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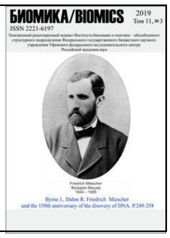

FRIEDRICH MIESCHER AND THE 150th ANNIVERSARY OF THE DISCOVERY OF DNA

Jonathan Byrne[1] and Ralf Dahm[1,2]

[1]Institute of Molecular Biology, Ackermannweg 4, 55128, Mainz, Germany
[2]Department of Biology, University of Padua, Via U. Bassi, 58/B, Padova, 35121, Italy
E-mails: J.Byrne@imb-mainz.de R.Dahm@imb-mainz.de

Resume

150 years ago, in October 1869, Friedrich Miescher completed his work on one of the great scientific discoveries of our time; the isolation and identification of DNA, "Nuclein", as a central cellular component. However, just how important DNA is in the biology of the cell would not be demonstrated for another 75 years, when in 1944 Avery, MacLeod and McCarthy showed that it is the molecule of inheritance. From here, DNA rapidly went on to capture the scientific and public imagination and over the next 75 years became integral to our understanding of life. Yet the man who first discovered it still lingers in relative obscurity, often not even remembered by scientists who work closely with nucleic acids. On this sesquicentennial anniversary, we look back to just how this monumental discovery was accomplished, who the man behind it was, and how he tried to understand Nuclein's role in the cell in the context of his day. Perhaps now is the right time for Miescher's legacy to return to the spotlight.

Keywords: Friedrich Miescher, nuclein, DNA

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Introduction

When we think of modern times compared to those 100 years ago, two standout changes are the rapid pace of technological development and the growing ability to manipulate living organisms at the most fundamental level. While the pioneers in the development of computing, such as Charles Babbage and Alan Turing, are still well remembered as part of the cultural zeitgeist, the same cannot be said for that early biological adventurer who first isolated and characterized the most iconic symbol of modern biology: DNA. The irony is that even many molecular biologists who work with DNA on a daily basis editing it, reading it or analyzing it do not know who the first person to isolate DNA actually was. When people think of DNA it is usually in the context of the discovery of its structure by James Watson and Francis Crick in 1953 (Watson & Crick, 1953; Dahm, 2010a). It might, therefore, come as a surprise to know that the story of DNA had already begun in 1869, with the young Swiss physician Friedrich Miescher (Figure 1) who discovered DNA while working in the biochemistry laboratory of Tübingen University (Dahm, 2005).

With this year being the 150th anniversary of this monumental discovery, this essay aims to reintroduce a pioneer of modern biology and to share his route to starting one of the great scientific revolutions, completely changing our understanding of life and leading to medical breakthroughs that were unimaginable in his time.

Figure 1. Photograph of Johann Friedrich Meischer as a young man

Miescher's path to discovering DNA

Johann Friedrich Miescher was a man who was deeply influenced by some of the foremost scientists of the day. Born on 13 August 1844, he was named after his father, Johann F. Miescher and Miescher's home life often involved visits from a range of scientists that, at a young age, exposed him to a variety of scientific ideas and concepts. Both, his father and uncle (Willhelm His) were well-respected physicians and renowned professors of anatomy and physiology at the University of Basel in Switzerland. Following their footsteps, at the age of 17, Miescher studied medicine in Basel and graduated in 1867 at the age of 23. However, his aspirations to move from the study of medicine to its practice were limited, not least because he suffered from poor hearing that would have made the examination of patients difficult (Dahm, 2005). Despite his fears that he lacked the necessary knowledge and training to work as a scientist, Miescher's fascination with scientific endeavors, combined with prompting from his uncle convinced him to pursue scientific research as a career. Indeed, his uncle's view that the "last remaining questions concerning the development of tissues can only be solved on the basis of chemistry" led Miescher to begin his studies in the newly developing field of biochemistry, or 'physiological chemistry' as it was known at the time (His, 1897a; Dahm, 2005).

In 1868, one of the pre-eminent centres of the Natural sciences was located at the Eberhard-Karls-University in Tübingen, Germany. Amongst a diverse faculty, which featured Chairs for Mathematics, Physics, Astronomy, Mineralogy, Pharmacology, Botany and Zoology, were some of the pre-eminent biochemists of the day, including Adolf Strecker, Felix Hoppe-Seyler, Hugo von Mohl and Franz von Leydig, and was thus a natural fit for Miescher. With the goal of exploring the chemistry of cells and tissues, Miescher spent his first semester at Tübingen in the laboratory of Adolf Strecker, the first person to synthesize an amino acid (alanine) (Strecker, 1850), where he learnt the fundamentals of organic chemistry, in particular how to determine the chemical composition of samples through elementary analysis. Keen to apply his newly acquired knowledge to unravel the biochemistry of life, in autumn 1868, Miescher chose Felix Hoppe-Seyler (Figure 2) as his supervisor and moved to his laboratories in the town's medieval castle, which is situated high above the old town and the surrounding river valley (Figure 3). Hoppe-Seyler was already a well-established name in the field when Miescher joined him, having, among other things, helped uncover the nature and role of hemoglobin (which he named) within the cell (Hoppe-Seyler, 1864).

Figure 2. Photograph of Felix Hoppe-Seyler, mentor to Friedrich Miescher and one of the first pioneers of Biochemistry

Figure 3. Tübingen Castle as it overlooks the town to the south. Miescher's laboratory, where he first isolated Nuclein, was located in the former kitchen of the castle on the ground floor.

In many ways, the supervision of Hoppe-Seyler proved serendipitous for Miescher. In his quest to understand the secrets of cellular life, Miescher began with lymphocytes. "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 and most independent form of animal cell." (Miescher, 1869a; Dahm, 2008). However, lymphocytes proved impossible to purify in the quantities needed for chemical analyses. It is likely that Hoppe-Seyler, who had a focus on the constituents of blood, suggested that Miescher instead use closely related leukocytes as his starting material. This focus on a single cell type as opposed to whole organs or tissues significantly reduced the complexity of Miescher's starting material and allowed him to rapidly investigate specific cellular components.

To collect the needed starting material, Miescher turned to a readily available source; pus from fresh surgical bandages, which was available from the local surgical clinic. "[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; Dahm, 2008). The switch to this cell type provided Miescher with the sufficiently large quantities of material he needed for further biochemical analysis and set the stage for his breakthrough discovery. While examining the various types of proteins found in leukocytes, Miescher noticed the presence of a substance that precipitated from solution when acid was added but disappeared again when the solution was neutralized. This behavior was not standard for proteins and, indeed, suggested that this was an altogether different type of substance. Miescher had, in fact, just performed the first crude precipitation of nucleic acids (Dahm, 2008).

Isolating and identifying DNA as a new substance

Intrigued by this enigmatic substance, Miescher's first task was to determine what part of the cell it came from. He had been painstaking in his efforts to develop a robust protocol for the isolation and examination of leukocytes. When extracting cells from the pus on surgical bandages, he took great care in ensuring that the samples were fresh, discarding anything that showed signs of decomposition, either in terms of smell, appearance under the microscope, or by having turned acidic (Dahm, 2008). With these fresh samples, he had to concoct a way to wash the pus off the bandages, as simple salt solutions caused the cells to swell in a manner that prevented further processing. It was only when he turned to a dilute solution of sodium sulphate (a mixture of one part cold saturated Glauber's salt and nine parts water) combined with filtration that he managed to successfully isolate distinct leukocytes without damaging them.

His protocol then exposed the cells to several washes of very dilute (1:1000) hydrochloric acid over a period of several weeks at 'wintry temperatures' to prevent degradation of the sample. This saturated the cells such that "Through prolonged exposure of the cells to diluted hydrochloric acid it is possible to reach a point where the acid will not take anything up anymore. The residue consists partially of isolated [nuclei] and partly of nuclei surrounded by a shrunken contour. The contour can no longer be stained yellow with iodine [indicating that the protoplasm and the proteins therein had largely been extracted]" (Miescher, 1869a; Dahm, 2008). When neutralising this suspension of nuclei with alkaline solutions, the nuclei would swell and sometimes fade from visibility under the microscope but did not break apart or dissolve. It is at this stage, if the solution was re-acidified that Miescher could "obtain precipitates that could not be dissolved either in water, acetic acid, very dilute hydrochloric acid, or solutions of sodium chloride, and which [Miescher concluded] thus could not belong to any of the hitherto known proteins. [….] According to these facts, surely known to histologists, the substance could only belong to the nuclei and therefore captivated my interest" (Miescher, 1869a; Dahm, 2008). Miescher repeated and refined this technique, including adding steps to remove lipids and other hydrophobic molecules by shaking the material in a mixture of ether and water, and once again obtained a white, flocculant precipitate. Although he was still somewhat uncertain about the nature of the substance he had discovered, due to contaminating protein in his early tests, Miescher named the substance "Nuclein" for its origin in nuclear material, a term still preserved in today's name deoxyribonucleic acid.

Findings confirmed

The first time we know of that Miescher shared his discovery of this novel substance was in a letter to his uncle Wilhelm His in Basel, dated the 26th of February, 1869, 150 years ago this year (Miescher, 1869a). He gave a detailed account of his initial analyses on the cryptic precipitate and mentioned the difficulties he was having in removing contaminating cell constituents from the isolated nuclei. He was, however, hopeful that these obstacles could be overcome.

Miescher's first protocol, while successful, gave quantities of isolated nuclei that were too few for more detailed analyses. As he wrote, "the minimum quantity of nuclei that can be obtained through the described procedure hardly permits the few reactions mentioned; elementary analysis could not even be considered" (Miescher, 1871; Dahm, 2008). In developing a second protocol to address these issues, one of Miescher's major concerns was to ensure complete removal of the contaminating cytoplasm, which would have obscured his ability to reliably analyze the composition of the novel substance. Miescher, thus, turned to a cutting-edge protocol of the time, which had in fact only been published that year, in which Wilhelm Kühne described the use of pepsin solutions to digest a cell's cytoplasmic contents (proteins) while sparing the nuclei (Kühne, 1868a).

Lacking a readily available source of pepsin Miescher isolated it himself by rinsing out pig stomachs with hydrochloric acid to extract the proteolytic enzymes therein (Miescher, 1871). One great advantage when using pepsin was that it failed to digest Nuclein, thus finally proving to Miescher that his extract was definitively not a proteinaceous substance. In his revised protocol, Miescher began by directly washing the leukocytes with warm alcohol. This not only broke open the cells, removing most of the cytoplasm, but also extracted lipids and hydrophobic proteins. He digested the extracted nuclei in his home-made pepsin solution for 18 – 24 hours, which resulted in a fine grey sediment that separated from a yellowish supernatant. To ensure that residual lipids were also removed he shook the sediment in ether and warm alcohol and then washed them in water.

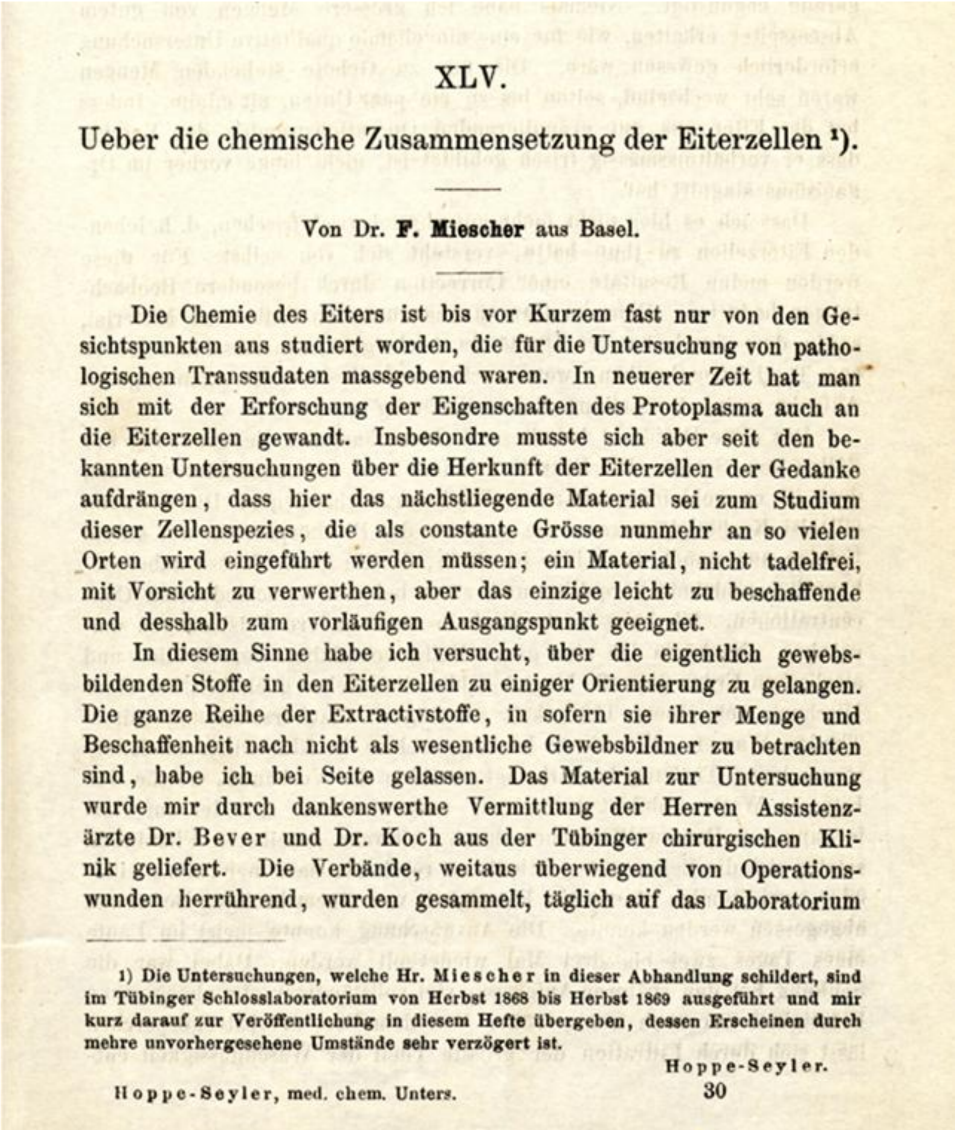
The nuclei obtained this way had the same chemical profile as the extracts from his previous protocol and showed no signs of contaminating cytoplasm under a microscope. Miescher subsequently washed the sample in very mild alkaline solutions, such as highly diluted sodium carbonate, to which he then added excesses of acetic or hydrochloric acid, causing his fluffy white flocculate to appear once more. This precipitate could be re-dissolved with the addition of alkaline solutions and was the first-ever comparatively clean preparation of DNA (Miescher, 1871).

With enough material now in hand, Miescher could finally begin to examine the elements that comprised this brand new substance. Influenced by his time in Strecker's laboratory, Miescher began to quantify the amount of each individual element present in Nuclein. By burning the precipitate in the presence of various chemicals that reacted specifically to the different chemical components of the sample and then weighing the subsequent reaction products, Miescher was able to determine that Nuclein contained the elements normally found in organic molecules – oxygen, carbon, hydrogen, and nitrogen – but, in contrast to proteins, it was rich in phosphorous and lacked sulfur. This, along with Nuclein's contrary physical properties led Miescher to state "We are dealing with an entity *sui generis* [of its own kind] not comparable to any hitherto known group" (Miescher, 1871; Dahm, 2005).

Miescher the man

That Friedrich Miescher is not a household name, despite having discovered the substance at the root of all life, is, in large part, due to his personal nature. From what is known about Miescher, it is clear that he was introverted, preferring seclusion to interaction with others. This was particularly problematic when it came to interacting with students. His reclusive behavior often repelled them and meant that there were few young researchers becoming enamoured with Nuclein and making their own discoveries on the molecule. Considering his role model in Hoppe-Seyler, who advanced his research and reputation through numerous successful students including Miescher, it is perhaps a pity that Miescher was not able to follow the same pattern.

It has been suggested that his introversion may have come from his poor hearing, which he had suffered since childhood after a severe illness (His, 1897a, Dahm, 2008), as this made it difficult for him to communicate easily with those around him. Miescher's isolationism also extended to his written communications, whereby from the letters surviving to this day it is clear that he wrote only to a limited number of colleagues and did not spread his findings widely within the community of researchers who focused on the nucleus, fertilization or heredity.



XLV.

Ueber die chemische Zusammensetzung der Eiterzellen 1).

Von Dr. **F. Miescher** aus Basel.

Die Chemie des Eiters ist bis vor Kurzem fast nur von den Gesichtspunkten aus studiert worden, die für die Untersuchung von pathologischen Transsudaten massgebend waren. In neuerer Zeit hat man sich mit der Erforschung der Eigenschaften des Protoplasma auch an die Eiterzellen gewandt. Insbesondre musste sich aber seit den bekannten Untersuchungen über die Herkunft der Eiterzellen der Gedanke aufdrängen, dass hier das nächstliegende Material sei zum Studium dieser Zellenspezies, die als constante Grösse nunmehr an so vielen Orten wird eingeführt werden müssen; ein Material, nicht tadelfrei, mit Vorsicht zu verwerthen, aber das einzige leicht zu beschaffende und desshalb zum vorläufigen Ausgangspunkt geeignet.

In diesem Sinne habe ich versucht, über die eigentlich gewebsbildenden Stoffe in den Eiterzellen zu einiger Orientierung zu gelangen. Die ganze Reihe der Extractivstoffe, in sofern sie ihrer Menge und Beschaffenheit nach nicht als wesentliche Gewebsbildner zu betrachten sind, habe ich bei Seite gelassen. Das Material zur Untersuchung wurde mir durch dankenswerthe Vermittlung der Herren Assistenzärzte Dr. Bever und Dr. Koch aus der Tübinger chirurgischen Klinik geliefert. Die Verbände, weitaus überwiegend von Operationswunden herrührend, wurden gesammelt, täglich auf das Laboratorium

1) Die Untersuchungen, welche Hr. Miescher in dieser Abhandlung schildert, sind im Tübinger Schlosslaboratorium von Herbst 1868 bis Herbst 1869 ausgeführt und mir kurz darauf zur Veröffentlichung in diesem Hefte übergeben, dessen Erscheinen durch mehre unvorhergesehene Umstände sehr verzögert ist.

Hoppe-Seyler.

Hoppe-Seyler, med. chem. Unters. 30

Figure 4. The first page of Miescher's article describing his discovery of Nuclein. The article's title translates as "On the Chemical Composition of Pus Cells". The article was published in 1871 in the journal of *Medicinisch-chemische Untersuchungen* (Medical-chemical Investigations), which Hoppe-Seyler was the publisher of. The note at the bottom of the text reads "The investigations, which Mr Miescher describes in this treatise, were carried out in the Tübingen Castle Laboratory from autumn 1868 to autumn 1869 and handed over to me shortly thereafter for publication in this booklet, whose publication is delayed by several unforeseen circumstances." This text was added at Miescher's request to ensure that his place as the discoverer of Nuclein would be preserved even if there was a subsequent discovery of Nuclein by another researcher in the time it took to publish the manuscript.

It is clear that Miescher was not a gifted communicator. He did not promote his work effectively, meaning his findings failed to reach a wide audience (Dahm, 2005; Dahm and Banerjee, 2019). Even his seminal paper on the discovery of a new cellular substance was, in effect, a poor attempt at communicating his results. The paper is 20 pages of dense text, in which the description of Nuclein's discovery is deeply buried. Miescher instead lists his experiments in the order they were performed rather than building a story around his key results (Dahm and Banerjee, 2019). Even the title fails to highlight his discovery. Miescher gives no indication of the importance of his finding, indeed as a title "On the Chemical Composition of Pus Cells" (Figure 4) hardly draws someone into reading the text.

Miescher is often seen as being unaware of the significance of his discovery (discussed in Dahm, 2010b). This view no doubt comes from the paucity of his publications and public lectures, 15 in all, of which only 5 discussed investigations into the properties of the nucleus. Indeed, Miescher did lament his failure to grasp the significance of some of his findings, writing "Only when I read, here and there, an immature fragment of what [data] I have accumulated, published by another author, do I realize what I could have made of my material" (His, 1897d, Dahm and Banerjee, 2019). Yet to assume he was unaware of the potential of what he had uncovered is a misunderstanding. Miescher was a perfectionist. He would discard samples that looked even slightly aberrant, he obsessively checked his isolated nuclei under the microscope to ensure there were no deformities during the extraction, he was highly critical of his findings, and he only published after exhaustive verification of his results (Dahm, 2005, 2008, 2010b). This careful nature meant he was slow to release his findings and reluctant to speculate freely about their importance. However, when he did make statements about his discoveries they were often eerily prescient. Indeed, he was so convinced Nuclein was a critical discovery that he suggested in his first publication that 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, 1871; Dahm, 2005). This type of bold statement may be commonplace in scientific publications today, but for someone as cautious and reticent as Miescher, it demonstrates a remarkable conviction in his discovery.

From molecule to mechanism

At the time of Miescher's discovery, the role and function of the nucleus as an independent organelle was only beginning to be unravelled. First formally identified in sketches in 1802 by Franz Bauer, speculation on the role of the nucleus was one of intense controversy. Three years prior to Miescher's discovery, the renowned German biologist Ernst Haeckel proposed that the nucleus was the source of factors responsible for the transmission of heritable traits (Haeckel, 1866). This led to a renewed interest in the nucleus and gave Miescher a chance to be at the right place at the right time to weigh in on the question of the nucleus's function and Nuclein's role therein.

Although he knew nothing about the function of Nuclein, Miescher almost immediately recognized that its presence could be used as a way of chemically defining the nucleus (Miescher, 1870). Furthermore, in a letter to his uncle in 1869 he suggested that "Based on the relative amounts of nuclear substances, proteins and secondary degradation products, it would be possible to assess the physiological significance of [cellular] changes with greater accuracy than is feasible now" (Miescher 1869b; Dahm 2010b). Miescher hoped that assessing such changes would ensure that the nucleus would be defined by variations in its physiological activity, which he believed was closely linked to Nuclein. He also suspected that such a definition might aid in the distinction of cellular processes. For example, "nutrative progression", which he characterized by an increase in the cytoplasmic proteins and the enlargement of the cell; "generative progression", which he defined as an increase in "nuclear substances" i.e. Nuclein as a preliminary phase of cell division in proliferating cells and possibly in tumours; and "regression", defined as an accumulation of lipids and degenerative products (Miescher, 1869c; Dahm 2010b). Although these ideas closely mirror what we now know today as cell growth, cell proliferation and senescence or cell death, Miescher missed the physiological relevance of these processes, notably the relationship of increasing Nuclein content and the doubling of hereditary information prior to cell division. Had he made this leap it is very likely that his name would not linger in obscurity today.

Miescher in Basel

Following his discovery of Nuclein and completion of his experiments on the new substance in October 1869, Miescher moved to the Physiology Institute of the University of Leipzig, Germany for one year to work under the supervision of the famous philosopher Carl Ludwig. During this time, he wrote up his manuscript and submitted it to Hoppe-Seyler for review and inclusion in the journal *Medicinisch-chemische Untersuchungen* (Medical-chemical Investigations) of which Hoppe-Seyler was the publisher. Although initially sceptical, Hoppe-Seyler and a student in his laboratory Pal Plósz were able to replicate Miescher's findings and Miescher's publication titled "*Ueber die chemische Zusammensetzung der Eiterzellen"* (On the Chemical Composition of Pus Cells) was finally published in 1871 (Miescher, 1871) (Figure 4). In the opening statements of his own companion 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, Dahm, 2008).

With his paper now published, Miescher moved back to Basel where, in 1872, he was offered the Chair of Physiology at Basel University. This position had previously been occupied by both his father and his uncle and possibly out of fears of claims of nepotism, Miescher's equipment and space at the university were greatly limited. 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. [...] You can imagine how it must feel to be hindered in the energetic pursuit of an endeavour on account of the most miserable conditions, knowing that I may never have such a fine opportunity again." (His, 1897b; Dahm, 2005). However, Basel had one significant advantage that was not available to Miescher in Tübingen; plentiful supplies of fresh salmon from the river Rhine.

Figure 5. The original glass vial containing Nuclein extracted from salmon sperm by Friedrich Miescher in Basel. The vial is part of materials preserved in the Miescher-Museum in Tübingen, Germany. The label has become severely faded but reads "*Nuclein aus Lachssperma, F. Miescher"* (Nuclein from salmon sperm, F. Miescher).

As it turned out, salmon and salmon sperm in particular, proved to be an ideal source for the isolation of large quantities of very pure Nuclein. These cells are simple in composition with a head that is almost entirely nucleus. Also, during mating season, as the salmon swim back up the Rhine, the amount of sperm in their bodies increases dramatically, greatly facilitating extraction. This along with increasingly sophisticated protocols for the isolation of Nuclein allowed Miescher to purify significant quantities of the purest Nuclein he had ever isolated (Dahm, 2005). Indeed, a glass vial containing Nuclein isolated from salmon sperm (Figure 5) is on display at the recently opened Miescher-Museum in Tübingen, Germany (Figure 6), and still contains intact nucleic acids.

Using this highly pure Nuclein, Miescher repeated his elementary analysis and confirmed an absence of sulfur (Miescher, 1872a,b,c) and determined the P_2O_5 content to be 22.5% of the total mass (Miescher, 1872b), a value very close to its actual proportion. He also determined that all the phosphorous contained in Nuclein was in the form of phosphoric acid (Miescher, 1872b). Along with these discoveries, Miescher was able to determine that Nuclein was "at least [a] four basic acid" and that it must be a molecule with a high molecular weight (Miescher, 1874a, Dahm, 2008).

In the spring of 1872, Miescher reported one of his most interesting discoveries to the Naturalist Society in Basel. He reported that the heads of salmon sperm were comprised almost completely of the "multi-basic" acid Nuclein, bound in a salt-like state to a basic molecule he called "*protamin"* [protamine in English]" (His 1897a). Protamines, a name that is still in use today, are small proteins that replace histones during spermatogenesis and Miescher determined that they bind to DNA in constant ratios. (Miescher, 1874b). This was Miescher's first foray into openly speculating about the role that Nuclein, or the nucleus in general, might play in fertilisation. As he stated "aside from the mentioned substances [protamine and Nuclein] nothing is present in significant quantity. As this is crucial for the theory of fertilisation, I carry this business out as quantitatively as possible right from the beginning" (Miescher, 1872a; Dahm, 2010b).

Miescher's speculations about how the nucleus and its contents could be involved in fertilization were quite varied and often off the mark. Nevertheless, some of his ideas had intriguing elements, which, had he followed up on them, may have led to a better understanding of Nuclein's function. In 1872 and 1873 Miescher moved beyond his studies of salmon sperm to the sperm of carp, frogs, chicken and bulls, in which, for each species, he found Nuclein to be the primary component (Miescher, 1874a,b). Miescher reasoned that perhaps the oocyte might be held in a suspended state through the absence of a crucial component, which would then be provided by the sperm allowing the oocyte to develop into an embryo. As he wrote “if now the decisive difference between the oocyte and an ordinary cell would be that from the roster of factors, which account for an active arrangement, an element has been removed? For otherwise all proper cellular substances are present in the egg” (Miescher 1872c; Dahm, 2010b). Miescher initially preferred protamine as the missing driver for initiating development as he was unable to detect it within oocytes. However, he was later unable to detect protamine in the sperm of other species, such as bulls, leading him to change his mind. He wrote, “Nuclein by contrast has proved to be the constant so far; to it and its associations I will direct my interests from now on” (Miescher 1872 c; Dahm, 2010b).

Figure 6. The entrance from the courtyard of Tübingen Castle leading to the former laboratories of Friedrich Miescher and Felix Hoppe-Seyler. Through this door and within the castle is also the Miescher-Museum. To the left of the door are three plaques that commemorate four notable biochemists who worked in the laboratories. Inset left of the image is a close-up view of the bottom plaque which translates to read “Here discovered / Friedrich Miescher / 1869 Nuclein”.

The intense speculation at the time on the mechanisms controlling fertilisation and the nature of how hereditary traits could be transmitted had led scientists, such as Wilhelm Kühne, to speculate that sperm carry specific substances whose chemical properties are necessary to achieve fertilisation (Kühne, 1868b). Miescher's own findings regarding the ubiquity of Nuclein in the sperm of multiple species matched well with this view and led him to state that "if we were to assume at all that a single substance, as an enzyme or in any other way […] could be the specific cause of fertilisation, we would without a doubt first and foremost have to consider Nuclein." (Miescher, 1874a; Dahm, 2005).

Despite these ideas, which came very close to unlocking the true nature of Nuclein, Miescher was unable to conceive that a single substance could be responsible for fertilization or the transmission of hereditary traits. In this, he was strongly influenced by his uncle, Wilhelm His, who favoured the notion that sperm imparted a motion stimulus to the egg, which triggered subsequent embryonic development (Dahm, 2010b). This was a popular notion of the time and Miescher did speculate that Nuclein might be the molecule to transduce this stimulus. However, he did not include the idea of fusion between the egg and the sperm and hence the transfer of actual molecules that would explain inheritance. His had been an instrumental factor in fostering Miescher's interest in biochemistry at a young age, even prompting Miescher into his search for the chemical secrets of life, and he remained a strong influence throughout Miescher's life. Therefore, it is truly unfortunate that His held this view due to its influence on Miescher's thoughts. Indeed, Miescher's unwillingness to break from his mentor's views ultimately prevented him from seeing Nuclein for what it really was, the fundamental molecule of hereditary.

Miescher's legacy

Over the next 20 years, in part due to his increasingly onerous responsibilities as a member of faculty at Basel University, Miescher never again managed to obtain conclusive results on Nuclein. At the beginning of the 1890s, his health had deteriorated significantly and he contracted tuberculosis. As a result, he had to abandon his work and moved to a clinic in Davos in the Swiss Alps (His, 1897a; Dahm, 2008). He attempted to write one last summary of his work including all his unpublished findings on Nuclein (Miescher, 1894) but could not find the strength to finish it. His former mentor in Leipzig, Carl Ludwig, wrote to console him: "As hard as it may be, you have the comfort of having achieved everlasting accomplishments; you have made the centre, the core of all organic life accessible to chemical analysis; and however often in the course of centuries to come, the cell will be studied and examined, the grateful descendant will remember you as the groundbreaking researcher" (His, 1897a; Dahm, 2005). Unfortunately, although many of Miescher's findings would form the basis for further development on the understanding of Nuclein, the man himself would largely be forgotten. Today Miescher is largely unknown, not only amongst the public but more startlingly even amongst the scientific community.

Friedrich Miescher died in 1895 at the age of just 51, with a feeling of a promising career unfulfilled (His, 1897a; Dahm, 2010b). After his death, his uncle Wilhelm His collated and published his nephew's papers. In the introduction, he wrote, "The appreciation of Miescher and his works will not diminish; on the contrary, it will grow and his discoveries and thoughts will be seeds for a fruitful future." (His, 1897c; Dahm, 2008).

The tributes from both Ludwig and His are all the more remarkable for their grasp of just how important Miescher's discovery would turn out to be for the future of biology. It took another 50 or so years for the true role of Nuclein, or as it had become known nucleic acid, to be fully realized. In 1944 Oswald Avery, Colin MacLeod and Maclyn McCarty published their landmark paper suggesting that DNA rather than protein, was the carrier of genetic information (Avery et al., 1944). Then in 1952, Al Hershey and Martha Chase confirmed these findings by observing that viral DNA –but not protein– enters bacteria during infection by a bacteriophage and, that this DNA is also present in new viruses produced by infected bacteria (Hershey & 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 & Crick, 1953). And finally, in 1966 the genetic code was ultimately cracked (Nirenberg et al., 1966). All of which has ushered in the current era of genome editing, genetic disease testing, and DNA as a central part of our modern lives. Indeed, the future of DNA is still evolving. Synthetic biologists modify DNA to insert unnatural amino acids into cells, ecologists hijack the DNA of invasive species to eradicate them, and geneticists use precision molecular scissors to modify genes in ways unthinkable just 10 years previously.

In the end, Miescher's story is about an obsessive, brilliant but retiring scientist whose personality and intellectual and working environment prevented one of the greatest scientific discoveries being fully realized in its time. Though Miescher's legacy may still languish in obscurity, the importance of DNA today suggests that the 150th anniversary of Miescher's discovery could be the right time for him to re-enter our public memory.

References

Avery O.T., MacLeod C.M., McCarty M. (1944) Studies of the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from *Pneumococcus* Type III. *J. Exp. Med.* V.79(2). P.137–158. DOI: 10.1084/jem.79.2.137

Dahm R. (2005). Friedrich Miescher and the discovery of

DNA. *Dev Biol* 278: 274–288. DOI: 10.1016/j.ydbio.2004.11.028
Dahm R. (2008). Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. *Hum Genet* 122: 565–581. DOI: 10.1007/s00439-007-0433-0
Dahm R. (2010a). A slip in the date of DNA's discovery. *Nature* 468: 897. DOI: 10.1038/468897d
Dahm R. (2010b). From discovering to understanding. Friedrich Miescher's attempts to uncover the function of DNA. *EMBO Rep* 11: 153–160. DOI: 10.1038/embor.2010.14
Dahm R., Banerjee M. (2019). How we forgot who discovered DNA: Why it matters how you communicate your results. *Bioessays* 41:1900029. DOI: 10.1002/bies.201900029
Haeckel E. (1866). Generelle Morphologie der Organismen. Reimer, Berlin, pp 287–288.
Hershey AD and Chase M. (1952). Independent functions of viral proteins and nucleic acid in growth of bacteriophage. *J Gen Physiol* 36:39–56. DOI: 10.1085/jgp.36.1.39
His W. (1897a). F. Miescher. In: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 1, Vogel F.C.W., Leipzig, pp 5–32.
His W. (1897b). F. Miescher. In: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 1, Vogel F.C.W., Leipzig, p 543.
His W. (1897c). Einleitung. In: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 1, Vogel FCW, Leipzig, pp 1–4.
His W. (1897d). F. Miescher. In: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 1, Vogel F.C.W., Leipzig, p 109.
Hoppe-Seyler F. (1864). Ueber die chemischen und optischen Eigenschaften des Blutfarbstoffs. *Archiv pathol Anat* 29: 597–600.
Hoppe-Seyler F. (1871). Ueber die chemische Zusammensetzung des Eiters. *Medicinisch-chemische Untersuchungen* 4:486–501.
Kühne W. (1868a). Männliche Geschlechtsabsonderungen. In: Lehrbuch der physiologischen Chemie. Engelmann W, Leipzig, pp 49–50.
Kühne W. (1868b). Männliche Geschlechtsabsonderungen. In: Lehrbuch der physiologischen Chemie. Engelmann W., Leipzig, pp 555–558.
Miescher F. (1869a). Letter I; to Wilhelm His; Tübingen, February 26th, 1869. In: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 1, Vogel F.C.W., Leipzig, pp 33–38.Miescher F. (1869b). Letter V; to Wilhelm His; Tübingen, December 20th, 1869. In: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 1, Vogel F.C.W., Leipzig, pp 39–41.
Miescher F. (1870). Nachträgliche Bemerkungen. In: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 2, Vogel F.C.W., Leipzig, pp 32– 34.
Miescher F. (1871). Ueber die chemische Zusammensetzung der Eiterzellen. *Medicinisch-chemische Untersuchungen* 4: 441–460.
Miescher F. (1872a). Letter XXVI; to Hoppe-Seyler; Basel, Summer 1872. n: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 1, Vogel F.C.W., Leipzig, pp 64– 68.
Miescher F. (1872b). Letter XXVII; to Hoppe-Seyler; Evolena, Juli 20th 1872. n: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W *et al* (eds), Vol 1, Vogel FCW, Leipzig, pp 68– 69.
Miescher F. (1872c). Letter XXVIII; to Dr. Boehm; Basel, May 2nd, 1872. n: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 1, Vogel F.C.W., Leipzig, pp 70–73.
Miescher F. (1874a). Die Spermatozoen einiger Wirbelthiere. Ein Beitrag zur Histochemie. *Verh Nat forsch Ges Basel* 6: 138–208.
Miescher F. (1874b). Das Protamin—Eine neue organische Basis aus den Samenfäden des Rheinlachses. *Ber Dtsch Chem* Ges 7: 376.
Miescher F. (1894). Letter LXXXI; to His; Davosplatz, December, 1st 1894. I In: Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher - Aus dem wissenschaftlichen Briefwechsel von Friedrich Miescher, His W. *et al.* (eds), Vol 1, Vogel F.C.W., Leipzig, pp 124–126.
Nirenberg M, Caskey T, Marshall R, Brimacombe R, Kellogg D, Doctor B, Hatfield D, Levin J, Rottman F, Pestka S, Wilcox M, Anderson F. (1966). The RNA code and protein synthesis. *Cold Spring Harb Symp Quant Biol* 31: 11–24. DOI: 10.1101/sqb.1966.031.01.008
Strecker A. (1850). Ueber die künstliche Bildung der Milchsäure und einer neuen, dem Glycocoll homologen. *Liebigs Ann Chem* 75: 27–45.
Watson J.D., Crick F.H.C.. (1953). A structure for deoxyribose nucleic acid. *Nature* 171: 737–738. doi:10.1038/171737a0