

The Molecule from the Castle Kitchen

Last year marked the 50th anniversary of the historic scientific breakthrough of American biologist **JAMES WATSON** and British physicist **FRANCIS CRICK**. On April 25, 1953, the two scientists published a brief article in *Nature*, revealing for the first time the structure of DNA. However, the DNA story actually begins with a man who was less well known: **FRIEDRICH MIESCHER**, who discovered the genetic material – 135 years ago. Today, the **FRIEDRICH MIESCHER LABORATORY** of the **MAX PLANCK SOCIETY** in Tübingen continues to commemorate this scientist.

Tübingen Castle, February 26, 1869. A young Swiss physician, Friedrich Miescher, wrote a letter to his uncle describing a recent discovery he had made. Unaware of the significance of his work, Miescher was about to initiate one of the greatest scientific revolutions of all time. As a consequence of his discovery, large areas of medicine and biology would change fundamentally. But first things first.

Johann Friedrich Miescher was born on August 13, 1844 into a family of scientists. His father and uncle 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 a very early age. At 17, he began his medical studies in Basel, concluding them at the age of 23. However, the practice of medicine was difficult for him due to his poor hearing. Inspired by his uncle's interest in biochemistry, he turned to research.

In the spring of 1868, Miescher relocated to Tübingen to work in the laboratory of distinguished biochemist Felix Hoppe-Seyler, who was one of the pioneers in this new discipline, then known as "physiological chemistry." His labora-

tory was housed high above the Neckar river valley in the former kitchen of Tübingen Castle. Here, a few years earlier, Hoppe-Seyler had made groundbreaking discoveries regarding the properties of the red blood pigment hemoglobin. This achievement was a significant step for later investigations into the structure and function of this and other proteins.

As Hoppe-Seyler's only student, Miescher's first task in Tübingen was to determine the chemical composition of cells. White blood cells served as the source material for his studies. With this "most simple and independent cell type," he hoped to unravel the mysteries of the life of cells. And so the discovery of DNA was off to a rather unappetizing start: Miescher obtained the cells for his experiments from pus-covered bandages procured from a nearby hospital.

The young scientist initially investigated the components of the cell body, focusing particularly on the various types of proteins that made up the pus cells. In Miescher's time, proteins were considered the most promising part of a cell, and scientists analyzed them hoping to understand how cells worked. Miescher described their properties in detail and attempted to classify them. However, his work was marked by setbacks: the combination of proteins in the cells was too complex for the simple analytical methods and equipment available at the time.

Miescher ultimately turned to the nucleus – a part of the cell about which hardly anything was known at the time. This decision had a far-reaching impact: the young scientist noticed a precipitate in nucleic extracts that did not ap-

pear to be made of proteins (see box on page 54). Enzymes that cleave proteins had no effect on it. Moreover, Miescher realized that this precipitate was different from proteins in other ways, as well: Proteins are almost exclusively composed of the elements carbon, hydrogen, oxygen and nitrogen. In the new substance, however, Miescher also detected large amounts of phosphorus, which occurs only in small quantities in proteins. Since Miescher discovered the compound in the cells' nuclei, he called it "nuclein."

A completely new type of substance

On February 26, 1869, Friedrich Miescher reported on the discovery of this mysterious substance for the first time. In a letter to his uncle, Wilhelm His, he wrote: "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 salt solution, and therefore do not belong to any known type of protein." At that point, Miescher recognized that he had discovered a completely new type of substance, one that was equal in importance to proteins. Still, his mentor Hoppe-Seyler was skeptical of the unusual results and wanted to re-examine them. Finally, a year later, he was convinced. Miescher's findings were released in early 1871 in a journal published by Hoppe-Seyler himself. At the time, however, neither Miescher nor his contemporaries fully comprehended the significance of the discovery. ▶

ILLUSTRATION: ROHNER

1865: Gregor Mendel discovers through breeding experiments with peas that traits are inherited based on specific laws.

1882: Walther Flemming discovers the chromosomes.

1889: Richard Altmann renames "nuclein" to "nucleic acid."

1902: Theodor Boveri and Walter Sutton postulate that the heredity units (called "genes" after 1909) are located on chromosomes.

1908: Archibald Garrod proposes that genetic defects may result in the loss of enzymes and hereditary metabolic disorders.

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

1929: Phoebus Levene identifies the building blocks of DNA, including the four bases A, C, G and T.

1944: Oswald T. Avery, Colin MacLeod and Maclyn McCarthy demonstrate that Griffith's "transforming principle" is not a protein, but rather DNA.

1869: Friedrich Miescher is the first person to isolate DNA.

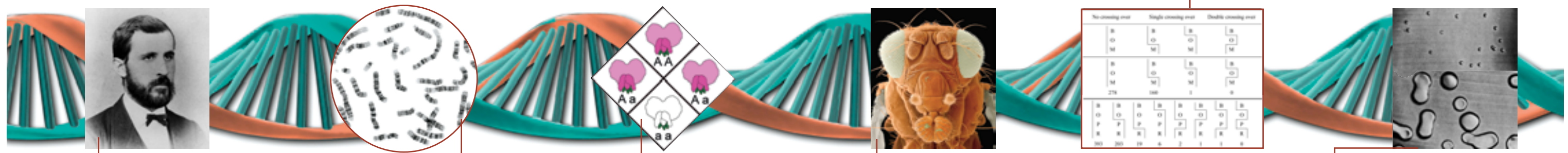
1887: Theodor Boveri establishes the individuality of the chromosomes.

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

1910: Thomas Hunt Morgan finds the first mutant fruit fly (with white eyes).

1928: Frederick Griffith postulates that a "transforming principle" permits properties from one type of bacteria to be transferred to another.

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



Following his time in Tübingen, Miescher spent one year at the University of Leipzig. In order to expand his horizons, he devoted himself to new subjects: under the direction of Carl Ludwig, he investigated, among other things, the nerve tracts that transmit pain signals in the spinal cord. In 1871, he returned to his hometown of Basel and within a year was offered the Chair of Physiology at the university there – the position previously held by his uncle, Wilhelm His.

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 (...) wanted to assume that a single substance (...) in some way (...) is the specific cause of fertilization, then one should undoubtedly first and foremost consider nuclein.”

Yet Miescher was not convinced that a single substance could be responsible for transmitting hereditary traits. He discarded the idea because, among other things, it seemed implausible to him that the same substance could result in the diversity of the various animal species whose sperm he had examined. He conceded that “differences in the chemical structure of these molecules will occur,” but then went on to say that “they will do so only in a limited range.” Too limited, Miescher believed, for this phenomenon to even explain the slight differences between individuals of the same species. Instead, he believed that mechanical stimuli – triggered by the movement of the sperm – and electrochemical processes, such as those that occur when nerves and muscles are stimulated, were responsible for the development of the fertilized egg cell.

But his fellow scientists were happy to use his findings in their continued investigations into nuclein. 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 molecules. Botanist Eduard Zacharias showed that nuclein was an integral part of chromosomes, and Theodor Boveri proved that genetic

information was contained in the cell’s nucleus. Several biologists gradually became convinced that nuclein played a key role in heredity. Due to the fact that nuclein behaved like an acid, Richard Altmann renamed it “nucleic acid” in 1889, which it is still called today (refer to the timeline at the bottom of the pages for more details on the history of DNA research).

However, Miescher increasingly turned his attention to other matters and never again published anything on this subject. In the mid-1870s, he began researching the changes that occurred in the bodies of salmon that migrated from the ocean to their spawning grounds in the Rhine River. Over entire winters, he would get up in the middle of the night and spend the early morning hours on the banks of the Rhine to catch salmon. He took thousands of them to his laboratory, measured and weighed them, and examined their muscles, internal organs and blood. Above all else, however, he was fascinated by the extent to which the reproductive organs of the salmon grow during this time – up to a quarter of their body weight.

He also spent a great deal of time preparing lectures for his students. The government even asked him to assess the nutrition of inmates in Basel’s prison – a task Miescher found less than appealing. Nevertheless, the authorities were so impressed with his work that other Swiss penal institutions also asked him for advice. Amused by this development, his uncle wrote: “Every jail wanted his special menu.” But Miescher had finally had enough. “I have made myself too green, now the goats are eating me up,” he not-

ed with dismay. He added, “Investigation into the nutrition of the Swiss people, a cookbook for workers, nutrition charts for the national fair, controversies with the Chamer Milk Company – to put it in a nutshell, I am well on my way to becoming the Guardian of Zion for the stomachs of my three million fellow countrymen.”

Early death precedes late fame

Instead, Friedrich Miescher once again took on a new challenge and founded the city’s first institute for anatomy and physiology. After some initial stumbling blocks, it was decided in 1883 to construct the new building; just two years later, in 1885, the institute was officially opened. Miescher took his job as head of the new institute very seriously. He made sure it had a lively scientific atmosphere, and attracted several renowned precision mechanics who devised innovative tools and instruments for physiological measurements, permitting a previously unattained degree of precision.

However, Miescher’s numerous obligations began to wear on him. His obsession with his work and his propensity for perfectionism left him less and less time to rest. He slept little, hardly fulfilled any of his social obligations and even spent most of his vacation time in the laboratory. Completely exhausted, he grew weaker and weaker. Finally, at the beginning of the 1890s, he contracted tuberculosis. He was forced to abandon his work and move to a clinic in Davos. One last time, he attempted to write a summary of his work (including his findings on nuclein),

“... in a small corner of the laboratory ...”

In Basel, Miescher continued his research on nuclein, although, owing to poor working conditions, his progress was slow. In a letter to a friend he complained: “In the past two years, I have avidly yearned for the meat pots of the laboratory in Tübingen Castle again, for I had no laboratory here and was (...) merely tolerated in a small corner of the chemistry laboratory where I could hardly move, as the small room is already more than overcrowded with students, and the professor of chemistry also works there.” 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...”

Fortunately, Miescher was ultimately able to complete his work. In 1874, he published his results on the occurrence of nuclein in the sperm of various vertebrates – a publication that caused a considerable sensation. Researchers at the time were already trying to understand how

1950: Erwin Chargaff determines that 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 (bacteriophages) to confirm DNA as the genetic material.

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 decode 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 to 1966: Robert W. Holley, Har Gobind Khorana, Marshall W. Nirenberg and colleagues crack the genetic code.

1968 to 1970: Werner Arber, Hamilton Smith and Daniel Nathans use restriction enzymes to sequence-specifically cut DNA 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 based on recombinant DNA appears on the market (human insulin).

1983: Kary Mullis invents PCR, a method for duplicating DNA in vitro.

1990: Sequencing of the human genome begins.

How Miescher Discovered Nuclein

Miescher obtained the cells (white blood cells) for his experiments from the pus on fresh surgical bandages, which he procured from the nearby surgical clinic in Tübingen. Very discriminating in his choice of source material, Miescher discarded everything that showed signs of decay, based on either smell or appearance.

Miescher first separated the cells from the bandaging material. To do this, he washed the bandages with a mixture of one part cold saturated Glauber's salt solution and nine parts water, and filtered the liquid through a sheet to remove the cotton fibers of the bandaging. Afterwards, he let the washing solution stand for one to two hours so that the cells could sink to the bottom. He then poured off the supernatant and filtered out the remaining washing solution. Finally, he checked whether the cells were still intact.

Miescher initially examined the proteins in the pus cells. During these tests, he noticed that a substance precipitated from the solution when acid was added, and dissolved again when alkali was added. Miescher said that, "according to known histo-chemical facts, I had to ascribe such material to the nuclei." He thus decided to examine the cells' nuclei more closely. He wrote: "A material made up of only cells, such as this one, would, above all else, finally call for a serious study of the chemical constitution of the cell's nucleus." However, he was not able to separate this substance from the proteins in his cell extracts in order to better analyze it. Miescher thus had to develop two different protocols, one after the other, to first separate the cells' nuclei from the cytoplasm and then isolate the nuclein.

FIRST PROTOCOL

Over a period of weeks at "wintry temperatures," Miescher first washed the cells by rinsing them several (6 to 10) times with fresh solutions of diluted hydrochloric acid. This procedure removed most of the cells' cytoplasm (protoplasm), leaving behind the nuclei. He then vigorously shook the residual matter for an extended period of time with a mixture of water and ether. This caused those nuclei that were still attached to cytoplasm to collect at the boundary layer between the water and ether. In contrast, clean nuclei without contaminating cytoplasm remained 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 and sharply defined nucleolus, somewhat smaller in comparison to their original volumes."

Using highly diluted alkaline solutions, Miescher then isolated a "yellowish solution of a substance" from these nuclei. By adding acetic acid or hydrochloric acid in excess, he could obtain an insoluble, flocculent precipitate. Without knowing it, Miescher had isolated DNA for the first time. He noticed that he could dissolve the precipitate again only by adding alkaline solutions. He realized that, although the substance was similar to proteins in certain ways, it was not a protein. However, he did not have a sufficient amount of material to conduct a further analysis of the novel substance. He wrote: "The minimum quantity of nuclei that can be obtained through the described procedure barely permitted the few reactions mentioned; elementary analyses could not even be considered." Miescher had to develop a new protocol.



The former kitchen of the castle in Tübingen housed one of the world's first biochemistry labs.

SECOND PROTOCOL

"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." Pepsin is an enzyme that occurs in the stomach and digests proteins. Miescher used it to separate the DNA from the proteins of the cytoplasm. He extracted the pepsin from pig stomachs by washing the stomachs with a mixture of 10 cc of fuming hydrochloric acid in a liter of water. He filtered the resulting solution until it was clear.

In contrast to his earlier protocol, Miescher first washed the pus cells 3 to 4 times with warm alcohol to remove the lipids. He then let the residual material digest with the pepsin solution for 18 to 24 hours at 37 to 45 degrees Celsius. After only a few hours, a fine gray powdery sediment separated from a yellowish liquid. Miescher continued the digestion process, changing the 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 any remaining lipids. Afterwards, he filtered out the nuclei and washed them with water until there was no longer any trace of protein.

He described the nuclei isolated in this way as "completely naked (...). The contours, smooth in some cases, were as if slightly eaten away in others." Miescher washed the nuclei again several times with warm alcohol and noted that the purified "nuclear mass" exhibited the same chemical behavior as the nuclei isolated using hydrochloric acid. Adding a diluted soda solution created a yellowish liquid again; adding an excess of acetic acid or hydrochloric acid to this liquid produced an insoluble precipitate of nuclein. And Miescher once again determined that he could dissolve the precipitate by adding a base, and cause it to re-precipitate by adding an excess of acid. Following these tests on the solubility of the nuclein, Miescher set out to determine its composition. To do so, he burned the precipitate to confirm the presence of various elements through the chemical reactions they exhibited. His tests showed that the nuclein "contained a large amount of phosphorus." He noted: "I think that the given analyses – as incomplete as they may be – show that we are not dealing with some random mixture, but (...) rather with a chemical entity or a mixture of very closely related compounds." For Miescher, the large amount of phosphorus in the nuclein was an indication that it could not be a protein or any other known molecule. He concluded: "We are dealing with a sui generis entity that is not comparable to any hitherto known group." Miescher also discovered nuclein in the cells of other tissues. He suspected that, upon further investigation, an "entire family of such phosphorus-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."

Without knowing anything about how nuclein functioned, Miescher nevertheless suspected that it played a central role in cells. 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, particularly 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 internal processes of cell growth."

PHOTO: UNIVERSITY LIBRARY, TÜBINGEN

but did not have the strength. Friedrich Miescher died on August 26, 1895, at the age of 51. After his death, his uncle, Wilhelm His, wrote: "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." Not even His himself knew how accurate his words would prove to be.

A molecule becomes an icon

Long after Miescher's death, the vast majority of scientists remained convinced that the more complex proteins were the carriers of genetic information. Proteins are comprised of twenty 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 1940s, when Oswald T. Avery, Colin MacLeod and Maclyn McCarthy demonstrated that DNA is the carrier of genetic information. In 1953, Watson and Crick deciphered the structure of DNA and provided the first insight into how it works. For their work, the scientists were awarded the Nobel Prize in 1962. Eight years later, Robert W. Holley, Har Gobind Khorana and Marshall W. Nirenberg finally cracked the genetic code, which likewise earned them the Nobel Prize in 1968. At this point it had become clear how the information for creating the various organisms could be encoded in a single molecule with just four bases. This information served as the point of departure for the development of a completely new

THE DNA ERA

DNA is the most important molecule we know. As the basis of all life on earth, no bacterium, fungus, plant, animal or human being could exist without it. Its discovery and the unraveling of its structure has fundamentally changed our world. Our understanding of bodily processes, as well as many areas of medicine, would not be possible without it. But not only biomedical research now has possibilities that were inconceivable before. Our understanding of life's evolution has been expanded by molecular phylogenetic trees. In forensic medicine, suspects can now be convicted based on information obtained from a single strand of hair, a piece of skin or a tiny drop of blood. Extremely accurate paternity tests are now possible and the food industry is able to determine the origin of plant and animal products. And the invention of the DNA computer a few years ago may even turn the "molecule of life" into the "molecule of the information era" one day.

biological discipline, namely, molecular genetics. Today, DNA is considered to be far more than just a molecule. It has become the icon of the modern biosciences. It has been immortalized in numerous paintings and sculptures, postage stamps have been dedicated to it, a perfume has been named after it, and stairways and even entire buildings are being designed after it. The most recent breakthrough in the history of DNA research was the mapping of the human genome in 2001. However, despite the impressive advances of the past decades, our understanding of how DNA works is still far from complete. Nearly 150 years after its discovery, no one has yet solved the remaining mysteries of DNA.

RALF DAHM

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

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

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

2002: The sequence of the mouse genome is published.



1998: The complete sequence of the genome of the nematode *C. elegans* is published.

2000: The complete sequence of the genome of the fruit fly *Drosophila* is published.