

Review

Friedrich Miescher and the discovery of DNA

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Abstract

Over the past 60 years, DNA has risen from being an obscure molecule with presumed accessory or structural functions inside the nucleus to the icon of modern bioscience. The story of DNA often seems to begin in 1944 with Avery, MacLeod, and McCarty showing that DNA is the hereditary material. Within 10 years of their experiments, Watson and Crick deciphered its structure and yet another decade on the genetic code was cracked. However, the DNA story has already begun in 1869, with the young Swiss physician Friedrich Miescher. Having just completed his education as a physician, Miescher moved to Tübingen to work in the laboratory of biochemist Hoppe-Seyler, his aim being to elucidate the building blocks of life. Choosing leucocytes as his source material, he first investigated the proteins in these cells. However, during these experiments, he noticed a substance with unexpected properties that did not match those of proteins. Miescher had obtained the first crude purification of DNA. He further examined the properties and composition of this enigmatic substance and showed that it fundamentally differed from proteins. Due to its occurrence in the cells' nuclei, he termed the novel substance "nuclein"—a term still preserved in today's name deoxyribonucleic acid.

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Introduction

The year 2003 marked the 50th anniversary of the historic characterization of DNA by James Watson and Francis Crick with an article in the journal *Nature* on April 25, 1953, that revealed the structure of DNA (Watson and Crick, 1953). Their discovery was the culmination of a decade of intense research following Oswald T. Avery, Colin MacLeod, and Maclyn McCarty's demonstration that DNA, not protein as previously thought, is the hereditary molecule (Avery et al., 1944). Today, the history of DNA is often told as though it started with these fundamental discoveries. However, the description of DNA actually began 135 years ago with its discovery by Friedrich Miescher, a much less known man who isolated the hereditary material in 1869.

The second half of the 19th century was a period in which many key concepts in biology were established. For

one, the focus of biologists was shifting from studying organisms, organs, or tissues to their component cells. Matthias J. Schleiden and Theodor Schwann had just shown that all tissues have a cellular origin and that both animals and plants consist of the same fundamental units of organization, cells, which interact to give rise to complex organisms (reviewed in Mayr, 1982). Experiments by, among others, Louis Pasteur and Rudolph Virchow demonstrated that new cells can only arise from other cells (Virchow, 1855)—rebutting the notion of spontaneous generation of new cells from lifeless matter, which had prevailed for a long time.

In parallel to these breakthroughs in cytology, the basic concepts of heredity and evolution were being worked out. The publication of Charles R. Darwin and Alfred R. Wallace's theories of evolution by natural selection occurred in 1858 (Darwin and Wallace, 1858), and 1 year later, Darwin's famous book *On the Origin of the Species by Means of Natural Selection* appeared (Darwin, 1859). In 1865, Gregor Mendel discovered the laws of heredity through his breeding experiments with peas (Mendel,

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1866), which were “rediscovered” in 1900 by Carl Correns (Correns, 1900), Hugo de Vries (Vries, 1900a,b) and Erich von Tschermak (Tschermak, 1900; see also Dunn, 1991; Sturtevant, 2001).

Observations by Theodor Boveri, Walther Flemming, Ernst Haeckel, and Edmund B. Wilson—to name but a few of the protagonists—combined the emerging fields of cytology and genetics and laid the foundation of cytogenetics: For example, in 1866, Haeckel proposed that the nucleus contained the factors responsible for the transmission of hereditary traits (Haeckel, 1866)—causing increased interest in this organelle. In the 1870s, Flemming described the morphology and behavior of the chromosomes during mitosis (Flemming, 1879), coining both the terms “chromatin” and “mitosis.” And in the late 1880s and 1890s, Boveri began advancing the theory that the chromosomes not only harbor the genetic information in a cell, but that individual chromosomes carry different parts of the hereditary material (Boveri, 1888, 1892, 1907).

While most of these discoveries and concepts were met with great interest by the scientific community at the time, the discovery of DNA was generally underappreciated. Although uncovering the molecular basis of cellular life had become one of the most fundamental problems of the time, no one grasped the real significance of Miescher’s findings to answer this problem until the middle of the 20th century. Having died half a century earlier, Friedrich Miescher was largely forgotten and is rarely credited for his discovery.

Miescher was a passionate scientist who gleaned a lot from DNA despite the relatively simple tools and methods available to him. He had an instinct for identifying the key questions and then choosing the appropriate objects of study to address them. But what gifts he had in tackling difficult experiments, he lacked in communicating and promoting the results of his work. Too much of a perfectionist, he conscientiously repeated his experiments and analyses and often hesitated for long periods before publishing his results.

During his scientific career, which extended nearly three decades, Miescher published just nine scientific papers and only a handful of lecture manuscripts were printed. A substantial part of Miescher’s results and, in particular, his ideas have been passed down only through letters he wrote to friends and colleagues.

Most of the materials that have been preserved come from the efforts of Miescher’s uncle Wilhelm His, who was both a close friend and important partner for scientific discussions. Together with Miescher’s friends and colleagues, His compiled a two-volume collection of his nephew’s work, which was published 2 years after Miescher’s death (His et al., 1897a,b). These volumes comprise Miescher’s scientific publications, nearly 100 letters by Miescher on various aspects of his work and theories, the manuscripts of his key lectures, as well as subsequent papers compiled and published posthumously by coworkers based on Miescher’s laboratory notes.

The beginnings of Miescher’s scientific career

Johann Friedrich Miescher (Fig. 1A) was born in Basel, Switzerland on August 13, 1844, into a family of scientists (His, 1897b). His father, Johann F. Miescher, and more notably his uncle, Wilhelm His (Fig. 1B), were renowned physicians and professors of anatomy and physiology at the University of Basel. As a result of growing up in this environment, Miescher developed a keen interest in the sciences at an early age.

At 17, he began his medical studies in Basel—interrupted only by a semester in Göttingen, Germany—and concluded them in the spring of 1868 at the age of 23. To accommodate his father’s wish for “practical competence,” Miescher specialized as an otologist after finishing his basic medical training. However, Miescher only had a very limited interest in the practice of medicine—not least as it



Fig. 1. Friedrich Miescher and his mentors. (A) Friedrich Miescher (1844–1895) as a young man. (B) Wilhelm His (1831–1904), Miescher’s uncle. His still is famous for his work on the fate of cells and tissues during embryonic development and for his insights into neuroembryology. He, for example, discovered neuroblasts and coined the term “dendrite” (Finger, 1994; Shepherd, 1991). (C) Felix Hoppe-Seyler (1825–1895), one of the pioneers of physiological chemistry (now biochemistry). Hoppe-Seyler performed seminal work on the properties of proteins, most notably hemoglobin (which he named), introduced the term “proteid” (which later became “protein”), and worked extensively on fermentation and oxidation processes as well as lipid metabolism (Perutz, 1995). He was instrumental in founding Germany’s first independent institute for physiological chemistry (in 1884) and in 1877 founded and edited the first journal of biochemistry, the *Zeitschrift für Physiologische Chemie*, which still exists today as *Biological Chemistry*. (D) Adolf Strecker (1822–1871), a leading figure in chemistry in the mid-19th century and professor at the University of Tübingen from 1860 to 1870. Among other achievements, he was the first to synthesize an amino acid (alanine from acetaldehyde via its condensation product with ammonia and hydrogen cyanide) in a reaction known today as Strecker synthesis (Strecker, 1850). (E) Carl Ludwig (1816–1895), a protagonist in the field of physiology in the second half of the 19th century. His focus was the physiology of the nervous system and its sensory organs. In 1869, he founded Leipzig’s Physiological Institute.

was difficult for him to examine his patients due to poor hearing that resulted from an ear infection he had suffered during childhood (His, 1897b). His strong interest in the “theoretical foundations of life” suggested he pursue a career in research instead.

Soon after he had passed his boards exam in the spring of 1868, Miescher relocated to Tübingen, Germany to study histochemistry. Inspired by His’ conviction that the “last remaining questions concerning the development of tissues could only be solved on the basis of chemistry” (His, 1897b), he intended to work in the laboratory of the distinguished biochemist Felix Hoppe-Seyler (Fig. 1C). However, prior to joining Hoppe-Seyler’s lab, Miescher spent a semester in the chemistry laboratory of Adolph Strecker (Fig. 1D) to familiarize himself with the techniques of organic chemistry. Only after Miescher had acquired a solid background did he join Hoppe-Seyler’s laboratory in the autumn of 1868.

Working toward the discovery of DNA

Hoppe-Seyler was one of the pioneers in a new discipline, then referred to as “physiological chemistry.” His laboratory was housed high above the Neckar river valley in Tübingen’s Castle (Figs. 2 and 3). As Hoppe-Seyler’s only student, Miescher wanted to determine the chemical composition of cells. Lymphocytes were to serve as the source material for these studies. By studying this “most simple and independent cell type,” he hoped to unravel the fundamental principles of the life of cells (Miescher, 1869a).

Initially, Miescher tried to isolate the cells for his experiments from lymph nodes, but it was difficult to purify the lymphocytes and impossible to obtain sufficient quantities for analysis (Miescher, 1869a). On Hoppe-Seyler’s suggestion, Miescher changed to examining leucocytes and obtained the cells for his experiments from the pus on fresh surgical bandages, which he collected from the nearby surgical clinic in Tübingen. In pus, he found the ideal base material for his analyses, and its “histological purity” allowed him to achieve the most complete purification of the chemical building blocks that constitute cells (Miescher, 1869a).

At first, Miescher focused on the various types of proteins that make up the leucocytes, as proteins were considered to be the most promising targets for understanding how cells function. Miescher showed that proteins (and lipids) were the main components of the cells’ cytoplasm, described their properties in some detail, and attempted to classify them (Miescher, 1869a, 1871d). However, his work was hampered by the simple protocols and equipment available to him and the diversity of proteins within the cells surpassed his analytical methods.

Yet during these tests, Miescher noticed that a substance precipitated from the solution when acid was added and dissolved again when alkali was added (Miescher, 1869a, 1871d). He had, for the first time, obtained a crude precipitate of DNA. Miescher stated that “according to known histochemical facts, I had to ascribe such material to the nuclei” and he decided to examine the cells’ nuclei more closely—a part of the cell about which very little was known at the time.

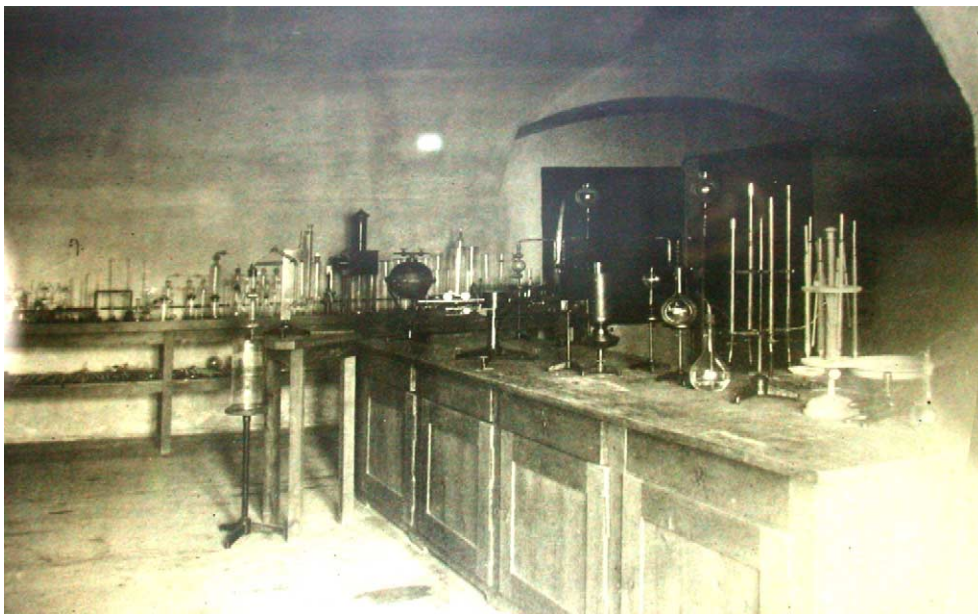


Fig. 2. Photograph of Felix Hoppe-Seyler’s laboratory around 1879. Prior to becoming the chemical laboratory of Tübingen University in 1823, this room was Tübingen castle’s laundry. Here, Hoppe-Seyler had made ground-breaking discoveries regarding the properties of hemoglobin. This achievement was a significant step for later investigations into the properties and functions of this and other proteins. Photography by Paul Sinner, Tübingen.



Fig. 3. Tübingen castle. (A) Historic photograph of Tübingen castle (seen from the east) overlooking the old town. The picture was taken around the time of Friedrich Miescher's stay in Tübingen. Photography by Paul Sinner; possession of Stadtarchiv Tübingen. (B) Tübingen castle today (seen from the south). The picture was taken at roughly the same time of year as when Miescher first isolated DNA. The laboratories of Friedrich Miescher and Felix Hoppe-Seyler were located next to each other on the ground floor of the main building (lower row of windows in the façade of the main building facing the viewer). Photography by Benjamin Saur, Tübingen.

Realizing a potential significance, he wrote, “A material made up of only cells, such as this one, would above all finally call for a serious study of the chemical constitution of the cell's nucleus” (Miescher, 1871d). However, Miescher still needed to separate the novel substance from the proteins in his cell extracts in order to better analyze it. He thus had to develop new protocols to first separate the cells' nuclei from the cytoplasm and then isolate the enigmatic precipitate (see Box 1).

Miescher's initial characterization of DNA

By working out the isolation conditions, Miescher realized that despite having similar properties to proteins, the new substance was not a protein. On February 26, 1869, he reported the discovery of this mysterious substance in a letter to Wilhelm His (Miescher, 1869a),

“In my experiments with low alkaline liquids, precipitates formed in the solutions after neutralization that could not be dissolved in water, acetic acid, highly diluted hydrochloric acid or in a salt solution, and therefore do not belong to any known type of protein.” Due to its presence in the nuclei, Miescher termed the enigmatic compound “nuclein”.

Miescher was intrigued by the properties of his novel substance. However, his first protocol failed to yield enough material to conduct a further analysis. He wrote, “The minimum quantity of nuclei that can be obtained through the described procedure [see Box 1] hardly permits the few reactions mentioned; elementary analyses [one of the few methods available to analyze novel substances at the time] could not even be considered” (Miescher, 1871d). Miescher thus had to develop a second protocol in order to obtain larger quantities of purified nuclein (see Box 2).

Box 1**Miescher's first protocol to isolate DNA**

Before attempting the isolation of cells from the pus on surgical bandages, Miescher took great care to ensure that his source material was fresh and not contaminated. He painstakingly examined it and discarded everything that showed signs of decomposition, either in terms of smell, appearance under the microscope, or by having turned acidic. A great deal of the material he could obtain did not meet these strict requirements (Miescher, 1871d). Those samples that did were subsequently used to isolate leucocytes.

In a first step, Miescher separated the leucocytes from the bandaging material and the serum (Miescher, 1869a, 1871d). This separation posed a problem for Miescher. Solutions of NaCl or a variety of alkaline or alkaline earth salt solutions used to wash the pus resulted in a “slimy swelling” of the cells, which was impossible to process further (His, 1897b). (This “slimy swelling” of the cells was presumably due to high-molecular-weight DNA, which had been extracted from cells that had been damaged.) Only when Miescher tried a dilute solution of sodium sulfate [a mixture of one part cold saturated Glauber's salt ($\text{Na}_2\text{SO}_4 \cdot 10 \text{H}_2\text{O}$) solution and nine parts water] to wash the bandages did he manage to successfully isolate distinct leucocytes, which could be filtered out through a sheet to remove the cotton fibers of the bandaging. Miescher subsequently let the washing solution stand for 1–2 h to allow the cells to sediment and inspected the leucocytes microscopically to confirm that they did not show any signs of damage.

Having isolated the cells, Miescher next had to separate the nuclei from the cytoplasm. This had never been achieved before and Miescher had to develop new protocols. He washed the cells by rinsing them several (6–10) times with fresh solutions of diluted (1:1000) hydrochloric acid over a period of several weeks at “wintry temperatures” (which were important to avoid degradation). This procedure removed most of the cells' “protoplasm,” leaving behind the nuclei. The residue from this treatment consisted in part of isolated nuclei and of nuclei with only little fragments of cytoplasm left attached. Miescher showed that these nuclei could no longer be stained yellow by iodine solutions, a method commonly used at the time for detecting cytoplasm (Arnold, 1898; Kiernan, 2001).

He then vigorously shook the nuclei for an extended period of time with a mixture of water and ether. This caused the lipids to dissolve in the ether while those nuclei, still attached to cytoplasm, collected at the water/ether interface. By contrast, the clean nuclei without contaminating cytoplasm were retained in the water phase. Miescher filtered these nuclei and examined them under a microscope. He noticed that in this way he could obtain “completely pure nuclei with a smooth contour, homogeneous content, sharply defined nucleolus, somewhat smaller in comparison to their original volumes” (Miescher, 1871d).

Miescher subsequently extracted the isolated nuclei with alkaline solutions. When adding highly diluted (1:100,000) sodium carbonate to the nuclei, he noticed that they would swell significantly and become translucent. Miescher then isolated a “yellow solution of a substance” from these nuclei. By adding acetic acid or hydrochloric acid in excess, he could obtain an insoluble, flocculent precipitate (DNA). Miescher noted that he could dissolve the precipitate again by adding alkaline solutions.

Although this protocol allowed Miescher for the first time to isolate nuclein in appreciable purity and quantities, it was still too little and not pure enough for his subsequent analyses. He consequently improved on this protocol until he established the protocol detailed in Box 2, which enabled him to purify sufficient amounts of nuclein for his first set of experiments on its elementary composition.

With his second protocol, Miescher had shown that nuclein is not digested by the protease pepsin and he once again determined that he could dissolve the precipitate by adding a base and cause it to reprecipitate by adding an excess of acid (Miescher, 1871d). Following these tests on the solubility and digestibility of the nuclein, Miescher focused on determining its composition and realized that it was different from proteins in other ways too.

He burned the precipitate and confirmed the presence of various elements commonly found in organic molecules—carbon, hydrogen, oxygen, and nitrogen—through the chemical reactions they exhibited. These tests showed that nuclein, unlike proteins, lacked sulfur (Miescher, 1869a, 1871d) but contained a large amount of phosphorus, which he first reported in a letter to his parents dated August 21, 1869, (Miescher, 1869b; see also Miescher, 1871d).

Based on his analyses, Miescher noted that the novel substance he had isolated was different from the known types of protein (Miescher, 1869a). He went on to state “I think that the given analyses—as incomplete as they might be—show that we are not working with some random mixture, but ... with a chemical individual or a mixture of very closely related entities.” For Miescher, the large amount of phosphorus in the nuclein was another indication that it could not be a protein or any other known molecule. He concluded, “We are dealing with an entity *sui generis* not comparable to any hitherto known group” (Miescher, 1871d).

Following these experiments with leucocytes, Miescher also discovered the presence of nuclein in the cells of other tissues (Miescher, 1869b, 1871d). He suspected that upon further investigation, an “entire family of such phosphorus-

Box 2**Miescher's second protocol to isolate DNA**

A key concern of Miescher's was to get rid of contaminating proteins, which would have skewed his analyses of the novel substance. "I therefore turned to an agent that was already being used in chemistry with albumin molecules on account of its strong protein-dissolving action, namely, pepsin solutions" (Miescher, 1871d). Pepsin is a proteolytic enzyme present in the stomach for digesting proteins. Miescher used it to separate the DNA from the proteins of the cells' cytoplasm. He extracted the pepsin for his experiments from pig stomachs by washing the stomachs with a mixture of 10 cc of fuming hydrochloric acid and one liter of water and filtering the resulting solution until it was clear.

In contrast to his earlier protocol, Miescher first washed the pus cells (leucocytes) three or four times with "warm alcohol" to remove lipids. He then let the residual material digest with the pepsin solution between 18 and 24 h at 37–45°C. After only a few hours, a fine gray powdery sediment of isolated nuclei separated from a "yellow liquid." Miescher continued the digestion process, changing the pepsin solution twice. After this procedure, a precipitate of nuclei without any attached cytoplasm formed. He shook the sediment several times with ether in order to remove the remaining lipids. Afterwards, he filtered the nuclei and washed them with water until there was no longer any trace of proteins.

He described the nuclei isolated in this way as "completely naked (...). The contours were smooth in some cases or slightly eaten away in others" (Miescher, 1871d). Miescher washed the nuclei again several times with warm alcohol and noted that the "nuclear mass" cleaned in this way exhibited the same chemical behavior as the nuclei isolated with hydrochloric acid.

Miescher subsequently extracted the isolated nuclei using the same alkaline extraction protocol he had previously employed on the intact cells (see Box 1) and, when adding an excess of acetic acid or hydrochloric acid to the solution, again obtained a precipitate of nuclein.

containing substances, which differ slightly from one another, will reveal itself, and that this family of nuclein bodies will prove tantamount in importance to proteins" (Miescher, 1871d).

Without knowing anything about how nuclein functioned, Miescher nevertheless assumed that it played a central role in cells. In a letter to Wilhelm His dated December 20, 1869 (Miescher, 1869c), he speculated that analyses of the quantitative ratio of nuclein to proteins in cells would allow a better distinction of pathological processes. For example, he believed that an increase in "nuclear substances" represented a preliminary phase to cell division in proliferating tissues, such as tumors.

Slow acceptance of nuclein

Miescher completed his initial set of experiments on the nuclein in the autumn of 1869 (His, 1897b). In order to expand his horizons, he decided to spend 1 year at the Physiology Institute of the University of Leipzig, Germany—a highly renowned institute at the time—and dedicate himself to new areas. Under the direction of Carl Ludwig (Fig. 1E), he mainly wanted to improve his knowledge of experimental techniques employed in physiology and investigated, among other things, the nerve tracts that transmit pain signals in the spinal cord.

In Leipzig, Miescher wrote up his first scientific publication, detailing the results he had obtained when studying the leucocytes in Hoppe-Seyler's laboratory (Miescher, 1871d). In a letter dated December 23, 1869, to his parents he wrote, "On my table lies a sealed and addressed packet. It is my manuscript, for whose shipment I

have already made all necessary arrangements. I will now send it to Hoppe-Seyler in Tübingen. So, the first step into the public is done, given that Hoppe-Seyler does not refuse it" (His, 1897b).

However, Hoppe-Seyler was skeptical of Miescher's results and opted to repeat the experiments for himself (Hoppe-Seyler, 1870; Miescher, 1870). Finally, after 1 year he was convinced. In early 1871, the manuscript was included in an issue of a journal published by Hoppe-Seyler himself (Miescher, 1871d)—together with a two-page article by P. Plósz (another student of Hoppe-Seyler's) demonstrating the presence of nuclein in the nucleated erythrocytes of birds and snakes (Plósz, 1871) and Hoppe-Seyler's own article (Hoppe-Seyler, 1871) in which he confirms Miescher's findings on nuclein, including its unusually high phosphorous content. Like Miescher, Hoppe-Seyler concluded that nuclein is unlike any other substance isolated before. He also excluded the possibility that it is merely a degradation product of the isolation procedure, but instead a novel substance of its own kind (Hoppe-Seyler, 1871).

In the first paragraphs of his own article in this issue, Hoppe-Seyler notes, "The analyses by Mr. F. Miescher presented here have not only enhanced our understanding of the composition of pus more than has been achieved in the past decades; for the first time they have also allowed insights into the chemical constitution of simple cells and above all their nuclei. Although I am well acquainted with Dr. Miescher's conscientious proceeding, I could not suppress some doubts about the accuracy of the results, which are of such great importance; I have therefore repeated parts of his experiments, mainly the ones concerning the nuclear substance, which he has termed nuclein; I can only emphasize that I have to fully confirm all of

Miescher's statements that I have verified" (Hoppe-Seyler, 1871).

Miescher himself was also confident about the importance of his discovery and claimed that he had found a completely new type of substance, equal in importance to proteins. He concluded his publication with the following words: "This is how far I have come based on the material at my disposal (...). However, I believe that the given results, however fragmentary, are significant enough to invite others, in particular, chemists, to further investigate the matter. Knowledge of the relationship between nuclear substances, proteins and their closest conversion products will gradually help to lift the veil which still utterly conceals the inner processes of cell growth" (Miescher, 1871d).

Miescher also realized that the presence of nuclein in the nucleus created an important chemical difference that set the nucleus apart from the cytoplasm. He was so convinced of the importance of nuclein for the identity of the nucleus that in an unpublished addendum to his 1871 paper, he even suggested that nuclei should no longer be defined based on their morphological properties, but by the presence of nuclein as this more closely correlates with the nuclei's physiological function (Miescher, 1870). However, neither Miescher nor his con-

temporaries could at that time fully grasp the significance of this discovery.

Return to Basel and resumption of work on nuclein

In 1871, Miescher returned to his hometown of Basel and prepared for his habilitation to become a professor. Inspired by his time with Hoppe-Seyler and his stay at Ludwig's laboratory, he chose the physiology of respiration as its topic. His aim was to combine physiological aspects of respiration with comparative anatomy to study the absorption of oxygen by blood and hemoglobin and the use of oxygen by different tissues—topics that would increasingly become a focus of his research. He concluded his habilitation with a lecture in 1871 (Miescher, 1871a) and in the following year was offered the Chair of Physiology at Basel University—a position previously held by Miescher's father and Wilhelm His who had accepted a position at the University of Leipzig.

In Basel, Miescher resumed his research on nuclein, which had rested during his stay in Leipzig. However, owing to poor working conditions, his progress initially was painfully slow (Miescher, 1872b). In a letter to a friend he complained, "In



Fig. 4. The laboratory in the former kitchen of the castle in Tübingen as it was in 1879. It was in this room that Miescher had discovered DNA 10 years earlier. The equipment and fixtures available to Miescher at the time would have been very similar, with a large distillation apparatus in the far corner of the room to produce distilled water and several smaller utensils, such as glass alembics and a glass distillation column on the side board. Photography by Paul Sinner, Tübingen.

the past two years, I have avidly yearned for the meat pots of the laboratory in Tübingen Castle again (also see Fig. 4), for I had no laboratory here and was (...) merely tolerated in a small corner of the chemistry laboratory, where I could hardly move...” He continued, “You can imagine how it must feel to be hindered in the energetic pursuit of an endeavor on account of the most miserable conditions, knowing that I may never have such a fine opportunity again...” (His, 1897b).

Nonetheless, he worked on and discovered that sperm cells proved to be an ideal source material for the isolation of large quantities of very pure nuclein (Miescher, 1871c, 1874b). Miescher chose these cells due to their simple composition with their heads comprised almost exclusively of a nucleus (Miescher, 1872a). Finally, he saw a possibility of obtaining sufficient amounts of nuclein to perform the exhaustive quantitative experiments he had already intended to do in Tübingen.

Basel’s location on the Rhine river with its annual upstream migration of salmon to their spawning grounds had a flourishing salmon fishing industry and there was an abundance of freshly caught salmon at Miescher’s disposal. Thus, in the autumn of 1871, he started to work on salmon sperm and developed numerous, ever more sophisticated protocols for the isolation of nuclein [see Miescher, 1874b, as well as the paper by Miescher’s coworker Oswald Schmiedeberg (Schmiedeberg and Miescher, 1896), which was published after Miescher’s death], which allowed him to obtain considerable quantities of the purest nuclein he had ever isolated (Fig. 5).

With this nuclein, he repeated the initial analyses of the elementary composition carried out in Tübingen. He confirmed that nuclein contained carbon, nitrogen, and hydrogen atoms and was indeed devoid of sulfur but rich in phosphorous (Miescher, 1872a,b,c). When having achieved the highest purity in isolating nuclein, he determined the proportion of P_2O_5 in salmon nuclein to be 22.5% of its total mass (Miescher, 1872b)—a figure very close to the actual proportion of 22.9%—and correctly stated that all the phosphorous contained in the nuclein is present in the form of phosphoric acid (Miescher, 1874b).

Further analyses of the nuclein isolated from sperm confirmed its acidic properties, showing that it must be a “multibasic acid” (Miescher, 1872b), a statement, which he refined to “at least three basic acid” (Miescher, 1874d) and eventually “at least four basic acid” (Miescher, 1874b). Miescher also noticed that nuclein was not well diffusible and concluded that it must be a molecule with a high molecular weight (Miescher, 1872c; see also Miescher, 1874b). Later however, Miescher determined an approximate atomic weight of 5–600 for nuclein (Miescher, 1873e) and postulated several approximations of an atomic formula, including the formulae of $C_{22}H_{32}N_6P_2O_{16}$ (Miescher, 1874d) and $C_{29}H_{49}N_9P_3O_{22}$ (Miescher, 1874b).

In the spring of 1872, Miescher presented his results on sperm to the Naturalist Society in Basel (His, 1897b). Among descriptions of the spermatocyte morphology, he reported



Fig. 5. Glass vial containing nuclein isolated from salmon sperm by Friedrich Miescher while working at the University of Basel. The faded label reads “Nuclein aus Lachssperma, F. Miescher” (*Nuclein from salmon sperm, F. Miescher*). Possession of the Interfakultäres Institut für Biochemie (Interfaculty Institute for Biochemistry), University of Tübingen, Germany; photography by Alfons Renz, University of Tübingen, Germany.

that in the heads of salmon spermatocytes, the “multibasic” acid nuclein is bound in a saltlike state to a basic molecule, which he called “protamin” (see also Miescher, 1872a,c, 1874b) and that together nuclein and protamin made up almost the entire mass in the sperm heads (Miescher, 1874b).

In the years 1872 and 1873, Miescher extended his studies to the sperm of carp, frogs, chicken, and bulls (Miescher, 1872a, 1873a,b,c,d,f), but with less success than he had previously had with salmon sperm. However, in all sperm examined he did find nuclein (Miescher, 1873b). The complete account of these analyses was published in 1874 (Miescher, 1874a,b,c).

Miescher owed a great deal of his success in isolating and characterizing DNA to his choice of cells for his experiments. Both leucocytes and spermatozoa are not embedded in a tissue or extracellular matrix and can thus easily be purified. Moreover, in both, but especially in the spermatozoa, the nuclei are large compared to the cytoplasm, facilitating an enrichment of nuclear components in purification protocols.

Theories on the role of DNA, gametogenesis, and fertilization

Miescher’s 1874 publication on the occurrence of nuclein in the sperm of various vertebrates (Miescher, 1874b) caused some interest in the scientific community at the

time. Embryologists then were already trying to understand the mechanisms controlling how an embryo develops and how characteristics and traits are passed on from one generation to the next. Miescher came very close to finding the answer himself. In his article he wrote, “If one (...) wants to assume that a single substance (...) is the specific cause of fertilization, then one should undoubtedly first and foremost consider nuclein” (Miescher, 1874b).

Yet, Miescher was not convinced that only one substance could be responsible for transmitting hereditary traits. He discarded the idea because, among other reasons, it seemed implausible to him that the same substance could result in the diversity of different animal species whose sperm he had examined. He conceded that “differences in the chemical structure of these molecules [*the different types of nuclein*] will occur,” but then went on to say that “they will only do so in a limited diversity.” Too few, Miescher believed, for this phenomenon to even explain the slight differences between individuals of the *same* species (Miescher, 1874b).

Over time, Miescher’s engagement with sperm as a source material for the isolation of nuclein led him to turn his attention to other matters. Although nuclein always played a role in his studies, his focus increasingly shifted to investigating the morphology and chemical nature of spermatozoa (Miescher, 1874b, 1890a, 1892d) and oocytes (Miescher, 1871b, 1877b) as well as the physiological and chemical processes accompanying their differentiation. Among other things, Miescher tried to discover the origin and changes in the nuclein as germ cells differentiate (Miescher, 1876b,c,d). He hoped that by examining the morphological and chemical changes underlying gametogenesis he could understand the process of sexual reproduction. Owing to his previous work on salmon sperm, he primarily investigated these processes in this species (Miescher, 1890a, 1892d).

Based on his results on the differentiation of oocytes and spermatozoa, over the years Miescher developed a range of theories attempting to explain the processes of fertilization (Miescher, 1872c, 1874b, 1892b,c, 1895) and the transmission of hereditary traits (Miescher, 1892c, 1893c). However, due to the lack of knowledge at the time, many of these theories were quite speculative.

For example, he believed that the key to understanding the requirement for sexual reproduction (i.e., the fusion of an oocyte with a spermatozoon) in order that normal development ensues was “stereochemistry” (Miescher, 1892c, 1893c). He supposed that the hereditary information was encoded in the countless asymmetric carbon atoms in organic molecules. These could be changed to a different stereochemical state by environmental factors. These errors could be corrected, Miescher postulated, by the fusion of information from two germ cells. The enormous numbers of asymmetric carbon atoms (mainly in the proteins) would allow such a great number of stereoisomerisms that the diversity of hereditary information might well be encoded in these, much as an alphabet of 24–30 letters is enough to express all words and concepts in a number of different

languages (Miescher, 1892c). Instead of containing a large number of different molecules, where each confers a specific inherited trait (reflecting de Vries’s pangenesis theory), Miescher believed the nucleus and protoplasm of germ cells to be composed of very few molecules with maybe a very complex chemical composition (Miescher, 1892c).

However, Miescher also developed theories that came surprisingly close to our modern day understanding of sexual reproduction. Wondering why the unfertilized egg is kept in a state of physical and chemical suspense, like a “watch that has not been wound up” (Miescher, 1895), he speculated that the two kinds of germ cells—the oocyte and the spermatozoon—develop in different directions. When fully differentiated, they have developed such that each on its own is lacking the physiological completeness, which is the basis of cellular life. The missing part in the oocyte, he hypothesized, could be the complete nucleus. Oocytes would thus have to be regarded as entities consisting predominantly of a well-developed cytoplasm whereas the sperm imported the “nuclear life” (Miescher, 1895), which has developed unilaterally in it. Only a fusion of one of each makes for a complete cell with the potential to develop (Miescher, 1895).

Miescher gradually moves away from DNA

From the mid-1870s, Miescher became increasingly absorbed in researching the changes that occurred to the bodies of salmon as they migrate from the ocean to their spawning grounds in the Rhine River. Coming from his interest in the development of sperm and oocytes, he was primarily fascinated by the size to which the gonads of the salmon grow at the expense of other parts of the body and he performed seminal experiments on the turnover of body constituents during this process (Miescher, 1881, 1897b). In addition to his work on the development of the gonads in salmon, Miescher continued to work on the physiology of respiration, for instance, investigating how the composition of the blood changes as a function of altitude (Miescher, 1885a, 1888a, 1897a; see also the papers by his students, which were published posthumously, Egger, 1897; Karcher et al., 1897; Suter and Jaquet, 1897; Veillon, 1897).

Despite always returning to his analyses of nuclein and spermatozoa (Miescher, 1887, 1890b,c, 1891, 1892a,b, 1893a,b,c,d, 1894), Miescher never again managed to obtain conclusive results on this molecule. He tried, for example, to understand its chemical structure, which he found to be “very peculiar and very different from that of proteins” (Miescher, 1890b), however, without ever publishing on the subject again or elaborating in lectures or letters on exactly what he had found.

Miescher’s working conditions had improved, but the responsibilities of his new position began to tax him, particularly as he took his various responsibilities very seriously. He spent a great deal of time preparing lectures for his students. Furthermore, from the mid-1870s, Miescher

was repeatedly asked to conduct surveys on nutrition—a cumbersome task for Miescher that diverted a great deal of his time and energy away from his research goals (Miescher, 1876a, 1877a, 1882, 1883, 1884, 1885a). In the early 1880s, he once again took on a new challenge and founded Basel's first institute for anatomy and physiology, which was opened in 1885 (His, 1897b). Miescher took his job as head of the new institute very seriously. He provided for a lively scientific atmosphere and attracted several renowned precision mechanics, who—together with Miescher—devised innovative instruments for physiological measurements (Miescher, 1888a,c).

However, Miescher's numerous obligations began to wear on him. His obsession with his work and tendency towards perfectionism left him less and less time to rest (Miescher, 1878, 1885b, 1888b, 1891). He slept little, hardly fulfilled any of his social obligations, and even spent most of his vacations in the laboratory. Completely exhausted, he began to show signs of depression and also weakened physically. Finally, at the beginning of the 1890s, he contracted tuberculosis. As a result, he had to abandon his work and move to a clinic in Davos in the Swiss Alps (His, 1897b).

One last time, he attempted to write a summary of his work including his unpublished results on nuclein (Miescher, 1894), but did not have the strength. Carl Ludwig, his former mentor in Leipzig, wrote in a consoling letter to Miescher, “As hard as it may be [to have to abdicate such promising work/projects], you have the comfort of having achieved everlasting accomplishments; you have made the center, the core of all organic life accessible to chemical analysis; and however often in the course of centuries to come, the cell will be studied and examined, the grateful descendant will remember you as the groundbreaking researcher” (His, 1897b).

Friedrich Miescher died on August 26, 1895, at the age of only 51 years. After his death, Wilhelm His wrote in the introduction to the collected works of Miescher: “The appreciation of Miescher and his work will not diminish; on the contrary, it will grow and his discoveries and thoughts will be seeds for a fruitful future” (His, 1897a) (Fig. 6). However, not even His himself knew how accurate his words actually were.

DNA after Miescher

After Miescher's initial description in his 1871 paper (Miescher, 1871d), other scientists also started investigations into nuclein. Mostly chemists, often through personal contacts with Miescher or Hoppe-Seyler, were intrigued by its potential as a novel kind of cellular substance—among them Albrecht Kossel (1879, 1891), Jules Piccard (1874), and Jakob Worm-Müller (1874). Most notably, Albrecht Kossel—another scientist in Hoppe-Seyler's laboratory and later winner of the Nobel Prize in Medicine—discovered that nuclein was comprised of four bases and sugar



Fig. 6. This picture of Friedrich Miescher in his later years is the frontispiece on the inside cover of the two volume collection of Miescher's scientific publications, his letters, lecture manuscripts, and papers published posthumously by Wilhelm His and others (His et al., 1897a,b).

molecules. Gradually, also histologists became interested. The botanist Eduard Zacharias showed that nuclein was an integral part of chromosomes and thus in 1881, according to His (His, 1897b), was the first to combine the histological concept of chromatin with the chemical substance nuclein.

However, for long after Miescher's death, nuclein still received comparatively little attention. The vast majority of scientists remained convinced that the more complex proteins must be the carriers of genetic information. Proteins are comprised of 20 different amino acids, while DNA is made up of only four different nucleotides—too few, it was believed, to store the enormous amount of genetic information. Widespread interest in DNA was not rekindled until the mid-1940s and early 1950s, when Oswald T. Avery, Colin MacLeod, and Maclyn McCarthy on the one hand and Al Hershey and Marta Chase on the other demonstrated in classical experiments that DNA is the carrier of genetic information (Avery et al., 1944; Hershey and Chase, 1952).

In 1953, Watson and Crick (1953) deciphered the structure of DNA and thus provided the first insight into how it works. A decade later, Robert W. Holley, Har Gobind Khorana, Marshall W. Nirenberg, and colleagues finally cracked the genetic code (Singer, 1968). At this point, it had become clear how the information for creating the variety of organisms could be encoded in a single molecule composed of only four different building blocks. This information served as the point of departure for the development of a completely new biological discipline: molecular genetics (for further information on the history of DNA and genetics, see also Box 3 and Mayr, 1982; Olby, 1994).

Box 3**Timeline of DNA**

1865: Gregor Mendel discovers through breeding experiments with peas that traits are inherited based on specific laws (later to be termed “Mendel’s laws”).

1866: Ernst Haeckel proposes that the nucleus contains the factors responsible for the transmission of hereditary traits.

1869: Friedrich Miescher isolates DNA for the first time.

1871: The first publications describing DNA (“nuclein”) by Friedrich Miescher, Felix Hoppe-Seyler, and P. Plösz are printed.

1882: Walther Flemming describes chromosomes and examines their behavior during cell division.

1884–1885: Oscar Hertwig, Albrecht von Kölliker, Eduard Strasburger, and August Weismann independently provide evidence that the cell’s nucleus contains the basis for inheritance.

1889: Richard Altmann renames “nuclein” to “nucleic acid.”

1900: Carl Correns, Hugo de Vries, and Erich von Tschermak rediscover Mendel’s Laws.

1902: Theodor Boveri and Walter Sutton postulate that the heredity units (called “genes” as of 1909) are located on chromosomes.

1902–1909: Archibald Garrod proposes that genetic defects result in the loss of enzymes and hereditary metabolic diseases.

1909: Wilhelm Johannsen uses the word “gene” to describe units of heredity.

1910: Thomas Hunt Morgan uses fruit flies (*Drosophila*) as a model to study heredity and finds the first mutant (*white*) with white eyes.

1913: Alfred Sturtevant and Thomas Hunt Morgan produce the first genetic linkage map (for the fruit fly *Drosophila*).

1928: Frederick Griffith postulates that a “transforming principle” permits properties from one type of bacteria (heat-inactivated virulent *Streptococcus pneumoniae*) to be transferred to another (live nonvirulent *Streptococcus pneumoniae*).

1929: Phoebus Levene identifies the building blocks of DNA, including the four bases adenine (A), cytosine (C), guanine (G), and thymine (T).

1941: George Beadle and Edward Tatum demonstrate that every gene is responsible for the production of an enzyme.

1944: Oswald T. Avery, Colin MacLeod, and Maclyn McCarty demonstrate that Griffith’s “transforming principle” is not a protein, but rather DNA, suggesting that DNA may function as the genetic material.

1949: Colette and Roger Vendrely and André Boivin discover that the nuclei of germ cells contain half the amount of DNA that is found in somatic cells. This parallels the reduction in the number of chromosomes during gametogenesis and provides further evidence for the fact that DNA is the genetic material.

1949–1950: Erwin Chargaff finds that the DNA base composition varies between species but determines that within a species the bases in DNA are always present in fixed ratios: the same number of A’s as T’s and the same number of C’s as G’s.

1952: Alfred Hershey and Martha Chase use viruses (bacteriophage T2) to confirm DNA as the genetic material by demonstrating that during infection viral DNA enters the bacteria while the viral proteins do not and that this DNA can be found in progeny virus particles.

1953: Rosalind Franklin and Maurice Wilkins use X-ray analyses to demonstrate that DNA has a regularly repeating helical structure.

1953: James Watson and Francis Crick discover the molecular structure of DNA: a double helix in which A always pairs with T, and C always with G.

1956: Arthur Kornberg discovers DNA polymerase, an enzyme that replicates DNA.

1957: Francis Crick proposes the “central dogma” (information in the DNA is translated into proteins through RNA) and speculates that three bases in the DNA always specify one amino acid in a protein.

1958: Matthew Meselson and Franklin Stahl describe how DNA replicates (semiconservative replication).

1961–1966: Robert W. Holley, Har Gobind Khorana, Heinrich Matthaei, Marshall W. Nirenberg, and colleagues crack the genetic code.

1968–1970: Werner Arber, Hamilton Smith, and Daniel Nathans use restriction enzymes to cut DNA in specific places for the first time.

1972: Paul Berg uses restriction enzymes to create the first piece of recombinant DNA.

1977: Frederick Sanger, Allan Maxam, and Walter Gilbert develop methods to sequence DNA.

1982: The first drug (human insulin), based on recombinant DNA, appears on the market.

1983: Kary Mullis invents PCR as a method for amplifying DNA in vitro.

1990: Sequencing of the human genome begins.

1995: First complete sequence of the genome of a free-living organism (the bacterium *Haemophilus influenzae*) is published.

1996: The complete genome sequence of the first eukaryotic organism—the yeast *S. cerevisiae*—is published.

1998: Complete genome sequence of the first multicellular organism—the nematode worm *Caenorhabditis elegans*—is published.

1999: Sequence of the first human chromosome (22) is published.

2000: The complete sequences of the genomes of the fruit fly *Drosophila* and the first plant—*Arabidopsis*—are published.

2001: The complete sequence of the human genome is published.

2002: The complete genome sequence of the first mammalian model organism—the mouse—is published.

Today, DNA is considered far more than just a molecule. It has become the icon of the modern biosciences. Understanding its structure and how it functions has fundamentally changed our world. Most of modern biology relies heavily on molecular genetics techniques, be it directly to elucidate the functions of cellular components or indirectly, for example, in the form of molecular phylogenetic trees that aid in reconstructing the evolution of life. Also other disciplines, such as psychology, criminology, and most notably medicine benefit increasingly from our knowledge of DNA.

The most recent breakthrough in the history of DNA research has been the publication of the very nearly complete sequence of the human genome in 2001 (Lander et al., 2001; Venter et al., 2001)—an achievement deemed utterly impossible only two decades ago. However, despite impressive advances in the past decades, our understanding of how DNA works is still far from complete. Nearly 150 years after Miescher's first experiments, there still remains a lot to discover.

Great discoveries often result from a combination of serendipity and an openness to accept (and follow up) an unexpected result. However, the breakthroughs in thought that follow great discoveries depend both on a mind prepared to change previously held concepts and a context of preexisting knowledge. This context determines if the significance of a discovery can be appreciated. In the case of Miescher, serendipity and the prepared mind were there: He had set out to characterize proteins and discovered DNA, which he recognized as being very worthy of further investigation. However, the breakthrough in thought that his discovery deserved only occurred half a century after his death, when the data necessary to fully grasp the significance of DNA's function were emerging. In many ways, Miescher's discovery was well ahead of its time.

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