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Bi4025en
Molecular Biology

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Content of the Course

- 1. Definition and brief **history** o the molecular biology discipline.
- 2. **Nucleic acids**: primary, secondary and tertiary structure of nucleic acids, conformation of DNA and RNA, different conformations of DNA and their significance for biological systems, genetic information and genetic code.
- 3. Molecular **structure** and **replication** of prokaryotic and eukaryotic genomes.
- 4. **Transcription** of prokaryotic and eukaryotic genomes, posttranscriptional modifications and processing of RNA, mechanisms of RNA splicing and self-splicing.
- 5. **Translation** of prokaryotic and eukaryotic mRNAs.

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Content of the Course

- 6. **Posttranslational processing** of proteins.
- 7. **Regulation of gene expression** in prokaryotes and eukaryotes.
- 8. Molecular mechanisms of **mutagenesis** and **recombination**.
- 9. Molecular basis of **cancerogenesis** (oncogenes, antioncogenes).
- 10. **DNA Repair** mechanisms.
- 11 **Mobile** genetic **elements**, transposons and retrotransposons.
- 12. Basic principles of **genetic engineering**.

Content of the Course

- the subject of study of the molecular biology, its origin and the main stages of development, structure and function of macromolecules, nucleic acids and proteins
- basic concepts of molecular biology: genetic information, genetic code, gene definition, types of genes
- characteristic of Prokaryotic and Eukaryotic genomes
- DNA replication, regulatory proteins and mechanism
- Prokaryotic and Eukaryotic transcription, posttranscription modification of RNA
- translation, cotranslation and posttranslational processes, selfassambly

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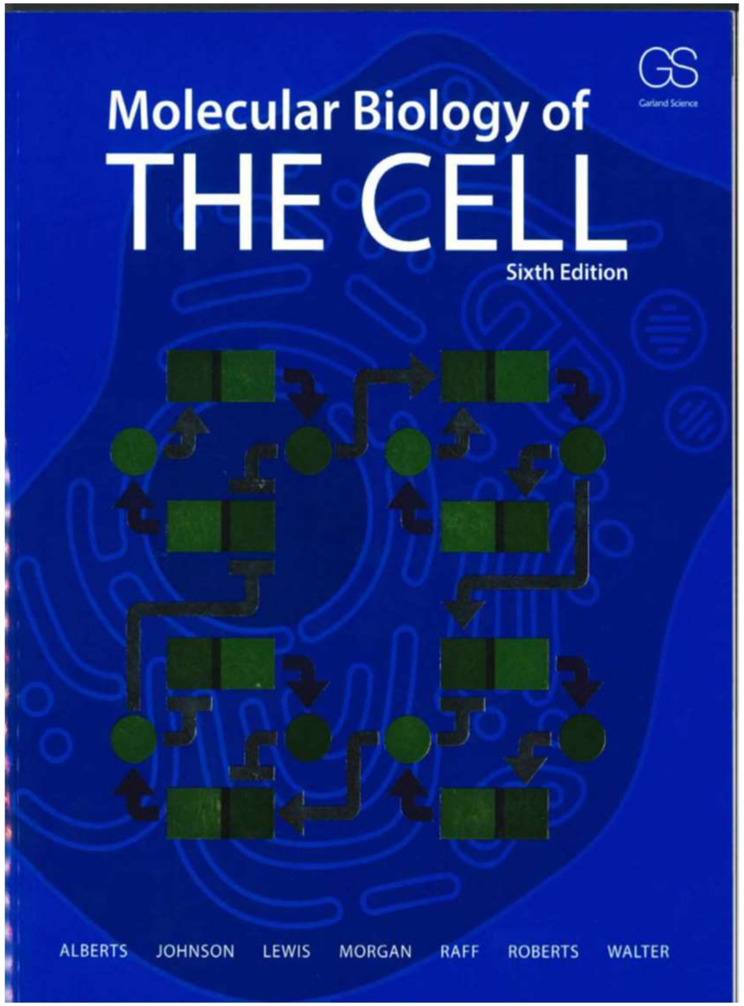
Studying sources

- PowerPoint presentations
- Literature

W. W. Norton & Company, Inc., 500 Fifth Avenue, New York, New York 10110

 Alberts et al.: Molecular biology of the cell.

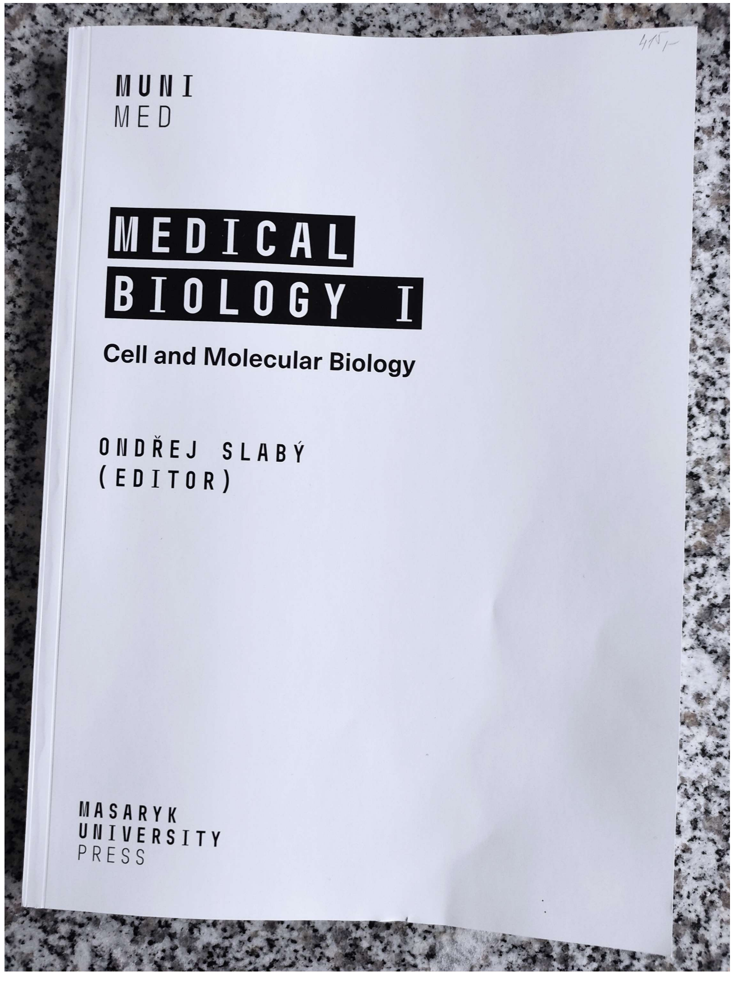
2014



Masaryk University Press, Brno, 2023

 Slabý et al., Medicical Biology I.

2023



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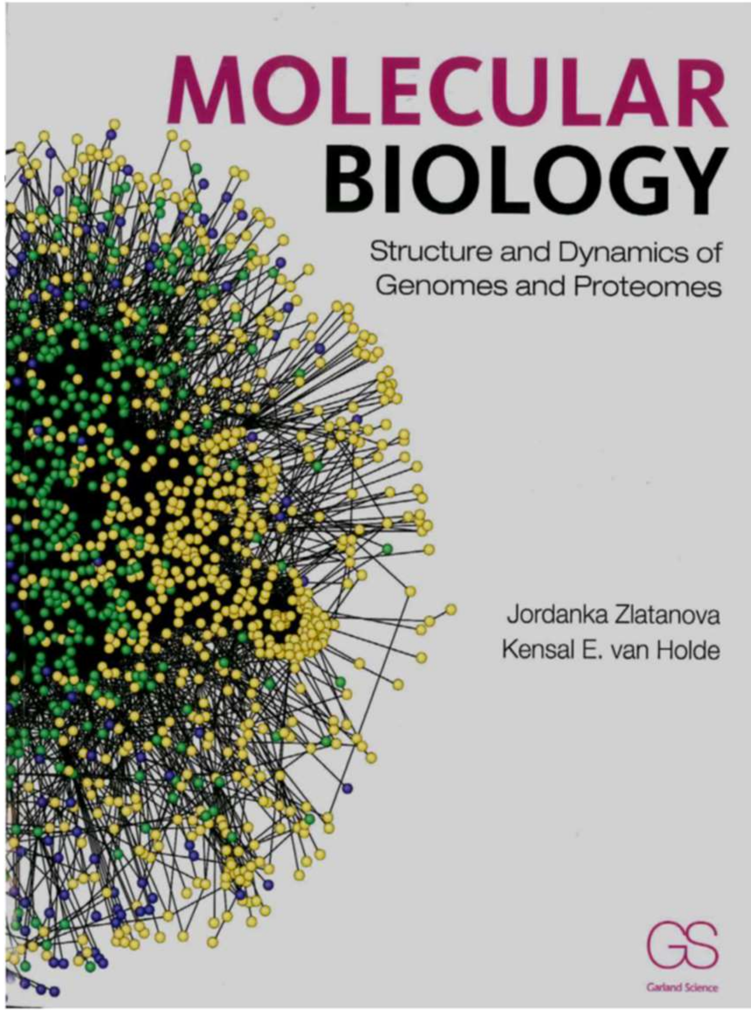
Garland Science, Taylor & Francis Group: New York, USA and Abingdon, UK.

 Zlatanova and van Holde: Molecular Biology: Structure and Dynamics of Genomes and Proteomes

2016



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Exam requirements

- Final Exam – written test and oral exam – 50% + 50% of final grade

- Written Test
  - 50 questions
  - 60 % to pass
  - Score
    - A – 100 – 92
    - B – 92 - 84
    - C – 84 – 76
    - D – 76 – 68
    - E – 68 – 60

- Oral Exam
  - 2 questions

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Lecture 1

- Definition and brief history of the Molecular Biology

Aim of Molecular Biology

- Clarify the relationship of the structure and interactions of biomacromolecules, in particular, the informational biomacromolecules, on the functions and properties of living systems.

- Explanation of functions and properties of living systems based on structure and interaction of their molecules.

- Integration of physical, chemical, biological and bioinformatical approaches.

- Knowledge of the processes that take place in the living systems at the molecular level in the realization of genetic information.

Definition of Molecular Biology

- Study of the structure, interaction and function of biological macromolecules.
- Elucidation of the molecular properties of the life.
- Deciphering the molecular entity/constituency of the cell.
- Elucidate the genetic information and the mechanisms of its impact on living organism.

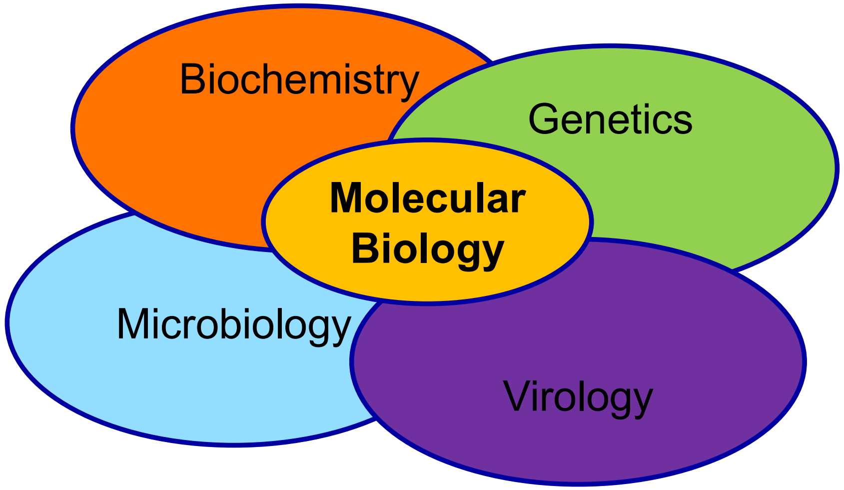
Molecular biology is not Biochemistry

Biochemistry

- It studies chemical processes in living biological organisms.
- Description of nucleic acid and protein as well as organic molecules (lipids, sugars and carbohydrates).

Origin of Molecular Biology

- The history of the Molecular Biology begins in 1930s with the union of various, previously distinct biological disciplines, such as
  - Biochemistry
  - Genetics
  - Microbiology
  - Virology.
- In the modern sense, <u>molecular biology attempts to explain the phenomena of life</u> starting from the macromolecule properties that generate them.



https://www.slideshare.net/indrajay/history-of-molecular-biology-134296287

Origin of Molecular Biology

- Molecular biologists focus primarily on two macromolecules.

- Nucleic acid
  - DNA – deoxyribonucleic acid propagating genes in time
  - RNA - ribonucleic acid – sustaining gene propagation
  - sncRNA, miRNA, piRNA… - regulatory function

- Proteins
  - active agents of the life

- Scope of the Molecular biology is to seek, characterize and interpret the structure, function and relationships between these types of macromolecules.

https://www.slideshare.net/indrajay/history-of-molecular-biology-134296287

Definition of Molecular Biology

- Director of the Natural Sciences Division of the Rockefeller Foundation Warren Weaver.

- In 1938 he coined the term „Molecular biology“ to describe the use of techniques from the physical sciences (X-rays, radioisotopes, ultracentrifuges, mathematics, etc.) to study living matter.

- In the same year the Rockefeller Foundation awarded research grants to Linus Pauling for research on the structure of hemoglobin.

- Under Weaver's direction the Rockefeller Foundation became a primary funder of early research in molecular biology.

Warren Weaver
(1894-1978)

https://www.slideshare.net/indrajay/history-of-molecular-biology-134296287
Molecular biology: origin of the term, Science 170 (1970) 591-2.
https://www.historyofinformation.com/detail.php?id=3962

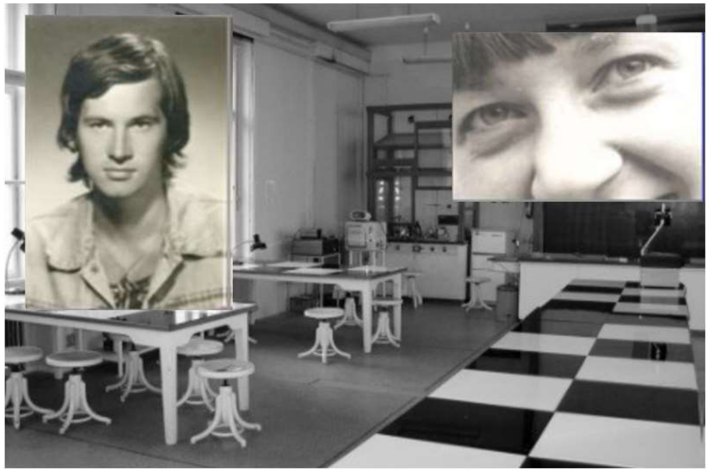
History of Molecular Biology

- Molecular biology arises in the form of molecular genetics synthesis of the functionalist and structuralist "school„ in protein and nucleic acid research.

- Structuralist (physicists, chemists)
- focus on <u>structure of biomacromolecules</u> (proteins, NK), <u>not on function and inheritance</u>
  - W. T. Astbury
  - J.D. Bernal
  - L. Pauling
  - E. Chargaff
  - M.H.F. Wilkins
  - F.H.C. Crick

- Functionalists (biochemists, virologists, microbiologists, geneticists)
- focus on <u>preservation and transfer of genetic information</u> (bacteria and bacteriophages)
  - M. Delbrück, E. Schrödinger
  - G.W. Beadle, E.L. Tatum
  - O.T. Avery, C.M. MacLeod,M. McCarty, J. Lederberg
  - A.D. Hershey
  - J.D. Watson

School of MB in Brno - prof. Stanislav Rosypal, DrSc.



History of Molecular Biology

- Relatively young science.
- The origin is established by many, but four fundamental discoveries:
  - Understanding the Structure and Function of Nucleic Acids (1944, 1953)
  - Deciphering the Genetic Code (1966)
  - Description and understanding of the processes by which genetic information is not only inherited but propagates in live (transcription, translation, regulation of gene expression)
  - Discovery, description, development and donation of approaches for gene editing (2011, 2013).

History of Molecular Biology

- 1865: Gregor Mendel discovers through breeding experiments with peas that traits are inherited based on specific laws (later to be termed „ Mendel's laws or principles“).
- 1866: Ernst Haeckel proposes that the nucleus contains the factors responsible for the transmission of hereditary traits.
- 1866: Felix Noppe-Seyer – identifies hemoglobin and its ability to bound oxygen.
- 1869: Friedrich Miescher isolates DNA for the first time.
- 1871: The first publications describing DNA (nuclein) by Friedrich Miescher, Felix Hoppe-Seyler, and P. Plo´sz are printed.
- 1882: Walther Flemming describes chromosomes and examines their behavior during cell division.
- 1884 – 1885: Oscar Hertwig, Albrecht von Kflliker, Eduard Strasburger, and August Weismann independently provide evidence that the cell's nucleus contains the basis for inheritance.
- 1889: Richard Altmann renames nuclein to nucleic acid.
- 1885 – 1901: Albrecht Kossel describes pyrimidines and purines in nucleic acids.

R. Dahm / Developmental Biology 278 (2005) 274–288

History of Molecular Biology

- 1900: Carl Correns, Hugo de Vries, and Erich von Tschermak rediscover Mendel’s Laws.
- 1902: Theodor Boveri and Walter Sutton postulate that the heredity units (called genes as of 1909) are located on chromosomes.
- 1905: William Bateson as first person uses the term „ Genetics“ in order to describe the study of heredity.
- 1909: Wilhelm Johannsen uses the word „ gene“ to describe units of heredity.
- 1910: Thomas Hunt Morgan uses fruit flies (Drosophila) as a model to study heredity and finds the first mutant (white) with white eyes.
- 1913: Alfred Sturtevant and Thomas Hunt Morgan produce the first genetic linkage map (for the fruit fly Drosophila).
- 1928: Frederick Griffith postulates that a transforming principle permits properties from one type of bacteria (heat-inactivated virulent *Streptococcus pneumoniae*) to be transferred to another (live nonvirulent *Streptococcus pneumoniae*).
- 1929: Phoebus Levene identifies the building blocks of DNA, deoxyribonucleic and ribonucleic acid, as well as four bases adenine (A), cytosine (C), guanine (G), and thymine (T). The Tetra nucleotide hypothesis.

R. Dahm / Developmental Biology 278 (2005) 274–288

History of Molecular Biology

- 1934: Caspersson and Hammersten determined that DNA is polymer.
- 1935: Max Delbrück, Nikolai V. Timofeeff-Ressovsky, and Karl G. Zimmer suggested that chromosomes are very large molecules, its structure can be changed by treatment with X-rays leading to changes of heritable characteristics.
- 1941: George Beadle and Edward Tatum demonstrated that every gene is responsible for the production of an enzyme.
- 1944: Oswald T. Avery, Colin MacLeod, and Maclyn McCarty demonstrated that Griffith's transforming principle is not a protein, but rather DNA, suggesting that DNA may function as the genetic material.
- 1949: Colette and Roger Vendrely and Andre´ Boivin discover that the nuclei of germ cells contain half the amount of DNA that is found in somatic cells. This parallels the reduction in the number of chromosomes during gametogenesis and provides further evidence for the fact that DNA is the genetic material.
- 1949–1950: Erwin Chargaff finds that the DNA base composition varies between species but determines that within a species the bases in DNA are always present in fixed ratios: the same number of A's as T's and the same number of C's as G's.

R. Dahm / Developmental Biology 278 (2005) 274–288

History of Molecular Biology

- 1952: Alfred Hershey and Martha Chase use viruses (bacteriophage T2) to confirm DNA as the genetic material.
- 1953: Rosalind Franklin and Maurice Wilkins use X-ray analyses to demonstrate that DNA has a regularly repeating helical structure.
- 1953: James Watson and Francis Crick discover the molecular structure of DNA: a double helix in which A always pairs with T, and C always with G.
- 1956: Arthur Kornberg discovers DNA polymerase, an enzyme that replicates DNA.
- 1957: Francis Crick proposes the „ central dogma“ (information in the DNA is translated into proteins through RNA) and speculates that three bases in the DNA always specify one amino acid in a protein.
- 1958: Matthew Meselson and Franklin Stahl describe how DNA replicates (semiconservative replication).
- 1960 – Jacob and Monod – determined the mRNA as a carrier of genetic information which is propagated in to the protein structure.
- 1961–1966: Robert W. Holley, Har Gobind Khorana, Heinrich Matthaei, Marshall W. Nirenberg, and colleagues crack the genetic code.

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History of Molecular Biology

- 1975: Sanger and Coulson the termination chain sequencing method.
- 1977: Maxam and Gilbert the chemical method for sequencing.
- 1986: Mullis established specific enzymatic amplification of DNA in vitro – polymerase chain reaction.
- 1995: First complete sequence of the genome of a free-living organism (the bacterium *Haemophilus influenzae*) is published.
- 1996: The complete genome sequence of the first eukaryotic organism - the yeast *Saccharomyces cerevisiae* - is published.
- 1998: Complete genome sequence of the first multicellular organism - the nematode worm.
- 1998: Fire and Mello pull out RNA interference concept.
- 2000: The complete sequences of the genomes of the fruit fly Drosophila and the first plant - Arabidopsis - are published.
- 2001: The complete sequence of the human genome is published.
- 2011 - 2012: Charpentier and Doubna introduce CRISPR editing approach to the science.
- 2013: Zhang develops tools to edit genomic DNA in various organisms.

R. Dahm / Developmental Biology 278 (2005) 274–288

Biochemistry foundation

- German physiologist and chemist, and the principal founder of the disciplines of biochemistry and molecular biology.

- He also recognized the binding of oxygen to erythrocytes as a function of hemoglobin, which in turn creates the compound oxyhemoglobin. Hoppe-Seyler was able to obtain hemoglobin in crystalline form and confirmed that it contained iron.

- He performed important studies on chlorophyll.

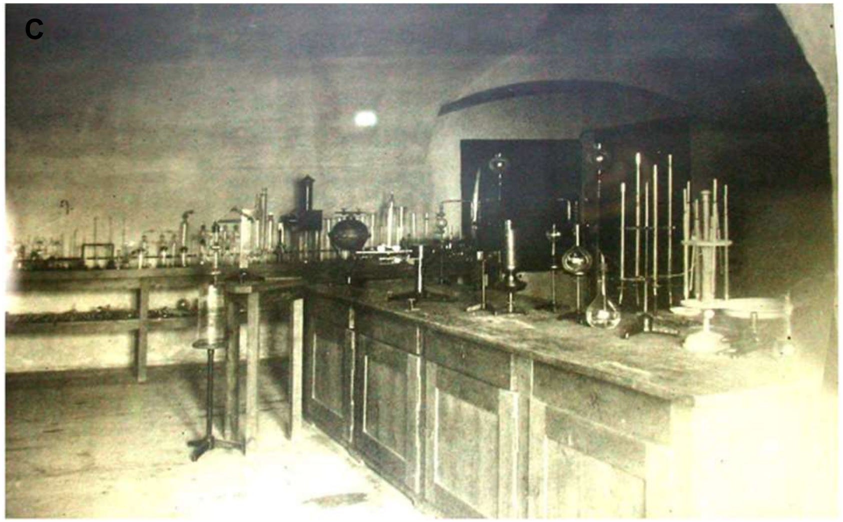
- He is also credited with the isolation of several different proteins (which he referred to as proteids). In addition, he was the first scientist to purify lecithin and establish its composition.

- His students Friedrich Miescher and Nobel laureate Albrecht Kossel.

Felix Hoppe – Seyler
(1825 – 1895)

https://en.wikipedia.org/wiki/Felix_Hoppe-Seyler

Biochemistry foundation



(A) Historic photography of Tübingen castle overlooking the old town.
(B) Tübingen castle today.
(C) Photograph of Felix Hoppe-Seyler’s laboratory around 1879. Prior to becoming the chemical laboratory of Tübingen University in 1823.

Biol. 2005 Feb 15;278(2):274-88. doi: 10.1016/j.ydbio.2004.11.028.

Discovery of Nuclein

- Swiss naturalist and physician.
- Miesher works as the doctoral student in the lab of prof. Hoppe-Seyler.
- He isolates leukocytes from pus (on bandages), breaks down nuclear proteins by pepsin (a proteolytic enzyme isolated from the stomach of pigs) in order to disrupt the structure of cells and to describe released ingredients.
- He subjected the purified nuclei to an alkaline extraction followed by acidification, resulting in the formation of a precipitate that Miescher called nuclein, which is resistant to proteases and lipases
- The function of the nuclein remains unclear for a long time, but Miescher proves, that it is present in the nuclei of all cells and suggests that it could play a role in inheritance.

Johannes Friderich Miescher
(1844 – 1895)

Biol. 2005 Feb 15;278(2):274-88. doi: 10.1016/j.ydbio.2004.11.028.

Discovery of Nuclein

(A)

(B)

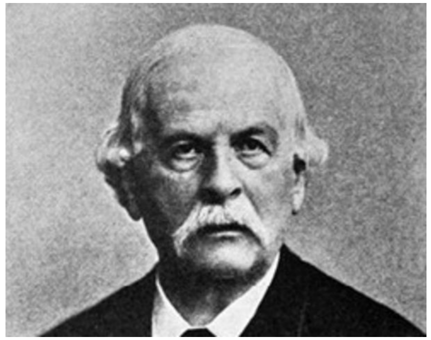
(A) Glass vial containing <u>nuclein isolated from salmon sperm</u> by Friedrich Miescher while working at the University of Basel.

(B) 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 and several smaller utensils, such as glass alembics and a glass distillation column on the side board.

Biol. 2005 Feb 15;278(2):274-88. doi: 10.1016/j.ydbio.2004.11.028.

Nuclein is Nucleic Acid

- German pathologist and histologist.
- 1889 named Miescher´s term „ nuclein“ by the term „ nucleic acid“, when he demonstrated that nuclein was acidic.
- He is also recognized for observation of filaments in the nearly all cell types, developed from granules. He named the granules „ bioblasts“.
- He explained them as the elementary living units, having metabolic and genetic autonomy, it is believed he decribed the mitochondria.

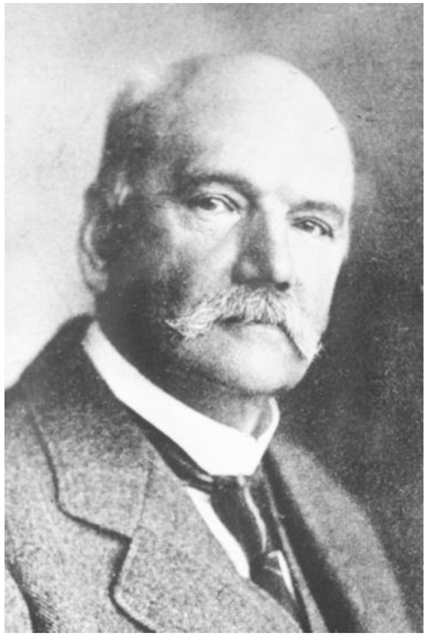


Richard Altmann
1852 – 1900

https://alchetron.com/Richard-Altmann

Nucleic Acid contains Nucleobases

- German biochemist, who studied under Felix Hoppe-Seyer.
- He described chemical composition of nucleic acids having pyrimidines and purines.
- Between 1885 - 1901, he was able to isolate and name its five constituent organic compounds:
  adenine, cytosine, guanine, thymine, and uracil.
- These compounds are now known collectively as nucleobases, and they provide the molecular structure necessary in the formation of stable DNA and RNA molecules.
- 1910 – Nobel Prize for Physiology or Medicine.

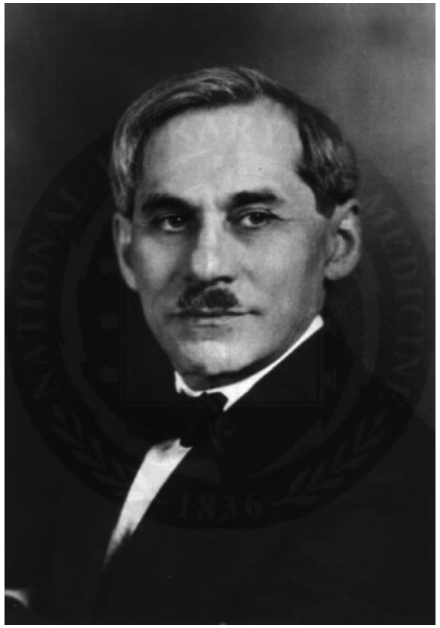


Albrecht Kossel
1852 – 1927

https://alchetron.com/Albrecht-Kossel

Nucleic Acid has two Forms

- In 1909, Levene and Walter Jacobs recognised D-ribose as a natural product and an essential component of nucleic acids.
- In 1929 Levene also discover the D-deoxyribose in nucleic acid.
- He identified components within the nucleic acids and showed that were linked together in the order phosphate-sugar-base to form units.
- He called each of these units a nucleotide, and stated that the DNA molecule consisted of a string of nucleotide units linked together through the phosphate groups, which are the backbone of the molecule.

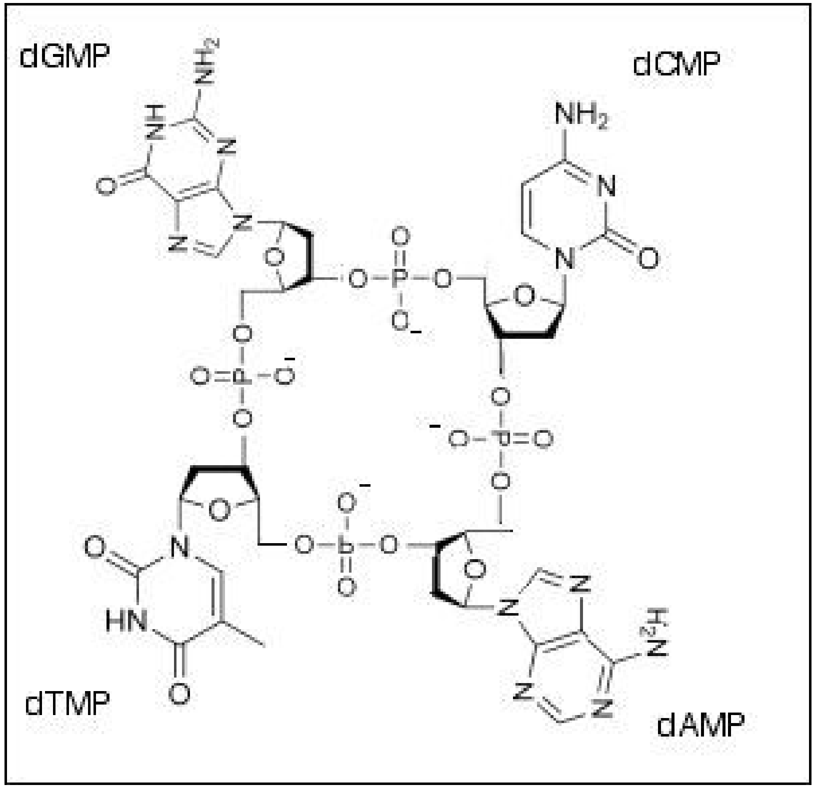


Phoebus Levene
(1869 – 1940)

https://en.wikipedia.org/wiki/Phoebus_Levene

Nucleic Acid has two Forms

Levene's Tetranucleotide Hypothesis (1910)



- He called the <u>phosphate</u> - <u>sugar</u> - <u>base unit</u> a nucleotide.

- Note that adjacent sugar molecules are connected by a 3'-5' phospho-diester linkage, and bases are attached to the 1'-C of the sugar, just as in the Watson-Crick model. However, each <u>four-nucleotide component is a separate</u> molecule, and the <u>bases are directed to the outside</u>.

- The simplicity of this structure implied that nucleic acids were too uniform to contribute to complex genetic variation. Attention thereafter focused on protein as the probable hereditary substance.

https://www.mun.ca/biology/scarr/Tetranucleotide_Hypothesis.html

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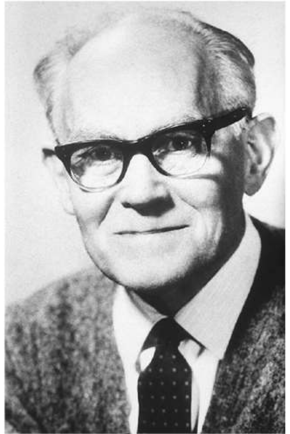
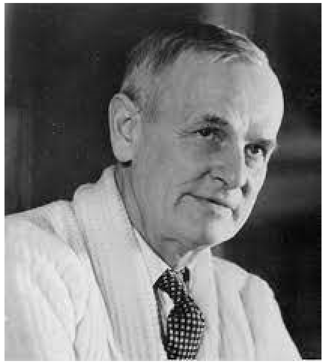
Nucleic Acid is a polymer - macromolecules

- Swedish biochemist Einar Hammersten, conducted investigations into the molecular
mass of DNA (deoxyribonucleic acid). This research led to the discovery that DNA was a polymer, or macromolecule, made up of small, repeating units.



Tjorborn Caspersson
(1910 – 1997)

- In the 1934 he and Einar Hammarsten showed that DNA was a polymer. Previous theories suggested that each molecule was only ten nucleotides long.



Einar Hammersten
(1889 – 1968)

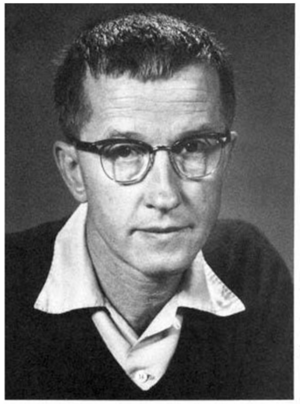
https://www.jbc.org/article/S0021-9258(19)60918-X/pdf

Chromosomes are macromolecules and carry heritable traits

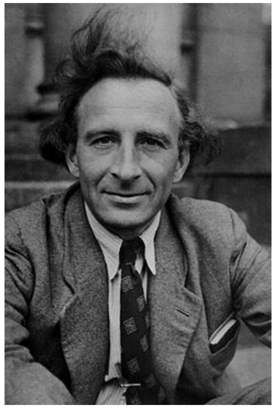
- Max Delbrück, Nikolai V. Timofeeff-Ressovsky, and Karl G. Zimmer published results in 1935 suggesting that chromosomes are very large molecules.

- The structure of chromosomes can be changed by treatment with X-rays.

Max Delbrück



NV Timofeeff-Ressovsky



KG Zimmer

- Alteration of chromosome´s structure led to change of the heritable characteristics governed by those chromosomes.

- It was thought as a major advance in understanding the nature of gene mutation and gene structure.

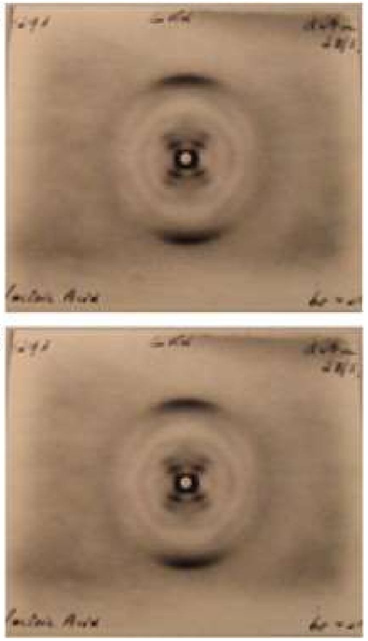
https://alchetron.com/Max-Delbrück
https://alchetron.com/Nikolay-Timofeev-Ressovsky
https://www.mdc-berlin.de/karl-guenther-zimmer

Nucleic Acid has regular structure

- William Astbury was an English physicist and molecular biologist who made pioneering X-ray diffraction studies of biological molecules.

William Astbury
(1898 – 1961)

(i)
(ii)

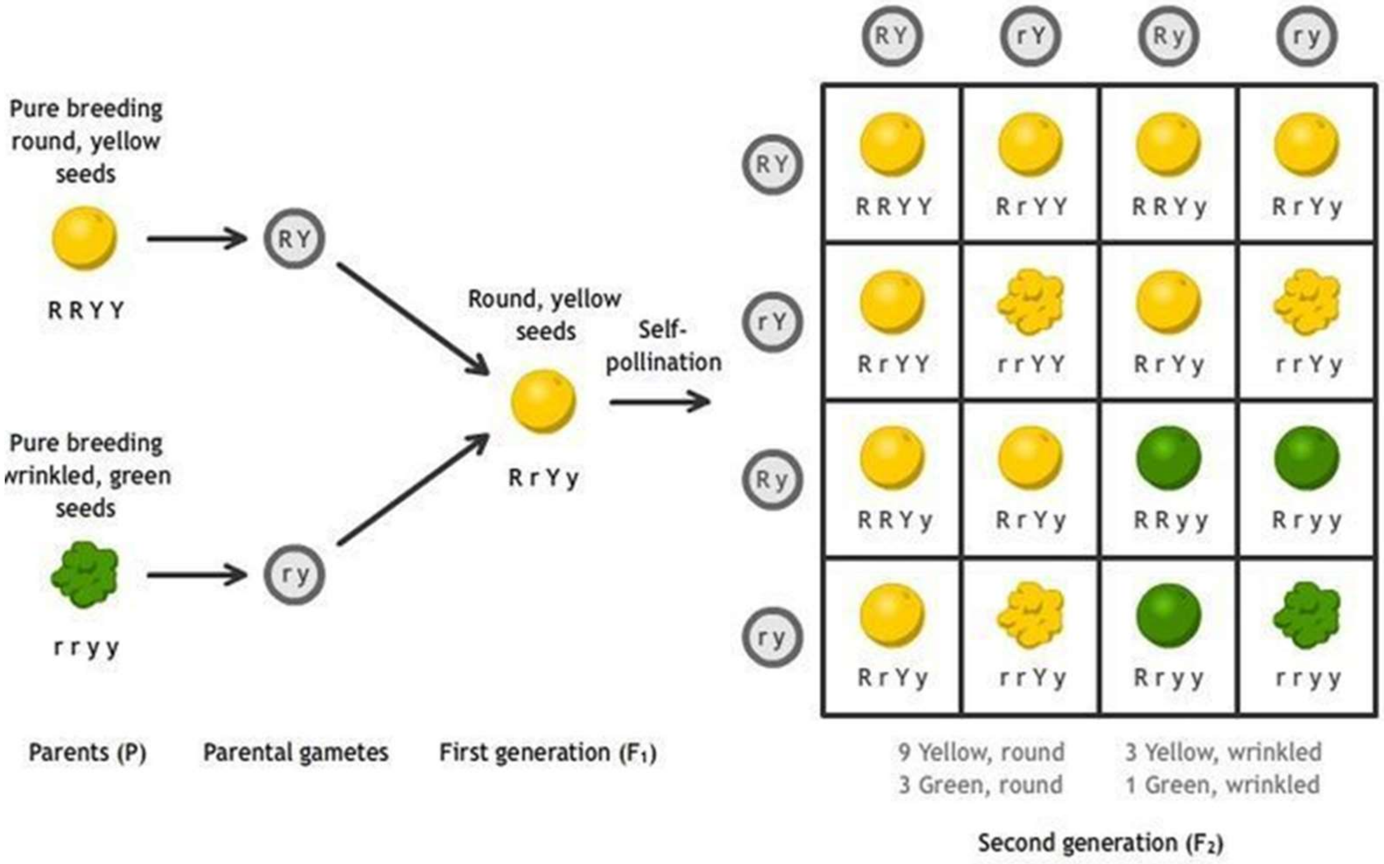


- 1937 he studied the structure for DNA.
- Tjorborn Capersson prepared DNA for his first studies.
- The patterns showed that DNA had a regular structure and therefore it might be possible to deduce what this structure was.
- X-ray diffraction photographs taken by Elwyn Beighton in Astbury's laboratory of B-form sodium thymonucleate fibres on (i) 28th May 1951 and (ii) 1st June 1951.

Studies in History and Philosophy of Biological and Biomedical Sciences 42 (2011) 119–128

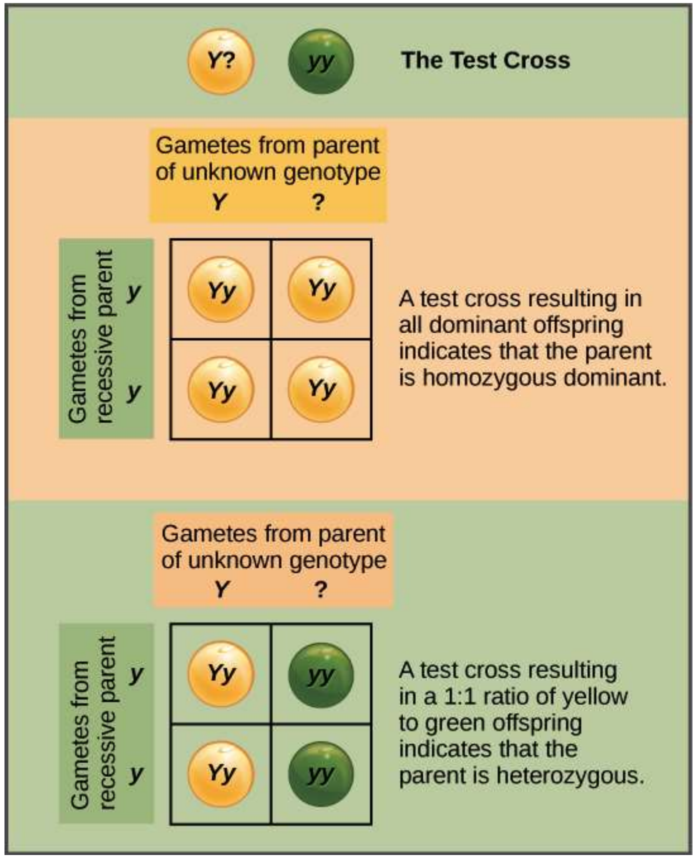
Johann Gregor Mendel principles

- 1. Dominance
- 2. Segregation
- 3. Independent assortment



https://www.sciencelearn.org.nz/resources/2000-mendel-s-principles-of-inheritance

Johann Gregor Mendel principles



- Mendel also came up with a way to figure out whether an organism with a dominant phenotype was a heterozygote (Yy) or a homozygote (YY).
- Test cross is an experimental cross of an individual organism of dominant phenotype but unknown genotype and an organism with a homozygous recessive genotype (and phenotype).
- Test cross is still used by plant and animal breeders today.

Johann Gregor Mendel
(1822 – 1884)

https://www.khanacademy.org/science/ap-biology/heredity/mendelian-genetics-ap/a/the-law-of-segregation

Rediscovery of Mendel´s principles

- 1990 – Huge de vries, Carl Correns, Erich von Tschermak rediscovered Mendel´s principals of heridity.
- 1901 – Hugo de Vries – introduce term „ Mutation?

H. De Vries
(1848 – 1935)

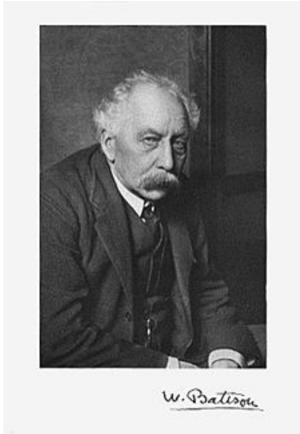
C. Corens
(1864-1933)

E. Von Tschermak
(1871 – 1968)

https://www.eucarpia.eu/tschermak-seysenegg

Rediscovery of Mendel´s principles

- First person to use the term „ Genetics“ in order to describe the study of heredity.
- Based on Mendel's findings, he said, we can develop a new theory that is the correct way to study heredity and will further shed light on the nature of evolution.



William Bateson
(1861 – 1926)

Wilhelm Johansen
(1857 – 1927)

- 1909 - plant physiologist, and geneticist. He is best known for coining the terms gene, phenotype and genotype.
- Gene – unit of heriditary material.

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Chromosomes carry heritable traits

- Boveri–Sutton chromosome theory, also known as the chromosome theory of inheritance is a fundamental theory of genetics proposing that the behavior of chromosomes during meiosis can explain Mendel's laws of inheritance and identifies chromosomes as the carriers of genetic material.

- Boveri studied sea urchins - all the chromosomes had to be present for proper embryonic development to take place.

- Sutton's work with grasshoppers showed that chromosomes occur in matched pairs of maternal and paternal chromosomes which separate during meiosis and "may constitute the physical basis of the Mendelian law of heredity".

Watler Sutton (1877 – 1916)

Theodor Boveri (1862 – 1915)

https://en.wikipedia.org/wiki/Boveri%E2%80%93Sutton_chromosome_theory
https://www.khanacademy.org/science/ap-biology/heredity/chromosomal-inheritance-ap/a/discovery-of-the-chromosomal-basis-of-inheritance

Chromosomes carry heritable traits

- 1910 Morgan noticed a white-eyed mutant male among the red-eyed wild types.

- 1911, he concluded that:
  - (1) some traits, white-eye, were sex-linked,
  - (2) the trait was probably carried on one of the sex chromosomes,
  - (3) other genes were probably carried on specific chromosomes as well.

Thomas Hunt Morgan
(1866 – 1945)

- He and his colleagues combined Mendelism and the chromosome theory of inheritance.

- They established the Mendelian genetics - the inheritance patterns may be generally explained by assuming that genes are located in specific sites on chromosomes.

https://www.mun.ca/biology/scarr/4270_Sex-linkage_in_Drosophila.html

Discovery of bacterial Transformation

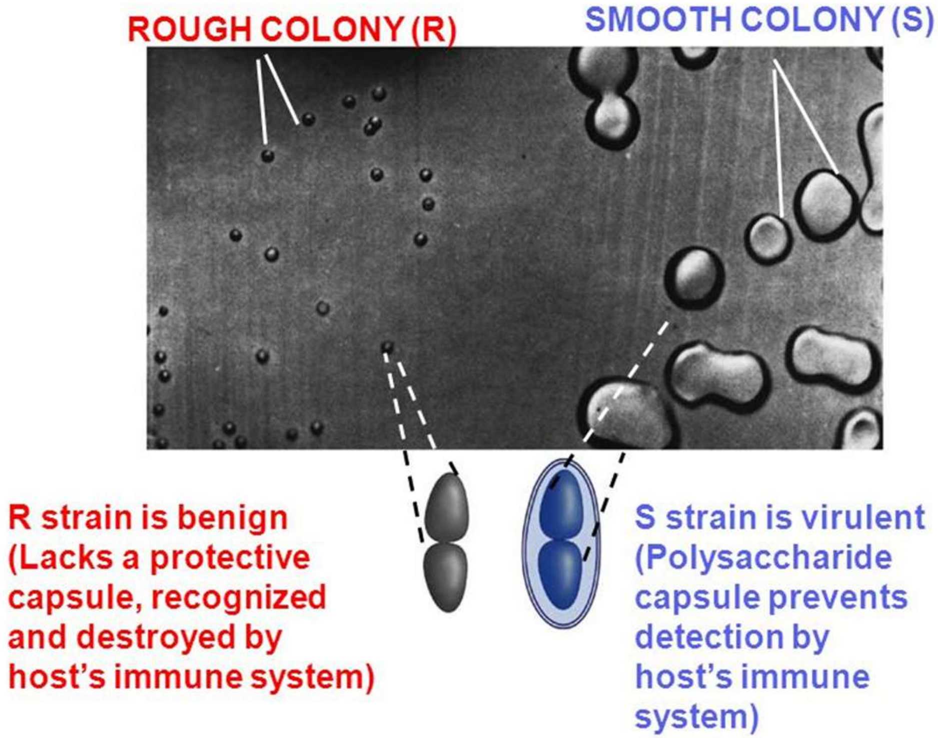
- Frederick Griffith - English bacteriologist.
- In the 20s of the 20th century, he examines the bacterium *Streptococcus pneumoniae*, as a consequence of the Spanish flu in 1918,often accompanied by pneumonia caused by this bacterium.
- The Ministry of Health requires research on *S. pneumoniae* and the creation of a vaccine.
- In January 1928 he reported his work, what is now known as Griffith's Experiment, the first widely accepted demonstrations of bacterial transformation, whereby a <u>bacterium distinctly changes its form and function</u>.

Frederick Griffith
(1877 – 1941)

https://www.mun.ca/biology/scarr/4270_Sex-linkage_in_Drosophila.html

Discovery of bacterial Transformation

- There are 2 related strains *S. pneumoniae*, which differ morphologically and the degree of pathogenicity:
  - the R strain forms rough colonies, avirulent, not lethal
  - the S strain forms smooth colonies, virulent, after injection kills experimental mice.



https://slideplayer.com/slide/4955288/

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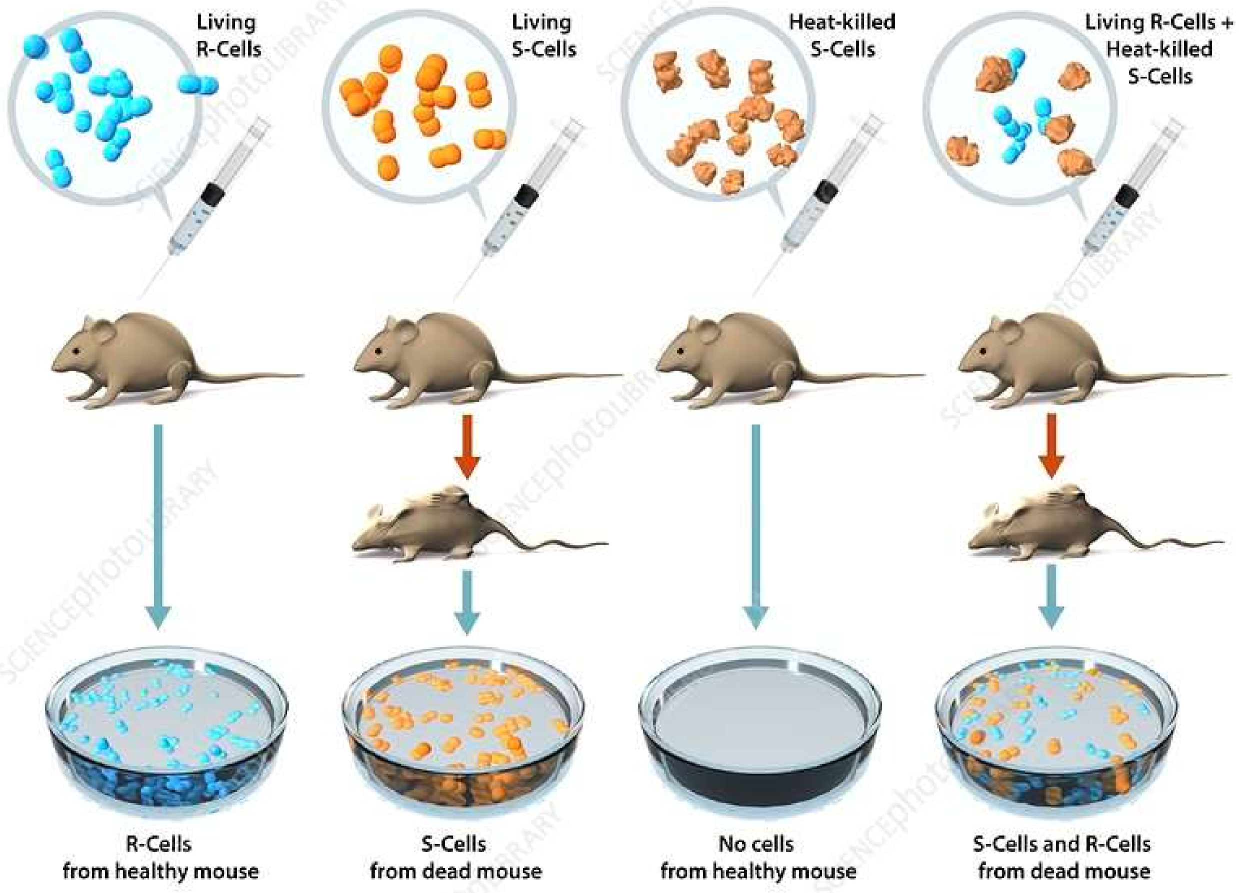
Discovery of bacterial Transformation



- The R strain forms <u>rough colonies</u>, avirulent, not lethal.
- The S strain forms <u>smooth colonies</u>, virulent, after injection kills experimental mice.

https://www.sciencephoto.com/media/717359/view/griffith-s-experiment-illustration

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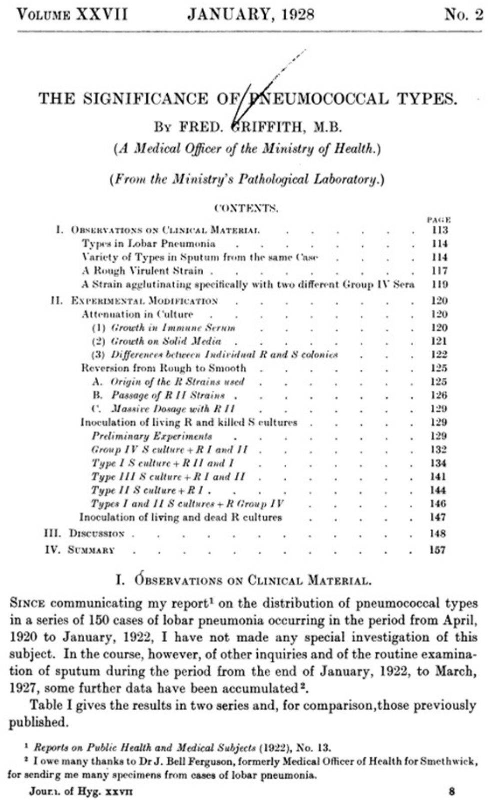
Results of Griffith´s Experiments

- There is a chemical compound capable of transmitting hereditary instructions between organisms „ gene molecule“.
- Restrained Griffith delays the publication of this revolutionary conclusion.
- In January 1928 under pressure from friends he publishes its experiments in unknown journal „ Journal of Hygiene“.
- Article written in an remorseful style for the turmoil, which it causes to the genetics.



VOLUME XXVII JANUARY, 1928 No. 2

THE SIGNIFICANCE OF PNEUMOCOCCAL TYPES.

BY FRED. GRIFFITH, M.B.
(A Medical Officer of the Ministry of Health.)
(From the Ministry's Pathological Laboratory.)

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I. OBSERVATIONS ON CLINICAL MATERIAL.

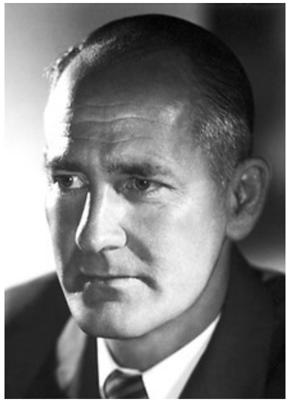
SINCE communicating my report[1] on the distribution of pneumococcal types in a series of 150 cases of lobar pneumonia occurring in the period from April, 1920 to January, 1922, I have not made any special investigation of this subject. In the course, however, of other inquiries and of the routine examination of sputum during the period from the end of January, 1922, to March, 1927, some further data have been accumulated[2].

Table I gives the results in two series and, for comparison,those previously published.

[1] *Reports on Public Health and Medical Subjects* (1922), No. 13.
[2] I owe many thanks to Dr J. Bell Ferguson, formerly Medical Officer of Health for Smethwick, for sendirg me many specimens from cases of lobar pneumonia.

Genes and enzymatic activity

- The were using mold *Neurospora crassa* model, new to the molecular biologists.
- They x-rays *Nerospora creassa* and induced mutations.
- In a series of experiments, 1941, they showed that these mutations caused changes in specific enzymes involved in metabolic pathways.



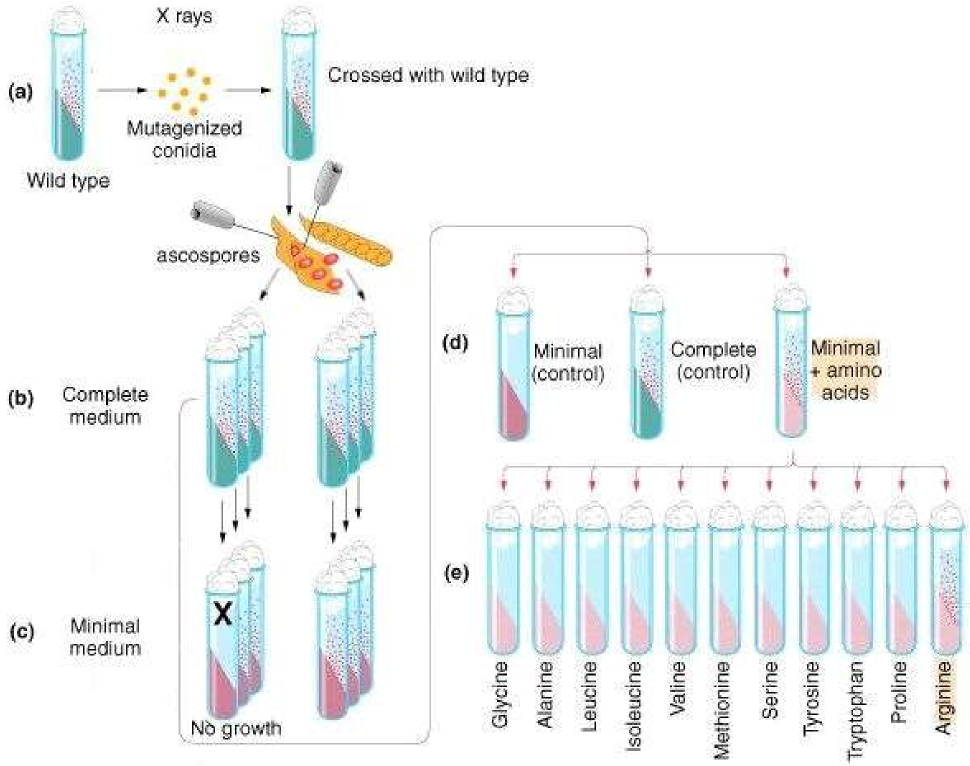
George Beadle
(1903 – 1989)

Edward Tatum
(1909 – 1975)

The implementation and exploitation of novel model to the Molecular Biology becomes a recurring theme.

Genes and enzymatic activity

- 1941 – The direct link between genes and enzymatic reactions leading to postulation of „ One gene - one enzyme hypothesis“.

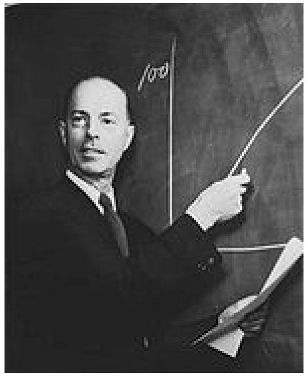


https://www.mun.ca/biology/scarr/Beadle_&_Tatum_Experiment.html

DNA harbors the genetic information

- Confirmation of Griffith's experiment.
- DNA is responsible for the transformation of *Streptococcus pneumoniae* bacteria, 1944.
- Adding purified DNA to bacteria changes their properties (shape of colonies, ability to cause disease, etc.).
- Acquired properties are transferred to subsequent generations.

Oswald Avery
(1877 - 1955)



Colin MacLeod
(1909 – 1972)

Maclyn McCarty
(1911 – 2005)

STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES

INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III

BY OSWALD T. AVERY, M.D., COLIN M. MACLEOD, M.D., AND MACLYN MCCARTY,* M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research*)

PLATE 1

(Received for publication, November 1, 1943)

https://www.mun.ca/biology/scarr/Beadle_&_Tatum_Experiment.html

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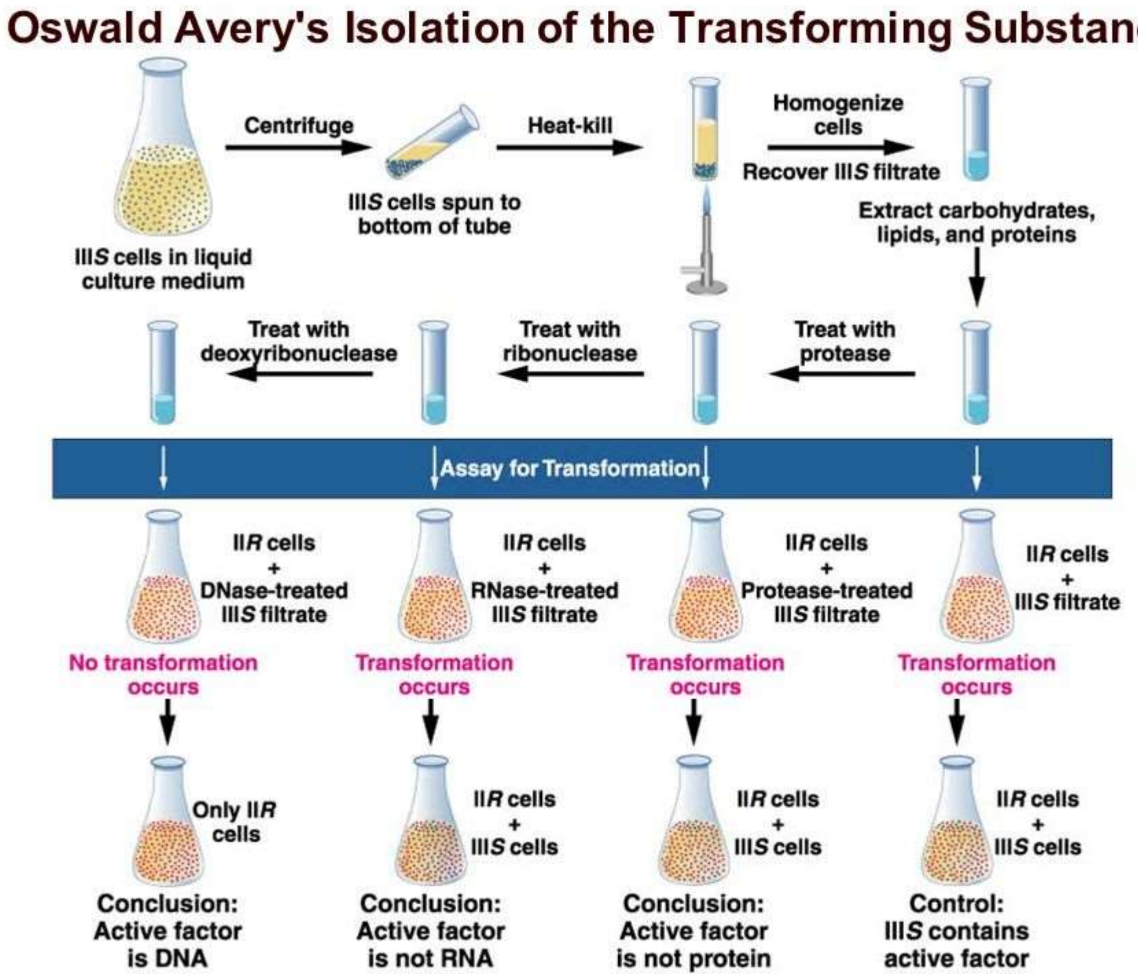
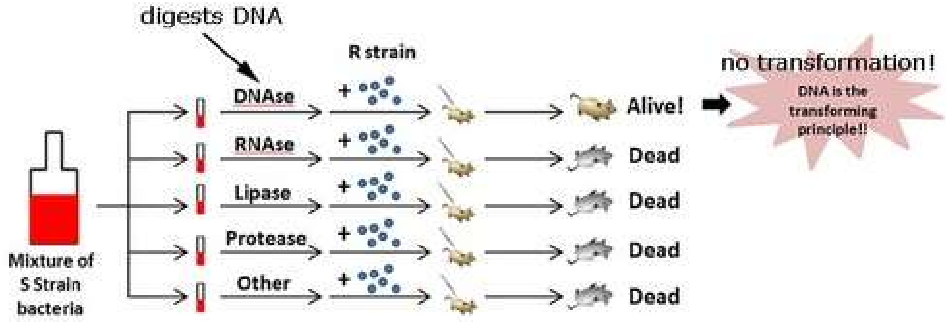
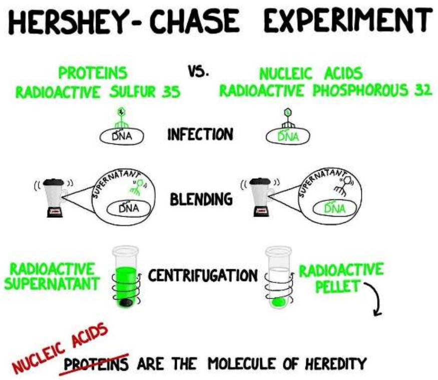
DNA harbors the genetic information





Hereditary genetic information is carried by DNA

- Phage DNA and proteins are separable.
- The phages inject their DNA into the host bacteria.
- The phages inject their DNA into the bacteria and then the DNA serves as replicating element genetic element of phages.



Martha Chase and Alfred Hersey
1952 CSHL USA

https://www.cshl.edu/from-phages-to-faces/
https://twitter.com/bogobiology/status/947161622019280898

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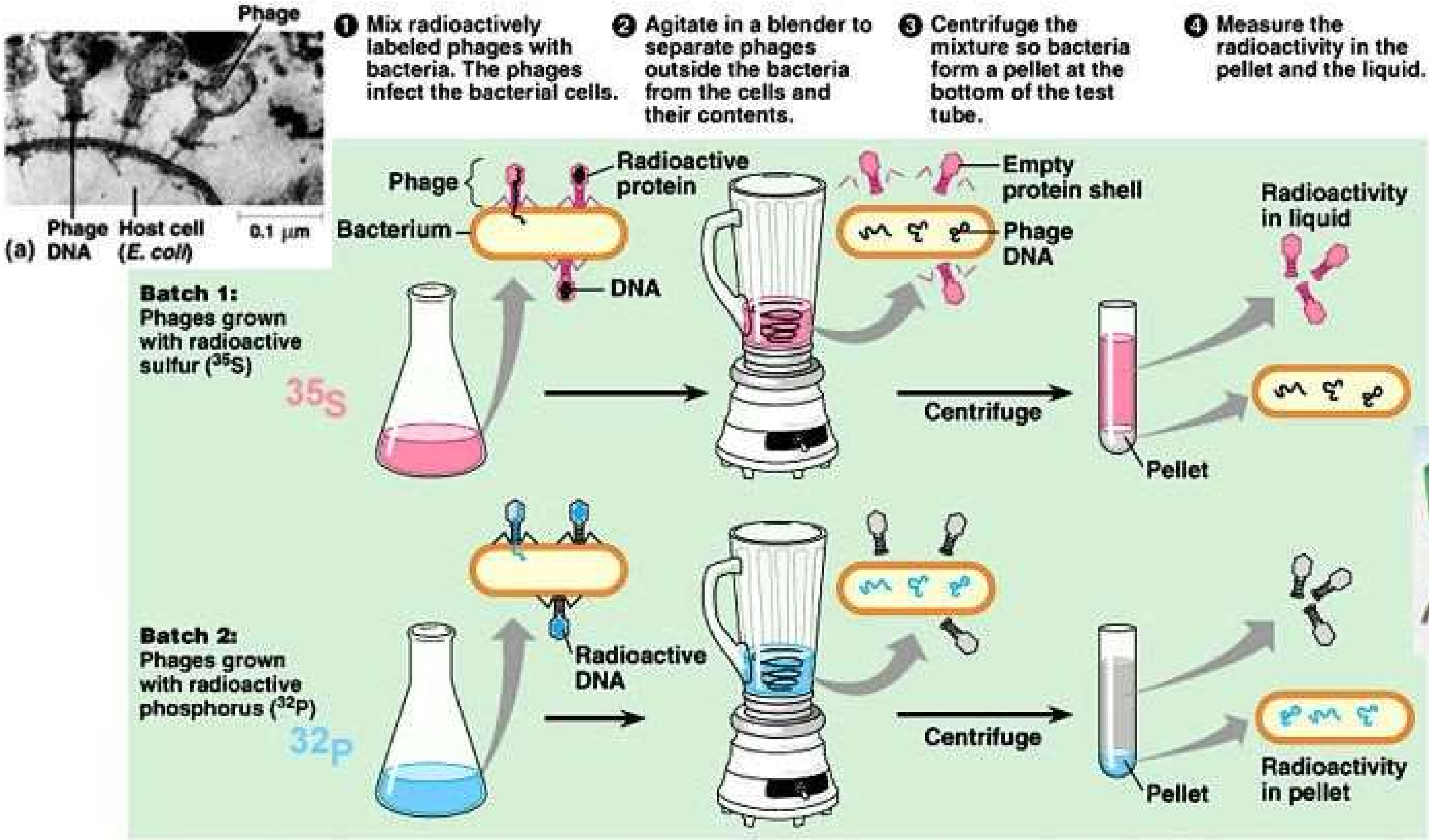
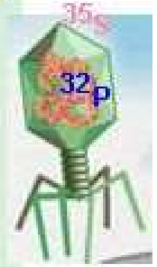
SCI

Hereditary genetic information is carried by DNA



(b) The experiment showed that T2 proteins remain outside the host cell during infection, while T2 DNA enters the cell.

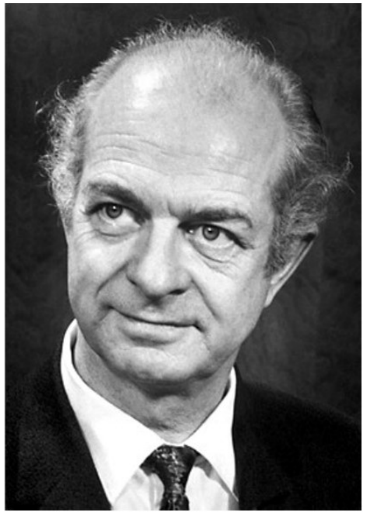
- Waring blender experiment.



https://embryo.asu.edu/pages/hershey-chase-experiments-1952-alfred-hershey-and-martha-chase
https://i.pinimg.com/originals/c9/62/82/c96282125ff929437ae46adb23bc0301.jpg

Mutation in the DNA causes disease

- He applied principles of quantum mechanics in chemistry and also participates in on the study of the spatial structure of proteins.
- He formulates a hypothesis, that the cause of sickle cell anemia could be abnormal form of hemoglobin.
- The hypothesis successfully verified by electrophoretic techniques, 1949.
- For the firts time he linked specific genetic mutation with the sickle cell disease to a demonstrated change in an individual protein, the hemoglobin in the erythrocytes of impacted individuals.
- Nobel Prize in Chemistry in 1954.



Linus Pauling
(1901 – 1994)

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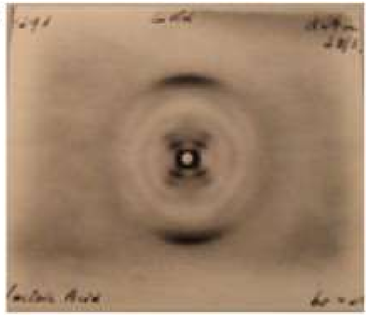
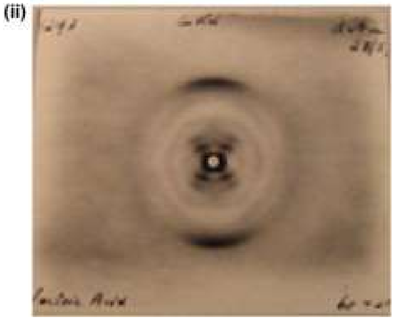
Hunt for the structure of DNA

- In the 1950s, <u>three groups</u> made it their goal to determine the structure of DNA.
- The first group to start was at King College London and was led by Maurice Wilkins and was later joined by Rosalind Franklin.
- Another group consisting of Francis Crick and James D. Watson was at Cambridge.
- A third group was at Caltech and was led by Linus Pauling.

(i)



(ii)



- The fourth group was in Leeds led by William Astbury and Elwyn Beighton.

Hunt for the structure of DNA

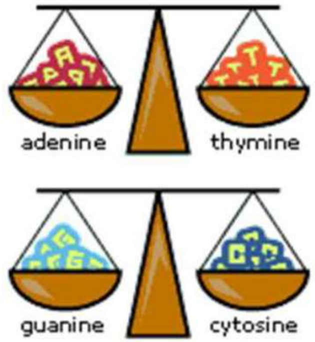
- 1948 Pauling discovered that many proteins included helical shapes. Pauling had deduced this structure from X-ray patterns and from attempts to physically model the structures.

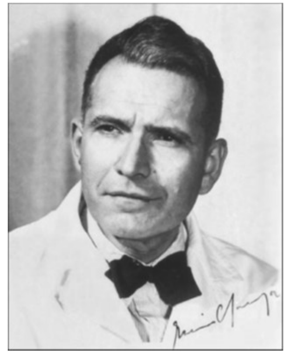
- There remained the questions of how many strands came together, whether this number of bases was the same for every alfa-helix, whether the bases pointed toward the helical axis or away, and ultimately what were the explicit angles and coordinates of all the bonds and atoms.

- Such questions motivated the modeling efforts of Watson and Crick.

Hunt for the structure of DNA

- Watson and Crick restricted themselves to what they saw as chemically and biologically reasonable.
- A breakthrough occurred in 1952, when Erwin Chargaff visited Cambridge and inspired Crick with a description of experiments Chargaff had published in 1947.
- Chargaff had observed that the proportions of the four nucleotides vary between one DNA sample and the next, but that for particular pairs of nucleotides
    - adenine and thymine
    - guanine and cytosine





- the two nucleotides are always present in equal proportions.

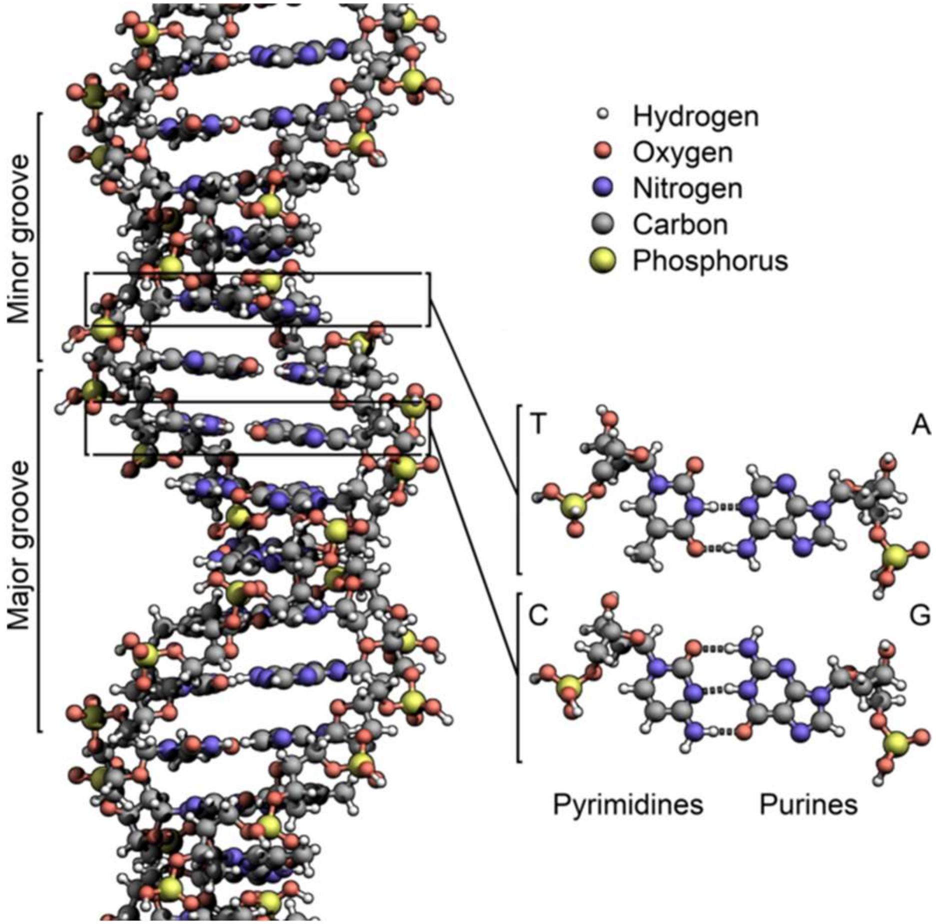
Discovery of DNA structure

- 1953: James Watson a Francis Crick derive the structure of DNA on the basis of the following data:

- Chemical data: Erwin Chargaff principles:
  - the concentration of T and A is the same
  - the concentration of C and G is the same

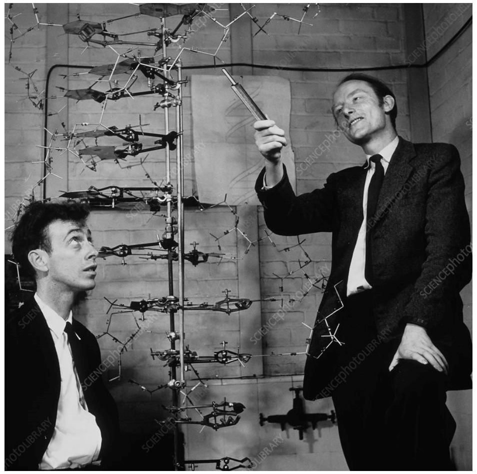
- Physical data: Maurice Wilkins a Rosalind Franklin after exposure of purified DNA molecules to X-rays, there is a characteristic scattering of rays that signal way of arranging DNA components into a helix.

Discovery of DNA structure



Rosalinda Franklin

James Watson a Francis Crick



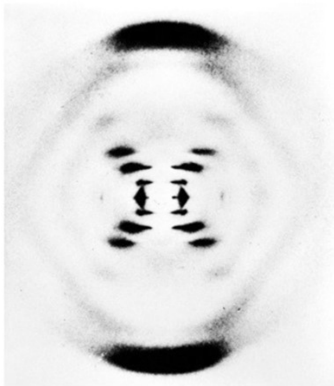
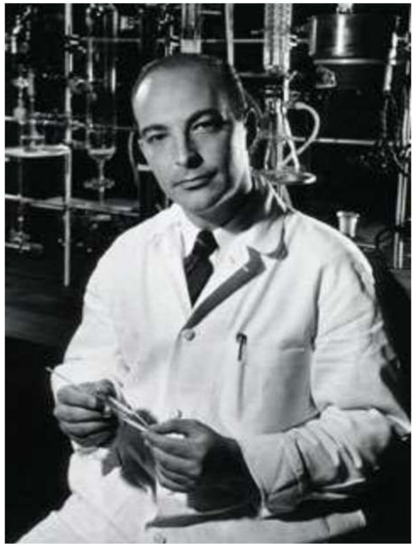


Photo 51

https://commons.wikimedia.org/wiki/File:DNA_Structure%2BKey%2BLabelled.pn_NoBB.png
https://www.sciencephoto.com/media/222783/view
https://en.wikipedia.org/wiki/Rosalind_Franklin

Discovery of DNA polymerase

- American biochemists.
- In 1956 he isolated the DNA-polymerase from *E. coli*, for the first time.
- Function
  - synthesis of short sections of DNA (filling in the gaps between Okazaki´s fragments)
  - component of reparation mechanisms
  - main function: removal of RNA-primers.
- 1959 - Nobel Prize in Physiology or Medicine.

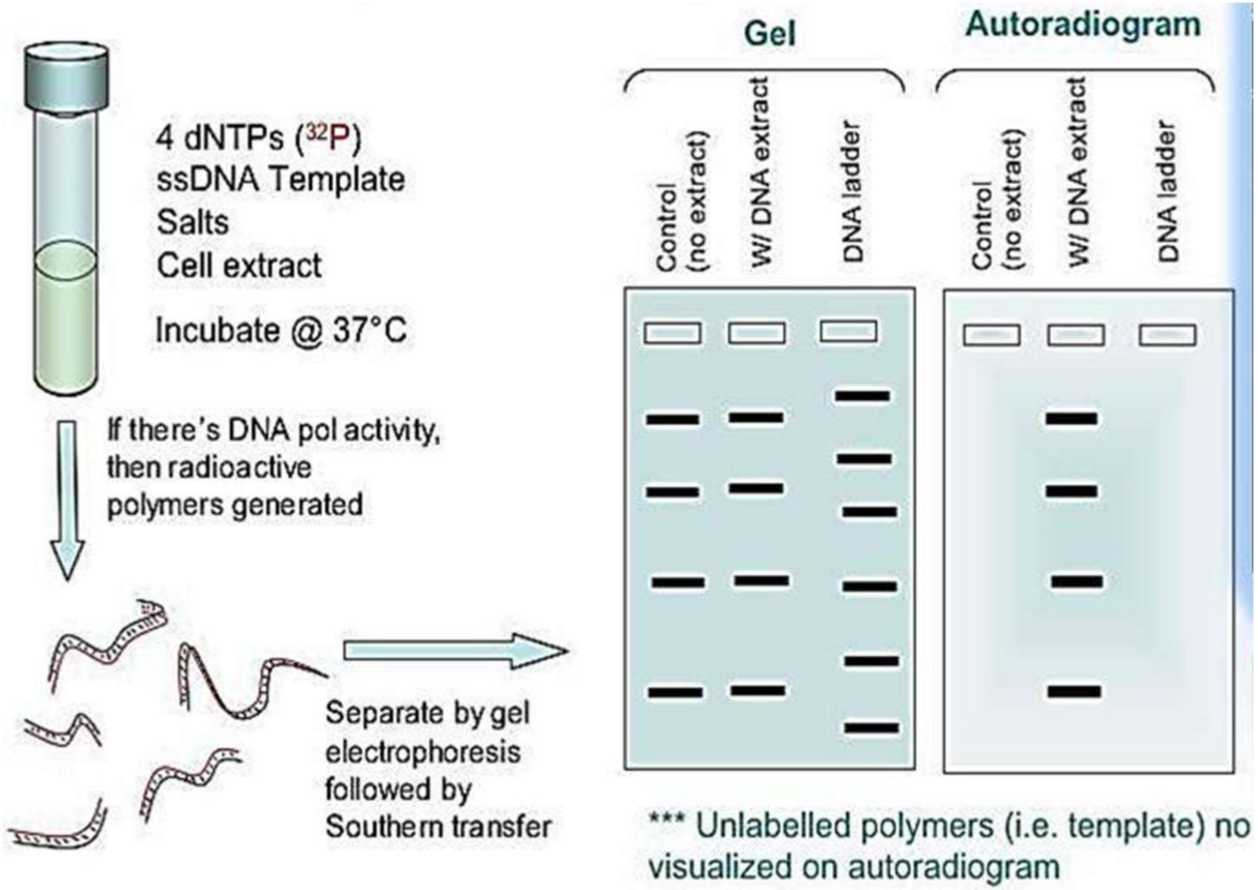


Arthur Kornberg
(1918 – 2007)

https://www.jbc.org/article/S0021-9258(20)59088-1/fulltext

Discovery of DNA polymerase

- The two reports describing "DNA polymerase," reaction were declined by the Journal of Biological Chemistry when submitted in the Fall of 1957.
- Among the critical comments were: "It is very doubtful that the authors are entitled to speak of the enzymatic synthesis of DNA"; "Polymerase is a poor name"; "Perhaps as important as the elimination of certain banalities..." etc.



https://slideplayer.com/slide/14061307/

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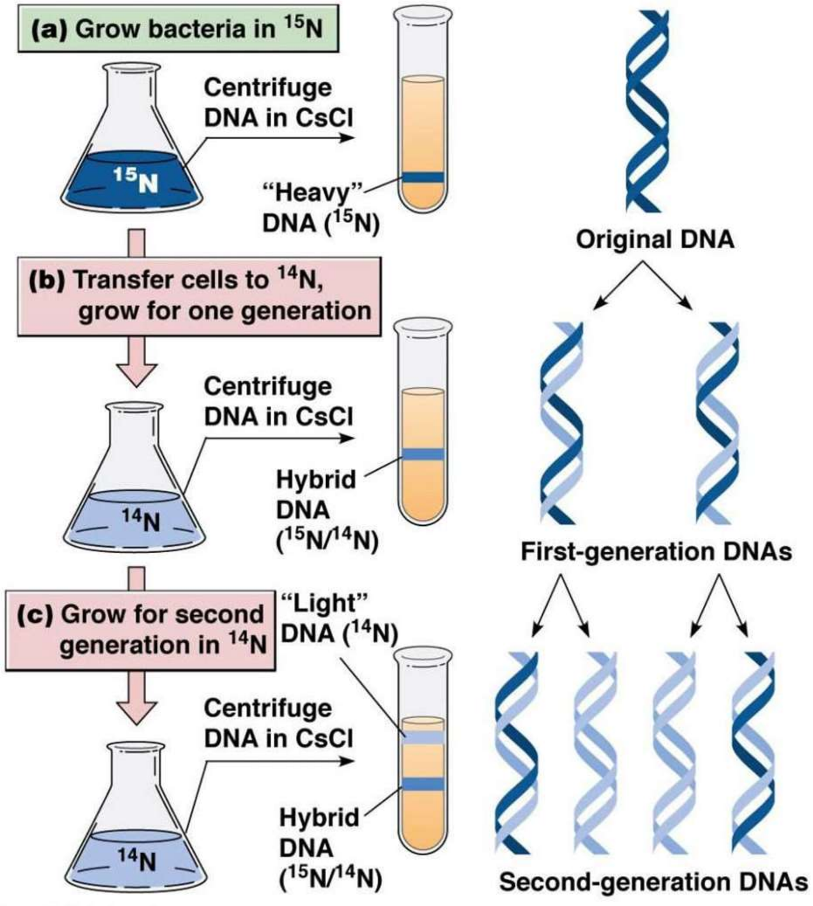
DNA replication is semi-conservative process

- In 1958 they proved the validity of semi-conservative model of replication proposed by Watson and Crick in 1953.
- Evidence based on the study of DNA density after marking with heavy nitrogen 15N.

Matthew Meselson
(1930 -

Franklin Stahl
(1929 -

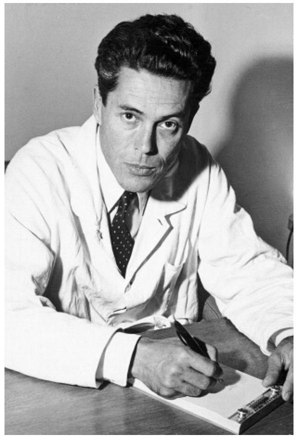


https://www.nature.com/scitable/topicpage/semi-conservative-dna-replication-meselson-and-stahl-421/

There is intermediate between DNA and protein

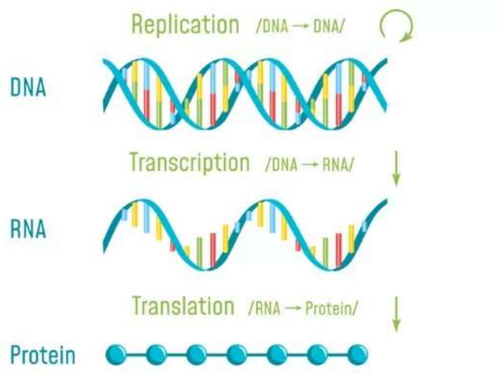
- At the beginning of 1960, Jacob and Monod observed regulatory proteins at the edges of the genes and control the transcription of these genes into messenger RNA, in other words they direct transition of these genes.

- Two concepts of utmost importance came out of those experiments in 1961:
  - that of messenger RNA
  - the operon.



Jacques Monod
(1910 – 1976)

Francois Jacob
(1920 – 2013)



Research in Microbiology Volume 161, Issue 2, March 2010, Pages 68-73
https://mubi.com/cast/francois-jacob
https://www.thoughtco.com/dna-transcription-373398

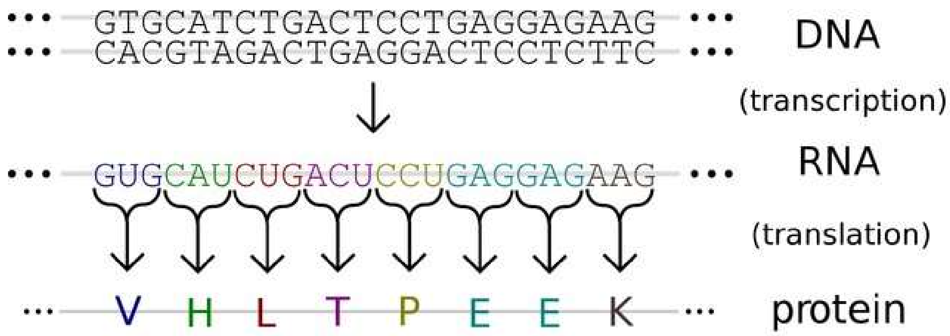
Cracking the genetic code

- Work by <u>Crick and coworkers</u> showed that the genetic code was based on non-overlapping triplets of bases, called codons.
- H. G. Khorana, R. Holley and M. Nirenberg and others deciphered the encoding the meaning of all codons in 1966.
- In 1968H.G. Khorana R. Holley M. Nirenberg were awarded by the Nobel Prize in Physiology or Medicine.

H. G. Khorana

R. Holley

M. Nirenberg



https://www.researchgate.net/publication/282279062_Computational_Statistics_for_the_Identification_of_Transcriptional_Gene_Regulatory_Networks

Cracking the DNA code

- Khorana synthesis of polynucleotides with <u>defined sequence of nucleotides</u>, repeated mostly → *in vitro* <u>transcription</u> → *in vitro* <u>translation</u> → polypeptide analysis. The technique works only for DNA, not for RNA.

- 1961 - Nirenberg and Matthaei discovered that poly-U RNA nucleotide makes phenylalanine polypeptide chain *in vitro*.

- Team of researchers workd to identify meaning for all 64 codons.

- 1966 – The complete Geneitc Code was deciphered.

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Marshall Nirenberg assembled a team of about 10 researchers and technicians who discovered the chart above — the genetic codes describing 20 amino acids.

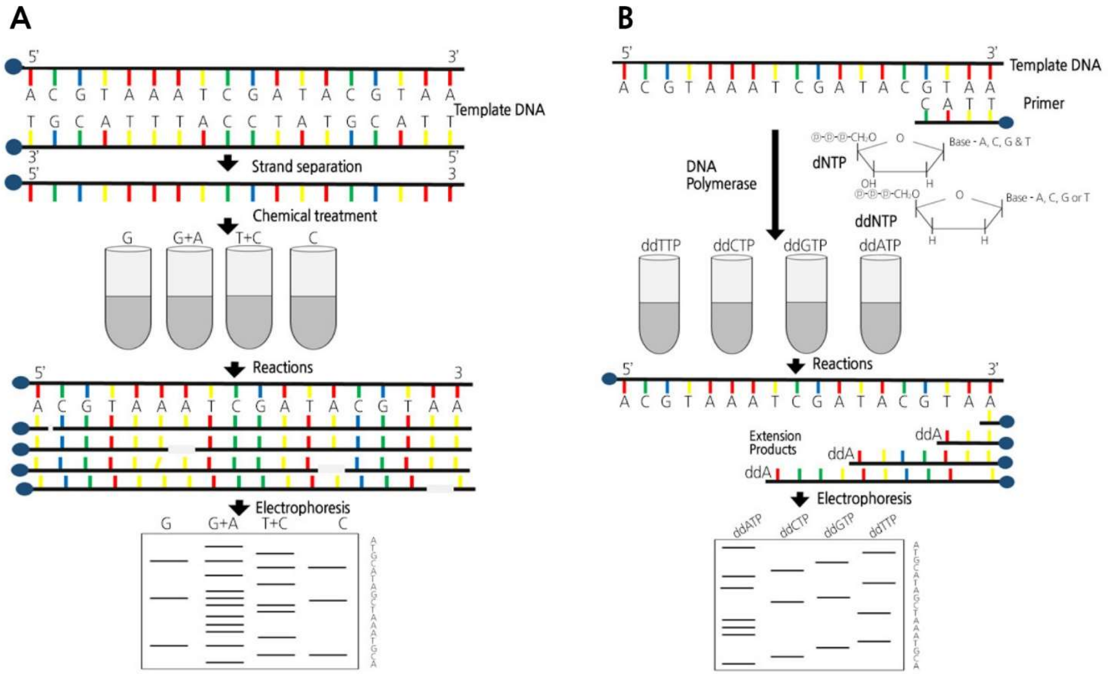
https://www.acs.org/content/acs/en/education/whatischemistry/landmarks/geneticcode.html

DNA sequencing

- Maxam–Gilbert technique depends on the relative chemical liability of different nucleotide bonds, 1977.

- Sanger method interrupts elongation of DNA sequences by incorporating dideoxynucleotides into the DNA strands, 1975.

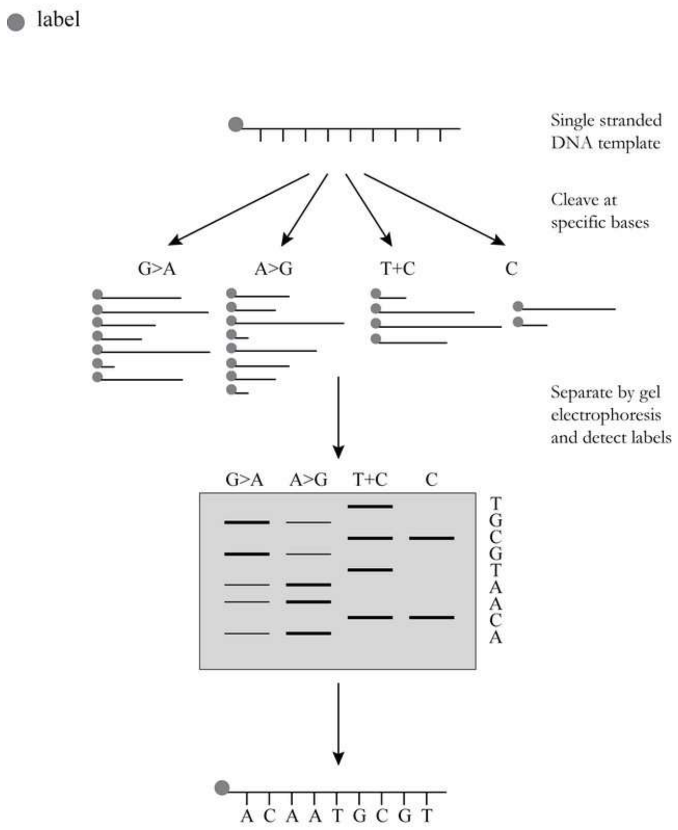
- Gilbert and Sanger were awarded by the Nobel Prize in Chemistry in 1980.

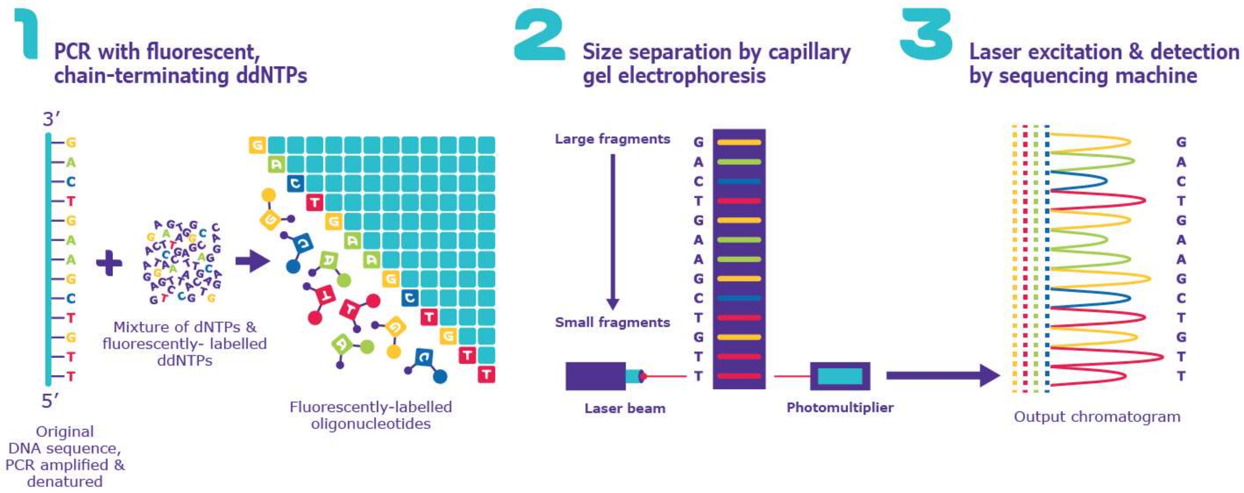


Horticultural Science and Technology. 31 October 2019. 549-558

DNA sequencing

- Maxam–Gilbert technique
- Sanger method



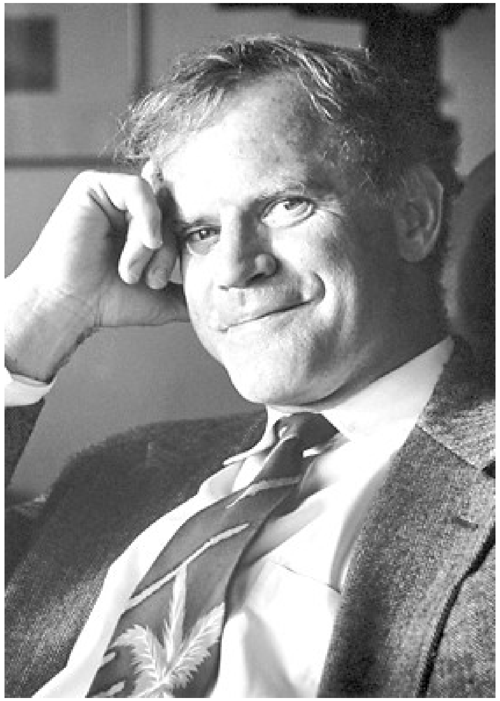


https://www.researchgate.net/publication/268048875_Strategies_for_de_novo_DNA_sequencing/figures?lo=1

https://www.sigmaaldrich.com/CZ/en/technical-documents/protocol/genomics/sequencing/sanger-sequencing

Polymerase Chain Reaction – PCR

- 1979 Cetus Corporation hired Kary Mullis to synthesize oligonucleotides.
- May 1983 Mullis synthesized oligonucleotide probes for a project to analyze a sickle cell anemia mutation.
- In the spring of 1985 the development group began to apply the PCR technique to other targets.
- Early in 1985, the group began using a thermostable DNA polymerase (the enzyme used in the original reaction is destroyed at each heating step).
- Nobel Prize in Chemistry 1993.



Kary Banks Mullis
(1944 – 2019)

Saiki RK et al. "*Enzymatic Amplification of β-globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia*" Science vol. 230 pp. 1350–54 (1985).

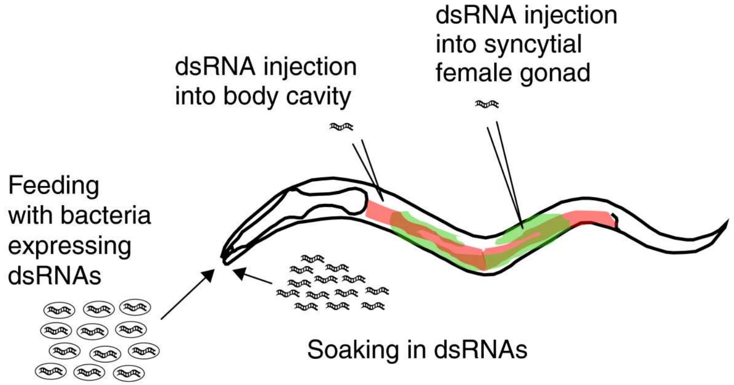
Dig Dis Sci. 2015 Aug; 60(8): 2230–2231.

RNA interference – non-coding RNA

- In 1998, Fire and Mello demonstrated that they could efficiently and selectively dial down the expression of various genes in the worm *Caenorhabiditis elegans* by injecting small quantities of short interfering RNA (siRNA) molecules, which comprise paired strands of RNA.
- Discovery of additional biologically active RNA followed:
  - siRNA
  - piRNA
  - sncRNA
- Nobel Prize in Physiology or Medicine in 2006.

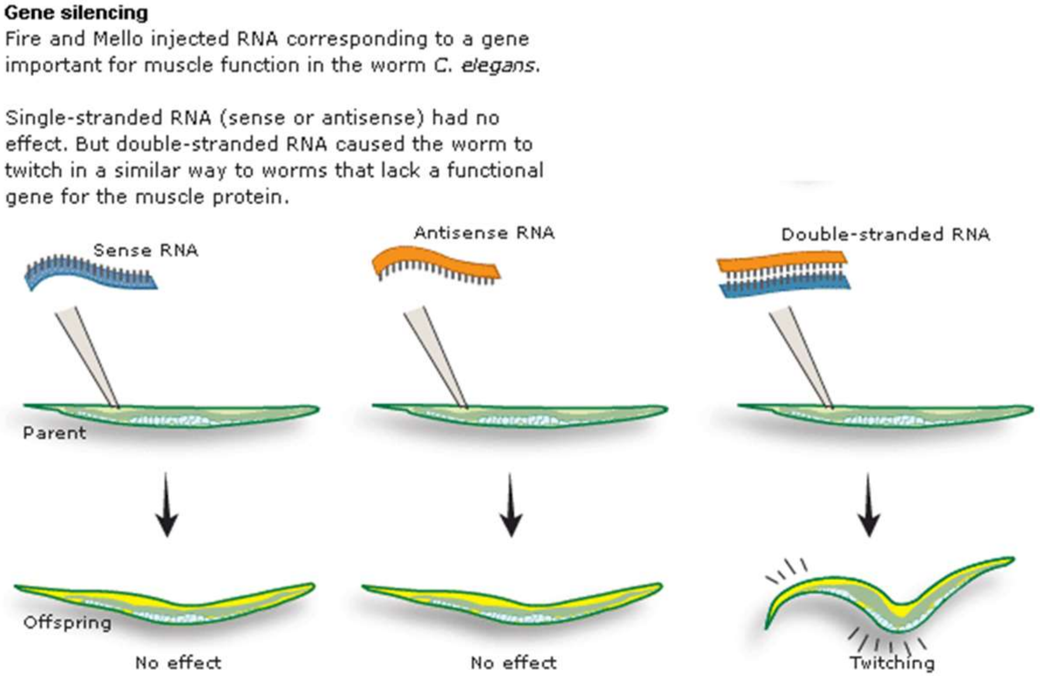
Andrew Z. Fire

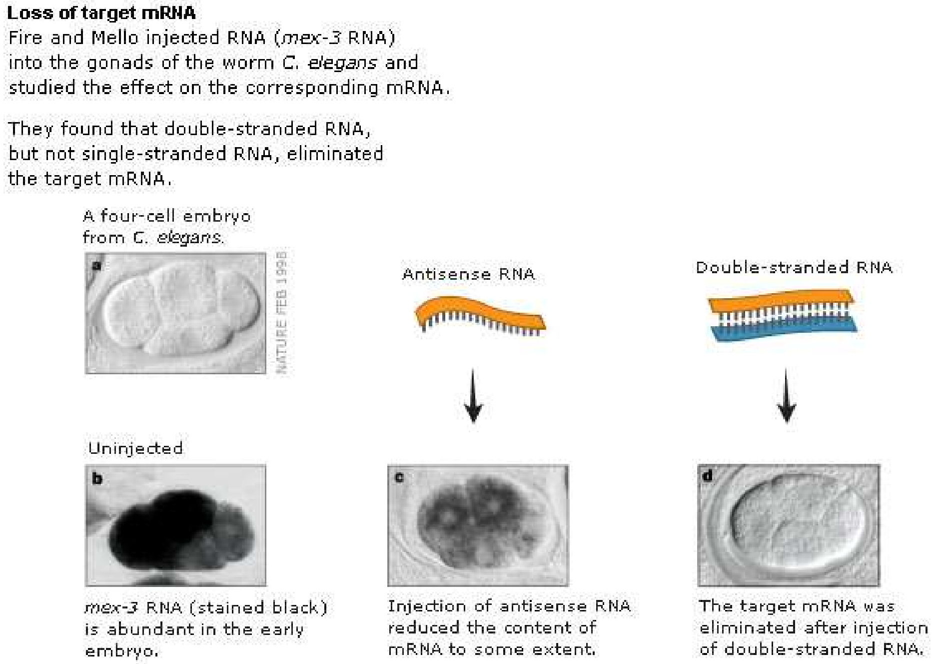
Craig C. Mello



https://jbiol.biomedcentral.com/articles/10.1186/jbiol97

RNA interference – non-coding RNA





Nature. 1998 Feb 19;391(6669):806-11. doi: 10.1038/35888.
https://bastiani.biology.utah.edu/courses/3230/db%20lecture/lectures/wormrnai.html

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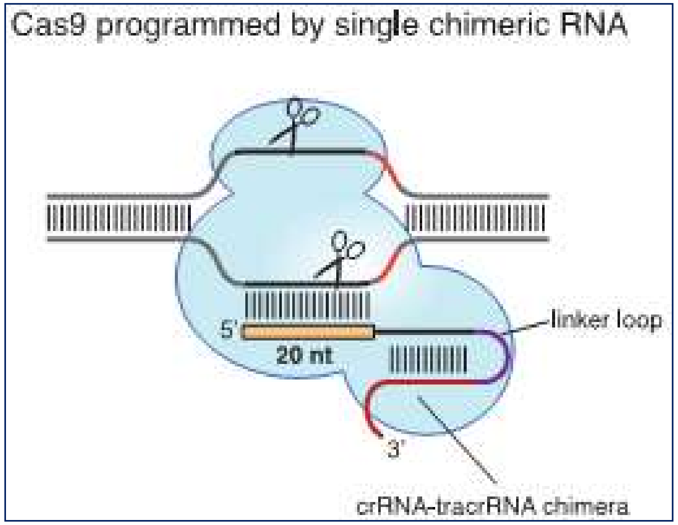
CRISPR method for genomic DNA editing

- Nobel Prize for Chemistry in 2020.

Emmanuelle Charpentier

Jennifer A. Doudna

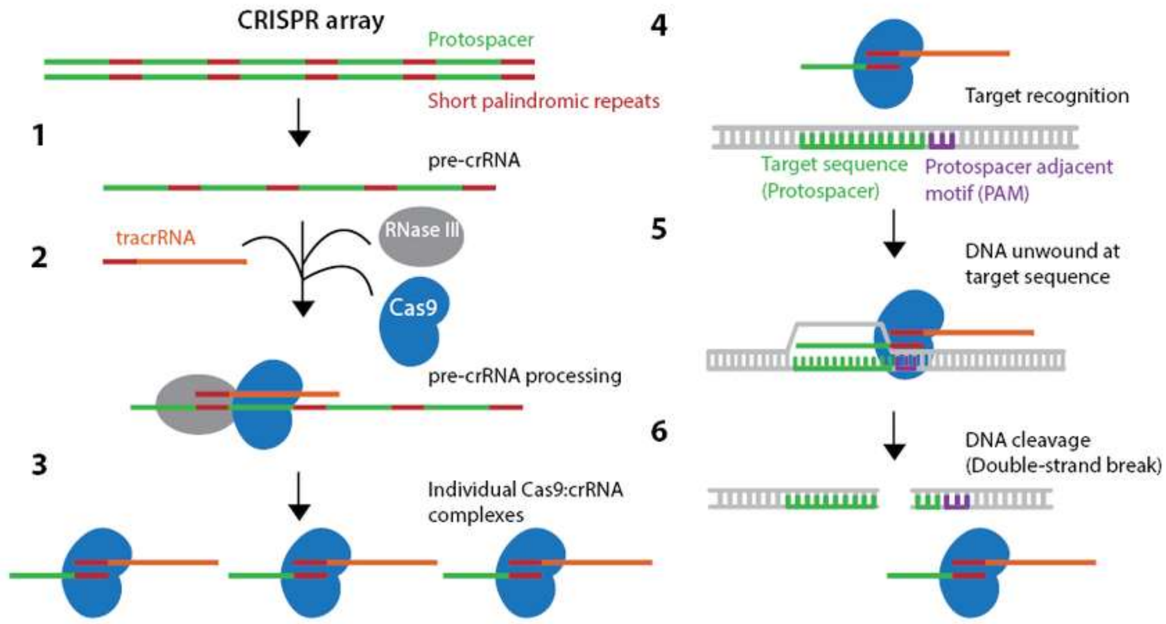


17 AUGUST 2012 VOL 337 SCIENCE

MUNI SCI

CRISPR method for genomic DNA editing
(Clustered Regularly Interspaced Short Palindromic Repeats)

- 2011 — Emmanuelle Charpentier, showed that tracrRNA forms a duplex with crRNA, and that it is this duplex that guides <u>Cas9 to its targets</u>.
- 2012 — Charpentier and Jennifer Doudna reported that the crRNA and the tracrRNA could be fused together to create a single, synthetic guide, further simplifying the system.

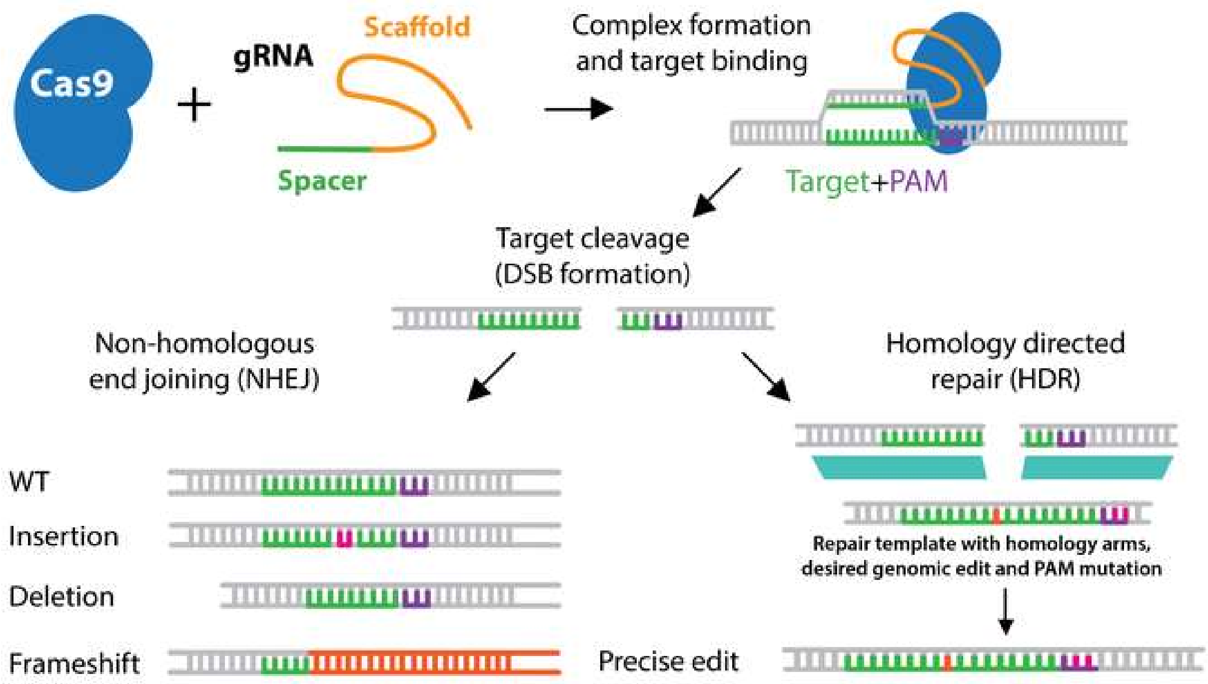


https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/crispr-timeline
https://www.addgene.org/crispr/history/

CRISPR method for genomic DNA editing

- 2013 – Zhang was first to successfully adapt CRISPR-Cas9 for genome editing in eukaryotic cells.

- They engineered two different Cas9 orthologs (*S. pyogenes* and *S. thermophilus*. They demonstrated targeted genome cleavage in human and mouse cells.
  - (i) could be programmed to target multiple genomic loci,
  - (ii) could drive homology-directed repair.



https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/crispr-timeline
https://www.addgene.org/crispr/history/

Current age of Molecuar biology

- <u>Research area</u>
- New separate disciplines within molecular biology:
- Transcriptomics, metabolomics, exposomics, microbiomics, secretomics, kinomics a “… omics“.
- Study of regulation of gene expression and cell differentiation processes (cell cycle, signaling pathways, regulatory disorders, stem cell research).
- Neurobiology.
- Use of the molecular methodology in a number of fields: molecular microbiology, virology, immunology, physiology, anthropology, evolution.

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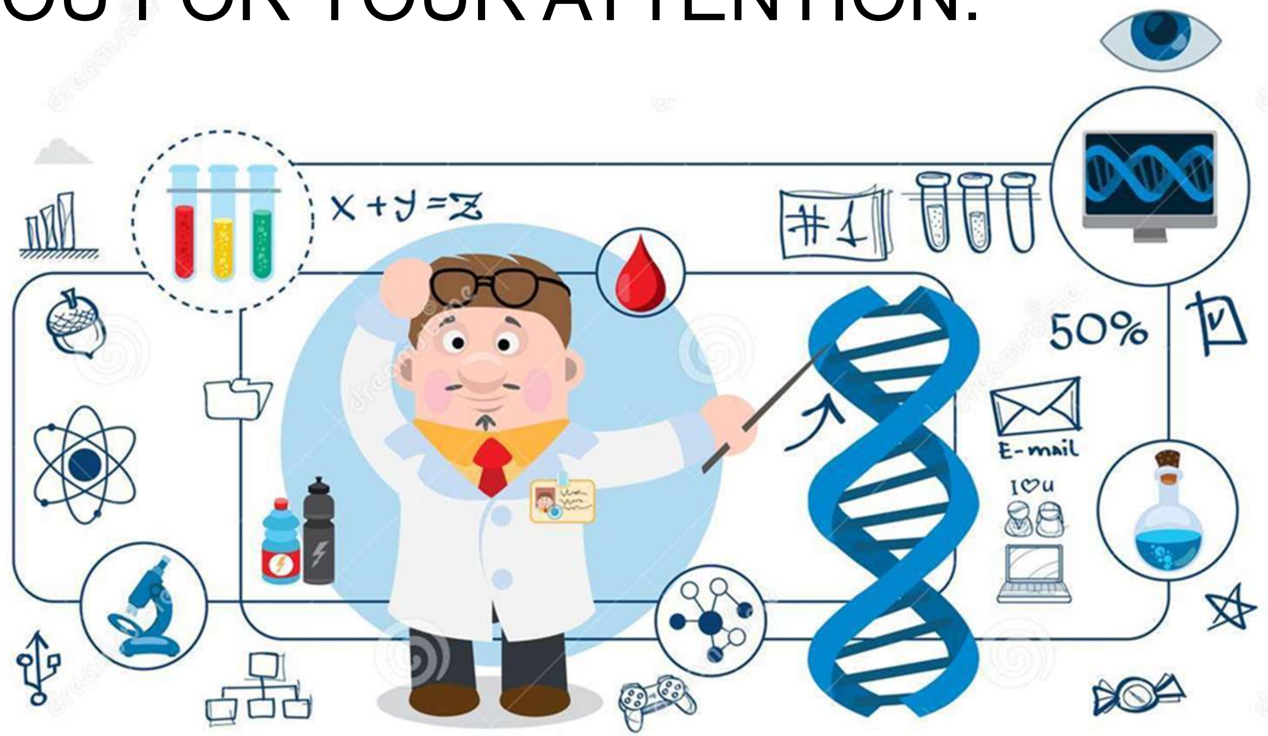
Current age of Molecuar biology

- Practical applications
- Gene engineering – overlaps into agriculture, pharmacy, medicine.
- Modern biotechnology – preparation of transgenic and genetic modified organisms and new substances by targeted gene repurchase.
- Genome editing – targeted changes in genomes *in vivo*, CRISPR/Cas.
- Molecular diagnostics of infectious, hereditary and cancerous diseases, new ways of their treatment (detection of latent pathogens, prenatal diagnostics).
- Pharmacogenomics – drugs "tailored" to the individual genetic constitution (allergies, susceptibility...).
- Gene therapy - treatment of genetic diseases (beginning in the 80s,but not yet too widespread, big risks).

- THANK YOU FOR YOUR ATTENTION.



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