Newsletter #4
February 2022

How to tackle the big challenges?

Enrique J. de la Rosa
Director at the CIB Margarita Salas

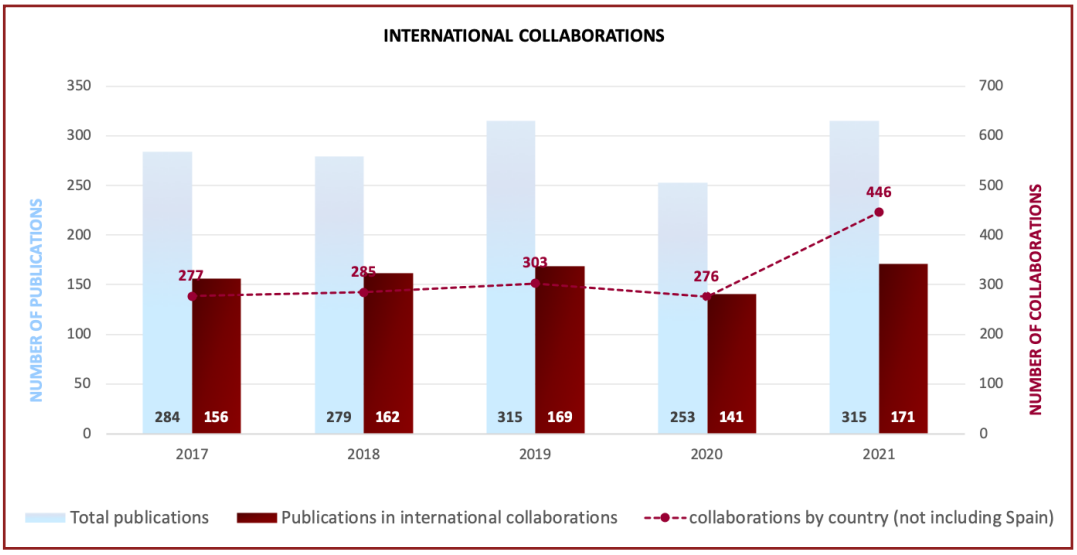
"For great ills, great remedies", as the saying goes. In the hope that this is the last mention of COVID-19 in an editorial in our newsletter, I refer once again to this disease, which has underscored the importance of multidisciplinary research to mitigate the personal and social impacts of the pandemic. We hope that we have learned our lesson, since challenges as great and as complex as COVID-19, if not more so, undoubtedly await us at some, as-yet-undetermined point in the future: bacterial resistance to antibiotics; climate change and all its possible consequences; the need for sustainable management of material resources, including food production, and energy; new zoonoses with the potential to cause new pandemics; etc. That these "great ills" also appear to be interconnected further emphasizes the importance of studying them from multiple perspectives. For example, excessive use of fossil fuels in energy production has fed climate change, which in turn, together with the destruction of ecosystems due to overexploitation, facilitates the spread of zoonoses and impacts food production. A comprehensive, multidisciplinary approach is necessary not only to tackle the pressing problems facing people, society, and the planet, but also some of the central issues in basic science, which comprise many more facets than a single discipline can unravel.

The scientific policy designed by the current CSIC presidency emphasized the need to improve our way of dealing with scientific issues long before COVID-19 emerged. Through the Interdisciplinary Thematic Platforms (PTI), the foundations of a new research framework were laid, with a view to providing superior approaches to investigate complex problems, and facilitating the transfer of research findings to society and to industry, one of the pending aspects of our R+D+i system. It is not that there were no previous examples of collaboration from different conceptual and experimental standpoints, but rather an absence of any such clearly defined framework within the CSIC.

In this same issue of the Newsletter we include the celebration of the 25th anniversary of the publication of a study by CIB groups directed by Santiago Rodríguez de Córdoba and Miguel Ángel Peñalva. From their different respective fields, and with the subsequent support of other researchers who complemented the skills of both groups, they joined forces for a new project that was far removed from their main interests: characterization of the molecular bases of the first disorder of genetic origin ever described: alkaptonuria. This resulted in an excellent publication, made the researchers pioneers in the field of full gene sequencing in Spain, and enabled the development of an important tool for the diagnosis of congenital metabolic disorders.

It is no coincidence that this happened in our center, nor is it surprising that various groups from the CIB Margarita Salas participate in several of the PTIs that emerged as a result of the EU's Recovery, Transformation and Resilience Plan: Susplast, Salud-Global, Trans-Ener, Neuro-Aging and, hopefully, the new Horizonte-Verde initiative. We have participated in and engaged with the initiatives of the PTIs+ and, more recently, that of *Conexiones CSIC*, in full coherence with the multidisciplinary nature of the CIB Margarita Salas since its inception, a fact not always well explained or well understood both outside and inside the center. This feature gives us the human resources and technical necessary skills to address complex issues, sometimes only laterally related to the specific lines of our research, but framed within the common goals of **Biology for Global Well-being**.

A few numbers



The publication of the results of our research in the form of articles in scientific journals, as well as reviews and specialized books that summarize the knowledge acquired in our fields of research, is the primary manner in which we share our findings with the scientific community around the world. In this way, we present our results for criticism and validation, and share them so that they can serve as the basis for future research to advance global knowledge and help resolve the problems faced by people, society, and the planet.

We are in the process of reviewing the publication quality assessment system in accordance with the recommendations of DORA (San Francisco Declaration on Research Assessment). For this reason, instead of bibliometric indices, we have decided to present the degree of internationalization of publications, in terms of collaboration with groups from other countries when conducting and publishing our work. This criterion has been, and will surely remain, a key benchmark. CIB Margarita Salas researchers published 1,446 publications in the 2017–2021 period, 55% of which include authors from other countries, resulting in a total of 1,587 references to those countries in the authorships. This constitutes documented proof not only of our knowledge generation activity, but also of our vocation for international collaboration.

Discovering the molecular basis of alkaptonuria: a first-hand account: CIB, 1995-1996

Miguel Ángel Peñalva
CSIC Research Professor at the CIB Margarita Salas

In September 2021 the CIB Margarita Salas celebrated the 25th anniversary of the discovery of the gene that causes alkaptonuria. The discovery, led by researchers Santiago Rodríguez de Córdoba and Miguel Ángel Peñalva, made the cover of the journal Nature Genetics in September 1996, and was the first time that the complete sequencing of a human gene had been performed in Spain.

Alkaptonuria is a rare disease that affects 1 per 250,000 people globally, and is the first genetic disorder ever described. It is characterized by urine that turns dark when it comes into contact with the air. Archibald Garrod, a British physician, studied this disease at the beginning of the 20th century and concluded that it was caused by malfunction of an enzyme due to defects in the instructions for its production. This was a very accurate estimation of the function of genes in living beings.

In the following piece, Miguel Ángel Peñalva describes his memories of how this recently commemorated discovery, and important example of interdisciplinary research, was conceived.

Biennium 1995-1996. Cornered against the narrow side of the parallelepiped on the fourth floor of the Joaquín Costa wing of the building on 144 Calle Velázquez, three groups of relatively young researchers were huddled together, but, in line with the interdisciplinary spirit of the CIB, each working on their very distinct research lines. "Los Peñalva" were at the corner, where we occupied two small laboratories, one facing calle Joaquín Costa, where my office (about 3 m²) was located, and the other facing the patio, where Teresa Suárez set up her lab. In the neighboring laboratory (towards the elevators), Flora de Pablo and Enrique J. de la Rosa investigated the role of growth factors in nervous system

development. On the other side of the corridor, with the windows facing the patio, was Víctor de Lorenzo's group, officially nicknamed "*Pseudomonas* Molecular Genetics", which had the invaluable support of Pepe Pérez-Martín. During that biennium, Pepe and Víctor published, in addition to several "minor" papers, two articles in PNAS and one in Cell, authored exclusively by Pérez-Martín and de Lorenzo!

A quarter of a century ago my laboratory worked on the transcriptional regulation of secondary metabolism in fungi, using as a model the penicillin biosynthetic pathway in *Aspergillus nidulans*, a close but genetically manipulable relative of *Penicillium chrysogenum*. We had signed a research contract with the firm Antibióticos SA, a company for which I had worked for six years. Penicillin G, a derivative of 6-aminopenicillanic acid in which the carboxylate of a molecule of phenylacetic acid forms an amide bond with the NH_2 group, was produced at its factory in León. To promote the biosynthesis of the desired product, the fermenters were supplemented with large amounts of phenylacetic acid. The drawback was that *Penicillium*, like its first cousin *Aspergillus nidulans*, was capable of degrading this compound. The objective of the project was to characterize the metabolic pathway for the degradation of phenylacetic acid in order to genetically inactivate it, first in the model organism as a proof of concept, and then in the production organism. To take charge of this project, I had hired José Manuel Fernández Cañón, one of the most brilliant people I have met in my scientific career. José Manuel had done his doctoral thesis supervised by José María Luengo at the University of León, working on the enzymology of the biosynthesis of β-lactam antibiotics, and therefore had the most appropriate experience for the project.

To clone the genes responsible for phenylacetic catabolism, we exploited the fact that, in fungi, many genes involved in the catabolism of carbon sources other than glucose are expressed at very high levels when the substrate of the pathway is added to the medium. For this reason, we used a subtractive RNA hybridization procedure, a technically complex process that was essentially based on the separation of double-stranded cDNA:RNA hybrids from single-stranded RNA, enriched in the transcripts of interest. In an ideal world, this separation would be performed in a hydroxyapatite column set up to recirculate 68°C water through an outer jacket. But that was a luxury we couldn't afford. Chema (José Manuel –I never understood why we call him Chema) had a house full of fish tanks and was a dab-hand at water recirculation, so he created a diabolical instrument with a conventional column inserted into a water bath full of algae and other micro and macroscopic slime. To my surprise, the RNA survived and the column worked, and when we characterized the retro-transcripts enriched by this system, we found that a large majority of the genes in the cDNA library corresponded to genes inducible by phenylacetic acid. When we began to characterize them, we realized that in addition to abundant cytochrome P450 cDNAs, potentially belonging to the phenylacetic catabolic pathway, there were others, the most abundant cDNAs, which clearly belonged to the phenylalanine and tyrosine catabolic pathway. We called the most abundant one fahA (pronounced facha). It encodes a protein whose amino acid sequence shares 47% sequence identity with human fumarylacetoacetase (hereafter FAH). There's a reason why fungi are first cousins to metazoans.

In humans, FAH deficiency caused by the presence of loss-of-function mutations in the homologous gene, either in homozygosis or compound heterozygosis, gives rise to a devastating disease called hepatorenal tyrosinemia type 1 (HT1). Cell damage is caused by compounds that specifically accumulate in the liver and kidneys when the phenylalanine and tyrosine catabolic pathway is interrupted due to enzyme deficiency. These compounds are both toxic and mutagenic, and therefore are the inevitable cause of liver cirrhosis and hepatocarcinoma. Until relatively recently, this very serious disease could only be treated by liver transplantation early in life. Today, the availability of a drug called nitisinone, which interrupts the catabolism of aromatic amino acids at the level of an enzyme upstream of FAH, has substantially improved the prognosis and treatment of children with this congenital metabolic deficiency.

The diagnostic metabolite sought in neonatal screening for this disease is a compound called succinylacetone (SA). In short order we had constructed a deletion mutant of the *Aspergillus* fahA gene, and Chema had used the "litmus test" (gas chromatography coupled with mass spectrometry) to demonstrate the accumulation of SA in the cultures of the mutant fungus supplemented with phenylalanine. This showed that the similarities between fungi and humans also extended to the biochemical level, since equivalent deficits led to the accumulation of identical compounds. In fact, when we grew the fahAΔ mutant fungus on culture plates supplemented with lactose as the only carbon source, it grew just as well as the wild-type fungus. However, when we added a mixture of lactose and phenylalanine to these culture plates, the mutant, unlike the wild type, was unable to grow because it accumulated toxic compounds derived from Phe/Tyr, which cause liver and kidney damage in humans.

Meanwhile, four floors below, at street level on Calle Joaquín Costa, three researchers shared a large laboratory. One of them was Santiago Rodríguez de Córdoba, who on his return from the United States had worked on

a project focusing on diseases associated with the complement cascade. In the late 1980s Santiago and I had arrived at the CIB, then directed by Pepe Gómez Acebo. We had shared a laboratory and, by extension, had shared the hardships and unmet needs of new arrivals to the CIB. Almost 10 years later, at the time of the HT1 discovery, Santiago and I also shared a love of road cycling, and practically every weekend we went out to ride together with a mutual friend, Tito Silva. During the hours we spent on the bicycle we had a lot of time to talk about everything, including tyrosinemia. We each were very familiar with the other's research projects because our laboratories were directly connected by the fire escape, which made it easy for us to have frequent meetings in which we chatted about our objectives.

Meanwhile, Chema and I were very excited, because among the genes of the phenylalanine catabolic pathway that were still uncharacterized, and possibly represented in our cDNA library, was the gene that encodes homogentisate dioxygenase (HGO), the protein that British physician Archibald Garrod at the beginning of the 20th century had proposed was deficient in alkaptonuria (AKU) patients, a fact subsequently proven by Bert La Du in 1955. Alkaptonuria, in addition to its medical importance, was of enormous historical importance: it was the disease with which Garrod coined the term 'inborn error of metabolism', and the first human disease that conformed to the pattern of recessive inheritance postulated by Mendel's laws, as the geneticist William Bateson had informed Garrod himself. Surprisingly, and despite this importance, the human gene had not yet been characterized in 1995, nor had the mutations that cause the disease been described. In other words, the molecular basis of alkaptonuria were unknown.

Miguel Angel Peñalva (left) and Santiago Rodríguez de Córdoba (right) along with their respective Pinarello and Colnago bicycles in Plaza Mayor de Brunete.

Chema and I were convinced that, because the phenylalanine metabolic pathway is so highly conserved, once the *Aspergillus* HGO transcript was identified in our cDNA library, with a protein sequence identity level of 50% between human and fungal enzymes that catabolize phenylalanine, it would not be very difficult for us to find RNAs encoding the homologous protein in the incipient databases of human transcripts. We arrived at the *Aspergillus* gene via two different routes. First, sequencing of the cDNA library revealed the existence of clones that potentially encoded dioxygenases. But molecular biology approaches would have been insufficient to identify HGO: this would have involved checking clone after clone to rule out the possibility that candidates encoded enzymes in the phenylacetate or parahydroxyphenylpyruvate catabolic pathways, and unequivocally identifying it as HGO belonging to the phenylalanine pathway. Genetics came to our aid. When plated on culture plates containing lactose and phenylalanine, the fahAΔ mutants were unable to grow. But if the plates were left on the table for several days, areas of arborescent mycelium appeared around the sites of inoculation, which we, as fungal geneticists, quickly identified as mutant clones that carried extragenic suppressor mutations that rescued viability.

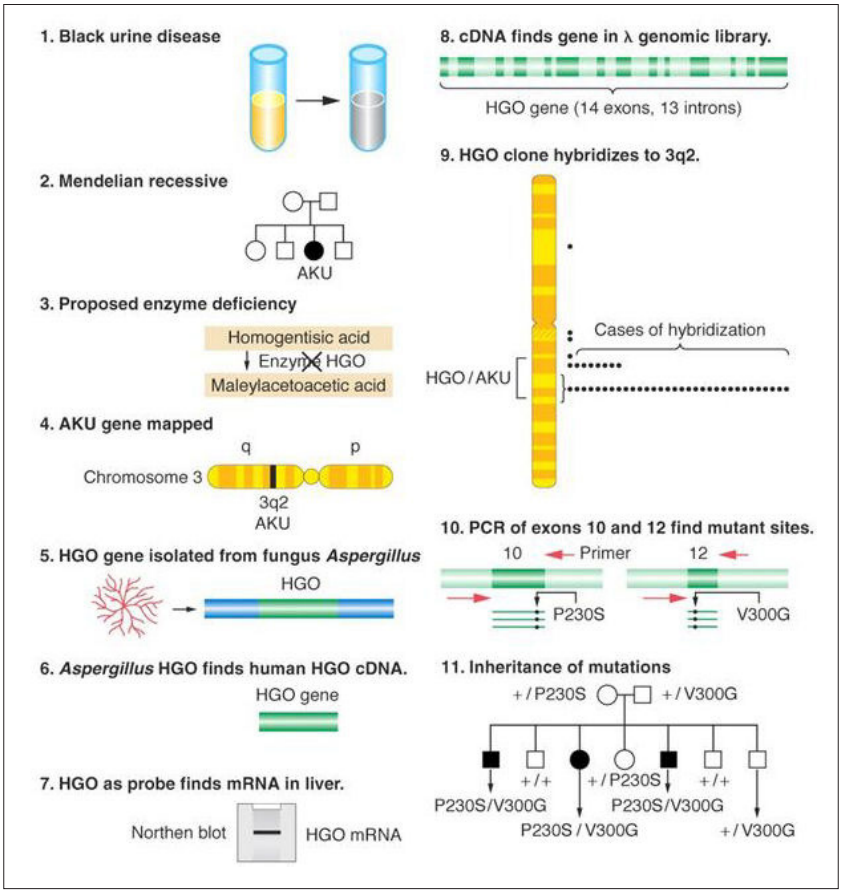
Human patients with alkaptonuria accumulate homogentisic acid as a consequence of enzyme deficiency. This acid is excreted in large quantities in the urine, and is easily oxidized in the air, giving rise to a series of coloured compounds characteristic of this disease. These coloured, phenolic compounds also accumulate in the cartilage of large joints and in other areas of connective tissue, giving rise to a progressive, disabling disease. The arborescent sectors that appeared in our culture plates accumulated a compound that gave the medium a colour similar to that of the urine of alkaptonuria patients. We thus realized that we had identified the gene.

Fortunately, through classical genetics experiments, we were able to determine that the FAH and homogentisate dioxygenase genes were closely linked, separated by less than a recombination unit, on chromosome VII of the fungus. So, walking a few hundred base pairs down the chromosome, we found a gene whose product was, eureka!, a dioxygenase. When we ablated the gene, which we called hmgA, using genetic engineering, the mutant strains accumulated homogentisic acid. By means of enzymatic assays we showed that the mutant strains, unlike the wild-type, lacked homogentisate dioxygenase activity.

The next step was obvious: with the help of Santiago,

using a TBLASTN search, we screened the human databases of short RNA sequences (expressed sequence tags; ESTs) available at the National Center for Biotechnology Information (NCBI), and identified RNAs that were expressed in the expected tissues and that allowed us to practically reconstitute the complete coding sequence of human homogentisate dioxygenase. Before the summer of 1995, Chema and I submitted papers to *PNAS* and *JBC* with the summary of our experiments and the identification of human ESTs, which were accepted almost immediately. The partial cDNA sequences were already published in the autumn, which was dangerous, as they were completely accessible to potential competitors.

Meanwhile, Santiago and I talked almost daily about the progress of this project. There was still a long way to go to identify the molecular basis of alkaptonuria. We would not have travelled this road without Santiago, for two reasons. First, because at that time we had next to no experience in human genetics; and second, because when Santiago realised that the project was not only exciting (which he already knew) but also feasible provided he was fully involved, he did not hesitate for a minute to abandon other projects and tackle the problem with all his intellectual and technical resources, which were many. Success was assured.



Analysis of alkaptonuria (black urine disease).
from: "An introduction to genetic analysis", Anthony Griffiths. Copyright © 2000, W. H. Freeman & Co Ltd.

Santiago took two approaches. The first was to map the position of the gene, using the cDNA probe, on a human karyotype. To do this, he requested assistance from a collaborator of his, Elena Fernández-Ruiz, at the Hospital de la Princesa, who was an expert in fluorescence *in situ* hybridization (FISH) of chromosomal spreads with fluorescent gene probes. Elena conclusively demonstrated that the gene that codes for the RNA that we had reconstructed mapped to the q2 band of chromosome 3. A couple of years earlier, a team from the Pasteur Institute had proposed that this was the position on which the causative gene for alkaptonuria should be located. Despite the fact that this region contains a good handful of genes, Elena and Santiago's experiments provided solid evidence that the gene we had identified was the one affected in alkaptonuria patients, and hence the cause of the disease.

However, it was the second approach that was most critical for our goals. We had to show that loss-of-function mutations in the gene located at 3q2 co-segregated with the disease, and that patients were homozygous or compound heterozygous for the causative mutations. The first step to achieve this goal was to be able to sequence the exonic regions of the human AKU gene. At that point, we knew the complete coding sequence, but we lacked information about the intronic regions, which was necessary for us to design the primers to amplify the exons by PCR. Begoña Granadino, at Santiago´s laboratory, tackled this challenge, and by screening a genomic library she was able to reconstruct the complete gene and determine its sequence. The HGO gene was the first human gene to be completely sequenced in Spain. It is 54,363 base pairs long. Its coding sequence is distributed over 14 exons ranging in size from 35 to 360 base pairs. This organizational complexity contrasts with the much simpler structure of the *Aspergillus* gene, which is interrupted by a single intron of a few dozen base pairs. In the intronic sequences, Begoña detected several simple sequence repeats (SSRs) that were highly polymorphic among the different patients. This finding was extremely useful in reconstructing the geographic origin of the different mutations. But that story is for later.

By this point, at the beginning of 1996, we were at a critical point. Reconstruction of the human cDNA had allowed Chema to demonstrate that the encoded protein had HGO activity, but we could not go any further without having the possibility of genetically characterizing patients. This task was far from easy, since the incidence

of the disease is one patient for every quarter of a million births. At this point we benefited from the enthusiastic and selfless help of Dr. Magdalena Ugarte, Professor of Biochemistry at the Autonomous University of Madrid and director of the Center for Diagnosis of Metabolic Diseases (CEDEM). Maleni used all her resources and influence to find patients who wanted to collaborate with our research. Finally, she found a family in Seville with healthy parents and, out of seven siblings, two sons and a daughter with the disease, and who kindly agreed to donate blood for genetic analysis. Santiago bought a train ticket to Seville to obtain blood samples. Soon a second family was located in Madrid consisting of three children, a man and a woman with alkaptonuria, and a third healthy sibling.

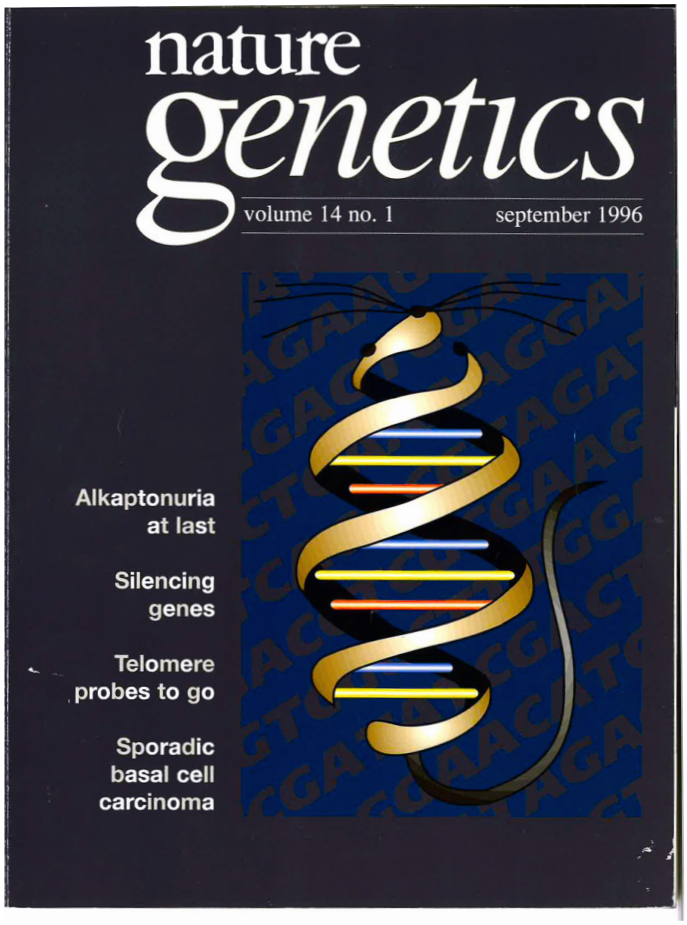
Sequencing of the exonic regions of the AKU gene in samples from all these families was carried out by Begoña, Chema, and a new student in Santiago's lab, Daniel Beltrán-Valero de Bernabé, who would write his thesis on the alkaptonuria project. This study revealed that, as expected based on Mendelian inheritance, both parents in the M family were heterozygous for a C>T transition at position 817 of the coding sequence, resulting in substitution of proline 230 by serine in the enzyme. Both the affected son and daughter were homozygous for this mutation, while the unaffected daughter was heterozygous, consistent with Mendelian recessive inheritance of the disease. The pathogenic nature of the P230S allele was underscored when we discovered that the three alkaptonuric children in the S family were compound heterozygotes, carriers of P230S and of a second mutation resulting in the V300G substitution, while the remaining four healthy siblings were either homozygous for the wild-type allele or heterozygous carriers of a potentially pathogenic allele (P230S, V300G) and the wild-type allele, which behaved as dominant. Thus, Mendel's second law in a medical context. To conclusively demonstrate that the P230S mutation was pathogenic, Chema introduced it into a plasmid expressing the human HGO enzyme in bacteria. Pure P230S protein was essentially devoid of enzymatic activity. The work was complete and ready to send for publication.

To avoid "surprises", we had previously established contact with Kevin Davies, Chief Editor of *Nature Genetics*, who had expressed interest in publishing the work if it passed the review process. In 1997, *Nature Genetics* had an impact factor of 38.8, which compared favourably to *Nature*'s 27.3, and even *Cell*'s 37.3. In 1996, the Internet and email were quite limited. The reports from the reviewers arrived by fax, signed by the deputy editor Lauri Goodman, who told us the good news that they had rated the manuscript highly, as well as her own impression that the manuscript represented a "milestone in the history of alkaptonuria". The manuscript, reference NG-3891, was proofread and returned to the Washington, D.C., office on July 8. On July 12, 1996, we received news by fax of definitive acceptance of the article. The study was published in the September 1 issue, with a sentence on the cover that read 'Alkaptonuria at last'. Inside was a highly complimentary News & Views piece by Charles Scriver, the most respected figure in the field of metabolic disease. Scriver, a medical doctor by training and profession like Garrod, is a fervent admirer of the latter's scientific career and had shone a light on the figure of Garrod, who was frequently ignored, to ensure he received appropriate recognition in the history of medicine. He had been responsible for the reissue of Garrod's key book, published in 1931, entitled "Inborn Factors in Disease". The book had anticipated key concepts such as genetic susceptibility to disease, and impressively predicted the future of personalized medicine. Indeed, Scriver was most likely the reviewer who had signed off his evaluation of our paper with the following words: "Thank you for this elegant paper. Garrod would be delighted. Certainly this reader is".

From that point, two new stories began. One was international recognition for such an original approach and the soundness of the experiments with which we had demonstrated the molecular basis of alkaptonuria. This success was retold by Matt Ridley in his famous bestseller "Genome, the autobiography of a species in 23 chapters", by Jim Watson himself in his book "DNA, the secret of life", and even in a book on the philosophy of science "The Missing Moment: How the Unconscious Shapes Modern Science", by Robert Pollack. The seventh edition (2000) of the classic genetics textbook "An introduction to Genetic Analysis" also retells the story, to which it dedicates two full-page figures. Finally, my post-doctoral mentor, Claudio Scazzocchio (then at Paris-Orsay), published a complimentary editorial in *Trends and Genetics* entitled "Alkaptonuria, from human to moulds and back".

The second story is that of a success of which much of the Spanish scientific community may be unaware. Santiago founded a mixed molecular pathology unit at the prestigious Fundación Jiménez Díaz, where he moved in the second half of 1996. As the reader will no doubt have guessed, Santiago received very little material support for that initiative, and in a relaxed talk between friends reminded me that he had to paint the lab himself.

Despite these "minor" drawbacks, Santiago resumed work on alkaptonuria with his student Daniel Beltrán-Valero de Bernabé and through their work the AKU gene, which up to then had been poorly characterized, became one of the best-studied human genes. During this adventure, Santiago and his group demonstrated that the alkaptonuria gene was populated with sequences hypersensitive to mutation and characterized



the gene in patients from practically all over the world, from Slovakia, which has a population with a much higher incidence of alkaptonuria than the rest of Europe, to Reunion Island, where there is an interesting phenomenon called "the Reunion paradox": a founder effect with multiple allelic variants, which seems paradoxical. These population studies have even proved important for the study of human migrations, traced through the movement of allelic variants of alkaptonuria and the haplotypes associated with them. By this stage, thanks to this work by Santiago and the methodical isolation and characterization of new mutants of the fungus carried out by my assistant Elena Reoyo, we had accumulated a remarkable list of mutations, which Santiago compiled in a table that was published on the CIB website under the name of AKUdb. This table was almost lost, but fortunately we were able to recover an intact version that we hope to soon add to a website/portal that will collect historical and scientific information on alkaptonuria for academic use.

In the meantime, José Manuel Rodríguez Rodríguez, Secho, had joined my laboratory and was purifying and analysing the enzymatic activity of all mutant HGO proteins that seemed particularly interesting to us. While Secho was analysing kinetic constants, David Timm, an American crystallographer then working at Indiana University, contacted us to help resolve the 3-dimensional structure of the enzyme, which turned out to function in a homohexameric manner. The corresponding work, which was published in *Nature Structural Biology* in 2000, allowed us to study the relationship between genotype and biochemical phenotype by correlating the kinetic constants that Secho was measuring with the predictable effect of mutations in a structural context. Perhaps this is the high point at which I should end the story.

But I can't resist. I haven't finished yet. I want to emphasize two fundamental aspects of this long history. First, the incalculable value of the principle of serendipity; that is, an accumulation of coincidences that I hope I have described with sufficient clarity in the preceding paragraphs, which led to the establishment of a powerful multidisciplinary team capable of tackling the great challenge of identifying the alkaptonuria gene in the mid-1990s. The second is that this cluster of coincidences would not have been possible if the CIB had not been a multidisciplinary center that accommodated groups with interests and experience as disparate as mine and that of my good friend Santiago.

"For us, multidisciplinarity always has been and will be a strength"

Carmen Fernández Alonso
PhD in Chemistry at CIB Margarita Salas

Since its creation in 1953 the CIB Margarita Salas has brought together researchers from diverse areas in microbiology, enzymology, endocrinology, metabolism, neurology, developmental biology, etc., giving the center a constantly evolving, multidisciplinary and dynamic character, with a mission of furthering our knowledge in biology in the broadest sense.

Traditionally, this multidisciplinarity has been perceived as a negative factor. However, in the context of scientific research, dealing with global problems requires the development of strategies that address these challenges from an interdisciplinary standpoint.

An example of interdisciplinary research carried out at the CIB Margarita Salas is the study recently published in the Journal of Experimental Botany by researchers Pilar S. Testillano, Ana Martínez, and Carmen Gil. They demonstrate for the first time in plants that certain small-molecule inhibitors the enzyme glycogen

Ana Martínez

Carmen Gil

Pilar Sánchez Testillano

synthase kinase 3β (GSK-3β) in mammals increase the rate of cell reprogramming and the production of somatic embryos in several cultivated species, specifically in rapeseed, barley, and cork oak, a finding with important technological implications.

Pilar S. Testillano leads the Pollen biotechnology of crop plants group at the CIB Margarita Salas and is currently deputy director of the center. Ana Martínez and Carmen Gil are principal investigators at the Translational Medicinal and Biological Chemistry group and co-founders of the CSIC spin-off Ankar Pharma SL. In this interview we talk to the researchers about this work, and ask their opinion on multidisciplinarity.

Question | To begin with, can you briefly describe the work carried out in your groups and your main lines of research?

Pilar S. Testillano (PST) | In our laboratory we investigate the mechanisms that regulate cell reprogramming in plants and the development of the pollen grain, as well as their biotechnological applications in sustainable agriculture. Among these regulators, we analyse the role of autophagy, proteases, epigenetic and hormonal factors, and cell wall remodelling. One of the main biotechnological applications of cell reprogramming is somatic embryogenesis, which allows selected plants to be regenerated *in vitro*, in a massive and controlled manner and in much shorter times than in the field. One benefit of this is that it can accelerate the improvement and development of new agricultural varieties that are more productive or better adapted to the stresses caused by climate change, and to the forestry sector. For example, it allows us to markedly shorten the production and propagation time of trees that are resistant to pathogens or adverse environmental conditions for reforestation and conservation programs in threatened ecosystems.

Carmen Gil and Ana Martínez (CG/AM) | Our group has extensive experience in the design, synthesis, and development of new drugs for neurodegenerative and infectious diseases, mainly. In these two large areas, there is currently a lack of effective treatments and, at the same time, a great need for them given the many people who suffer from them and the social and personal costs that they entail.

P | With this context in mind, how does a collaboration emerge from lines that are seemingly so far apart? What has motivated this work?

PST | *In vitro* plant regeneration technology still has many limitations. For example, it is very inefficient in many agroforestry species. So the search for new methods that promote cell reprogramming is an objective of our laboratory. I was aware of findings in the field of human cell biology that demonstrated the great potential of "small molecules" to promote reprogramming in mammalian cells, and I thought that perhaps some of these molecules could also enhance the same process in plants. So I asked Ana and Carmen what they thought of the idea and whether we could test some of the molecules they had designed for therapeutic purposes in our laboratory's *in vitro* plant embryogenesis systems. They loved the proposal and we started the collaboration, with a very innovative idea. The results indicate that it will be very useful.

CG/AM | As a result of our research, we now have our own library of highly valuable small molecules, which can be used as pharmacological tools for various emerging projects. Many of these molecules are characterized and we know some of the targets they modulate and their pharmacological action. One of the active research lines is the design of small molecules that can stimulate endogenous neurogenesis - that is, the production of new neurons from existing neural stem cells in specific areas of our brain. These molecules could have great therapeutic potential for the repair or modulation of many neuronal damage processes. When Pilar asked us if we had any compound that could help stimulate embryogenesis, we thought that perhaps these molecules could have this effect, since we had already tested their ability to modulate adult stem cells in animal models.

P | What are the main results you have obtained?

PST | Several molecules have worked, increasing the

rate of reprogramming and embryogenesis and the *in vitro* production of somatic embryos (from microspores and other cell types). In addition, the enhancing effects of these molecules have been demonstrated both in agricultural species, such as rapeseed and barley, and in a forest species, the cork oak. We have also shown that one of these molecules exhibits activity in plants that is analogous to that described in mammals: inhibition of the enzymatic activity of GSK3 in plants, which in turn activates the brassinosteroid hormonal signalling pathway and promotes embryogenesis. These findings are very novel since they suggest a certain homology of cell reprogramming signalling pathways in animals and plants, and also reveal a new pathway via which we can intervene in this process in plants.

CG/AM | It worked! In other words, treatment of plant embryos of very diverse species with our molecules increases the capacity for embryogenesis and therefore the number of new plants. This tells us that, in living beings, the cell signalling pathways for very basic processes such as cell reprogramming must be quite conserved.

P | What are the main applications of these findings?

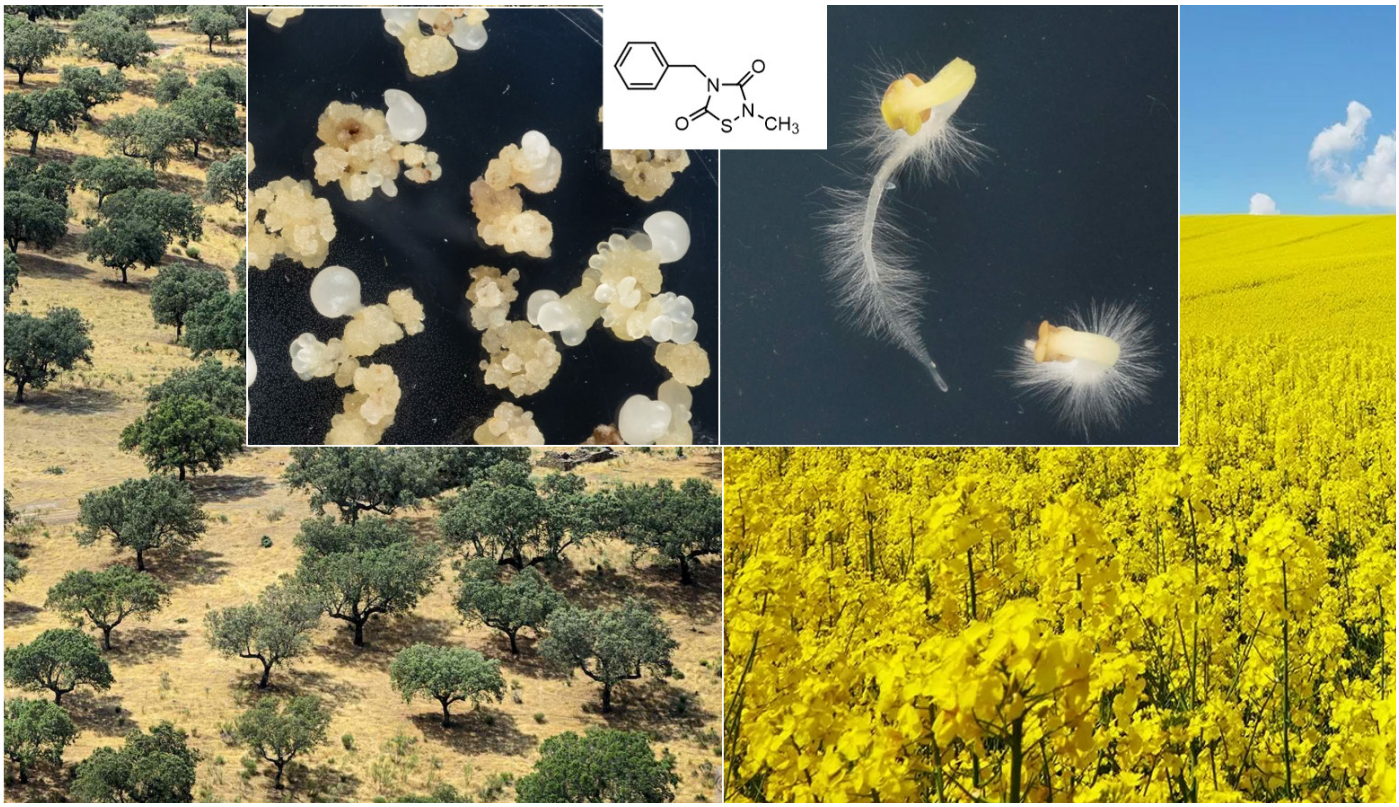
PST | These results constitute a totally new technological innovation in the field of *in vitro* plant cultivation, since this type of molecule and the protocols that we have developed had never been used previously: we should emphasize that these are molecules from chemical libraries in the field of biomedicine. Their application will increase the efficiency of *in vitro* production of embryos and plants in species of interest, in order to improve selection and propagation of genotypes with superior productive capacities, quality, or resilience to stress, which is currently a major problem in agricultural and forestry nurseries. The findings can also be used to improve *in vitro* regeneration of plants using gene editing techniques, which is also a major bottleneck in the agrobiotechnology sector.

CG/AM | The applications are very varied and all highly valuable in the fields of agriculture, forestry, and agrobiotechnology. Our first patent has been widely accepted and several national and international companies are already working with our compounds in different applications.

P | Are you going to continue with this line of research? What are the next steps?

PST | Of course, these results have encouraged us to test more molecules with which Ana and Carmen's group is very familiar and that exert interesting effects in mammals and could also promote *in vitro* reprogramming and regeneration in plants. We also want to extend the studies to other species and varieties of agricultural and forestry interest in which somatic embryogenesis is very inefficient, and which we hope these new molecules can improve. We are also investigating possible targets of these small molecules in plants, which is providing new information on the regulation of these processes in plants.

CG/AM | We are delighted with this collaboration, which is very close to market launch. Now we are going to take two important steps. First, we are attempting to



Chemical structure of a synthetic small molecule (center), originally designed for therapeutic purposes, which has been shown to promote cell reprogramming and somatic embryogenesis in plants. Left: cork oak somatic embryos (top) produced *in vitro* and panoramic image of cork oak (bottom). Right: *in vitro* germination of somatic embryos of rapeseed microspores (top) and flowering rapeseed field (bottom)

modulate the embryogenesis of several forest species, which entails selecting or modifying the molecules so that they can be prepared easily and economically, since they must be used in a solid, gel-like culture medium, which hinders diffusion. This is the most common system in which embryos and seedlings are developed *in vitro* in the laboratories of nursery companies in this sector. The second direction represents the start, in our laboratory, of the search for epigenetic modulators, which we know modulate plant embryogenesis. We will also explore their therapeutic potential in various human diseases.

P | This study is an example of how interdisciplinarity enables pioneering and innovative results. What is your opinion on the negative perception of multidisciplinarity in some quarters?

PST | To me it is very clear that multidisciplinarity is a strength, and the results that we are discussing demonstrate this, since they would never have been obtained without an approach combining two disciplines that, *a priori*, are very distant from each other: plant biotechnology and medical chemistry. Sometimes it is not easy to find collaborators in other areas, even if you have an idea that can only be developed with the assistance of an expert in another discipline. The CIB Margarita Salas houses multiple research groups working in very diverse areas, which makes it easier to create these synergies. Furthermore, in our case, the level of understanding and enthusiasm in this collaboration has been excellent from the start.

CG/AM | In reality, we are very used to multidisciplinary research because medicinal chemistry combines biology and biomedicine: this is the only way to generate molecules with high added value, such as drugs. For us, multidisciplinarity has always been and will be a strength, and we have always believed in it.

Well-being and knowledge, intermixed

Mercedes Jiménez Sarmiento
CSIC Scientist at the CIB Margarita Salas

It seems obvious that knowledge generates well-being.

How many times has a lack of knowledge of a piece of information or an address filled us with uncertainty or anxiety, only to evaporate in an instant once that knowledge is acquired?

The definition of well-being covers three meanings: a set of things necessary to live well; a comfortable life, in which one has everything they need to live well, in peace; and, finally, a state of being whereby the individual is conscious of the proper functioning of their own mind and body.

This last meaning seems important to me: "to be conscious". To feel, in short, a direct allusion to the senses, which inform our brain of what is happening outside and become conscious of it.

We have evolved thanks to our curiosity

Curiosity has been part of our evolution. We need to be informed not only to satisfy curiosity but also because it broadens our points of view: the more one knows, the better one can argue.

Although it may be difficult to admit, uncertainty is part of life itself, and in this context we have an innate need for knowledge. Without it we would not have evolved or shaped the world around us as we have.

The process of knowing, of learning, entails honing one's skills in order to be able to perform an activity with greater ease. This knowledge also makes it possible to differentiate and categorize, favouring the development of critical thinking. Knowledge of alternatives allows us to make informed opinions, with greater decision-making power, far from the simple slogans of leaders or snake-oil sellers, emotional manipulators who shun reflection and considered reasoning.

Can we live without learning?

Well, it seems not, because even the smallest or least developed (or both) organisms contain well-described molecular mechanisms to extract knowledge from their environment and decide what to do when faced with a stimulus. Sporulate? Attack? Flee? Just look at the SARS-CoV-2 virus and the adaptation of its S protein to the human ACE receptor. The evolutionary development of the senses and their subsequent processing in the brain is sufficient proof that we must explore the environment and draw conclusions to survive, learn and then act, decide and live better.

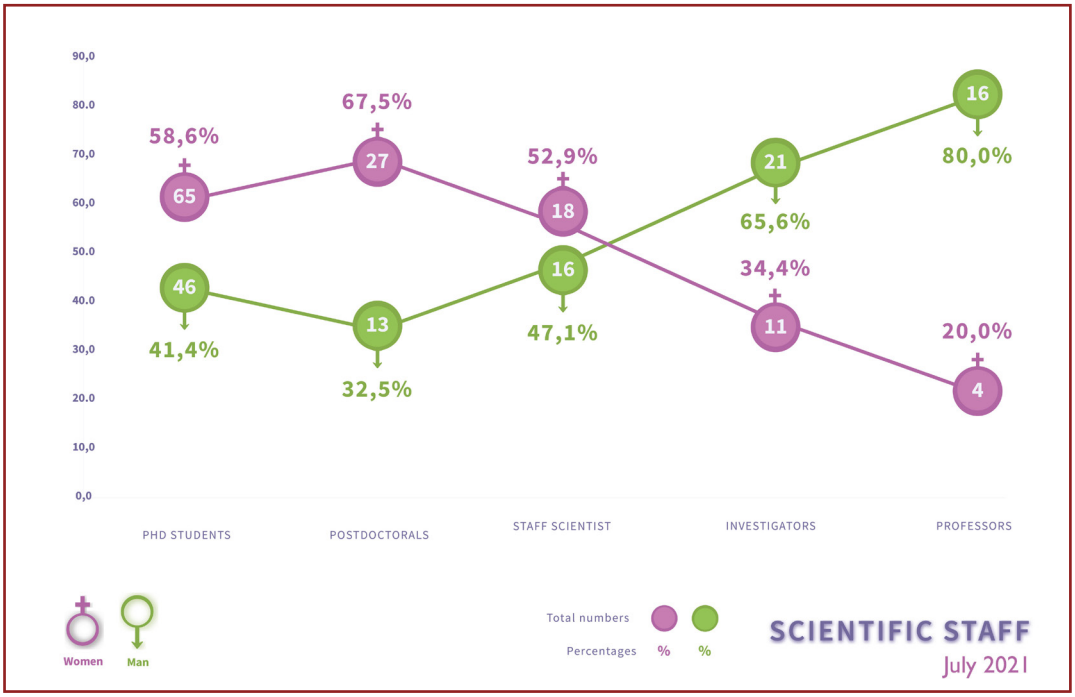
Scientific knowledge and well-being

If we limit our definition of scientific knowledge to the conclusions obtained after investigating a process using the scientific method, we would be limiting the definition of well-being to the curiosity of the scientist engrossed in his work.

And it is worth asking ourselves whether scientific knowledge leads to well-being for the average person. Science emerged as a rational response to the questions of how and why things are the way they are, and the desire to understand the world and be able to transform it. If we stop to observe what surrounds us, everything we use on a daily basis is derived from science: medicine, drinking water, food, energy, appliances, vehicles, technology. In short, an endless list of items that contribute to our well-being. And these are not exclusively the products of applied science: basic research is indeed the basis of well-being. Nowadays, no one wonders whether there is any point in doing research on phage Φ29. Mario Bunge, physicist and philosopher of science who died in 2020, was adamant: "We must abandon the idea that basic science is a luxury or only serves to produce technology, tolerating it when it produces immediate results but not when it simply explores the world".

Mario Livio studied what makes us curious and argued that one of the reasons was to obtain a reward. Well-being is a reward for knowledge.

The CIB Margarita Salas creates its Gender Equality Commission



The CIB Margarita Salas has always been committed to the advancement and promotion of women's careers in science, and the development of initiatives to inspire and nurture female vocations in disciplines within STEMM.

To avoid any discrimination related to gender, age, nationality, class, sexual orientation, or disability, the CIB Margarita Salas has been presenting statistics disaggregated by sex for more than 15 years and all the center's selection committees and boards fulfil parity requirements. It has taken steps to comply with current regulations on maternity/paternity leave, supporting optimal working conditions for pregnant women and offering a private lactation room. A specific protocol against sexual harassment has been implemented and awareness has been raised about gender imbalance in the institute.

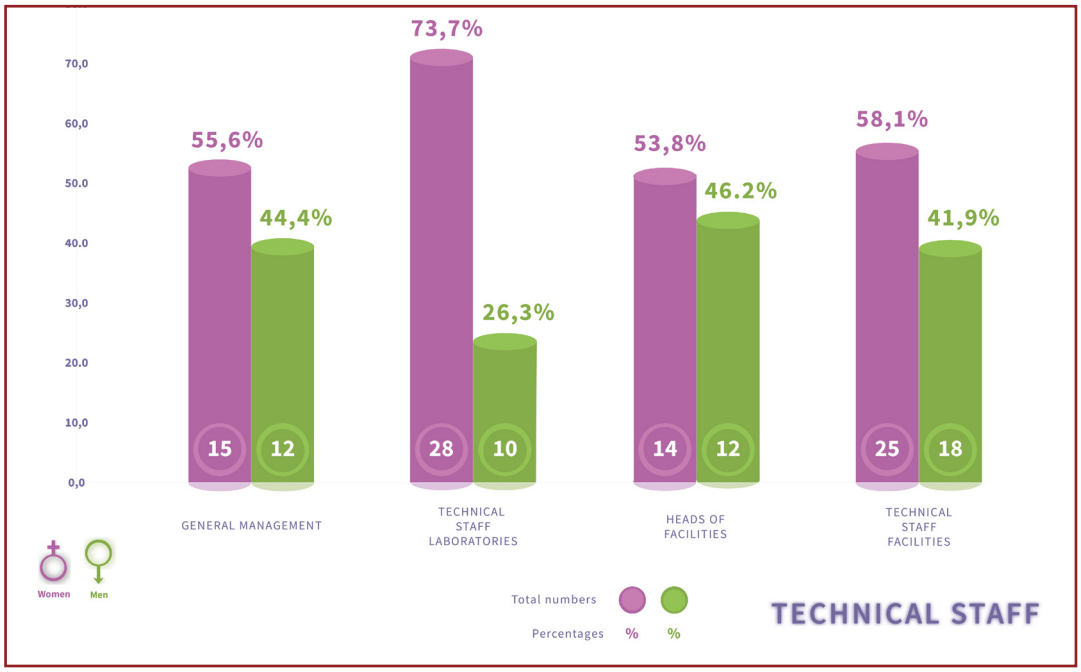
To coordinate efforts and strengthen these measures to reduce the gender gap and promote women's participation at all professional levels, the **CIB Margarita Salas Gender Equality Commission** was created in February 2021. The Commission is made up of eight members from the different scientific and administrative levels of the center: Enrique J. de la Rosa (Director of the CIB Margarita Salas), Teresa Suárez (Head of the Commission), Javier Redondo, Aurora Gómez-Durán, Gema Elvira, Francisco J. Sánchez, Carmen Fernández, and J. Ignacio Jiménez.

The Commission will hold regular meetings to assess the degree of compliance with the CSIC Equality Plan, to which the CIB Margarita Salas subscribes, review the actions carried out, and propose new actions and tools, which include the following:

- Prepare statistical data, always disaggregated, on the center's personnel from a gender-based perspective.
- Promote the establishment of flexible work schedules and appropriate programming of meetings or scientific events.

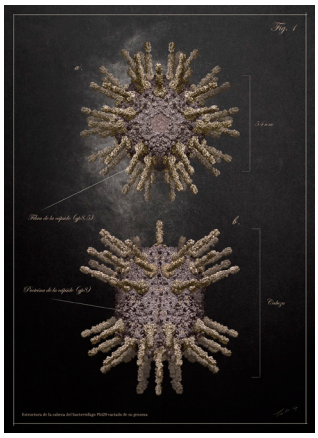
• Create an email address (igualdad@cib.csic.es) to gather suggestions from all the center's staff, and to identify any inequality-related issues, providing the center with a mechanism for the prevention, detection, and follow-up of situations of workplace sexual harassment and gender violence.

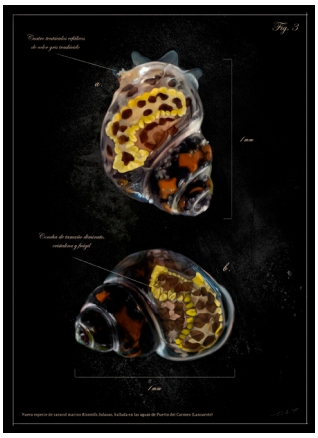
• Promote meetings and discussion forums to analyse and reflect on the situation and visibility of women in research and science. Promote a specific programme aimed at schools to educate on gender stereotypes, increase the visibility of women scientists, promote vocations within STEMM, etc.

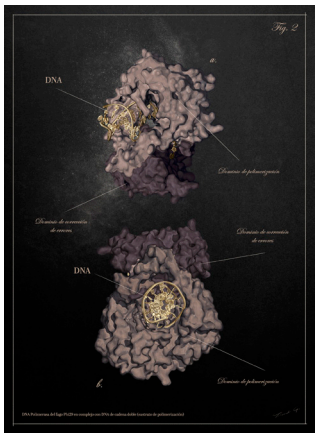


Margarita Salas was not alone at the CIB on Calle Velázquez. Thus, we have depicted her here along with four of her contemporaries, also pioneers of Spanish science: Consuelo de la Torre and Gabriela Morreale to her left, and Sara Borrell and Gertrudis de la Fuente to her right. Yolanda González, a scientific illustrator, created these digital oil portraits, printed on Hahnemühle Photo Rag cotton paper, along with three illustrations of the Φ29 phage and the phage polymerase, the focus of Margarita Salas' research; the *Rissoella salasae* snail, which was named in her honour; and three photos of her during her career at the CIB, which now decorate the lobby of the Margarita Salas Center for Biological Research.

Art and science come together to honour the trajectory of these scientists at the CIB Margarita Salas, CSIC, in the hope that they will inspire and motivate the researchers who pass through this building every day. If you want to know more about the history of these researchers and their time at the CIB, you can read this article written by Flora de Pablo, Pilar S. Testillano, and Enrique J. de la Rosa.

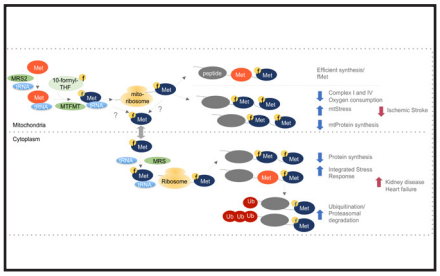




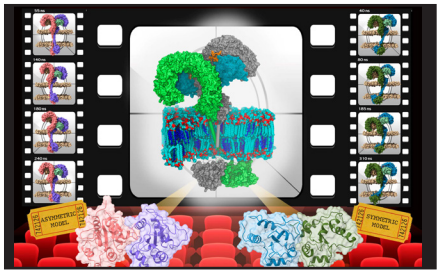


Recent months at the CIB Margarita Salas

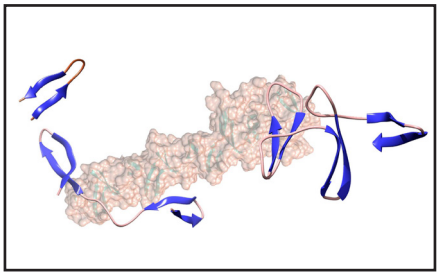
NEWS



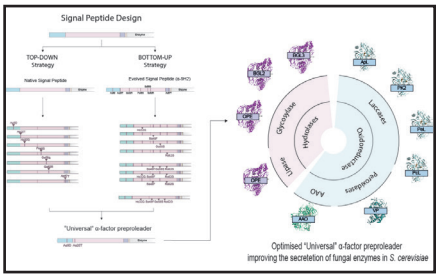
A link between amino acid and a range of common diseases could help predict personal risk



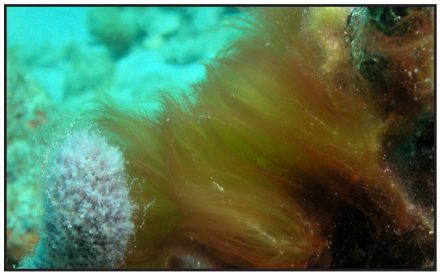
Full-Atom Model of the Agonist LPS-Bound Toll-like Receptor 4 Dimer in a Membrane Environment



Determined the minimal structure of the ββ-solenoid fold in choline-binding modules of the pneumococcal LytA protein



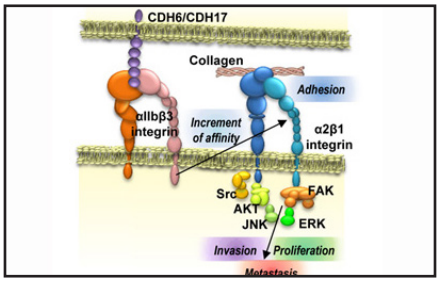
Design of an improved universal signal peptide based on the α-factor mating secretion signal for enzyme production in yeast



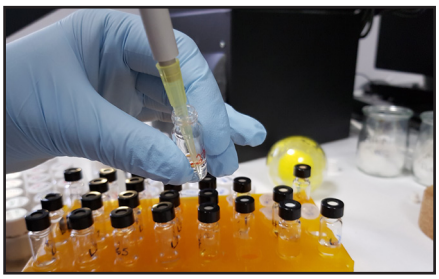
New tubulin drugable site identified using novel natural compound

CSIC and Reina Sofía Foundation sign an agreement to promote "Vanguard biotechnology for the sustainable management of plastics"

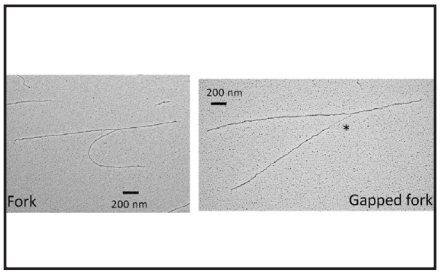
The CIB commemorates World Rare Disease Day and contributes to its visibility



Identified a new therapeutic target against metastatic ovarian cancer



Speeding up thiol-disulfide molecular networks

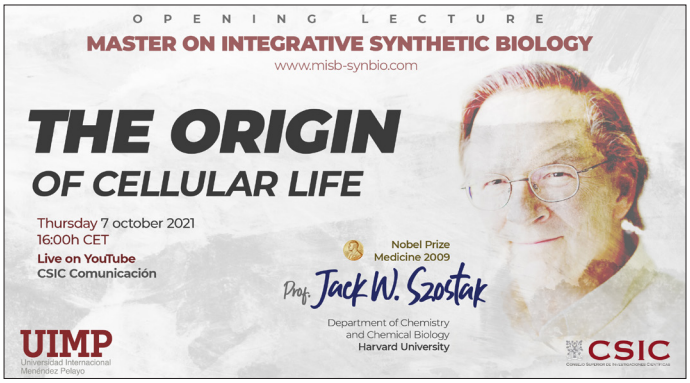


The mechanism by which the checkpoint-mediated DNA polymerase ε exonuclease activity curbing preserves the integrity of replication forks unveiled

Two projects led by researchers from the CIB Margarita Salas have been selected in the CAIXARESEARCH Call for Health Research 2021

CO2SMOS, new European project on biotechnological production of advanced chemicals from biogenic CO2 emissions for circular bio-based industries

EVENTS



Jack W. Szostak, 2009 Nobel laureate in Physiology and Medicine, inaugurated the first edition of the new Master's degree in Integrative Synthetic Biology coordinated by the CSIC and UIMP and organized by CIB Margarita Salas, the CNB, and I2SysBio.

His talk, "The origin of cellular life", was broadcast live on the CSIC's YouTube channel.

The CIB Margarita Salas celebrated the 25th anniversary of the discovery of the gene that causes alkaptonuria with various commemorative activities.

The discovery was led by researchers Santiago Rodríguez de Córdoba and Miguel Ángel Peñalva and was featured on the cover of the prestigious journal Nature Genetics in September 1996. It was the first time that complete sequencing of a human gene was performed in Spain.

The Spanish Society for Biochemistry and Molecular Biology (SEBBM) held the awards ceremony for the Best Doctoral Thesis in Biochemistry and Molecular Biology in the auditorium of the CIB Margarita Salas.

The center, which has been related to the SEBBM since its origins, was the proud host of this act, which was broadcast live on YouTube.

The CIB Margarita Salas has participated once again in the annual Science and Technology Week. Between November 1 and 15, the center offered a total of 12 activities, three held online and nine face-to-face, with various themes and formats: talks, workshops, escape room, board games and even a microbiological gymkhana at the National Museum of Natural Sciences in Madrid.

The traditional Open Days have been replaced by videos of researchers and services to publicize the center's scientific activity.

HM Queen Sofía visits the CIB Margarita Salas / Vinca Page (CSIC)

Her Majesty Queen Sofia visited the CIB Margarita Salas in January.

She toured in the Polymer Biotechnology laboratories, home of the cutting-edge ReCrea biotechnology project for the sustainable management of plastics through bioplastics production. Researchers Isabel Pardo and Auxiliadora Prieto work there. She then visited the Molecular Parasitology laboratory of Vicente Larraga, principal investigator on a project developing a vaccine against SARS-CoV-2.

The Reina Sofía Foundation supports Vicente Larraga's project and, together with the Primafrío Foundation, finances the ReCrea project.

OUTREACH

CIB Margarita Salas researchers Nuria E. Campillo, Carmen Fernández, and Mercedes Jiménez have published the book *Nuevos usos para viejos medicamentos* (New uses for old medicines). It belongs to the collection *¿Qué sabemos de?* (What do we know about...?) series, edited by the CSIC and the publishing house Los libros de la Catarata.

The book explains one of the strategies used to try to reduce the cost and accelerate the production of new medicines: repositioning.

Throughout the year, the CIB Margarita Salas ran the workshop "Do masks really work? Let's prove it!". The activity, financed by the Fundación General CSIC, sought to provide a better understanding of the measures related to the control of the transmission of COVID-19. The workshop attracted more than 2,000 participants from educational centers throughout Spain, and has been included in the CSIC's Science in the Neighbourhood programme, thus ensuring its continuity.

As part of the activity, students had to create informative proposals based on what they had learned, and we have already received some very interesting submissions. The best have been exhibited on the social networks of the CIB Margarita Salas.

Another outreach project carried out this year is "Do you want to discover a drug?", also financed by the Fundación General CSIC.

Members of the Translational Medicinal and Biological Chemistry group toured cities organizing a gymkhana through which they describe the process of manufacturing a medicine. The participants role-play scientists, who must pass a series of tests until they bring the medicine they have developed to the market. The activity has been included in the CSIC Science City initiative, and will thus continue to run into the future.

CONGRATULATIONS!

María Platón Cochado. *Participación de las proneurotrofinas y sus receptores en la degeneración retiniana en modelos murinos de retinosis pigmentaria*. February 2021.

Loreto Martínez González. *Modulacion de TDP43: una nueva estrategia terapeutica para la esclerosis lateral amiotrofica*. May 2021.

Roberto Vázquez Fernández. *Nuevas estrategias para el diseño y desarrollo de antimicrobianos proteicos basados en productos fágicos*. July 2021.

Alicia Villacampa Calvo. Effects of microgravity and partial gravity and the influence of photostimulation on plant adaptation to spaceflight. September 2021.

Rocío Benítez Fernández. *Descubrimiento de nuevas terapias y técnicas de diagnóstico no invasivas en esclerosis múltiple*. October 2021.

Yolanda Pérez Pérez. *Efectores positivos y negativos de la embriogénesis somática y de microsporas de especies cultivadas y forestales: regulación hormonal, pared celular y muerte celular*. November 2021.

Ignacio Bravo Plaza. *Mecanismos reguladores del tráfico en la interfaz retículo endoplasmático/Golgi*. December 2021.

Álvaro Diezma Poyatos. Cysteine modifications of glial fibrillary acidic protein and impact in Alexander disease. December 2021.

The CIB Margarita Salas wishes to congratulate the new doctors as well as the students who presented their Master's or Degree dissertation at the center in 2021, particularly given the circumstances surrounding the COVID-19 crisis.

Meet...

Angélica Horrillo, Animal Facility

Angélica Horrillo, a graduate in Veterinary Medicine from the Complutense University of Madrid and a PhD in Biochemistry and Molecular Biology from the University of Seville, is the head veterinarian of the Animal Facility of the CIB Margarita Salas.

She wrote her thesis on epigenetic processes related to the maintenance of stem cell pluripotency and their differentiation into different cell types at the Andalusian Center for Molecular Biology and Regenerative Medicine. She subsequently carried out postdoctoral research based on the use of animal models of human diseases at the CIB Margarita Salas and the Josep Carreras Research Institute, later joining the Gregorio Marañón Hospital Research Institute where she worked in the animal service with large animals, rodents, and lagomorphs (rabbits). In 2017 she began her work in the Animal Facility Unit of the CIB Margarita Salas, where she is currently Head of Animal Welfare and the Designated Veterinarian.

Angélica is responsible for the running of the animal unit and compliance with current legislation on the protection of experimental animals, ensuring that the animals with which she works meet the highest quality and health standards, and are suitable for use in studies carried out at the center. She also participates in the evaluation of research projects and advises the staff at the center on experimental design in projects involving the use of rodent and lagomorph species.

Animal Facility, CIB Margarita Salas / Marcos del Mazo

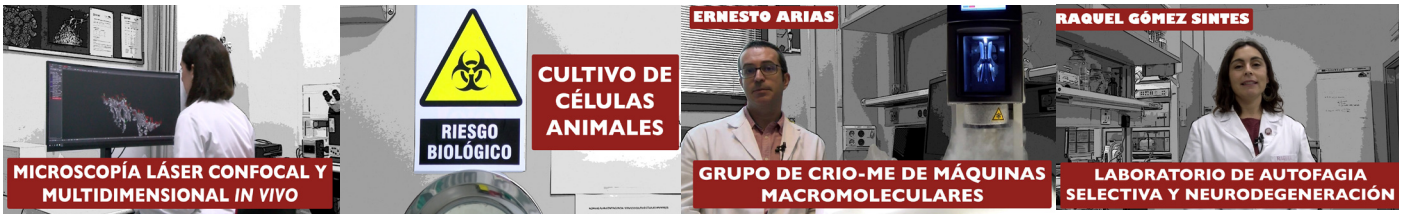
The latest on CIB TV

On January 26 the president of the CSIC, Rosa Menéndez, inaugurated a mural at the entrance to the center paying homage to Margarita Salas.

After the unveiling of the mural a short but emotional ceremony was held inside the facilities in which Enrique J. de la Rosa, director of the CIB Margarita Salas, Lucía Viñuela, daughter of Margarita Salas and Jesús Ávila and Miguel A. Peñalva, disciples of Margarita Salas, participated. The event was drawn to a close by the president of the CSIC.

The event was broadcast live on YouTube, where it can be viewed.

New videos in the "Get to know the CIB Margarita Salas" section



There is more! Don't miss the latest on our YouTube channel!

Do you have a question that you want our scientists to answer? Do not hesitate to write to us:
difusion@cib.csic.es

CSIC
Consejo Superior de Investigaciones Científicas

Margarita Salas

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