Fundamental biology at the CIB Margarita Salas

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At the Margarita Salas Center for Biological Research we are celebrating because one of our researchers, Ana Martínez Gil, was recently awarded the "Juan de la Cierva" National Transfer Research Award for her contributions to the design and development of drugs for neurodegenerative and infectious diseases. This award is a reaffirmation of our Center's motto, "Biology for Global Well-being", which encompasses the scientific character and objectives of the CIB. Our research also demonstrates how the generation of basic knowledge translates into applications with a social impact, as highlighted in an interview in this issue of the newsletter.

The advancement of biological research that seeks to improve global well-being is aided by the rich multidisciplinarity of the CIB's research groups, which allows it to address biological questions from different perspectives and at different levels of biological complexity. A historical example of the search for answers to complex biological problems presented in this edition of the newsletter is the internationally recognized Spanish school of developmental biology, founded by Antonio García Bellido at the CIB in the 1970s. This school was one of the pillars on which the Severo Ochoa Center for Molecular Biology was founded in the mid-1970s.

Investigating in both directions, starting on the one hand with atoms, molecules, and organized supramolecular entities and on the other with organisms and their cells, organelles, and compartments, the aim is to understand the elements that make up biological systems, determine how and when they carry out their functions, and to identify and understand the causes and consequences of structural and functional alterations in order to be able to develop modulatory or corrective strategies.

In this two-pronged effort to advance our fundamental knowledge of biological systems, the CIB Margarita Salas is very well positioned. On the one hand, the center has appropriate scientific equipment and the accumulated experience of various research groups working with techniques such as nuclear magnetic resonance, X-ray crystallography, electron microscopy, and molecular modelling, which enable the study of atoms that form molecules, their structures and dynamics, and their interactions in biological systems, ultimately allowing us to "visualize" biological molecules and understand how they work both in space and time. In this issue we summarize the key future challenges surrounding these techniques.

On the other hand, working from another level of complexity, at the CIB Margarita Salas we also study fundamental processes to understand how a cell or organism is capable of adapting and responding to changing stimuli, whether internal or external, in order to complete its biological cycle For example, the ability to recycle organelles and subcellular structures that are involved in autophagy processes; or the mechanisms that guarantee stability of the genome and thus ensure the reliability of duplication, transcription, and translation processes, ultimately linking them to the molecules involved in these processes.

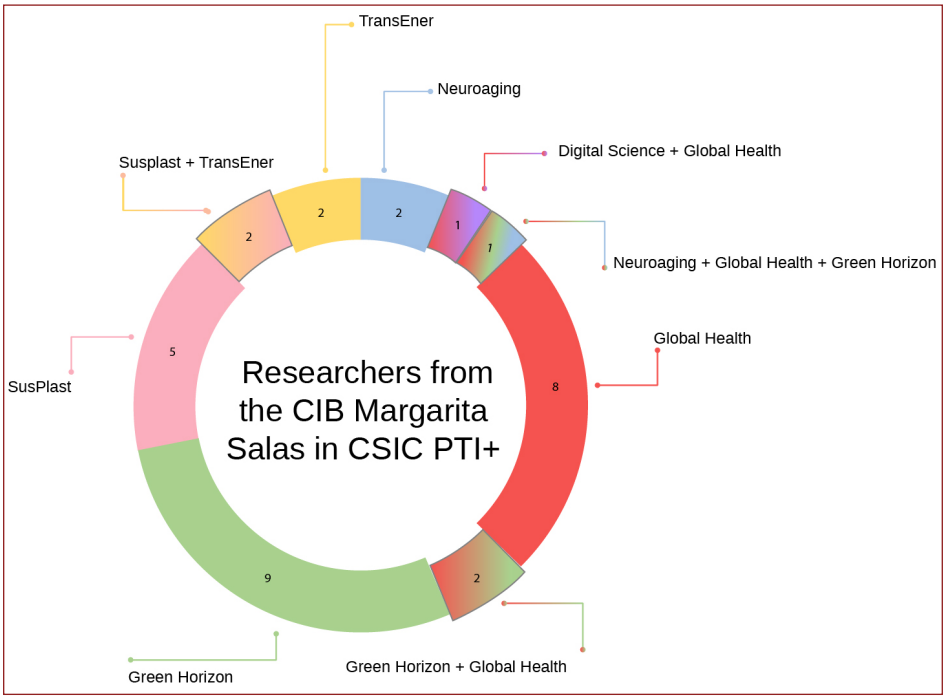
This basic information is essential to be able to modulate activities and improve biological functions. In turn, this facilitates the development of strategies to correct diseases affecting the biological systems studied, and to design applications of biomedical, biotechnological, and environmental interest. The case of phage therapy, discussed in the interview with Dr. Pedro García, is an example of how the convergence of distinct basic research perspectives can lead to applications that benefit society: progress in our basic understanding of

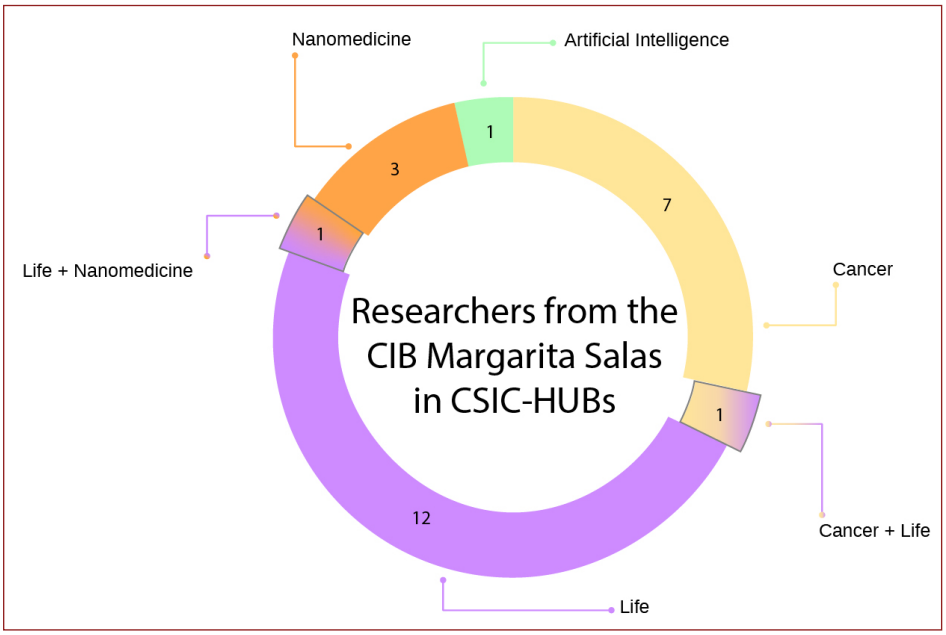
microorganisms and how they interact with other external agents (bacteriophages); structural and functional characterization of the biomolecules involved in these interactions; biomimicking of these mechanisms and translation to the design of possible therapeutic applications to combat bacterial infections; and development of new tools to fight global challenges such as bacterial multiresistance.

In this newsletter we summarize several lines of fundamental biological research at the CIB Margarita Salas that illustrate how this multidisciplinary scientific approach helps achieve the goal of global well-being.

A few numbers

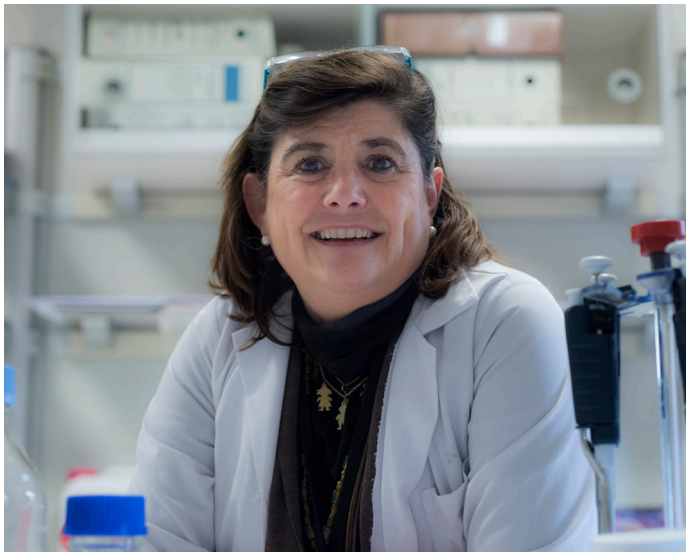
The editorial of the previous newsletter discussed the key roles played by researchers from the CIB Margarita Salas in recent collaborative and interdisciplinary initiatives of the CSIC. These initiatives, the Interdisciplinary Thematic Platforms (ITPs) and the "Conexiones" are designed, respectively, to seek solutions to global challenges that have a significant impact on society and address research issues at the frontiers of knowledge. Here, we present some numbers to provide an overview of our participation in these initiatives. Of the 78 permanent researchers at the Center, 53 contribute via the work of their research teams to the objectives of these research initiatives, in some cases participating in several of them. A total of 38 researchers in 6 ITPs are seeking to develop solutions to global health problems, design new forms of plastics and sustainable energy, tackle population aging and cognitive deterioration, and improve the sustainability of agricultural and forestry systems in context of climate change. They also seek to innovate in all areas of digital science and data lifecycle management and promote open and innovative science. For their part, 27 researchers in 4 "Conexiones" are seeking to create networks that advance knowledge of different aspects of life, from its origin to the possibility of creating synthetic life, furthering understanding of cancer to improve preventive and curative strategies, advancing the development of nanotechnology for medical applications, and integrating all fields, from philosophy to microelectronics, necessary for socially acceptable and beneficial development of artificial intelligence. A true reflection of our commitment to Biology for Global Well-being.





Ana Martínez, National Research Award 2022: "Being in a multidisciplinary environment is of great help in obtaining applied results"

Begoña García Sastre
Journalist at the CIB Margarita Salas



Q| Who is Ana Martínez?

A| Well, currently Ana Martínez is a Research Professor of the Spanish National Research Council. Personally, I would define myself as a long-time science enthusiast. I love medicinal chemistry, designing drugs, being with my team… and I love my family. So I would also define myself as a mother.

P| In which lines of research are you most involved?

R| Since 1996, when I started my own group, I have been working on Alzheimer's disease, and since then I have expanded to include neurodegenerative diseases and also some infectious diseases. Even before I had my own group, I directed theses on antivirals, and that provided an additional focus for the group, although another colleague, Dr. Carmen Gil, mainly leads the antiviral research line. My involvement mainly centers on neurodegenerative and neurological diseases, and I provide some support in the infectious diseases area.

Q| You have recently been awarded the Juan de la Cierva National Research Award in the area of technology transfer. What does this recognition mean to you?

A| It's wonderful. I am very happy to be recognised in this way. In truth, it was something unexpected. I never expected it! And I am tremendously grateful to my research group, to all those who have passed through and formed part of the group for many years, and of course to the national and international collaborators, my family, my husband, and my children. I believe that this is the result of teamwork, working with a large team that has to help you balance both the scientific and the personal: without them this would not have been possible. So many thanks to them. Thanks also to the CSIC for believing in me and presenting me as a candidate, thanks to the Ministry for selecting me, and thanks again to my research team and my family for always being by my side.

Q| How did working in a center like the CIB Margarita Salas contribute to this award?

A| I think it has had a very positive influence because this is a multidisciplinary center. Despite the importance of multidisciplinarity, there are times when it is not appropriately valued. But it is what we were looking for in our group. And we have achieved it. We have been able to advance very quickly in our research because we have access to all the know-how required for molecular modelling, drug design and synthesis. Indeed, we had access to all these areas in the previous institute, but now we also have access to biological assays, cell cultures, animal models, etc. We also have an environment rich in different technologies, and therefore this has allowed us to make advances in the areas of nanotechnology, structural biology, etc. Overall, this context has allowed us to generate results much faster and more efficiently, and have them ready for transfer more quickly. And this transfer has indeed occurred. We have also been able to move forward with the spin-off that we created. I think that being in a multidisciplinary environment is of great help in obtaining applied results and ultimately transferring them.

Q| What were the most important results that were taken into account when awarding this prize?

A| These awards come at the end of a long career. I think that the award was based on the entire trajectory of my career. Primarily, they have taken into account technology transfer, i.e. my contributions to industrial property patents. Our group holds many industrial property patents, and quite a few have been transferred and are being actively exploited. We have also founded

a spin-off, which is now accepting the transfer of some of the patents. I am an advisor to several biotechnology companies, thereby helping to also advance valuable results in science and research, and not just those of our group: the most important aspect is that our science advances society and reaches the patients. And above all, as we do is develop new drugs, our dream is that they reach the patients that need them.

Q| Do you have to give up a life outside the laboratory in order to obtain a National Research Award, the most important scientific recognition in our country?

A| I don't think so. Also, I would not recommend anyone to renounce their personal life for anything. I believe that, in the end, we have a personal and family life that we have to balance with our work, not the other way around. In my mind, if you want to achieve an appropriate balance at work you have to have a proper family balance. So I don't think that's something that should be abandoned.

When I finished school, the COU, I didn't know exactly what I wanted to study or do. In fact, I really liked medicine, but what I really wanted was to be a mother and a good wife; and I thought that pursuing medicine could take a very long time, and that maybe I wasn't suited to it. So I thought about biochemistry because it was closer to biomedicine. I was advised that I would do better if I had a background in chemistry rather than biology, and that's why I enrolled in Chemical Sciences. In the end what I did was organic chemistry. I dedicated myself to medicinal chemistry, and I am really grateful that I had this opportunity and was able to pursue it.

However, I did not give up my first vocation either: I have been married for 36 years, I am a mother to seven children, and I have eight grandchildren. So I have a very full and complete professional life and a family life… which also gives me a lot of work! And I'm very happy.

Q| In this last call you were the only woman to receive an award in the senior categories, and only 22% of the admitted applications were women. Why do you think that is?

A| Actually, I'm not really sure. It's probably because this vocation of mine is one shared by many other women, some of whom feel more strongly about it and therefore sacrifice other opportunities. Or, perhaps some women are not lucky enough to have the support necessary to find the right balance. But I don't know either. I know many very bright women who do very good science. I don't really have a very clear explanation. There is the concept of the glass ceiling for women, which may be true. But it's not because the woman isn't good enough: maybe it's because she is more focused on her family than a man might be. Ultimately, we will find the appropriate balance in society, where all are considered capable of working professionally, carrying out research, and where both men and women are considered essential within the family environment. That is something that must be transmitted, and that I try to transmit to my children. I think that we need to achieve this balance in society.

Q| Your scientific career is inspiring for upcoming generations of researchers. What advice would you give them?

A| They should follow their dreams. Everyone should pursue their own dream. That's what I would tell everyone. And this can be achieved with enthusiasm, perseverance, continuity, and joy, without becoming overwhelmed by challenges.

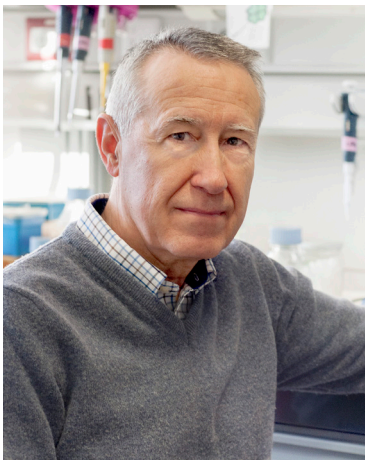
Pedro García: "Phage therapy is a line of research that holds great promise in the fight against multiresistant bacteria"

Begoña García Sastre
Journalist at the CIB Margarita Salas

One of the main health problems we are currently facing is the resistance of certain bacteria to antibiotics. Misuse and abuse of these medications over the years has caused some bacteria to develop resistance mechanisms, which make it very difficult to fight the infections caused by them.

This problem already poses a great threat to global health and solutions must be sought.

In this issue of the Newsletter we interview Pedro García, CSIC scientific researcher at the CIB Margarita Salas (retired in June 2022). He directed the Host-Parasite Interactions in Pneumococcal Infections group, now known as the Protein Engineering Against Antimicrobial Resistance group, which seeks to identify new antimicrobials that prevent cases of resistance, focusing specifically on pathogens that cause respiratory diseases.



We talked to him about phage therapy as a method to combat multiresistant bacteria, and also about his research career and his 44 years at the Center for Biological Research.

Q| How did your relationship with science begin? Was it vocational?

A| At the end of school it was clear to me that I was going to dedicate myself to some field of science, and I started studying Chemistry at Complutense University, convinced that it was for me. Therefore, I graduated in biochemistry under the great professor Don Ángel Municio. We had some very good teachers, and in the end I decided that my main aim was, if possible, to enter a research laboratory to do my Ph.D. That was in April 1978 and I joined his lab initially to do a dissertation and then the thesis.

Q| When and why did you direct your research towards phage therapy as an alternative to antibiotics against multiresistant bacteria?

A| For my thesis, the bulk of my research focused on the study of an enzyme, murein-hydrolase, which is encoded by the Dp-1 phage and degrades the peptidoglycan of the susceptible bacterium, in this case *pneumococcus*. That was the initial and fundamental objective of the thesis. Subsequently, my main lines of research have involved the study of the structure, function, and mechanism of other murein-hydrolases, both from pneumococci and their phages. And so it was for many years, with the exception of my two postdoctoral stays. Curiously, around 2000 an American researcher, Vincent Fischetti, from the Rockefeller University in New York, wrote to Rubén López García asking him for the exact plasmid that encoded the enzyme that I had studied in my thesis, the murein-hydrolase of the phage Dp-1, called Pal. Initially we didn't really know what he wanted it for, and after much thought we gave it to him. Then in 2001 he published an article in Science, no less, in which this enzyme Pal was used to kill the susceptible bacteria (*pneumococcus*). So then we, as well as other groups that were working on this subject, realized the potential that these enzymes had and that we had not been able to exploit. From that point, we set out to take advantage of this series of bacterial peptidoglycan lytic enzymes for this purpose.

That's a brief summary of this entire line of research.

Q| What exactly is phage therapy?

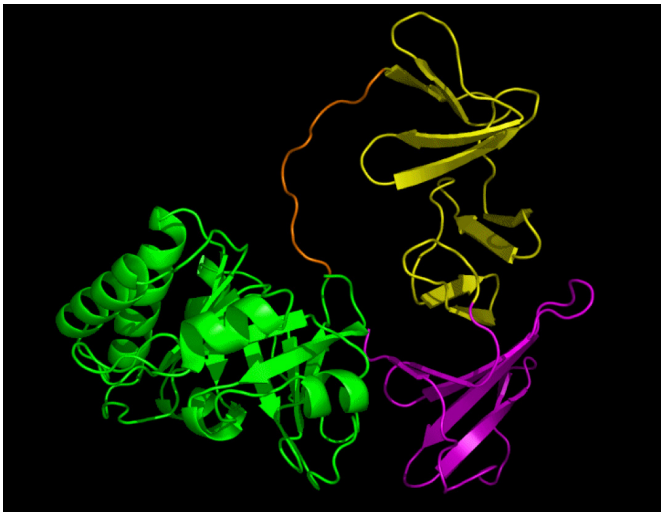
A| Phage therapy refers to the use of whole phages, so-called virions, as well as some phage-encoded products such as murein hydrolases. As mentioned before, these enzymes have enormous potential because they degrade bacterial peptidoglycan rapidly and specifically.

Q| Why is prior knowledge provided by basic science important when proposing a therapeutic use of these phages?

A| I would answer this question with a saying: I can't remember where it came from originally, but I have made it mine: And sooner or later, quality basic science always gives rise to different applications, many of which arise unexpectedly or serendipitously. Phage therapy is no exception. Phage therapy dates back nearly 100 years ago, although the initial applications were rather inexact. Subsequently, this area was enriched with newly acquired knowledge on mechanisms of action, etc., enabling a series of advances and the emergence of a line of research with very clear applications, both in clinical practice and other disciplines, making it a very promising tool in the fight against multiresistant bacteria.

Q| Why has the potential of bacteriophages as a therapy against bacterial infections traditionally been ignored?

A| Somewhat related to the previous question, the history of phage therapy has gone through several stages since it was first described a little over a century ago by Félix D'Herelle, a French-Canadian researcher, who actually used phages for therapeutic purposes. This approach was almost abandoned, at least in the Western world, with the discovery of penicillin and other antibiotics. During the decades of great advances in the field of antibiotics, phage therapy practically disappeared, except in the eastern part of Europe. In the Soviet Republic of Georgia and in Poland this line of work was consistently maintained. But in the West, and in the bulk of English language scientific literature, phage therapy was all but forgotten, until the alarming and increasingly frequent appearance of multiresistant strains, beginning in the 1980s. This means that these strains are resistant to all known antibiotics. Therefore, there is no valid treatment, hence the urgent need for therapeutic alternatives. This is where phage therapy plays, and will undoubtedly continue to play, a very important role in combating multiresistant bacteria.



Schematic representation of the three-dimensional structure of Cpl-1, murein hydrolase encoded by the Cp-1 phage. In green, the catalytic module; in yellow and purple, the two regions of the module for anchoring to the wall; in orange, the region that connects domains (Structure, 2003)

Q| What advantages can phage therapy have over classical antibiotics?

A| Phage therapy refers to the use of whole virions (whole phages) and also their products, basically enzybiotics. In general, the mechanisms of both are quite specific as they attack only the susceptible bacteria. This offers a great advantage over antibiotics, which kill practically the entire bacterial population within their reach. When the objective is to eliminate an infection caused by a specific bacterium, it is important to do this as effectively as possible, while also leaving the other bacterial populations intact. And that is what both whole phages and enzybiotics do.

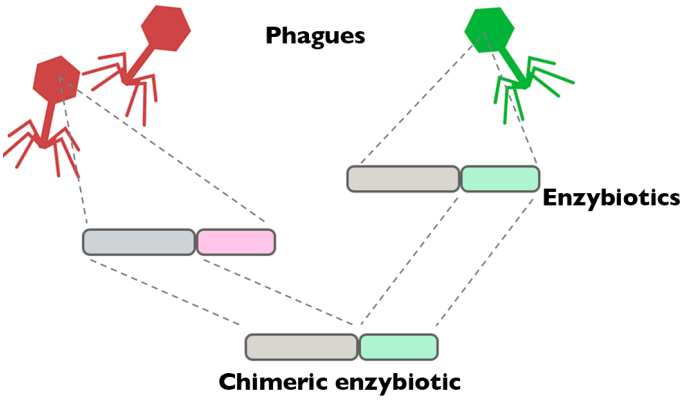
There are several other very important advantages. Also, I do not want to finish without mentioning the issue of resistance. It is very clear that the use and abuse of antibiotics has led to this situation of multiresistance, because bacteria very easily develop mutations that confer resistance to these drugs. It is true that mutations that confer resistance to whole phages can also arise, but this can be circumvented by using a cocktail of phages, normally three or four. This ensures that the development of resistant bacteria is greatly minimized, partly because phages act very quickly. As soon as they interact with susceptible bacteria, they eliminate them relatively fast. In the case of enzibiotics the data are even better because so far, and they have been searched very intensively, no resistant mutants have been described. Resistant mutants have not been found probably because of the mechanism of action, which targets a polymer that is highly conserved among bacteria: peptidoglycan. Therefore it is unlikely, but not impossible, that resistant mutants will be found.

Undoubtedly, other key advantages are the ease with which new phages target practically any bacterium can be found, and the fact that they are much cheaper to develop than any other antibiotic drug. Among other things, because lately it is already known that the big pharmaceutical companies have almost stopped researching and investing in this field

Q| At what stage are we currently at, and what is the future of phage therapy? Have phages already been used clinically to treat antibiotic-resistant infections?

A| Regulation of the clinical use of phages remains poorly defined. European, Spanish, and as far as I know United States agencies have no regulations that allow the use of all currently available phage therapies, because phages are living beings and their use is more complex than that of a conventional drug. What is common, since there are more and more severe or very severe patients infected with different multiresistant pathogens, is the use of phage therapy as so-called last-resort or compassionate therapy. This means that, although it is not absolutely regulated, its use is always permitted with the approval of the corresponding hospital's ethics committee and the patient's informed consent. In those cases, there are and will continue to be more examples of the compassionate use of phages. This has not happened with enzybiotics, but I expect that this will occur in the near future. In Europe, the example to follow, because it is very well studied and very well developed, is Belgium. But also in the United States. In the case of Spain, there have already been some cases of successful use of phage cocktails to treat patients with very serious infections caused by multidrug-resistant bacteria. That is the path that must be followed.

Within Spain we have, and my group is part of, a thematic network called FAGOMA, which is a consortium of laboratories that work on various aspects of phage therapy. Within FAGOMA is a smaller group of experts



In phage therapy, whole phages (virions) or the murein-hydrolases they encode, also called enzybiotics, can be used. These enzymes can be wild type or chimeric, formed by fusion of different domains.

in everything related to phage therapy. What do we want to do? We want to copy the approach used up to now in certain other countries, especially Belgium. In other words, having a space, a single or centralized phage center, a kind of "phage library", to carry out characterization and phagograms of a series of phages with activity against different pathogens. Then, when Spanish doctors receive patients with serious infections caused by multi-resistant pathogens, they do not have to call their colleagues in Belgium or the United States asking for phages to target pathogenic strain "X". I believe that in Spain we have a very good level of knowledge about everything related to the purification and characterization of phages, but it must be centralized. This work must be done, but we want to do it from FAGOMA, and I hope that other appropriate institutions realize the importance of this issue for public health and provide adequate funding. That is the current context in Spain in the field of phage therapy.

Q| Finally, on a more personal note, now that you are about to retire, what will you do to fill the time that up to now was dedicated to science? Will you miss the CIB Margarita Salas?

A| After 44 years in this center, of course I'm going to miss it. Although I am going to start another stage, I am not going to retire from science. I want to maintain my interest in my line of work, and specifically in phage therapy, via other activities. But of course I have quite a few plans outside of science - leisure, travel. These will keep me occupied. I don't expect to get bored in my retirement.

Future challenges of structural biology

Future challenges and opportunities in cryo-electron microscopy

Ernesto Arias Palomo
Senior CSIC Scientist at the CIB Margarita Salas

Electron microscopy has been used for the study of biological systems for more than 80 years. During this time, there have been constant improvements in the instruments and classical applications. Here, we will briefly describe how these techniques are contributing to different scientific areas, including fundamental biology, as well as some of the main challenges that will be faced in the coming years.

Micro-diffraction, for example, uses the diffrantion patterns obtained by illuminating tiny crystals with the electron beam to determine the three-dimensional structure of molecules (Figure A). The small size of the crystals is very useful in organic chemistry, where the structure of new compounds can be easily solved by simply by examining crystalline powder. It has also generated some expectation in the case of large macromolecular complexes, due to the difficulty in obtaining large crystals of these often flexible and dynamic samples..

On the other hand, cryo-electron tomography offers great promise for the study of the structure of unique objects such as sub-cellular organelles and compartments, and even complete cells (Figure B). Furthermore, correlative microscopy allows the use of fluorophores to locate biological events of interest that occur with low frequency, so that attention can be focused on these areas to reconstruct tomograms and analyse these processes with higher level of detail. This methodology is providing new insights into different fields of research,

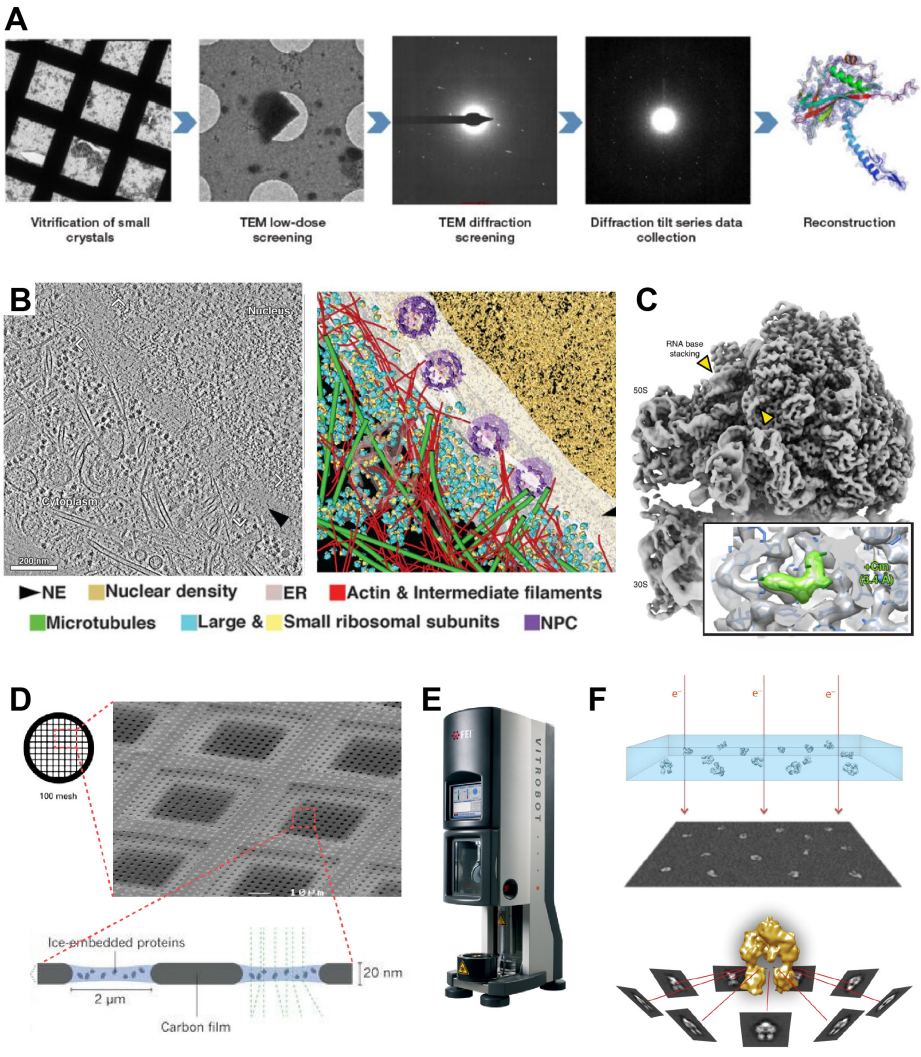
such as the study of the organization of the cytoskeleton, the morphology and development of different cell organelles, or numerous viral infection processes. Overall, the global resolution is often limited between 15-40 Å. However, this resolution can be improved if hundreds or thousands of identical copies of the same object can be detected within the tomogram, extracted, and averaged. Recent advances in this procedure have allowed to achieve unprecedented levels of detail, including the reconstruction of ribosomes inside the cell at 3.5 Å, which allowed the direct examination of the effects of antibiotic agents *in situ* (Figure C).

However, despite the recent breaktrhoughs in tomography, it is not easy to identify and extract the different macromolecules present in the cellular context if they are not large and well-defined molecular machines (e.g. ribosomes, proteasomes, etc). Furthermore, many procedures used in cryo-electron tomography, particularly when used in combination with fluorescence microscopy, can be difficult to perform and require highly qualified personnel. Although during the last years new molecular recognition algorithms -some based on artificial inteligence- and instruments are being implemented, numerous procedures required for this technique are still performed in a rather tedious and non-automated way.

Single particle analysis is perhaps the most widely used cryo-electron microscopy technique in the last decade. This methodology was chosen by the journal Nature Methods as the technique of the year in 2015, and some pioneers received the Nobel Prize in Chemistry in 2017. This method allows to determine of the three-dimensional structure of macromolecules at the atomic level. Briefly, the purified complexes are applied on cryo-EM grids, which contain regularly spaced 1-2 µm wide holes (Figure D). The sample is then snap-frozen in liquid nitrogen so that a one-molecule-thick layer of vitreus ice, in which hundreds of macromolecules are suspended, remains over each hole (Figure E). These grids are inserted into the microscope to collect thousands of micrographs that, together, contain millions of individual molecules. Finally, these images are computationally processed to find their realitvie angles and reconstruct the three-dimensional structure (Figure F).

One of the main advantages of this technique is that it does not require large amounts of sample: in some cases the structure of macromolecules can be obtained directly from native sources. Moreover, the formation of ordered crystals is not necessary. All this has revolutionized a wide variety of fields within fundamental biology. One example is the study of large macromolecular machines such as the complexes involved in protein translation, gene expression, splicing, or ATP production, which are often flexible and dynamic.

In our laboratory we use single-particle analysis to characterize the structure and function of macromolecular machines involved in essential cellular processes, such as the transmission of genetic information and the spread of antibiotic resistance factors. These macromolecules undergo large conformational changes, sometimes assisted by the binding



A) Diagram showing steps involved in electron micro-diffraction (figure adapted from Thermo Fisher). Science. 2016). C) Structure of M. pneumoniae ribosome at 3.5 Å bound to an antibiotic (in green in the panel insert), obtained by averaging of sub-tomograms (adapted from Tegunov, D. et al. Nature Methods. 2021). D) Cryo-electron microscopy grid. E) One of the guillotines traditionally used to vitrify the sample (Vitrobot, Thermo Fisher). Microscopy. 2016)

and hydrolysis of ATP, to copy, remodel and reshuffle the DNA. The inherent flexibility of these systems, thus, makes cryo-electron microscopy an ideal technique to analyze these molecular machines.

However, while the rapid advances seen in recent years have allowed to achieve increasingly higher levels of resolution and study a greater number of biological systems, there still are numerous challenges. For example, although it should be theoretically possible to analyze small proteins (~40 kDa), despite great efforts to develop tools to study this type of molecules (e.g. phase-plates), it is still difficult to solve the structure of complexes smaller than 150 kDa. The elevated costs of the initial purchase and regular manteinance of the electron microscopes is an additional challenge. Fortunately, significant efforts are being made in redesigning microscopes and cameras to operate at lower voltages, which should have lower associated costs. Interestingly, although the time required to solve a structure has decreased considerably, (for example, the reconstructions of the polymerase and the spike proteins of the coronavirus were obtained just 2 or 3 months after the publication of the genome of the virus), the speed of data collection and processing remains significantly lower compared to other structural determination methods. Notably, sample vitrification is one of the main bottlenecks of the technique. This process is still carried out in a very manual fashion, and during vitrification numerous samples can be denatured or undergo alterations that can make structural determination difficult. Although numerous groups are working in this area and automatic freezing devices using novel procedures are beginning to be commercialized , future work will be required to make this process more robust and reproducible.

It should also be noted that experimental techniques will have to work together with powerful new protein structure prediction algorithms, such as AlphaFold and RoseTTAFold. Specifically, microscopy maps are already used to guide and iteratively improve the predictions made using these algorithms, and the results are increasingly being used as starting point to help build and refine the atomic models determined with this technique.

Overall, cryo-EM has experienced and exponential development in the last decade. In the next years it will have overcome new challenges that will allow it to remain a powefull techinique to analyze biological systems at the cellular, molecular and atomic level.

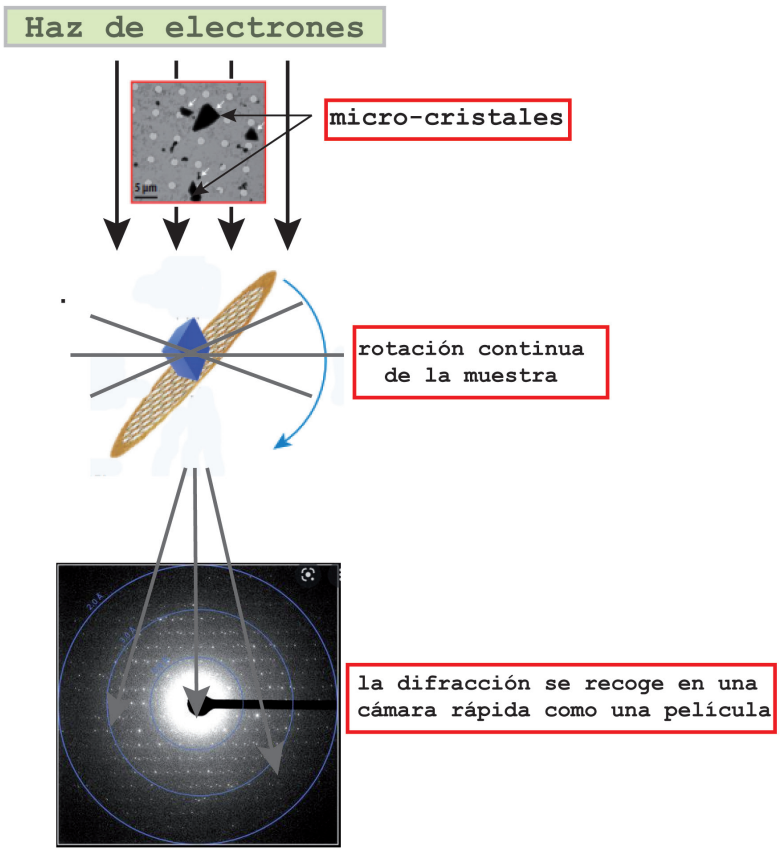
Latest Advances in Structural Biology: X-ray Crystallography

Antonio Romero
CSIC Research Professor at the CIB Margarita Salas

The advance of knowledge in Biology together with the development of several techniques and the technological innovations in computation is allowing an enormous progress in the understanding of the diverse biological systems. The biological function of most proteins is essentially determined by their 3D structure and by the physical characteristics of their environment. Detailed knowledge of a protein's 3D structure is a key requirement to begin to understand its mechanism of action at the atomic level. Experimentally determining 3D structure remains a major challenge despite major advances in the three techniques widely used in Structural Biology: crystallography, nuclear magnetic resonance (NMR), and electron microscopy (EM). To date, of the almost 192,000 structures deposited in the Protein Data Bank (PDB), 87% have been resolved by X-ray crystallography, 7% by NMR, and around 6% by cryo-EM.

However, the need to obtain crystals of sufficient size for structure determination by conventional X-ray crystallography is often the bottleneck for many biological samples among which we would highlight membrane proteins and macromolecular complexes. To solve this problem and address new challenges in Structural Biology, new methods have been developed that, together with access to large facilities, such as X-ray free electron lasers (XFEL) and microfocus beamlines in synchrotrons, can provide data from micro- or nano-crystals, which are too small for conventional crystallography. Notable major milestones in structural biology include serial X-ray crystallography and electronic crystallography of micro- and nano-crystals (MicroED).

The development of X-ray Free-Electron Lasers (XFEL) opened up new avenues of development for the crystallographic study of proteins and nucleic acids. This new XFEL technology led to the emergence of an important technological advance: serial femtosecond crystallography (SFX). In SFX, diffraction is obtained through hundreds of thousands of micro- or nano-crystals that are injected at room temperature into the XFEL beam, randomly oriented, and an image is recorded per crystal and X-ray pulse before being destroyed. Because these experiments are carried out at room temperature, dynamic study of proteins is possible, enabling capture of atomic movements on different time scales. SFX inspired the adaptation of methods and technologies for serial data collection on synchrotron beamlines. Since the brightness of synchrotron light is not as intense as that of XFELs, exposure at the order of milliseconds is needed to produce sufficiently intense diffraction. In this scenario, known as SMX (serial millisecond crystallography), detection of the diffracted beams is carried out continuously without a shutter on a millisecond time scale, incident on the crystals that are injected into high-viscosity matrices (LCP; lipid cubic phases), or high molecular weight polymers (PEO; polyethylene oxide). This facilitates structural determination at room temperature from thousands of diffraction patterns with crystal sizes ranging from 5 to 20 microns. SMX offer advantages over SFX given the global scarcity of XFEL lines compared to the greater number and easy access to synchrotron lines. By means of this SMX technique we can obtain a high structural resolution without the danger of radiation damage, the study by fast diffusion of ligands and inhibitors and the possibility of obtaining experimental phases.



Microcrystal electron crystallography (MicroED)

Electron crystallography of micro- and nano-crystals (MicroED) has been developed as a hybrid method that combines the advantages of cryo-electron microscopy and X-ray crystallography. Solutions containing micro-crystals are deposited on carbon-coated electron microscopy grids and are vitrified by immersion in ethane or liquid nitrogen. Data are collected for each selected crystal, applying an attenuated electron beam, through a continuous rotation in which we can collect up to 140º. The data are recorded as a movie using direct detectors, and each frame contains reciprocal space information. MicroED data can be processed using standard X-ray crystallography software. A key advantage of MicroED over XFEL, for which hundreds of thousands of crystals are required, is that a single nano-crystal may be sufficient to resolve a structure. In addition, there is the difficult access to large XFEL facilities compared to the increasing presence of transmission electron microscopes in a large number of laboratories. Finally, this technique is broadly applicable to the resolution of the structure of globular proteins, peptides, membrane proteins, and organic and inorganic compounds.

Probably one of the most recent and innovative advances in the field of structural biology is the prediction of 3D protein structures using neural network models. Thus was born AlphaFold, an artificial intelligence program developed by DeepMind, which has shown great accuracy in predicting 3D structures from their primary sequence alone. In the words of Edith Heard, director of EMBL, the achievement is "a true revolution for the life sciences, just as genomics was decades ago".

Our line of work at the Margarita Salas Center for Biological Research has focused on antibiotic resistance, a global public health problem and a growing phenomenon with important economic and social implications. We launched a new strategy to control bacterial populations, focusing on the secretion system 6 (T6SS). Activation of this complex machinery could play a decisive role in infectious diseases. We have successfully characterized several components of this system: VgrG1, Hcp, TssL, and TssK, as well as one of the effectors of *Acinetobacter baumannii* (Tse1).

The next years of Nuclear Magnetic Resonance in Biology

Francisco Blanco Gutiérrez
CSIC Scientific Researcher at the CIB Margarita Salas

Most scientists doing research in biology identify NMR as the technology to determine the three-dimensional structure of proteins in solution. This is its most visible contribution since it was first done in 1985 by Swiss chemist Kurt Wüthricht. It is useful to remember that all methods, both experimental and computational, generate models of protein structures. In the case of NMR spectroscopy, many frequencies, distances, and angles are measured, and the spatial arrangement of the polypeptide chain that best explains the set of measurements is modelled. The method is efficient for small proteins, with a resolution comparable to crystallographic structures. With improvements in the technique, equipment, and training, structural determination of proteins and nucleic acids by NMR developed to the point of contributing almost 1000 new structures to the Protein Data Bank in 2007. This boom occurred when structural genomics consortia were highly active, tackling the easiest proteins to produce and analyze: small or fragments of large proteins with autonomous folding. Since then, the number of NMR structures has been decreasing (about 350 in 2021). The main reason is that the procedure loses efficiency as the protein size increases and becomes impractical. The largest protein for which the structure was determined in solution by NMR is 723 amino acids long. The procedure may be further compounded by certain structural features, such as oligomerization and symmetry, which instead facilitate modelling from crystallographic or electron microscopy data. The size is not limiting in the case of solid samples, but the spectral complexity, at the resolution that can be reached with the techniques available in solid-state NMR, is. Other limitations are the need for a large amount of pure, stable, and isotope-enriched proteins, and the laborious procedure. NMR has enabled the determination of 7% of all structures deposited in the PDB, and this proportion will likely decrease. Conversely, the proportion of structures determined by electron microscopy, which currently stands at 6%, is rapidly growing. The advent of AlphaFold, which provides highly reliable models for most of the sequences capable of autonomous folding, will increase this trend.

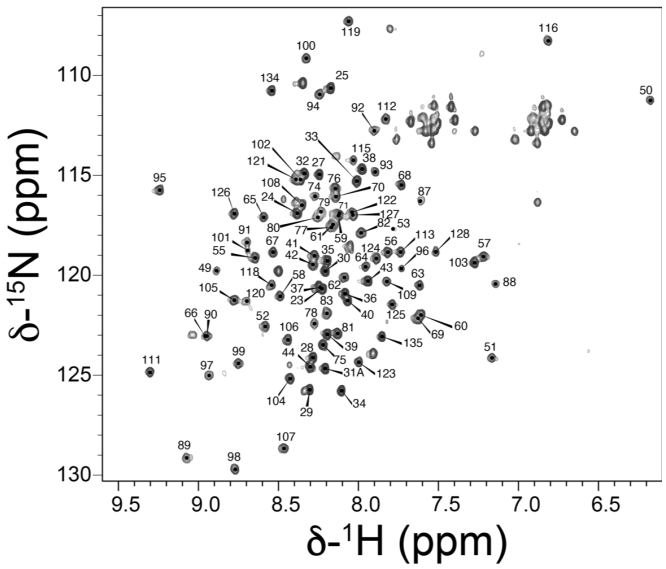
NMR is the most efficient experimental technique for obtaining high-resolution structural information on folded biopolymers that do not crystallize or do not provide sufficient contrast in microscopy, and on those that are not folded and lack a persistent structure (e.g. intrinsically disordered proteins and polysaccharides). The expected trend is an increase use of NMR to analyze these molecules, particularly in the areas in which it is most powerful: molecular recognition and internal dynamics.

Due to its high sensitivity to changes in the chemical environment of atoms, NMR in solution is very useful for the study of molecular interactions. In favourable cases, an interaction can be detected, the binding site identified, and the equilibrium constant measured quickly and easily, with much lower sample requirements than for structural determination. The methodology is especially efficient if a protein enriched in nitrogen isotope 15 and with known assignment (the correspondence between the signals in the NMR spectrum and each amino acid of the protein) is available. Being able to predict the NMR spectrum based on the structure would constitute a huge step forward, circumventing the costly process of spectral assignment. Attempts so far have been unsatisfactory, but a tool analogous to AlphaFold may soon be able to achieve this. This would speed up the identification and characterization of ligand binding to proteins of interest.

We tend to imagine proteins as having structures fixed in time, while under physiological conditions proteins structures are dynamic. In the buffered aqueous solutions used in NMR, information on these dynamics can be obtained over a wide range of time scales and with atomic resolution, which is difficult to obtain otherwise. This information is especially relevant for proteins with catalytic activity or that regulate other proteins by binding to them.

Intrinsically disordered proteins have a particularly dynamic behavior, as they adopt very different structures on very short time scales (nano-microseconds). NMR is especially useful in identifying and characterizing this behaviour, as well as the changes that occur when they fold upon binding to a ligand (often another protein or a nucleic acid). The highly dynamic behaviour of these proteins favours observation of their

NMR signals, to the point that they can be studied at very low concentrations, and even inside living cells (by introducing them enriched in the nitrogen 15 isotope).



Assigned two-dimensional NMR spectrum of a 117 amino acid protein

Structure of a protein, the spectrum of which is shown in the previous figure, studied in the biomolecular NMR laboratory of the CIB Margarita Salas

For these proteins without persistent structure, it would be very useful to predict the spectrum from the amino acid sequence. This is hard to do precisely enough to be useful, but we might be pleasantly surprised in the future if the machines learn enough. Of great interest are the phase separation phenomena (coacervate formation) in which this type of proteins frequently participate, and which are related to cellular sub-compartments without membranes. Although there are strategies to study these coacervates by NMR, their high viscosity severely limits the ability to achieve the same level of detail as obtained for the non-condensed phase. At the other extreme in terms of dynamic behaviour are proteins that assemble in the form of amyloids, either in functional or pathological contexts. Over the last 20 years, solid-state NMR has provided high-resolution information on these amyloids through the analysis of samples prepared from synthetic or bacterially produced polypeptides. However, recent cryo-electron microscopy studies of amyloids obtained from natural sources (the brains of deceased patients) cast doubt on the relevance of some of these pioneering studies, which also showed great structural variability depending on the history (preparation) of the amyloid sample.

NMR has great potential to continue contributing to research in fundamental biology, especially in molecular recognition and biopolymer dynamics, and to a lesser extent in the study of large, membrane, coacervate or amyloid complexes. The main limitation is low sensitivity relative to other techniques. Strategies are being developed to increase sensitivity, although single-molecule measures useful in biology are not expected to be feasible. In the Biomolecular NMR laboratory of the CIB Margarita Salas, we use NMR in solution to study the structure and interactions of tumour suppressors of the ING family, DNA replication machinery, and the alpha subunit of trimeric G proteins.

Autophagy: Recycle or Die

Patricia Boya
CSIC Scientific Researcher at the CIB Margarita Salas (until August 2022)

Juan Ignacio Jiménez Loygorri
Postdoctoral researcher at CIB Margarita Salas

Taken from the Greek αὐτόφαγος (αὐτό, "oneself"; φαγος, "to eat"), autophagy is the main recycling mechanism at the intracellular level. It is a dynamic and sequential process that begins with the formation of a double membrane structure (the phagophore) around

the material to be degraded, in a process mediated by the initiation machinery. Through a process of elongation, this double membrane will eventually engulf and completely isolate the cargo from the rest of the cytoplasm, forming what is known as an autophagosome. Finally, the autophagosome will fuse with the lysosome, where the degradation of the material will take place until it is reduced to its essential components, which are then released again into the cytoplasm.

This process was first observed in the 1960s thanks to Christian de Duve's efforts to define the various cell organelles using electron microscopy. In his experiments, he observed "cytoplasmic sequestration" in the liver of rats treated with glucagon, characterized by distinct phases of maturation of the vesicles, and ending with their degradation within the lysosome. However, it was not until the 1990s that the mechanism of induction and regulation of autophagy was elucidated with the discovery of the ATG (AuTophaGy-related) genes, thanks to studies performed in yeast by Yoshinori Ohsumi, among others. Ohsumi received the Nobel Prize in Physiology or Medicine in 2016 "for his discoveries on the molecular mechanisms of autophagy".

Initially associated with a response to fasting or stress, it has now been shown that autophagy is also essential for maintaining homeostasis within cells under physiological conditions, helping to eliminate damaged organelles, protein aggregates or components that are not useful at a given time. In recent years, different autophagy routes have been described, each differing in the way that the material to be degraded is delivered to the lysosome: macroautophagy (through an autophagosome), microautophagy (direct entry through invagination of the lysosomal membrane) or chaperone-mediated autophagy (CMA; degradation of specific proteins recognized by the chaperone Hsc70 and entering the lysosome through a channel formed by the LAMP2A protein). The discovery of the selectivity of autophagy has also revolutionized the field, giving rise to processes such as mitophagy (mitochondria), lipophagy (lipid bodies) or ribophagy (ribosomes). For example, at the Autophagy Laboratory of the CIB Margarita Salas we have described the essential role of mitophagy during neuronal development and differentiation, where it is necessary to readjust cell metabolism and produce sufficient energy to carry out this process[1].

Studies in cohorts of centenarians have shown that autophagy is particularly well preserved, or even increased, in these individuals, implying that it may be a key aspect of healthy aging. In the case of the central nervous system, neurons are a post-mitotic (non-dividing) cell type that also has one of the most active metabolisms in the body. In these circumstances, autophagy is an essential process to keep these cells healthy throughout the organism's life. Our group has found that during physiological aging macroautophagy in the retina decreases progressively, an effect that is partly compensated for by the activation of other intracellular degradation mechanisms such as CMA[2]. Supporting these findings, we have also shown that mice with deficient autophagy show accelerated aging, with alterations in visual function and metabolism already evident at middle age, making them more susceptible to stress and various pathologies[3].

Paradigms that increase autophagy levels such as calorie restriction or intermittent fasting have gained importance in recent years, with several studies highlighting their health benefits. These include a lower incidence of age-associated pathologies such as metabolic syndrome, cardiovascular disease, type 2 diabetes mellitus and cancer. Although increased life expectancy has also been described in various animal models, the effect in humans is still a topic of discussion in the scientific community. For example, rapamycin, described as a robust autophagy inducer, also has a potent immunosuppressive effect that limits its long-term use. In order to find more specific drugs, several compound discovery and medicinal chemistry programs are underway to modulate autophagy in physiological or pathological conditions. Recently, in collaboration with the groups led by Ana María Cuervo and Evris Gavathiotis (Albert Einstein College of Medicine, New York), we described a new family of compounds that selectively induce CMA and that, when administered by intravitreal injection, reduce vision loss caused by retinitis pigmentosa[4]. This rare disease (incidence, 1 per 3500) is hereditary in origin and causes progressive loss of visual function and,

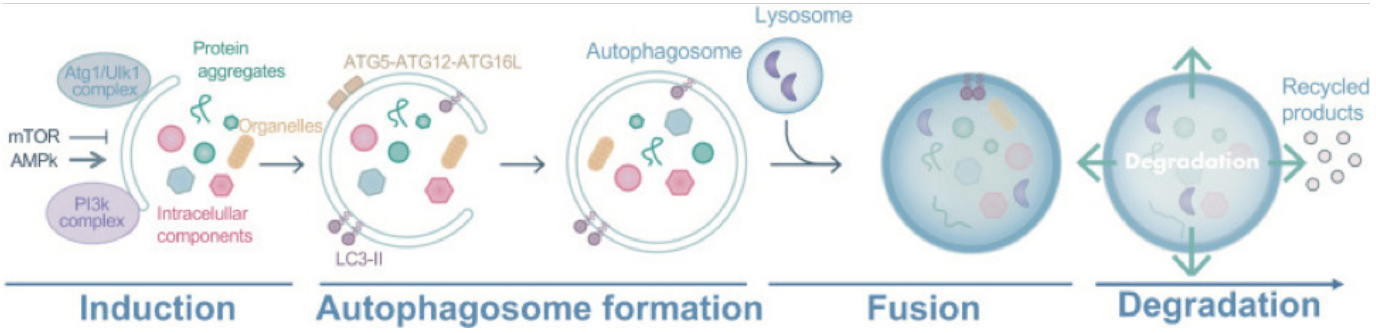
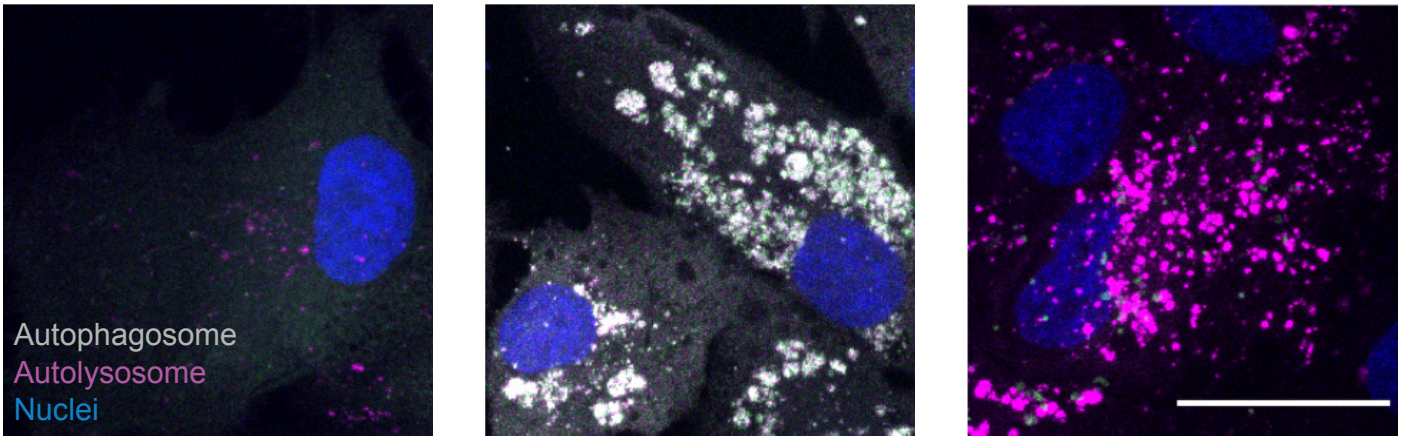


Diagram representing the steps of the autophagy process, as well as the main modulators involved, including the ATG genes and the LC3 protein.

eventually, blindness. Currently, there is no pharmacological treatment.

Much remains to be explored and elucidated in the field of autophagy, but the central role of this intracellular process, and the butterfly effect on the organism's health when autophagy is modulated, highlight the importance of basic biological research. Once this beneficial process for the body is more fully understood, targeted drug development will help accelerate the process of moving knowledge from the laboratory to the clinic.



Retinal pigment epithelium cells expressing an autophagy reporter fusion protein (mCherry-GFP-LC3), cultured in complete medium (Control), with lysosomal inhibitors blocking autophagic degradation (Baf-A1) or fasting (EBSS). Scale 25 µm.

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DNA Replication: a Process Fundamental for Life and Risky for Health

Rodrigo Bermejo
Senior CSIC Scientist at the CIB Margarita Salas

DNA replication is an essential process for the maintenance and development of life. It allows both prokaryotic and eukaryotic organisms to generate virtually identical copies of genetic information for transmission to daughter cells. In addition to being essential for the preservation of genetic material during the propagation of organisms, proper functioning and temporal control of replication is required for development and differentiation programs in higher organisms. Consequently, mutations in genes involved in replication are associated to human diseases, including developmental syndromes and cancer.

In eukaryotic cells, most of the genetic information is contained in chromosomes. Chromosome replication takes place in macromolecular structures known as replisomes. In these, DNA replication is verified, together with associated processes such as the transmission of epigenetic information and the detection and repair of damage. The two fundamental functions of replication are to open the parental DNA helices, a process mediated by the CMG helicase complex (Cdc45-MCM-Gins), and to synthesize new DNA strands, complementary to the parental strands, a process carried out by DNA polymerases Polα, Polδ, and Polε. Polα, after forming a complex with the enzyme primase, synthesizes short RNA and DNA primers that are used by Polδ and Polε to extend the nascent strands. Through its association with the functional core of the replisome (the CMG helicase), Polε continuously synthesizes a leading strand. Polδ synthesizes a lagging strand in a discontinuous fashion, generating short fragments of DNA (Okazaki fragments) that are later edited and ligated by specialized machi-

nery. Chromosomal DNA replication is initiated at sites known as origins of replication and occurs only once per cell division. This is strictly controlled by separating the process into 2 phases: origin licensing, which occurs in G1, with low cyclin-dependent kinase (CDK) activity, in which the origin is enabled by recruitment of the MCM complex; and origin firing, in which CDK activity promotes the recruitment of additional factors that lead to assembly of the replisome and opening of the parental helix in structures known as replication forks.

In addition to the basic factors required for DNA synthesis, the replisome has accessory factors that guarantee the integrity of the replication process, avoiding the formation of DNA mutations and breaks or structural alterations in the chromosomes. These additional factors are of great importance, since they allow cells to respond to problems of replication in situations that interfere with the opening of the parental helix or with the synthesis of new DNA, known globally as replicative stress. For example, the presence of proteins strongly bound to DNA (such as topoisomerases, or proteins chemically entangled with DNA) or DNA damage (e.g. stable interstrand crosslinks) prevents the activity of CMG helicase and requires the action of specialized nucleases and proteases that remove these barriers (by degrading proteins or cleaving them from DNA) or of accessory helicases that help CMG to progress past the obstacle. In turn, different situations can interfere with DNA synthesis. Certain DNA sequences tend to organize themselves into secondary structures or have a high number of base repetitions, and therefore can give rise to errors due to polymerase slippage. Chemical damage to DNA can also block replicative polymerases, and metabolic disturbances (such as decreases in nucleotide triphosphate levels) can lead to a global cessation of replication. Eukaryotic cells possess specialized helicases that are activated in response to replicative stress and remove these secondary structures from template DNA, as well as factors that facilitate continued synthesis by bypassing DNA damage (e.g. replacing replicative polymerases with trans-lesion polymerases, which are more robust and capable of replicating damaged bases).

In general, a prolonged halt in the opening of the parental helix or in the synthesis of nascent strands causes the replisome to become unstable and may undergo a collapse leading to the breakage and degradation of the nascent strands, which when mistakenly repaired can generate mutations and structural alterations of the chromosomes. There is a signalling pathway (the DNA damage checkpoint) mediated by highly conserved protein kinases (e.g. ATR, ATM, and CHK1). These can detect situations of replicative stress and impose a series of controls on essential cell processes (cell cycle arrest, replisome stabilization, inhibition of triggering of additional origins) to ensure that the integrity of the information and structure of the genome is not altered.

DNA replication is related to the development of human diseases, especially when it takes place under abnormal conditions. Replicative polymerases can introduce erroneous bases that differ to those of the parental DNA template, generating mutations that are important drivers of cancer development. The frequency of these alterations is strongly limited, both by the intrinsic error-correcting activity of the polymerases that removes erroneously incorporated nucleotides "on the fly", and by the mismatch repair system that recognizes the alteration introduced by the erroneous nucleotide in the newly synthesized DNA helix and is able to repair it. Mutations that affect the corrective activity of human replicative polymerases (especially POLE1) or that of factors necessary for nucleotide mismatch repair are associated with increased susceptibility to cancer. Moreover, activation of some oncogenes and dysregulation of tumour suppressors cause chronic replicative stress through mechanisms that remain poorly understood. It is believed that this continued replicative stress eventually generates mutations of genes involved in replisome protection or DNA repair, leading to a state of "genomic instability" (an acceleration in the generation of mutations and chromosomal alterations) in pre-malignant cells. Genomic instability acts as a motor of malignant transformation, accelerating the acquisition of genetic characteristics that promote the proliferative and invasive capacity of the neoplasia. Paradoxically, chronic replicative stress can also represent a specific weakness in cancer cells. Indeed, studies are investigating the ability of drugs that inhibit checkpoint kinases (ATR, CHK), responsible for stabilizing the replisome in response to stress, to selectively induce cytotoxicity in these cells.

There are a number of rare Mendelian-transmitted diseases associated with mutations in factors related to replication or its coordination with other cell cycle processes. Biallelic mutations in genes involved in origin licensing and firing (ORC1-6, CDT1, CDC6, GMMN, CDC45, MCM10) cause Meier-Gorlin syndrome, characterized by primordial dwarfism and specific alterations in skeletal development. Mutations in genes that encode the ATR kinase (ATR) or its cofactor ATRIP (ATRIP, ATR-interacting protein) cause Seckel syndrome, characterized by developmental delay with severe microcephaly and intellectual retardation. Recently, the development of whole exome sequencing (WES) techniques has revealed mutations in other replication genes implicated in Seckel syndrome such as DNA2 and CTIP nucleases, which process abnormal structures generated during replicative stress. Mutations in helicases and DNA repair factors have been associated with rare genetic syndromes, characterized by developmental abnormalities

and predisposition to cancer. Mutations in RECQL4 are associated with Rothmund-Thomson, RAPADILINO and Baller-Gerold syndromes. Genes that encode helicases of the RECQ, BLM, WRN, and FANCM family are associated with Bloom, Werner, and Fanconi anaemia syndromes, respectively, which are characterized by predisposition to cancer development and premature aging (Bloom, Fanconi anaemia) and developmental abnormalities (Werner and Fanconi anaemia). Mutations in DDX11, which encodes a helicase involved in replisome protection and coordination with sister chromatid cohesion, are involved in Warsaw breakage syndrome and Roberts syndrome, also characterized by genetic instability and developmental abnormalities.

The discovery in the last decade of various genetic conditions caused by mutations in the replication machinery has greatly advanced our understanding of the importance of DNA replication in genome stability, as well as human development and health. The coming years should yield further advances in our knowledge of the health impact of other key aspects of replication (e.g. the maintenance of epigenetic information).

The DNA Replication and Genome Integrity group at the Margarita Salas Center for Biological Research studies the molecular function of many of these factors in conditions of replicative stress using genomic approaches. Perhaps in the near future, the knowledge generated can be translated into effective strategies to intervene in the pathophysiology of these diseases and to treat cancer.

The CIB Margarita Salas: the Cradle of the Spanish School of Developmental Biology

Carmen Fernández Alonso
PhD in Chemistry at CIB Margarita Salas

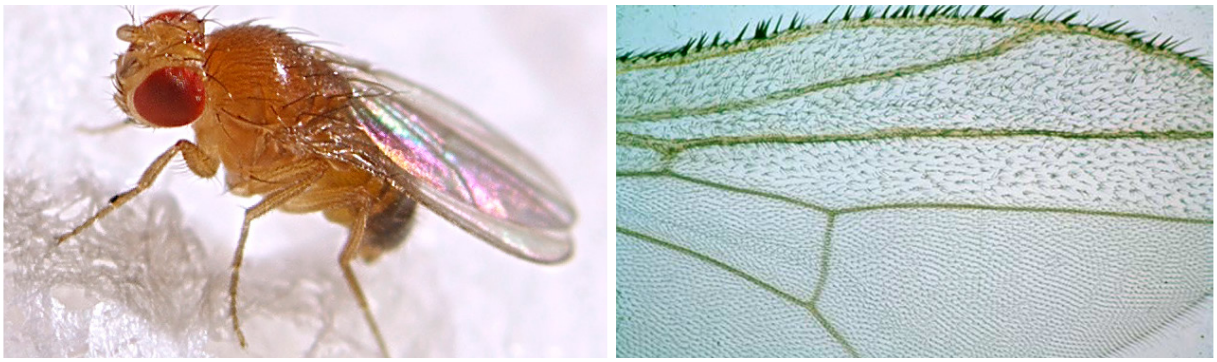
A key player in the evolution of biological research in Spain, the Center for Biological Research (CSIC) played a fundamental role establishing developmental biology research. At the CIB between 1969 and 1975, new methodologies for working with *Drosophila* were developed, helping to define key concepts in developmental biology and resulting in recognition from the international scientific community. This planted the seed that gave rise to the so-called Spanish school of developmental biology.

The historical beginnings of this area of research in the CIB can be traced back to the group of Eugenio Ortiz de Vega, whose laboratory studied genetics in *Drosophila melanogaster*. Antonio García Bellido, considered the father of modern developmental biology, worked on his doctoral thesis in this lab between 1958 and 1962. During this period he analysed the effects of mutations in the *furrowed* gene on development in *Drosophila*, an animal model that is very useful for studying physiology owing to its simplicity and its 65% genetic similarity to humans.

A postdoctoral stay at Ernst Hadorn's laboratory in Zurich provided García Bellido with fundamental knowledge and techniques for the experimental analysis of the development process. Specifically, he delved into methods for culturing cells from the imaginal discs of *Drosophila* larvae (precursors of the structures of wings, legs, antennae, eyes, etc.) in the abdomen of sterile adults, a system he perfected in later years in order to study the properties of these cells in mutants of the bithorax complex, which is responsible for the differentiation of the posterior thorax and abdominal segments of the fly.

Antonio García Bellido (Prince of Asturias Award for Scientific and Technical Research, 1984)

In 1967, he moved to the laboratory of Edward Lewis at Caltech. Lewis was one of the pioneers of *Drosophila* genetics and focused on the study of mutagenesis, cytogenetics, and evolution. Lewis was working with the bithorax system, whose mutations produced flies with four wings, eight legs, etc., showing that the developmental process was severely affected, but the role of this system in the process was unknown. García Bellido began using somatic cells to create genetic mosaics in mutant and normal cells, combining physiology, cell biology, and genetics.



(Left) Drosophila melanogaster. (Right) Wing compartments of Drosophila melanogaster

The key period, 1969-1975

In 1969 Antonio García Bellido returned to Spain and established his laboratory at the CIB-CSIC. The experience acquired during his successive international stays allowed him to establish a new approach for the analysis of the developmental process. He investigated the role of the bithorax complex through two distinct lines of research: 1) the analysis of cell lineages, which establishes the origin of tissues and allows us to understand the evolution of precursor cells during various phases of development; and 2) the study of mutations in genes that regulate development.

The imaginal discs are the most accessible tissues for lineage analysis. Through clonal analysis it is possible to mark a single cell at one point in its development and to study all its descendants in the form of marked clones. García Bellido's group improved on these techniques by identifying markers that could be recognized in individual cells and by making extensive use of X-ray-induced mitotic recombination, which allowed them to perform detailed clonal analysis of the various imaginal discs.

Important findings from that period include the work published in 1971 by García Bellido and John Merriam showing that the marked clones did not cross the border that separates the dorsal and ventral regions of the wing, suggesting specific determination of these structures.

In 1975, Ginés Morata and Pedro Ripoll, pre-doctoral students in Bellido's group at the time, published an article describing the development of the Minute technique, which improved upon the X-ray-induced mitotic recombination method, allowing the group to demonstrate that these clones filled large areas of the wing, but never crossed certain lines: the dorso-ventral demarcation already highlighted in the 1971 work, and a line dividing the wing into two approximately equal halves, an anterior and a posterior zone. This border does not correspond to a morphological accident or to a change in cell differentiation; the cells on either side were morphologically indistinguishable, yet had distinct lineages.

These regions, called compartments because they are distinct cell blocks, were described in an article published in Nature (García-Bellido, Ripoll and Morata, 1973), which marked the first international success of the Madrid group. Analysis of the developmental regulatory genes, engrailed and bithorax complex, revealed the genetic significance of the compartments.

Peter Lawrence, who at that time was collaborating with Francis Crick on more theoretical aspects of development, visited the CIB-CSIC group during these years. Lawrence became convinced of the importance of the ideas developed by Spanish researchers, resulting in a publication by Crick and Lawrence in the journal Science in 1975 that drew attention to the results, increasing the group's international reputation and the expansion of the Spanish school of developmental biology.

Ginés Morata and Pedro Ripoll began their work as independent group leaders after their postdoctoral stays and, together with García-Bellido, joined the Center for Molecular Biology (CBM) after its creation in 1977. Scientists working in other research areas were attracted towards the developmental biology in *Drosophila*, enriching the field and contributing new experience and methodologies. New topics were addressed and experimental animal models beyond *Drosophila* were incorporated into research practice, contributing to the growth of the discipline throughout Spain.

FOR MORE INFORMATION:

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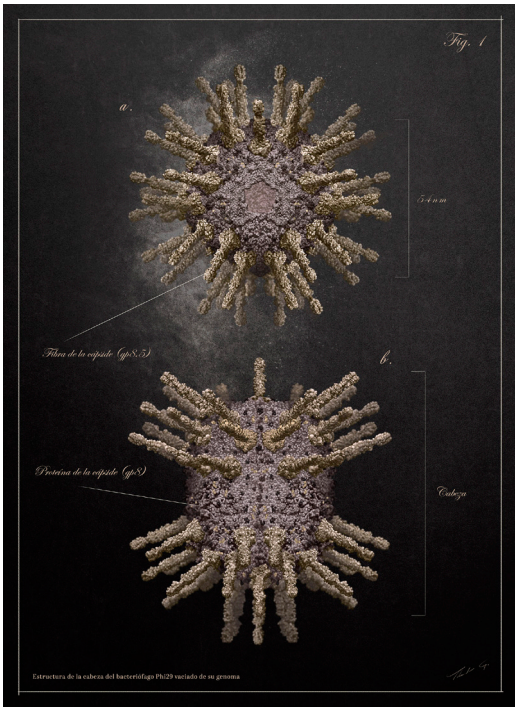
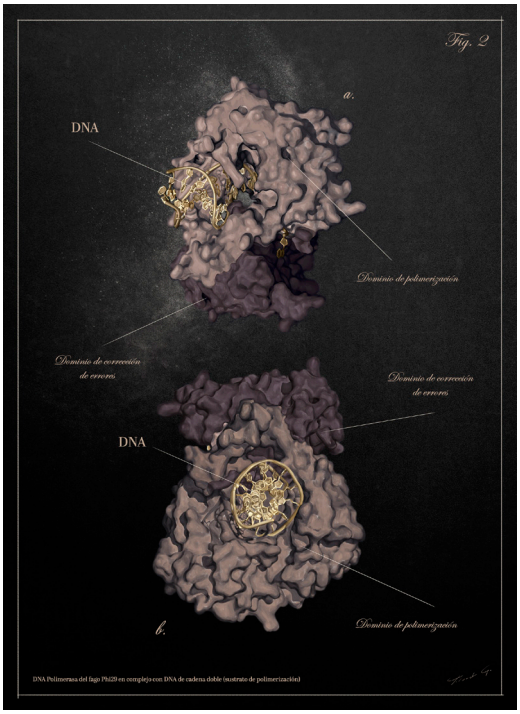
Phi29 DNA polymerase decorates the CIB Margarita Salas





Illustrations by Yolanda González for the lobby of the CIB Margarita Salas

The DNA polymerase of the bacteriophage Phi29 will soon be 40 years old. It was first characterized at the beginning of the 1980s[1], in a study that already hinted that this was an exceptional enzyme: not only could it catalyse copying of individual DNA strands (as did other DNA polymerases, few of which had been identified at that time), but it was unique in its ability to use a small protein from Phi29 itself as a primer to mediate replication of the entire viral genome[2] (19285 base pairs). Moreover, unlike Phi29 replicase, other known DNA polymerases could not copy a genome without the assistance of another type of enzyme, a "helicase", which separated the strands. Nor could they do this in one go, without dissociating from the template until copying was complete, as Phi29 DNA polymerase does. This enormous "synthesis processivity" and its intrinsic capacity to open the double helix[3] formed the basis of the Phi29 replicase patent[4] and its subsequent development as an ideal tool in isothermal DNA amplification processes, both for metagenomic studies and for diagnosis or sequencing techniques. No other DNA polymerase is capable of synthesizing very long DNA chains with very high copy fidelity, and doing so at room temperature, without the need for thermal denaturation cycles that separate the two DNA strands to enable copying.

The molecular structure of this small enzyme[5], resolved by Nobel Prize winner Thomas A. Steitz, is beautiful and exquisite, and explains its unique processivity properties and ability to open DNA as it copies each strand. In addition, the resolved 3D structure confirmed the presence of an evolutionarily conserved 3'-5' exonuclease domain[5] that mediates the enzyme's error correction mechanism (editing), guaranteeing high copy fidelity.

We are grateful to Luis Blanco (CBMSO-CSIC), who discovered together with Margarita Salas the DNA polymerase of bacteriophage Phi29, for writing this text and for his assistance to prepare the illustrations.

1. L. Blanco and M. Salas (1984). Characterization and purification of a phage ø29 coded DNA polymerase required for the initiation of replication. Proc. Natl. Acad. Sci. USA 81, 5325-5329.

2. L. Blanco and M. Salas (1985). Replication of ø29 DNA with purified terminal protein andDNA polymerase: synthesis of full-length ø29 DNA. Proc. Natl. Acad. Sci. USA 82, 6404-6408.

3. L. Blanco, A. Bernad, J.M. Lázaro, G. Martín, C. Garmendia and M. Salas (1989). Highly efficient DNA synthesis by the phage ø29 DNA polymerase. Symmetrical mode of DNA replication. J. Biol. Chem. 264, 8935-8940.

4. Patent título: 29 DNA polymerase. Inventors: Luis Blanco, Antonio Bernad y Margarita Salas. Priority number: 328462. Country of priority: EE.UU. Priority date: 24th/April/1990 Owner: CSIC. Extended countries: whole world. Licenced to: General Electric Healthcare. Explotation: ended; Other related patents: US5001050; US5198543; US5576204; JP2907231.

5. S. Kamtekar S, A.J. Berman AJ, J. Wang, J.M. Lázaro, M. de Vega, L. Blanco, M. Salas & T.A: Steitz (2004). Insights into strand-displacement and processivity from the crystal structure of the protein-primed DNA polymerase of bacteriophage ø29. Mol. Cell 16, 609 -618.

The CIB Margarita Salas makes a successful debut at Researchers' Night

The CIB Margarita Salas has participated for the first time in the Community of Madrid's European Researchers Night (2022 edition).

The center opened its doors on Friday, September 30, offering two outreach activities:

"The bugs that surround us", a microbiology workshop for primary school boys and girls to learn about the microbes that surround us and our interactions with them. Through different activities participants learn the importance of washing their hands and check whether they did so correctly, and the difference between beneficial microorganisms such as yeasts and pathogens such as rotten fruit fungus. In addition, they can view these microbes under a microscope and even make their own "bug" preparations.

"Bacterial polymers: Let's give our planet a breather" is a workshop for ESO and Baccalaureate students in which they learn about the importance of polymers produced by bacteria (biopolymers) in the fight against plastic of petrochemical origin. After an introductory talk and display of some images and experiments, attendees play an escape-room-type game in which they have to solve logical tests using what they previously learned.

Both activities had high levels of participation and were later repeated in Science Week 2022, among others.

The European Researchers' Night is a project in which research centers and other scientific and technological institutions from all over Spain and Europe open their doors or take to the streets to show their work to the public. It is one of the most important dissemination events and this year the CIB Margarita Salas has participated.

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

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