BASIC PRINCIPLES IN NUCLEIC ACID CHEMISTRY

Edited by

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VOLUME I

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PREFACE

About one hundred years ago, a young Swiss physician, Friedrich Miescher, published the first paper on "nuclein" (or nucleohistone in current terminology) and thus launched chemical research on nucleic acids. Nearly twenty-five years ago, nucleic acid was identified as the physical basis of genes, and since that time the quest for knowledge on genes rightfully has become a major thrust in modern biological research. In fact, the tremendous progress in nucleic acid research has raised the possibility that advancements in this field may exert a profound influence on the future of man.

We, as researchers in nucleic acid chemistry, have prepared this multivolume treatise in honor of this historic event: the centennial anniversary of the discovery of nucleic acid. Our view is that progress in nucleic acid chemistry has been substantial and sufficient to justify an attempt to formulate certain basic principles in this field. We hope that these basic principles will not only endure the test of time but will serve as a foundation for further advancement in nucleic acid research as well. Not only have we critically examined the achievements of the past, we have also contemplated the future: the momentum of nucleic acid research and its contribution and influence on the destiny of man. Knowledge of nucleic acid chemistry will be utilized more extensively than ever in biomedical research areas such as cell biology, differentiation, microbiology, virology, oncology, genetic therapy, and genetic engineering. Hopefully, this treatise will serve as reference and resource material for many workers in biomedical research and as teaching material for instructors in institutions of higher learning.

The chapters in this treatise fall into three categories. The first of which comprises about 70% of the work consists of chapters written by scholars who have expert knowledge in a particular area of research in nucleic acid chemistry. In Volume I, these are Chapters 2, Chemical Synthesis and Transformations of Nucleosides; 3, Mass Spectrometry; 4, Excited States of Nucleic Acids; and 5, Infrared and Raman Spectroscopy; they deal with specific problems and approaches in nucleic acid research. The second category is comprised of chapters written by the editor; they describe current knowledge and concepts of nucleic acid chemistry at four levels of complexity. In this volume, Chapter 6, Bases, Nucleosides, and Nucleotides, belongs in this second category. Finally, Chapter 1, In the Beginning, falls into the third category which concerns the broad implications of nucleic acid research. Not only is the early history of nucleic acid research described briefly in this chapter, but both the involvement of nucleic acid in past chemical and biological evolution as genetic material and the beginning of the influence of nucleic acid research on the future of the human race are discussed. In the coming age, scientists, especially workers involved in research on genes, should seriously consider the implications of their work to the progress of man. It is urged that the problems and challenges raised in this chapter about the destiny of man be widely and vigorously discussed by all scientists, especially biologists, and indeed by all concerned persons. While scientific research and scholastic inquiry need not be relevant to immediate gains in wealth and comfort, they should be deeply involved in the search for the future of our own race. In general, the contributions in the earlier volumes will deal with the study of small units of nucleic acid; chapters in later volumes will be concerned with the investigation of nucleic acid as macromolecules.

Chapters in the first and second categories have been reviewed by scholars in their fields: Chapter 2, Leroy Townsend, University of Utah; Chapter 3, Catherine Fenselau, The Johns Hopkins University; Chapter 4, James W. Longworth, Oak Ridge National Laboratory; Chapter 5, George J. Thomas, Jr., Southeastern Massachusetts University; Chapter 6, Muttaiya Sundaralingam, University of Wisconsin. We are grateful for their assistance and suggestions in the preparation of this treatise. Finally, the help and assistance from many colleagues, friends, and from the staff of Academic Press are gratefully acknowledged.

Onward to the second hundred years!

PAUL O. P. TS'O

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Paul O. P. Ts'o

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IN THE BEGINNING

PAUL O. P. TS'O

> Following the powerful course of Nature,
> The Superior Man revitalizes himself ceaselessly.
>
> IMAGE OF CHIEN, I CHING

At this pivotal juncture looking back into the past and ahead into the future, we soon find ourselves contemplating three beginnings...the beginning of the scientific investigation of nucleic acid; the beginning of the formation of nucleic acid as genetic material and its progressive change as reflected in the evolution of the biological kingdom; finally, the beginning of the influence of nucleic acid research on the future of the human race.

I. The Beginning of the Scientific Investigation of Nucleic Acid

A. Friedrich Miescher, 1844–1895*

In 1871, at the age of 28, Friedrich Miescher published the first scientific paper on the finding of "nuclein," a discovery which he had made two years earlier in the laboratory of Hoppe-Seyler. The first isolation of the "nuclein," the nucleic acid–protein complex, was not an historical accident, but was brought about by the determined effort of this young scientist.

Miescher, who was born on August 13, 1844, almost entered the field of theology, but instead studied medicine in Basel, Switzerland, and Göttingen, Germany, and qualified in 1868. Instead of following his father's advice to go into medical practice, he became deeply interested in the chemical study of the cell. Miescher's uncle Wilhelm His, who was a fervent believer in this new development, stated that "in my own histological studies I came to the conclusion that the final solution of the problems of tissue development could be solved only by chemistry." Miescher was first pupil, then colleague, then life-long friend of His, and was greatly influenced by him. The letters from Miescher to His reveal much about the thoughts which motivated his career. Following the advice of His, after obtaining his doctor's degree in 1868 in Basel, Miescher entered Hoppe-Seyler's laboratory at the University of Tübingen, which became the first laboratory devoted entirely to the new field of biochemistry. Initially, he was trained in general and organic chemistry in the laboratory of Strecker and Wöhler. This experience must have been very useful to him in his later research.

Soon he was applying this chemical knowledge to the study of nuclei from cells. As source material for this investigation, Miescher chose pus cells collected from the bandages discarded from the adjacent Tübingen surgical clinic. Miescher considered the white blood cells present in pus to be the simplest of animal cells and noted that the lymphoid cells had a very large nucleus to total cell volume ratio; thus, he decided to work on this material in spite of the hardship in collecting it, due to the strong stench and the slimy, clumping nature of the pus. He succeeded in preserving the cells by using dilute sodium sulfate solution (instead of ordinary saline solution) for washing. In this procedure, the cells can be sedimented rapidly and isolated from other materials in the pus. In his trials to determine the chemical composition of the white cells, he extracted them with various solvents, following the tradition at that time. Using strong salt solution, as did Hoppe-Seyler and other workers earlier, he obtained a gelatinous material which reminded him and others of myosin. This gelatinous substance turned out to be mostly due to the highly polymerized DNA. In the absence of a centrifuge,

* The discussion on Miescher is based on the material in References 1–4.

Miescher really had no way to handle such viscous material and had to use other procedures which yielded DNA in highly degraded form, but which could be studied at that time.

In extracting pus cells with dilute alkali, he obtained a substance which can be precipitated on the addition of acid, but which is dissolvable on reintroduction of alkali. Miescher supposed this material to originate from nuclei and, therefore, dedicated himself first to the task of the isolation of nuclei, a task which had never been attempted before. The primary observation of Miescher on which the isolation of nuclei depended was that the dilute hydrochloric acid dissolves most cellular materials away, leaving behind the nuclei. This finding was still the basis for a procedure for the isolation of nuclei (the citric acid procedure) widely employed 10 to 20 years ago. To remove the remaining contaminated protein from the acid-precipitated nuclei, Miescher included the use of the proteolytic enzyme pepsin in the acidic treatment. In fact, he made a hydrochloric acid extract of pig's stomach (remember that it was in 1868) and applied it to pus cells. After the solution was shaken with ether, the nuclei settled to the bottom and could be filtered off. Miescher was thus the first to recognize that the nucleus possessed a higher density than the rest of the cell. The nuclei so isolated (rather than the whole cells) were now ready for the extraction with dilute alkali. The extracted material precipitated on addition of acid and redissolved readily on readdition of alkali. Elementary analysis of this material was carried out by Miescher who found it to consist of 14% nitrogen and 2.5% phosphorus. Up to this time, organic phosphorus was observed only in lecithin. Miescher knew that it was a new substance and named it "nuclein." The analytical data indicated that this first preparation of nuclein by Miescher consisted of somewhat less than 30% DNA. Miescher completed his work in Hoppe-Seyler's laboratory in the fall of 1869 and submitted the manuscript to Hoppe-Seyler for its publication in Hoppe-Seyler's *Medizinisch-chemische Untersuchungen*. Miescher himself then left for Leipzig to study physiology in the Institute of Carl Ludwig, then a world center of physiology.

Hoppe-Seyler was skeptical of the work done by his only pupil in 1869 and hesitated to publish it. He therefore wanted to repeat the work and at the same time to extend such a study to avian and reptilian erythrocytes. In a note, Hoppe-Seyler explained his position to Miescher and suggested that if the latter were in a hurry to publish, he could send a brief communication to Pflügers Archives. In reply, Miescher indicated that he was quite willing to wait until Hoppe-Seyler was ready. Then he added casually that Hoppe-Seyler had already inserted the description of the nuclein technique in the new edition of Hoppe-Seyler's book. Finally, in the fourth volume of *Medizinisch-chemische Untersuchungen*, Miescher's classic paper "Über die chemische Untersuchung von Eiterzellen" was published in 1871 together with a confirmation by Hoppe-Seyler and two supplementary papers by two of his pupils [5]. Hoppe-Seyler, in the introduction to his own paper in this volume, confessed his former doubts about Miescher's results, as well as his personal confirmation of these results at every point. In a letter to His, Miescher expressed his appreciation of his former teacher and concluded that "Hoppe-Seyler is master of the entire field which encloses histology on one side and pure chemistry on the other."

By then, Miescher had become Privatdozent in physiology at the University in Basel, and a year later, in 1872, he was appointed successor to His (who left Basel when he was appointed to a chair in Leipzig) as professor of physiology. Upon his return to Basel, Wilhelm His introduced Miescher to the salmon fishery then flourishing along the banks of the Rhine at Basel. These sexually mature salmon, after swimming all the way from the North Sea to spawn, contained a huge quantity of ripe sperm. The salmon spermatozoon has a very large nucleus, about 90% of the cell mass. Immediately, Miescher worked hard on this study. He had little space, time, equipment, or help. His work was performed in a small corner of the general chemistry laboratory, for he had none of his own; his analyses were carried out in the corridors of another building. Nevertheless, his finest and most enduring work was performed under these conditions. In the spring of 1872, he notified the Natural History Society in Basel that he had isolated "nuclein" of high molecular weight from sperm heads, as well as a basic substance which he called "protamine." The protamine was extracted by dilute acid from the defatted sperm leaving behind the "nuclein" in the residue. This "nuclein" had a phosphorus content of 9.59%, corresponding to the phosphorus content of pure nucleic acid. As for protamine, he thought that it was simple in structure ($C_9H_{21}N_5O_3$), a substance between urea and protein, and that this basic substance of high nitrogen content combined with nuclein, which is a "polybasic" acid in the sperm. While Miescher never did isolate DNA in undegraded and fibrous state, he did get the idea that the nuclein was of high molecular weight since it would not pass through a parchment filter, whereas protamine would do so readily.

Miescher's paper published in the sixth volume of *Verhandlungen der Naturforschenden Gesellschaft in Basel* in 1874 was a landmark in which the studies on the testicles, the sperm head, and the isolation of nuclein and protamine were described. Miescher described the sperm head as "an insoluble, saltlike compound of a highly nitrogenous organic base with a phosphorus-rich, acidic nuclein [nucleic acid]." He thought that this complex in the sperm head was not a static but a highly fluid system, whose composition and properties varied markedly in response to the condition present. He pointed out that sodium chloride, protamine, and nuclein formed a three-component, dynamic system, the equilibrium of which was governed by the relative concentration of each of the components as well as by *Alkalescenz* (pH in modern terms). Miescher recognized that the basic reason for this dynamic state is due to the polyvalent character of both nucleic acid and protamine, and the ionic dissociation of the protamine–nucleic acid complex by salt.

This understanding of the polyvalent characteristics of protein led him to the following statement in a letter to His, dated May 1876: "The thought always occurs to me that the proteins are really both strong acids and strong bases, which possess a neutral reaction only because of an inner neutralization. If one mixes sodium chloride with protein, there must occur protein chloride, sodium proteinate, and protein proteinate. Different proteins have different affinities, and even the insoluble proteins are not unreactive." Thus, Miescher was the first to understand and to describe the amphoteric character of the proteins long before it was established by the work of Küster, Bjerrum, and others, some 50 years later.

The work on ripe salmon sperm was subsequently extended to the sperm of frog, bull, and carp. In the preparation of nucleic acid from various sources, Miescher recognized the lability of the material, and he spared no effort to obtain preparations as unaltered as possible. He stated that all work was performed in a room at 2–3°C or less. He described his working day in a letter. "When nucleic acid is to be prepared I go at five o'clock in the morning to the laboratory and work in an unheated room. No solution can stand for more than five minutes, no precipitate more than one hour before being placed under absolute alcohol. Often it goes on until late in the night. Only in this way do I finally get products of constant phosphorus proportion." Miescher's analyses of his preparations compare favorably with the best of the modern analyses of nucleic acids. Yet by temperament Miescher was not an analyst. Nevertheless, he appreciated (as so few did in his time) the importance of this chemical tool which he brought to the investigation of biological materials. In a letter he remarked, "Always I ask myself if histochemistry could only be conducted otherwise [without analysis], and always I return to my phosphorus, fat and other determinations, as a necessary control and assurance against disappointments with the work with the microscope."

The long years of hard work in cold rooms with little help began to exact their toll. By 1885, even though the government had constructed for him a fine research institute in Basel (nicknamed "Vesalianum"), the creative period of his life was over. He suffered from fits of lassitude and depression, forerunners of the tubercular disease to which he was to succumb at the age of 51. Depressed by the waning of his strength, he wrote to His "that my work is only the preliminary study to a future Histochemistry." During the last few years of his life, he devoted his remaining strength to the inspiration of his pupils in the field of physiology. He died on August 26, 1895. After Miescher's