Early and late stages of Developmental Biology in Argentina

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**Abbreviations used in this paper:** CEBBAD, Center for Biomedical, Biotechnological, Environmental and Diagnostic Studies; CEBICEM, Center for Cellular and Molecular Biology; CNBP, cellular nucleic acid
binding protein; CNTF, ciliary neurotrophic factor; CONICET, National Council for Scientific and Technical Research; Dil8, Drosophila insulin-like peptide; EP, extracellular matrix proteins; FIL, Leloir Institute Foundation; HGP, highly glycosylated protein; IAL, Institute of Agrobiotechnology of Litoral; IBBEA, Institute of Biodiversity and Experimental and Applied Biology; IBCN, Institute of Cell Biology and Neurosciences; IBioBA, Biomedicine Research Institute of Buenos Aires; IBR Institute of Cellular and Molecular Biology of Rosario; IFIBYNE, Institute of Physiology, Molecular Biology and Neurosciences; IIB-Intech Biotechnological Research Institute- Chascomús Institute of Technology; IIBYT, Institute of Biological and Technological Research; IMBICE, Multidisciplinary Institute of Cellular Biology; INGEBI, Research Institute in Genetic Engineering and Molecular Biology; INIBIBB, Biochemical Research Institute of Bahía Blanca; LABINE, Embryology Research Laboratory; Lgr3, leucine-rich repeat-containing G protein-coupled receptor 3; L-HGP, highly glycosylated low molecular mass protein; MMPs, matrix metalloproteases; PKA, protein kinase A; Shh, sonic hedgehog; TGF-β, transforming growth factor beta; UBA, University of Buenos Aires; UNC, National University of Córdoba; UNCuyo, National University of Cuyo; UNDP/UNESCO United Nations Development Programme / United Nations Educational, Scientific and Cultural Organization; UNER National University of Entre Ríos; UNL National University of Litoral; UNS, National University of the South; UNSAM, National University of San Martin; UNT, National University of Tucumán; uPA, urokinase type plasminogen activator; uPAR, urokinase type plasminogen activator receptor
Abstract

The history of science in Argentina is based on the enormous contribution that the great immigration of the 19th and 20th centuries produced in the country. The scientific and philosophical ideas and the role played especially by Italian scientists who arrived in the country produced a great impact on the different disciplines including development biology in emerging universities. The University of Tucumán pioneered the study of experimental biology, making important contributions to reproductive biology and to the early development of amphibians. The contribution of the Italian embryologist Prof. Armando Pisanó and the Argentinian Francisco D. Barbieri expanded the field to other universities and research centers located in Córdoba, La Plata, Bahía Blanca and Rosario. Given its strategic position, laboratories located in Buenos Aires city reached technological advances faster than others. Indeed, these laboratories saw the evolution from experimental biology to developmental genetics, renewing interest in this area. Currently, Developmental Biology brings together young researchers eager to consolidate regional and global collaboration networks that seek to help solve specific problems such as fertility, epigenetics, stem cells and tissue engineering.
The history of science in Argentina would be unimaginable without the contribution of the great immigration of the late 19th and early 20th centuries. The migratory avalanche coming mainly from southern Europe constituted the largest human contingent that entered the country during that period. The rapid transformation of Argentina from then on was significant and included numerous areas -habits, way of life and conception of the world, economy, letters, science, art, language, religion and politics (UNT 2002). Immigrants came mainly from Europe. The scientific and philosophical ideas and the role that foreign scientists who arrived in the country played in the academic research community marked a change of paradigms in the knowledge and the development of many scientific disciplines, including experimental embryology, which would lead to developmental biology. In this sense, it is important to know what the historical framework in which the transformations in this area of knowledge were taking place in Europe was, especially in Italy, at the end of the 19th century.

The influence of Italian laboratories

Most studies in embryology in Italy were conducted in the south of the country, in Palermo and Naples, where the Stazione Zoologica di Napoli played an important role in the formation of several generations of embryologists and developmental biologists (Fantini, 2000). Among them were Giuseppe Reverberi (1901-1988), Silvio Ranzi (1902-1996), Giuseppe Montalenti (1904-1990), and Alberto Monroy (1913–1986). They made important contributions to embryogenesis from a morphological, physiological and metabolic point of view using models of echinoderms, amphibians, agnates, ascites, birds and insects. The Stazione Zoologica di Napoli was a central point of an ever-growing international scientific network, promoting independent research, exchange and diffusion of novel practices and techniques that brought together numerous Nobel Prizes (Groeben and De Sio 2006). The most important biologists of the time as Hans Adolf Eduard Driesch (1867-1941), Otto Warburg (1883-1970), Kurt Herbst (1866-1946), Hans Spemann (1869-1941), Charles Otis Whitman (1842-1910) and Charles Manning Child (1869-1954), spent long research stays at the Stazione Zoologica, initially focused only on morphological studies, later on a new experimental methodology. In fact, it was in Naples that the proponents of the new experimental approach to development problems made some of their most important discoveries, thus beginning a new era in the study of development. In the 1940s, Naples was one of the main centers of experimental embryology: research traditions in systematics, cytology, physiology and biochemistry survived and genetics and biophysics became rooted there.

Italian immigration in Argentina

The political changes that shook Europe in the second half of the 19th century led a large part of the Italian population to emigrate not only to Argentina but also to other countries in the Americas such as Brazil,
Uruguay, and the United States. Most emigrants were academics, managers, businessmen, diplomats, merchants and career officers in search of a place for both material and cultural survival and where their children could grow and study freely (Terracini, 1989). Italian professionals were welcomed by Argentine universities, which offered generous contracts to incorporate teachers and researchers, thus promoting the internationalization of the institutions. Italian scientists received from the Argentine environment not only hospitality and affection, but also a remarkable cultural stimulus. It was a fortunate encounter between local interests and European energies, a forced but extremely fertile adaptation.

**Experimental embryology in Argentina**

The history of science in Argentina cannot be separated from the events that took place in the recently created university institutions. The first national university in northwestern Argentina, the National University of Tucuman, UNT, was created in 1914; the National University of Cordoba had already been founded in 1613 and the University of Buenos Aires in 1821. From the beginning, these institutions suffered from the difficulties of the surrounding environment and the contemporary historical period. During the first decades of the 19th century, the universities had as their main objective the training of professionals who would form the ruling class and serve the needs of a country that exported raw materials, while scientific knowledge played only a minor role. In the mid-nineteenth century, according to the enlightened and encyclopedic conception of science that prevailed in the country, the greatest activity of research in the area of biology was carried out in natural science museums and dedicated to the classification and survey of plant and animal species. This state of things began to change at the end of that century with the emergence of the first experimental laboratories connected with the teaching of medicine (Tagashira 2009).

During its first four decades, the UNT carried out an important scientific activity where important government projects were executed to establish academic models committed to the geographical region and to the development of knowledge. The most memorable academic administration was that of Horacio Descole, who chaired the UNT between 1846 and 1951. Descole was a dynamic, creative and very ambitious scientist who focused his management on the development of new disciplines and institutes and setting up of undergraduate and graduate degrees. His policy of adding to each Institute an academic with the capacity to strengthen scientific research led to the hiring of outstanding Argentinian and foreign researchers in mathematics, architecture, philosophy and natural sciences in special.

**José Miguel Alfredo María Cei (1918-2007) and Armando Pisanó (1914-2008)**

As a result of Descole's efforts to the development of natural sciences in Tucumán, a group of leading scientists were transferred to the UNT and joined either the School of Pharmacy and Biochemistry or an academic center -the current Institute Miguel Lillo-, which became the most important natural sciences
institution in the country during the third decade of the 20th century (Willink 1999). José Miguel Alfredo María Cei (1918-2007) and the Armando Pisanó (1914-2008) were among the Italian biologists who arrived in Argentina after 1945.

José Cei, born in San Miniato (Pisa, Italy) in 1918, finished his university studies at the Faculty of Natural Sciences-Biology branch- and the Faculty of Medicine of Florence and Pisa. In 1940, he obtained his Doctorate in Biological Sciences with specialization in Zoology and worked at the University of Florence from 1942 to 1947. In 1945, he participated in the US University Training Command in Florence, conducting research on sexual and hormonal cycles in vertebrates, the comparative anatomy of the organs of vision in mammals and general studies on biogeography and evolution. In World War II, he was transferred to North Africa, where he conducted anthropological studies with the various ethnic group’s native to Libya, Somalia and Ethiopia. In 1948, he joined the teaching staff of the Faculties of Biological Sciences and Pharmacy and Biochemistry (UNT) as a Professor in the Department of General Biology and Director of the Zoology area of the UNT. In 1950, he was part of the group of founders of the School of Medicine, which later became the Tucumán Faculty of Medicine. Until 1954, he was also the head of the Institute of General Biology and Experimental Embryology (the current Institute of Biology). His scientific contributions during this period focused on amphibian sexual cycles and biology. He also published a Biology text for university students.

From 1955 to 1980, Cei was a professor at the School of Medicine and the Director of the Institute of Biology of the National University of Cuyo (UNCuyo). He was a visiting professor in Angola, Chile, Italy, Portugal and the United States and specialized in amphibian systematics, ecology and biogeography. Cei’s extensive scientific production was an extraordinary contribution to the knowledge and development of Herpetology. A tireless traveler and a privileged explorer of nature, Cei discovered and described numerous amphibian and reptile species in the search for links that would allow us to decipher the great enigma of evolution, the current focus of evolutionary developmental biology.

In 1948, the embryologist Armando Pisanó (Fig. 1) joined the UNT zoology research team. Born in Catania, Italy, in 1914, Pisanó earned his PhD in Natural Sciences from the National University of Palermo, Italy, in 1936. In 1938, the Italian Ministry of Public Instruction granted him the title of Professor in Natural Sciences, Chemistry and Geography in a National Contest. He completed his scientific training at the Universities of Palermo and Bologna and at the prestigious Stazione Zoologica di Napoli (Pisanó 1939, 1942). While still very young, he became a Professor of Comparative Anatomy and Physiology at the University of Palermo and of General Biology and Zoology at the University of Siena. The UNT hired him as an extraordinary professor of Embryology for medical students at the professor of embryology at the Institute of General Biology and Experimental Embryology of the Faculty of Biochemistry, Chemistry and Pharmacy. He stayed in Tucumán until 1955, when he left in order to continue his scientific career at the Department of Scientific Research of the UNCuyo and later at the Institute of Marine Biology of the National University of La Plata. He also worked as a visiting professor in universities in Latin America, for instance in Colombia and Venezuela, and made numerous study trips abroad which allowed him to establish links with laboratories in Italy, France,
the Netherlands, England, Chile, Colombia, Paraguay and various African countries. He even conducted his research work at the Esperanza Base in Argentine Antarctica (Pisanó 1975), where he wrote "Topics on Embryology", one of his works where he develops several topics concerning comparative and experimental embryology.

His organizational ability was shown when he participated in the organization and creation of the Department of Biology for embryological studies at the University of Quindío, Colombia, as well as in the Pontificia Universidad Javeriana de Bogotá. In 1972, at his suggestion, the Embryology Research Laboratory (LABINE), in Buenos Aires was created by the National Council for Scientific and Technical Research (CONICET), where he continued to carry out his research work. Among his successors are mentioned Dante Paz, Leandro A. Miranda, Enrique Mariano, Jorge Herkovits, Marta Mariano and Clara L. Valdez Toledo.

Few researchers have been able to preserve and transmit their enthusiasm for naturalistic research as Pisanó did, who, though attentive to new trends, always considered it essential to have a broad vision of nature, loving it and not considering it an abstract topic for research. His total dedication to embryological studies and his intense driving action in all these activities were reflected throughout his work. He co-authored several books and monographs on Comparative Anatomy and Embryology and on Biology of Amphibian Development.

Pisanó’s enthusiasm and dedication to the study of biological sciences was transmitted to several generations of students, as reflected in the large number of fellows and thesis candidates whom he directed at the National Universities of Tucumán, of Buenos Aires and of the Northeast and in the Latin American Universities of Colombia and Venezuela as well as in his numerous lectures aimed not only at specialists but also to a wider and more varied audience.

Francisco D. Barbieri (1930-1993)

The teachings of Cei and Pisanó attracted general biology students interested in the study of nature. In 1949, one of them, Francisco Barbieri (1930-1993) (Fig. 1), was appointed assistant student at the Institute of Zoology of the UNT, and started his career as a researcher conducting cytological studies in native amphibian species (Barbieri 1950, 1954).

At that time, the teaching of biology and developmental anatomy were not clearly outlined concerning their objectives or their teaching methodologies. Pisanó’s enthusiasm led Barbieri to the study of embryology, and he collaborated in the writing of the chapter of Elements of Embryology in the General Biology textbook (Fig. 2), one of the first books in Spanish written by Cei and Pisanó for the students of Biochemistry at the UNT (Cei 1953). In 1952, Barbieri graduated with a biochemistry degree and, in 1954, he earned a PhD in Biochemistry. His histophysiological studies in the field of systematic biology, which he started in 1950,
allowed him to define two forms within the same batrachian species, *Leptodactylus ocellatus* and *Leptodactylus chaquensis* Cei (Barbieri, 1950). From then on, Barbieri started a series of research stays in Italian laboratories such as the Istituto di Gentica Lazaro Spallanzani in Pavia, the Istituto di Anatomia comparata in Florence, Palermo and Torino, the Istituto di Genetica and Institute of Histology in Naples and at the Stazione Zoologica di Napoli, where he came in contact with prestigious researchers who influenced his future research that would pave the way for experimental biology in Argentina. Having been awarded a fellowship by the International Rotary Foundation, he conducted a research stay at the Animal Morphology Laboratory of the Free University of Brussels, Belgium, under the direction of Prof. Jean Louis Auguste Brachet (1909-1988). Brachet, the son of the famous embryologist Albert Brachet (1869-1930), after extensive activity in the field of cell differentiation, in 1933 demonstrated that RNA plays an active role in protein synthesis. Brachet’s interest and enthusiasm in elucidating the role of nucleic acids and their metabolism during embryonic development and regeneration attracted a number of Belgian and foreign researchers, Barbieri among them. Having learnt more precise methods, Barbieri reconsidered some aspects of the metabolism of nucleic acids during avian embryo development. In Brachet’s laboratory, Barbieri studied the role of DNA synthesis in initial development, following the incorporation of adenine-C14 into the chick embryo. The autoradiographic method allowed him to observe that adenine was incorporated mainly in the neural tube and then in the somites, according to two gradients: caudo-cephalic and ventro-dorsal. Using autoradiographic techniques, he was able to determine that low doses of UV light inhibited morphogenesis, preventing the closure of neural folds in the chick embryo as a result of an alteration in the metabolism of nucleic acids, and not by an alteration of steroids - particularly vitamin D precursors - as had been suggested by James O. Davis (Davis, 1944). Years after his return to Argentina, these works were published in the Journal of Embryology and Experimental Morphology (now Development) (Barbieri, 1960; 1961) and led to new studies on the morphogenesis of the neural tube (Brauckmann and Barbieri 1957), the interaction between the notochord and the neuroectoderm in chick embryos (Torres and Barbieri 1987) and other studies on the biosynthesis of deoxyribonucleic acid in amphibians (Barbieri and Fernandez 1962). During his stay in the Netherlands (Belgium, 1956), Barbieri also worked at the Institute of Anatomy of the Free University of Brussels led by Prof. Albert Dalcq. Dalcq was interested in elucidating the role of calcium cations in the maturation, activation and fertilization of sea urchin eggs in order to lay the physiological basis of fertilization and parthenogenesis. His studies on morphogenesis in amphibians gave rise to a new concept about morphogenetic potential during embryo development. Barbieri’s stay in Albert Dalcq’s laboratory in 1957 had introduced him to the experimental manipulation of amphibian embryos. Upon his arrival in Brussels, Barbieri was already an expert in experimental embryology methods and began studies of reproductive biology and various aspects of embryonic development in an indigenous amphibian, the common toad *Bufo arenarum* (now *Rhinella arenarum*). By 1958, he had already published several articles on endocrine control of embryo development and regeneration.

During those years, his interest had been focused on the study of the energy metabolism of *B. arenarum* eggs, which, like other amphibian eggs, could be cleaved at a normal rate in the absence of oxygen or in the
presence of cyanide - an inhibitor of the respiratory chain - during the first days after fertilization the egg. Together with his disciples Arnaldo Legname (1927-2017) and Hortensia Salomón (1936-2007), Barbieri showed that *R. arenarum* eggs displayed an intense glycolytic activity under anaerobic conditions, which was inhibited when the eggs returned to oxygen (Pasteur effect) (Barbieri and Legname 1957; Barbieri and Salomón 1963). They also observed an increase in oxygen consumption during the first 2h of recovery (payment of oxygen debt) which was not involved in the lactate oxidation (Barbieri and Valde Toledo de Moreno 1966). These results supported Brachet's idea concerning the presence of an 'oxidative reserve' in vertebrate eggs since a similar respiratory response had been described in eggs of *Rana fusca* (Brachet, 1934), *R. pipiens* (Cohen, 1955) and *R. arenarum* (1964).

In 1958, Barbieri was appointed to the Chair of Animal Histology and then to that of Embryology and Histology at the Faculty of Natural Sciences and the Miguel Lillo Institute. In 1960, he became a professor of General Biology and, in 1971, a professor of Comparative Anatomy in the Faculty of Biochemistry.

One of the most important contributions to the experimental analysis of nuclear differentiation at the time had been the method developed by Briggs and King for the transplantation of embryonic nuclei of amphibians (Briggs and King 1952). In 1967, Barbieri extended the nuclear transplantation experiments to the *R. arenarum* toad, transplanting blast nuclei to enucleated oocytes of the same species in different culture media, managing to develop embryos until the beginning of the tadpole stage (Legname and Barbieri 1967, 1968). Years later, Barbieri, along with his disciple Mario Manes, studied the determination of the bilateral symmetry plane of *R. arenarum* at the time of fertilization. They found that the injection of sperm homogenate, without requiring the intact structure of the male gamete, is enough to activate the oocyte and determine the bilateral symmetry plane of the future embryo. The results suggested that microtubules could play several roles in gray crescent formation and pronuclear movement and orientation in the oocyte (Manes and Barbieri 1976; 1977; Manes et al., 1978).

Barbieri was also interested in aspects of amphibian reproduction. Together with Villecco, observed that diffusible components of the jelly surrounding *R. arenarum* oocytes ("egg water") were necessary for fertilization to take place (Barbieri 1976). Thus, with the help of Raisman, Barbieri determined that this factor was produced by dialyzable and heat stable oviductal cells (Barbieri and Villecco 1966; Barbieri et al., 1977), suggesting that this factor induce a pH increase in the inseminating medium (Barbieri and Cabada 1969).

This hypothesis was soon ruled out, although studies that attempted to determine the molecular identity of the diffusible factor and its mechanism of action continued. In 1977, Barbieri and Miceli observed that the epithelium of the most cephalic region of the oviduct, the pars recta, secreted a fluid that allowed the fertilization of coelomic oocytes. The factor involved, a protein displaying a molecular weight of 47000, was purified and called oviductal pars recta factor. It was hypothesized that this protein was a Ca²⁺-dependent trypsin-like enzyme which effect on the egg yolk envelope increase the susceptibility to sperm lysine by facilitating the penetration of the vitelline envelope by sperm. Later studies showed that the secretion of the pars recta factor is hormonally reduced (Miceli et al., 1987).
In 1978, at Barbieri’s suggestion, the Department of Developmental Biology was created at the Faculty of Biochemistry, Chemistry and Pharmacy. This was the first research unit under the CONICET and the UNT. In 1980, this Department led to the creation of the INSIBIO (CONICET-UNT).

In 1983, Barbieri was hired by the Rockefeller Foundation to work on amphibian fertilization together with Asa A. Humphries, JR. (Fig. 3), who, at that time, was Vice President and Dean of the Transylvania University, Lexington, Kentucky. The meeting took place at the Bellagio Study and Conference Center, which was the venue for numerous meetings for academics from all over the world where they reviewed subjects of international importance for the development and strengthening of universities and institutes in developing countries. Humphries was the PhD mentor of Eugenia del Pino at Emory University, Atlanta, GA (see article by del Pino in this issue).

Towards the end of the 20th century, the study of developmental biology sought explanations based on cellular or tissue structure and function, considering the interactions between its components. Thus, a modern vision in this discipline, similar to the one outlined in the world, appeared in the UNT. In the 80s, research in Barbieri’s laboratory was oriented to the study of cell interactions during primary embryonic induction in R. arenarum, analyzing the role of the cell surface and the extracellular matrix during the determination of the neuroectoblast in gastrulation. A first approach aimed at determining whether primary induction was accompanied by a modification on the surface of epiblast cells. Thus, it was observed that epiblast cells change the composition of surface glycoproteins as a result of neural induction (Barbieri et al., 1980). Whether this alteration of the surface was causally related to the inductive action of the primary organizer remained an open question. With radiochemical studies, synthesis of polyadenylated RNA was observed at the interaction interface of cordomesoblast with the epiblast during neural induction (Sánchez et al., 1983). Years later, the identification by recombinant DNA techniques of the signals released by the Spemann organizer (cordina, cerberus, noggin, follistatin, nodal, etc.) and their mechanism of action by blocking the ventralizing signals verified this hypothesis (De Robertis 2009).

In a second approach, Barbieri and Sánchez determined that the inhibition of glycosylation of proteins in the blastocoel of R. arenarum embryos produced an alteration in morphogenetic movements, leading to embryo exogastrulation (Sánchez and Barbieri 1983). One of these molecules in close correlation with cell migration was identified as fibronectin (Sánchez et al., 1984). Sánchez observed that when epiblast cells were cultured with dorsal extracellular matrix (located mainly at the interface between the dorsal lip of the blastopore and the overlying ectoblast), they differentiated into neural, mesenchymal and pigmentary cells. In contrast, cells grown in saline solution or with extracellular matrix of the ventral region differentiated into ciliated and secretory (non-neural) cells. These results evidenced a differential biological activity of the extracellular material located in the dorsal and ventral regions of R. arenarum (Sánchez and Barbieri 1988). This once again confirmed the regulatory role of surface and extracellular matrix proteins in the migration of cordomesoblastic cells during gastrulation.
The energy and passion of Barbieri for the enigmas of development led him to organize and promote numerous postgraduate courses and conferences, spreading his knowledge of development biology in different universities of the country such as the National University of Salta (1959), the National University of Buenos Aires (1961, 1978, 1980) and the Universidad del Nordeste (1966). Thus, he encouraged interest in the study of embryology in Argentina as well as in neighboring countries such as Chile (Santiago de Chile 1971, Concepción, 1981). His integration with Chilean groups led him to organize international courses sponsored by UNDP/UNESCO between 1978 and 1985, with the participation of prestigious researchers in the area of development biology (Fig. 4 and 5). Two outstanding figures among these researchers were Luis Izquierdo (1928-1992), devoted to the early development of the mammalian embryo, and Juan Andrés Fernández Hidalgo (?-2018), who studied cytoskeleton dynamics and their role in the formation of cytoplasmic domains during early embryogenesis of zebrafish and *Theromyzon rude*. The participation of Claudio Barros (1936-2008), from Chile, was very important given his interest in the different steps of sperm-egg interactions during mammalian fertilization, particularly during the acrosome reaction. Many of the participants in these courses spread the concerns of developmental biology to numerous scientific research centers in Argentina and Latin America. Barbieri's passion in the study of the morphogenetic movements of gastrulation has left its mark on renowned researchers such as Eugenia del Pino (Ecuador) and Roberto Mayor (Chile). In 1973, thanks to Barbieri's effort, the VI Argentine Congress of Biology (Fig. 6) was held in Tucumán, with the participation of the prestigious developmental embryologist Sir John B. Gurdon (Nobel Prize in Medicine in 2012), who would lay the foundations of cloning in experiments conducted on frogs in 1962, and of embryologist Rubén Adler, who had begun his studies on neurodevelopment. Luis F. Leloir (Nobel Prize in Chemistry in 1970), also participated in the event.

**Barbieri’s successors in Tucumán**

Several young researchers who had been Barbieri’s students continued investigating aspects of reproduction and embryogenesis, forming new research groups within the Department of Developmental Biology in Tucumán and in other provinces in Argentina. By the end of the 80s, the Biology Institute already had numerous lines of research, the reproduction and embryology of amphibians being the primary focus of research.

Arnaldo Legname (1925-2017) and Hortensia Salomón de Legname (1936-2007), who studied in greater depth the intermediate metabolism during the embryo development of *R. arenerum*, characterized a pregastrular embryonic metabolism where carbohydrates are broken down mainly through the cycle of pentose phosphate with a relatively low participation of the Embden Meyerhof glycolytic pathway. As ontogenesis progresses, this metabolic pathway increases its participation, until in tadpoles, and especially in adult tissue, almost the whole carbohydrate content is metabolized through glycolysis. They also demonstrated that during the segmentation of *Rhinella* embryos this pentose cycle is mainly associated with...
the glutamic-aspartic cycle, producing intermediates such as fumarate or malate, which would be used as precursors of pyrimidine and purine salts (Salomón de Legname 1969; Salomón de Legname et al., 1975). As embryonic development progresses, the participation of the glutamic-aspartic cycle decreases by focusing on the classic tricarboxylic acid cycle (Salomon de Legname et al., 1971). Although coelomic oocytes have a metabolism similar to that of adult tissues, during their passage through the oviduct they undergo biochemical and metabolic transformations influenced by pituitary hormones that give them an embryonic metabolism (Salomón de Legname et al., 1979). One of the members of the laboratory, Marta Bühler, continued with the study of the processes that take place during maturation, activation of amphibian oocytes and ability to initiate development. They analyzed seasonal changes in the oxidative metabolism and the signaling pathways involved in these processes (Zelarayan et al., 1995; Toranzo et al., 2010). In recent years, they extended their studies to mammals by adopting the Chinchilla lanigera Grey model to study the signaling pathways that participate in the capacitation and acrosomal reaction of epididymal sperm, as well as the location and importance of some sperm proteins involved in gamete interaction (Gramajo-Bühler et al; 2014; Zapata et al., 2016).

Jorge Raisman, while working toward his doctorate degree with Barbieri, made a research stay at the Stazione Zoologica di Napoli under the direction of Alberto Monroy. In 1972, he worked at the Emory University in Atlanta, Georgia, US, in the laboratory of Asa Alan Humphries, Jr., where he studied the role of the jelly coat in amphibian fertilization. On his return, after having earned his Doctor’s degree, he continued to work on morphological and biochemical aspects of \textit{R. arenarum} fertilization (Miceli et al., 1977).

Marcelo Cabada, during a stay in the laboratory of Chris Darnbrough at the University of Edinburgh, Scotland, UK, demonstrated the existence of two different classes of “maternal” poly (A) + RNA in \textit{Xenopus levis} oocytes during different oogenesis periods (Cabada et al., 1977). Upon his return to Tucumán, Argentina, he continued with studies on the metabolism of oocyte RNA during the maturation stage. He found that progesterone does not modify RNA synthesis (Sánchez Riera et al., 1988a), and that follicle cells transfer RNA to the oocyte (Sánchez Riera et al., 1988b). He continued studying different factors participating in gamete interaction during \textit{R. arenarum} fertilization such as the effect of sperm concentration on the fertilization rate (Cabada 1975), the influence of different lipids (cholesterol and phosphatidylcholine) on the acrosome reaction (Díaz Fondevila et al., 1988), the role of glycosidic residues of the vitelline envelope in gamete interaction and the exocytosis of oocyte cortical granules (del Pino and Cabada 1987).

Dora Miceli, from the Barbieri laboratory, continued studying the function of oviductal molecules involved in fertilization and embryo development of amphibians and various mammalian species (hamster, rat, sow, cow, llama and vicuña). Following her theory about the participation of proteases in the oviductal environment, she and her team evaluated the activity and expression of components of the plasminogen/plasmin system, enzymes that belong to the serine protease family in the mammalian oviduct (Roldán-Olarte et al., 2012). They studied different matrix metalloproteases (MMPs) and their inhibitors, which participate in fertilization and early embryo development of the llama, a camelid native to the Andes.
Sara Sánchez continued analyzing the participation of surface molecules and extracellular matrix during the migratory processes that take place in the embryonic development of *R. arenarum*. She identified and characterized six extracellular matrix proteins (EP1-EP6) that are synthesized de novo during gastrulation (Sánchez et al., 1988). In those days, cell lineage studies represented a strong component of developmental biology in France. During her stay at the Laboratoire de Biologie Expérimentale of Jean-Claude Boucaut at the Université Pierre et Marie Curie, Paris, Sánchez studied morphogenetic movements during the early gastrula stage of *Pleurodeles walt*, establishing the map of the fate of superficial and deep circumblastoporal cells (Delarue et al., 1992). Upon her return to Argentina, she and her team determined that the blocking of EP3-EP4 with specific antibodies injected into the blastocoel causes severe alterations in the gastrulation process, affecting the convergence extension processes of the dorsal marginal zone and the epiboly of the blastocoel roof (Genta et al., 1997). Similar effects had been described for fibronectin in amphibians (Boucaut et al., 1985). Using inhibitors of glycoprotein and glycolipid synthesis, the importance of these molecules in the processes of radial intercalation and migration was also established (Peralta et al., 1995). Sánchez and her team also determined that the inhibition of glycosphingolipid synthesis or its blocking by antibodies causes alterations in the contacts of mesodermal cells, affecting cytoplasmic processes (filopodia and lamellipodia), inhibiting gastrulation movements (Aybar et al., 2000a; 2000b). Following the study of extracellular proteins, the expression of a vitronectin-like adhesion protein that is stored in vitelline platelets during *R. arenarum*’s oogenesis was determined. Years later, this protein was cloned and its implication in organogenesis was suggested. The need to use molecular biology techniques to try to solve these biological approaches led to the substitution of the native experimental model of the amphibian *R. arenarum* for *Xenopus laevis*, an organism which genome was at least partially known at the time. The Department of Developmental Biology created its first *Xenopus* bioterium and, using *Xenopus laevis* as a model organism, the enzymes Xlgd3 and Xlcgt, responsible for ganglioside synthesis, were cloned, studied, and found to be involved in convergent extension movements during *Xenopus* early development (Luque et al., 2008).

The economic instability in 2000 caused biomedical research and biology in Argentina fall behind in the spectacular worldwide advance that the use of molecular biology represented in numerous scientific research fields. The new technologies had been applied to only a few either areas, while other fields such as cellular and molecular neurobiology, developmental genetics, structural biology, genomics and computational biology were represented poorly or not at all (Sabatini et al., 2000).

Given the recent interest in studying the genes and signaling pathways that control the development processes, young researchers from the Sánchez group conducted different research stays at the Millennium.
Nucleus in Developmental Biology, the Faculty of Science, University of Chile, with Roberto Mayor, who had made great contributions related to the molecular mechanisms that underlie the development of the neural crest in *Xenopus laevis* (Mayor et al., 1999). These young researchers determined that a balance in the activity of the transcription factors Slug and msx1 controls apoptosis in certain areas of the neural folds, defines the precise boundaries of the territory of the neural crest, and accurately controls the number of cells among neural crest derivatives (Tribulo et al., 2004). They also cloned characteristic transcription factors of the progenitors of the migratory multipotent neural crest, as well as several of its differentiated derivatives such as *sox10* and *paraxis*, a regulator of the development of paraxial mesoderm and the formation of somites (Honoré et al., 2003; Carpio et al., 2004). At present, in Sánchez’s laboratory, researchers determined that *paraxis* participates in the cellular rearrangements that take place during *Xenopus* somitogenesis through the regulation of cell adhesion and the differentiation of somites (Sánchez and Sánchez 2015). They continue studying the processes and genes involved in the formation and establishment of the anuran spine pattern, considering the structural divergence that this group presents with respect to other vertebrates (Sánchez and Sánchez 2013).

Manuel J. Aybar, during his postdoctoral stay in Roberto Mayor’s Laboratory, studied the complex sequence of inductive events responsible for the generation of *Xenopus* neural crest. He determined that the Snail transcription factor is required for the specification and migration of neural crest cells and that the early activation of this gene defines the competent territory of the neural crest (Aybar et al., 2003). Upon his return to the INSIBIO (UNT-CONICET), Aybar leads a new research group aimed in studying the role of different genes and cell signaling pathways (BMPs, Ihh/gli, Endothelins, Notch) in the molecular, cellular and morphogenetic regulation of the processes that take place during neural crest development in vertebrates (Bonano et al., 2008; Tribulo et al., 2012; Agüero et al., 2012; Vega Lopez et al., 2015). In this group, Celeste Tribulo also assesses the molecular aspects of epidermal development in amphibians (Tribulo et al., 2012).

**Developmental Biology throughout the country**

**Buenos Aires**

Until the mid-twentieth century, the teaching of embryology and histology at the Faculty of Medicine of the University of Buenos Aires (UBA), Buenos Aires city, was integrated into a single subject. The tenured professor of the chair of histology, Julio Lacoste, was a strong promoter of both subjects and, in 1922, he created the Institute of General Comparative Anatomy and Embryology, thus laying the foundations for the creation of the Embryology Museum.

Between 1922 and 1957, the Institute of General Comparative Anatomy and Embryology was run successively by Pedro Rojas, who returned to the country after having worked with the prestigious
embryologist Albert Brachet, Manuel Varela, who created the Chemical Embryology section of the Institute, Jorge Porto and Eduardo De Robertis (1913-1988). De Robertis started teaching cytology, describing the subcellular ultrastructure not only from a morphological but also from a biochemical and genetic point of view, thus laying the foundations of Cellular Biology in Argentina.

In the 1960s, De Robertis invited Bradley M. Patten (1889-1971), Emeritus Professor of the University of Michigan, to spend a season at the Chair in his charge. During this period, Patten promoted the training of Roberto Narbaitz as an embryologist, who then began teaching embryology to medical students. Narbaitz focused his research on the analysis of sexual differentiation in birds and their hormonal control, analyzing sexual inversion from a morphological and functional point of view (Narbaitz and Sabatini 1963; Narbaitz and Kolodny 1964; Narbaitz and Teitelman 1965). Their findings confirmed the bisexuality and asymmetry of the embryonic gonads of birds (Rahil and Narbaitz 1972). Together with his disciple Rubén Adler (1940-2007), he studied the differentiation of germ cells in embryonic gonads and described submicroscopic aspects during the differentiation of fetal Leydig cells from rodents (Narbaitz and Adler 1966). Narbaitz made important contributions to the knowledge of the parathyroid gland and its role in Ca²⁺ homeostasis during chicken embryogenesis (Narbaitz and Gartke 1975).

Adler graduated from the Faculty of Medicine of the UBA in 1963. In 1968, he made a postdoctoral stay at the embryology school of the Hubrecht Institute of the University of Utrecht, one of whose most prominent members was the researcher Pieter Nieuwkoop (1917-1996), a pioneer in the induction analysis of the mesoderm and in the processes that lead to neural induction. There Adler started his research in a field that would become his scientific passion, developmental neurobiology (Adler 1968; Adler et al., 1976; 1977), establishing the basis for modern experimental analysis of development processes at the morphogenetic, cellular and genetic level. On his return to Argentina, Adler became Assistant Professor of Histology, Cytology and Embryology at the Faculty of Medicine of the UBA, where he wrote a book dealing with the teaching of embryology titled “Developmental biology and congenital malformations” and published in 1974. One of the main aims of the book was to bridge the gap between morphology of development, the predominant viewpoint taught at that time, and the biology and genetics of development, which expanded its influence in the world under the enthusiastic leadership of Conrad H. Waddington (1905-1975), Joseph Needham (1900-1995), Gregory Bateson (1904-1980) and Pieter Nieuwkoop (1917-1996), among others. From the beginning of his career, Adler focused his interest on ocular development with novel approaches to identify genetic factors and microenvironmental signals that regulate neuron survival. In 1977, because of political conditions in Argentina, Adler, Narbaitz and other researchers left the country. After obtaining a Guggenheim Fellowship, Adler held research scientist positions on the campus of the University of California, Berkeley and San Diego in the US. In 1982, he joined the Wilmer Eye Institute at Johns Hopkins University, where he spent the rest of his remarkable career. Adler’s work revealed key molecular pathways that regulate the survival and differentiation of retinal cells both in normal development and in degenerative retinal disease, and played a key role in the discovery of the CNTF neurotrophic factor (Adler et al., 1979;
Wahlin et al., 2000; Canto Soler et al., 2008). His laboratory is currently under the direction of one of his successors, Valeria Canto Soler.

The current Institute of Cell Biology and Neurosciences (IBCN) of the UBA School of Medicine originated from the Institute of Comparative General Anatomy and Embryology. Vladimir Flores started his research in the IBCN Electronic Microscopy laboratory. There he earned his doctor’s degree, spending most of his time in the study of nervous system regeneration under the direction of Amanda Pellegrino de Iraldi. Flores completed his postgraduate training at the University of Cambridge, working at Downing College under the direction of professors J. Treherne and N.J. Lane, and specializing in techniques applicable to the study of nervous system development. Upon his return to the country, he went back to the IBCN, where he studied the development of local circuits and synaptogenesis during the development of the dorsal midbrain of the chick embryo, the *tectum opticum*. In the IBCN, he created the Laboratory of Developmental Neurobiology which up to now is directed by his former student Gabriel E. Scicolone (Scicolone et al., 1995). Later, at Favaloro University, Flores created an Interdisciplinary Group of Theoretical Biology devoted to the analysis of cell behavior dynamics involved in the development of the central nervous system (Rapacioli et al., 2012; 2016). At present, Scicolone’s research is focused on neuronal determination and differentiation of progenitor cells and on the acquisition of competence to respond to signals that guide axonal growth, using the retinotectal system of the chick embryo as an experimental model. He is investigating the molecular bases of the development of topographically ordered connections in the central nervous system. His team studies the role of the Eph tyrosine kinase receptor system and its ligands, ephrins, uPA/uPAR and neurotrophic factors in growth and axonal guidance (Ortalli et al., 2012; Lino et al., 2014).

At the IBCN, Andrés Carrasco (1946-2014) worked as a professor of physiology, cell biology and embryology at the UBA. In 1981, he made a postdoctoral stay in Edward De Robertis’ laboratory at the University of Basel, Switzerland. He cloned and sequenced the first vertebrate homeobox gene involved in development known as *AC1/Hoxc 6* (Müller et al., 1984). In 1984, Carrasco started working at the Institute of Cellular and Molecular Biology at the University of Indiana, where he carried out the first in situ hybridization in *Xenopus laevis* embryos. It was the first demonstration of the spatial distribution of a *Hox* gene, a low abundance mRNA in amphibian embryos (Carrasco and Malacinski 1987). Then he moved to the Department of Molecular Genetics at the University of Texas to work with antisense RNA. In 1990, when he returned from his postdoctoral stay, Carrasco settled in the Institute of Cellular Biology and Neurosciences of the Faculty of Medicine of the UBA. He continued investigating the role of *Hox* genes in the development of the nervous system of *Xenopus laevis*, initiating studies on gene function using molecular biology methods (Lopez and Carrasco 1992; Paganelli et al., 2001). Silvia López, a disciple of Carrasco, continues to investigating the signaling pathways with *Xenopus laevis*, especially focusing on the Notch pathway and other factors in the segregation of embryonic layers and their impact on the structures derived from them (Revinski et al., 2010; Favarolo and Lopez 2018).
In 1968, Marta Fernández de Recondo, who developed her doctorate by studying *R. arenarum* oogenesis under the direction of Barbieri in Tucumán (1967), started teaching at UBA as Professor of Embryology at the Faculty of Natural and Exact Sciences-UBA. Together with Graciela Guerrero and María Cristina Maggesse (1944-2014), formed a new Developmental Biology group (Maggesse et al., 1976; Cukier et al., 1979). Maggese and Guerrero were trained in the study of the amphibian model in the Postgraduate Courses run by Barbieri and also during several stays in his laboratory. For several years, together with other research teachers, Maggese and Guerrero taught the postgraduate course “Topics in Developmental Biology” at the UBA. At the same time, Guerrero started to study the implication of the germinal epithelium in the reversal of fish sex (Lo Nostro et al., 2003). In the meantime, Maggese continued her studies on the hormonal control of the reproduction of native fish, training numerous researchers who analyzed various aspects of fish biology at the Institute of Biodiversity and Experimental and Applied Biology (IBBEA) (Vissio et al., 2002; Pandolfi et al., 2002; Ravaglia and Maggesse 2003; Cánepa et al., 2006; Genovese et al., 2011).

Her former students developed independent lines of research, giving rise to three autonomous working groups, one of them being the Laboratory of Neuroendocrinology of Growth and Reproduction, run by Paula Vissio. This group is dedicated to the study of morphological and adaptive changes during the early development of regulated teleosts at the neuroendocrine level. Vissio continued teaching Animal Embryology which, in 2019, changed its name to Biology of Growth and Reproduction.

Dante A. Paz did his doctoral thesis with Pisanó, analyzing the processes of differentiation of the heart area of *R. arenarum*. With his team, he continued working on the structuring of the olfactory system in anuran amphibians, during larval development and in adults, studying the signaling pathways involved in the perception of olfactory stimuli, neuronal projections and their connections with the central nervous system.

He was also interested in the study of the regenerative processes of the olfactory epithelium, analyzing the participation of different growth factors and the participation of stem cells, the differentiation of olfactory neurons and of sustainable cells (glial cells) (Jungblut et al., 2010; Frontera et al., 2015).

At the Institute of Physiology, Molecular Biology and Neurosciences (IFIBYNE), Flavio Silva Junqueira de Souza is currently leading the Embryonic Development Neurobiology Laboratory, which is devoted to the study of the genes controlling the early patterning of the hypothalamus and the pineal gland in mammals and amphibians. In the same laboratory, María Cecilia Cirio investigates the role of cofactors of the transcriptional complex Ldb1-Lhx1 in the development of vertebrate kidneys (Espiritu et al., 2018).

Alfredo Vitullo, made a post-doctoral stage at the Centre de Recherches de Jouy-en-Josas, Institut National de la Recherche Agronomique -INRA-, France, working on mechanisms of oocyte electroactivation and cloning of mammalian embryos. Subsequently, he served as a Guest Researcher at the Lerner Research Institute of the Cleveland Clinic Foundation, Cleveland, USA, in the molecular biology of T-box genes in breast cancer and its role in mouse embryological development. Nowadays he works at the Center for Biomedical, Biotechnological, Environmental and Diagnostic Studies (CEBBAD) of the Maimonides University, conducts research on the specification of germ cells in eutherian mammals (Proietto et al., 2019).
At the Leloir Institute Foundation (FIL), Luis A. Quesada Allué began his scientific research with ecological studies in yellow clams and scallops, two mollusks found on Argentine shores, and then moved on to investigate the behavioral physiology of honeybees. His main scientific contributions are the biochemical and molecular studies in the development of insects concerning the mechanism of synthesis and deposition of cuticle proteins of insects, which were later extended to crustaceans, trypanosomatids and somatic embryos of plants (Quesada-Allue 1981; 1982; Pérez et al., 2002). In recent years, his extensive work on the biology of insects become focused on two little explored aspects worldwide: the study of functional senescence (aging) and a new mechanism of regulation of neurotransmitters in insect brains (Pujol-Lereis et al., 2016).

Since 1998, Pablo Wappner leads the Laboratory of Genetics and Molecular Physiology at the Leloir Institute using Drosophila melanogaster as a model system for the study of molecular and cellular mechanisms that mediate stress adaptation. Since its creation, the group has focused on the elucidation of the mechanisms that mediate hypoxia adaptation (Centanin et al., 2005; Galagovsky et al., 2014), developing transgenic lines to characterize physiologically and biochemically the elements that take part in the response to hypoxia. In recent years, the group has expanded its studies to elucidate hypoxia genes linked to the metabolic reprogramming that occurs under certain pathologies such as tumor development in cancer, a line of work that might lead to future therapeutic applications (Gândara and Wappner 2018).

In recent years, young researchers who conducted postdoctoral studies and training in centers of great relevance in La Jolla, California, acquiring significant experience in new technologies, have conducted their research at the Leloir Institute. Among them is Maria Fernanda Ceriani, who, after a stay in the laboratory of Steve Kay at The Scripps Research Institute, made important contributions to the knowledge of the neural control mechanisms of the generation of circadian rhythms in Drosophila while directing the Behavioral Genetics laboratory (Franco et al., 2018). Another team member is Marcelo J. Yanovsky, who also carried out postdoctoral studies in the laboratory of Steve A. Kay, and is currently focusing his research on the genetic plot that regulates the daily and seasonal rhythms of plant growth and development in response to the environment. His work is conducted in the laboratory of Comparative Genomics of Plant Development (Mora-Garcia et al., 2017; Hernando et al., 2019). Pablo D. Cerdán, who carried out his postdoctoral studies at the Plant Molecular and Cellular Biology Laboratory of the same Institute, has devoted his research to analyzing the gene cascade involved in Arabidopsis thaliana’s response to temperature and light, in the Plants Molecular Biology laboratory at the Leloir Institute (Jaskolowski et al., 2019). At the FIL, Guillermo Lanuza, upon his return from the Laboratory of Molecular Neurobiology at the Salk Institute for Biological Studies, started to direct the Laboratory of Genetics and Neural Development. He is working on the ontogenic mechanisms that control the diversification of neuronal and glial types in the central nervous system in experimental models in mice and birds (Petracca et al., 2016; Di Bella et al., 2019).

At the Research Institute in Genetic Engineering and Molecular Biology “Dr. Héctor N. Torres” (INGEBI), Lucia F. Franchini is studying the genetic basis that underlies the evolution of the primate brain, especially in humans and in the inner ear of mammals in the Laboratory of Genetics and Molecular Evolution (Pisciottano...
et al., 2019). Jorge Muschietti, in the Laboratory Molecular Mechanisms of Fertilization in Plants of INGEBI, is at present investigating the bases that regulate the development of pollen grain. He is also conducting research on the molecular mechanisms that control the specificity of pollination in plants (Sede et al., 2018).

At the Biomedicine Research Institute of Buenos Aires (IBioBA), Luis Morelli is studying the principles that control the processing of information that cells receive from their environment and use it to make decisions, during morphogenesis and in adult tissues, using the zebrafish as an experimental model (Uriu et al., 2017).

La Plata

Nestor Carri, who at the beginning of his career had worked with Adler and Suburo (Suburo et al., 1979), completed his PhD with Ted Ebendal in the Department of Developmental Biology, Uppsala University, Sweden, studying the influence of axon terminal fields of the optic tectum on the selective stimulation of the growth of neurites of the chick retina. Upon his return to Argentina in 1983, he created the Molecular Development Biology laboratory within the Multidisciplinary Institute of Cellular Biology (IMBICE-CONICET) of the city of La Plata in Buenos Aires, which was devoted to the study of the embryological mechanisms of nervous development. There he worked on trophic molecules and signaling cascades that activate the "gene tandem" of survival and neuroprotection of nerve and glial cells in primary cultures and established cell lines (Blanco et al., 2001; Villegas et al., 2006).

Upon his return from a postdoctoral stay with Herbert Jäckle at the Abteilung Molekulare Entwicklungsbioiogie, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany, Rolando Rivera-Pomar began working on gene regulation in the Drosophila embryo (Rivera-Pomar et al., 1995). For some years now, Rivera-Pomar has been working on the comparative genomic study of insects. In the Laboratory of Genetics and Functional Genomics, Regional Center for Genomic Studies, UNLP, he investigates early development genes and their regulation with a focus on the segmentation process, genes related to resistance to insecticides and small peptides, and neuropeptides in different species of insects, some of them of medical and agricultural interest (Lavore A, et al., 2015).

Bahía Blanca

In 1963, Nicólas G. Bazán began to study developmental biology in Tucumán as a Barbieri fellow, being trained in the embryonic model of amphibians (Brauckmann and Bazán 1963; Barbieri and Bazán 1964). In 1970, on his return from a stay abroad (Harvard Medical School, Boston, MA, US, and a postdoctoral stay at the University of Toronto, Canada), he moved to the National University of the South (UNS), and organized the Biochemical Research Institute of Bahía Blanca (INIBIBB) and the Department of Biology. In addition to his managerial skills, his enthusiasm for biological studies led him to focus his research on the central nervous system, including the retina, and on early embryo development on vertebrate models, with a strong interest in lipid composition and metabolism (Bazán 1971; Crupkin et al., 1973; Aveldano and Bazán, 1975).

During his stay in the country until 1980, Bazán trained a significant group of developmental biologists
interested in the lipid metabolism of amphibians, among them Ana M Pechen, Ida C. Bonini and Telma Alonso (Pechen et al., 1974; Bonini de Romanelli et al., 1981; Alonso et al., 1982), who made important contributions in this area. Telma Alonso continued to explore Development Biochemistry using the *R. arenarum* toad as an experimental model. She focused on the biochemical mechanisms involved in oocyte maturation, fertilization and early embryogenesis (Alonso et al., 1986; Buschiazzo et al., 2008), establishing close collaboration with other related research groups, especially with members of the Biology Institute of Tucumán and in particular with Armando Pisanó. Pisanó taught postgraduate courses in Bahía Blanca and members of INIBIB also participated in courses taught by him in Buenos Aires and in Neuquén. The INIBIB Development Biology Laboratory was named after him as a tribute to his outstanding scientific career.

Luis E. Politi, one of Rubén Adler’s students (Politi and Adler 1986; Politi et al., 1989), on his return from the Wilmer Eye Institute in Johns Hopkins created in the INIBIB the Developmental Neurobiology group, devoted to the search for cellular or molecular trophic factors involved in the survival and differentiation of retinal neurons. Politi was also interested in the use of stem cells during neurodegenerative diseases that cause blindness, such as retinitis pigmentosa or age-related macular degeneration (Garelli et al., 2006; De Genaro et al., 2013).

In recent years, the study of neuroendocrine control of *Drosophila* morphogenesis during pupariation was also addressed by Andres Garelli. His work sheds new light on the function and evolution of the relaxin receptors, revealing a novel neuroendocrine circuit responsive to growth aberrations mediated by *Drosophila* insulin-like peptide (Dil8) and leucine-rich repeat-containing G protein-coupled receptor 3 (Lgr3) (Garelli et al., 2012; 2015).

*Chascomús*

In 2013, Pablo H. Strobl Mazzulla and Juan I. Fernandino returned from their postdoctoral training abroad and created the Development Biology Laboratory at the Biotechnological Research Institute (IIB-Intech, CONICET-UNSAM). Their research is aimed at understanding the epigenetic basis that regulates the development and differentiation of cells in vertebrates, analysing the dynamics of the epigenetic-microRNA regulation network during delamination and condensation of neural crest cells in vertebrates using chicken as animal model. They also analyse the transgenerational epigenetic effect of folic acid deficiency during embryo development in vertebrates (Alata Jimenez et al., 2018; Sánchez-Vasquez et al., 2019). Another line of research focuses on the study of the molecular control of gonadal development in an embryonic model in *Oryzias latipes* (medaka fish) and the molecular pathways mainly involved in the proliferation of primordial germ cells (Fernandino and Hattori 2019; Castañeda Cortés, 2019).

*Mar del Plata*

At the Institute for Biological Research, IIB-CONICET- National University of Mar del Plata, Gabriela C.
Pagnussat is studying the molecular basis of gametophytic development and fertilization in plants using *Arabidopsis thaliana* as a model. She also studies iron-dependent cell death in response to adverse environmental conditions (Distéfano *et al.*, 2017; Jaskolowski *et al.*, 2019).

**Córdoba**

In the early 1980s, Roberto A. Rovasio, initially trained by Pisanó in the embryological area and by Benito Monis in cell biology, returned to the Institute of Cell Biology of the Faculty of Medical Sciences from the National University of Córdoba (UNC), after a stay at the Institut d'Embryologie Cellulaire et Moléculaire (CNRS et Collège de France). During his training in France, under the direction of Nicole M. Le Douari and Jean Paul Thiery, Rovasio made pioneering contributions on the role of fibronectin extracellular glycoprotein as an essential factor in the migration of neural crest cells (Rovasio *et al.*, 1983), which marked the focus of his future research on birds.

In the late 1980s, Rovasio worked as a Professor in the Faculty of Exact, Physical and Natural Sciences of the UNC. In 2002, he created the Center for Cellular and Molecular Biology (CEBICEM, UNC), and in 2011 he joined the new Institute of Biological and Technological Research (IIBYT, CONICET-UNC) with his group. In Argentina, Rovasio oriented his studies toward the mechanisms involved in the dynamic behavior of avian embryo cells as well as their epigenetic disturbance. His research in vitro and in vivo showed that the cells of the cranial neural crest are oriented in relation to gradients of several molecules of their microenvironment.

Rovasio's global contribution was the demonstration of a chemotactic mechanism in the emerging neural crest cells of different levels - brain and trunk - of the neural tube, induced by trophic factors and by the sonic hedgehog morphogen (Shh), proposing the convergence of molecular signals in the modulation of cellular directionality during embryo development (Zanin *et al.*, 2013; Tolosa *et al.*, 2016). On the other hand, he investigated the effects of the administration of ethanol on the proliferation, migration and distribution in the embryo of neural crest cells and its relationship with the Fetal Alcoholic Syndrome (Jaurena *et al.*, 2011). In his laboratory, Rovasio also conducted research on mammalian sperm-oriented motility (Fabro *et al.*, 2002).

Ana Macias, conducted her postdoctoral studies at the Morata G’s laboratory at the Molecular Biology Center, Autonomous University of Madrid, Spain. There, she studied the functional hierarchy and phenotypic suppression among *Drosophila* homeotic genes (Macias and Morata 1996). Since her return to Argentina, she studied the Genital disc growth in *Drosophila* in the Genetics laboratory of the Universidad Nacional de Córdoba. Her group analyzes the contribution of apoptosis to growth regulation and the participation of Jun-NH2-terminal kinase (JNK) pathway in the regulation of these events (Benitez *et al.*, 2010; Arias *et al.*, 2015; 2019).
**Entre Ríos**

Valeria Sigot, at the Laboratory of Microscopy Applied to Molecular and Cellular Studies of the National University of Entre Ríos (UNER), has started a new group interested in studying the dynamic remodeling of intercellular contacts in embryogenesis of zebrafish.

**Santa Fe**

In 1987, Marcelo Cabada, a former Barbieri student, started to work at the National University of Rosario (UNR), Rosario, Santa Fe, as Professor of Biology at the Faculty of Biochemical and Pharmaceutical Sciences. He created a developmental biology laboratory in this academic unit. His work continued to focus on the elucidation of the molecular mechanisms of recognition between gametes in amphibians and mammals. In 1999, together with other local scientists, created the Institute of Cellular and Molecular Biology of Rosario (IBR, CONICET-UNR). Together with his student Silvia E. Arranz, he isolated and biochemically characterized two highly glycosylated proteins from the jelly coats from *Rhinella arenarum* (previously known as *Bufo arenarum*) oviduct, the highly glycosylated protein (HGP) and the highly glycosylated low molecular mass protein (L-HGP). They demonstrated that HGP forms a stable fiber matrix around the oocyte and is the main structural component of the jelly coat, while L-HGP is present in the jelly coat and is released into the incubation medium, protecting the integrity of the acrosome until sperm contact the vitelline envelope (Arranz et al., 1997; Krapf et al., 2006). Cabada’s team determined the glycoproteins composition of the vitelline envelope of *R. arenarum* and its participation in the spermatozoid-oocyte interaction during the early stages of fertilization (Scarpeci et al., 2008; Barisone et al., 2009). Arranz continued the studies on amphibian fertilization (Morales et al., 2012) and on the development of teleost fish, with particularly interest in growth hormone response of *Odontesthes bonariensis* (Di Prinizio et al., 2010; Botta et al., 2016). Dario Krapf, another Cabada’s disciple, leading a group at the IBR, focusses his efforts to understand the molecular events that underpin sperm capacitation. He has shown that PKA activity acts as a key player during this process. His studies aim to elucidate how a highly promiscuous kinase manages to activate different signaling cascades at different time points, and at different locations (Stival et al., 2015; Stival et al., 2018; Ritagliati et al., 2018).

Nora Calcaterra began her studies in Developmental Biology by identifying and characterizing mRNA binding proteins differentially expressed during oogenesis and early developmental stages of *R. arenarum* embryos (Calcaterra et al., 1999; Armas et al., 2001). Later, Calcaterra incorporated *Danio rerio* (zebrafish) as a study model to analyze the molecular mechanisms controlling gene expression during the specification and differentiation of craniofacial structures. Calcaterra´s lab is pioneer in Argentine in the use of zebrafish as animal model for studying developmental biology. Her research group analyzes the role of CNBP (cellular nucleic acid binding protein) in the folding/unfolding of non-canonical DNA structures (guanine quadruplexes) and its possible role in controlling the balance between proliferation and cell death of cranial neural crest
cells (Armas et al., 2004; Weiner et al., 2007; Armas et al., 2008; David et al., 2019). They also study the participation of miRNAs in the differentiation of melanocytes as an experimental approach to clarify the molecular basis of some genetic diseases (Weiner et al., 2019). The laboratory is also focused in deciphering the molecular bases of the Treacher Collins Syndrome (Rosas et al., 2019), a neurocristopathy that arise from defects in the development of the cranial neural crest.

At the IBR, a group of scientists led by Javier Palatnik studies the biogenesis and the role of microRNAs in the development of Arabidopsis thaliana. They analyze the participation of miR396 and miR319 in the complex regulatory plot of proliferative processes in the generation and growth of the different organs throughout the plant life cycle (Palatnik et al., 2003; Rodriguez et al., 2016; Ercoli et al., 2018). The laboratory seeks to develop tools for practical use that can be applied to species of agronomic importance.

In the city of Santa Fe, in the Institute of Agrobiotechnology of Litoral (IAL, CONICET-UNL), Andrés Dekanty leads the branch of the Molecular Biology laboratory devoted to the study of the cellular and molecular mechanisms that control cell growth and proliferation during the development of in vivo organs and tissues, using Drosophila melanogaster as a model system (Sánchez et al., 2019).

Developmental Biology promotion in Argentina

Since 2013, and on a biannual basis, a “Workshop on Cellular and Development Biology” is been held with great enthusiasm at the city of Chascomús. This workshop aims to generate a discussion space for researchers from the developmental biology field. Throughout the different editions (2013, 2014, 2016 and 2018) there was a growing participation of consolidated researchers with outstanding experience, as well as undergraduate students, doctoral and post-doctoral fellows from various research centers throughout Argentina and abroad. This broad participation generated an active scientific discussion and favored genuine collaboration among the participants. The active scientific discussion was achieved through the implementation of a dynamic format of the Workshop activities where many young scientists from the country and abroad present various themes and experimental models of animals and plants.

Final considerations

As can be seen through the evolution of Developmental Biology in Argentina, studies and the interest in solving biological development issues changed over the years. In the beginning, investigations were centered on the analysis of the large biological questions such as the reproduction, early development, gastrulation, the organogenesis that would lead them to understand the origin and formation of a new organism. Using the tools available during those first years, the biological processes were analyzed from morphological, descriptive and chemical and functional points of view, trying to answer the great questions of
biology. The studies initiated in Tucumán and Buenos Aires pioneered works and caused the migration of researchers who settled in different parts of the country, constituting the bases for the growth of developmental biology in Argentina, despite the great ups and downs of the socio-political situations of the country. Since the 1970s, the great technological advances with the great expansion of cellular and molecular biology and genetics allowed investigators to study the molecules involved in the different processes and their gene regulation. These very exciting facts are sometimes so difficult to contextualize within developmental biology that it would seem that it is difficult to determine the boundaries between the various areas of knowledge. At present, thanks to the enthusiasm of many young researchers, developmental biology has made considerable progresses, albeit still rather smaller compared to what happens in the most advanced countries. Finally, mention should be made to the active participation of the different research groups in the LASDB, the Latin American Society of Development Biology.

Last, but not least, the authors would like to apologize for facts and colleagues whose names and relevant work have not been cited here due to space limitations.
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Fig1. Prof. Armando Pisanó (1914-2008) and Prof. Francisco D. Barbieri (1930-1993)
Fig. 2. Pisano's drawings about amphibians gastrulation (toad) (from the chapter "Embriología" in the book "Biología General", by José. M. Cei and Armando Pisanó, 1951).
Fig. 3. Prof. Asa A. Humphries, JR (left) and Prof. Francisco D. Barbieri (right) at the Bellagio Study and Conference Center, The Rockefeller Foundation, Villa Serbelloni on the shores of Lake Como in Bellagio, Italy. 1983.
Fig. 5. Participants of the International Training Course "Initial embryonic development in vertebrates" sponsored by UNDP / UNESCO. Tucumán, Argentina, 1980. Else Brauckmann (1), Clara L. Valdez-Toledo (2), Juan A. Fernández Hidalgo (3), Claudio E. Barros (4), Arnaldo H. Legname (5), Francisco D. Barbieri (6), Armando Pisanó (7), Marcelo O. Cabada (8), Nestor G. Carri (9), Sara S. Sánchez (10), Alicia N. Sánchez Riera (11), Mario E. Manes (12), Marta I. Gomez (13), Marta I. Bühler (14), Luisa Peralta (15), Teresa Petrino (16), Liliana I. Zelarayán (17), Luis Izquierdo (18), Jorge Herkovitz (19), Silvia N. Fernández (20), Dora C. Miceli (21), Inés C. Ramos (22).
Fig. 6. Prof. Francisco D. Barbieri (top left), Prof. Luis F. Leloir (Nobel Prize, 1970) (top right), Prof. Sir John B. Gurdon (Nobel Prize, 2012) (down left) and Prof. Ruben Adler (down right) during the VI Argentine Congress of Biology, held on Tucumán in 1973.
Fig. 7. Distribution of the main Developmental Biology laboratories in Argentina.