The molecular biology of the mammalian genetic apparatus

Volume 1

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1977

North-Holland Publishing Company
Amsterdam · New York · Oxford

PREFACE

Investigations on the mammalian genetic apparatus were initiated about one hundred and five years ago when Friedrich Miescher published his findings on the preparation and characterization of chromatin (termed nuclein at that time) from human pus cells in 1871. Not until about thirty years ago did nucleic acids, particularly DNA, gain recognition as the genetic material which is reponsible for the physical transfer of hereditary information. Only about 25 years ago, the basic features of the double helix of DNA were formulated, which provided a structural basis for its function. During the ensuing years from 1955 to 1970, intensive efforts were made to decipher the universal genetic code of nucleotide sequences in nucleic acids specifying the amino acid sequences in proteins, and to investigate the translational machinery, i.e. the transfer RNA and the ribosomes. Concurrently, the replication and transcription enzyme systems for nucleic acids were beginning to be revealed. It was then generally acknowledged that the elucidation and understanding of the programming/control of the expression/replication of the genetic apparatus as related to differentiation/development must be the next most important challenge to be faced in our research.

Thus, the first World Conference on Histone Biology and Chemistry was organized by Professor James Bonner and myself in 1963; this conference, together with the published proceedings, 'The Nucleohistones', have provided an impetus to the research on chromatin. In the years following this conference, tremendous progress has been made in the purification of histones; determination of the primary structure of histones; characterization of their secondary modification, i.e. phosphorylation and acetylation; studies on conformation and mutual interaction of histones, as well as studies of interaction of histones with DNA; elucidation of the organization of DNA sequences; investigation of the synthesis, structure, and function of messenger RNA; and active research on the possible role of chromatin-associated nonhistone proteins; as well as in other areas. In

addition, it was generally recognized that an understanding of the cell can only be attained through knowledge of the genetic apparatus. Furthermore, to reach an understanding of many medical problems such as genetic diseases, cancer and aging rests on knowledge of the mammalian cells, which in turn depends on elucidation of the mammalian genetic apparatus. Thus, the prevention and control of these major medical problems are indeed directly dependent on the research on the mammalian cell and its genetic apparatus.

With this background, the 'International Symposium on the Molecular Biology of the Mammalian Genetic Apparatus – Its Relationship to Cancer, Aging and Medical Genetics' was held at the California Institute of Technology in December of 1975. This symposium not only provided an opportunity to examine the recent achievements and future developments in the research on the mammalian genetic apparatus, but also recognized the contributions made to this field by the Caltech people (faculty, students, alumni, associates, and friends) and particularly those from Professor James Bonner's laboratory on the occasion of his 65th birthyear. This symposium was organized by Roger Chalkley, Norman Davidson, Ru-chih Huang and Jerome Vinograd*, with myself as chairman. We are grateful for the support and assistance of the following organizations and the people in these organizations principally responsible for this support: Energy Research and Development Administration (Dr. James Liverman), National Cancer Institute (Dr. Gary Flamm), National Science Foundation (Dr. Herman Lewis), National Institute on Aging (Dr. Richard Greulich and Dr. Donald Murphy), Fogarty International Center (Dr. Fred McCrumb* and Dr. Maureen Harris), California Institute of Technology (Dr. Robert Sinsheimer and Dr. John Baldeschwieler) and Johns Hopkins University (Dr. John C. Hume).

The proceedings of this symposium are divided into three parts to be published in two volumes: Volume 1 contains – Structure; Volume 2 contains Part A – DNA organization and gene expression, and Part B – Relationship to somatic genetics, cancer and aging. Encouraged by the rapid growth in this important field and recognizing the need for reference and educational materials, a continuing series of books on this subject, entitled 'Molecular Biology of the Mammalian Genetic Apparatus – Its Relationship to Cancer, Aging and Medical Genetics' has been planned. Thus, these two volumes will be the initial publication of this series. In the coming decades, armed with the confidence that we have made enormous strides in the elucidation/control of the genetic apparatus of microorganisms such as phages, bacteria and other prokaryotic protozoans, we are destined to understand the genetic apparatus of the mammalian cells. Through this knowledge, we hope to control the mammalian cells and eventually our own cells and ourselves. This symposium and this series of books may provide

* Recently deceased.

an impetus to the effort and progress in this direction, as did the first World Conference on Histones and the book 'Nucleohistones'.

Together with the organizing committee, I would like to acknowledge the valuable assistance of Ms. Betty Lodge and Mr. Richard Schuster of the California Institute of Technology to the Symposium and the valuable contributions of Miss Christine Dreon and Mr. Robert Moyzis from the Johns Hopkins University to the Symposium and the publication of the proceedings.

Paul O.P. Ts'o

THE LIFE AND TIMES OF JAMES BONNER

James Bonner was born on 1 September 1910, the son of Grace Gaylord and Walter Daniel Bonner, both chemists. Both of his parents received their Bachelor's Degree in Chemistry from Nebraska Wesleyan University. His father was subsequently a graduate student at Princeton and later at the University of Toronto, where he received his Ph.D. in Physical Chemistry. James lived in Kingston, Ontario where his father held his first professional position as an Assistant Professor at Queen's University. At the age of five, James, his parents, his younger brother, Lyman and younger sister, Priscilla, moved to Salt Lake City where Walter Sr. had been invited to become head of the Chemistry Department of the University of Utah. His father remained in this position for almost 40 years.

James Bonner grew up in an environment dominated by chemistry. He remembers that until he went to school at the age of eight, he believed that all people were chemists, scurrying off to their laboratories in the morning. At the Bonners' table, salt was always referred to as sodium chloride, sugar as sucrose, water as H_2O and so forth. At the age of seven he received lessons in test tube cleaning in the laboratory from his father. From that time on he was hooked on the laboratory.

James was a Chemistry major, as were all of his six siblings. He received his A.B. in Chemistry from the University of Utah with a minor in Mathematics in 1931. Biology at that time was essentially nonextant at the University of Utah, and he would have known nothing about Biology except for the fact that he spent one year as a transfer junior student at the California Institute of Technology – one year after the founding of the Division of Biology at Caltech. Although a Chemistry major at Caltech, he had an opportunity to learn something about Biology from Thomas Hunt Morgan, Theodosius Dobzhansky, C.B. Bridges, Alfred Henry Sturtevant and others. He worked as a summer assistant for Theodosius Dobzhansky and learned that biologists take long trips to the mountains in the summer and go to the beach when they are worn out from their hard

work in the lab. This convinced him that Biology is the proper life for a scientist.

In 1931 James Bonner came to Caltech as a graduate student in Biology. He decided to try to apply his chemical knowledge to a biological problem by working with Herman E. Dolk and Kenneth B. Thimann on the nature of the newly discovered plant hormone, auxin. This problem and related research engaged his interest over a period of many years. At the same time, he retained an active interest in Genetics and worked on, among other things, successfully making revertants at the bar locus of *Drosophila*. During the period of his graduate work, 1931–34, all sorts of exciting things happened in Biology at Caltech. George Wells Beadle, a postdoctoral fellow at that time, decided to invent biochemical genetics. C.B. Bridges found polytene chromosomes in *Drosophila* following the lead of an old Caltechian, Hans Bauer, in Germany who had previously found them in Chironomus. In fact, he remembers Dr. Morgan telephoning a paper of Bridges' to the editor of SCIENCE so that it could be published in time to scoop the competing group at the University of Texas – Morgan did scoop them.

James Bonner's graduate work was concerned mainly with the mechanism of action of the plant hormones, with a minor in Genetics. During his third year as a graduate student, Professor Morgan said, 'I think you ought to go to Europe as a postdoctoral fellow'. It therefore happened. He received a National Research Council Fellowship to study in Europe at the University of Utrecht, and as it turned out, the University of Leiden Medical School and finally at the Swiss Federal Institute of Technology in Zurich (ETA) to work with Professor Frey-Wyssling. While in Europe, he worked on making lipid bilayers at Leiden and on the biophysical problems of cell extension in plant tissues as controlled by plant hormones at Zurich. He also traveled throughout Europe on a bicycle as one was supposed to do during those days, and enjoyed the trip thoroughly.

In the winter of 1935 James Bonner returned to Pasadena, having received only one offer of a position, namely, from Thomas Hunt Morgan, to return to Caltech as a Research Assistant. He was a Research Assistant to F.W. Went, a new Professor of Plant Biology at Caltech. After one year, James became an instructor (in reminiscing, he jovially describes himself as a slave to Professors Henry Borsook and Thomas Hunt Morgan who taught the introductory Biology course). He subsequently became an Assistant Professor, then Associate Professor and in 1946 full Professor.

It is interesting to note the informality of the early days at Caltech. James had no formal letter of appointment, no formal written understanding with the authorities of the Institute as to salary, status, or anything of the sort. All such information was stored in Dr. Millikan's head. Only in 1946 or thereabouts did the Dean of the Faculty, Dean Watson, come around and say, 'Well, so far as I can determine by reading the rules you probably have by now acquired tenure'. Dr. DuBridge and Dean Watson regularized all faculty planning procedures and since that time James has received a letter of appointment each year.

During the years of the Second World War James Bonner was requested by the Federal government to work on the problem of solving the rubber emergency. Prior to the Second World War all U. S. rubber was imported from Southeast Asia. The 'Emergency Rubber Project' of the Federal government totally engaged his time. This led him to an interest in the biosynthesis of rubber, a problem which he continued to work on after the Second World War. As a result, the general path of carbon in rubber biosynthesis was worked out and published in 1958 (more of this below).

In 1949 he was invited by the government of Australia to attend a Commonwealth Conference on Agriculture to be held in Adelaide. After the meeting he was asked to travel around the Commonwealth of Australia. As a consequence, he was invited to become a consultant to the CSIRO (Commonwealth Scientific & Industrial Research Organization) of Australia and also to become a consultant to the Commonwealth Bank, which is the federal bank of Australia. This consultantship concerned educational and developmental problems. He continued in this capacity from 1949 to 1962, making frequent visits to Australia. In 1960, however, the lure of rubber expertise brought him an invitation to visit Malaysia. Since 1966 he has been a consultant to the Rubber Research Institute of Malaysia, and has visited Malaysia almost yearly. These trips to Kuala Lumpur have made it possible for him to travel widely in Southeast Asia. In fact, all kinds of opportunities to travel have come to him and one of his principal problems is how to stay home.

After many years of research related to plant hormones, James Bonner came, with the advice and help of Paul O.P. Ts'o, to view the problems of macromolecules as being of more, and indeed of major importance. After one short interlude in the study of cytoplasmic actin and myosin (in this case *Physarum Polycephalum*), he started, with Paul Ts'o, the study of ribosome structure and function. This began in 1956 and led directly to an interest in how RNA gets made in general. In 1960, James Bonner and R.C. Huang embarked on the study of how RNA is synthesized. They soon produced an RNA synthesizing system from pea seedling interphase chromosomes (chromatin). The chromatin contained chromosomal RNA polymerase and synthesized RNA from the four riboside triphosphates. This led to the preparation of more highly purified chromatin in 1962 and to the continuing study of the physical, chemical and biological properties of chromatin, which have continued to this day. Some of the important contributions from his laboratory are delineation of the role of histones (1962), the demonstration of the limited number and universality of histones in eucaryotes (1964–68), the first determination of the primary structure of one particular histone, H4 (1968) and the demonstration of the conservation of this structure in evolution. A further significant contribution has been the development of a method for the separation of expressed from non-expressed chromosomal sequences. In 1963, together with Paul Ts'o, he organized the

First World Conference on Histone Biology and Chemistry. This conference and its subsequent proceedings gave great impetus to this field. In reflecting over his life as a pioneer in research on plant science and on chromatin, he has made the following comments.

'I only regret that I did not start upon the study of chromosomal chemistry in the period of 1931–34 as a graduate student, when it was clear that there was a great deal to be done and no one doing it'. Unluckily, the technological tools for the study of chromatin, of transcription of DNA by RNA polymerase, etc., were not then available. It was not until much later that these studies could be undertaken, in fact, 20 or more years later. But that is the story of life.

'One important item which I wish to note is a tribute to my wife, Ingelore, whom I have known since the first came to Caltech as a Research Assistant to Professor Henry Borsook in 1947. She has worked also as a colleague of Professor Ts'o and has worked for many years with me, my helpmate in every way – my helpmate and my love'.

'Finally, it is my hope and wish that before I must give up my career as a chromosomologist I can finally solve the problem of how particular genes get turned on or off at particular times in the developmental sequence. We know that they do and we know a lot about the structure of chromosomes. We also know a lot about chromosome components and physical chemistry, but we do not yet really understand how a specific DNA sequence, a particular gene, gets turned on. Let us hope that this insight and knowledge will come to us in the near future'.

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