area to which they project *also* differ from one another in terms of the degree of inhibitory innervation they receive at the level of their soma and axon initial segment. The project required a quantitative analysis, via electron microscopy, of the inhibitory synapses received by neurons that we previously labelled during very long surgical processes in which we injected a tracer molecule intracerebrally.

This analytic process was both arduous and tedious, and the data generated were to be presented in only one original paper that was eventually turned into two separate ones together with a review that is to this day a most cited reference in the field.[1–3] I have always believed that the process of writing my thesis helped me to cultivate patience, in addition to being a test of my fortitude and humility. But my hypothesis had been proven correct: the corticocortical neurons received a significantly higher number of synaptic contacts than the neurons that projected from the cortex to the thalamus.

At the time we did not know exactly what that meant – molecular studies of the nervous system were only just getting off the ground. But to this day the data remain incontrovertible. Just a few years ago, Javier called to tell me that, together with a group of engineers from the Polytechnic University of Madrid, he had used a new electron microscope prototype to refine a methodology that, once the surface of a block of cerebral tissue had been photographed with ultrastructural resolution, allowed him to remove just a few nanometers of tissue using ionizing radiation. The new surface was then photographed, and the whole operation was repeated over and over until the entire block could be reconstructed photographically. With this new methodology, all the research I did for my entire doctoral thesis could be accomplished in a matter of months.

When my thesis was complete, I returned to Barcelona. If I was going to continue to seek out scientific adventures, I wanted to do so together with Ignacio, who was finishing up his own thesis at UAB. Thus, in 1989 I took a post as an assistant professor in the Department of Cell Biology and Pathological Anatomy at the University of Barcelona. For three years I interwove teaching histology to medical students with research on the release process for a neurotransmitter called acetylcholine. The research group that I joined, directed at that time by Jordi Marsal, was studying the electric organ of the *Torpedo* ray.

This organ consists of a sort of modified skeletal muscle, formed of lengthy cells called electrocytes, which grow one on top of another and are capable of generating an electric shock when stimulated by the acetylcholine that is released by the thousands of synaptic endings that innervate them. Unlike the synaptic connection between motor neurons and skeletal muscle fibres, which consists of a single neuromuscular junction, the electric organ has countless synapses that can be isolated by cell fractionation techniques. By homogenizing the organ, we were able to isolate a suspension of so-called "synaptosomes", which are nerve endings separated from their axons that naturally seal themselves up, thus turning themselves into structures in suspension that contain all the machinery needed for the release of acetylcholine.

Furthermore, the group had access to a luminescence-based system that could determine, in real time, the quantity of acetylcholine that had been released into the medium in response to a given series of manipulations and stimuli. Back then, my research was focused on using pharmacological approximations to determine the implications of voltage-gated calcium channels in neurotransmitter release. The terminals were too small to allow us to use electrophysiological measurements to determine which specific ionic currents were involved in vesicular release. However, it was around that time that toxins capable of

blocking every known subtype of voltage-gated calcium channel were obtained; the toxins came from *Conus*, a Pacific sea snail with a beautiful elongated, patterned shell. This snail devours fish after using its retractable harpoon to paralyze them with a mortal cocktail of nerve conductance inhibitors.

I was thus able to study the channels that were involved in the release of acetylcholine in the synaptosomes of my electric fish.[4–5] My research may not have produced any great scientific advances, but it taught me other ways of thinking about synaptology, and it toughened me up as well, showing me what it means to be a professor/researcher who must combine the two activities without giving short shrift to either. It provided me with a period of time in which to think about the direction in which I wanted my career to head. I learned to be autonomous, which as it happens was a perfect fit for my personality, and I learned a great deal about histology that would serve me extraordinarily well in the next stage of my professional life, in the world of mice with specific mutations created via homologous recombination techniques – a methodology that was only then beginning to be used.

At that time, as the 1990s were beginning, both Ignacio and I had posts as assistant professors at the university. In spite of that institutional bond, and of how uncertain the future would be without it, we both felt that we needed to head abroad. We were especially interested in going to the United States, and there were three cities in particular that had caught our eye, each with a great deal of high-quality research being done, each with research groups and projects that we found fascinating. We were ready for a change, and while our career history to that point did not serve particularly well as a calling card, we thought that if we could each get just one high-level research group in the area that interested us to accept us, we would definitely take advantage of the opportunity.

Perhaps because counting synapses had helped me to see the importance of the concept of populations in terms of the regulation of processes, I then decided that I wanted to study how the size of neuronal populations were regulated. And I wanted to do so using organisms on which genetic manipulations could be performed – that is to say, not cats, and not electric fish. Finally, I wanted to incorporate molecular biology into my research. We wrote to approximately twenty different laboratories, and in the end I had two offers from which to choose. To this day I am all too aware of what a luxury it was to have been offered even one of those opportunities. Simply because my body could only be in one place at one time, I turned down the offer to go to Boston to join the lab of Robert Horvitz, who would go on to win the 2002 Nobel Prize in Physiology or Medicine for his work on apoptosis in the nematode *Caenorhabditis elegans*. Instead, with the help of a Fulbright fellowship, in 1993 I joined the laboratory of Louis F. Reichardt at UCSF (the University of California at San Francisco), in order to work on neurotrophin-based regulation of neuronal survival in mice. My stay there lasted five years thanks to a second fellowship, this one from the HFSPO (Human Frontier Science Program Organization), and to a research contract with UCSF.

Lou Reichardt's laboratory was dedicated to researching the development of the nervous system, especially in terms of the establishment of neural connections as well as the involvement of molecules that regulate adhesion and neuronal survival. Above and beyond

his stature as a world-class scientist, Lou is also an American hero. He was part of the first expedition to climb Mount Everest's most difficult face, known as Kangshung; the face's reputation is easily understood when one sees the topography, jagged as a row of knife blades, in the photographs Lou took from base camp. He was one of the few climbers in that expedition who reached the top, an event documented in the photograph on his desk in which he is shown holding up an American flag, his beard heavy with stalactites, his face a study of extreme exhaustion. He later successfully climbed K2, "the Savage Mountain", and his expeditions were turned into films and documented in *National Geographic* articles.

Knowing these things, it is not as if you can pretend you are working for some run-of-the-mill scientist. One of the postdocs who joined the lab after I had been there for a couple of years asked me how I managed to have such a great working relationship with Lou. I told him that whenever I went into Lou's office to talk about my projects, I reminded myself that he had been to the peaks of Everest and K2, which meant that I did not get to complain about experiments that were not working out as planned: in this lab, cowardice was not an option.

Lou's laboratory was a very large one, with a dozen postdoctoral fellows. And while the lab's projects were very important to him, and his opinion was always illuminating, the overall zeitgeist was such that each of us was responsible for climbing our own scientific Everest, and our individual autonomy was all but absolute. Personally, I loved having the ability to build my own histories. The research group was financed by the Howard Hughes Medical Institute, which guaranteed that we would be able to run practically any experiment, and we were located in a terrific enclave of scientific interaction, near to Stanford University, where Ignacio worked, and UC Berkeley as well, not to mention a large number of biotechnology companies such as Genentech.

In my opinion, Lou was an excellent supervisor. What most impressed me about him was his vast scientific knowledge; beneath his somewhat absent-minded appearance was a man who was very well learned in many subjects, and extraordinarily well learned in biology. He accepted postdoc researchers from a wide range of biological subfields, creating a sort of crucible of widely varied professional experiences, which led to group meetings and interactions that were highly stimulating for all of us.

I joined the research line investigating neurotrophins, a family of proteins related to NGF (nerve growth factor). The seminal work that Viktor Hamburger and Rita Levi-Montalcini accomplished in the 1950s at Washington University in St Louis on the development of motor neurons in the spinal marrow of chicken embryos demonstrated that many of the neurons produced within the nervous system during fetal development die before the fetus is born, and that the death rate depends directly upon the size of the target tissue innervated by the neurons. The neurotrophic hypothesis postulated the existence of molecules that promoted neuronal survival, and that were produced by the target tissues in limited quantities, such that the neurons would compete for them, and only those neurons exposed to them would survive; this was the mechanism with which the size of the pre- and post-synaptic populations could be regulated. However, the nature of said molecules was completely unknown.

Rita Levi-Montalcini united forces with the biochemist Stanley Cohen in order to isolate the first known neurotrophic factor, NGF, an achievement for which they were awarded the Nobel Prize in 1986. NGF was shown to be essential in regulating the number of neurons in the sympathetic and sensory ganglia, having been produced in areas protected by these neurons. When more NGF was added, more neurons survived. If the protein was injected into gestating rats, the rats produced antibodies that were then passed on to their fetuses, whose sympathetic and sensory ganglia shrunk as a result. All the evidence appeared to support the neurotrophic hypothesis, but NGF only explained the survival of a few neuronal populations; thus, the idea was that there had to be other factors involved.

Following up on this notion over in Germany, Yves Barde and Hans Thoenen decided to isolate activities that promoted neuronal survival. Working with dozens of pig brains, they were able to identify a new protein they named BDNF (brain-derived neurotrophic factor). Comparing it to NGF, they saw that the two molecules were closely related in terms of their sequence variation. As it was by now the end of the 1980s, the information the two men obtained and the cloning of the two genes involved allowed for the identification of new molecules that were seen to be related at nucleic acid sequence level. Subsequent discoveries included NT-3 (Neurotrophin-3, discovered in Lou's own laboratory) and NT-4/5, completing what was by this time known as the neurotrophin family.

For quite some time it had been generally agreed that the only molecule at the plasma membrane that appeared to bind NGF, and that thus potentially could act as a receptor, was one known as p75: a protein that seemed to possess none of the enzymatic activity needed to transmit a growth factor signal to the interior of a cell. But it was only now – still the end of the 1980s, the world still aflutter at the discovery of oncogenes – that famous molecular oncologist Mariano Barbacid discovered a membrane-based tyrosine kinase-type receptor that only appeared in the sensory and sympathetic ganglia of the peripheral nervous system. In his brief but significant incursion into the world of neurobiology, Mariano had identified the receptor for NGF, a receptor that would soon be known as TrkA; he also showed that it is similar proteins that transmit signals from BDNF and NT-4 (TrkB), as well as from NT-3 (TrkC).[6]

Given my field of interest – the control of neuronal population size, in this case as regulated by survival rates – this was an ideal topic in a period in which the entire field was making great strides. On the culture plate in the lab, each neurotrophin appeared to be acting upon specific neuronal populations. However, no functional studies had been done on an intact organism. Given this context, it was clear that using the latest methodologies for eliminating specific genes in mice would be extremely useful for my research. Lou's laboratory had begun to produce two mutant mouse strains: one that lacked the gene that encodes BDNF, and one that lacked the gene for NT-3. When I arrived, they had just achieved the first mutations; we did not know which phenotype was on its way, but those mice would afford us the extraordinary opportunity to analyse the basic activities of both neurotrophic factors. And my training in neuroanatomy and my theoretical knowledge of histology allowed me to design an integral analysis of the mice.

It was soon evident that factors promoting neuronal survival could potentially be of use in fighting neurodegenerative diseases and, given worldwide interest in that subject, it was obvious to us that Lou's laboratory was not going to be the only one at work producing these sorts of mutations. And in fact it was not. Our competition with Rudolf Jaenisch's laboratory in Boston was heated indeed; happily, my efforts paid off with an article published simultaneously with theirs. Other research groups simply fell behind. With the two strains I was able to identify the essential neurotrophic requirements of specific neuronal populations, to show that the neurons are dependent upon the neurotrophins while the neurons' axons are developing prior to the innervation of the target tissue, and to establish that NT-3 was able to exert its effects via any one of the three Trk receptors.[6]

It was a period of frantic activity every step of the way. At around this same time, a second family of neurotrophic factors was discovered, this one related to GDNF (glial cell derived neurotrophic factor), a protein discovered in a glial cell line, which promoted the survival of dopaminergic neurons – the neurons that degenerate in the case of Parkinson's disease. The receptor for this trophic factor was unknown, but Arnon Rosenthal's laboratory at Genentech had created a strain of mice that lacked GDNF, and they were asking for my assistance. In this case, we were going to compete directly with Mariano Barbacid, whose own laboratory had created exactly the same mutant strain. The two laboratories published the mouse phenotype simultaneously, and it led to the identification of the receptor of this factor as c-Ret, a membrane tyrosine kinase-type receptor that had theretofore been an orphan receptor. There was a striking concordance between the phenotypes of the mutants that lacked GDNF and those that lacked c-Ret, the latter of which had been produced several years before; this observation was essential in designing the biochemical experiments that would lead to proof of our conclusions.[7]

Ignacio and I thought long and hard before deciding to return to Spain. It would have been easy to stay in the United States; many of my colleagues did not understand why I never even bothered to try to get a permanent post at an American university. But Ignacio and I felt that our work could actually mean more in Spain, and that it was time to head home. In 1998 opportunities for Spanish researchers to return from abroad were scant; the nation's institutes had only recently begun to build themselves up, with the Spanish National Cancer Research Centre (CNIO) having been founded that same year by Mariano Barbacid; and throughout Spain, science could only be done through the Spanish National Research Council (CSIC) or at universities.

In spite of my accomplished postdoctoral career and my personal resolve to succeed, the return to Spain was anything but easy. Opportunities and support that perhaps should have materialized somehow never did. In those years, the neuroscientific community was rather standoffish and fatalistic as regarded welcoming new professors. Ignacio and I sought a professional home that would allow us to live together in the same city, so we applied together for assistant professor positions much like the ones we had left five years before, and found jobs at the University of Valencia.

The truth of the matter is that it has never been easy for researchers to incorporate themselves into a Spanish university of which they are not themselves graduates. For one thing,

you are an unknown person who has taken a position for which others already working there had hoped to be hired. For another, there are no resources whatsoever dedicated specifically to your incorporation into the university, nothing like the sort of start-up package that is common in more scientifically advanced countries. I did not even have any lab space; my first laboratory bench in Valencia was one I built with a sheet of wood and two cabinets behind my desk in my half of the shared office my new department had provided me. Even as late as the end of the 1990s, Spain did not recruit scientists so much as grudgingly allow them to join university faculties. A few years later, the new institutes, created with a more proactive, pro-contract mentality, would demonstrate the great impulse that can be given to scientific research simply by applying the criteria of focused recruiting and logistical support that were already part of the process in other countries.

I was not afraid of the double role of professor/researcher into which I was stepping – I had had plenty of experience with it already. But I needed to build a laboratory, the kind that I had always wanted. Back then it was extremely difficult to find funding for a project requested by an employee working under contract; nowadays, of course, with the Ramón y Cajal research programme, ICREA (the Catalan Institution for Research and Advanced Studies) and our growing number of scientific foundations, such difficulties seem unthinkable. I had to decide if I wanted to take the risk of leading the way myself, with the possibility that I would not get the funding I needed, or to accept the fact that my project would be directed by one of the tenured colleagues that surrounded me.

Good advice from a good friend led me to take the risk, and it paid off with a project funded through Plan Nacional, an associate pre-doctoral fellowship and a second project funded by the Ramón Areces Foundation. With that funding in place I fought for more space, and was rewarded with sixty square metres in a laboratory that the school had granted for internal use to the Department of Cellular Biology and Parasitology of which I was a part. Two other professors, both recently incorporated from elsewhere much like me, decided to join forces with me. The two, Francisco Pérez and Martina Kirstein, have been with me ever since. Together we remodelled that laboratory, for all intents and purposes with our own bare hands, and the funding I had acquired allowed us to stock it with a bare minimum of the equipment we would need to begin work. We advanced little by little, following in the footsteps of so many Spanish scientists who had done likewise as part of the nation's precarious university system of the 1970s and 1980s. But as the new millennium began, the scientific world outside was churning madly; I had just come in out of those heady, whirling winds, and it was not at all clear what future there could be for someone taking tiny steps in a place that felt so isolated, so distant from the science of which I had been a part over the previous several years.

In 1999, with my new neurotrophin projects underway, I made my way to the NGF Conference in Stockholm. It was a trip I will never forget. I had been invited to give a talk at the meeting; my current situation notwithstanding, I was still a researcher of some standing in the field of neurotrophic factors. During my flight back home, as we passed over the clouds that I could see out of my window, I understood that there was simply no way to keep my current work on neurotrophins up to the standards I had previously set for myself. Parts of my projects and many of my ideas had stayed back in Lou's laboratory. Though our

relationship was a good one, there was no way I could do the kind of work that all my former colleagues were still doing there; my personal Everest had grown much higher, and I was stuck at base camp. In that moment of clarity, and of anguish, it was clear to me that I had to change course: I had to begin an entirely new line of research. I thought that if I began something genuinely new, no one would have any concrete expectations for my work, and I would be able to build my laboratory with a greater degree of freedom.

I had previously studied neuronal population size controls that used elimination mechanisms that worked via programmed cell death. I decided that I would study the production of neurons, on the other side of the equation where the final number of neurons in a given population is decided. It was an extremely risky decision. We could easily fail, and all my previous scientific production, achieved at a cost of so much effort and dedication, would be of no help as I sought to make our laboratory a key part of a new scientific community.

Back then, the process by which new neurons are produced in the brains of adult mammals, now known as postnatal neurogenesis, had only recently been discovered, and the nature of the neural stem cells (NSCs) present in two specific zones in the mammalian brain had only recently been identified. It was believed that there had to be mechanisms that helped sustain the NSCs throughout an organism's life (a process we call self-renewal) while the requisite number of new neurons are being produced. I decided to focus our research on the more active of those two zones, known as the neurogenic niche of the subependymal zone (SEZ) or ventricular-subventricular zone (V-SVZ), located on the wall of the lateral ventricles; this is the production site for neurons that migrate rostrally towards the olfactory bulb into whose circuitry they will incorporate themselves as they begin taking part in the work of olfactory discrimination.

The NSCs can be isolated from this zone, and grown in a culture system consisting of free-floating NSC clusters known as "neurospheres"; doing so allows us to combine the analysis of mice *in vivo* with experiments done *in vitro*. The best thing about this system is that it replicates the processes that occur during the development in a separate closed, accessible system within an adult organism. We were able to study population dynamics while controlling for aspects of neurogenesis, neuronal differentiation and neuronal survival that I had previously studied in the context of the developing peripheral nervous system. Nonetheless, the territory that seemed most interesting to me was that of the self-renewal of stem cells – the process by which these cells sustain the size of their populations, their properties, their very existence.

And this is the territory on which we have focused our efforts over the past fifteen years. Self-renewal is the result of a stem cell dividing in conditions such that at least one of the resultant new cells is retained as a stem cell. In order for this to occur, the activation of these relatively quiescent cells must be carefully coordinated according to the type of division they perform (either symmetrical or asymmetrical) so as to perpetuate the stem cell's own characteristics of multipotency and undifferentiation. It is a fascinating cell process whose complex control depends upon the integration of signals received by stem cells with their own intrinsic control programmes.

I must note here that our early work in this area would hardly have been the same if Sacri R. Ferrón, who at the time had just taken a degree in Biology at the university, had not taken an interest in my offer to sign a pre-doctoral contract. Given our still-tenuous laboratory situation, our new and difficult research topic and the fact that she only had a two-month stay at Angelo Vescovi's laboratory at the National Neurological Institute of Milan to learn how to cultivate stem cells, Sacri played a crucial role in getting our laboratory off the ground. In her footsteps followed a series of students and postdoc fellows, all talented and some of them extremely so, but it was her influence and generosity in those early years that made it possible to build the kind of laboratory that I wanted. I wanted to do high-quality science, and to publish relevant articles that would serve to advance our knowledge of the field, much as I had been doing in the United States. I wanted to find answers to our questions about the processes regulating self-renewal, and I wanted the answers to bear our seal, to demonstrate the creativity that every scientist hopes to bring to bear. Thanks to the individuals who have passed through our laboratory, and to those who are working there now, all of whom adopted our work philosophy with enthusiasm, ambition, and a tremendous amount of hard work, we have managed to push forward, seeking to confirm our hypotheses, and tell our stories.

As I have said, in those years we were focused on learning how the stem-cell processes of activation and self-renewal were regulated, and how the stem cells were able to integrate signals from their micro-environment into their intrinsic programmes controlling the activation of the self-renewal process. Of the many intrinsic stem-cell mechanisms, we have chosen those that regulate the cell cycle; those which, in our stem cells, have shown themselves to possess functions that go beyond their canonical function as cell-cycle regulators, to regulate the programmes of gene expression associated with the state of the stem-cell state.[8,9]

However, our most intense efforts have been invested in researching the effects of interactions that take place within a given micro-environment. One could say that our laboratory has specialized in what are known as "niche effects". In particular, we have worked actively to identify and characterize signals produced by the vasculature, seeking to understand the cellular strategies that respond to these signals. Thus, in 2006 we were the first lab ever to describe the molecule produced by endothelial cells that regulates the self-renewing divisions of the NSCs in the SEZ: pigment epithelium-derived factor (PEDF).[10,11] This factor is a protein with neuroprotective and anti-angiogenic properties that also promotes the symmetrical NSC division that increases the size of their population.

More recently we discovered that the NT-3 I had spent so much time working with in the United States is, somewhat surprisingly, also produced by endothelial cells, and regulates the quiescence of the NSC, a function that had never been witnessed before in a neurotrophin.[12] It seems that I will never be able to escape the world of neurotrophic factors. Currently we are exploring NSC self-renewal that occurs in response to other processes in the neurogenic niches such as innervation and inflammation, proces ses that are apparently altered in the course of neurodegenerative ailments such as Parkinson's disease, which is another field of interest for us. Understanding the regulation of the plastic potential and self-renewal capabilities of stem cells in adult tissue such as neural tissue could help us to

improve the conditions under which we cultivate these cells for their subsequent *ex vivo* expansion. This in turn could help optimize our cultivation of these cells, particularly those we intend to transplant in accordance with any of the therapeutic strategies that form a part of what we currently refer to as regenerative medicine.

In reference to the brain in particular, our current strategies for restoring neural function via cell transplants do not appear to be working; we have been unable to achieve functional integration between the transplanted cells and the pre-existing circuitry. Fortunately, however, the concept of activating endogenous NSCs to restore neural function is currently emerging as a strong candidate for success. Such a strategy is in many ways an attractive one, but it requires a great deal of knowledge regarding how these cells function within their natural micro-environments. Such knowledge is of transcendental importance not only for cell therapy, but also for our understanding of how our tissues age as the stem cells gradually lose their potential for self-renewal.

My years as a lead researcher have been fascinating ones, but they have also been difficult. It has not been easy breaking new ground, particularly under conditions that frankly could have been better, and without any true institutional support. But, as Lou taught me, science is not for cowards. Two years after joining the University of Valencia, I passed the exam to become a tenured professor. Next came the nationwide exams, which I passed in 2007, just as my first doctoral students were defending their theses. Despite having been the lead researcher on various projects already, I was not planning to compete for a full professor chair without first having demonstrated the ability to train young researchers. In 2008 I was awarded the chair in Cellular Biology at the University of Valencia.

One could say that I am, fundamentally, a university-based researcher. The teaching load in Spanish universities is too heavy to combine well with research, but the process of transmitting one's love of the profession and training students to do research is extremely gratifying work. I believe that if the university system were better organized, research could be done better within the universities than anywhere else. But there is too much inertia within the institution, weighed down as it is by an inefficient bureaucratic system in which vocational absenteeism leads to overloading the few in the name of some false sense of democracy.

Our understanding of how NSCs behave has become ever clearer; many of the young researchers who have worked with me went on to win positions either abroad or with Spanish research groups, and they have acquitted themselves well, some of them extremely well. Sacri returned to Spain after a successful postdoc at the University of Cambridge, joined the Ramón y Cajal contract programme, and is directing her own project alongside me at the University of Valencia. Nowadays, my research group is a hub for the RETIC (Network for Cooperative Research in Health) for Cell Therapy, the Centre for Networked Biomedical Research dedicated to Neurodegenerative Diseases, and is Prometheus group of excellence of the Community of Valencia.

The personal honours that have meant the most to me include having been chosen to join the European Molecular Biology Organization (EMBO) in 2013 and, more recently, having

been selected for recognition by the Botín Foundation. These two honours have made me feel that the leap into the void that I took in 1999 from high above cloud-covered Sweden did not turn out so badly after all. The past fifteen years have held far more than just those two honours, of course; other accomplishments range from starting a laboratory from scratch to building a superb team of young professionals who work together with great enthusiasm; from solidifying my position at the university to building a family with Ignacio and our two children. And the best thing is, there is still more to come.

This is just the story of one individual, and it would be easy to think that, given that it turned out to be a story of success, there must be some magical formula inside that can be applied to the lives of others. Nothing could be further from the truth. Aside from the talent concerned, there is always a great deal of luck involved in one's scientific career. One aspect of this luck is the degree of support that one's society and leaders may or may not provide. I have always wanted to see my country move forward, and have always wanted to contribute to that advancement; I understand the enormous social responsibility that is shared by all of us who have received training and jobs thanks to the efforts of the Spanish taxpayer. Science is a wonderful profession, but much as a majority of scientists must work to serve society, society must be conscious of the value of the work of its researchers, and not allow the frivolities and distractions of short-term politics to endanger the solidity of the structures that generate knowledge, or the strength of public education at every level. An educated society is a society that demands that things be better than they are. An educated society is a richer and more united society. And that is how ours can be, if everyone gets involved.

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GLOSSARY

A

Acetylcholine. One of the main neurotransmitters in the autonomic nervous system. It works at the central level (memory) and peripheral level and is the only transmitter used by the motor part of the somatic nervous system.

Adenosine. Molecule with important functions in biochemical processes such as transferring energy in the form of ATP (adenosine triphosphate) or as a signal transducer such as cAMP (cyclic adenosine monophosphate). It also plays an important role as a central and peripheral neuromodulator.

Adhesion molecule. Transmembrane protein that plays a role in processes for cellular bonding with other cells or the extracellular medium, such as cadherins, integrins and selectins.

Advanced medical technology. Concept that includes those techniques used in diagnostic or therapeutic procedures characterized by their complexity, use of apparatuses and high price. They include "large" machines, such as magnetic resonance installations, lithotripsy devices and linear accelerators.

Algorithm. Set of instructions that are used to execute a task or solve a problem in a finite number of steps or operations. The term algorithm is not exclusively related to mathematics, computational science or computers. Actually, in everyday life we use algorithms on a wide range of occasions to solve various problems. For example, the use of a washing machine (by following the instructions) or cooking (by using a recipe). An algorithm must be defined, finite and precise.

Amino acid. Each of the twenty organic acids that make up the basic building blocks of proteins. A protein is, in principle, a long chain of different amino acids (polypeptide chain) that undergoes folding several times to form a complex three-dimensional structure.

Amyloidosis. Generic term referring to the pathological extracellular deposit of an amorphous, insoluble protein material that is resistant to proteolysis.

Angiogenesis. Process of vascular neoformation. The process of neovascularization is necessary to feed solid tumours and their later growth and dissemination. Under normal conditions, angiogenesis is limited to the processes of scarring, healing from ischemic damage, the development of breasts during pregnancy and muscular development when practicing sports.

Aquaporin. Integral membrane protein that forms pores on the cell membranes through which water flows faster than it would if it did so by diffusing through the phospholipid bilayer that basically forms the cell membrane.

B

Biomedicine. Clinical medicine based on the principles of the natural sciences: cellular and molecular biology, genetics, biochemistry or biophysics.

Biomimetics, biomimesis, biomimicry. The study of nature as a source of new and innovative technologies to solve those problems that nature has already resolved.

Bionics. 1. In the medical world, this refers to the development of artificial organs: skin and synthetic hearing organs, blood vessels, heart, kidney, liver, limbs, etc. **2.** The term is also used for any mechanical design that emulates the behaviour of a living organism. Robots of various types that move using artificial limbs and chess players that use AI are referred to as "bionic machines".

Biopolymer. Each of the types of polymers that play a fundamental role in the maintenance of structures and the functions of living beings. Their chemical units are different (amino acids, nucleotides and monosaccharides) and they form, respectively, proteins, nucleic acids and polysaccharides (glycogen, etc.).

Bioreactor. Any system, at the micro- or macroscale, used to carry out enzymatic reactions.

Biosensor. 1. Any sensor that transmits data on a biological process (arterial pressure, blood sugar level, etc.). **2.** System that uses immobilized biological material to detect and measure a chemical compound.

Bisphosphonate. Non-hormonal agent used to prevent and treat diseases with bone resorption, such as osteoporosis and cancerous bone metastases.

Blastocyst. Hollow, preimplantational embryonic structure made up of fifty to a hundred cells. It consists of a trophoectodermal layer surrounding a cavity, or blastocoele, in which an internal cellular mass made up of blastomeres is located eccentrically.

Blastomere. Pluripotent embryonic cell derived from the internal cell mass of the blastocyst resulting from the first divisions of the fertilized ovum or zygote; it is able to produce cellular lineages of any of the three embryonic cell layers (ectoderm, mesoderm and endoderm). Therefore, it is a totipotent cell.

C

Cancer. A series of diseases characterized by the rapid, uncontrolled division of a set of cells in the body. Their growth rate is so fast that the cancer cells begin to interfere with the patient's normal functions, which can lead to death. Cancer cells have the properties of losing inhibition by contact, invasiveness and the ability to metastasize.

Capacitative current. Unlike a resistance, in the case of a cell, the voltage response varies more slowly than the change in current. This delay produced in the voltage's stabilization is due to capacitance.

Catecholamine. Each of the metabolites of the amino acid tyrosine: dopamine, adrenaline (epinephrine) and noradrenaline (norepinephrine). Dopamine and noradrenaline are neurotransmitters (the first at the central level, a lack of which causes Parkinson's disease; the second is the peripheral effector of the sympathetic nervous system); adrenaline, produced in the adrenal medulla, performs important metabolic activities.

Cell cycle. Set of processes necessary for a cell to shift from a state of rest to cellular division. The cellular cycle is divided into four stages: G1 (leaving the state of quiescence), S (synthesis of new DNA), G2 (preparation for mitosis) and M (mitosis). The shift from one stage to another is controlled by the so-called checkpoints, which are irreversible, and therefore, once they have occurred, the cells must move into the subsequent stage. The different stages in the cycle are regulated by a family of proteins called cyclines.

Chemokine. Each of the members of a family of chemotractor mediators (which attract different types of cells to a specific site in tissue).

Chromaffin cell. Neuroendocrine cell that is located in the adrenal medulla and in the ganglia of the autonomic nervous system, though also in the intestinal, vesical and prostatic mucus membranes. They secrete various neuropeptides (adrenaline or epinephrine, noradrenaline or norepinephrine and enkephalin).

Clinical trial. Type of clinical study in which an important question in medicine is researched so as to increase knowledge. Most clinical trials that are carried out evaluate new drugs or experimental medical treatments with a strictly controlled research protocol. A clinical trial must be controlled, prospective, random and blind and have a sufficient sample size.

Clonality. Characteristic of tumours whereby they gradually select groups of cells (clones) with a more aggressive ability to divide. Each new mutation that provides an advantage to the cell that undergoes that mutation and allows it to divide more rapidly than before suffering the mutation is a new process of clonality, and therefore this event occurs several times throughout the tumour's development.

Clone. Exact replica of an animate or inanimate object. **1. Genetics.** Set of genetically identical cells produced by an original cell's mitotic divisions. Set of genetically identical organisms, all of which descend from one single parent organism through an asexual process. Set of DNA molecules derived from an original sequence and produced using genetic engineering techniques. **2. Virtual reality.** Virtual body that is an exact replica of a real body, based on morphofunctional data transmitted remotely and reconstructed in a virtual environment.

Cloning. 1. Process of inserting DNA into a vector for its later amplification and/or manipulation. **2.** Replica of an individual using nuclear transference techniques.

Cryofracture. Technique used in transmission electron microscopy that consists of freezing the sample with liquid nitrogen, after cutting or fracturing and shading the surface of the sample.

Cytokine. Each of the soluble polypeptide factors produced and secreted by the different types of cells, which act as autocrine, paracrine or endocrine chemical messengers. Lymphokines, interleukins and growth factors are all cytokines.

Cytoskeleton. Structural network displayed by eukaryotic cells responsible for maintaining cell shape, intracellular transport, cellular motility and, in general, the cell's internal organization. It braches out through the entire cell using microtubules, microfibrils and microfilaments, with a protein structure.

D

Dendrimer. Three-dimensional macromolecule with a synthetic branching construction.

Dendron. Concept established by John C. Eccles (Nobel Prize in Physiology for Medicine in 1963) to explain the phenomenon of consciousness. Eccles proposed that the mental world is microgranular, giving the name "psychons" to mental units. Ideally, each psychon should have a discrete anatomic base (dendron). Mind-brain interaction would be produced within the realm of each psychon-dendron unit.

DNA (deoxyribonucleic acid). Biopolymer arranged into strands made up of a large number of units known as deoxyribonucleotides. Its bases carry the genetic information, whereas the rest of the molecule plays a structural role. It constitutes the genetic material of all cells and many viruses. The macromolecule's skeleton is constant throughout and is made up of deoxyribose groups linked by phosphodiester bonds. The variable portion is made up of a sequence of four bases. In cells, DNA is organized into long structures known as chromosomes: twenty-three pairs in the human species, twenty-two of which are autosomal (one inherited paternally and the other maternally) and one that is sexual (X and Y). They contain approximately 20,500 genes. *Coding DNA.* Sequences that code for

proteins. *Junk DNA.* Portions of DNA that do not code for proteins (the vast majority of the genome), though they may play a regulatory role. This type of DNA contains microsatellites (SSR or SRT, for the acronyms in English "simple sequence repeat" and "short tandem repeat", respectively), sequences of DNA in which a fragment containing two to six base pairs is repeated consecutively; they have a high rate of mutation and are used as molecular markers with a wide variety of applications in the field of genetics, such as paternity studies.

Dopamine. Neurotransmitter whose decrease in the dopaminergic cells of the midbrain's *substantia nigra* causes Parkinson's disease.

Drug. 1. Pharmaceutical. Any product used as a medicine. **2. Narcotic.** Substance or preparation that circumstantially produces a sensation of well-being and euphoria. Repeated administration causes psychological and physical addiction to the substance (dependence or drug addiction).

E

Electrophoresis. Method of molecular fractionating based on differences in mobility in an electrical field, which is a function of the molecules' charge.

Enzyme. Catalyst of specific biochemical reactions whose structure has been universally considered as belonging to the category of proteins. However, this catalytic activity has also been found in some ribonucleic acids (ribozymes). The name isoenzyme is given to each of the forms of an enzyme with the same specific activity but different chemical or immunological characteristics. For example, glucokinase, an enzyme involved in the metabolism of carbohydrates; phospholipase, in the metabolism of the phospholipids that form biological membranes, and proteases, in those of proteins.

Epidermolysis bullosa or ampollosa. A series of skin diseases, though they are also found in the mucous membranes, transmitted genetically; they are expressed by the appearance of blisters, wounds or ulcers in the event of minimal trauma (for example, slight abrasion).

Epigenetics. Study of the modifications in gene expression that affect phenotype but are not caused by mutations.

Epitaxial growth, epitaxia. Process of producing integrated circuits by crystalline deposition, in which fine layers of material are made to grow onto a substrate.

Exon. Fragment of DNA in the sequence of a eukaryotic gene that contains the specific codons of a sequence of amino acids of a specific polypeptide; it also contains the information at the beginning and end of the sequence.

F

Field-effect transistor (FET). Family of transistors that are based on the electric field to control the conductivity of a channel in a semiconductor material.

Functional genomics. Study of the genome to determine the biological function of genes and their products.

G

G protein. Type of cell receptor.

Gene. Basic functional unit of genetic material located at a specific site on a chromosome. Originally, it was considered a unit of inheritance and mutation, but at present it is defined as a sequence of DNA that acts as the unit that controls the formation of a polypeptide chain. In diploid organisms, including the human species, the genes appear in the form of a pair of alleles. A gene can be divided into a structural region and a regulating region. The structural region contains sequences that transcribe messenger RNA that will translate proteins. The regulating region consists of two types of elements. One is responsible for ensuring basal expression and has two components: the proximal component directs contact with RNA polymerase and the specific distal component (promoter) the initiation frequency. The second is in charge of regulating expression (element of response) and consists of two elements that stimulate (enhancers) or silence expression, and others that mediate the response through various signals. The specific expression in tissue involves sequences of this last type.

Genetic engineering. A set of techniques that make it possible to isolate and fractionate a cell's DNA into specific fragments, and to assemble the obtained fragments with another macromolecule of carrier DNA (recombinant DNA). This makes it possible to transfer genetic information among organisms and produce specific proteins at an industrial scale.

Genome. Set of genes that specify all of the potentially expressible characteristics of a given organism, with no connotation whatsoever of the allelic nature of the genes that make it up. The sequencing of all the bases that make up the genome was the final objective of the Genome Project.

Genomics. Comprehensive study of all genes and their interactions.

Genopathy. Disease caused by genotype. A disease is monogenic when it is caused by a change in one single gene, and polygenic if several genes are involved.

Genotype. The set of genes in one individual. Normally this refers to the pairs of alleles that a person has in one region of the genome. The external expression of a genotype in a specific environment is referred to as a "phenotype".

Glomus cell. A class of neuron cells that form part of the glomus or carotid body, located at the branching of the carotid artery. It is a chemical receptor organ sensitive to changes in the pO2 of arterial blood. Glomus cells produce dopamine, a neurotransmitter that causes Parkinson's disease when lacking in the central nervous system; they also produce GDNF (glial line-derived neurotrophic factor), a neurotrophic factor.

Growth factor. 1. Each of the agents able to induce the proliferation, development and differentiation of various cell types. For example, the angiogenic factor, which induces the neoformation of blood vessels. **2.** Each of the members of a superfamily of proteins responsible for cellular development and survival.

H

Hyaluronic acid. A complex polysaccharide that is distributed throughout the connective tissue.

I

Immunoassy. Immunological analysis in which an enzyme is used as a marker to indicate the presence of specific antigens or antibodies.

Inflammation. Process unleashed due to aggression by different agents (biological, chemical or physical) on the organism. In the beginning it is localized and temporary, and the inflammatory response tends to neutralize the aggressive agent (micro-organisms) and limit tissue damage. The vessels and intravascular and tissue cells in the affected area play a role in the response, in a process coordinated by various chemical mediators. In specific situations, the inflammatory process can become generalized (inflammatory shock), causing terminal multiple organ failure, or it may become chronic (for example, rheumatoid arthritis); in this last case, there is always an associated immunological process.

Inotrope. A drug that strengthens function (for example, a cardiac inotropic drug).

Intron. Fragment of DNA that is unable to express itself in the form of a protein. In genes, they are located between the corresponding exons, and the ensemble of introns and exons is transcribed in the form of a large messenger RNA molecule that is later split off and spliced into a new messenger RNA molecule that then translates the proper protein.

Isomer. Each of the compounds that have an identical molecular formula but differ in the way their atoms bond or in their spatial layout. The former are referred to as "structural isomers", and the latter as "stereoisomers".

K

Kinase protein. Each of the enzymes involved in the processes of protein phosphorylation.

Kinome. Set of kinase proteins in a genome.

Knock out. 1. Procedure to eliminate the presence or activity of a specific gene. It is used to create specific animal models of diseases in which a gene is defective or missing. **2.** Organism in which a gene has been silenced (knocked out).

L

Lab-on-a-chip (LOC). Device that includes one or more functions typical of a conventional laboratory on one single chip, which makes it possible to handle extremely small volumes. They belong to the field of microelectromechanical systems (MEMS) or micro total analysis systems (μTAS). LOCs are very closely related with microfluidics.

Leptin. Appetite-inhibiting hormone produced mainly by adipocytes in the fatty tissue. It works as a lipostat: when the amount of fat stored in the fat cells increases, they release leptin, which informs the hypothalamus that the body has sufficient energy reserves and must moderate the ingestion of foods.

Leukaemia. Cancerous disease of the haematopoietic organs characterized by the uncontrolled proliferation of leukocytes and their precursors. Based on their evolutionary characteristics, they are classified as acute or chronic; in accordance with the affected cellular lineage, they are divided, in general terms, into lymphoids (single-nucleus cells or lymphocytes) or myeloids (multinuclear cells or polymorphonuclear leukocytes).

Ligand-receptor. Complex that results from the interaction of various ligands (growth factors, hormones) with their corresponding specific membrane receptors. This interaction begins the sequence of stages in the signal transduction mechanism, which leads to the final regulation of nuclear transcription.

Luciferase. Enzyme that catalyses the use of luciferin in bioluminescent processes.

M

Malignant tumour. This is the name given to a solid tumour that has the ability to invade adjacent tissues and organs and to spread to distant regions within the body.

Meiosis. Succession of two cell divisions with one single chromatidic reproduction, which gives rise to four sexual cells with the number of chromosomes reduced to half. In the first of these divisions, the recombination of paternal genetic information with the maternal genetic information takes place.

Metastasis. Set of cancer cells that have migrated from the site of tumour origin to other areas in the body, where they grow, creating a new tumour or metastatic tumour.

Metastasized tumour. See "metastasis".

Microarray. Microdevice based on fixating probes (genes, proteins, metabolites) onto a solid substrate (silicon, plastic) exposed to target (the sample).

Microfluidics. Cross-cutting field (engineering, physics, chemistry, biochemistry, biotechnology, electronics, nanotechnology) that encompasses everything from the applications to the design of microsystems that process minimal volumes of fluids with very low energy use.

Mitochondrial DNA (mtDNA, mDNA). DNA located in the mitochondria that forms a circular chromosome that codes thirty-six genes. It is only inherited maternally.

Mitosis. Process of cell division through which a cell produces two genetically identical daughter cells. The nucleus's division (karyokinesis), which is carried out in four phases (prophase, metaphase, anaphase and telophase), is followed by the division of the cytoplasm (cytokinesis) to form the two daughter cells.

Monogenic disease. Disease caused by a lack of or defects in one single gene and that follow a Mendelian inheritance pattern. Most diseases involve several genes (polygenic diseases), as well as environmental conditioning factors.

Morphogen. Substance that controls the pattern of tissue development and, in particular, the positions of several types of specialized cells within a tissue. Its effect spreads from a localized source, forming a concentration gradient alongside a tissue undergoing development. The definition of the morphogen is conceptual, not chemical.

Mutation. Copying error (random or induced) in the process of DNA replications that the systems for verification and repair of DNA have not caught. They may be neutral, deleterious or positive.

N

Nanophotonics. This field studies the interactions between matter and light at the nanometric scale, as well as the production of nanostructured material, modified in either a natural or artificial manner, in terms of its physical, chemical or structural properties, to explore and enhance the reactions at this scale when it interacts with laser light.

Nanotechnology. Manipulation of matter with dimensions of the order of 10-9 at the atomic and molecular levels to produce devices at a supramolecular scale.

Neurofibrils. Bundles of neurofilaments measuring approximately 10 nm in diameter; they form part of the neuronal cytoskeleton.

Neurotransmitter. Neurons have the ability to produce and secrete specialized chemical messengers known as neurotransmitters. They are usually classified as small molecule neurotransmitters, generally metabolites of amino acids (tyrosine, histamine, serotonin), such as catecholamines or gamma-aminobutyric acid, and neuropeptides, chains of approximately two to forty amino acids, such as ACTH and endorphins (endogenous opioids).

Neurotrophic factor. Neuronal growth factor.

Nociception. Nerve system process (initiated by nociceptors or pain receptors) through which stimuli potentially damaging to the body are coded and processed.

Nucleic acid. A biopolymer whose constituent units, nucleotides, are made up of one of four different nitrogen bases (adenine, guanine, cytosine and thymine in DNA; and adenine, guanine, cytosine and uracil in RNA), one pentose molecule and a phosphoric acid group. Depending on the nature of the pentose, nucleic acids are divided into ribonucleic acids (RNA) and deoxyribonucleic acids (DNA): the former have many biological functions, while the latter are the site of informational content in cells.

Nucleosome. Basic unit for packaging DNA. It consists of segments of DNA that surround one central protein element.

O

Omics. Generic term that refers to one of several new fields in today's biology: genomics (study of the structure and function of an organism's genes), proteomics (study of the structure and function of an organism's proteins) and metabolomics (global study of an organism's metabolism), which are creating new opportunities for the application of molecular-biological knowledge in medicine.

Oncogene. A gene which, in one or more of its forms, is oncogenic; in other words, it is able to direct the transformation of a normal cell into a cancer cell. In its normal form (proto-oncogene), it is involved in regulating the signalling routes of cell proliferation.

Oxidative stress. Structural or functional disorders in an organism caused by an imbalance between the production of reactive types of oxygen (derived from oxygen produced in the cell metabolism very reactive to different biological components) and the ability to neutralize them.

P

Parkinson's disease. Late-appearing, slowly advancing progressive neurodegenerative disease, characterized by tremors while at rest, facial inexpressiveness, short, rapid gait, slow movement and muscular weakness. It is due to the inability to biosynthesize dopamine (a neurotransmitter) in the neurons of the brain's *substantia nigra,* a neuronal nucleus located in the brain stem whose components produce dopamine as a neurotransmitter. Along with pharmacological treatment (administration of dopa, a precursor of dopamine) neuronal transplant has been suggested.

Patch clamp. In electrophysiology, the local fixation or patching of membranes is a laboratory microtechnique that makes it possible, through the use of micropipettes, to perform individual or multiple study of ion channels, especially in excitable cells such as neurons and cardiac myocytes.

Pharmacogene. Transfected gene with therapeutic uses.

Pharmacogenetics. Study of the effect of an individual's genetic variability on that individual's response to specific drugs. It is the basis of personalized medicine.

Phosphorylation-dephosphorylation. The process of introducing a phosphate group into a molecule and its elimination, in the reverse, is a fundamental event in the transmission of biological signals.

Photonics. Study of the generation, control and detection of photons.

Plasmid. Genetic element, in the form of a small, circular DNA molecule that remains separate from the bacterial chromosome and is replicated independently from it.

Plasmonics. A branch of nanophotonics that is based on studying the processes of interaction between electromagnetic radiation and the conducting electrons in metal-dielectric interfaces. The behaviours that are observed as a result of this interaction may be interpreted on the basis of the existence of plasmons (collective oscillations of conducting electrons) that possess characteristics related to the metal, the light wavelength and the surrounding medium.

Polymerase chain reaction (PCR). Technique for *in vitro* enzymatic amplification of specific nucleotide sequences.

Primary tumour. This is the tumour made up of the initial group of cells on the basis of which the cancer has developed.

Progeria. Genetic disease that is expressed in childhood, characterized by sudden premature ageing.

Prosthesis. Artificial device that replaces or provides a part or function of the body that is missing or lacking for any of various reasons.

Proteostasis. Process that includes the cell's biological pathways controlling the biogenesis, folding, transit and breakdown of an organism's proteins.

Purinergic. Type of membrane receptor whose ligands are different nucleotides or adenosine.

R

Radionucleide. Each of the chemical elements with an unstable configuration that undergoes radioactive breakdown manifested in the emission of radiation in the form of alpha or beta particles and X-rays or gamma rays.

Ras protein. Each of the members of a family of proteins which, along with the gene bearing the same name, form an ensemble of very important molecular switches/regulators in a wide variety of cell signal transmission pathways that control different phenomena: cytoskeleton integrity; proliferation, differentiation, cell adhesion and migration, and apoptosis. Both the gene and the related Ras proteins are often altered in malignant tumours, causing an increase in the capacity for invasion and metastasis, and a decrease in apoptosis.

Recombinant DNA technology. Set of tools used to identify, cut, isolate, combine and recombine segments of DNA.

Recombination. Normal process through which segments of DNA are exchanged between homologous chromosomes during gametogenesis. The resulting gametes contain chromosomes that are taken from both homologues. In general, the more separate the loci, the greater the possibility of recombination; the highest recombination rate is equal to fifty per cent for two loci located on separate chromosomes.

Reflectivity. Fraction of incidental radiation reflected by a surface.

Retrovirus. RNA virus whose main characteristic is the presence of an RNA-dependent DNA polymerase known as reverse transcriptase. This enzyme allows the RNA to direct the synthesis of DNA that is integrated into the genome of the infected cell, after which it can be transcribed and give rise to various messenger RNAs coding for the viral proteins. Some of them contain sequences with oncogenic potential (viral oncogenes) able to cause tumours in different species.

Reverse transcriptase. Enzyme that acts as a catalyst for the transcription process in the reserve direction; in other words, the synthesis of DNA directed by RNA. It may be of viral or cellular origin and takes part in processes for differentiation and genetic or immunogenetic amplification. Synonym: RNA-dependent DNA polymerase.

RNA (ribonucleic acid). Nucleic acid whose constituent units are ribonucleotides. Their nitrogen bases are adenine, guanine, cytosine and uracil, or some types of derivatives. The presence of the 2'-OH group in the carbonate skeleton gives the molecule lesser flexibility, while at the same time more reactivity. Ordinarily, it makes up one single chain, but in certain cases it may have the form of a double helix with a complementary chain or DNA chain. In accordance with its function, it is classified as: RNA involved in protein synthesis (messenger, transfer, ribosomal), regulatory RNA (interference, microRNA, antisense) or that with catalytic activity (ribozymes, spliceosomes). It makes up the genome of many viruses and bacteriophages.

S

Screening. Strategy applied to a population in order to detect a disease in individuals with no signs or symptoms thereof. For example, screening for congenital metabolopaths in newborns (the blood drop test at the heel).

Signal transduction. A complex mechanism based on protein-protein interactions that lead to phosphorylations and dephosphorylations of the proteins themselves; the action of signals external to the cell causes a series of actions in a cascade, through chemical messengers (hormones, growth factors, cytokines, etc.) that act on receptors, leading to the activation of one or more genes that end in the final expression of a biochemical nature.

Signal transduction cascades. A set of proteins in the cell responsible for transmitting information from the cell's surface to the nucleus. This information can be quite varied; for instance, signals are transmitted for proliferation, differentiation or apoptosis, as well as many other purposes.

Solid tumour. That which affects a solid tissue or organ, contrary to what occurs with haematopoietic tumours, referred to in general as "leukaemia".

Spliceosome. Complex molecular machine that carries out the mechanism of cutting and splicing in the processing of eukaryotic messenger RNA. It consists of more than thirty proteins and several molecules of specialized RNA.

Splicing. See "spliceosome".

Statin. Group of drugs used to inhibit the synthesis of endogenous cholesterol, compared with another that blocks the intestinal absorption of exogenous cholesterol.

Stem cell. Cells that can divide asymmetrically, giving rise to a replica of themselves (self-renovation) and a cell that can be differentiated into various cell types. If this cell can produce a complete organism, it is classified as totipotent (morula). Pluripotent cells (blastomeres) cannot create a complete organism but can create any cell corresponding to the three embryonic lineages (ectoderm, mesoderm or endoderm). If the ability to differentiate is restricted to one of the embryonic lineages, they are called multipotent.

Stereotactic therapy. Technique for endocraneal therapy using an external device with three-dimensional coordinates to locate deep structures in the brain and access them with the required precision.

Structural genomics. Study of the three-dimensional structure of proteins and their structural and functional domains using experimental and simulation techniques through the use of computers.

Synapse. Intercellular space through which the transmission of signals and sharing of information occurs between nerve cells (neural synapse) or between cells in the immune system (immunosynapse).

Synaptosome. Isolated synaptic terminal of a neuron.

T

Tauopathy. The tau protein is an essential component of the neuron's cytoskeleton. The presence of anomalous forms of this protein causes neurodegenerative diseases, especially Alzheimer's.

Telomere. Repetitive region of nucleotide sequences located at the ends of the chromosomes. Excluded from the replication process of the rest of the chromosome, they are cut on each cell division. Maintaining them requires the presence of an enzyme (telomerase) that decreases with the cell's age and is related to ageing and processes like cancer.

Transcription. Biosynthesis of ribonucleic acid (RNA) on the basis of deoxyribonucleic acid (DNA), which acts as a mould for the four types of triphosphate nucleosides serving as substrates, and of the enzyme RNA polymerase, which is a catalyst for the process. The synthesized RNA has different functions, with ribosomal (rRNA), transference (tRNA) and messenger (mRNA) RNA types.

Transcriptome. All the messenger RNA or RNA transcribed in a tissue and at one time in particular.

Translation. Exchanging the information contained in the sequence of the four messenger RNA

nucleotides for that required for the ordering of the twenty amino acids in the structure of the polypeptide chains. The process takes place within ribosomes, in which the interaction of messenger RNA codes with the region of the anticodon in the aminoacyl-tRNA takes place. Distinguished therein are the phases of initiation, elongation and termination, in which different protein factors play a role.

Translational research or from laboratory to clinical practice. Research oriented towards solving clinical problems.

Translocation. Type of mutation in which part of a chromosome is transferred to another place on the same chromosome, or to a different chromosome. This movement of genes may lead to major genetic disorders.

Transplant. Any implant (graft) of a tissue or organ into an individual. Based on their location, they are categorized as homotopic (implant at the corresponding anatomic site) and heterotopic (in a place other than the anatomic site). Based on their origin, they are classified as a self-transplant or autologous transplant (transplant from one site to another in the same individual); isotransplant or syngenic transplant (between genetically identical individuals); allotransplant or homotransplant (between compatible individuals of the same species who are genetically different); and xenotransplant or heterotransplant (transplant between individuals of different species).

Tumour. 1. Anomalous growth of a normal tissue with no functional purpose whatsoever. A limited, non-invasive growth is referred to as "benign tumour"; unlimited growth that invades neighbouring structures is classified as a "malignant tumour" or cancer. **2.** One of the so-called cardinal signs of inflammation. Any inflamed tissue (for example, an arthritic joint) shows an increase in volume (tumour), redness, heat and pain.

Tumour-supressing gene. Gene whose elimination favours tumour development. When it recovers its function, the phenotype of the transformed cells is reversed.

V

Valproate. Drug with anticonvulsive properties indicated in the treatment of epilepsy.

Vector. Genetic element (plasmid) that can be used to build recombinant DNA molecules for introduction into living cells.

Virus. In biology, a non-cellular entity able to replicate inside specific living cells. They are categorized as either DNA virus or RNA virus, in accordance with the nature of the nucleic acids that make up their genome and, in turn, can be divided into one (single-stranded) or two (double-stranded) chains. The viral cycle includes a reservoir and a vector immune to its effects, and a final host sensitive to its effects. Viruses can give rise to epidemics or become endemic, and their consequences can range from mild to deadly. They are responsible for common epidemics and pandemics of greater or lesser severity (influenza), and others referred to as emerging, which are characterized by their high mortality rate (AIDS, haemorrhagic diseases). One special type, the lentiviruses (for example, the AIDS virus), have incubation periods that can even last years.

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