

## Margarita Salas returns to the CIB

**Enrique J. de la Rosa**

*Director at the CIB Margarita Salas*

Margarita Salas left us on November 7, 2019. She had a long scientific career that began at the Centro de Investigaciones Biológicas (Center for Biological Research; CIB-CSIC) and ended at the Centro de Biología Molecular Severo Ochoa (Center for Molecular Biology; CBMSO-CSIC-UAM), where she worked in her laboratory until shortly before her death, as surely was her wish.

On November 21, our president Rosa Menéndez signed off on a resolution to change the name of the CIB, which will henceforth be known as the CIB Margarita Salas. It took just 2 weeks to fulfil the necessary formalities, an effort that Margarita, as both an individual and a scientist, more than deserved.

Throughout her career, Margarita participated in all the types of work that we who dedicate our lives to science can and, in my opinion, should undertake. The fruitful line of basic research that she established based on a model system, the *Bacillus subtilis* bacteriophage *Phi29*, made her a pioneer of molecular biology in Spain. She was an excellent teacher to her doctoral and postdoctoral students, and created an outstanding scientific school. She did not shy away from the transfer of knowledge, participating in the creation and subsequent commercialization of an essential tool for genome sequencing, which was the best known patent to emerge from the CSIC. Moreover, she assumed

the social responsibility that comes with being a scientist, serving as a role model for researchers in general and female researchers in particular, underscoring the importance of our work. While there are many other reasons to justify this lasting tribute to Margarita, those outlined above are more than sufficient.

For this inaugural issue of the Newsletter of the CIB Margarita Salas, we have asked our colleagues at the CIB who trained under Margarita to share their memories. In a separate article, we review her career and discuss its impact, highlighting in particular the 13 years that Margarita spent at the CIB at the beginning of her career, a period that we as researchers know crucially influences future trajectories. Margarita's name will be associated with a research center of the CSIC. The center where she began her illustrious scientific career now bears her name in her remembrance.



Credit: European Patent Office

# Margarita Salas, a pioneer of molecular biology in Spain and a role model for female scientists

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Margarita Salas, an exceptional scientist, a pioneer of molecular biology in Spain, and a role model for Spanish female scientists and for several generations of researchers, passed away on November 7 in Madrid, aged 80. She retained her passion for science right up to her passing, working tirelessly in her laboratory at the Centro de Biología Molecular Severo Ochoa (CBMSO), where she served as Professor *Ad honorem*.

Her encounter with Severo Ochoa at a conference in Asturias in the summer of 1958 convinced her to pursue a career in biochemistry. Ochoa subsequently became her mentor and role model in many aspects.

**A firm defender of a high-quality basic research,** she personified the words of the Nobel laureate Ochoa: “A country without research is a country without development.” She emphasized the need to promote basic research, the basis for the development of a country and the source of many discoveries that benefit mankind. She put this belief into practice throughout her entire scientific career, focusing on the study of the bacteriophage *Phi29*, more recently known as *Salasvirus*.

Specifically, her discoveries relating to the mechanisms of action of the DNA polymerase of phage *Phi29* revolutionized molecular genetics, promoting the development of simple techniques for the amplification of DNA from small samples.

This discovery formed the basis of several international patents with important applications in biotechnology and biomedicine. Indeed, the first of these patents has generated more benefits for the CSIC than any other. In the years that the patent

was active (2003–2009), it accounted for more than half of the total income generated by CSIC patents.

## Doctoral thesis at the CIB (1961–1964)

Margarita Salas, born in Canero (Asturias) in 1938, graduated in Chemical Sciences at the Universidad Complutense de Madrid, and began her scientific career at the CIB, where she completed her doctoral thesis on carbohydrate metabolism between 1961 and 1964, under the supervision of Alberto Sols. During this period, she made her first scientific discovery: the enzymatically catalyzed anom-erization of glucose-6-phosphate.

She arrived at Alberto Sols’ laboratory recommended by Severo Ochoa, with the promise that upon completing her doctorate she could pursue postdoctoral research in Ochoa’s laboratory at New York University School of Medicine, where she subsequently worked between 1965 and 1967. She was accompanied to New York by her



Margarita Salas and Eladio Viñuela in their CIB laboratory in 1962.



husband, the researcher Eladio Viñuela. She often remarked that during this period Severo Ochoa separated husband and wife in the laboratory to ensure that she would be viewed as an independent researcher in her own right, and not merely Eladio's wife.

### Return to the CIB (1967–1977)

Upon returning to Spain in September 1967, she rejoined the CIB, where she founded her first research group with her husband. There, they began their systematic genetic analysis of a bacterial virus, the phage *Phi29*, although shortly after Eladio Viñuela created a new line of research on African swine fever virus. From then on, Margarita independently oversaw the bacteriophage research line. Among other findings, she characterized RNA polymerase, the viral enzyme that transcribes viral DNA to RNA, forming the basis for an entire line of research. Moreover, she discovered that the double-stranded linear DNA of the virus contained a specific protein, p3, bound at one end of either strand, that acted as a starting point for viral genome replication. Later, while working at the CBMSO, Margarita and her team discovered the viral enzyme responsible for replication of the viral genome: DNA polymerase of *Phi29*.

During the first 10 years of her career as an inde-

pendent researcher at the CIB, she contributed to the creation of Spain's first department of molec-

ular biology. She was also key figure, together with her husband and other leading researchers, in the creation of the CBM (now CBMSO), Spain's first molecular biology research center, to which she definitively transferred in 1977. There, for several decades up until her death, she led one of the most important and productive research groups in the country, and oversaw the training of tens of researchers, many of whom are currently directing leading research groups and research centers. Her wide-reaching and outstanding scientific career earned her numerous national and international scientific prizes and awards. She was the first Spanish member of the American Academy of Sciences and the first female researcher to enter the Royal Spanish Academy. She has been awarded honorary doctorates by more than a dozen universities, and was a recipient of the King Jaime I Prize, the Santiago Ramón y Cajal National Research Prize, and the UNESCO L'Oréal Award for Women in Science. In 2017 she received the Nature Award for Mentoring in Science. She was awarded her last international prize in June 2019 from the European Patent and Trademark Office, which presented her with the 2019 European Inventor Award in two categories: Popular Prize and Lifetime Achievement Award.

### Role of women in science

She never neglected the dissemination of science, participating in interviews and public events defending basic research and the role of women in science. She herself recognized that she had not always been aware of gender biases in research. When she was, she started to explain how she had experienced sexism at the beginning of her career and highlighted her role as a woman of science, serving as a model for many girls and young women with a scientific vocation.

The path she followed, as both a person and a scientist, has made her one of the most important female scientists in the history of Spain and, without doubt, the most well known, with streets,



Eladio Viñuela, Antonio Talavera, Jesús Ávila, José Gómez-Acebo, Lola Hermoso, Roberto Parrilla, Juanjo López Fando, Matilde Salinas, **Margarita Salas**, Enrique Méndez, and José Luis Rodríguez Candela, among others (CIB, 1968).

pendent researcher at the CIB, she contributed to the creation of Spain's first department of molec-

schools, and institutes named in her honour. Now, she will also lend her name to a research center, the CIB, part of the CSIC, the scientific research institution in which she spent her entire career.

The **Centro de Investigaciones Biológicas Margarita Salas** proudly bears her name since November 21.

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## Spontaneous and Enzymatically Catalyzed Anomerization of Glucose 6-Phosphate and Anomeric Specificity of Related Enzymes\*

MARGARITA SALAS,<sup>†</sup> ELADIO VIÑUELA,<sup>†</sup> AND ALBERTO SOLS

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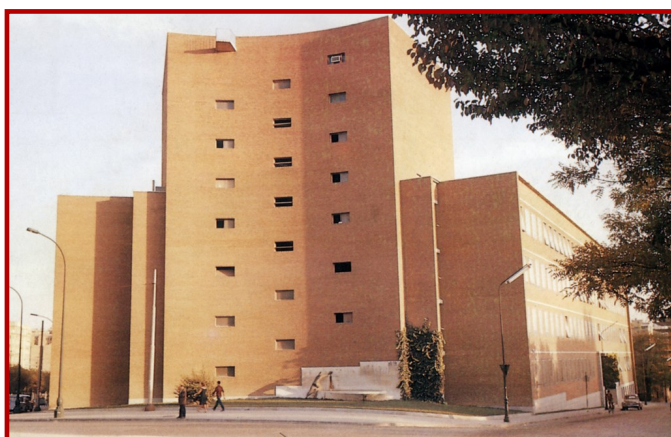
Publication of Margarita Salas' first scientific discovery, obtained during her PhD in Alberto Sols' group at CIB

## My thesis with Margarita Salas: between the CIB and the CBM

**Miguel Ángel Peñalva**

*CSIC Research Professor at the CIB Margarita Salas*

It was so long ago that not even the bricks on the façade had started to fall. It was a spring morning in 1977 when I first climbed the stairs of the Centro de Investigaciones Biológicas at Velázquez 144. I had just completed a course in Molecular Genetics taught by Marta Rodríguez Inciarte at the Faculty of Biology of the Universidad Complutense de Madrid.



The Center for Biological Research, at Velázquez 144.

Marta was my mentor, and the person who convinced Margarita to let me join her laboratory.

During her doctoral thesis, directed by Eladio Viñuela, Marta had generated the second ever-published complete restriction map of a viral genome, ordering the five restriction fragments that the *EcoRI* enzyme generated from the 18 kb of the *Phi29* genome! This had not been a trivial task, since 'the enzyme' (the sole restriction enzyme available at that moment) had to be purified through the use of fermenters that enabled the large-scale growth of the enzyme-producing bacterium. That's how I met José María Lázaro, the laboratory technician who oversaw this task, the best protein purifier I've ever met, and a faithful collaborator who worked with Margarita until his retirement a few years ago. As soon as I arrived at the laboratory, which I'll describe in detail shortly, Marta threw a lead apron on me and told me to keep my eyes open and soak up all the necessary know-how about phage purification with <sup>32</sup>P-labelled DNA. Labelling was performed using 5 mCi in a single experiment. I opened my very pores, not just my eyes! The laboratory was located in the "Viñuelas wing",

on the fourth floor of the old CIB, to the right as you emerged from the elevator, opposite the so-called “Davides” wing (after David Vázquez), also on the fourth floor, but with windows facing Calle Joaquín Costa. Located in front of the library of the Gregorio Marañón Institute and the office of Dr. Rodríguez Candela, director of the institute at that time, it consisted of three windows (the unit of measure of space in the former CIB), all facing Calle Velázquez, two corresponding to the laboratory and one to the office. The office was shared by Margarita and Eladio, and the laboratory space, where I also worked for a few months before we moved to the campus at the Autonomous University, was shared by Marta and Margarita.

If Marta was my “school master” in matters of incipient molecular biology, the person who taught me phage microbiology was the late-lamented Rafa Perez Mellado, who worked in the next laboratory of the same corridor. Even further away, at the end of the corridor, was the ‘large’ laboratory, where 10 years later I would meet two new arrivals to the CIB, the research fellows Santiago Rodríguez de Córdoba and Javier Paz-Ares, two of the brightest researchers ever to have passed through the CIB. The large laboratory housed a group of people who worked on virus morphogenesis, including Juan Antonio García Álvarez, who had begun his doctoral thesis one year before me and went on to become my inseparable phage companion.

**Two key features of the CIB** were lost after the move to the Centro de Biología Molecular: the “confession” and the “seminars/exams”. The latter were held in a small room adjacent to the dining room of “Casa Visi” (which years later was incorporated into the dining room itself). These meetings consisted of conventional group seminars in which each researcher periodically presented his/her work. Eladio, who sat in the front row, would frequently interrupt the speaker and turn around to pose theoretical questions about the subject at hand to other researchers in the room. It goes

without saying that these questions generated much uneasiness in the audience. The “confession”, on the other hand, consisted of the summoning of a doctoral student to the office of Margarita and Eladio where, sitting between the two, the student would present an account of their experiments and conclusions. Margarita monitored daily the progress and planning of all researchers under her supervision, but Eladio also participated actively in the discussion of the results. The moment of the confession was feared, not because Margarita was at all aggressive with her students, but because it fully revealed the true extent of one’s limitations and how much more there was to learn. The practice of “confession” ended when we moved at the end of that same year to the CBM, where Margarita and Eladio had independent offices. Nonetheless, Margarita continued to monitor my experiments daily, designing infinite controls that made the conclusions rock-solid, planning work on the calendar many days in advance, teaching me how to write the protocols so that the experiments could be reproduced by others at any time, and pointing me towards essential reading material.

For several years Juan Antonio and I were her only doctoral students. We enjoyed the luxury of her availability and we received our training through what today would be called “personalized attention” from a supervisor who, in addition to always treating us with almost maternal affection, was already a legend of molecular genetics.

In that room in Velázquez 144 I saw Margarita working for the first time at the bench. Before starting, she cleared her workplace and laid out on the wooden bench a mosaic of immaculately white filter papers that stood out against the black paint. Then, she took from her office some written protocols on which she had detailed all the stages of the procedure to be followed. After consulting the protocol, she placed on the filter papers all the material required: flasks, test tubes, conventional pipettes, micropipettes (which were then made of

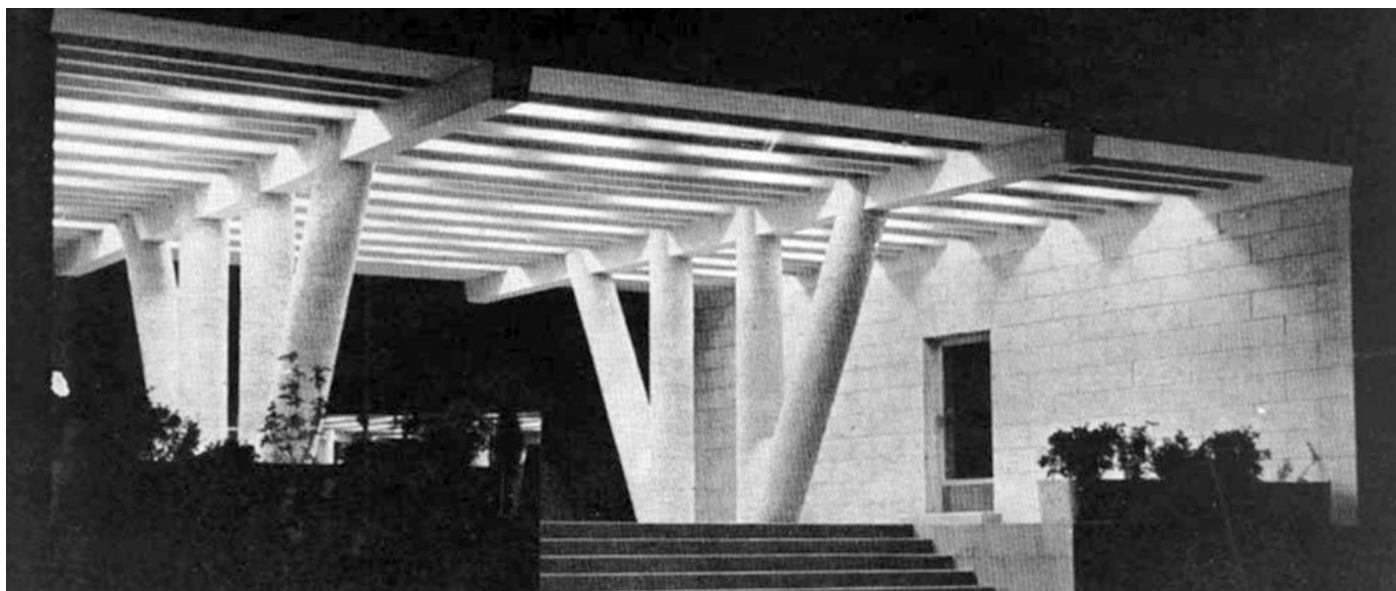


glass and were reusable). Only when this ceremony had concluded would the experiment begin. It reminded me of an orchestra warming up before performing a complicated symphony, under the direction of a true master. I have never met a person who showed such a level of skill or concentration when executing complex scientific protocols.

**I would like to end with what for me is an unforgettable memory of Margarita** that sums up the extraordinary generosity with which she supported me on numerous occasions throughout my work. It was during the final stage of my doctoral thesis, towards the end of 1980, after we had transferred to the CBM. Ten years before, Juan Ortín had demonstrated (in the CIB) that phage DNA was covalently bound to a protein, and subsequent laboratory studies had identified this protein as the product of the *P3* gene. All available data pointed to that protein as an initiator of replication at the ends of *Phi29* DNA, but the definitive evidence was missing: synthesizing a complex consisting of p3 and dAMP that would serve as primer for the DNA polymerase at the ends of the DNA. After many unsuccessful efforts we managed to detect *in vitro*, through the incubation of protein extracts from phage-infected cells with dATP- $\alpha$ - $^{32}\text{P}$ , the formation of the p3-dAMP complex. While this was a promising start, much work remained to make the conclusions sufficiently solid for publication. During the following months we conducted a series of radiolabelling experiments

in order to study all of the factors necessary for the reaction to occur. For each reaction, the protocol required prior gel filtration of the sample to remove the non-incorporated nucleotide before analysis by electrophoresis. Often I found myself with eight samples that needed to be urgently purified once the reaction was completed. Margarita realized right away that I simply couldn't handle so many samples, and offered to help me. Thus, the days that the radioactive nucleotide arrived I had to prepare two methacrylate screens and eight Sephadex columns packed in Pasteur pipettes. I knocked on the door of her office and Margarita came out, stood behind one of the screens and we passed the samples through, four per person, counting the drops that we collected in plastic tubes. I don't know how, but she always managed to finish before me. And she even had time to place the tubes in vials for the scintillation counter, so that I could rapidly analyse them. And so I was almost certainly the last person to work with Margarita Salas at the bench.

I recount this story because it is often noted, and rightly so, that one of Margarita's great achievements was creating an outstanding school. To do this it is necessary not only to have many (often very bright) students, but to also have a teacher that supports their students with generosity and unflagging dedication, qualities that she always exhibited.



# My postdoctoral stay at Margarita Salas' laboratory

**Pedro García**

*CSIC Investigator at the CIB Margarita Salas*

My stay at Margarita Salas' laboratory, between 1983 and 1984, began shortly after I finished my thesis at the CIB with Rubén López, and was my first postdoctoral experience. Although I was there less than 1 year, my time there was enriching in every aspect thanks to the personal touch that Margarita lent to all her activities.

From a scientific point of view, there was a constant requirement to be consistently up-to-date on one's specific line of work: we had to examine newly published articles in order to stay familiar with the work of other groups and to be aware of progress made by scientific rivals working in common areas. Margarita encouraged daily discussion of these topics, either personally with each member of the laboratory, or as a group during our frequent seminars. This created an environment in which there was a great demand to "measure up" to the scientific level that Margarita maintained throughout her life.

In addition to Margarita the scientist, the best known and most recognized by everyone, there was the less well known personal side of Margarita. This aspect of her character was evident whenever you met her at any meeting, conference, or event. She was always affectionate, interested in the progress in your laboratory, the struggle to obtain more funding, and mentioning, frustrated,

the lack of common sense among politicians, who didn't (and still don't) prioritize science. She was always acutely aware of the day-to-day problems we faced and her constant encouragement gave us the strength to continue fighting and improving. This side of her character was evident in her handling of a Ministry of Science-funded project that began in 1985 and was co-led by Margarita and Rubén; throughout the course of the project her spirit and leadership guided us all.

More recently, researchers throughout Spain with a shared interest in the study of phages, albeit from very different perspectives, once again witnessed Margarita's dedication through her participation in the FAGOMA network. This group of "phagologists" has already held five meetings. Margarita participated in all of them, either in person or, if this was not possible, by providing interesting contributions describing her recent findings about the enzymes encoded by *Bacillus subtilis* phages. My last personal encounter with her was just before the summer at the Centro Nacional de Biotecnología (National Center of Biotechnology; CNB-CSIC), in the context of a project that involved several colleagues and to which she had many ideas to contribute. As always, and until the end, she was in the thick of it.

All that is left for me to say is, with the deepest affection, "goodbye Margarita and thank you for the lasting example you have set".

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## Subunit Composition of *B. subtilis* RNA Polymerase

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# Margarita Salas, endless constancy

**Alicia Bravo**

*CSIC Scientist at the CIB Margarita Salas*

It was 1989 when I met Margarita Salas in the *Bromfietsen Meeting on Molecular Biology of Bacillus and its Phages*, which was held at the National Parador in Almagro. At that point, I was finishing a postdoctoral stay at the Max-Planck-Institut für Molekulare Genetik (Berlin). Margarita's group presented several communications and I found them so interesting that I did not hesitate to ask if I could work with her on returning to Spain.

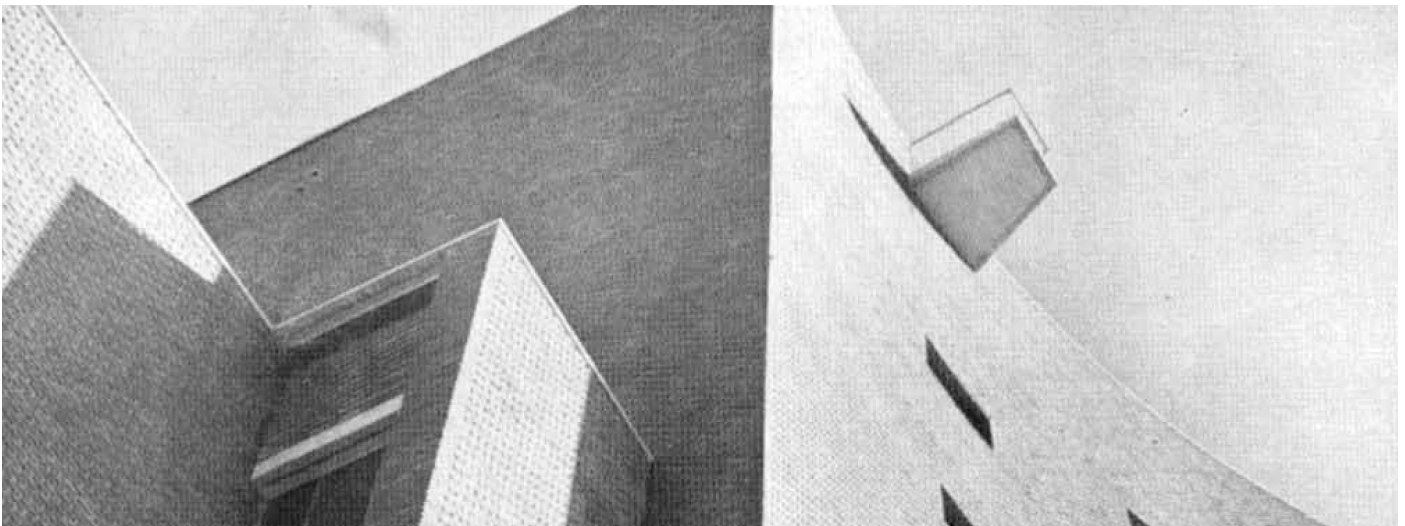
I had the great privilege of sharing 15 years of basic research with Margarita. They were years of much enthusiasm, a lot of work, and rewarding scientific research. Today, I remember Margarita with much love and admiration.

**Margarita Salas was an unstoppable fighter** in very difficult times for female scientists. Tenacity was undoubtedly a key feature of her personality. Her enthusiasm for science and her capacity for work always motivated those of us who were lucky enough to be close to her. She had a great ability to organize and maintain a dynamic research group, in which senior researchers, post-doctoral researchers with excellent scientific backgrounds in other national or international centers,

and PhD students from different universities all contributed to create a highly enriching scientific environment.

Despite her multiple academic and institutional responsibilities, which multiplied as she was recognized and rewarded for her scientific work, Margarita Salas always managed to remain abreast of daily activities in her laboratory. She always found a place in her busy schedule to discuss the latest results, no matter how preliminary they were. She always opened the doors of her office to talk about science. The weekly group seminars were sacred, and discussion and scientific criticism were highly motivating for all involved. Students quickly learned that working in science required a lot of dedication, that keeping up to date with one's field was essential, and that rigor, honesty, and credibility are fundamental values in science.

Margarita Salas is not gone. She will remain among us because she has made history in the field of molecular biology. She has achieved recognition and respect from the scientific community, both nationally and internationally. She succeeded in joining that still small list of women leaders in science.





# Selected publications by Margarita Salas during her time at the CIB

## Doctoral thesis under the supervision of Dr. Alberto Sols (1961–1964)

- E. Viñuela, M. Salas and A. Sols (1963) Glucokinase and hexokinase in liver in relation to glycogen synthesis. *J. Biol. Chem.*, 238, 1175-1177.
- M. Salas, E. Viñuela and A. Sols (1963) Insulin-dependent synthesis of liver glucokinase in the rat. *J. Biol. Chem.*, 238, 3535-3538.
- M. Salas, E. Viñuela, J. Salas and A. Sols (1964) Muscle fructose-1, 6-diphosphatase. *Biochem. Biophys. Res. Commun.*, 17, 150-155.
- M. Salas, E. Viñuela and A. Sols (1965) Spontaneous and enzymatically catalyzed anomerization of glucose-6-P and anomeric specificity of

related enzymes. *J. Biol. Chem.*, 240, 561-568.

## Period from 1967 to 1977

- J. Avila, J.M. Hermoso, E. Viñuela, M. Salas (1970) Subunit composition of B. subtilis RNA polymerase. *Nature*; 226:1244-5
- J. Ortín, E. Viñuela, M. Salas, C. Vázquez (1971) DNA-protein complex in circular DNA from phage *Phi29*. *Nature New Biology*; 234:275-7
- F. Jiménez, J. Ávila, E. Viñuela, M. Salas (1974) Initiation of the transcription of *phi29* DNA by *Bacillus subtilis* RNA polymerase. *Biochim Biophys Acta.*, 349(3):320-7.
- J.L. Carrascosa, A. Camacho, E. Viñuela, M. Salas (1974) A precursor of the neck appendage protein of B. subtilis phage *Phi29*. *FEBS Lett.* Aug 30;44 (3):317-321



Margarita and Eladio at the 1969 FEBS Congress



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Margarita Salas

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