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Lessons from the Organizer - an interview with Edward M. (Eddy) De Robertis

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ABSTRACT In this interview, we talk with developmental biologist Eddy De Robertis about his wider scientific career and the history of developmental biology in Latin America. We discuss the early days of the homeobox, the discovery of the mechanism of the Spemann-Mangold organizer function in *Xenopus* embryos, and related Evo-Devo. De Robertis reflects on trends of how conducting biological research has changed over the years and he provides advice for young scientists.

KEY WORDS: *Argentina, chordin, developmental biology, goosecoid, homeobox, Spemann organizer*

Il n'y a qu'un animal.

Honoré de Balzac (1799 – 1850)
paraphrasing E. Geoffroy Saint-Hilaire

In the face of the immense reality in which we find ourselves, the old researcher is always seized by the same feeling that already lured the young researcher out into nature, the feeling of a deep reverent wonder.

Hans Spemann (1869 - 1941)

Like his forerunner Hans Spemann, Edward De Robertis, whom everyone calls Eddy, has been driven in his career as developmental biologist by a deep sense of wonder and curiosity. This spurred Eddy's major discoveries in two topics of historic importance in developmental biology, that are associated with the illustrious names of French naturalist Étienne Geoffroy Saint-Hilaire (1772 – 1844) and German zoologist Hans Spemann.

Education in Uruguay and Argentina

Eddy was born in Boston in 1947 to Argentinian parents exiled during the Perón era and had to become a researcher almost by default. His father, Eduardo De Robertis was a famous Argentinian electron microscopist and cell biologist, who co-discovered synaptic vesicles; he also became Eddy's scientific mentor in early childhood. Eddy was three when the family moved to Montevideo, Uruguay. When his parents divorced two years later, Eddy stayed in Uruguay with his mother but saw his father regularly. He showed a keen interest in Biology at high school, which was run by American Methodist missionaries who provided a good education mostly in English. He read scientific journals such as *Scientific American*, where he was to publish himself decades later, and he was given the key to the school lab. With highest expectations from and encouraged by his parents, he studied medicine and in 1971 received a degree and the Gold Medal as the top student from the University of Uruguay's School of Medicine. There, Roberto Narbaitz became his advisor in developmental biology and Eddy at only 21 years of age co-authored two papers with him on chick gonad development. Shortly after graduation, he got married to his wife Ana; they have three children, of whom his son Alex De Robertis became a marine biologist, continuing the biology tradition in the De Robertis family in the third generation. Attesting to the saying that *behind every successful man is a woman*, Ana De Robertis later worked as a secretary in Eddy's lab at UCLA, shielding Eddy from administrative burden for decades, reading every wish off his eyes, and providing a life buoy for foreign postdocs, so that they too, could focus on their research.

For his PhD, Eddy moved to Buenos Aires, Argentina, where he worked with biochemist Héctor N. Torres. His main thesis theme was the mechanism of bacterial growth control by cAMP (De Robertis, 1974). An exercise in signal transduction and gene regulation, these where to become two central topics of Eddy's later research in developmental biology. In addition to biochemistry, Eddy also received important training in cell biology by re-editing the renowned textbook *Cell and Molecular Biology*, which his father had written. Working in a neighboring laboratory was one of

Abbreviations used in this paper: HHMI, Howard Hughes Medical Investigator; LMB, Laboratory of Molecular Biology (Cambridge, UK); UCLA University of California, Los Angeles (USA).

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Eddy's early mentors, Nobel Laureate Luis Federico Leloir. Leloir impressed Eddy with his disciplined schedule, conducting one experiment in the morning and one in the afternoon. It was at the Instituto Leloir, where he met a future Nobel Laureate who would determine his career path: John Gurdon. On his way back from a lecture at the Instituto Leloir, Eddy picked up Gurdon at a bus stop and gave him a ride to his hotel. Impressed by the young talent, Gurdon had the British Embassy inform Leloir that if De Robertis ever wanted training in Britain, there would be a fellowship available for him. Eddy was not told about this prestigious offer until years later, when searching for a postdoctoral position, "so that it would not go to your head", he was told. With Leloir's blessing, Eddy and his family moved to Cambridge in 1975, where he started as a Royal Society fellow in John Gurdon's laboratory at the Medical Research Council (MRC) Laboratory of Molecular Biology (LMB).

LMB Cambridge: reprogramming and nucleocytoplasmic transport

With Max Perutz, Sydney Brenner, Francis Crick, John Kendrew, Aaron Klug, Cesar Milstein, and Fred Sanger, the LMB hosted a Who-is-Who of Nobel Prize winners and was the world's leading research institute in molecular life sciences. When asked about the secret of the institutes' success, Max Perutz, chairman of the LMB said: "*Creativity in science, as in art, cannot be organized. It arises spontaneously from individual talent. Well-run laboratories can foster it, but hierarchical organizations, inflexible bureaucratic rules, and mountains of futile paperwork can kill it. Discoveries cannot be planned, they pop up, like Puck, in unexpected corners.*" (Rhodes, 2002). The canteen on the top floor of the LMB building acted as the central station for meeting people to discuss the latest results and the institute's seminars were an intellectual firework, all of which was highly inspiring for young research fellows like Eddy.

One of his projects was nuclear reprogramming of somatic nuclei to a pluripotent state via oocyte nuclear transplantation. Gurdon had previously pioneered the concept of embryonic reprogramming, which is of paramount importance for developmental biology and the stem cell field, and eventually earned him the Nobel Prize in 2012. Eddy aimed at characterizing this fascinating process molecularly. Using the then-new 2D protein gels, Eddy demonstrated that by injecting kidney cell nuclei from *Xenopus laevis* into oocytes of a newt, *Xenopus* genes normally expressed in the oocyte became reactivated (De Robertis and Gurdon, 1977). Eddy and a co-worker also showed that recombinant DNA could be translated into protein using the frog oocyte (De Robertis and Mertz, 1977).

The central topic, which he was to continue subsequently as a staff member, was nucleocytoplasmic segregation of proteins and RNAs; he wondered how the components of the nucleus and the cytoplasm become segregated and what retains some macromolecules selectively within the nucleus. Since John Gurdon's initial finding that microinjected iodinated histones move into the nucleus, a large variety of nuclear migrating proteins have been found by him and his colleagues. The main conclusion from their research was that essentially all proteins isolated from nuclei have the ability to migrate and accumulate in the nucleus, while cytoplasmic proteins do not (De Robertis *et al*., 1978), paving the way towards identification of nuclear localization signals. He also showed that *Xenopus* oocytes transcribed a cloned yeast tyrosine tRNA gene, correctly spliced the tRNA (De Robertis and Olson, 1979), and that the splicing enzymes resided in the nucleus (De Robertis *et al*., 1981).

Biozentrum Basel: The Homeobox in vertebrates

In 1980, after 6 years at the LMB, Eddy became the youngest professor at the Biozentrum Basel, Switzerland – he was only 33. He continued his research on nucleocytoplasmic transport and trained one of the future leaders in the field, Iain Mattaj. The major questions on the nucleocytoplasmic transport of proteins and RNAs were still unanswered and he was keen to find out more about the chemical nature of the karyophilic signal. Moving from proteins to RNAs, he microinjected ^{32}P-labelled RNA from HeLa cells into the cytoplasm of *Xenopus* oocytes, showing that snRNAs migrate into the cell nucleus, tRNA and 7S RNA remain in the cytoplasm, while 5S RNA becomes concentrated in the nucleolus (De Robertis *et al*., 1982). This study opened a route for the characterization of the selective RNA targeting mechanisms (Zeller *et al*., 1983; Mattaj and De Robertis, 1985).

However, Eddy abandoned this line of research when he and his friend Walter Gehring discovered the first vertebrate homeobox gene. Gehring was a pioneer in *Drosophila* molecular developmental genetics, who came out of the Boveri-Spemann-Baltzer-Hadorn lineage and was deeply familiar with classical embryology. His laboratory had discovered a gene sequence conserved in several *Drosophila* homeotic genes, which regulate anteroposterior cell differentiation. Back then, *Drosophila* was the only organism in which genes that control early embryonic development had been

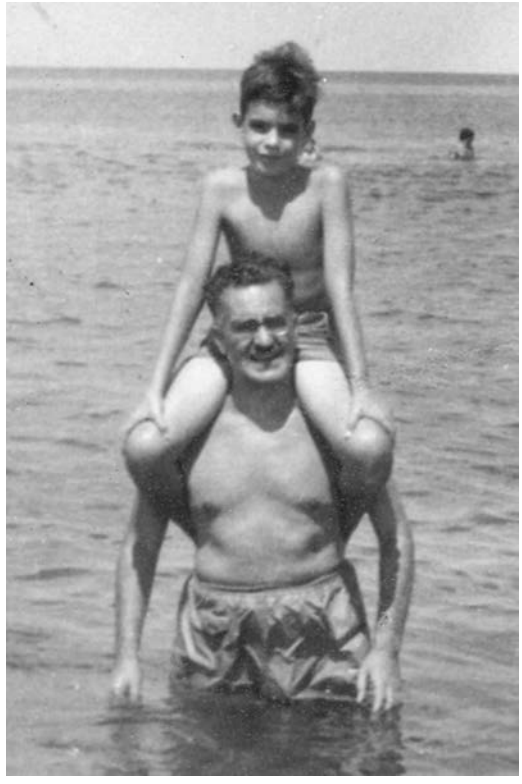

Fig. 1. On the shoulders of giants. *Eduardo D. P. De Robertis and Edward M. F. (Eddy) De Robertis in Playa Pocitos, Montevideo, Uruguay, circa 1957. Photo: Cristina De Robertis.*

Fig. 2. Eddy De Robertis and his mentor Sir John Gurdon. *Summer 2019, Pacific Palisades, California. Photo: Ana De Robertis.*

identified. Eddy and Walter decided to test whether such a gene sequence may exist in another evolutionary clade, the vertebrates. At the time, this was an outrageous idea, given that embryonic development of frogs and flies appear to be so different: Homeotic genes were known to control the anteroposterior identity of fly segments, structures lacking in vertebrates. Andres Carrasco in Eddy's lab isolated HoxC-6 and in a seminal paper, Eddy's lab reported the first development-controlling gene in a vertebrate (Carrasco *et al*., 1984).

While his lab was not involved in the subsequent discovery of the conserved collinearity of *Hox* complexes, he designed in a 1990 Scientific American article (De Robertis *et al*., 1990) the now iconic diagram of the conserved *Hox* complexes between *Drosophila* and mouse embryos, which can be found in most textbooks. The discovery of the conserved homeobox and the realization that many more developmental regulators and gene regulatory networks are evolutionary conserved eventually gave rise to the sub-field of Evo-Devo.

UCLA: The molecular nature of the Spemann-Mangold organizer

In 1985, Eddy became the Norman Sprague Professor of Biological Chemistry at the University of California, Los Angeles (UCLA), School of Medicine. He now focussed completely on homeobox genes, considered as the Rosetta stone of developmental biology. His laboratory discovered additional homeobox genes and characterized their role in frog and mouse development in a series of papers. Among these studies, two years before the first mouse *Hox* knockout mice were reported (Chisaka and Capecchi, 1991; Lufkin *et al*., 1991), Eddy's lab published the first evidence for the requirement of a *Hox* gene in vertebrate anteroposterior development using microinjected neutralizing antibodies (Wright *et al*., 1989).

A momentous discovery in Eddy's lab was the first molecular regulator of the Spemann-Mangold organizer, the homeobox gene *goosecoid* (Cho *et al*., 1991), which catapulted the decades-old organizer embryology into the modern era. Hans Spemann was a German developmental biologist from the Boveri school who after World War I carried out systematic transplantation experiments between salamander embryos to establish when during development the anlagen for certain organs become committed to their final fate. If the anlagen were transplanted sufficiently early, they would incorporate smoothly into the new host environment differentiating according to their new neighborhood. Later during development, the transplanted anlagen became committed and differentiated according to their original fate. One of these many experiments became historic, the transplantation of the dorsal blastopore lip from a gastrula stage embryo to the ventral side of a host embryo, fated for epidermis. This transplantation led to the twinning of the host body axis, where a small central nervous system with underlying notochord and somites had formed (Spemann and Mangold, 1924). It became clear that the dorsal lip had instructed the surrounding tissue to take on a different fate and to orchestrate proper axial patterning. Few experiments in biology had such enormous repercussions as this transplantation and Spemann consequently received the Nobel Prize for his discovery of the organizer and embryonic induction. Generations of developmental biologists have tried to identify the chemical nature of the organizer inducers but given the small quantities of embryonic material and the lack of modern protein biochemistry techniques no specific factors were identified. Worse, since unspecific reagents such as methylene blue and even sand particles could induce neural tissue, the Spemann-Mangold organizer fell in disrespect. When Eddy was a student in the 1970s, it was said that "Spemann's organizer set developmental biology back by 50 years."

The spectacular discovery of *goosecoid* (*gsc*) by Eddy's lab resuscitated Spemann's work. His postdocs Ken Cho and Bruce Blumberg had isolated *gsc* while screening a *Xenopus* dorsal lip cDNA library with a homeobox probe. This author was present when the first *in situ* wholemount hybridization of *gsc* was carried out successfully by the late Herbert Steinbeisser. The organizer was the holy grail of developmental biology of sorts and hence all lab members gathered around the microscope while the staining reaction went on, electrified to see for the first time the organizer lighting up using a molecular probe. Suddenly the dorsal blastopore lip showed an ever so faint blue staining and we felt that we were witnessing something of historic importance. If that was not enough, when Ken Cho later microinjected full length *gsc* mRNA into the ventral side of early frog embryos he could induce twinning of the embryonic axes, recapitulating the Spemann transplantation, indicating that the gene plays a central role in executing Spemann's organizer phenomenon (Cho *et al*., 1991). When Ken showed these embryos to Eddy, he was overjoyed and embraced Ken enthusiastically. The cloning and analysis of the *gsc* homologues in mice and chick allowed for the first time to identify the homologous structures in these species (Blum *et al*., 1992; Izpisúa-Belmonte *et al*., 1993), a conceptual milestone in Evo-Devo.

The *gsc* breakthrough spurred the discovery in rapid succession

Fig. 3. Eddy De Robertis and his friend and collaborator Walter Gehring. *Sydney 2005, at the ISDB Congress. Photo: Ana De Robertis*

of other molecules mediating organizer function and over the next 10 years the Spemann-Mangold organizer emerged like a phoenix from the ashes. But *gsc* was not a secreted factor and left unanswered the molecular nature of the inducers emitted from the organizer. In a series of seminal papers, Eddy and his lab discovered the molecular mechanism of embryonic induction along the D-V axis in the *Xenopus* embryo. His group found that tissue differentiations are regulated by secreted antagonists of growth factors - such as Chordin, Frzb, Crescent, Cerberus, and others - that prevent their binding to cell surface receptors (Sasai *et al*., 1994; Leyns *et al*., 1997; Bouwmeester *et al*., 1996). The most important gene was Chordin, which provided a new paradigm for understanding cell-cell signaling over long distances through the establishment of a morphogen gradient. Chordin is a Bone Morphogenetic Protein (BMP) antagonist expressed in the dorsal side (Piccolo *et al*., 1996). Independently, Richard Harland reported that another Spemann-Mangold organizer inducer, Noggin, also encodes a BMP antagonist (Zimmerman *et al*., 1996). What prepared these discoveries was the key observation by the Melton and Ueno labs that BMP signaling antagonizes dorsal mesoderm formation and that experimental inhibition of BMP signaling induces Siamese twins in *Xenopus* embryos (Graff *et al*., 1994; Suzuki *et al*., 1994).

Eddy's lab went on to unravel the regulatory network of BMP signaling in early embryos. Chordin levels are regulated by the activity of a Tolloid metalloproteinase (Piccolo *et al*., 1997) and a protease inhibitor (Sizzled) secreted by the ventral pole of the embryo (Lee *et al*., 2006). More recently, Eddy was able to visualize the Chordin morphogenetic gradient in the extracellular matrix that separates the ectodermal and endomesodermal germ layers (Plouhinec *et al*., 2013). This long-range facilitated diffusion is driven by the proteolysis of Chordin in the ventral side of the embryo. The Chordin/Tolloid/BMP biochemical pathway, together with the discovery of Hox genes in vertebrates, had profound implications for Evo-Devo because these gene networks are ancestral to all bilateral animals (De Robertis and Sasai, 1996; De Robertis, 2008; Bier and De Robertis, 2015).

Much like Eddy resuscitated Spemann, he also reanimated French anatomist Geoffroy Saint-Hilaire. Through his work in comparative anatomy, Geoffroy Saint-Hilaire recognized an underlying unity of organismal design pervading the animal kingdom. Decades before Darwin, Geoffroy followed Lamarck's proposition that species are not static but can "transmutate" in time. Geoffroy's claims were in sharp contrast to the prevailing theory at the époque, notably of French naturalist and zoologist Georges Cuvier: Species were static in time and created by the almighty in a fashion unconstrained by apparent design principles. One of Geoffroy's famous examples for the unity of animal anatomic design was his bold claim of the similarity between the D-V axis of the lobster and mammals. "*What was my surprise, and I add, my admiration, in perceiving an ordering that placed under my eyes all the organic systems of this lobster in the order in which they are arranged in mammals*" (De Robertis and Sasai, 1996). A historic debate between Geoffroy and Cuvier went on for two months at the French Academy of Sciences (Appel, 1987), which was closely followed by intellectuals not only in Paris but also in Europe, including Alexander von Humboldt and Goethe. The great Balzac supported Geoffroy's idea of unity of biological design and famously wrote in his foreword to La comédie humaine: *Il n'y a qu'un animal* (There is but one animal).

In the post-Darwin era this idea was formulated as the axis inversion hypothesis by German zoologist Anton Dohrn (Dohrn, 1875). He proposed that deuterostome and protostomes evolved from a common ancestor, which possessed a body plan similar to that of modern annelids with a ventral nerve cord and dorsal heart. During evolution towards chordate deuterostomes an axis inversion occurred, which placed the nerve cord dorsally and the heart ventrally. The discovery of *chordin* and its fly homolog short gastrulation (*sog*) (Francois *et al*., 1994) provided strong molecular support for the largely forgotten axis inversion theory. It became clear that the deuterostomian frog and the protostomian fly share an ancient Chordin-BMP signaling regulatory network, which patterns their D-V axis, but whose polarity is inverted with respect to each other. The evolutionary conservation of the chordin-BMP antagonism and inversion of its polarity supported their origin from a common bilaterian ancestor of invertebrates and vertebrates, which Eddy and the late Yoshiki Sasai in an influential article termed 'Urbilateria' (De Robertis and Sasai, 1996).

Howard Hughes Medical Investigator: BMP and Wnt signaling

While at UCLA, Eddy was appointed Howard Hughes Medical Investigator, allowing him to move to a modern building and giving him greater freedom to pursue his research. Eddy became increasingly interested in the molecular mechanisms regulating BMP signaling in development and he discovered how ADMP and BMPs at opposite embryonic poles of the embryo generate a self-regulating morphogen field (Reversade and De Robertis, 2005) and that secreted Frizzled-related proteins act as inhibitors of Tolloid proteinases (Lee *et al*., 2006). He discovered an important cross-talk between Wnt and BMP signaling whereby Wnt/GSK3 signaling regulates the duration of the BMP/Smad1 signal (Fuentealba *et al*., 2007). This discovery led him to focus on Wnt signaling, a highly complex pathway with great relevance not only for development but also for cancer.

At the heart of canonical Wnt signaling is the transcription factor β-catenin, which is rapidly targeted for degradation by phosphoryla-

tion by Glycogen Synthase Kinase 3 (GSK3). Wnt signaling inhibits GSK3 and β-catenin becomes dephosphorylated and stabilized and enters the nucleus to regulate Wnt target gene transcription. Exactly how Wnt signaling suppresses GSK3 activity remains one of the outstanding questions in Wnt signaling. Eddy's lab surprised the field by showing that Wnt receptor trafficking sequesters GSK3 in multivesicular bodies. Even more surprisingly, this "imprisoning" of GSK3 protects many other protein substrates from phosphorylation besides β-catenin (Taelman *et al*., 2010), thought to be the only essential downstream mediator of Wnt signals. This study led to the formulation of Wnt/STOP signaling as a new branch downstream of the Wnt/GSK3 pathway (Acebron *et al*., 2014). Eddy realized that Wnt signaling functions as a general regulator of cellular endocytosis and protein stability (Albrecht *et al*., 2018; Albrecht *et al*., 2019). Eddy's and another lab discovered that the Wnt pathway activates macropinocytosis, an elementary cell biological process important for growth of normal and malignant cells (Redelman-Sidi *et al*., 2018; Tejeda-Muñoz *et al*., 2019). These recent discoveries highlight Eddy's profound training not only as developmental- but also as a cell biologist. His encyclopedic knowledge and keen interest in cell biology were certainly triggered by co-authoring as a young man with his father the famous De Robertis & De Robertis textbook on Cell Biology.

Looking back

Eddy's recent findings that there is much more to canonical Wnt signaling in mammalian cells than β-catenin provide another example of his scientific creativity and great intuition for groundbreaking discoveries that are so characteristic for him. At an age where others retire, Eddy continues to make landmark discoveries.

Having early on realized that the big questions in biology stay the same, just the methods are changing, Eddy has cultivated a rare interest for scientific history. He avidly studies old scientific literature, also in French and German, and he has developed an acute sense for - and quotes forgotten pioneers. Eddy is a pioneer himself in the profound realization of modern developmental and evolutionary biology that the molecular mechanisms of dorso-ventral and anteroposterior cell differentiation are common to all animal embryos. Likewise, Eddy is a pioneer in solving a holy grail of developmental biology by elucidating that a self-organizing morphogen gradient underlies embryonic induction by the Spemann-Mangold organizer. Thus, Eddy De Robertis can be considered the modern-day successor of both Hans Spemann and Geoffroy Saint-Hilaire.

Fig. 4. Eddy De Robertis and Christof Niehrs in the jacuzzi overlooking the Pacific Ocean at the De Robertis home. *This warm bath has been honored by the presence of great developmental biologists such as Nicole Le Douarin, Walter Gehring, Herbert Jäckle, Antonio Garcia-Bellido, Yoshiki Sasai, Stefano Piccolo, Denis Duboule and many others. Photo: Ana De Robertis*

Eddy has had a spectacular career and at each promotion step of his career, he was precocious. He and his laboratory have published more than 200 papers, among them a large number in leading journals. Not all of these contributions could be adequately described in this article. Eddy has received important recognitions, such as honorary doctorates from the Sorbonne, Paris, and his *alma mater* Universidad de la República, Uruguay, the Medal of the Collège de France, a MERIT Award of the National Institutes of Health, and the Ross Harrison Prize in Developmental Biology. He has presented innumerable keynote lectures. Eddy De Robertis is a member of the Pontifical Academy of Sciences of The Vatican, EMBO, the National Academy of Sciences, the American Academy of Arts and Sciences and the European Molecular Biology Organization. He is also a corresponding member of the Latin American Academy of Sciences, the Buenos Aires National Academy of Sciences and the Academy of Sciences of Uruguay. De Robertis received *honoris causa* doctorates from the Universités Sorbonne and his *alma mater* the University of the Republic of Uruguay. From 2002 to 2006 he was President of the International Society of Developmental Biologists. He received the Ross Harrison prize in developmental biology, the Society for Developmental Biology (USA) Lifetime Achievement Award, and the Kowalevsky Medal in Evolution and Development.

Beyond his own research, Eddy has been generous in his service to the organizations that contribute to scientific progress. As President of the International Society of Developmental Biologists (2002-2006), his unremitting efforts revitalized international exchanges in his field and guided the creation of new scientific societies such as the Asian-Pacific Developmental Biology Network and the Latin American Society of Developmental Biologists. He trained many distinguished scientists that have influenced life science, such as Juan Carlos Ispizua Belmonte, Iain Mattaj, Stefano Piccolo, Yoshiki Sasai, and Rolf Zeller.

The following interview was held with Eddy De Robertis by telecommunication via Skype in the summer of 2019 and provides a firsthand account of his scientific career and his connection to Latin American developmental biology.

Your father Eduardo De Robertis was a renowned South American electron microscopist and cell biologist. Having a famous father in your own profession can be both, a great help but also a burden. How was it for you and what do you owe him as a scientific teacher?

Indeed, my father was very famous. He discovered synaptic vesicles together with Stanley Bennett in 1955 and correctly predicted their function. I never considered it a burden but rather a great advantage. Since I was a child, it was assumed at home that I would study medicine and would get the Gold Medal to the top student just like my father had done before me. I always

Fig. 5. The De Robertis postdoctoral machine at the *Xenopus* meeting in the Mosel valley, Germany, 2008. *Top row from left to right: Herbert Steinbeisser, Yoshiki Sasai, Juan Larraín, Christof Niehrs, Eddy De Robertis, Oliver Wessely, Stefano Piccolo and Martin Blum. Front row: Edgar Pera, Vincent Taelman, Abraham Fainsod and Veronica Sander. Photo: Herbert Steinbeisser.*

seeked to please and was not much of a rebel. However, I must tell you that getting that Gold Medal was one of the most difficult things to achieve in my life. My parents divorced when I was five and my father moved to Argentina after the fall of General Perón in 1957. I remained in Montevideo, Uruguay with my mother who was a well-known poet in those parts, and who provided a very nurturing home for my sister and me. Growing up in Montevideo in the 1950s was idyllic as Uruguay was then a rich country with strong cultural traditions. Although there were at times economic difficulties in our household, I was a very lucky and happy child. I remember doing my homework on the console of an electron microscope with strict instructions not to touch any of the very appealing knobs. Another moment I remember is walking along the beach in Montevideo and my father explaining that the pH was the negative logarithm of the concentration of Hydrogen ions. I was probably less than 9 years old and I remember asking what was a logarithm, while admiring a nearby statue of the Venus de Milo that seemed more interesting at the time.

My Dad always promoted my career. When I was a first year medical student, he took me on one of his seminar tours throughout the United States. At Vanderbilt his friend Earl Sutherland, who got the Nobel Prize for the discovery of cyclic AMP, recommended all the textbooks I should read during my medical studies. I ended up studying two sets of textbooks, one in Spanish and one in English. I should say that we had a great medical school in Montevideo, a six-year course in the French tradition. In 1969, the year of the moon landing, I had the privilege of spending the whole summer at Woods Hole Marine Biological Laboratory working in a lab of a friend of my Dad's purifying the Golgi complex of the endocrine pancreas of the goosefish. The project was a complete failure but brought me into the most exciting environment imaginable for a biologist. When I was close to completing my Ph.D. my father was writing a new edition of his Cell Biology textbook, which he had been writing since a year before my birth. I wrote him two chapters on gene regulation. When the book came out, I was shocked to find I was included as a co-author. This bit of nepotism was very useful in my life. In later editions, I invested enormous amounts of work in this book, which was widely used in Latin America and the world. In my case, it also gave me a small but critical income when my family, one wife and three children, were on a very tight budget during postdoctoral studies in Cambridge, England. Not having to take difficult decisions about career paths proved a great plus in my life.

What was the state of developmental biology in Latin America during your PhD student days and who were the key figures in cell & developmental biology?

At that time in Latin America, there were very few schools of science at universities. If you wanted to study biology, you went into medicine. If physics, engineering and if chemistry, pharmacy. Fortunately, every Faculty of Medicine had a department of Histology and Embryology. Developmental Biology was very important because it was an experimental, less descriptive science. A key event in my development was when an anti-democratic military coup by General Onganía expelled many scientists from Argentinian universities in 1966. One of them was embryologist Dr. Roberto Narbaitz who went into exile to Uruguay. Argentina and Uruguay have this interesting relationship such that when times are difficult in one country, people are welcomed in the other. I was an assistant of histology, and my Professor put me to work under Narbaitz. We worked on the steroid-producing cells of the developing chick ovary and experimentally induced intersex gonads. We managed to publish two papers in international journals, which was very rare at the time from the Faculty of Medicine of the Universidad de la República del Uruguay (Narbaitz and De Robertis, 1968, 1970). There was a lot of cell biology in Latin

America, and I trained while in high school during the summers with cytogeneticist Francisco Sáez working on *Chironomus* salivary gland giant chromosomes. Many of the students of Cajal migrated to South America in the wake of the Spanish Civil War, greatly enriching the subcontinent. One famous disciple was Pío del Rio Ortega, who emigrated to Argentina, forming many neuropathologists.

Related to the last question, sometimes countries develop specialty themes in developmental biology, such as the Finnish school focusing on kidney development or the Soviet school focusing on morphogenesis. Are there, or have there been, Latin American schools that focused on certain themes?

In terms of a school of developmental biology, I would say that the most important example is the editor of this IJDB volume, Dr. Eugenia del Pino. She has trained many students in Ecuador studying the multinucleated giant oocytes of tropical frogs. There are other groups that blossomed in the rich environment of Iberoamerican culture. In Argentina important schools were established around Bernardo Houssay in physiology and Luis F. Leloir in biochemistry. Notably, both earned Nobel prizes for work carried out entirely in Buenos Aires, and share the distinction of having been expelled from the University of Buenos Aires by General Perón in the great purge of 1946. In other disciplines, neurophysiology had enormous impact from Chile with ion channel studies on the giant axon of the von Humboldt squid, centering at the marine station in Montemar. In physiology, Arturo Rosenblueth created an important school of cardiology in Mexico, and Roberto Caldeyro-Barcia one of perinatology in Uruguay.

In this context, what would be your advice to Latin American granting agencies and Academies how to best serve the advancement of developmental biology in their nations with their limited resources?

The most critical problem is to facilitate the creation of new independent laboratories for young investigators trained in Europe and the USA. In this, independence is the key word for much too often young postdocs must return to their laboratories of origin, neutering creativity. The most important factor is to have truly open calls for applicants at all universities and research institutes with set-up funds to start a new lab. Chile has been a leader in implementing this policy in their universities and it has made a world of difference. Another important factor is to ensure open grant peer review procedures, with the most successful example being the NIH. Many Ibero-American countries have established councils of science and technology and this is a great progress. Competitive review also might be achieved by involving international institutions such as Mercosur. One big regret is that the Organization of American States (OAS) does essentially nothing for science in the subcontinent, beyond having annual meetings of science ministers. In the past, there were OAS fellowships for exchanges of graduate students between countries, which unfortunately have been phased out. Some countries such as Mexico have excellent programs for short-term graduate student exchanges at a horizontal level between Latin American countries. Although most students choose to remain in Mexico, at least they have a good framework in place.

A program I have been involved in for the past 26 years is the PEW Latin American Postdoctoral Fellows Program from the PEW Charitable Trusts. They offer 10 fellowships per year for studies in the US and U$D 70,000 to set up an independent laboratory upon return to Latin America. This program has had an enormous impact and we how have about 160 independent labs seeded throughout Latin America that keep networked together. By reviewing many applications during many years, I have seen enormous progress in the quality of human capital in Latin America. One of the elements in this improvement, I think, is that many graduate students apply for short stays in laboratories in Europe and the USA during their Ph.D. studies. Many laboratories apply for joint projects with the European Union. In this respect, EMBO has been fantastic and I expect the new agreement between EMBO and Chile will yield enormous fruits. Hopefully, the recently signed trade agreement between the European Union and Mercosur will further open up international possibilities. Spain may have a larger role to play, in particular now that the President of the International Society of Developmental Biologists is Angela Nieto from Alicante.

You asked about Academies of Science. I am a strong supporter of these institutions for they are created by scientists to promote scientific progress independently of governments. We do have a Latin American Academy of Sciences (ACAL) which is active despite being located in Venezuela. The resilience of Iberoamerican peoples is astonishing. Two years ago I had the privilege of organizing a meeting between the Pontifical Academy of Sciences and ACAL at the Vatican. We brought together the top 25 Latin American scientists in the field of Cell Biology and Genetics. There is a lot of excellent science going on. A book of proceedings was published including many suggested policies for improving science in Latin America. The book is available in print and at the ACAL, Vatican, and LASDB websites. However, convincing the OAS or National Academies to apply some of these suggestions is a very difficult task. Yet we are trying and hope springs eternal.

During your 50 years in science you have performed pioneering work on a remarkably broad variety of themes, e.g. transcription, nucleo-cytoplasmic traffic of noncoding RNAs, the role of Homeobox proteins, the Spemann-Mangold organizer, BMP signaling, and Wnt signaling. You also published twice a review article in Scientific American, an honor reserved to authors who have greatly advanced a field of research. What motivated the topic changes?

In science, questions require the appropriate technologies to be answered. I studied *E. coli* RNA polymerase enzyme kinetics with Héctor N. Torres at the Leloir Institute in Buenos Aires for my Ph.D thesis (De Robertis, 1974). During my postdoc with Sir John Gurdon, the best mentor anybody could hope for, I used the newly developed O'Farrell 2-dimensional gels to study translation (De Robertis *et al*., 1978). With Janet Mertz we were able to demonstrate, for the first time, transcription followed by translation of a cloned gene using the oocyte as a living test tube (De Robertis and Mertz, 1977). This was my first *Cell* paper and one interesting bit is that John Gurdon performed all the oocyte DNA microinjections, yet he was only thanked in the acknowledgements! After three years as a postdoc, I became Staff Scientist at the renowned Medical Research Council Laboratory of Molecular Biology in Cambridge. The MRC-LMB in the 1970s was a wonderful place with luminaries such as Sanger, Crick, Perutz, Klug, Milstein, Brenner and Gurdon doing some of their best work during the exciting period

of my training. To become a bit more independent from Gurdon, I microinjected proteins into the cytoplasm of oocytes and by manually isolating the nucleus and cytoplasm could follow the transport of proteins into the nucleus. This experimental approach had been pioneered by John Gurdon, but no one else was studying it at the time. Using 2-D gels I could show that essentially all nuclear proteins contained in their mature sequence a nuclear localization signal. Once appointed Full Professor (Ordinarius) of Cell Biology at the Biozentrum in Basel, Switzerland, at the tender age of 33, I switched to the study of migration of radiolabeled RNAs into the oocyte nucleus. That was to change completely with the discovery of the vertebrate Homeobox in 1984.

My late colleague on the second floor was Walter Gehring, the famous *Drosophila* developmental biologist. His group had cloned *Antennapedia*, a homeotic gene that they found contained a conserved segment called the Homeobox. We had common seminars, and it is fair to say that I completed my education in developmental biology with Walter as a colleague. On a memorable day, Richard Garber presented a seminar showing specific expression of *Antennapedia* mRNA specifically in the CNS of the second thoracic segment. I immediately suggested that this would be some type of neuropeptide instructing other cells of their position in the body. Walter explained that this could not be because *Antennapedia* was a cell autonomous gene. Fortunately, I did not know what cell autonomous meant, and followed Walter into his office and said, "let's do the experiment anyway because we have genomic libraries of *Xenopus laevis* DNA already plated and ready to go". Iain Mattaj and Rolf Zeller were cloning snRNA genes for our nuclear transport studies. My postdoc Andrés Carrasco, who was Argentinian and later established a lab in his home country, on the first try isolated two phages that hybridized with *Antennapedia* and also with two other homeobox probes isolated by Bill McGinnis in Walter's lab. Carrasco sequenced one phage, now called Hox C6, and I the other. My sequence was correct, but since we did not have computers at the time, I missed the homology with *Antennapedia*. A few years later Christopher Wright revisited the sequence and it revealed a now famous gene called *Pdx-1*, a master regulator of pancreatic and duodenal development. The homeobox in vertebrates was a truly important discovery. In our paper (Carrasco *et al*., 1984) I ended the abstract saying: "This gene could perhaps represent the first development-controlling gene identified in vertebrates". And it proved true. Statements of priority are considered poor form in scientific papers, but in this case, I am so glad I did it and that Benjamin Lewin, the editor of Cell that was to publish so much of our later work, let it slide by. At that time, we all believed that the development of a fruit fly and of a vertebrate would be completely different. This discovery opened up a new era in developmental biology. I immediately stopped all work on oocytes and dedicated my lab purely to the developing embryo. These findings also resulted in a call to an Endowed Chair at UCLA, where I have been since 1985, living in the same house overlooking the Pacific Ocean.

Fig. 6. Eddy De Robertis being inducted into the Pontifical Academy of Sciences by Pope Benedict XVI in 2010 at the Vatican. *In the background Bishop-Chancellor Marcelo Sánchez Sorondo. De Robertis attends every biennial plenary meeting of this very stimulating Academy. Photo: Foto Vaticana.*

As the techniques of molecular cloning became practical, Bruce Blumberg and Ken Cho in our lab at UCLA generated cDNA libraries from which they isolated the first Spemann-Mangold organizer-specific gene, *goosecoid*. I distincly remember the day Herbert Steinbeisser developed an *in situ* hybridization that overlapped exactly with the position of the Spemann-Mangold organizer. Previously, the organizer was defined by its inductive properties after transplantation. We now had a marker that defined the existence of the organizer as a real molecular structure. This was followed by the cloning of Chordin by Yoshiki Sasai in 1994 (Sasai *et al*., 1994). Chordin opened up for us the study of morphogen gradients. It was shortly thereafter shown by Ethan Bier at UCSD, and independently by Chip Ferguson at the University of Chicago, that Chordin had a *Drosophila* homologue called *short gastrulation* (*sog*). Chordin induced the neural plate on the dorsal side of the frog and Sog induced CNS on the ventral side of the fruit fly. We had a lot of fun reviving the old theory of Etienne Geoffroy Saint-Hilaire that a unity of the body plan existed among animals, and that this plan had been inverted between vertebrates and arthropods. With Sasai we wrote in 1996 an influential review in Nature proposing that the common ancestor between the protostomes and the deuterostomes had been a genetically complex sea bottom dwelling organism that we named *Urbilateria* (De Robertis and Sasai, 1996). The discoveries of the conservation of Hox genes and Chordin/Sog initiated the young science of Evo-Devo. The passing of Yoshiki Sasai, and that of Herbert Steinbeisser, at a young age was a great loss to our field of Developmental Biology.

Stefano Piccolo purified Chordin protein and showed that it directly bound to BMP, antagonizing its activity (Piccolo *et al*., 1996). He also discovered that inhibition by Chordin could be reversed by a metalloproteinase called Tolloid/Xolloid that cleaves Chordin at specific sites, releasing BMP for signaling through its receptors (Piccolo *et al*., 1997). Chordin protein diffuses from the dorsal side to the ventral pole within a narrow region of extracellular matrix that separates ectoderm from endomesoderm in the gastrula. On the ventral side, high BMP signaling leads to the expression of Sizzled, a protein that we showed was dedicated to the competitive inhibition of Tolloid. The BMP/Chordin/Tolloid/Sizzled regulatory system represents probably the best understood self-organizing morphogen gradient. We cloned many other genes from the organizer, such as *Cerberus* and *Frzb*. Many were Wnt antagonists, leading to our interest in the integration between the BMP and Wnt pathways at the level of phosphorylations of the Smad transcription factors (Leyns *et al*., 1997; Piccolo *et al*.,

Fig. 7. Eddy De Robertis at work in his laboratory at the University of California, Los Angeles (UCLA) in 2009. *Photo: Ana De Robertis.*

1999). We are currently returning to cell biology, having found that canonical Wnt triggers macropinocytosis and major endolysosomal rearrangements (Tejeda-Muñoz *et al*., 2019).

As for the Scientific American articles, the first one retold my postdoctoral work with John Gurdon, with whom we had found that *Xenopus* kidney nuclei transplanted into salamander oocytes were reprogrammed to express oocyte proteins in the absence of DNA replication (De Robertis and Gurdon, 1979). This was one of the first studies analyzing nuclear reprogramming at the molecular level. For the second Scientific American article, I contacted the same editor because I felt our discovery of the vertebrate homeobox needed emphasizing (De Robertis *et al*., 1990). This was a good thing, because parts of this article were later picked up by the great Steven Jay Gould in the chapter on Evo-Devo of his treatise on Evolution. Scientific American was of great importance in my life. A close childhood friend was the son of Baptist missionaries to Uruguay and returned to the USA. As a gift, he sent me a subscription to Scientific American. I was only 11 years old. I understood a tiny fraction of what the articles said, yet my father renewed the subscription until I finished medical school. I was a lucky boy.

The one constant throughout your career seems to have been the frog *Xenopus laevis* as model system. What explains your continued usage of *Xenopus*?

The *Xenopus* embryo is a fantastic material to study development in a vertebrate. John Gurdon had the brilliant insight that it was possible to use the oocyte as a living test tube to study the biological effects of purified microinjected macromolecules. If you are interested in dorso-ventral patterning, as I am, the *Xenopus* embryo is indispensable. Right from the first cleavage division a less pigmented dorsal crescent marks the future organizer region as result of a rotation of the egg cortex along microtubules. This dorsal crescent has fascinated embryologists since the end of the 19th century. From a practical point of view, we can accurately target macroinjections or transplantations to the dorsal or ventral sides all through development. This predictable cleavage pattern is also very useful for lineage tracing experiments in which blastomeres change fates, such as you yourself showed in the case of microinjected homeobox mRNAs as a postdoc in our lab. Recently, we completed an analysis of twinning resulting from bisecting blastula embryos along the sagittal midline (Moriyama and De Robertis, 2018). Formation of twins has fascinated embryologists since the time of Hans Driesch, Spemann and Thomas Morgan. It turns out that upon bisection the BMP/Chordin/Sizzled morphogen gradient is rotated by 90 degrees, regenerating the missing half of the embryo. You could never do such experiments without knowing the location of the dorsal side.

Unlike many of your peers who have switched to other model systems, you have remained faithful to the frog. Where will the frog continue to be useful as a model system in the future?

There is a famous quote from Jean Rostand: "*Les théories passent, la grenouille reste*". Yoshio Masui and Marc Kirschner pioneered the use of *Xenopus* egg extracts to study the cell cycle and many other processes (Lohka and Masui, 1983; Murray and Kirschner, 1989). These extracts are proving invaluable to determine macromolecular complexes by proteomics. It is not that I have not tried being unfaithful. At times, I worked on the mouse and even on *Drosophila*, and found that outsiders are not warmly embraced. Each field has its own evolving technical requirements and reviewers are unforgiving. Most of my original ideas arise while I am manipulating frog embryos, trying to imagine how this amazing machine might signal between cells during the early stages of development. Now that I work on the stimulation of pinocytosis by canonical Wnt, I must confess I sometimes lament that *Xenopus* embryos are not transparent. By now, my relationship with the *Xenopus* embryo is so deep that if I abandon it, I fear the revenge of the embryo.

You are a pioneer in the discovery of the molecular mechanisms underlying the Spemann-Mangold organizer. For years, the Spemann-Mangold organizer field had a bad name. What exactly was the state of research into the Spemann-Mangold organizer when you entered the field? What changed the perception?

It was common in the 70s and 80s for our teachers to say that "Spemann set back developmental biology by 50 years". He had discovered that the dorsal lip of the gastrula had this amazing property of inducing both neural tissue and dorsal mesoderm such as somites and kidney in neighboring cells. By the time he received his Nobel Prize in 1935, a large number of biochemists, particularly in Cambridge, England, set out to purify the inducing substance. They chose to use salamander ectodermal animal cap explants, instead of whole embryos, and scored a substance as positive if it induced neural tissue rather than epidermis. They found that many substances, natural and artificial, could induce CNS. Even sand particles could induce neural in this assay. Finally, Lester Barth and Johannes Holtfreter showed that CNS could form in the absence of any inducer at all. Holtfreter mentioned that if the animal cap explant was cultured attached to glass it would form CNS at high frequency. Decades later, Cecilia Hurtado and I confirmed neural induction in the absence of organizer, and found that neural induction in the American salamander *Ambystoma maculatum* was due

to the activation of MAPK in these culture conditions (Hurtado and De Robertis, 2007). CNS organoids containing an eye and olfactory placodes connected by nerves to a small brain could be formed just by cultivating ectoderm explants attached to glass. Spemann had retired and could not respond to the attacks that the organizer was a non-specific experiment. What was unspecific was the use of salamander animal caps, ignoring the fact that organizer also induced dorsal mesoderm. In the basement of the Biozentrum I found a video tape of a teaching assistant performing the Spemann dorsal lip transplantation experiment. We repeated the experiment in our practical course for graduate students. Even today, I always show a movie of the Spemann graft in my seminars, having learned the procedure from a movie.

What really changed the field was an electrifying short book of memoirs about the Spemann laboratory by Viktor Hamburger published in 1988 (Hamburger, 1988). He was a very good friend of Hilde Mangold and I think the book might have been written to honor her memory. I gave a seminar for our "Embryology Club" on Hamburger's and Spemann's books. Other labs were also inspired by Hamburger's monograph; he was 88 when he wrote this, showing that it is never too late in science. The cloning of *goosecoid* demonstrated that the Spemann-Mangold organizer was real. The subsequent isolation of secreted factors such as Noggin by Richard Harland, Chordin by Sasai, and Dickkopf 1 by Christof Niehrs established that one of the main functions of the Spemann-Mangold organizer was to secrete growth factor antagonists that generate morphogen gradients.

Where has the stem cell field benefitted from your work on the Spemann-Mangold organizer?

The main benefit they received was through my training of Yoshiki Sasai in embryology. His pioneering work showed that one could generate mouse and human eyes, brains and pituitary glands out of embryonic stem cells. The field of organoids he initiated is the most exciting aspect of modern stem cell research. The factors that govern stem cell differentiation, such as Wnt, BMP and FGF are the same ones used by the embryo. For years, I was very worried that the field of stem cell biology was growing separately from developmental biology while in reality they were the same discipline. Only recently, with the amazing progress with organoids, I came to realize that it is all part of the same circle of biological progress. Initially, there were cytologists studying chromosome behavior, such as Theodor Boveri and E. B. Wilson. They were followed by histologists studying tissue organization, such as Camillo Golgi and Santiago Ramón y Cajal. Then came the experimental embryologists such as Hans Spemann and Nicole Le Douarin. Then there were stem cell biologists such as Martin Evans and Shinya Yamanaka. Recently, we had the organoid makers, such as Yoshiki Sasai and Hans Clevers. With the organoids, we are returning to the beginning, discovering new cell types in tissues by single cell sequencing that were not even imagined by histologists. Thus, it is all part of the one circle of life.

Have we answered all major questions surrounding the Spemann-Mangold organizer?

I do not think so. Progress comes when new technologies are developed. Gene cloning was the great equalizer that allowed the molecular dissection of the Spemann-Mangold organizer phenomenon. As new techniques arise, new questions follow. Metabolism and endolysosomal trafficking are two examples of understudied aspects. The most unappreciated aspect is, I think, the role of the ventral side of the embryo, which you, Christof Niehrs, pioneered with the cloning of the *Xvent* genes in your independent lab upon return to Germany. For every action of the organizer, there is a reaction on the ventral side. In the BMP/Chordin gradient, the most interesting patterning genes are those transcriptionally induced at the extreme ends of the gradient. I think that for many other signaling systems the same rules will apply. With Juan Aréchaga we published a large volume of the *Int. J. Dev. Biol.* on the Spemann-Mangold organizer 75 years on. For the 100th anniversary, Wolfgang Driever and Martin Blum are organizing a meeting in 2024 in Freiburg and I expect many new findings will continue to emerge from this incredibly fertile experiment in embryology.

Few modern scholars have studied the work of Hans Spemann as intensely as you did. What impressed you most about him?

The principles of embryonic induction were worked out during short seasons in which the embryos of *Triturus* were available. During the rest of the year, they analyzed the embryos and wrote extremely detailed papers on topics such as lens induction, twin formation and induction by organizer. Year after year steady progress was done and recorded meticulously. The entire Spemann and Mangold 1924 paper (Spemann and Mangold, 1924) concerned only 5 embryos, of which 2 were the good ones. The use of pigmented versus unpigmented transplants combined with the amazing drawing talent of Hilde Mangold with the *camera lucida* brought forth an incomparable discovery in embryonic induction. Statistics did not matter, for the experiment proved true.

What would be your advice to young scientists as to which topic in developmental biology to choose? Where do the future and main unanswered questions lie? Where do you expect the greatest breakthroughs?

If you are a young person searching for an interesting topic, the place to look for ideas are the old textbooks. A good place to start is always the E. B. Wilson *Cell in Development and Inheritance* book (Wilson, 1896). The problems, as does the frog, remain the same. An area where we can expect great breakthroughs is in the field of human organoids. Humans develop much slower than other mammalian model systems. With organoids it is now possible to study human embryology and all the intermediate stages during cell differentiation in healthy and diseased conditions, as we could not ever do before. Perhaps with luck we might be able to save the mouse and go directly to human tissue studies.

You have witnessed the progress of life science research over half a century. Which aspects about conducting research have changed for the better and which for the worse?

Developmental Biology has become much more quantitative. Huge data sets are generated from single cells, and tissue culture conditions have greatly improved. Interest is now turning into more cell biological aspects. There is enormous progress in cell signaling with genome-wide screens with siRNA or CRISPR/Cas9. I hope there will still be some room left to study one gene at the time as I have done all my life. Older people tend to think that things were always better in the past. One thing that was certainly much better

was scientific publishing. We now have useless supplementary figures that no one looks at, and reviewers with the nerve to try to change the interpretation of the results presented. Postdocs and even undergraduates have to write long grants to get funding, while their energy should be better focused at experimenting at the bench, which is what generates unexpected discoveries. One thing that has improved immensely is that we no longer have to justify to society that our work is useful. Biological sciences have generated entire new industries generating enormous wealth and improvement of human health. Undoubtedly, science is necessary if Ibero-American nations are to prosper in the modern world. It was my great privilege to watch how so many marvelous biomedical advances developed during just one lifetime. A life in science brings great joy and I recommend it highly.

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