

BIOLOGY / GENETICS

A deep-rooted lineage

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Neuronal embryonic stem cells
forming neural networks, seen through
fluorescence microscopy

**The institutionalization of human genetics
at USP has helped shape excellent
research teams in the Biosciences Institute**

“T

hey're here!” shouted a student of geneticist Mayana Zatz upon bursting into the classroom at the Human Genome Research Center (HGRC), in an annex to the Biosciences Institute of the University of São Paulo (IB-USP). The student was referring to the newborn pups of two female golden retrievers, both carriers of muscular dystrophy, a neuromuscular disease that leads to progressive weakening of the muscles and an inability to move. The dogs are part of a stem cell study, an important component of HGRC research efforts, along with more traditional studies such as those associated with the human genome. “We injected the stem cells into dogs with dystrophy for the purpose of trying to reverse the effects

of the disease,” Zatz explains. In a previous study on the same breed, her group observed that some of the animals carried genes that neutralized the negative effects of the mutation responsible for the dystrophy. The dogs had a genetic alteration associated with the disease, which prevented them from producing dystrophin, an essential protein for maintaining muscle integrity. None of the dogs presented the classic signs of dystrophy, such as difficulty in walking, however.

Zatz believes neuromuscular diseases are perhaps the first to benefit from stem cell studies. In cases of muscular dystrophy, she explains, there is muscle degeneration and, despite the fact that the body has many muscles, it is easier to replace an area of affected tissue than it is to make a new muscle through the use of stem cells. In 2012, Zatz’ group concluded that injecting adult human stem cells together with administering daily doses of a growth factor could be a possible alternative to the treatment of progressive muscular dystrophies in mice. In the study published in the journal *Stem Cell Reviews and Reports*, the group reports that while the combination did not generate new muscles, it reduced the inflammation and fibrosis in the existing muscle.

Zatz’ successful research studies in the field of stem cells and in other areas of genetics have made her one of the most visible Brazilian scientists in the world. As professor at the Biosciences Institute, the geneticist has headed up the HGRC, one of the FAPESP-funded Research Innovation and Dissemination Centers (RIDC), since 2000. In all, the group led by Zatz has already cared for 20,000 people suffering from neuromuscular diseases. “It is the largest sample in the world studied at a single center,” she says. “Today, we’re following the second generation of these patients.” The research and patient care conducted at the HGRC is one of the legacies left by geneticist Oswaldo Frota-

Pessoa, who died in 2010 at the age of 93. One of Brazil’s pioneers in human and medical genetics, he was Zatz’ undergraduate advisor in the 1960s. At the time, he was already caring for people with a variety of genetic diseases. “In his view, the patients helped generate new research studies, so the new studies had to help the patients,” Zatz explains. “Frota-Pessoa trained all the medical geneticists of my generation as well as the generation that preceded me.”

Born in Rio de Janeiro in 1917, Frota-Pessoa studied natural history at the University of the Federal District. In 1941, he graduated from what was then known as the University of Brazil. Still in Rio de Janeiro at the time, he began to collaborate with geneticists from USP, led by André Dreyfus, one of those responsible for bringing Russian biologist and naturalized American Theodosius Dobzhansky to Brazil from Columbia University where he had intro-

duced the genetic study of *Drosophila* (fruit flies). Dobzhansky is remembered as a researcher that made numerous requests for study trips, resources and equipment. As a condition for coming to Brazil, he demanded a research trip to the Amazon region. Dobzhansky’s visit influenced Frota-Pessoa to return to studying the Brazilian species of fruit flies during his doctoral program. In the 1960s, Frota-Pessoa was invited by Crodowaldo Pavan, another important member of the group that helped institutionalize genetics in Brazil, to set up a human and medical genetics unit in the Department of Biology at the Biosciences Institute. “This unit later became the HGRC,” Zatz says.

HISTORY OF EXCELLENCE

The network of individuals who later enabled the establishment of groups of excellence at USP in human genetics and genomics began to take shape in the 1930s, a decade marked by establishment of activities at USP in 1934. The history of these individuals is often mixed with the history of that university — the

Crodowaldo Pavan helped start the center for human genetics and medicine at the Biosciences Institute

IB-USP building, which houses the Department of Genetics and Evolutionary Biology, is named after André Dreyfus, for example. Its halls display signs of history at every turn. Among them are scattered the pieces of the collection of the Institute’s memory, including photographs, fossil collections, statues and even a cabinet belonging to Crodowaldo Pavan.

The Biosciences Institute was established in 1969. It included the departments of biology, botany, physiology and zoology, established in 1934 together with the Chair of General Biology, occupied by André Dreyfus at the USP School of Philosophy, Literature and Human Sciences (FFLCH). Dreyfus was among members of the committee tasked with developing the project to establish São Paulo’s first public university. It was admiration for him that led Harry Miller Jr. of the Rockefeller Foundation to fund the purchase of laboratory equipment and research, and to bring Dobzhansky to Brazil to give a course on evolution, attended by nearly all biologists of USP, the Biological Institute of São Paulo and the Campinas Institute of Agronomy (*see Pesquisa FAPESP Issue No. 168*).

Today, the third floor of the IB building is the site of the Molecular Genetics Laboratory, created in 1996 through a FAPESP-funded project and since then led by geneticist Lygia da Veiga Pereira. A physics graduate of the Pontifical Catholic University of Rio de Janeiro (PUC-RJ), Pereira came to the Biosciences Institute motivated by the work



Pavan in his office in the 1950s, in the department’s attic on Alameda Gleite

of the groups led by Mayana Zatz and biologist Angela Morgante, whose master's and doctoral advisor was Frota-Pessoa. In 2008, Pereira's group announced that it had obtained the first Brazilian lineage of embryonic stem cells, BR-1, from a single embryo frozen three years earlier. To get to the BR-1 lineage, Pereira says, 250 embryos had to be defrosted, of which only 35 developed to the fifth day, the stage at which the cells are able to be extracted. "This is how we replace imported stem cells and develop our own technical competence to obtain and maintain these lineages," Pereira says.

Up to now, her laboratory is the only one in Brazil to produce human embryonic stem cells. Pereira's group recently obtained funds for construction of the National Laboratory of Embryonic Stem Cell Research (LaNCE), which will produce lineages for clinical use. "The other part of the stem cells produced in the future laboratory will be used in testing for drug response," she says. Another important project developed in the laboratory involves what are known as induced pluripotent stem cells, mature cells that can be reprogrammed to again become able to generate a number of different body tissues. "We have developed a technique used to produce these cells here in the laboratory," Pereira says. "Using these, we plan to compile a library of pluripotent cells that represent Brazilian genetic diversity. In the future, we would like to be able to test the drug response of cells derived from the pluripotent cells in *in vitro* experiments."

Since 1996, Pereira's group has dedicated a portion of the laboratory resources to the study of Marfan syndrome, a genetic disorder characterized by the development of very long limbs, along with such things as cardiovascular, ocular and bone complications. Clinical manifestations of the syndrome in humans are quite varied, the geneticist explains. "Members of a single family with the same mutation can develop dif-



A biomedical researcher handles stem cells grown in a greenhouse at 37°C

The golden days may have been sequencing the genome of the *Xylella fastidiosa* bacterium

ferent problems. One person might have a cardiac problem, while another develops an ocular complication." In studies using genetically modified mice, the researchers are trying to identify the genes that interact with the gene responsible for the syndrome, which causes two people with the same disease to develop different complications.

RECOGNITION

The golden days of the Biosciences Institute, according to biologist and professor in the USP Botany Department, Marie-Anne Van Sluys, perhaps came during the time of sequencing the genome of the *Xylella fastidiosa* bacterium, responsible for citrus variegated chlorosis, also known as yellowing disease, which at the time affected 34% of the orange groves in the state of São Paulo. Van Sluys and her husband, molecular biologist Carlos Menck of the USP Biomedical Sciences Institute, were part of a group of 190 researchers from various institutions and fields that, through a virtual network of 60 laboratories, worked on what was considered to be the largest

science undertaking ever carried out in Brazil, targeting the genetic sequencing of an organism.

Launched in 1997 through the FAPESP Genome Program, the work of sequencing the 2.7 million chromosomal bases of *Xylella*, according to Menck, enabled USP researchers to enter a new field of knowledge: bioinformatics. "At the time, it wasn't clear whether the sequencing would constitute progress for us. There was a lot of criticism!" the researcher recalls. "But we became adept in terms of sequencing and data analysis technologies, especially with regard to bioinformatics, which had no community up to that point in Brazil," he says. "All of this represented a huge advance for USP research groups," he concludes. The project received international recognition in 2000 with publication of a cover story in the journal *Nature* about sequencing of the bacterium's genetic code (see Pesquisa FAPESP Issue No. 55). "It is important to mention the work of Andrew Simpson, the project's DNA coordinator, whose enthusiasm never faded, and who never cut corners, allowing us to move forward," Menck recalls.

For the ICB researcher, despite the important legacies left by the project, such as Alellyx, the biotechnology company founded in 2002 by Votorantim Novos Negócios, the USP researchers did

not continue to make contributions to the field of bioinformatics. “I believe one of the reasons for this was the difficulty in developing the field of genome bioinformatics that is still in its infancy at USP.” In any case, “the genome projects had a tremendous impact on USP, but we could have gone much further if we kept groups working on sequence analysis studies, especially from the evolutionary perspective,” he says. “Dobzhansky would have loved this!”

Successful sequencing of the *Xylella* genome broadened the scope of the FAPESP Genome Program, which was later engaged in other projects of significant social and economic interest. One of them was the Sugarcane Genome Project known as the FAPESP SUCEST Project, which was responsible for mapping 238,000 functional sugarcane gene fragments. “The SUCEST project paved the way for the use of molecular markers in crop improvement,” says molecular biologist Glaucia Souza, professor at the USP Chemistry Institute and SUCEST participant. Nearly 240 researchers from 22 institutions worked from 1999 to 2002 to identify the expressed sequence tags (EST) of sugarcane.

“The project enabled us to learn about sugarcane metabolism,” says Souza, who today coordinates the FAPESP Program for Research on Bioenergy (BIOEN) and SUCEST-FUN, which focuses on the functional analysis of sugarcane genes and the identification of genes associated with agronomic traits of interest. The group’s work is directed towards such topics as generating transgenic plants and investigating genes associated with sucrose content, biomass, drought tolerance, phosphate deficiency and climate change.

The researchers still want to understand how these genes work. Souza explains that initially, the project focused only on the functional DNA sequencing of sugarcane, ignoring the genes that had no known function. “Now we’re trying to identify strands of DNA known as promoters,” she says. Under an agreement with the Microsoft Research Institute for a research study on sugarcane genomics, her group is working on the annotation and analysis of the gene activity, which could allow the cultivation of varieties with higher or lower quantities of sugar in areas with little water. ■

BIOLOGY / ZOOLOGY

Evolutionary history in progress

Studies of vertebrates and invertebrates on land and sea aim to understand the processes of species diversification

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or over 40 years, zoologist Miguel Trefaut Rodrigues has been studying snakes and lizards to understand their biology and evolution. But sitting in the sand dunes of the São Francisco River or in areas of the Atlantic Forest or Amazônia to examine the shape and size of scales and measure animals captured by him or his colleagues never gets old. The research study he leads is monitoring advances in evolutionary science, a focus that pervades the Biosciences Institute and its Department of Zoology.

Currently heading up a large FAPESP-funded project that seeks nothing short of investigating the evolutionary history of reptiles and amphibians within the context of environmental changes, his group aligns traditional analysis of physical characteristics with genetic markers and models that take into account climate fluctuations that took place thousands of years ago. One example of the breadth of the research focus is the indication, from the analysis of 25 Brazilian vertebrates, that climate changes approximately 250,000 years ago had a different impact on endemic species diver-