Lecture 6:

The location and identity of the genetic material

Course 371

Aims

- Introduce the experiments localized the genetic material to the nucleus.
- Introduce the connection between Mendel's work and findings related to cell biology and chromosome theory.
- Cover the early terms used to refer to the genetic material.

Review

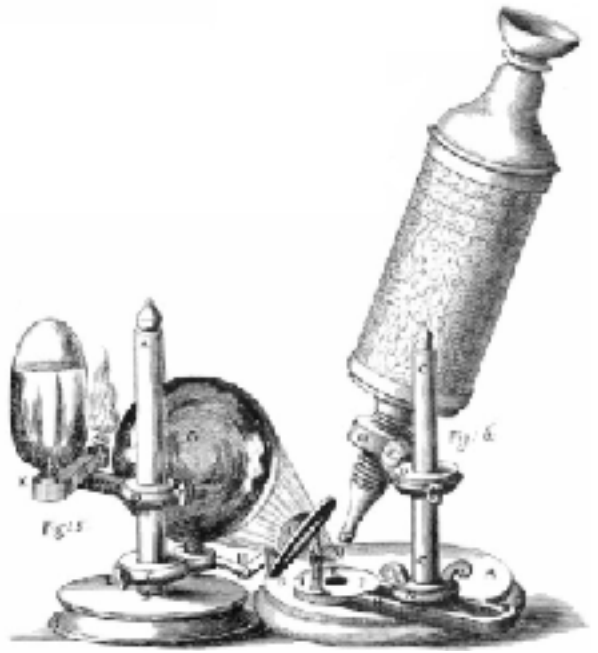
We learned by covering Mendel's experiments the general laws that govern the inheritance of characters.

The next question to ask is **where the genetic material is located**.

This will be the topic of today's lecture.

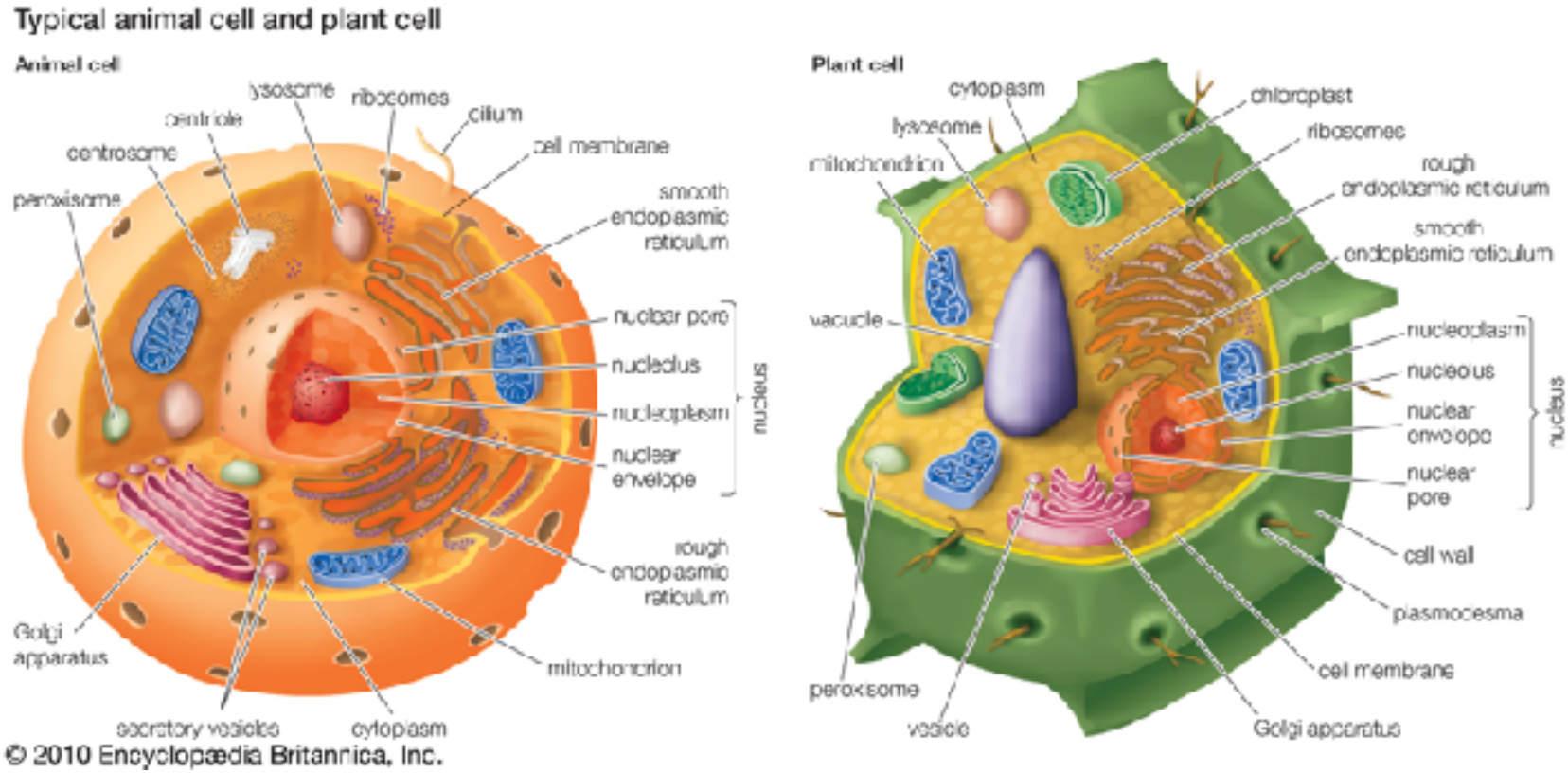
The cell

especially to the then radical new cell theory. Back in 1663, the English natural philosopher Robert Hooke had looked at cork, a tree-bark, through an early microscope. He observed the regular, empty spaces in the cork, which reminded him of the rows of tiny rooms in which monks lived, so he dubbed them 'cells'. The name



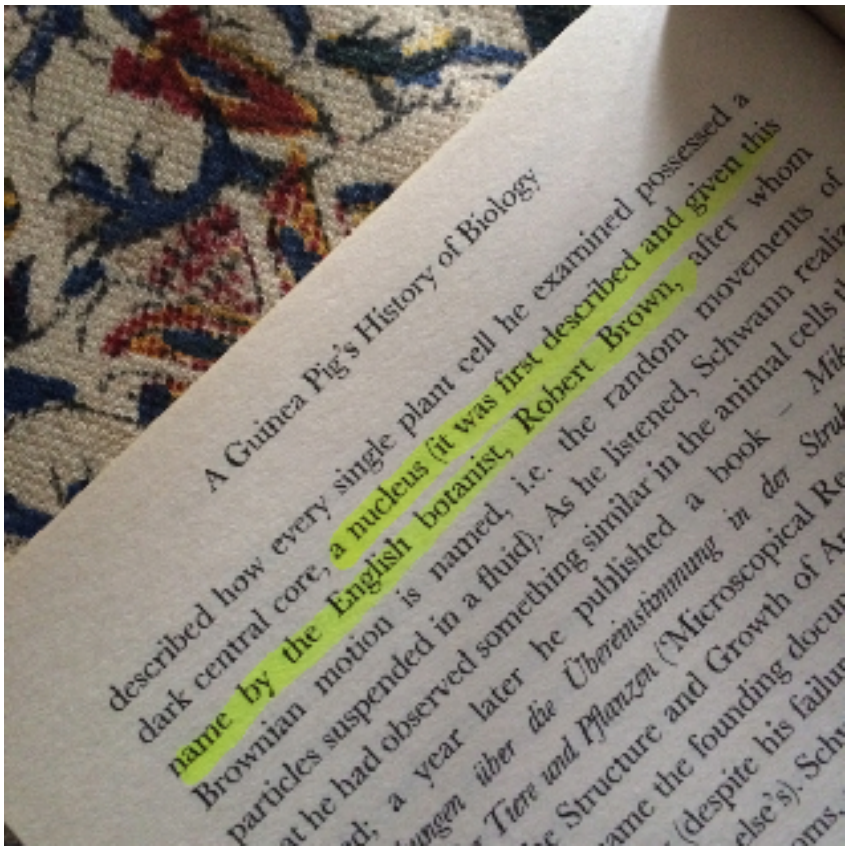
Cells

Mid 1800s, animals and plants are made of cells. Cells divide but do not know how.



The nucleus

A specific part was identified as the nucleus and it has specific chemical and physical characteristics.

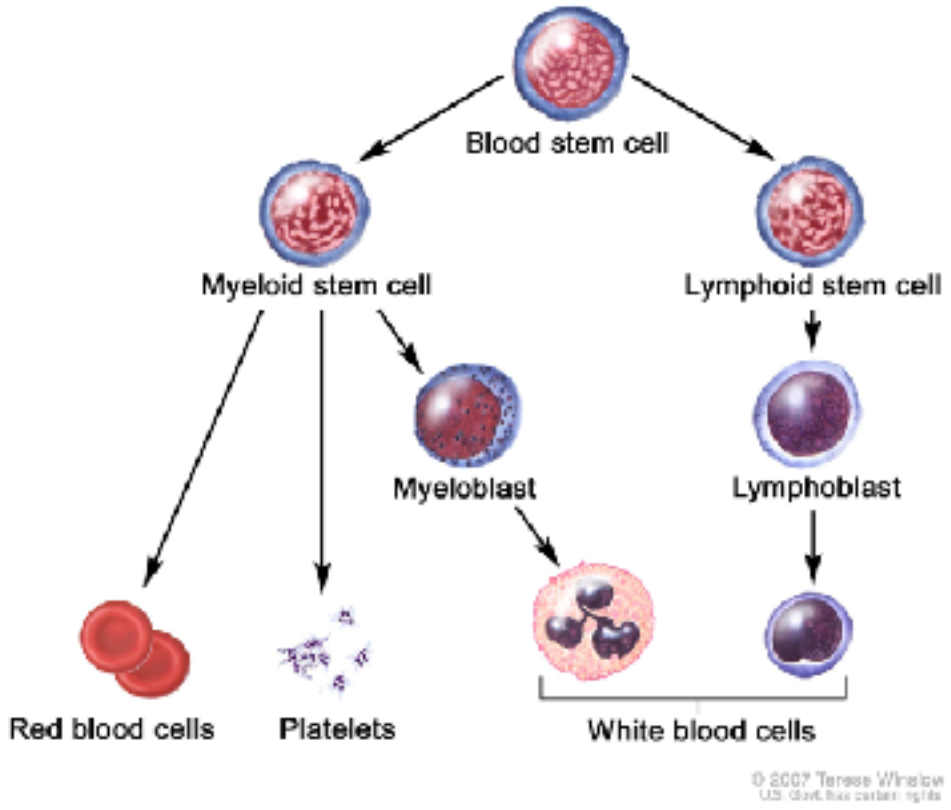


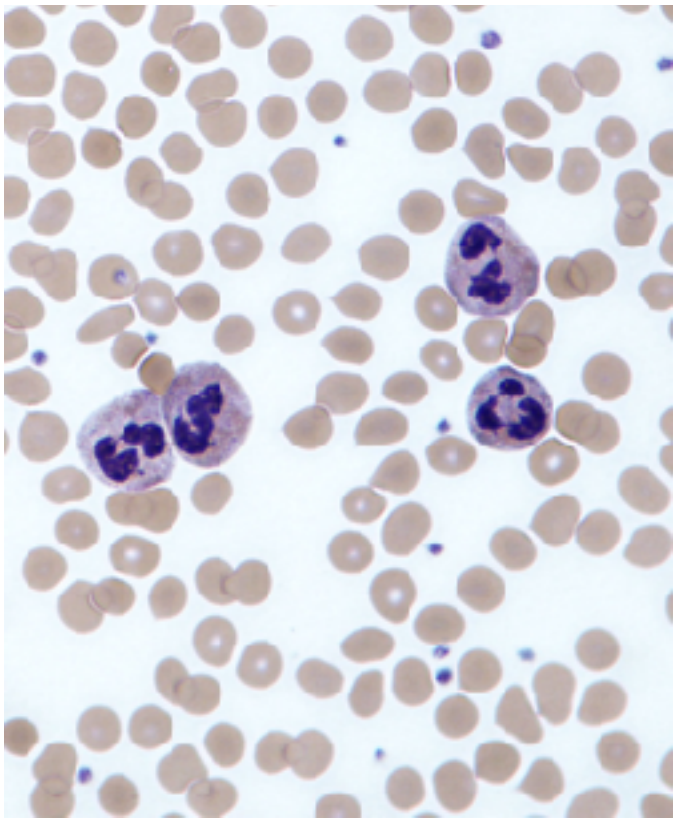
The nucleus and its content

What is in the nucleus?

Mammalian/ Human blood cells

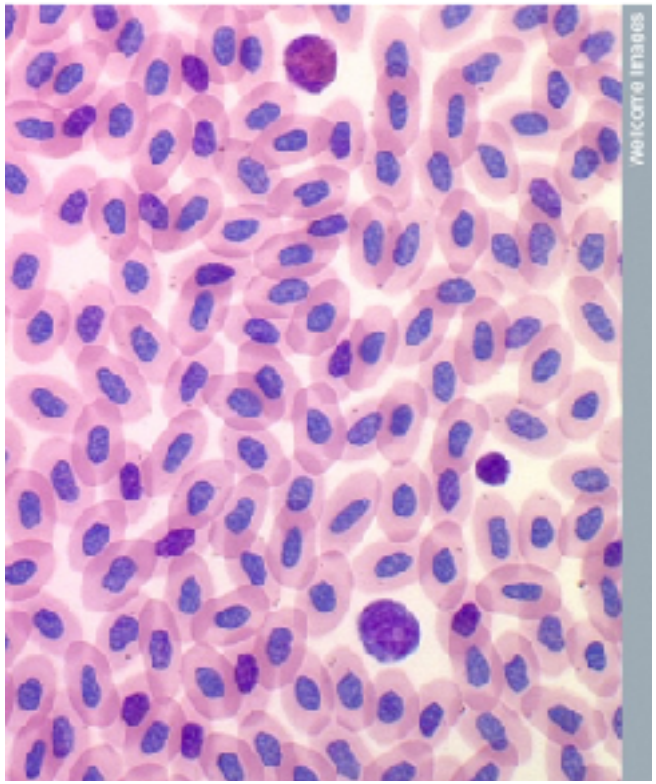
- Mammalian blood is composed of a variety of cells.
- Mammalian red blood cells lack nuclei and thus no genetic material.





Avian blood cells

- Avian blood is also composed of a variety of cells.
- Avian red blood cells contains nuclei and thus it is a source of genetic material.



Nuclein: the chemical of the nucleus

Fredrick Miescher bloody bandages (1869)

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 Zellenspecies, 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 Schlossladenlaboratorium 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.

Miescher, Friedrich (1871) "On the chemical composition of pus cells", Medicinisch-chemische Untersuchungen, 4 : 441–460.

Nuclein: the chemical of the nucleus

Great science can be done anywhere!
Castle's laundry room!

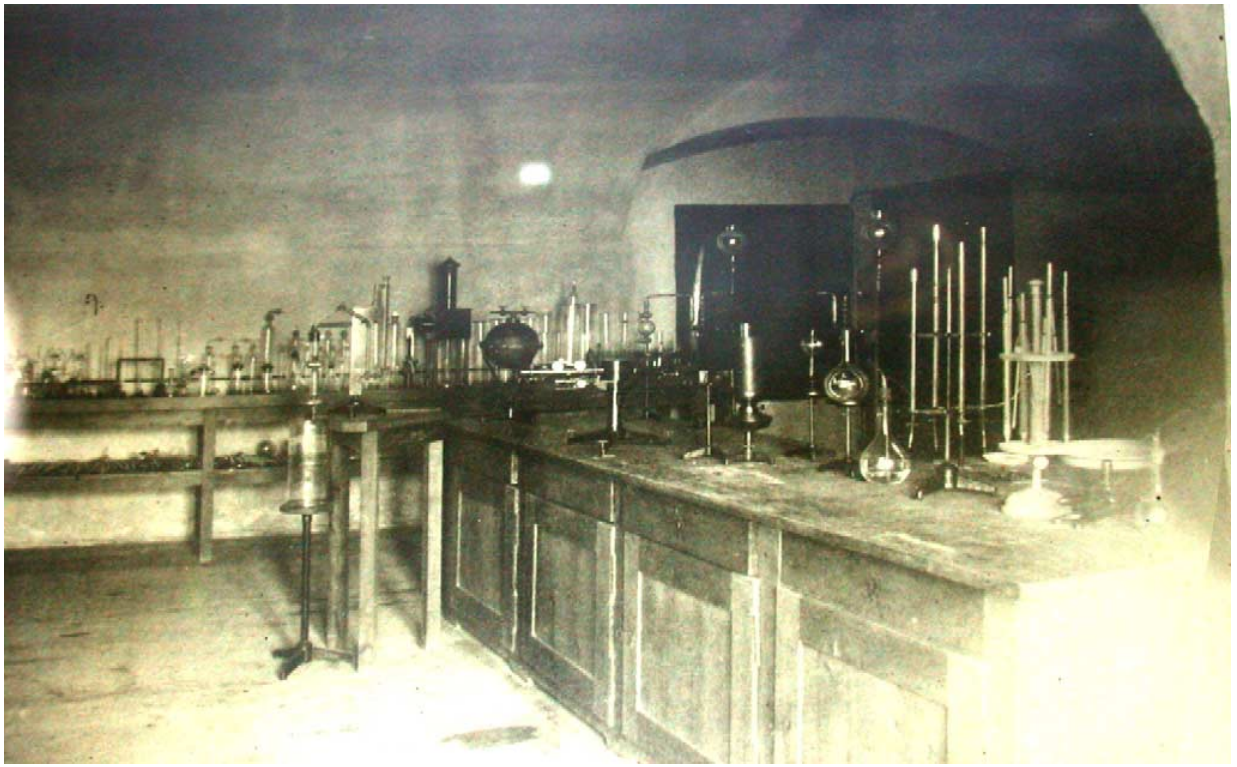


Fig. 2. Photograph of Felix Hoppe-Seyler's laboratory around 1879. Prior to becoming the chemical laboratory of Tübingen University in 1823, this room was Tübingen castle's laundry. Here, Hoppe-Seyler had made ground-breaking discoveries regarding the properties of hemoglobin. This achievement was a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

Ralf Dahm (2005), Friedrich Miescher and the discovery of DNA, Developmental Biology, 278(2) 274-288,

Nuclein: the chemical of the nucleus

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

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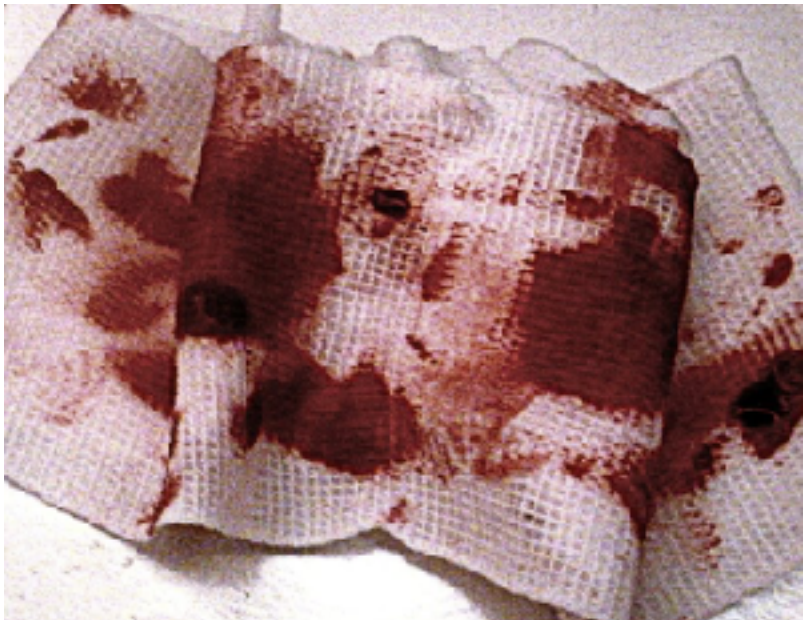
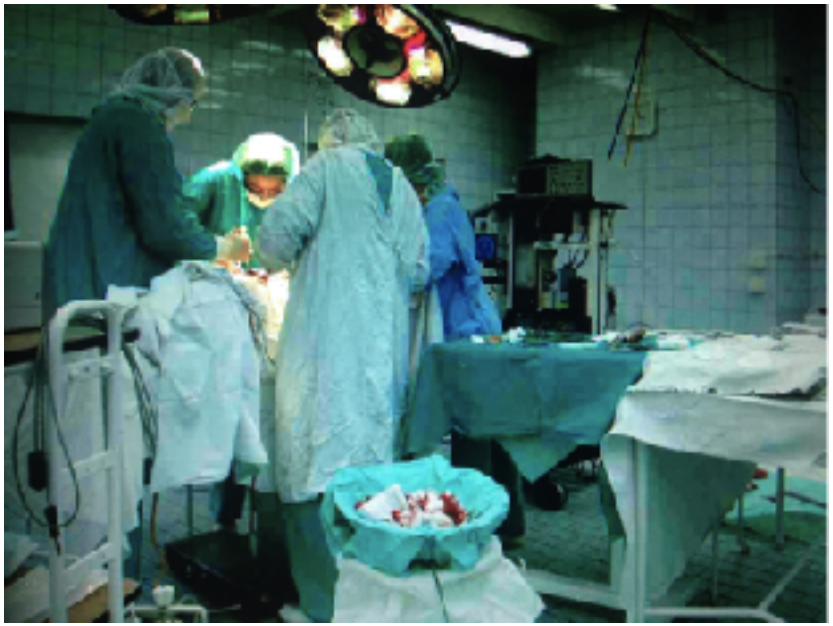
Miescher's samples

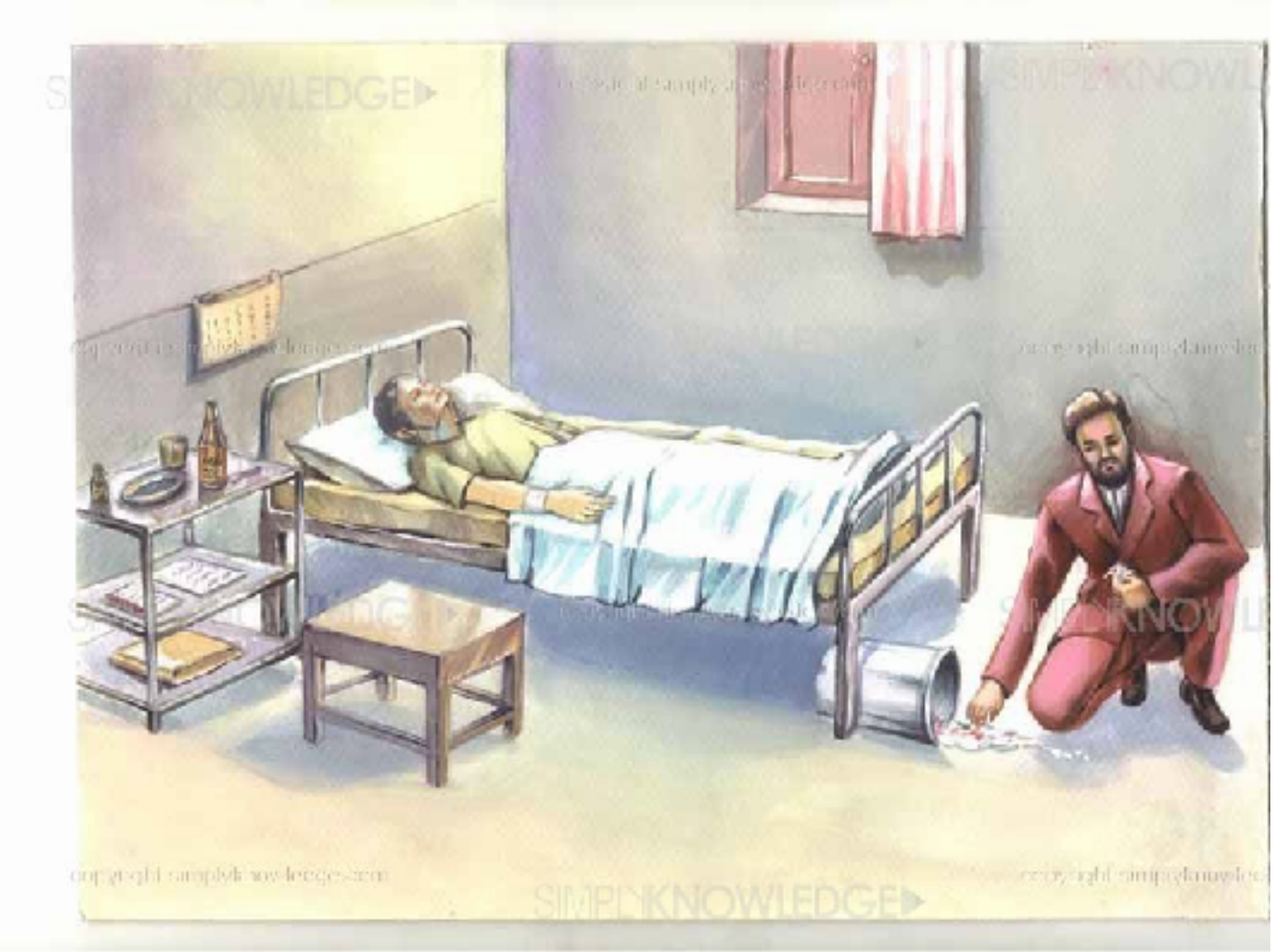




- Bloody bandages as a source of cells.

Where is the genetic material?

Miescher's samples



Miescher cell isolation protocol

Box 1
Miescher's first protocol to isolate DNA

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

In a first step, Miescher separated the leucocytes from the bandaging material and the serum (Miescher, 1869a, 1871d). This separation posed a problem for Miescher. Solutions of NaCl or a variety of alkaline or alkaline earth salt solutions used to wash the pus resulted in a "slimy swelling" of the cells, which was impossible to process further (His, 1897b). (This "slimy swelling" of the cells was presumably due to high-molecular-weight DNA, which had been extracted from cells that had been damaged.) Only when Miescher tried a dilute solution of sodium sulfate [a mixture of one part cold saturated Glauber's salt ($Na_2SO_4 \cdot 10\ H_2O$) solution and nine parts water] to wash the bandages did he manage to successfully isolate distinct leucocytes, which could be filtered out through a sheet to remove the cotton fibers of the bandaging. Miescher subsequently let the washing solution stand for 1–2 h to allow the cells to sediment and inspected the leucocytes microscopically to confirm that they did not show any signs of damage.

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

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

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

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

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Miescher's isolation of nucleus content

Box 2
Miescher's second protocol to isolate DNA

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

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

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

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

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Miescher's gray/white precipitate

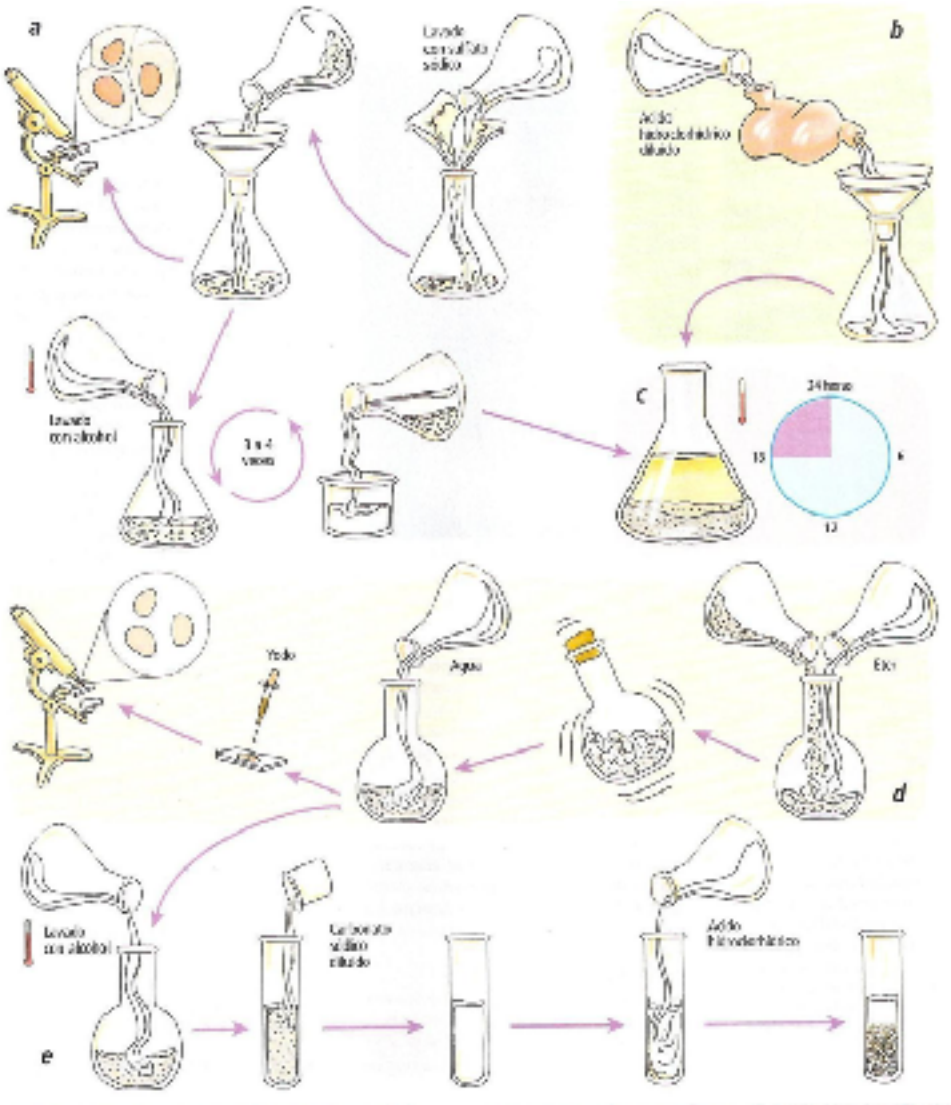


Fig. 5. Glass vial containing nuclein isolated from salmon sperm by Friedrich Miescher while working at the University of Basel. The faded label reads "Nuclein aus Lachssperma, F. Miescher" (*Nuclein from salmon sperm, F. Miescher*). Possession of the Interfakultäres Institut für Biochemie (Interfacultary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

Nuclein: the chemical of the nucleus

- The molecule is different than other molecules (contain C, O, N, H, and P – not known to be in proteins).
- Since the molecule came from nucleus, he called it **Nuclein (today called DNA)**.

daneben aber sehr reich an Phosphor. Die alte Tradition von den phosphorhaltigen Eiweissstoffen hat also doch einen reellen Hintergrund.

I. gr. 0,1915 lösliches Nuclein gaben 1811 Pt. = 13,47 N.
Die Kerne waren nach der Isolation nicht mit Alkohol extrahirt.
Die folgenden Versuche sind an ganzen, mit Alkohol heiss erschöpften Kernen gemacht.

Miescher, Friedrich (1871) "On the chemical composition of pus cells", Medicinisch-chemische Untersuchungen, 4 : 441–460.

Nuclein: the chemical of the nucleus

kleine Menge habe ich zu einer N bestimmung verwendet. Ich habe mich daher später mit meinen Versuchen an die ganzen Kerne gehalten, die Trennung der Körper, die ich einstweilen ohne weiteres Präjudiz als lösliches und unlösliches Nuclein bezeichnen will, einem günstigeren Material überlassend.

Therefore, in my experiments I subsequently limited myself to the whole nucleus, leaving to a more favorable material the separation of the substances, that for the present, without further prejudice, I will designate as soluble and insoluble nuclear material ("Nuclein").

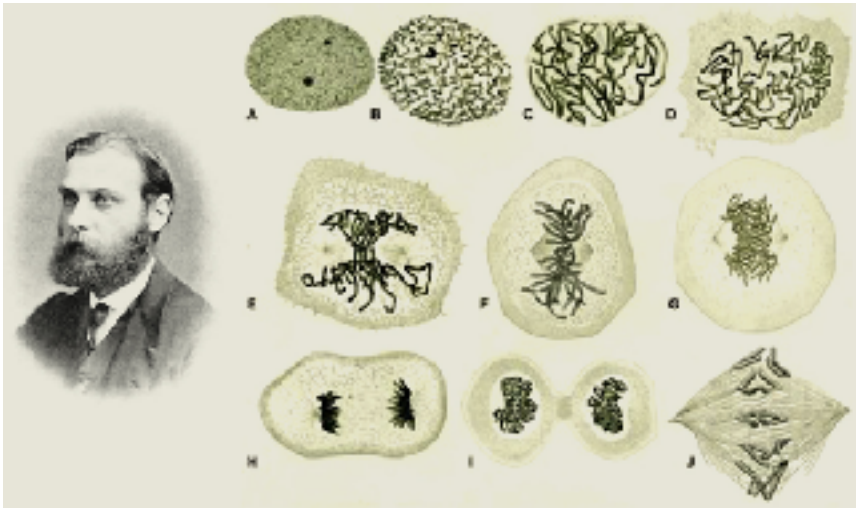
Miescher, Friedrich (1871) "On the chemical composition of pus cells", Medicinisch-chemische Untersuchungen, 4 : 441–460.

More evidence

Chromosome Theory

If Mendel factors segregate during meiosis, then something in the cell must do the same.

Chromatin



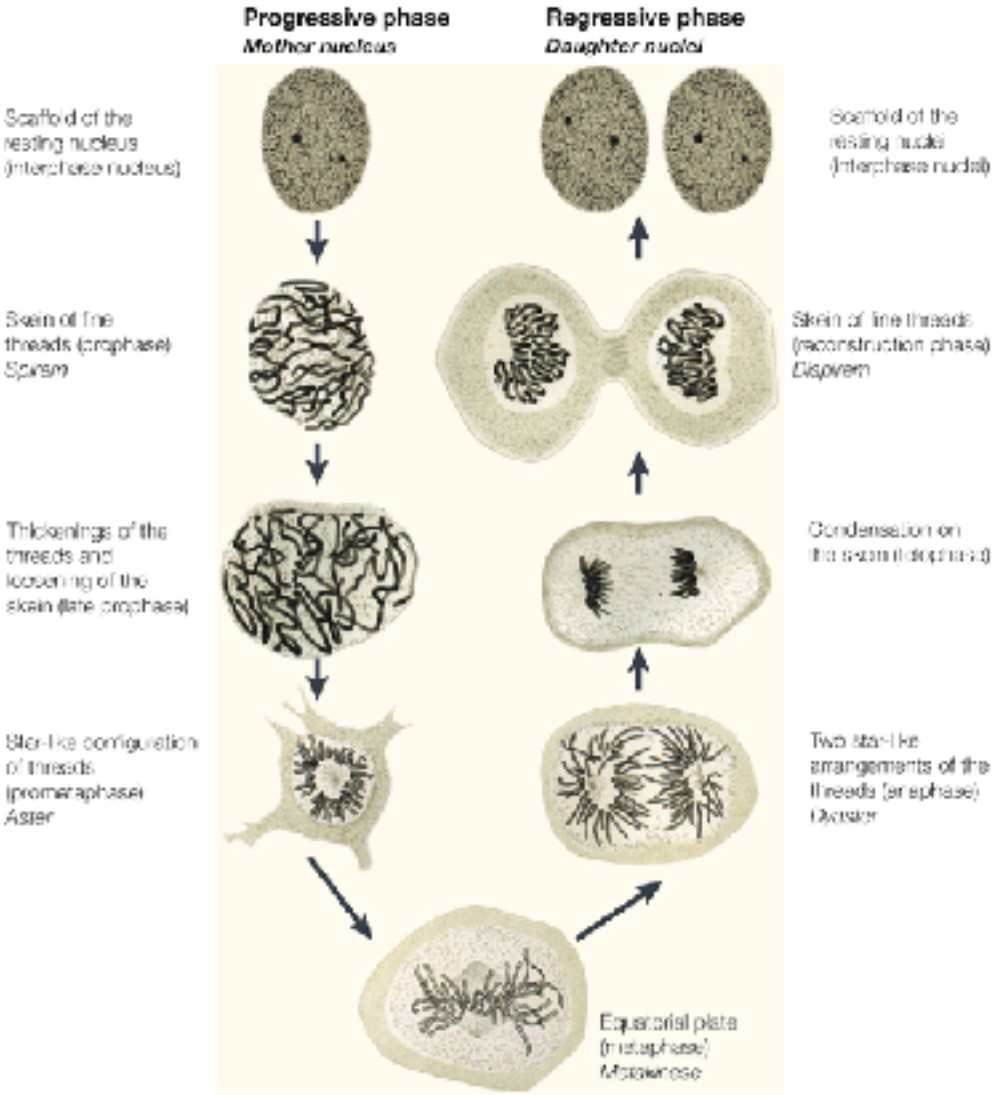
Walther Flemming band structure in dividing cells (1879)

- Used cells of salamanders and staining techniques to study cell division (he called it mitosis).
- The intensely stained parts of the nucleus he called **chromatin** (chroma is Greek for color).

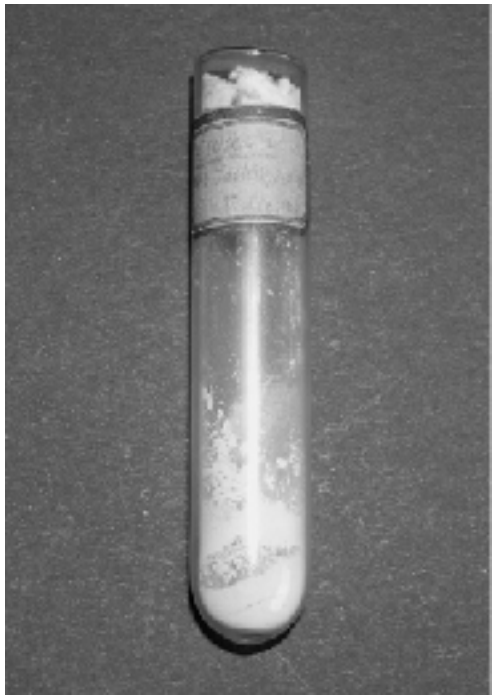
Chromatin

The nucleus content separates during cell division.

What is this substance?



Chromatin

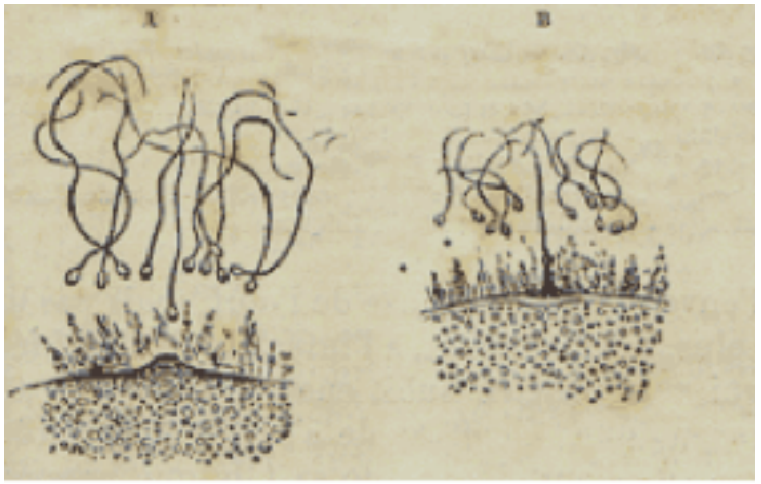


- Isolation of the chemical content results in the same gray precipitate.
- Conclusion: Miescher's nuclien and chromatin are the same.

Chromosomes

Hermann Fol and Oscar Hertwig
(1870-1880)





- Observed fertilization and fusion of the eggs and sperms nuclei.
- Chromatin is called **chromosomes**.

Chromosomes

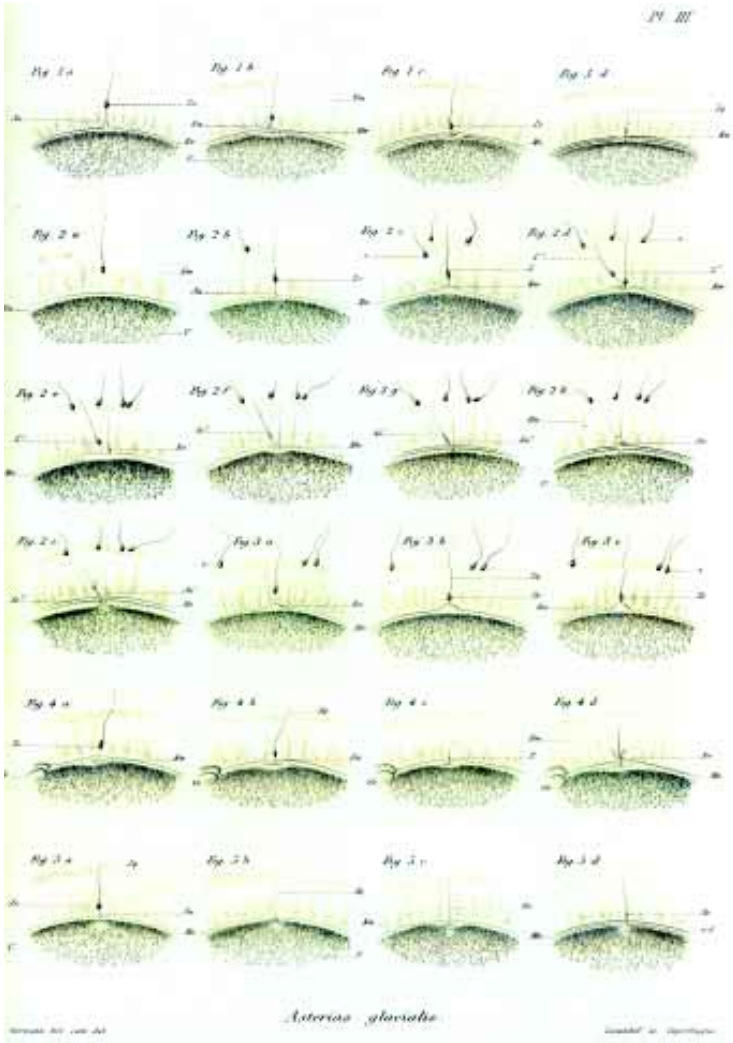


Fig. 8. Plate No III from the paper by Hermann Fol (1879) *showing* in vivo *drawings of the various steps in the fertilisation in* Asterias glacialis *(see Fig. 6).*

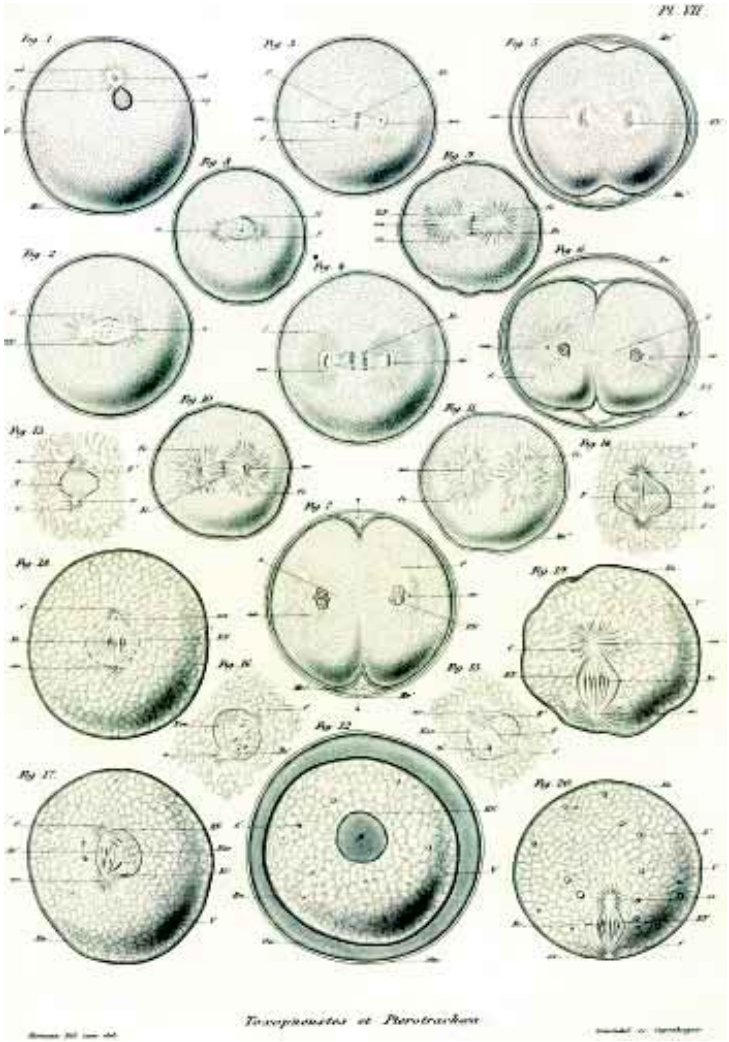


Fig. 7. Plate No. VII from the paper by Hermann Fol (1879), *showing nuclear amphimixy and the early cell divisions after fertilization in fixed material (see Fig. 6).*

Buscaglia M, Duboule D. Developmental biology in Geneva: a three century-long tradition. Int J Dev Biol. 2002 Jan;46(1):5-13

Chromosomes

- The study of fertilization using light microscopy led to:
    - Eggs and sperms have equal number of chromosomes.
    - Chromosomes get passed to future generations.
    - By 1890 almost everybody agreed that nuclien = chromatin = chromosomes are the basis of heredity.

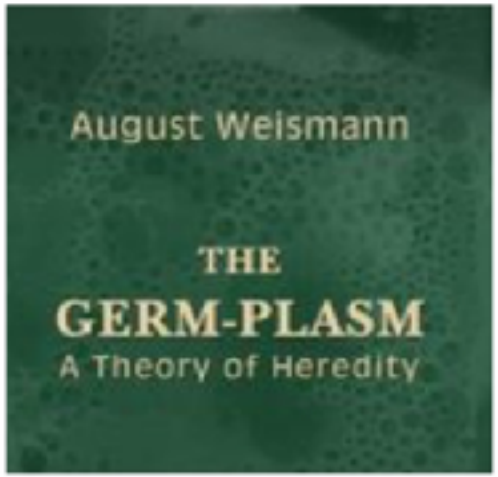
Chromosomes

How do these findings relate to Mendel's experiments?

Fun battle

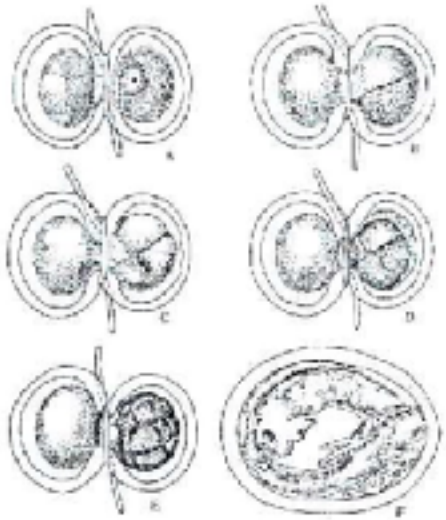
- Sperm and eggs form a zygote.
- **Hypothesis**: When zygote divides, it loses a half of the genetic molecular (instructions).

specialization, one led by Weismann.* Weismann studied zygotes, the fused product of a sperm and egg that formed an animal's first cell. He argued that this first cell obviously contained a complete set of molecular instructions, but that each time the zygote and its daughter cells divided, the cells lost half of those instructions. When cells lost the instructions for all but one type of cell, that's what they became. In contrast, other scientists maintained that cells kept the full set of instructions after each division, but ignored most of the instructions after a certain age.



Fun battle

German biologist Hans Spemann decided the issue in 1902 with a salamander zygote. He centered one of these large, soft zygotes in his microscopic crosshairs, waited until it divided into two, then looped a blond strand of his infant daughter Margrette's hair around the boundary between them. (Why Spemann used his daughter's hair isn't clear, since he wasn't bald. Probably the baby's hair was finer.) When he tightened this noose, the two cells



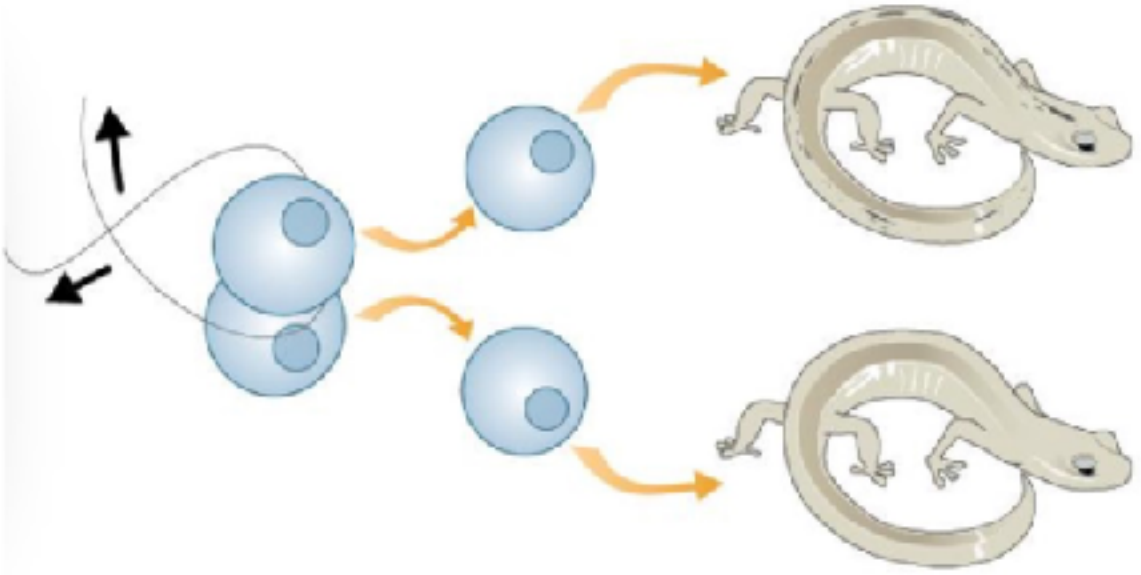
- Test the hypothesis that the genetic material gets reduced with every division.
- Need to separate the two cells resulting from the first zygotic division.
- **How?**

Fun battle

Using the thin hair of his daughter :-) to separate the two cells resulting from the first division of a salamander zygote.

Fun battle

develop separately. Weismann would have predicted two deformed half salamanders. But both of Spemann's cells grew into full, healthy adults. In fact, they were genetically identical, which means Spemann had effectively cloned them – in 1902. Scientists had re-discovered Mendel not long before, and Spemann's work implied that cells must retain instructions but turn genes on and off.



Characteristics of genetic material

The chemical content of the nucleus is the genetic material.

What characteristics this molecule must have?

Characteristics of genetic material

- **What should the hereditary molecule have?**
  - Contain the information in a stable form.
  - Able to self replicate and pass to future generations.
  - Can be changed (allowing for adaptation and evolution).

Chromosomes and heredity

• Chromosomes are composed of proteins and nucleic acid.

Which one is the genetic molecule?

• Many suspected proteins to be the hereditary molecule because of the high capacity to store info (20 amino acids – now 22) compared to 4 nucleotides in DNA.

The hereditary molecule

Proteins or DNA?

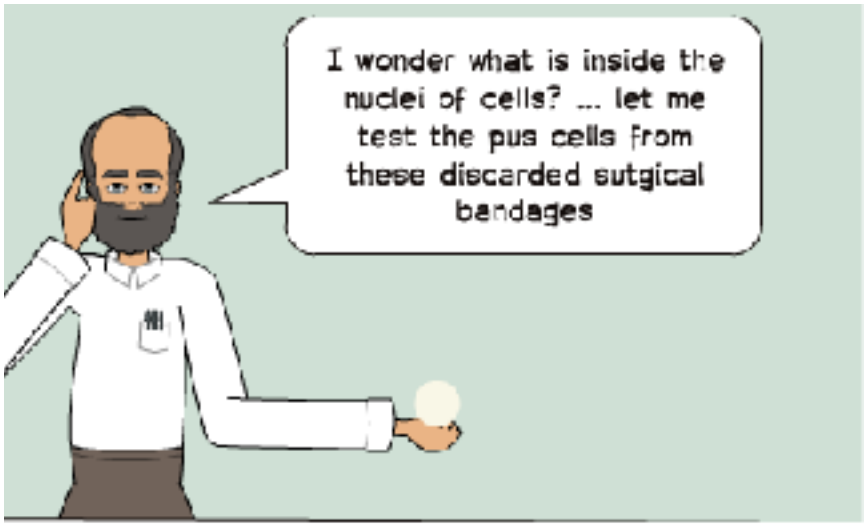
Need some cool experiments

We will go over next lecture.

Expectations

- Know the experiments that lead to the conclusion that the genetic material is located in the nucleus.
- Know the terms used to designate the genetic material before the discovery of DNA.
- Know how each experiment added to the previous knowledge.
- You know a bit of the story to tell your friends and family.

For a smile



1868 ... in the lab of Friedrich Miescher