Hum Genet (2008) 122:565–581
DOI 10.1007/s00439-007-0433-0

REVIEW ARTICLE

Discovering DNA: Friedrich Miescher and the early years of nucleic acid research

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Received: 16 July 2007 / Accepted: 15 September 2007 / Published online: 28 September 2007

Abstract In the winter of 1868/9 the young Swiss doctor Friedrich Miescher, working in the laboratory of Felix Hoppe-Seyler at the University of Tübingen, performed experiments on the chemical composition of leukocytes that lead to the discovery of DNA. In his experiments, Miescher noticed a precipitate of an unknown substance, which he characterised further. Its properties during the isolation procedure and its resistance to protease digestion indicated that the novel substance was not a protein or lipid. Analyses of its elementary composition revealed that, unlike proteins, it contained large amounts of phosphorous and, as Miescher confirmed later, lacked sulphur. Miescher recognised that he had discovered a novel molecule. Since he had isolated it from the cells' nuclei he named it nuclein, a name preserved in today's designation deoxyribonucleic acid. In subsequent work Miescher showed that nuclein was a characteristic component of all nuclei and hypothesised that it would prove to be inextricably linked to the function of this organelle. He suggested that its abundance in tissues might be related to their physiological status with increases in "nuclear substances" preceding cell division. Miescher even speculated that it might have a role in the transmission of hereditary traits, but subsequently rejected the idea. This article reviews the events and circumstances leading to Miescher's discovery of DNA and places them within their historic context. It also tries to elucidate why it was Miescher who discovered DNA and why his name is not universally associated with this molecule today.

Electronic supplementary material The online version of this article (doi:10.1007/s00439-007-0433-0) contains supplementary material, which is available to authorized users.

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Introduction

> "The double helix is indeed a remarkable molecule. Modern man is perhaps 50,000 years old, civilization has existed for scarcely 10,000 years and the United States for only just over 200 years; but DNA and RNA have been around for at least several billion years. All that time the double helix has been there, and active, and yet we are the first creatures on Earth to become aware of its existence."
>
> *Francis Crick (1916–2004).*

The middle of the twentieth century witnessed some of the most fundamental discoveries in DNA research. In 1944 Oswald T. Avery, Colin MacLeod and Maclyn McCarty published their landmark paper suggesting that DNA, not proteins as previously widely believed, was the carrier of genetic information (Avery et al. 1944). At the turn of that decade, Erwin Chargaff discovered that the base composition of DNA varies between species, but that within each species the bases are always present in fixed ratios: the same number of adenine as thymine bases and the same number of cytosine as guanine bases (Chargaff 1951; Chargaff et al. 1949). In 1952, Alfred Hershey and Martha Chase confirmed DNA as the genetic material (Hershey and Chase 1952). One year later, building on X-ray analyses by Rosalind Franklin and Maurice Wilkins, Francis Crick and James Watson famously solved the structure of DNA (Watson and Crick 1953b). Finally, by the mid-1960s the genetic code had been cracked (reviewed in Davies 2002).

Thus, over a period of around 20 years, many of DNA's secrets had been uncovered.

The importance of these breakthroughs often overshadows the fact that research into DNA had already begun 75 years before these events took place, with the discovery of DNA by Johann Friedrich Miescher in 1869. Miescher (Fig. 1), a recent graduate of Basel University's Medical School had moved to Tübingen in the spring of 1868 to be trained as a scientist (Dahm 2005). Miescher came to Tübingen to study the chemical constituents of cells. He had been inspired in his decision by his uncle, Wilhelm His (1831–1904), an eminent physician and professor of anatomy and physiology at the University of Miescher's native Basel. Throughout his life, His contributed important work to our understanding of the fate of cells during embryonic development, especially the development of the nervous system. He, for example, discovered neuroblasts and coined the term *dendrite* (Finger 1994; Shepherd 1991). It was his conviction that the key to the fundamental questions of biology lay in the chemistry of cells and tissues (His 1897e). Miescher, who showed limited interest in becoming a practicing physician, was easily persuaded by his uncle to devote his career to studying these questions.

At the time, Tübingen was a centre of the natural sciences. The city's Eberhard-Karls-University, founded in 1477, was home to Germany's first independent Faculty of Natural Sciences, which had been established in 1863. The founding of this faculty, which comprised Chairs for Mathematics, Physics, Astronomy, Mineralogy, Pharmacology, Chemistry, Botany and Zoology, reflected the growing recognition of the natural sciences at the University. Tübingen's pioneering position in the nineteenth century was also mirrored by the fact that the city was home to a roster of prominent scientists.

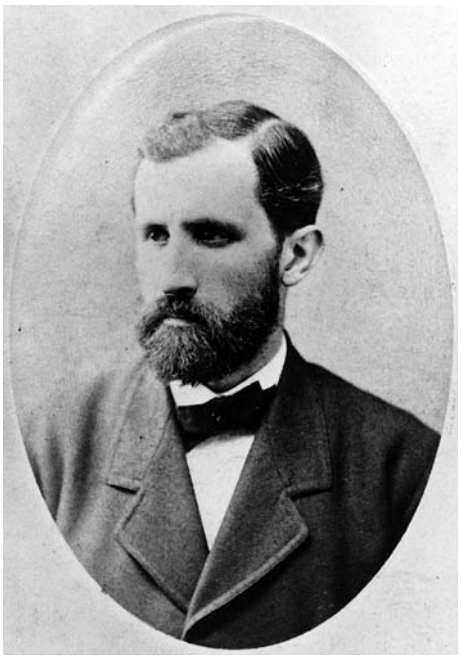

Fig. 1 Photograph of Johann Friedrich Miescher (1844–1895) as a young man

These included eminent biochemists, such as Adolf Strecker and Felix Hoppe-Seyler (see below), as well as Hugo von Mohl and Franz von Leydig. Von Mohl, an acclaimed botanist and cell biologist, coined the term protoplasm and contributed to establishing the concept that cells always originate from other cells (*omnis cellula e cellula*), which superseded Schleiden's theory that cells can form from an acellular origin. This and other achievements made von Mohl one of the founding fathers of the cell theory (reviewed in Ulshöfer 1964). Von Leydig was a noted cell biologist and histologist, whose textbook on the histology of humans and animals (Leydig 1857) was one of the most influential works of his time. Importantly, it contained an extensive review of the morphology of the cell. Today von Leydig is chiefly remembered for his discovery of the interstitial cells ("Leydig cells") in the testis.

In his first semester in Tübingen, Miescher worked with the chemist Adolf Strecker. Strecker (1822–1871) was a leading figure in organic chemistry in the mid-nineteenth century, who had achieved widespread renown for being the first person to synthesize an amino acid (alanine) in a reaction known today as Strecker synthesis (Strecker 1850). But Miescher was not so interested in organic chemistry as such and was keen to apply his newly acquired knowledge to explore the chemistry of cells and tissues. Thus following his training with Strecker, in the autumn of 1868, Miescher joined the laboratory of biochemist Hoppe-Seyler (His 1897e).

Hoppe-Seyler (1825–1895) was one of the pioneers of what was then referred to as *physiological chemistry*, a new field aiming to unravel the biochemistry of life (Anon 1970; Noyer-Weidner and Schaffner 1995). He performed seminal work on the properties of proteins, most notably haemoglobin (which he named), and introduced the term *proteid* which later became protein (Perutz 1995). Hoppe-Seyler's expertise and research interests were closely aligned with Miescher's aims and his laboratory proved to be a congenial place for Miescher to work (Figs. 2, 3).

The discovery of DNA

As Hoppe-Seyler's student, Miescher initially embarked on determining the biochemical composition of lymphocytes. Miescher wrote: "In full agreement with Hoppe-Seyler, I had set myself the task of elucidating the constitution of lymphoid cells. I was captivated by the thought of tracking down the basic prerequisites of cellular life on this simplest

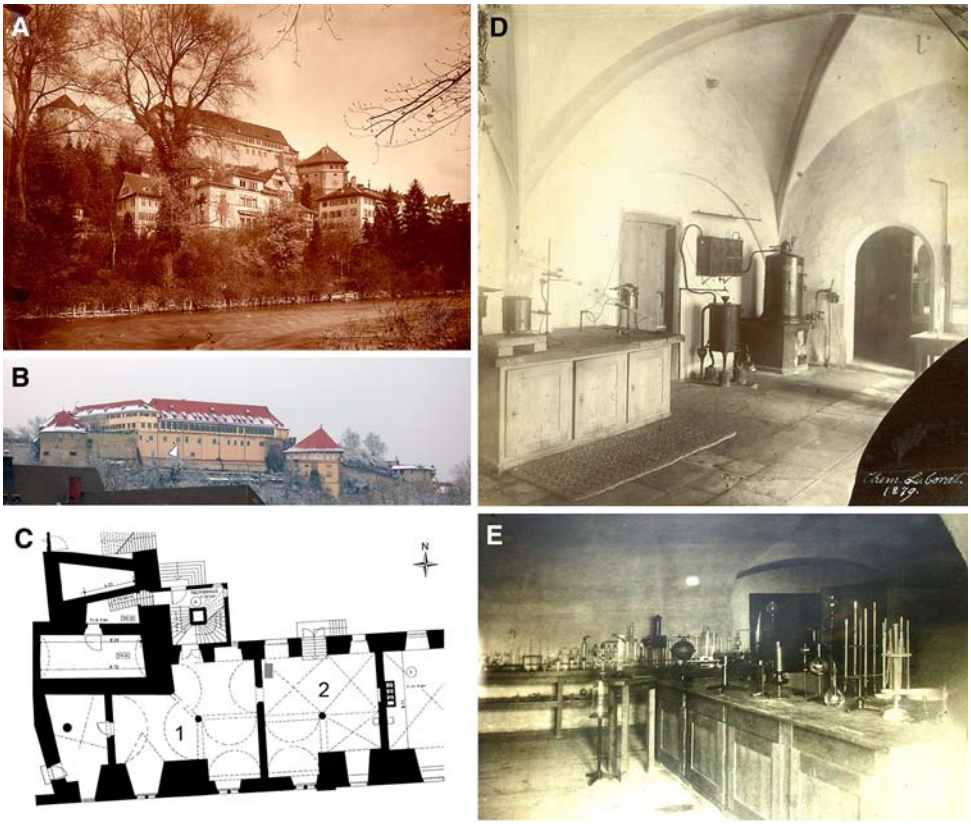

Fig. 2 **a** Historic photograph of Tübingen castle (viewed from the south-west) taken around the time when Miescher worked there. **b** Contemporary photograph of Tübingen castle (viewed from the south). The picture was taken at approximately the same time of year as when Miescher first isolated DNA. 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; the window to Miescher's laboratory is indicated by an *arrowhead*). **c** Presumed floor plan of the area of Tübingen castle then housing the laboratories of Felix Hoppe-Seyler (*1*) and Friedrich Miescher (*2*). These rooms, which had previously been the castles laundry and kitchen, had become laboratories of Tübingen University in 1823 and 1818, respectively. The place where Miescher presumably worked is indicated by a grey rectangle. **d** Miescher's laboratory in the former kitchen of Tübingen castle as it was in 1879. In this room ten years earlier, Miescher had discovered DNA. The equipment and fixtures available to Miescher at that time would have been similar to the ones seen in this photograph: a large distillation apparatus in the far corner of the room to produce distilled water and several smaller utensils including glass alembics and a glass distillation column on the side board. The hand-written note in the lower right-hand corner reads "Chem. Laborat. 1879." **e** Hoppe-Seyler's laboratory around 1879. Prior to becoming the chemical laboratory of Tübingen University in 1823, this room was the laundry of the castle. The two laboratories shown in (**d**) and (**e**) were adjacent to each other. The historic photographs in **a**, **d** and **e** are by Paul Sinner, Tübingen; the contemporary photograph in **b** was taken by Benjamin Saur, Tübingen; the floor plan of Tübingen castle was kindly provided by Alfons Renz of the Fundus Wissenschaftsgeschichte, University of Tübingen

and most independent form of animal cell." (Miescher 1869a). He tried to obtain the cells from lymph glands, but found that they were difficult to isolate in purities and quantities sufficient for chemical analysis. Instead Miescher soon moved on to using leukocytes as his model system. This cell type was readily available to him from the local surgical clinic. Pus from fresh surgical bandages was both a comparatively pure source of leukocytes and provided him with large quantities of material for his biochemical analyses, ideal conditions for Miescher to identify the fundamental components of cells. He wrote "[*I was*] faced with the task of determining, as completely as possible, the chemical building blocks whose diversity and arrangement determines the structure of the cell. For this purpose pus is one of the best materials. Hardly with anything else would it be possible to obtain such histological purity […]."(Miescher 1869a).

To begin, Miescher had to develop methods to wash the leukocytes and separate the protoplasm from the nuclei, such that he could analyse their constituents. He tested a variety of salt solutions, always checking the outcome of his trials under a microscope; "a very time-consuming task" as Miescher complained (Miescher 1869a). Once he had established the conditions, he set out to characterise and categorise the different proteins and lipids he isolated from the cells (Miescher 1871c; see also Wolf 2003b). During these experiments, however, he detected a substance with unexpected properties. It could be precipitated by acidifying

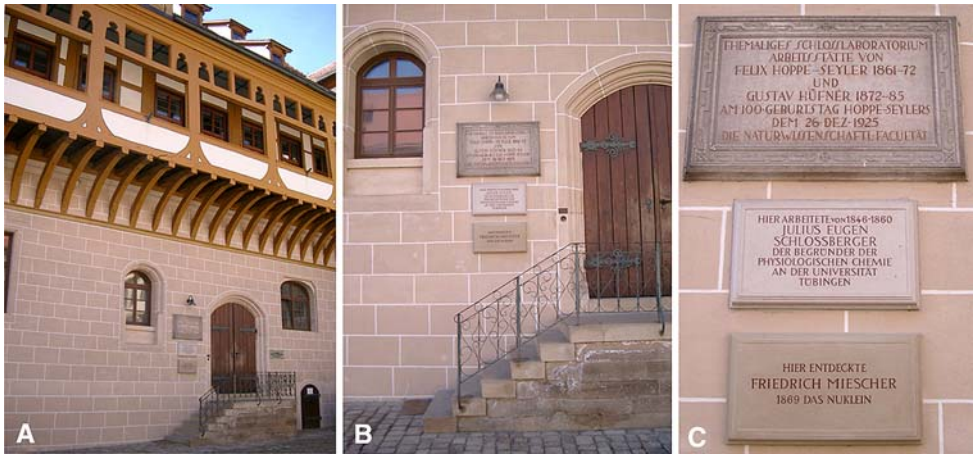

Fig. 3 **a, b** The entrance from the inner courtyard to the southern wing of Tübingen castle leading to the rooms formerly housing the laboratories of Felix Hoppe-Seyler and Friedrich Miescher. Left of the door are three wall-mounted plaques commemorating four notable biochemists who had worked there. **c** Detail showing the three commemorative plaques. The inscriptions are as follows: *Top plaque* "Former castle laboratory / workplace of / Felix Hoppe-Seyler 1861–72 / and / Gustav Hüfner 1872–85 / on [*the occasion of*] Hoppe-Seyler's 100th birthday / on 26 Dec. 1925 / [*installed by*] the Faculty of Natural Sciences"; *Centre plaque* "Here worked from 1846–1860 / Julius Eugen / Schlossberger / the founder of / physiological chemistry / at the University / Tübingen"; *Lower plaque* "Here discovered / Friedrich Miescher / 1869 the nuclein." All photographs are by the author

the solution and re-dissolved when alkaline solutions were added (Miescher 1869a, 1871c). "In my experiments with weakly alkaline solutions, when neutralising the solution, I could obtain precipitates that could not be dissolved either in water, acetic acid, very dilute hydrochloric acid, or in solutions of sodium chloride, and which thus could not belong to any of the hitherto known proteins." (Miescher 1869a). Unknowingly, Miescher had, for the first time, obtained a crude precipitate of DNA.

Where did this substance come from? Based on his observations of how the leukocytes behaved when he extracted them with acids, Miescher speculated that the new substance would likely derive from the nucleus. "Through prolonged exposure of the cells to diluted hydrochloric acid it is possible to reach a point when the acid will not take anything up anymore. The residue consists partly of isolated [*nuclei*] and partly of nuclei surrounded by a shrunken contour. The contour can no longer be stained yellow with iodine [*an indication that the proteins had been largely extracted*]. Very weak alkaline solutions […] lead to a strong swelling and fading of the nuclei, without them, however, dissolving into the solution. According to these facts [*i.e. behaviour of the extracted nuclei*], surely known to histologists, the substance could only belong to the nuclei and therefore captivated my interest." (Miescher 1869a).

At that time, little was known about this organelle. Although the nucleus had been discovered as early as 1802, its role inside the cell remained a matter of intense controversy and speculation (reviewed in Baker 1957; Harris 1999). However, since the influential German biologist Ernst Haeckel had suggested that the nucleus contained the factors responsible for the transmission of hereditary traits in 1866 (Haeckel 1866, see also Olby 1969), it received increasing attention. Miescher saw his serendipitous discovery as an opportunity to learn more about the chemistry and thus possibly about the function of the nucleus. As a first step he set out to develop protocols to isolate nuclei at higher purity (Miescher 1871c).

Miescher was very cautious in his choice of source material for his experiments. He only used clean pus from fresh surgical bandages and carefully examined it for signs of decomposition with a microscope before proceeding. Any material that did not meet his stringent criteria was excluded from the analyses. Miescher then set about washing the leukocytes off the bandages. After numerous attempts with different salt solutions, he discovered that if he used a dilute solution of sodium sulphate, a mixture of one part cold saturated Glauber's salt ($Na_2SO_4 \cdot 10\ H_2O$) solution and nine parts water, he could separate the cells from the bandages without damaging them. He then filtered the cell suspension through a sheet to remove any residual cotton fibres. Afterwards he let the washing solution stand for 1–2 h to allow the cells to sediment (Miescher had no centrifuges at his disposal). Finally, he examined his preparation again with a microscope to ensure that his isolation procedure had been successful and that the leukocytes were morphologically intact.

The next step was to isolate the nuclei. To do this, Miescher rinsed the cells several times with fresh solutions of a 1:1,000 diluted hydrochloric acid over a period of several weeks at "wintry temperatures" (which were important

to minimise degradation by DNases). This treatment lysed the cells and stripped most of the cytoplasm off the nuclei. To confirm the removal of contaminating cytoplasm, Miescher exposed the isolated nuclei to iodine solutions, a method commonly used during this period to stain the cytoplasm (reviewed in Kiernan 2001).

In this context, it is worth noting that Miescher was hesitant to use histological staining techniques to characterise his newly identified substance or to confirm its presence in the cells' nuclei (for a discussion of this issue, see Olby 1969). This may have stemmed from a general mistrust Miescher held against histologists who in his view employed procedures without understanding the underlying chemistry. As a consequence, he did not use carmine solutions, the only method to differentially stain the nucleus at the time.

Having isolated the nuclei, Miescher now had to obtain his enigmatic precipitate again to be able to better characterise it. He extracted the lipids as well as other hydrophobic molecules by vigorously shaking the material in a mixture of ether and water. After allowing the mixture to settle, Miescher observed that nuclei with cytoplasm still attached collected at the interface, whereas a fine powder of extracted nuclei sunk to the bottom of the aqueous phase. Adding alkaline solutions caused these nuclei to swell and fade. When adding acid, the reaction could be reversed and Miescher again obtained a white, flocculent precipitate (Miescher 1871c). This showed that the precipitate he had previously obtained was indeed derived from the nuclei. Due to its occurrence in the cells' nuclei, Miescher later named the novel substance *nuclein* (Miescher 1871c).

At this stage Miescher was still uncertain about the nature of the substance he had discovered. It was positive in the xanthoprotein colour reaction, suggesting at least a similarity with proteins (this was actually due to contaminating proteins in Miescher's early isolations of DNA). Its unusual behaviour in the extraction procedure was, however, unlike that observed for proteins, which could not readily be redissolved after acid precipitation. Also the substance would not coagulate when boiled (Miescher 1871c). Miescher noted: "According to these reactions it [*the precipitate*] does not seem to be a real protein, but rather correspond to mucin, albeit not precisely." (Miescher 1869a). On the 26 February 1869, Miescher wrote a letter[1] to his uncle His in Basel to share his discovery (Miescher 1869a). Following a detailed account of his initial analyses of the enigmatic precipitate, he reported on the difficulties he was experiencing in separating the different cell constituents from each other. But he was hopeful that he could overcome these problems and promised to inform His of his progress.

[1] Copies of Miescher's original handwritten letters are available from the Handschriftenabteilung of the University of Basel (see http://www.ub.unibas.ch or contact sekretariat-ub@unibas.ch). For Miescher's letter of the 26 February 1869 to Wilhelm His, as well as three other letters written by Miescher during his time in Tübingen, see supplementary figure 1 of Electronic Supplementary Material.

Miescher's first analyses of DNA

Miescher was eager to better characterise the precipitate. With his first protocol, however, the quantities of nuclei isolated were too small to allow further analyses. He wrote: "The minimum quantity of nuclei that can be obtained through the described procedure hardly permits the few reactions mentioned; elementary analyses could not even be considered." (Miescher 1871c). Miescher thus had to develop a second protocol in order to obtain larger quantities of purified DNA. A major concern of his was to get rid of contaminating cytoplasm which would have skewed his analyses of the novel substance.

Miescher decided to employ a recently developed protocol using proteases to remove the cytoplasm (as well as proteins). In his textbook on physiological chemistry, Wilhelm Kühne had described how the digestion of cells with pepsin solutions dissolved the cells' cytoplasm, but spared the nuclei (Kühne 1868). Miescher isolated the pepsin he needed for his experiments himself by rinsing out pig stomachs with a mixture of 10 ml of fuming hydrochloric acid per 1 l of water. The resulting solution, which contained the proteolytic enzymes secreted by the pig stomach, was filtered until it was clear (Miescher 1871c).

In contrast to his earlier protocols, Miescher began by washing the leukocytes several (3–4) times with warm alcohol. This not only lysed the cells but also removed most of the cytoplasm. The treatment also extracted the lipids as well as some of the hydrophobic proteins. He subsequently digested the nuclei with the pepsin solution at 37–45°C. Within a few hours, this procedure resulted in a fine, grey sediment separating from a yellowish supernatant. To ensure that all proteins had been removed, Miescher continued the digestion, changing the pepsin solution twice, until the extract had been digested for 18–24 h.

To remove residual lipids, Miescher took the sediment up in ether, shook it several times and subsequently washed it with water. He then stained and examined the "nuclei" under a microscope to ascertain that he could no longer detect (significant quantities of) contaminating proteins. Miescher next washed the extract several times with warm alcohol and noted that the "nuclear mass" cleaned in this way exhibited the same chemical behaviour as the nuclear extracts isolated with his previous protocol. Next, Miescher washed the preparation with alkaline solutions, for example highly diluted (1:100,000) sodium carbonate. When subsequently adding an excess of

acetic or hydrochloric acid, he got an insoluble, flocculent precipitate, which he could re-dissolve by adding alkaline solutions. Miescher had obtained the first comparatively clean preparation of DNA.

After having developed the protocol with which to isolate nuclein at a sufficient purity and in large enough quantities, Miescher set about determining its elementary composition. Elementary analyses were one of the few methods available to characterise novel substances at the time (Wolf 2003b; see also Hoppe-Seyler 1871). The procedure involved heating the substance to be analysed in the presence of various chemicals that selectively reacted with the different constituent elements. The resulting reaction products were weighed to quantify the amount of each element (see Wolf 2003b for details). Miescher was acutely aware that elementary analyses were crucial to help uncover the true nature of nuclein and whether it was actually distinct from other organic molecules. He noted: "I have tried to detect the essential peculiarities in [*nuclein's*] elementary composition, as best the sparse material at my disposal would allow me to." (Miescher 1871c). In addition to revealing the elementary composition of nuclein, repeated analyses also afforded Miescher with an approximation of how successful he was in reproducibly isolating pure nuclein.

In his experiments on the elementary composition of nuclein, Miescher detected various elements typically found in organic molecules: carbon, hydrogen, oxygen, nitrogen as well as some sulphur (an indication that his DNA preparation was likely still contaminated with proteins). However, his analyses also revealed that nuclein, unlike proteins, contained a large proportion of phosphorus, as Miescher reported in a letter to his parents on the 21 August 1869 (Miescher 1869b; see also Miescher 1871c).

Importantly, he noted that the ratio of phosphorous to the other elements in nuclein was different from that in any other organic molecule known at the time (Miescher 1869b, 1871c). To determine whether the phosphorous in nuclein was present as inorganic phosphate or organically bound, Miescher combusted samples of nuclein. When taking the ash up in water, it did not exhibit reactions characteristic of phosphoric acid (Miescher 1871c). This demonstrated that the phosphorous had evaporated during the combustion, indicating that it had been present in organic bonds rather than in an inorganic state.

Together with nuclein's behaviour in the isolation procedure, the presence of large amounts of organically bound phosphorous convinced Miescher that the substance he had discovered must be different from all known types of protein and other organic molecules. He wrote: "I believe that the given analyses, as incomplete as they may be, allow the conclusion that we are not dealing with some random mixture, but, apart from at most low levels of contamination, with a chemical individual or a mixture of very closely related entities." (Miescher 1871c). Miescher was confident that he had discovered a fundamentally new type of organic molecule with hitherto unknown properties and he went on: "We rather have here entities *sui generis* [= *of their own kind*] not comparable to any hitherto known group." (Miescher 1871c).

Following up on his discovery of nuclein in the nuclei of leukocytes, Miescher examined other tissues and cell types, for example liver, testes, kidney, nucleated erythrocytes and yeast cells, and could find it there too (Miescher 1869a–c, 1871c). Self confidently he speculated 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." (Miescher 1871c).

Miescher was uncertain about nuclein's function in the cell though. He contemplated that it might serve to store phosphorous or act as a reservoir for other molecules that could be derived from nuclein whenever needed. "The first thought will be that nuclein […] may be the mother substance of lecithin [*a phosphorous-containing molecule previously characterised by both Strecker and Hoppe-Seyler and which was under intensive investigation at the time*]." (Miescher 1871c). Miescher conjectured that lecithin may arise from nuclein when a nitrogen-rich moiety was cleaved off. He was, however, reluctant to engage in too much speculation. "But why discuss possibilities? These are questions that can be directly addressed from different angles. The analysis of cells at different stages of development will surly give good clues on the genetic [*sic, but meaning how they are "generated" here*] relationship between these two substances." (Miescher 1871c).

In a first attempt to find a function for nuclein, Miescher analysed its abundance in different tissues and under different pathological conditions. In a letter to Wilhelm His dated the 20 December 1869 (Miescher 1869c), Miescher believed that determining the quantitative ratio of nuclein to proteins in cells might aid in the distinction of pathological processes. He postulated, for example, that during "nutritive progression" and "regression" there would be "an increase in protoplasm proteins accompanied by an enlargement of the cell" and "an accumulation of lipids and products of degenerative processes", respectively. During "generative progression", however, he posited there is an "increase in nuclear substances [*as*] a preliminary phase to cell division in proliferating tissues, such as tumours" (Miescher 1869c). Although these observations capture the situation with astonishing accuracy, Miescher could not foresee the actual physiological relevance of the increase in nuclein that precedes cell division.

Difficulties publishing the discovery of DNA

In the autumn of 1869, Miescher had completed his initial characterisation of nuclein and returned to Basel for a short holiday. During this time he began writing up his first scientific publication: his analysis of the biochemical composition of leukocytes including his discovery and initial characterisation of nuclein. But he felt that his training as a researcher was not yet complete and he sought a new position and new projects to broaden his scientific education. He decided to move to the Physiology Institute of the University of Leipzig, Germany. The institute, under the direction of Carl Ludwig (1816–1895), was highly renowned and Ludwig, like Hoppe-Seyler, proved to be an inspiring mentor whom Miescher venerated throughout his life. In Ludwig's laboratory, Miescher investigated, among other things, the pain-transmitting nerve tracts in the spinal cord (reviewed in His 1897e).

While Miescher tackled his new tasks with his characteristic conscientiousness, he did not develop the same enthusiasm he had felt for his project in Tübingen. Nonetheless, the multitude of new topics Miescher encountered in Ludwig's laboratory and the internationality of the group—there were scientists from at least nine different countries, including Holland, Norway, Hungary, Germany, the UK, Switzerland, Russia, Egypt, and the USA (His 1897e; Miescher 1869d, 1869e)—greatly broadened his horizon. Moreover, a number of his Leipzig colleagues would remain close friends and collaborators long after Miescher had left the institute (His 1897e).

During his first months in Leipzig, Miescher also finalised the manuscript he intended to send to Hoppe-Seyler for his approval. Shortly before Christmas of 1869, he had finished it and on the 23 December 1869 he wrote in a letter to his parents: "On my table lies a sealed and addressed packet. It is my manuscript, for the shipment of which 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 1897e).

However, publication of Miescher's first manuscript was not to be a quick and straight forward process. For one, his former mentor Hoppe-Seyler was sceptical of Miescher's data and wanted to repeat the experiments for himself before consenting to their publication (see letters VI–XV in His 1897a). This was none too surprising given that only in 1868 Hoppe-Seyler's laboratory had seen a protracted argument over whether a putative phosphate-containing molecule from brain tissue, referred to as 'protagon', actually existed or not (Olby 1969). In this context, any claim by a young scientist of having discovered a new substance would have been viewed suspiciously by Hoppe-Seyler. A particular concern of Hoppe-Seyler's was that the pepsin digestion may have resulted in degradation products that could have combined with phosphorous-containing compounds to yield an artificial product with the atypical nitrogen and phosphorous content observed by Miescher (Olby 1969).

Moreover, starting in July 1870, a federation of German states was embroiled in a war with France that directed both resources and attention to matters other than the pursuit of science. But ultimately, after a year of anxious suspense for Miescher, Hoppe-Seyler, together with students of his, had been able to replicate Miescher's results on nuclein and was persuaded to allow the publication of his manuscript.

In early 1871, Miescher's manuscript entitled "Ueber die chemische Zusammensetzung der Eiterzellen" (*On the Chemical Composition of Pus Cells*) was included as the first paper in an issue of the *Medicinisch-chemische Untersuchungen*, a journal published by Hoppe-Seyler himself (Miescher 1871c) (Fig. 4). It was followed by two further articles dealing with nuclein: a two-page article by P. Plósz, another of Hoppe-Seyler's students, reporting the presence of nuclein in the nucleated erythrocytes of birds and snakes, but not in anuclear bovine erythrocytes (Plósz 1871) as well as an article by Hoppe-Seyler himself (Hoppe-Seyler 1871) in which he confirmed Miescher's findings on nuclein, including its unusually high phosphorous content.

In the opening paragraphs of his article, Hoppe-Seyler wrote: "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). Like Miescher, Hoppe-Seyler excluded the possibility of nuclein merely being a degradation product of the isolation procedure and concluded that it is unlike any other substance isolated before and thus a novel substance of its own kind (Hoppe-Seyler 1871).

In his manuscript, Miescher was also confident about the importance of his discovery. He stated that the new substance he had discovered would prove to be of equal importance to proteins. Concluding his publication he wrote: "This is how far I have come based on the material at my disposal. It is obvious that, elementary analyses apart, a number of simple and obvious experiments are missing, which would likely give essential information on the relationship between nuclein and the other hitherto known groups [*of molecules*]. I myself will, as soon as possible,

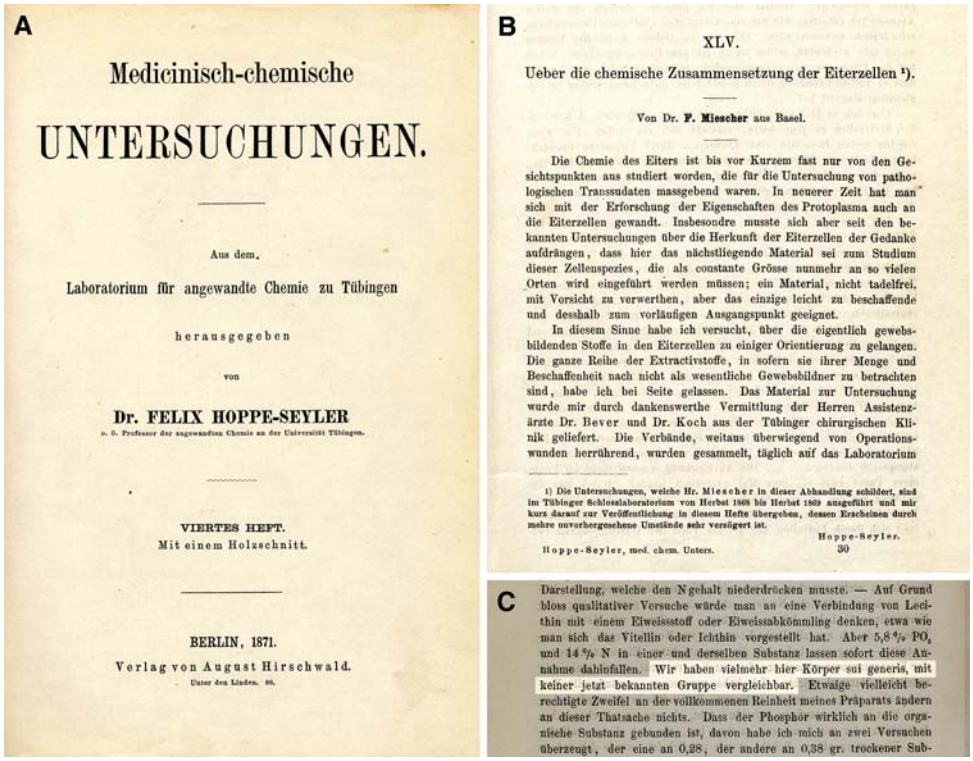

Fig. 4 **a** Front page of the issue of the journal *Medicinisch-chemische Untersuchungen* (*Medical-chemical Investigations*) in which Miescher's article describing his discovery of DNA appeared. The text underneath the journal's name translates as "From the / Laboratory of Applied Chemistry in Tübingen / edited / by / Dr. Felix Hoppe-Seyler / Professor of Applied Chemistry at the University of Tübingen / fourth issue / with one wood engraving / Berlin, 1871 / publishing house of August Hirschwald / Unter den Linden, 68 [street in the centre of Berlin]". **b** First page of Miescher's article. The title translates as "On the Chemical Composition of Pus Cells". A scanned version of the full article as it was re-printed in the collection of Friedrich Miescher's scientific publications, lecture manuscripts and scientific correspondence published by Wilhelm His and others (His 1897b, c) after Miescher's death is available as Supplementary Figure 2 of Electronic Supplementary Material. **c** Excerpt from Miescher's article in which he states that he has discovered a new substance. The highlighted sentence translates as "We rather have here entities *sui generis* [= *of their own kind*] not comparable to any hitherto known group." All photographs are by the author. At its end, Miescher's article bears the location (Basel) and date (October 1869) when he had finished analysing and writing up the experiments he had carried out in Tübingen. This addition was introduced at the request of Miescher (Miescher 1872a) to ensure that he would retain his place as the discoverer of nuclein even if someone had also found it in the period between Miescher completing his experiments and the publication of his manuscript

report further news. However, I believe that the given results, albeit 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 1871c).

Miescher also recognized that nuclein's exclusive presence in the nucleus meant an important chemical difference between this organelle and the cytoplasm. So convinced was he of the significance of nuclein for the identity of the nucleus that in an unpublished addendum to his 1871 paper, he suggested that nuclei should no longer be defined via their morphological properties, but by the presence of nuclein as this more closely correlates with their physiological function (Miescher 1870). These statements of Miescher suggest an astounding degree of foresight on his part. However, neither Miescher nor any of his contemporaries could at that time fully appreciate how close to the truth his speculations actually were.

Resumption of Miescher's work on nuclein in Basel

After less than 2 years in Leipzig, Miescher was offered the prospect of a professorship at the university in his hometown of Basel. He returned, submitted his habilitation and in 1872 was offered the Chair of Physiology at Basel University, a position previously held by both his father, Friedrich Miescher-His, and his uncle, Wilhelm His who had recently moved to take up a post at the University of Leipzig. It was a testimony to Miescher's achievements that at the age of only 28 he was offered a professorship. Despite highly commending recommendations from both Hoppe-Seyler and Ludwig in support of this appointment, however, the fact that both Miescher's father and uncle had held

positions at the institute led to rumours of nepotism. As a consequence, Miescher's working conditions in Basel were poor. He was allocated only a small space in a shared laboratory and had very little technical support (Dahm 2004, 2005; His 1897e; Wolf 2003b).

In his new position Miescher worked exceptionally hard, often to the point of exhaustion, not least driven by the desire to dispel any doubts that he may have been chosen due to his family ties rather than his accomplishments as a scientist. He also resumed his work on nuclein, which had all but rested during his stay in Leipzig. Working with Wilhelm His, who was studying the embryonic development of birds and fish, Miescher turned to analysing the eggs and sperm cells of different species (Wolf 2003b). He quickly noticed that sperm cells, consisting largely of nuclei, were an ideal source to isolate nuclein in large quantities and purity (Miescher 1871b, 1872b). And Basel proved to be well suited for these experiments. Being situated on the Rhine River with its annual upstream migration of salmon to their spawning grounds, Basel had a thriving salmon fishing industry and Miescher had an abundance of freshly caught fish at his disposal. Thus, in the autumn of 1871, he converted to using salmon sperm as his source material and developed successive, increasingly sophisticated protocols for the isolation of nuclein (see Miescher 1874b and the publication by Miescher's co-worker Schmiedeberg and Miescher 1896).

As before in Tübingen, Miescher took great care to only use fresh material and work rapidly during the isolation of nuclein. He also stressed the importance of handling the material in the cold to prevent degradation of the nuclein. As cold rooms were not available in those days, he could only perform the isolation during the winter months. Often he would get up in the middle of the night to catch salmon from the Rhine River, bring them to his laboratory and work away during the early hours of the day with the windows wide open to the freezing cold outside. Strenuous as they were, these protocols enabled Miescher to isolate substantial amounts of the purest nuclein that had ever been available to him. Thus, despite working conditions in Basel being inferior compared to those in Hoppe-Seyler's laboratory, the nearly inexhaustible supply of nuclein that Miescher obtained from salmon sperm allowed him to finally carry out the comprehensive quantitative analyses that he had already planned to do in Tübingen.

Miescher repeated the analysis of nuclein's elementary composition. He confirmed the presence of carbon, hydrogen, nitrogen and oxygen, as well as again the large proportion of phosphorous. Moreover, Miescher's new preparations of nuclein from salmon sperm no longer contained significant amounts of contaminating protein and, contrary to his earlier findings, he ascertained that it did not contain any sulphur (Miescher 1872b–d, 1874b). With his purest preparation of nuclein, Miescher determined the P_2O_5 content in salmon sperm nuclein to be 22.5% of its total mass (Miescher 1872c), a figure very close to the actual proportion of 22.9%. He later correctly established that all the phosphorous in nuclein was present as phosphoric acid (Miescher 1874b).

Further analyses of the nuclein isolated from sperm confirmed its acidic properties, which Miescher had already observed in Tübingen. He determined that it must be a "multi-basic acid" (Miescher 1872c), a statement which he later refined to "at least three basic acid" (Miescher 1874c) and eventually to "at least four basic acid" (Miescher 1874b). Miescher also noticed that nuclein could not diffuse across a sheet of parchment paper and concluded that it must be a molecule with a high molecular weight (Miescher 1872d); see also (Miescher 1874b). Later however, Miescher erroneously determined an approximate atomic weight of 500–600 for nuclein (Miescher 1873) and proposed several approximations of an atomic formula, including the formulae of $C_{22}H_{32}N_6P_2O_{16}$ (Miescher 1874c) and $C_{29}H_{49}N_9P_3O_{22}$ (Miescher 1874b).

In the spring of 1872, Miescher presented his results to the Naturalist Society in Basel (His 1897e). Amongst descriptions of the morphology of salmon spermatozoa, he reported that in their heads, the "multi-basic" acid nuclein is bound in a salt-like state to a basic molecule, which he referred to as "protamin" and that together nuclein and protamin made up almost the entire mass in the sperm heads (see also Wolf 2003b). In the years 1872 and 1873, Miescher extended his studies to the sperm of carp, frogs, chicken and bulls, but with less success than he had previously had with salmon sperm. However, in the sperm samples from all the species he examined he found nuclein. The complete account of these analyses was published in 1874 (Miescher 1874a, b).

Theories on the function of DNA

During the quarter century that Miescher worked on DNA, he developed several notions as to its putative function. His initial idea that it served to store phosphorous inside the cell or act as a precursor for the generation of other molecules, such as lecithin (see above), were gradually superseded by other theories. After having discovered nuclein in germ cells, for instance, especially the large abundance in spermatozoa, Miescher came to suspect an involvement in fertilisation.

The late nineteenth century was a time of intense research and speculation on the mechanisms controlling fertilisation and the transmission of hereditary traits from one generation to the next. In this context, Miescher's publication of 1874 on the occurrence of large amounts of

nuclein in the sperm of different vertebrate species caused some interest in the scientific community. Miescher himself came auspiciously close to uncovering the correct answer. In his publication 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).

Miescher could not conceive, however, that a single substance might be responsible for the transmission of hereditary traits. As many of his contemporaries, including Wilhelm His, Miescher favoured the notion that fertilisation and subsequent embryonic development was achieved by the sperm cell, which upon contact with the egg, transmitted a motion stimulus intrinsic in the sperm cell's molecular constitution. Miescher speculated that nuclein might be the molecule that transmits this motion stimulus. The fusion of the sperm cell and the egg and hence the transfer of actual molecules, however, was not part of this theory. Miescher also discarded the idea of nuclein carrying hereditary information because he thought it unlikely 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 continued that "they will only do so in a limited diversity", a diversity Miescher believed to be too restricted to explain even the slight differences between individuals of the *same* species, let alone those between species. He concluded that there is no specific molecule that could explain fertilisation (Miescher 1874b).

From the mid-1870s, Miescher increasingly began investigating topics not related to his work on nuclein (reviewed in Dahm 2004, 2005; Portugal and Cohen 1977). Initially, his work with sperm as a source of nuclein directed his attention to studying the overall chemical composition and morphology of spermatozoa (Miescher 1874b, 1890, 1892c) and also oocytes (Miescher 1871a; Miescher 1877). He became particularly interested in the chemical and physiological processes that lead to their differentiation. In this context, he attempted to uncover how nuclein originated and may change during the differentiation of the germ cells (for example, see letters XLIII–XLV in His 1897a). By investigating these as well as other chemical and morphological changes underlying gametogenesis he hoped to understand the process of sexual reproduction.

Due to the paucity of knowledge at the time though, many of the theories Miescher put forward were quite speculative. For instance, based on his work on the differentiation of spermatozoa and oocytes, Miescher developed a variety of theories trying to explain the processes of fertilization (Miescher 1874b; see also Miescher 1872d, 1892a, b, 1895) and the transmission of hereditary traits (Miescher 1892b, 1893). Later in life, he postulated, for example, that the key to understanding the need for an oocyte to fuse with a spermatozoon to ensure normal embryonic development was to be found in "stereochemistry" (Miescher 1892b, 1893). Miescher presumed that the hereditary information might be encoded in the stereochemical state of carbon atoms. Much like an alphabet of 24–30 letters is sufficient to represent all words and concepts in a range of different languages, these stereoisomers could be used to create molecules containing different information. The enormous numbers of asymmetric carbon atoms in large organic molecules, such as proteins, would allow an immense number of stereoisomers. A protein comprising a mere 40 asymmetric carbon atoms would, for instance, have 2^{40}, i.e. over one trillion, stereoisomers—a number large enough to envisage that they could encode the diversity of hereditary information for all the different forms of life (Miescher 1892b). To prevent errors, caused by environmental factors that might change the stereochemical state of atoms, from manifesting themselves in the developing embryo, Miescher further assumed that these errors had to be corrected by the fusion of information from two germ cells during fertilization. While genetic information is not encoded in the stereochemical state of atoms, Miescher's concept is still remarkably close to the actual way information is stored in biomolecules (see also Gehring 1998).

Amongst ideas now proven to be incorrect, however, Miescher later also proposed concepts that appear astonishingly modern. He speculated, for instance, that oocytes and spermatozoa developed in such a way that each lacked the full complement of what is required for the development of an organism to occur. He even went on to propose that, while the cytoplasm in the egg was well developed, what was lacking in the oocyte might be the "complete nucleus" or a "specific substance" (Miescher 1895). With this he seemed to anticipate what is known today: that during sexual reproduction each germ cell contributes one complement of DNA to the developing offspring. However, even towards the end of his work, Miescher was by no means convinced that the transfer of some molecule(s) from the spermatozoon to the oocyte was necessarily the explanation for fertilisation. He always also contemplated the notion that the sperm confers some sort of "movement impulse" to the oocyte which induced its development (Miescher 1895).

Miescher's work with salmon sperm also led him to examine the changes that occur in the fish as they migrate from the ocean to their fresh-water spawning grounds upstream of rivers such as the Rhine. Owing to his interest in the development of spermatozoa and oocytes, he was primarily captivated by the substantial increases in size which the salmons' gonads undergo at the expense of other parts of the body and he performed pioneering experiments on the turnover of body constituents during this process (Miescher 1881, 1897b). But Miescher also worked on topics

totally unrelated to nuclein and germ cells, such as the physiology of respiration. He investigated, for instance, how the composition of blood differs at varying altitudes and discovered that it is the blood's concentration of CO_2, rather than that of O_2, which regulates breathing (Miescher 1885, 1888, 1897a). Despite these varying interests, Miescher never fully gave up his research on nuclein. However, in his later years he no longer seemed to obtain conclusive results from his efforts and did not publish on the topic anymore.

Over the years, also Miescher's responsibilities outside of his research interests progressively grew (Dahm 2004, 2005). His teaching commitments required increasingly more time and he was repeatedly commissioned to conduct surveys on nutrition for various Swiss institutions (Dahm 2004). In the early 1880s, he founded Basel's first institute for anatomy and physiology (His 1897e) and became the new institute's first head, a responsibility he took very seriously. He strove to encourage a lively scientific atmosphere and hired several accomplished precision mechanics with whom he developed innovative instruments for physiological experiments (Miescher 1888). Yet over time his many obligations, allowing him ever less time to recover, began to wear on Miescher. When at the beginning of the 1890s he contracted tuberculosis, he fell seriously ill and had to cease working (His 1897e). Even a stay at a sanatorium in Davos could not restore his health and on the 26 August 1895 Friedrich Miescher died, only 51 years old.

After Miescher's 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 1897d). Yet not even His, a close friend and colleague of Miescher throughout his career, could possess the foresight at that time to know how true his words were to prove.

DNA research after Miescher's discovery

Already during his lifetime, others had continued to follow up the open questions Miescher's work had created. Initially it was largely biochemists influenced by Hoppe-Seyler who continued the investigations into the nature of nuclein. A prominent example is Albrecht Kossel who had worked with Hoppe-Seyler in the late 1870s and early 1880s. Richard Altmann also contributed fundamental work. Importantly, Kossel, Altmann and their co-workers developed new protocols that permitted them to isolate protein-free nucleic acid and thus enabled them to tease its chemical components apart.

Miescher's classification of nuclein as being distinct from proteins was by no means universally accepted at the time (Portugal and Cohen 1977; Wolf 2003b). This was largely due to the fact that it was very difficult to obtain pure preparations of DNA free of contaminating proteins with the isolation protocols available. Even Miescher's preparations failed to always be negative when exposed to staining tests for the presence of proteins (Olby 1969). As a consequence most scientists working on nuclein at the time did not draw a clear line between the two classes of molecules and even Miescher often regarded nuclein as being closely related to proteins.

Some further confusion stemmed from Miescher's work himself. He and his co-worker Jules Piccard failed to separate the protein protamine, isolated from salmon sperm, from contaminating DNA. This subsequently skewed their chemical analyses of the protein, suggesting that it shared chemical components, purine bases, with nuclein. It was only later that Kossel identified contaminating nucleic acid as the source of the purine bases in the analyses of protamine. When, in this context, Altmann finally succeeded in separating the DNA from proteins in his preparations, he believed that he had isolated a novel sub-component of nuclein. In 1889, based on the fact that it behaved like an acid, he named the substance "Nucleïnsäure" (*nucleic acid*) (Altmann 1889). Altmann as well as colleagues of his, failed, however, to realise that nucleic acid was in fact the same substance that Miescher had first described as nuclein in his seminal paper 18 years earlier (for example, see Kossel 1891).

Miescher was none too pleased about this change in nomenclature introduced by Altmann. He had already established that nuclein behaved like an acid in his earliest experiments with the molecule in Hoppe-Seyler's laboratory (Miescher 1871c) and corroborated this finding later in Basel when he had succeeded in extracting it at even higher purity from salmon sperm (Miescher 1874b). In March 1891, he wrote a letter to Wilhelm His with a slightly annoyed undertone complaining that Altmann's nucleic acid was of course identical to his nuclein and that it was he [Miescher] who had managed to obtain the purest samples of nuclein (Miescher 1891). Faced with the increasing interest and publications on nuclein by other scientists, Miescher at times even felt a little dispossessed of "his" nuclein.

Kossel in turn performed groundbreaking work identifying the fundamental building blocks of nuclein—the purine and pyrimidine bases, one sugar, and phosphoric acid—and confirmed that it is restricted to the nucleus (Portugal and Cohen 1977). Like the botanist Eduard Zacharias who in 1881 had linked the molecule nuclein to the cytological concept of chromosomes (reviewed in His 1897e), Kossel found that nuclein is a key component of chromatin, together with proteins such as histones which he discovered (reviewed in Olby 1994; Portugal and Cohen 1977). Kossel

further inferred from his experiments that nucleic acids do not serve as sources of energy or storage material, but are instead intrinsically tied to the synthesis of new protoplasm during growth and replacement (Kossel 1913). In 1910, he was awarded the Nobel Prize in Physiology or Medicine in recognition of his work on the chemistry of proteins and nucleic acids (Kossel 1910). Yet despite these early breakthroughs, the full importance of nucleic acids remained obscure for several decades.

Towards the end of the nineteenth century, also the views on fertilisation and the role of the nuclei in this process began to shift (reviewed in Portugal and Cohen 1977). In 1874, Leopold Auerbach published that fertilised worm oocytes contain two nuclei which fused prior to the first cell division. A year later, Oscar Hertwig, a student of Haeckel's, was investigating fertilisation in sea urchins. He confirmed Auerbach's observation of the presence and subsequent fusion of two nuclei in fertilised eggs and concluded that one of these nuclei must have come from a spermatozoon the other from the oocyte. Importantly, he found that the fused nucleus gave rise to all subsequent nuclei as the animal developed. With this the importance of the nuclei and their continuity during embryogenesis was established (Portugal and Cohen 1977).

Increasingly, through the work of, amongst others, August Weismann, Eduard Strasburger and Albert von Kölliker, it became clear that heredity had a molecular basis (Portugal and Cohen 1977). In this context, Miescher, who was aware of his colleagues work, also proposed his theories on the chemical basis heredity, for example, that information might be encoded in asymmetric atoms (see above). The realisation that hereditary information is transmitted by a molecule, or molecules, was a very important advance. It prompted intense research on the topic, which lead to, for instance, the re-discovery of Mendel's laws by Hugo de Vries, Carl Correns and Eric von Tschermak. Yet none at the time succeeded in defining the identity of this molecule.

Interest in nucleic acids gradually diminished. This decline was in part caused by the emergence of the tetranucleotide hypothesis advanced chiefly by Phoebus Levene and Hermann Steudel late in the first decade of the twentieth century (reviewed in Olby 1994). This hypothesis assumed that DNA was composed of identical units of tetranucleotides, which were thought to contain one of the four bases found in DNA each. Evidently this would have meant that the four bases would always have to be present in equimolar proportions, meaning that the DNA from any source would always contain exactly the same amount of each of the bases. Importantly, if true, the tetranucleotide hypothesis would also have implied that DNA, being a polymer composed of just one type of monomeric unit, could not encode information in the sequence of its building blocks. As a consequence of the popularity enjoyed by the tetranucleotide hypothesis, interest moved away from DNA and towards proteins. Being composed of 20 different amino acids, proteins appeared to be more promising candidates for molecules that encode all of the complexity and diversity of the different life forms.

The tetranucleotide hypothesis was eventually rebutted in the late 1940s and early 1950s by Erwin Chargaff and coworkers (Chargaff 1951; Chargaff et al. 1949). In keeping with the tetranucleotide hypothesis, they determined that DNA invariably contained as many purine (A, G) as pyrimidine bases (C, T) and ascertained that the molar ratios of the bases A/T as well as G/C were always (very close to) one. However, contrary to what was predicted by the tetranucleotide hypothesis, Chargaff and colleagues found that the ratios of A/C, A/G, T/C and T/G could deviate significantly from one. They also discovered that the composition of DNA—more precisely the relative proportions of bases in the DNA—was the same in individuals of a single species, but differed between species. These results finally proved Levene's hypothesis to be wrong.

In parallel to these analyses of DNA's chemical composition, experiments were carried out which proved that DNA, not proteins, is the carrier of genetic information. In 1944, Oswald T. Avery, Colin MacLeod and Maclyn McCarthy published their key paper proposing that Griffith's "transforming principle", an enigmatic substance that could transform an innocuous bacterial strain into virulent bacteria, is not a protein but DNA (Avery et al. 1944) and in 1952, Al Hershey and Marta Chase confirmed DNA as the genetic material by showing that during infection of bacteria with T2 bacteriophages viral DNA, but not viral proteins, enters the bacteria and that this DNA can be detected in new viruses produced by infected cells (Hershey and Chase 1952).

With the discovery that DNA contained the hereditary information key questions moved into the centre of attention, for example, how this information was stored in DNA and how it could be faithfully replicated prior to each cell division. When in 1953 Watson and Crick established the structure of DNA (Watson and Crick 1953b), the individual pieces of the puzzle fell into place. DNA now not only had a structure, but this structure could also explain how it functioned. As Watson and Crick put it in their second *Nature* paper on DNA in 1953, the double helix of antiparallel strands "is, in effect a pair of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken down and the two chains unwind and separate. Each chain then acts as a template for the formation onto itself of a new companion chain so that eventually we shall have two pairs of chains, where we only had one before […]. Moreover the sequence of pairs of bases will have been duplicated exactly." (Watson

and Crick 1953a). This arrangement elegantly explained how DNA replication could occur and genetic continuity be maintained. It further prepared the ground for our understanding of how the instructions for the synthesis of proteins can be read from DNA. But it was not until the mid-1960s that Robert W. Holley, Har Gobind Khorana, Heinrich Matthaei, Marshall W. Nirenberg and colleagues finally cracked the genetic code (reviewed in Davies 2002). This knowledge of DNA's structure and mechanism of function finally gave biologists the possibility to begin sequencing and manipulating it and thus ushered in the era of molecular genetics.

The context of Miescher's discovery of DNA

When Friedrich Miescher began his analyses of leukocytes, he had not set out to discover the molecular basis of hereditary information. However, his aim when joining Hoppe-Seyler's laboratory was no less ambitious. He wanted to tackle one of the most fundamental questions in biology of his time: What are the molecules that make up living beings? In other words, what is the chemical basis of life? While not living long enough to realise the full implications of his work, he did achieve just that: the first step towards understanding the common basis of all life on earth.

At the time, Miescher was by no means alone in trying to discover the chemical building blocks of cells and tissues and characterizing their properties. Why did it fall to him to discover DNA? Important discoveries often originate from a combination of fortuity and an open mind that is prepared to consider an unanticipated outcome of an experiment. This surely was the case when Miescher noticed his first crude precipitate of DNA with its unexpected properties. But new discoveries are also often the result of careful planning of the experimental approach and the intellectual context in which they are made. While it will be impossible to fully reconstruct the events and circumstances that lead to the discovery of DNA in retrospect, a few factors that may have been critical to Miescher's discovery stand out from the historic material.

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

Miescher's choice of lymphocytes and later leukocytes from pus (mostly neutrophile granulocytes) for his seminal experiments was very likely driven by Hoppe-Seyler's extensive occupation with blood and its constituents (for example, see Perutz 1995). Hoppe-Seyler had a long-standing interest in the composition of body fluids, mainly blood, and had recently also become interested in white blood cells and the formation of pus. In the second edition of his textbook on physiological and pathological chemical analyses, which had appeared just a couple of years before Miescher joined his laboratory, he included descriptions of current efforts of isolating and characterising the chemical components of various cell types, including spermatozoa and cells found in pus (Hoppe-Seyler 1865). Hoppe-Seyler's focus on blood is also evidenced by the fact that another student of his, P. Plósz, verified the presence of nuclein in the nucleated erythrocytes of birds and reptiles as part of the confirmation of Miescher's findings (Plósz 1871).

Miescher's isolation of the leukocytes for his studies may also have benefited from the fact that he performed his work at a time when antiseptic methods were only beginning to be introduced to prevent the infection of wounds (Lister 1867). Until the 1870s, an ample production of pus by wounds was considered essential to rid the body of harmful substances and to prevent internal damage (reviewed in Schadewaldt 1975). Pus secretion was thus not only seen as beneficial to wound healing, over centuries it was often actively induced. Similarly, wound dressings were not seen as a means to prevent the contamination of wounds with pathogens (Pasteur only established the concept of disease causing microorganisms in the late 1850s). Instead, in addition to stopping bleeding, they were mainly used to soak up the large amounts of pus produced by infected wounds. These conditions were likely conducive to Miescher's work in that pus was available to him in much larger quantities than could be obtained from hospitals today. Miescher may further have benefited from the invention of absorbent cotton as a wound dressing around the time when he worked in Tübingen (Klose 2007). This invention was made by Viktor von Bruns who was then the head of surgery at the clinic in Tübingen from which Miescher obtained his pus. The production process of the cotton wool likely resulted in a very clean, largely sterile and highly absorbent material. These factors may also have helped Miescher in obtaining large quantities of comparatively pure leukocytes.

Later, having returned to his native Basel, Miescher found an even better source to isolate nulein when he turned to spermatozoa, which are amongst the cells with the highest ratio of nuclei (and densely packed chromatin) to cytoplasm. Moreover, the sperm of fish lacks most of the secretions found in the sperm of other vertebrates. Instead the spermatozoa are immersed in a salt solution containing few molecules that could have contaminated Miescher's isolations. It was accordingly from salmon sperm that he

managed to extract the purest samples of DNA, which he characterized chemically with striking precision. Thus, as is often observed in the history of science, the choice of experimental system was also likely to be a crucial factor in the success of Miescher's experiments.

The fact that Miescher had previously been working in Strecker's laboratory and then under Hoppe-Seyler's guidance when he discovered and first characterized DNA may well have been instrumental in another way. Both Strecker and Hoppe-Seyler had been amongst the first to chemically characterise phosphorous-containing organic molecules, such as lecithin (see e.g. Anon. 1970; Strecker 1868). In this context, testing his newly discovered, enigmatic substance for the presence of phosphorous presumably suggested itself to Miescher. It was predominantly this presence of large amounts of phosphorous that convinced Miescher that nuclein was distinct from the proteins, which then were widely believed not to contain this element.

The advice and guidance provided by Hoppe-Seyler, an extremely experienced and successful biochemist, to a relatively inexperienced medical doctor undoubtedly also played a critical role in Miescher's success. Hoppe-Seyler was one of the most influential biochemists of his time. Groundbreaking discoveries aside, he developed numerous important procedures for biochemical analyses and his textbook (Hoppe-Seyler 1865) was a key reference for medical professionals and scientist alike. In addition to the immediate intellectual support by his mentor, the wider context in which Miescher carried out his work likely afforded valuable inspiration. Around the middle of the nineteenth century, the thriving University of Tübingen with its many notable scholars in the Natural Sciences would have been a stimulating environment for a young doctor to begin his scientific career.

Miescher's interest in the Natural Sciences was likely stimulated at a very early age. His father, Johann F. Miescher, and more notably his uncle, Wilhelm His, were renowned physicians and professors of anatomy and physiology at the University of Basel and his parent's home was frequently visited by a range of scientists (His 1897e). The lively discussions that ensued will have exposed the young Miescher to a broad range of the scientific ideas and concepts of the time. In 1861, at the age of 17, he began studying medicine at the University of Basel. His fascination with the sciences, however, prompted him to suspend his studies for a semester spent studying chemistry with, among others, Friedrich Wöhler at the University of Göttingen in Germany in 1865. A typhoid fever forced him to interrupt his studies further. In the spring of 1868, however, aged only 23, Miescher concluded his medical studies with what was considered an exceptional doctoral thesis.

Following his basic medical training, he initially considered becoming a practicing physician, not least because he was afraid that he lacked the necessary knowledge and training to work as a scientist. His aspirations to practice medicine were, however, limited. Moreover, his poor hearing, which resulted from an ear infection he had suffered as a child, made it difficult for him to examine patients. He briefly contemplated specializing as an otologist or ophthalmologist—professions that, unlike that of a general practitioner, did not require examining patients with stethoscopes. But his long-held passion for uncovering the "theoretical foundations of life", encouraged mainly by his uncle, finally made him embark on a career as a researcher. Hence, shortly after having passed his boards exam, Miescher relocated to Tübingen to study biochemistry. (For detailed accounts of Miescher's upbringing and early education in Basel as well as his scientific achievements, see the biography of Miescher published shortly after his death by Wilhelm His (1897e) or more recent reviews on the topic (Dahm 2004, 2005; Fruton 1999; Gehring 1998; Lagerkvist 1998; Portugal and Cohen 1977; Wolf 2003a, 2003b). Additional references, including contemporary appraisals of Miescher's life and work, can be found in (Fruton 1992)).

Appreciation of Miescher's achievements today

Why is Miescher's name not universally associated with DNA today? For one, unlike diseases, species, anatomical structures, cell types or even subcellular structures for which eponyms are common, molecules are not usually named after their discoverer. Moreover, soon after Miescher's discovery others began working on nuclein and tried to stake their claim on the new molecule, perhaps most notably Kossel and Altmann—the former by identifying the individual molecules DNA is composed of, the latter when he (likely in good faith) altered the designation "nuclein" to "nucleic acid", a term which is still part of DNA's modern name: deoxyribonucleic acid.

Already during his lifetime, Miescher felt that research on nuclein was increasingly being associated with other researchers. This may at least in part be also due to the fact that Miescher was not a gifted communicator and may have been too hesitant to promote his work (His 1897e; see also Wolf 2003 #136; Dahm 2004 #115, 2005 #113). He tended to be introspective and preferred working on his own to interacting with students. Miescher's introverted manner may well have been the result of the poor hearing he had suffered from since his childhood (His 1897e). Although those open enough to get involved with him described Miescher as a very knowledgeable and dedicated mentor, he repelled a lot of his students with his reclusive behaviour. Furthermore, the letters and other documents that have been handed down indicate that he communicated with a relatively limited number of colleagues.

Most importantly, however, a gap of 75 years between the discovery of DNA and the realisation of its importance may just have been too long. By contrast, Watson and Crick made their discovery at the right time. The realisation that DNA was indeed the carrier of hereditary information led to a mad rush to understand how a molecule with a relatively simple composition could encode the complex information that instructs the generation and functioning of all living beings. Determining DNA's structure solved this problem and showed how DNA works. It was these functional insights that—together with the powerful image of the double helix, which has since attained an almost iconic status extending well beyond the biological disciplines—that firmly associated Watson and Crick's names with DNA.

Not unlike Mendel's formulation of the laws of inheritance in 1865, which founded classical genetics, the discovery of DNA by Miescher could be viewed as the birth of molecular genetics. It is a curious parallel in the history of genetics that both discoveries occurred at roughly the same time. Both also went underappreciated for several decades, although it has been suggested that Mendel's insight and the significance of his findings have been over-interpreted retrospectively (reviewed in Sapp 1990). While today Mendel's work is mentioned in virtually every textbook dealing with genetics, Miescher's achievements are still widely unknown. Possibly, in contrast to Mendel whose work was "re-discovered" 35 years later, Miescher was actually just too far ahead of his time.

Acknowledgments The author is very grateful to Drs. Alfred Gierer, Rebecca Kirk, Markus Kunze, Alfons Renz, Helia B. Schönthaler, and John P. Vessey for helpful discussions and for critically reading this manuscript. The author would also like to thank Dr. Manuela Rohrmoser, Vienna University Library, for help in obtaining literature. I am particularly grateful to Dr. Ingmar Hörr for presenting me with a volume of Miescher's collected letters and publications.

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