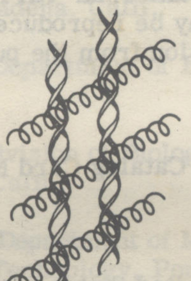
James Bonner and
Paul Ts'o, Editors

THE NUCLEOHISTONES



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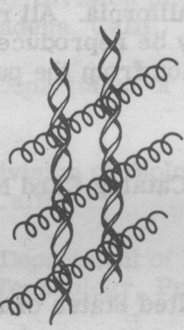

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James Bonner
Paul O. P. Ts'o

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and Paul O. P. Ts'o

INTRODUCTION

The giant strides which have been made in the study of the biophysics and biochemistry of the nucleic acids have clearly shown that these molecules are the ones entrusted by nature with the task of storage and transfer of genetic information. Rapid developments in the status of the coding problem give promise that the day is near when we shall have full understanding of the genetic language. What new frontier is there, then, for biologists to explore? One logical possibility is gradually to focus our attention upon the mechanisms which control the transcription of the genetic message, the mechanisms which by the exertion of such control bring about organized biological activity of the cell, and indeed cause the development of a single cell into a multicellular organism. What substances in the cell can be expected to serve in the control of genetic activity, to program the expression of the genetic command?

DNA of higher organisms, unlike that of bacteria, is not free within the nucleus of the cell, but is usually bound in varying degree to the histones, the biology and function of which have remained obscure until recently. Histones, because of their quantity, their exclusive location within the nucleus, and their strong interaction with DNA, might be expected to play a key role in the organization of DNA into the superstructure of the chromosome, and thus to regulate the properties and function of DNA. In order to discover this role and to discuss and think about histones from the standpoint not only of histone chemistry, but also of the interaction of histones with DNA and the place of histones within the framework of molecular biology, a first World Conference on Histone Biology and Chemistry was held from April 29 to May 2, 1963. The present volume is the outcome of that conference.

We trust that this volume will serve as a review of present knowledge of the histones and of their complexes with DNA, the nucleohistones. Even more, we hope that the present volume will serve as a preview of the problems important to the future course of research in nucleohistone biology and chemistry. Even now a variety of questions important to the future study of the nucleohistones are readily identifiable. For example, how many kinds of histones are there, and why are there this many? What is the distribution of the various kinds of histones among the different DNA molecules of a single nucleus? How and why is this distribution achieved? Is there a single universal structure for all nucleohistones, and if so, what, and why? How are histones synthesized and deposited on the DNA? Because the DNA of nucleohistone replicates in the nucleus prior to cell division, what happens to the histone during this process? Because the presence of appropriate histone so clearly represses the ability of DNA to support RNA synthesis in in vitro systems, how are nucleohistones related to repression or expression of genetic activity? In fact, it is this last question which confers upon the nucleohistones a place of such interest in molecular biology today. Much of the present volume, therefore, is concerned with the enzymology of the nucleohistones, and with consideration of the ways in which interaction between histones and DNA may influence both DNA-dependent RNA synthesis and DNA-dependent DNA synthesis.

It is a basic tenet of modern biology that the cellular differentiation of higher organisms results from an orderly and properly programmed sequential expression and repression of genetic activity. This volume includes discussion, then, of histones in relation to observable differentiation processes. It is clear that there is still a great gap between our knowledge of DNA-dependent RNA synthesis in the test tube and the influence of histone upon this process, and for example, the puffing of the giant chromosomes of flies. Still, the work presented here clearly points to areas which might profitably be explored. One such area is the implication of steroid hormones in the process of genetic derepression.

Chromosomes, giant aggregates of DNA and protein, have proved elusive to modern methods of structure analysis, although as the present volume indicates, we may be getting close to success. We do know, however, that histones staple individual DNA molecules together into chromosomes and are therefore indispensable to the establishment and preservation of chromosome structure. Histones may also play a role in that coiling and supercoiling of the chromosomes characteristic of the pre-mitotic stages of the cell. This volume suggests further steps which, in the light of our new knowledge of the nucleohistones, might be taken toward the understanding of chromosomal structure and of chromosomal activities such as coiling.

This volume is presented not as the conclusion to a finished subject, but rather as an introduction to a field which is just beginning to develop. It is in this sense that we hope the book may provide not only a summary of past knowledge, but also a guide to future inquiry. During the past decade we have witnessed the efforts and the successes of research on the structures and activities of a variety of cellular organelles, such as the mitochondria and the ribosomes, as well as of a variety of cytoplasmic molecules, for example, the soluble enzymes and the transfer RNA's. The structure and activity of the nucleus remains as a formidable challenge to the molecular biologist, both because it is complex and fragile and because well-defined nuclei exist only in the cells of higher organisms. Such nuclei are not found in bacteria, which were the experimental material chosen by the molecular biologist in the past. The time is now ripe for us to take up the challenge of the nucleus. Investigations of nucleohistones are now and will continue to be an important step toward the completion of a molecular interpretation of cell theory and cellular function.

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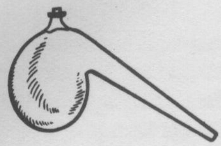

I. HISTORY

J. Murray Luck

HISTONE CHEMISTRY: THE PIONEERS

I would like to call to mind a few of the early students of the cell nucleus. One could start with Miescher, but to establish a pedagogic lineage I shall go back somewhat earlier, to Hoppe-Seyler, who 100 years ago was the uncrowned king of biochemistry [1]. Ernst Felix Immanuel Hoppe was born on December 26, 1825, the tenth child of pastor and superintendent Ernst Hoppe. Both of his parents died when he was a child, and he grew up in the home of Dr. Seyler, the husband of his oldest sister. Years later, in 1864, the relationship was formalized; he was legally adopted by the Seylers and thereafter used the name Hoppe-Seyler.

He had his early schooling, through gymnasium, in an orphanage in Halle. The educational standards were high, the rules were strict, and the environment was Spartan in all respects. Life therein exercised a decisive influence in shaping the future of the young man. Perhaps he even deserves the credit for discovering the value of the long walk. He and his friend Jahn, the great father of gymnastics, used to walk many miles together — Jahn warming up for the exercise by walking from Freiburg in Thüringen to Halle, a mere 30 kilometers. With all of this came a deep attachment to the outdoors. He had a great love for the mountains and was an expert mountain climber. With a pharmacist in Halle, he used to go on plant collecting expeditions and is credited with discovering in the Riesen mountains a new species of fern.

In later years he went regularly to the Bodensee on his vacations and was a very competent sailor. His last scientific publication reported on the distribution of absorbed gases in the water of the Bodensee and its relation to the plant and animal life of the lake. This followed earlier work with C. Duncan on the respiration of fish at different depths in the lake. His love of plants and his friendship with the Halle pharmacist led him, while still a boy, to carry out chemical experiments on plants and later to study the composition of plant ash. His love of the mountains developed his interest in geology, and he published several papers in this field.

When he finished gymnasium in 1846, he was admitted as a medical student in Halle. There he studied for two semesters and was properly initiated in chemical research by Steinberg, the Professor of Pharmaceutical Chemistry. He would probably have remained in Halle were it not for a hiking trip in the Riesen mountains in the fall of 1847. There he happened to meet Ernst Heinrich and Edward Weber, with whom he soon developed a close friendship, and who persuaded him to continue his studies in Leipzig. This he did, often visiting the Webers, three of whom — brothers — were professors in the University. All of them, but especially E. H. Weber, Professor of Physiology and Anatomy, profoundly influenced the young Hoppe.

Hoppe remained for five semesters in Leipzig. He then went to Berlin, where he finished his medical studies, published his dissertation with E. H. Weber on the composition of chondrin, and in 1851 received his certification as a physician. After a year of migratory study, mountaineering, and travel in Prague, northern Italy, and Vienna he took his state examinations in obstetrics and practiced medicine for a year or two. After another two years of service in the University, he accepted appointment as Prosector in Virchow's new Institute of Pathology. Here he directed the work of the chemical laboratory and gave a series of lectures on chemistry and physiological chemistry for doctors. His students and research associates grew rapidly in number and, thanks to Virchow's influence and his great interest in Hoppe, a second assistant was appointed. Hoppe was promoted to Professor Extraordinarius in 1860 and one year later moved to Tübingen, where in a short time he became Professor Ordinarius. It is evident that he was a stimulating teacher and, in association with Strecker and later with Fittig, was highly regarded for his lectures, carefully prepared experiments, and demonstrations in inorganic, organic, and physiological chemistry and toxicology. The physical facilities for his work were very poor, but this did not dampen his enthusiasm.

In 1872, Hoppe-Seyler was invited to the chair of physiological chemistry in the newly founded University of Strasbourg. He accepted with characteristic enthusiasm and for eleven years occupied quarters in the medical school building. The facilities were not completely suitable, and about 1883 a new building was erected, according to his design, for instruction and research in physiological chemistry. This was the first of its kind in Germany and can well be regarded as a landmark in development of the science as an expanding discipline in its own right. Here he gave lectures and conducted practical courses in physiological chemistry, toxicology, forensic chemistry, metabolism and nutrition, and hygiene. He attracted a great many students, notably Miescher and Kossel. His great textbook on physiological and pathological chemistry, which first appeared in 1858, ran into many editions and was translated into many languages. Every analytical method and every experiment described in this book and in its many revised editions were developed or checked by Hoppe-Seyler personally — a laudable practice that our many writers of present-day laboratory manuals might well emulate. Hoppe-Seyler's name is also indelibly associated with the Zeitschrift für Physiologische Chemie, which he founded in 1877, and with the 150