From Physical Chemistry to Molecular Biology: The Catalan Contributions to Nucleohistone Studies: 1965-1977

Xavier Calvó-Monreal[*]

During the 1960s, the Spanish Biochemistry and Molecular Biology received a decisive impulse for their development. The first generation of biochemists, trained abroad, had returned to Spain and set up their own research groups mainly with the support from the Spanish Science Council (CSIC). A new generation, their young graduates, completed their training following the same strategy, by postdoctoral research overseas.

A particular case, due to their initial training as chemists, was the group which became to be known as the "Catalan Structuralist School", led by Jaume Palau and Joan Antoni Subirana. Their postdoctoral training abroad represented a change in their scientific interests, from organic chemistry to structural molecular biology, particularly the study of the nucleohistone. The aim of this communication is to show how it has been possible to reconstruct and study their early years, taking into account the disciplinary identity of chemistry and its changing relationships with other fields, such as Molecular Biology, mainly studying their correspondence, analysing their scientific papers in addition to interviews with the main characters, placing their School in the framework of the historiography of Biochemistry and Molecular Biology in Spain, and also in the wider, international, context.

The Training Periods

Joan Antonio Subirana completed his degree in chemistry in Barcelona in 1958 and his PhD. in polymer chemistry and industrial engineering in Madrid in 1960. After a stay in Paris with Arnold Münster, the following year then moved to Harvard University to work with Paul Doty. More or less one year before his arri-

[*] Centre d'Estudis d'Història de les Ciències-Universitat Autònoma de Barcelona (CEHIC-UAB). Barcelona, Catalonia, Spain. *arjuna03@terra.es*

val in Harvard, the denaturation and renaturation of DNA had been discovered by Doty and his co-workers. Denaturation is the process by which the two strands of DNA become separated, and renaturation, the opposite. It is important to stress that the understanding of both processes was crucial in order to provide data to validate Watson and Crick's 1953 DNA structure. Subirana's stay in Harvard produced four papers; the first of them co-authored with Doty, concered phage DNA renaturation. Subirana became interested in the changes which could take place in DNA structure when interacting with other substances, such as proteins. As can be seen, Subirana's first interest was not in the biological field but in polymer chemistry. It was during his stay at Harvard when his shift to structural molecular biology took place.[1]

In 1963, after two years in Harvard, Subirana obtained a fellowship which allowed him to move to the polymer group at the Weizmann Institute in Rehovoth, to work under the leadership of Aaron Katchatsky. Subirana's work at Rehovoth was essentially theoretical, but it important to stress his contribution, in what was his first paper, on the X-ray diffraction of biological polymers, mainly in the preparation of DNA-spermine fibres. [2]

Jaume Palau took his degree in chemistry in 1959 and his PhD in 1963 in Barcelona. In 1964, Palau attended a course given in Madrid by Alberto Sols at the "Centro de Investigaciones Biológicas del CSIC" (from the Spanish Science Council). Sols was one of the Spanish researchers who were developing own groups after their postdoctoral stages in foreign countries.

The same year, Palau went to London, funded by the British Council, to the Chester Beatty Research Institute, in the Physical Chemistry department, led by John Butler, and began his first work on histones. Simultaneously, Palau went to London King's College, to assist in a course given by Maurice Wilkins, in the biophysics department, began his research on oriented fibres from DNA-histone complexes, which had to be studied with X-ray diffraction techniques.

Palau's studies in London produced three papers on histone fractions. As well as the papers, both Subirana and Palau apprenticeships also consisted of acquiring knowledge about department organisation, and laboratory skills concerning the molecular biology techniques and instruments. [3]

Briefly, their return to Barcelona was to jobs as research collaborators of the CSIC, the Spanish Science Council and the beginnings of the biopolymer section within the genetics department of the Science Faculty in the University of Barcelona, under the leadership of Antoni Prevosti. In 1967, Subirana was appointed to the Chair of Organic Chemistry Technology in the Engineers School.

In 1969, Palau was immersed in activities which led to the creation of the Basic Biology Institute. It is important to stress that, in 1968, Subirana met Alexander Rich from the Massachusetts Institute of Technology (MIT), and the idea of an X-Ray laboratory in the Engineers School in Barcelona came from Rich's suggestions. The research field chosen by Palau and Subirana was the characterisation of the histones from marine invertebrates, echinoderms and molluscs, as well as the structure of the related nucleohistones, that are the associations between DNA and these proteins.

In 1963, while Subirana was in Harvard and Palau in Barcelona, waiting for the grant that would allow him to go to London, an event took place which is important to the account being related, this was, The First World Conference on Histones, organised and coordinated by James Bonner, from Caltech's Biology Department, and Paul Ts'o, from the Johns Hopkins University. After knowing about this conference and the subsequent review in *Science*, Subirana and Palau began to specify the research field in which they would like to work.[4]

A quick look at the list of the contributors to this conference permits one to appreciate the influence which it exerted on our main characters, not only during their postdoctoral training but in future joint ventures in research. Among others, it is important to stress the contributions of John Butler, Ernest Johns and Derek Phillips, from the Chester Beatty Research Institute, London, Edward Morton Bradbury and Colin Crane-Robinson, from Portsmouth, Brian Richards, from King's College London, and Geoffrey Zubay, former researcher with Paul Doty, but working at King's at the time of the conference.

Why their interest in histones? In spite of the publication in 1953 of the collection of papers in *Nature* concerning the structure of DNA and the available experimental evidences, the 1957 paper on the semi-conservative replication of DNA by Meselson and Stahl and in 1960 by Doty and co-workers on the denaturation-renaturation of DNA, in which Subirana had taken part, the Watson-Crick structure was no more than a theoretical speculation, especially in their suggested genetic implications. It is important to take note that the *cracking* of the genetic code did not occur until 1967. Moreover, the RNA structure was not yet solved and it is important to stress the pioneering works of Watson and Alexander Rich from 1953 to 1956. It is also important to take into account that the main issue at the time was the chromosome structure and the understanding of the genetic regulation and to remember that the chromosome is constituted not only of DNA, but of proteins, which had to play some role in this regulation and in its structure, a matter that had been in Subirana's mind since his time at Harvard.

In order to start the research it was essential for Palau to obtain a grant which would allow him to go to London, to the Chester Beatty, to be precise, to John Butler's laboratory, a leader in histone research. Why the Chester Beatty and Butler's group? Shortly before Palau's arrival, Ernest Johns, one of Butler's co-workers had discovered that, in no matter which kind of living organism, there were always the five same kinds of histones associated to DNA in the chromosome, and had published an account of the techniques which allowed them to be isolated. A grant obtained from the British Council and subsequently another from the Wellcome Trust, allowed Palau to stay in London during 1964, at the Chester Beatty and King's College.

The research project

The next step was how to obtain funds for their research. Subirana's experience in the USA made him think in the Agriculture Department, which during these years was developing an aid program for underdeveloped countries called the Food for Peace Program, or Public Law 480. Their application was made during 1964 and approved in 1965, but the program was cancelled in the same year. These setbacks confirmed their decision to apply to the National Institutes of Health (NIH) extramural program. The grant was given in 1966 and allowed them to start their research group in Barcelona.

Apart from the fact that funding was guaranteed, the newborn group needed a physical space in which the research could be developed. While Palau was in London, Subirana had returned to Barcelona, to the Organic Chemistry Department, whilst waiting for the American grant, and applied for a collaborator post in the Spanish Science Council, insisting that Palau did the same.

Antoni Prevosti, Chair of Genetics At Barcelona University, who was interested in the setting up of a molecular genetics group in his department, was well disposed to host the new biopolymer's section, after applying for coordination with the Spanish Science Council. These circumstances allowed Palau and Subirana to begin with their research program on histones. Nevertheless, their economic situation was far from solved, because of their position in the Council structure. The "collaborator" was the lowest status. This potential instability caused Subirana's decision to compete for the Organic Chemistry Technology Chair in the Engineer's School in Barcelona, which he obtained in 1966.

It must be taken into account that Subirana, while studying chemistry, also took a degree in Industrial Engineering, which allowed him to obtain his second PhD

and that Subirana's first interests were not in the biological field but in the chemistry of polymers, mainly in plastics. Competing for a Chair was in Subirana's mind ever since he was at Harvard, according to him, in Spain, if anybody wanted to do research, the main requirements were independence and a global vision of the chosen research field, and a chair allowed the necessary independence. The fact of Subriana having the Chair and the NIH grant, allowed them to develop the biological research within the Engineer's School, at the same time dealing with the teaching and the industrial research.

In 1970 the group obtained a new coordination with the Council which allowed the formation of the Department of Macromolecular Chemistry. The grants, first from the NIH and, afterwards, from the Population Council, a Rockefeller Foundation subsidiary institution, allowed them since 1968 to buy the necessary instruments for the research program, to publish their papers and also to supervise their first Ph.D. students.[5] During their stay in the Genetics Department, Subirana and Palau published their first join paper on the histones of marine invertebrates, in 1966. From 1966 to 1973, Subirana published 23 papers, and Palau 10, five of which were co-authored. Their main interest was the study of these marine invertebrates, echinoderms and molluscs, because these organisms showed a wide range of basic proteins associated with DNA in the spermatozoid. They focused on these organisms after reading papers Maurice Wilkins and others from 1956 to 1960. Until the 1970s there were no papers published on the study of fish protamines, and little attention had been paid to other species' nucleoproteins which showed important differences in size and amino acid composition. Although the first research was on Sea urchin and Holothuria, a third kind of organism, molluscs, was included in the program.

Why did they study this kind of organism? Echinoderms have a special feature which is that their spermatozoids contents histones quite similar to those from somatic cells, but with some differences. One more advantage was the cell structure simplicity, related with its function, sperm cells are available and simple, because they contain the minimum components, among them the nucleoproteins, which allowed extrapolating the results to other species. Two important contributions were made by Palau and Subirana, firstly, the analysis of the proteins from invertebrate's sperm, particularly molluscs and echinoderms and, secondly, the structural studies of nucleohistone by X-Ray diffraction techniques. At this point, must be stressed that it was the methods developed by Johns, from the Chester Beatty Research Institute, which allowed the histone fractions to be isolated.[6]

The Chromosome Structure

In the mid 1950s, while DNA structure and properties had been elucidated in some detail, the clarification of the nature of histones and how could be linked with DNA, remained unsolved. According to John Butler, the problem lay in the extraction methods by which the fractions of different composition were obtained. It was concluded that the native structures were destroyed during the extraction. Since it was known that nucleoprotein could be obtained as an aqueous solution by chromosome dispersion in water, it seemed possible to study its molecular characterisation and to obtain evidences concerning the chromosome constituent units. Modifications introduced by Doty and Zubay in the procedures led to the isolation of deoxyribonucleoprotein (DNP) solutions and use of a wide range of techniques, among them, chemical analysis, infrared spectrometry, light dispersion and electron microscopy.

But Doty and Zubay's research showed that the aqueous solutions of DNP had a complex behaviour that challenged a simple analysis and suggested a gel like system, consisting in subunits which could not be identified at the time. The interest in its study vanished and only a few works on this issue were developed during the 1950s. The alternative was to develop chemical techniques which allowed the extraction of the unknown number of histone fractions, and this was the approach developed by Johns during the first half of the 1960s. These methods were developed using calf thymus, then were applied to other materials such as invertebrate sperm nuclei. The method is based on the extraction of the whole tissue with acid in the presence of a high concentration of ethanol, in combination with a whole set of techniques which allowed to obtain the histone fractions for further studies, such as the structural ones.

Palau and Subirana used this method and the modifications required by their research in order to characterise the proteins from invertebrates sperm, previous to the development of the X-ray diffraction techniques. Their landmark paper of this period of 1973 was published in *Biochimica et Biophysica Acta*.[7]

In 1970, the biopolymers group was replaced by the Department of Macromolecular Chemistry, again with assistance of the Spanish Science Council. At the same time with the characterisation of the histones, the X-Ray laboratory was in its trial period. When the X-Ray diffraction techniques began to be applied to the studies of the nucleohistone, they had provided a small amount of data, mainly because the material's nature which usually had gel, mainly non-crystalline, charcteristcs. Nevertheless, some features suggested the presence of a repetition which could represent some kind of structural unit in the chromosome.

From this speculation, three research lines arose among the community, physical studies of the shape of the histone fractions and their behaviour, X-Ray diffraction techniques and spectroscopy studies. How to focus the study of this supposed nucleohistone superstructure consisted in the reconstruction of nucleoproteins from a mixture of DNA and histones, obtained separately.

In Barcelona, the X-Ray diffraction techniques were considered the best approach, which implied the setup and optimisation of the X-Ray lab, between 1968 and 1973, including the modification of the standard instruments and the design of their own prototypes. The decision of the setting up of the X-Ray laboratory came, mainly, from Subirana's experience in Israel, were he had collaborated in his first paper on this issue and from the advice from Alexander Rich, then at the MIT, during the summer of 1968. The apprenticeship on these techniques for Subirana took place during that summer at the Massachusetts General Hospital. This stay led to the connections with Alexander Rich, who, knowing the interests of Subirana, suggested that he set up the X-Ray facilities in Barcelona and, at the same time provided him with an instrument which could be useful in the study of fibres. This was a small X-Ray diffraction camera, which in Barcelona became to be known as "RICH". It is important to stress that, in 1968, Rich was working on RNA structure, but in crystals. The first tests took place in Barcelona at the end of 1969, and the first paper was published in 1973. From 1973 to 1977, Subirana's group published 9 papers using of X-ray diffraction techniques and it must be considered that the laboratory was fully optimised. Since 1968, Subirana followed the same approach for the study of the possible conformational changes in DNA when associated with proteins. First of all, proteins mainly from marine invertebrate's sperm were characterised. A great variety of those proteins and the differences in their composition were described and fibres of DNA-protein complexes were prepared. The results confirmed that the double helix was very stable and that its conformation did not change when associated with proteins.

Concerning the suggested presence of a repetition in the chromosome structure, taking into account the data provided by electron microscopy and with enzyme digestion techniques, in 1974, Roger Kornberg proposed the nucleosome model. There was a problem in that X-Ray diffraction techniques applied to fibre studies could only provide a global vision of DNA structure. As the DNA sequence changes along the genome the question was, what was the influence of the sequence in DNA structure? Could these differences influence the organisation and the activity of the genome in the cell nucleus? In order to answer these questions, the only way to approach the problem was to determine the structure of DNA of different sequences.

In the late 1970s, organic chemists discovered how to synthesise crystalline DNA fragments of known sequence. This fact allowed the start of the study of DNA structure at the atomic level, with more precise crystallographic methods, the *single crystal* one, which was successfully applied to RNA studies ten years ago. The first structures obtained represented at the same time a surprise but also a long expected confirmation. In 1979, Alexander Rich's group at the MIT obtained artificial DNA which crystallised in a different way to that of the standard Watson and Crick model, and was called Z-DNA. While the Watson and Crick helix was dextrorotatory, Rich's structure was levorotatory, and this led to the fact that DNA could present different conformations as well as the A and B forms. The Z-DNA structure rarely appears in biological systems and its importance in cell life still remains unknown.

Thanks to the obtaining the sequences of crystallised DNA and with the available techniques, the same year 1979, Richard Dickerson's group at the University of California at Los Angeles synthesised a double helix structure in the B form, which gave practically identical diffraction data to that obtained by Franklin and Wilkins two decades before. This fact constituted a step further in the confirmation of Watson and Crick's model.

Coinciding with these discoveries, Subirana's laboratory shifted from the study of fibres to those of crystals. During the following years, their contributions to this issue were confirmation of the results obtained with fibre studies, and the attempts to obtain DNA crystals with new shapes. Moreover, since 1977, the group have continued to expand their use of a wide range of techniques and instruments, including ultracentrifugation, electron microscopy and so on, for their structural studies.

Conclusion

One of the issues raised in this communication has been the role of postdoctoral training in research centres abroad in the setting up of the new Spanish research groups in biochemistry and molecular biology as well as the maintenance of the established relationships. International recognition has been used as a legitimation strategy. Thus, Palau and Subirana developed a research programme, including the necessary infrastructures, and the scientific policies which allowed them success. In summary, the study of the Barcelona's structuralist school reinforces the idea that successful scientists help to construct the scientific policy together with advancing their disciplines, experiments and techniques.

Notes

[1] Juan Antonio Subirana, "The irreversible denaturation of bacteriophage deoxyribonucleic acid". *Biochim. Biophys. Acta* 103(1965):13-24; Juan Antonio Subirana and Paul Doty, "Kinetics of Renaturation of Denatured DNA. I- Spectrophotometric Results". *Biopolymers* 4 (1966): 171- 187; Juan Antonio Subirana, "Kinetics of Renaturation of Denatured DNA. II- Products of the Reaction". *Biopolymers* 4 (1966): 189-200; Juan Antonio Subirana, "La renaturalización de ácido desoxirrinonucleico (ADN) desnaturalizado: aplicaciones al estudio del genoma de los bacteriófagos". *Anales de la Real Sociedad Española de Física y Química. Serie B-QUÍMICA,* 62 (1966): 521-526.

[2] Mario Suwalsky, Wolfie Traub, Uli Schmueli & Juan Antonio Subirana, "An X-Ray Study of the Interaction of DNA with Spermine". *J. Mol. Biol.* 42 (1969): 363-373.

[3] Jaime Palau & John A.V. Butler, "Trout-Liver Histones" *Biochem. J.* 100 (1966): 779-783; John Butler, David Power, & Jaime Palau, "Countercurrent-Distribution Studies on Histones". *Biochem. J.* 102 (1967): 539-547; Jaime Palau, Pardon & Brian Richards, "The reversibility of the dissociation of nucleohistone by salt". *Biochim. Biophys. Acta,* 129 (1967): 633-636.

[4] James Bonner & Paul T'so (eds), *The Nucleohistones* (San Francisco and London: Holden-Day, Inc, 1964).

[5] Jaime Palau & Juan Antonio Subirana, "Histones of Marine Invertebrates". *Biochem. J., Proceedings of the Biochemical Society* 101 (1966): 34-35.

[6] Ernest W. Johns, "Studies on Histones. 7. Preparative Methods for Histone Fractions from Calf Thymus". *Biochem. J.* 92 (1964): 55-60.

[7] Juan Antonio Subirana, Carmen Cozcolluela, Jaime Palau & Mercedes Unzeta,"Protamines and other basic proteins from spermatozoa of molluscs". *Biochim. Biophys. Acta* 317 (1973): 364-379.