Historic nucleic acids isolated by Friedrich Miescher contain RNA besides DNA

Abstract: One hundred fifty years ago, Friedrich Miescher discovered DNA when he isolated “Nuclein”—as he named it—from nuclei of human pus cells. Miescher recognized his isolate as a new type of molecule equal in importance to proteins. He realized that it is an acid of large molecular weight and high phosphorus content. Subsequently, he discovered Nuclein also in the nuclei of other cell types, realized that it chemically defines the nucleus, and speculated on its role in proliferation, heredity and fertilisation. While now universally recognized as the discoverer of DNA, whether Miescher also discovered RNA has not yet been addressed. To determine whether his isolation also yielded RNA, we first reproduced his historic protocols. Our resulting modern Nuclein contained a significant percentage of RNA. Encouraged by this result, we then analysed a sample of Nuclein isolated by Miescher from salmon sperm. Assuming that the RNA present in this sample had degraded to nucleobases, we tested for the presence of uracil in the historic Nuclein. Detection of significant levels of uracil by LC-UV-MS demonstrates that Miescher isolated both forms of nucleic acid—DNA and RNA—and underlines the fundamental nature of his discovery for the field of molecular genetics.

Keywords: DNA in Nuclein; nucleic acid discovery; nucleic acid isolation; Nuclein; Nuclein analysis; RNA in Nuclein.

Introduction

In the mid-1800s, biochemistry was still an emerging discipline. One of the first laboratories dedicated to its study was located in the former kitchen of Tübingen’s medieval castle (Figure 1) (Bohley 2009, and references therein) (Beck 2015). Here, pioneers in this young discipline tried to discover the molecules that make up living things and to characterise their properties. Today, Tübingen’s biochemical laboratory is most renowned for the work of the Swiss physician Friedrich Miescher (1844–1895; Figure 1) and his discovery of what we now know as DNA. In 1868, Miescher joined the research group of Felix Hoppe-Seyler, the then head of the laboratory, to investigate the composition of cells. He chose leukocytes from human pus as his source material, hoping that analysing cells that are not embedded in a tissue would facilitate the identification of the molecular building blocks that make up cells. Over the course of the next months, Miescher proceeded to make his most important discoveries, culminating, in early 1869, in his isolation and characterisation of what he termed “Nuclein” (a name derived from the Latin word ‘nucleus’) (Dahm 2005, 2008a,b, 2010a; Miescher 1869, 1871).

Based on his analyses, Miescher realised that Nuclein was chemically distinct from all other types of molecules known at the time. Subsequent to his initial discovery and chemical characterisation of Nuclein, Miescher went on to show that Nuclein is present in the nuclei of all cell types he investigated and postulated that it chemically defines the nucleus. Importantly, he considered Nuclein to be “equal [in importance] to the proteins”. Later, he even speculated on a role for Nuclein in cell proliferation as well as in heredity and fertilisation. Alas, given the limited knowledge and experimental approaches available at this time, Miescher was unable to follow up on his foresights. It took until the middle of the 20th century for them to be confirmed and Miescher, despite the importance of his discoveries and insights, was largely forgotten (Byrne and Dahm 2019; Dahm 2010b; Dahm and Banerjee 2019).

When Miescher is remembered today, it is for his discovery of DNA. Given the fact that the nucleus also contains RNA, which likely behaved very similar to DNA in the protocols Miescher used to isolate his Nuclein, it seems likely that he was also the first to isolate RNA. Spurred by the upcoming 150th anniversary of Miescher’s publication...
of the isolation of Nuclein (Miescher 1871), this study addresses this question experimentally. First, Miescher’s isolation procedure was reproduced as closely as possible with a sample of feline pus, as detailed in Table 1. Unavoidable changes in starting material, laboratory equipment, and analytical methods to those used by Miescher are indicated. The “modern” Nuclein sample thus isolated contained a significant percentage of RNA, as shown by gel electrophoresis and fluorometric RNA-specific assays of digests with DNase and RNase.

Encouraged by these results, a historic sample of Nuclein isolated by Miescher from salmon sperm after he had moved to Basel was examined. If in this “historic” Nuclein RNA had been present, it may have degraded into

| Table 1: Comparison of the isolation protocols for Nuclein as isolated by Friedrich Miescher in 1869 and for the “modern” Nuclein as isolated in this study. |
|---|---|
| **Nuclein as isolated in 1869** | **“Modern” Nuclein** |
| Starting material: | Starting material: |
| Human pus, washed from bandages from a hospital in Tübingen | Feline pus, pyothorax from an injured cat obtained from a vet in the Tübingen area |
| Cells allowed to settle in 1.5% sodium sulfate solution at wintery temperatures; supernatant removed (several times) | Cells allowed to settle in 1.5% sodium sulfate solution at 4 °C; supernatant removed (three times over two days) |
| Filtration (unknown details) | Mild centrifugation (5 min, 250 × g, no break) |
| Extraction with warm alcohol (three times) | Extraction with pure ethanol for 90 min at 37 °C (twice) |
| In between filtration (supposedly) | In between mild centrifugation (5 min, 250 × g, no break) |
| Extraction of pepsin from porcine stomach with 1% hydrochloric acid | Preparation of a 0.1 g/L purified pepsin solution in 10 mM hydrochloric acid |
| Protease digestion by a crude pepsin extract for 18–24 h at 37 °C | Protease digestion with 0.018 g/L of pure pepsin for 1 h at 37 °C |
| Sedimentation of nuclei | Mild centrifugation (5 min, 250 × g, no break) |
| Repeated shaking with pure ether for 30 min | Repeated shaking with pure ether for 30 min |
| Collection of nuclei on filter paper | Collection of nuclei on filter paper |
| Extraction with warm alcohol (several times) | Extraction with pure ethanol for 60 min at 37 °C |
| In between filtration (supposedly) | Mild centrifugation (5 min, 250 × g, no break) |
| Suspension in sodium carbonate (6–24 mM) | Suspension in sodium carbonate (10 mM) |
nucleosides or even nucleobases that would be amenable to extraction. Accordingly, to determine the presence of RNA in the “historic” Nuclein, uracil as the RNA-specific nucleobase was quantified by high-performance liquid chromatography coupled to UV detection and mass spectrometry (LC-UV-MS) in aqueous extracts of the “historic” Nuclein. Successful quantification of significant levels of uracil strongly indicates the presence of RNA in the historic sample. The investigation presented here therefore shows that, 150 years ago, Friedrich Miescher had both DNA and RNA in his hands, isolating both types of nucleic acids.

Results

Isolation of “modern” Nuclein from pus cells using a protocol based on Miescher’s isolation method

The protocols used to obtain “modern” Nuclein and by Miescher to first isolate Nuclein in 1869 are juxtaposed in Table 1, see also supplementary methods. An illustrative graphic representation of the protocol Miescher used in 1869 has been published previously (Dahm 2008b).

Miescher’s starting material was fresh human pus from bandages obtained from the surgical department in Tübingen’s University hospital. Because such human material is essentially no longer available, we resorted to feline pus obtained from a curative veterinary practice. Intact cells were separated from debris and serum by suspension in 1.5% sodium sulfate solution followed by sedimentation for three times over two days. On the third day, the majority of pus cells were found to be intact, as indicated by a lack of trypan blue staining (Figure S1A).

Next, the lipids were removed from the pus cells with warm ethanol. Staining by trypan blue confirmed delipidation of the cells. Intracellular and structural proteins were then degraded with pepsin in acidic medium, yielding pure nuclei (Figure S1B). Finally, lipids were removed from the nuclei first with ether and then with warm ethanol to yield the final “modern” Nuclein. Its UV–Vis absorbance spectrum showed the maximum at 260 nm typical for nucleic acids (Figure S2), indicating that our procedure did isolate nucleic acids from pus cells.

Analysis of “modern” Nuclein

Miescher characterised his “historic” Nuclein by classical gravimetric elemental analysis. To his surprise, he found the content of phosphorus to be $2.56 \pm 0.06\%$ (Miescher 1871), much higher than in lipids or even proteins. It was this finding that led him to conclude that likely he had isolated a wholly new class of natural substance. Later, Miescher turned from human pus cells to salmon sperm as the source of Nuclein. Using refined protocols, he determined the phosphorus content of salmon Nuclein to be above 9% (Miescher 1874). As the phosphorus content of pure nucleic acid is 10% by mass, Miescher’s Nuclein isolated from salmon sperm consists almost exclusively of nucleic acid.

Using plasma optical emission spectroscopy, we determined the phosphorus content of our preparation. Consistent with Miescher’s analysis, we also found a high phosphorus content of $7.5 \pm 0.4\%$ relative to mass of nucleic acid, as calculated from absorbance. Having removed lipids extensively (the only other potential significant source of phosphorus), this reasonable agreement between the phosphorus content we found and the 10% phosphorus content of pure nucleic acid confirms that we did isolate nucleic acid. The phosphorus content is lower than the 10% expected, most likely due to an overestimate of the nucleic acid content of our Nuclein due to impurities also absorbing at 260 nm, e.g., protein, but is in line with Miescher’s results.

In order to discriminate between DNA and RNA in our “modern” Nuclein, we digested samples either with DNase I, RNase A, or both. Agarose gel electrophoresis shows that our sample contains predominantly DNA of high molecular weight (Figure 2). However, the DNase-only digest in lane 4 reveals that also RNA is present in our “modern” Nuclein.

To independently confirm that our “modern” Nuclein contained RNA, we quantified RNA using an RNA-specific fluorophore (Qubit RNA high-sensitivity assay) (Figure S3). Comparing the signal specific to RNA to a reference sample with RNA spiked in, approximately 1.5% of nucleic acid in our Nuclein is RNA.

Analysis of “historic” Nuclein

Finding RNA in our “modern” Nuclein set the stage for considering an analysis of a “historic” Nuclein preparation with regard to its RNA content. Miescher isolated Nuclein under non-sterile conditions and stored his sample in a glass test tube sealed with a cork stopper (see Figure 1). This type of storage allows constant replenishment of moisture to the sample. Thus, it seems likely that the nucleic acids in the “historic” Nuclein preparation will be significantly degraded. RNA, in particular, may partly have been degraded to nucleosides or even nucleobases. A historic sample may thus contain low molecular weight nucleic acid constituents.
that can be analysed for components specific to RNA. In particular, quantification of uracil as the RNA-specific nucleobase may enable an estimation of the lower bound of the RNA content of the “historic” Nuclein sample.

We analysed material from Miescher’s “historic” Nuclein preparation derived from salmon sperm in 1871, now on display at the museum in the former laboratory in Tübingen’s castle. Samples were taken – a loose yellowish powder – under sterile conditions. The cork stopper was carefully removed from the glass test tube and samples of several milligrams transferred into sterile glass vials. Samples of 7, 6, or 5 mg of “historic” Nuclein were suspended in water, aided by sonification, followed by centrifugation to remove all insoluble material. The supernatants, extracts containing the soluble low molecular weight nucleic acid constituents, were analysed by liquid chromatography-UV-mass spectrometry. Upon separation of the extracts on a reversed-phase column, a clear peak was observed at the retention time of uracil. The eluent was passed to the mass spectrometer and an extracted ion chromatogram at 113 Da (molecular mass of protonated uracil) showed a corresponding peak, confirming the identity of the chromatographic peak (Figure 3). As shown in Table 2, the extracts contained uracil at concentrations of 1.54, 3.11, or 1.71 µM (lower level of quantification: 24 nM), for a total of 3.08, 5.33, or 2.44 nmol of uracil, equal to 345, 597, or 273 ng of uracil extracted from the samples of “historic” Nuclein (slightly different extraction volumes need to be considered converting concentration to total amount).

The level of uracil we were able to extract from “historic” Nuclein of 1871 can be compared to the level of RNA/uracil in our “modern” Nuclein. In our “modern” Nuclein we found RNA to represent approximately 1.5% of its nucleic acid content. Assuming that the “historic” Nuclein, consisting almost exclusively of nucleic acids, contains RNA at a similar level relative to DNA as our “modern” Nuclein, the 7 mg of “historic” Nuclein extracted by us would have contained 77 nmol uracil (as a constituent of RNA originally). The 3.08 nmol uracil we were able to extract from the 7 mg of “historic” Nuclein would thus correspond to 1.5% of the nucleic acid content.

1 Seven milligram of Nuclein, almost all of which is DNA, corresponds to 20.6 µmol of nucleotide or 5.1 µmol of thymdin or uracil. If 1.5% of nucleic acids was RNA, 7 mg of Nuclein contain 77 nmol of uracil.
Table 2: Uracil content of “historic” Nuclein.

| Mass of samples taken from “historic” Nuclein [mg] | 7 | 6 | 5 |
|---------------------------------------------|---|---|---|
| Volume of water added to each sample for extraction [µL] | 200.0 | 171.4 | 142.9 |
| Dilution of each extract with water before injection into LC-UV-MS | 10 fold | 10 fold | 10 fold |
| Uracil concentration in 10-fold diluted extracts [nmol/L] | 1543 | 3108 | 1707 |
| Uracil per sample taken from “historic” Nuclein [nmol] | 3.08 | 5.33 | 2.44 |
| Uracil per sample taken from “historic” Nuclein [ng] | 345 | 587 | 273 |

Three samples of several mg each taken from “historic” Nuclein were analysed for their uracil content. Samples were extracted with water to solubilize low molecular weight nucleic acid constituents. After further 10-fold dilution with water, extracts were analysed by LC-UV-MS. Diluted extracts contained uracil in the µM range for a total of several nmol of uracil per sample taken from “historic” Nuclein.

extract from the 7 mg sample represent only a small fraction of the 77 nmol uracil expected to be present in the sample if it contained RNA at a similar level relative to DNA as our “modern” Nuclein. However, of course only a fraction of RNA may have been degraded to individual nucleobases over time, of which only a further fraction may have been preserved as uracil, not all of which may in turn be extractable. Thus, the amount of uracil found by us may be in line with the “historic” Nuclein containing RNA at a level not unlike our “modern” Nuclein. After our analyses, we can say with a high degree of certainty that Miescher isolated both natural forms of nucleic acids, at least a small fraction of RNA along with significant amounts of DNA.

Discussion

Friedrich Miescher’s discovery of a novel biochemical component of cells, “Nuclein”, was the first milestone in nucleic acid research. To commemorate this historic event, the laboratory in which he made his discovery has recently been converted into a museum open to the public (Thess and Dahm 2020). The most precious exhibit is the oldest sample of purified nucleic acid in the world (Figure 1). It contains Nuclein isolated from salmon sperm and carries the handwritten signature of F. Miescher from 1871. It is part of this sample used in the analyses described above.

Although for decades Miescher’s findings were largely forgotten, they represent the first steps towards identifying the programme underlying life. Where Miescher’s contribution is remembered, it is generally for the discovery of DNA (Byrne and Dahm 2019; Dahm 2005, 2008a, 2010b; Dahm and Banerjee 2019). Spurred by the 150th anniversary of the publication of his discovery of Nuclein, the objective of this investigation was to analyse whether Friedrich Miescher’s Nuclein contained, alongside its main component DNA, also much less stable RNA. This question was addressed by first determining if the isolation protocol used by Miescher in 1869 could support the extraction of RNA and then examining the oldest preserved Nuclein sample available, purified from salmon sperm in 1871, to determine if a residual RNA signal could be detected.

In order to find out whether the procedure for the isolation of Nuclein followed by Miescher also allows the isolation of RNA alongside DNA, Miescher’s historic laboratory protocols were repeated as closely as possible and the resulting “modern” Nuclein analysed for RNA. The isolation procedures as described by Miescher are summarised and compared to the “modern” isolation procedure used here in Table 1. It is shown that both preparations yielded nucleic acid: the “modern” Nuclein shows the same properties as Miescher’s Nuclein, it dissolves in diluted soda solution and precipitates in diluted hydrochloric acid. Both preparations contain a high content of phosphorus and show a high molecular mass. The differences in the starting materials and the use of mild centrifugation instead of filtration had no influence on the quality of the resulting Nuclein. The “modern” Nuclein was shown to contain RNA by gel electrophoresis of digests with DNase and RNase. Independently, RNA-specific fluorescence assays confirmed the presence of RNA in the “modern” Nuclein preparation, at a level of approximately 1.5% of total nucleic acid.

Finding that the procedures for the isolation of Nuclein followed by Miescher indeed support the isolation of RNA, it appeared promising to confirm the presence of RNA also in “historic” Nuclein. The authors turned to the historic sample of Nuclein isolated by Miescher from salmon sperm in 1871, as there is no extant sample from Miescher’s first Nuclein preparation in 1869. Considering the working conditions in biochemical laboratories at that time as well as the conditions and duration of storage of the sample, it appeared likely that RNA isolated by Miescher would be degraded by now. If degraded sufficiently, the “historic” Nuclein may contain the RNA-specific nucleobase uracil in its free form, which could then be detected in the sample. Indeed, analysis of an aqueous extract of the “historic” Nuclein sample by LC-UV-MS detected uracil at significant

2 https://www.unimuseum.uni-tuebingen.de/de/ausstellungen/besuchen-sie-die-mut-ausstellungen/schlosslabor.html.
levels, demonstrating that Miescher’s Nuclein contains at least a small fraction of RNA besides its high content of DNA. These results suggest that Friedrich Miescher had both DNA and RNA in his hands.

With his findings, Miescher laid the foundation for nucleic acid research, which later through the work of others was to develop into the field of molecular genetics; but it was only in 1944 that Oswald Avery and colleagues unequivocally correlated DNA with genetic information (Avery et al. 1944). Less than a decade later, Jim Watson and Francis Crick’s elucidation of DNA’s structure opened the door to our understanding of how DNA stores this information (Watson and Crick 1953). Shortly thereafter, in 1956, again in Tübingen, Alfred Gierer and Gerhard Schramm demonstrated for the first time that also RNA can function as genetic material (Gierer and Schramm 1956) (independently also found by Fraenkel-Conrat and Singer in 1957). Finally, RNA was found to serve as the direct template for the production of proteins in 1961 (Nirenberg and Matthaei 1961). Ultimately, these discoveries laid the foundation, amongst other things, for the development of RNA therapeutics today. Much recognized currently are, of course, mRNA vaccines. In 2000, less than a mile from Miescher’s former laboratory, researchers at Tübingen University demonstrated for the first time an mRNA vaccine inducing a complete adaptive immune response comprising antibodies and T cells (Hoerr et al. 2000). In the same year, similarly close to Miescher’s former laboratory, this work led to establishment of the world’s first company that began to develop mRNA-based therapeutics (Carralot et al. 2004; Weide et al. 2008).

Materials and methods

Sampling from “historic” Nuclein

To analyse “historic” Nuclein, samples were taken from a preparation derived from salmon sperm in 1871. The cork stopper was carefully removed from the glass tube in a PCR workstation. Samples of several milligrams of Nuclein were taken using PCR filter tips as small single-use spatulas and transferred into glass vials.

LC-UV-MS analysis of uracil in “historic” Nuclein

Samples were analyzed by reversed phase chromatography using a Hypercarb column (3 μm, 100 mm × 2.1 mm) attached to an RSLC U3000 HPLC system (both Thermo Scientific). Fifteen microliter of sample were injected and eluted with a linear gradient from 5% acetonitrile (up to 2.5 min) to 50% acetonitrile (14 min) at a flow rate of 200 μL/min at 40 °C. Eluents contained 0.1% formic acid either in water (A) or in acetonitrile (B). Analytes were detected at 260 nm in a “semi-Mic” UV cell (2.5 μL volume). Under these conditions, a dilution series of uracil standards was characterized by stable retention times and linear response. A standard curve was generated with quadruplicate measurements of 8 μM, 2 μM, 500 nM, 125 nM, and 31 nM uracil ($R^2 = 1$). The lower limit of quantification was 24 nM. The eluent was passed on to the mass spectrometer (Q-ToF, Maxis UHR, Bruker Daltonik) and ionized by electrospray. An extracted ion chromatogram at 113 Da (molecular mass of protonated uracil) was recorded. Seven, six or five microlgram of Nuclein were suspended in 200, 171, or 163 μL of water and sonified for 15 min in an ultrasound bath, followed by centrifugation for 10 min at 13,000 × g. The supernatant was diluted 10 fold with water and 15 μL were used for LC-UV-MS.

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