

M U N I
S C I

Bi4025en

Molecular Biology

prof. RNDr. Jan Šmarda, CSc.

Mgr. Jiří Kohoutek, Ph.D.

Content of the Course

- 1. Definition and brief history of molecular biology.
- 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.

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 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 procaryotic and Eucaryotic genomes
- DNA replication, regulatory proteins and mechanism
- Procaryotic and Eucaryotic transcription, posttranscription modification of RNA
- Translation, cotranslation and posttranslational processes, selfassembly

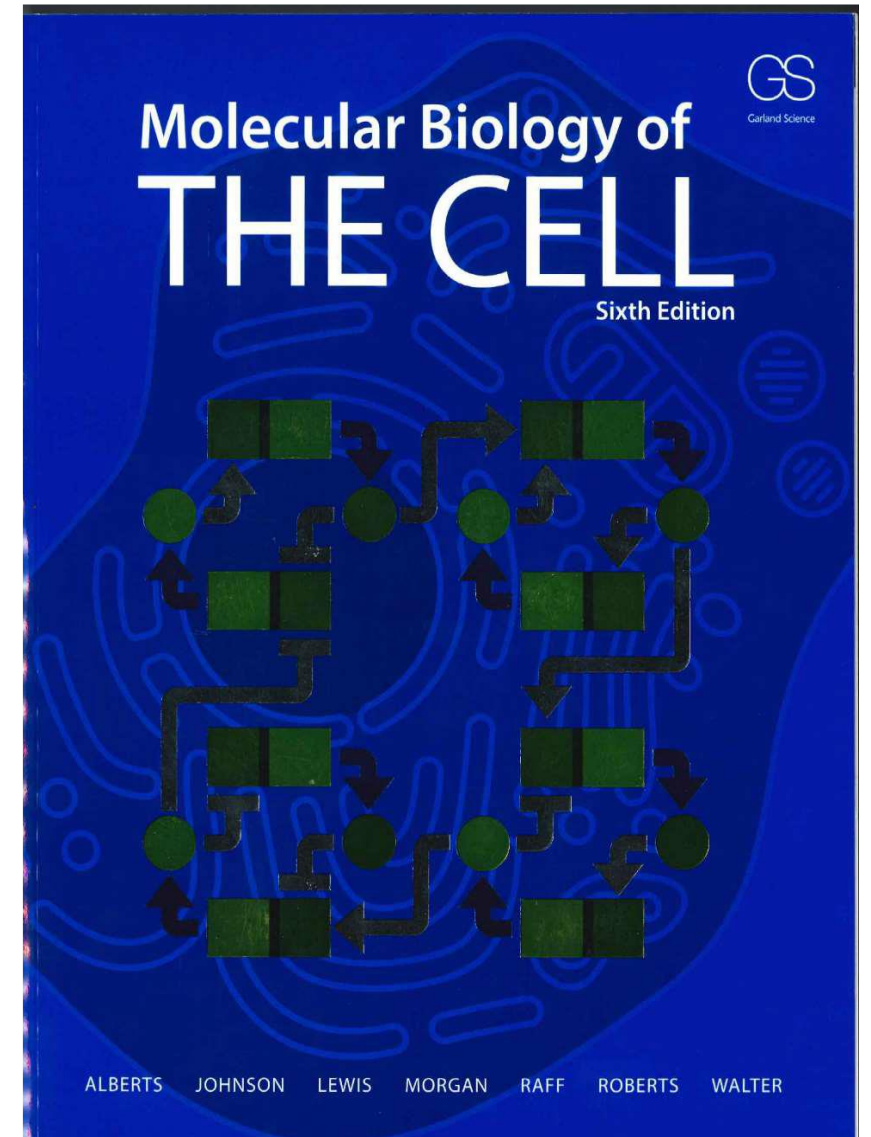
Studying sources

- Audio recording ?
- 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

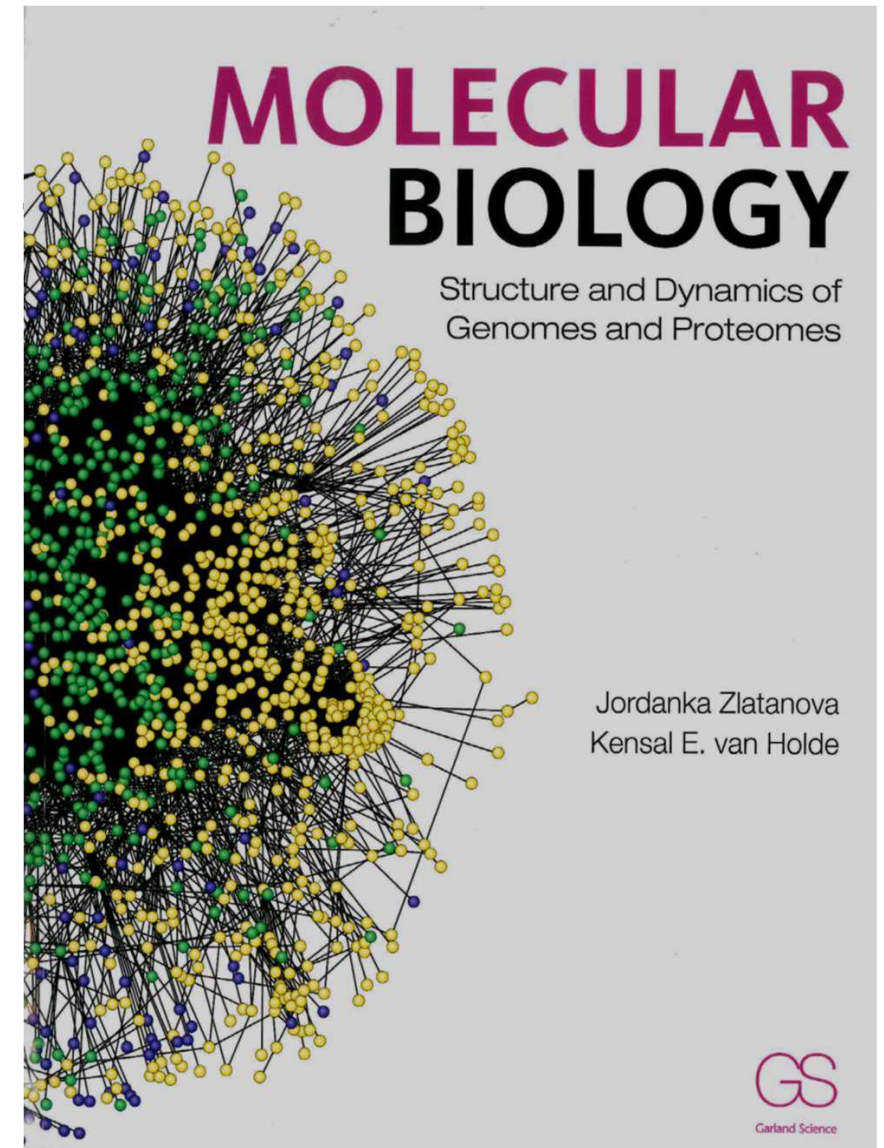


Garland Science, Taylor & Francis Group: New York, USA and Abingdon, UK.

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

2016

7 Department of Experimental Biology



MUNI
SCI

Exam rules

- Final Exam
- Written Test
 - 50 questions
 - 60 % to pass
 - Score
 - A – 100 – 92
 - B – 92 - 86
 - C – 86 – 78
 - D – 78 - 60

Lecture 1

- Definition and brief history of the Molecular Biology

Aim of the Molecular Biology

- Clarify the relationship of the **structure** and **interactions** of **biomacromolecules**, in particular 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.

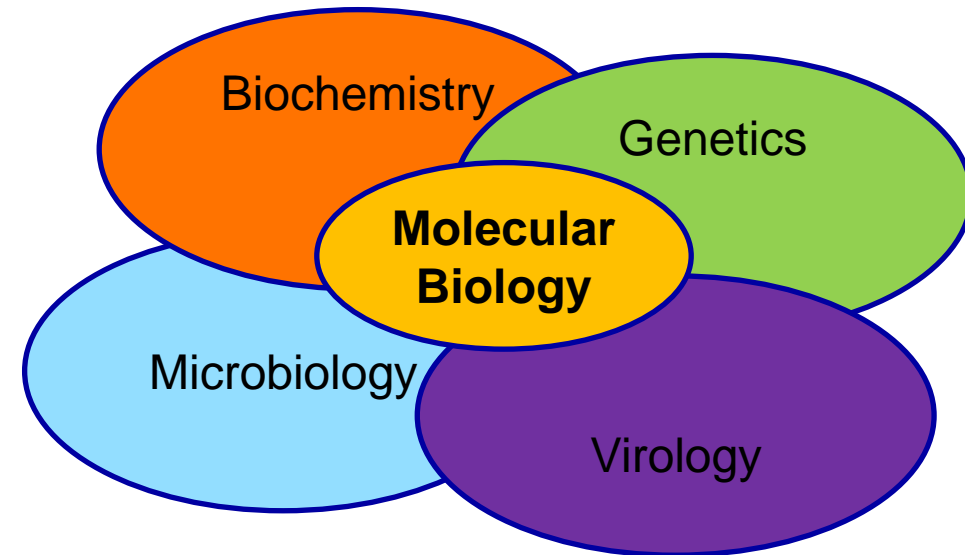
Molecular biology is not biochemistry

Biochemistry

- It studies chemical processes in living 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, molecular biology attempts to explain the phenomena of life starting from the macromolecule properties that generate them



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.

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)

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.
- **Strukturalist** (physicists, chemists)
 - focus on structure of biomacromolecules (proteins, NK), not on function and inheritance
 - 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 preservation and transfer of genetic information (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“).
- 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.
Tetranucleotides hypothesis.

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.
- 1902–1909: Archibald Garrod proposes that genetic defects result in the loss of enzymes and hereditary metabolic diseases.
- 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, including the four bases adenine (A), cytosine (C), guanine (G), and thymine (T).

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.

History of Molecular Biology

- 1952: **Alfred Hershey** and **Martha Chase** use viruses (bacteriophage T2) to confirm DNA as the genetic material by demonstrating that during infection viral DNA enters the bacteria while the viral proteins do not and that this DNA can be found in progeny virus particles.
- 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.

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 Doudna introduce CRISPR editing approach to the science.
- 2013: Zhang develops tools to edit genomic DNA in various organisms.

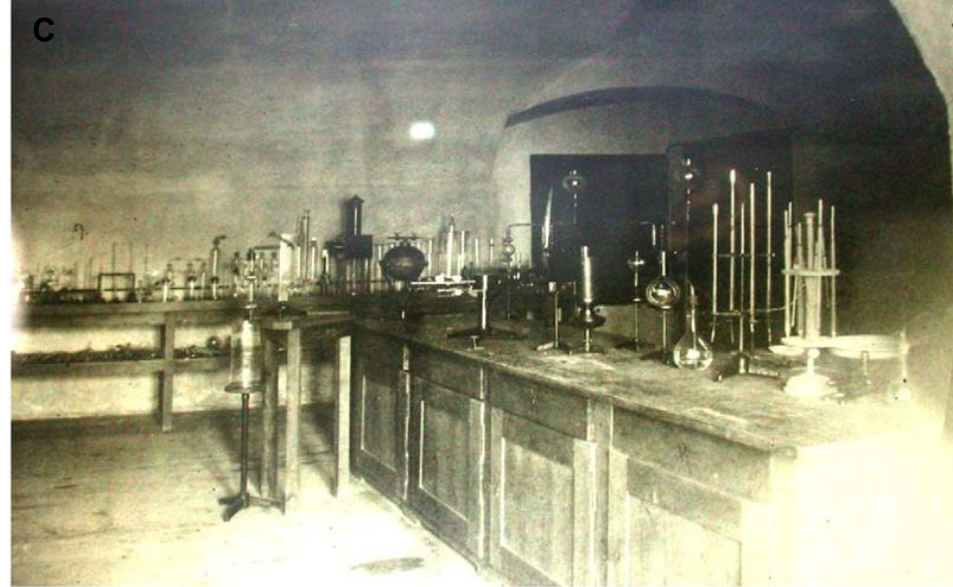
Biochemistry foundation

- 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 of 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)

Biochemistry foundation



(A) Historic photograph 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.

Discovery of Nuclein

- Swiss naturalist and physician.
- Miesher works as the doctoral student in the lab of prof. Hoppe-Seyer.
- 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)

Discovery of Nuclein



(A) Glass vial containing nuclein isolated from salmon sperm 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.

Nuclein is Nucleic Acid

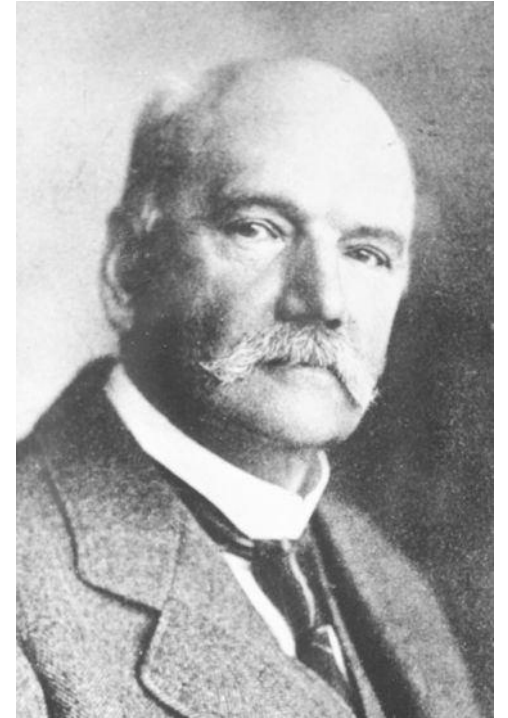
- German pathologist and histologist.
- 1889 named Miescher's term „**nuclein**“ by the term „**nucleic acid**“, when he demonstrated that nuclein was acidic.
- He is also recognized for observation of **filaments** in the nearly all cell types, developed from granules. He named the granules „bioblasts“.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



Richard Altmann
1852 – 1900

Nucleic Acid contains Nucleobases

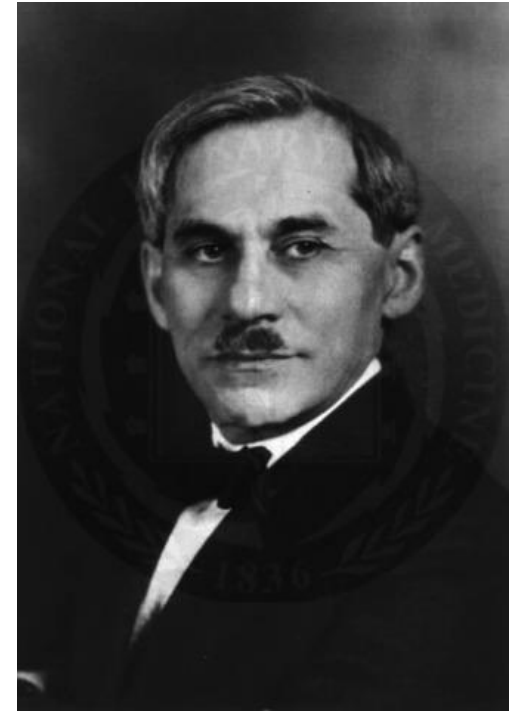
- German biochemist, who studied under Felix Hoppe-Seyler.
- He described chemical composition of nucleic acids having **pyrimidines** and **purines**.
- Between 1885 - 1901, he was able to isolate and name its five constituent organic compounds: **adenine**, **cytosine**, **guanine**, **thymine**, and **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

Nucleic Acid has two Forms

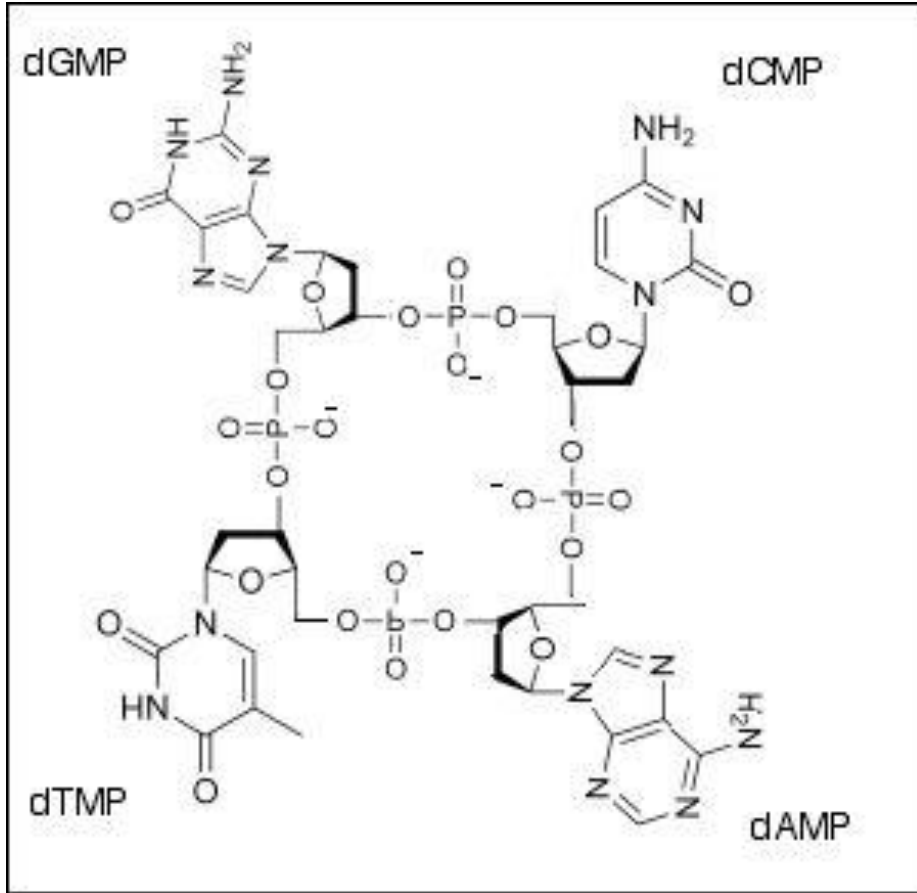
- In 1909, Levene and Walter Jacobs recognised **D-ribose** as a natural product and an essential component of nucleic acids.
- In 1929 Levene also discover the **D-deoxyribose** in nucleic acid.
- He identified components within the nucleic acids and showed that were linked together in the order phosphate-sugar-base 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)

Nucleic Acid has two Forms

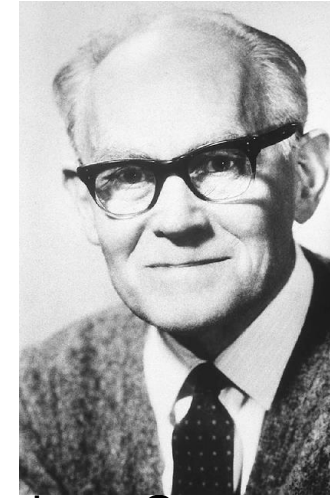
Levene's Tetranucleotide Hypothesis (1910)



- He called the phosphate - sugar - base unit 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 four-nucleotide component is a separate molecule, and the bases are directed to the outside.
- 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.

Nucleic Acid is a polymer - macromolecules

- Swedish biochemist Einar Hammersten, conducting investigations into the molecular mass of DNA (deoxyribonucleic acid). This research led to their discovery that **DNA was a polymer**, or macromolecule, made up of small, repeating units.
- In the 1934 he and Einar Hammarsten showed that **DNA was a polymer**. Previous theories suggested that each molecule was only ten nucleotides long.



Tjorborn Caspersson
(1910 – 1997)

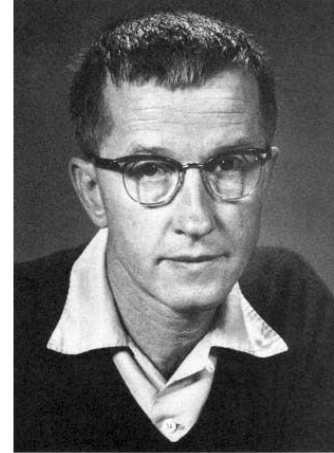


Einar Hammersten
(1889 – 1968)

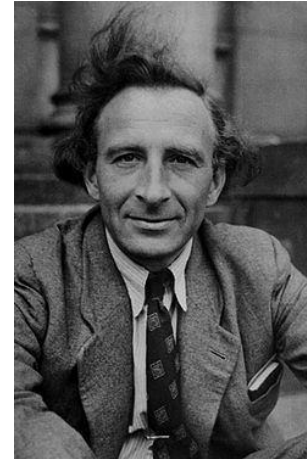
Chromosomes are macromolecules and carry heritable traits

- 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**.
- 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**.

Max Delbrück



NV Timofeeff-Ressovsky



KG Zimmer



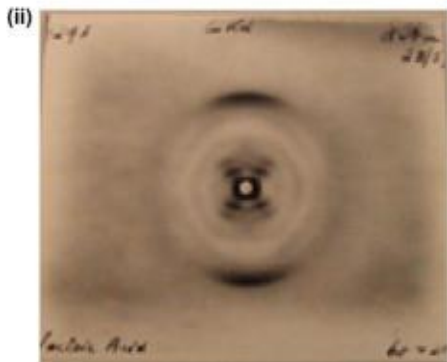
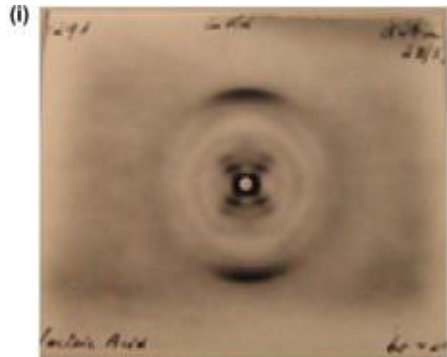
https://www.timofeoff.com/medias/History_of_Molecular_Biology/166fc34c-dedf-48ce-8318-7ad4a3eae585

Nucleic Acid has regular structure

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



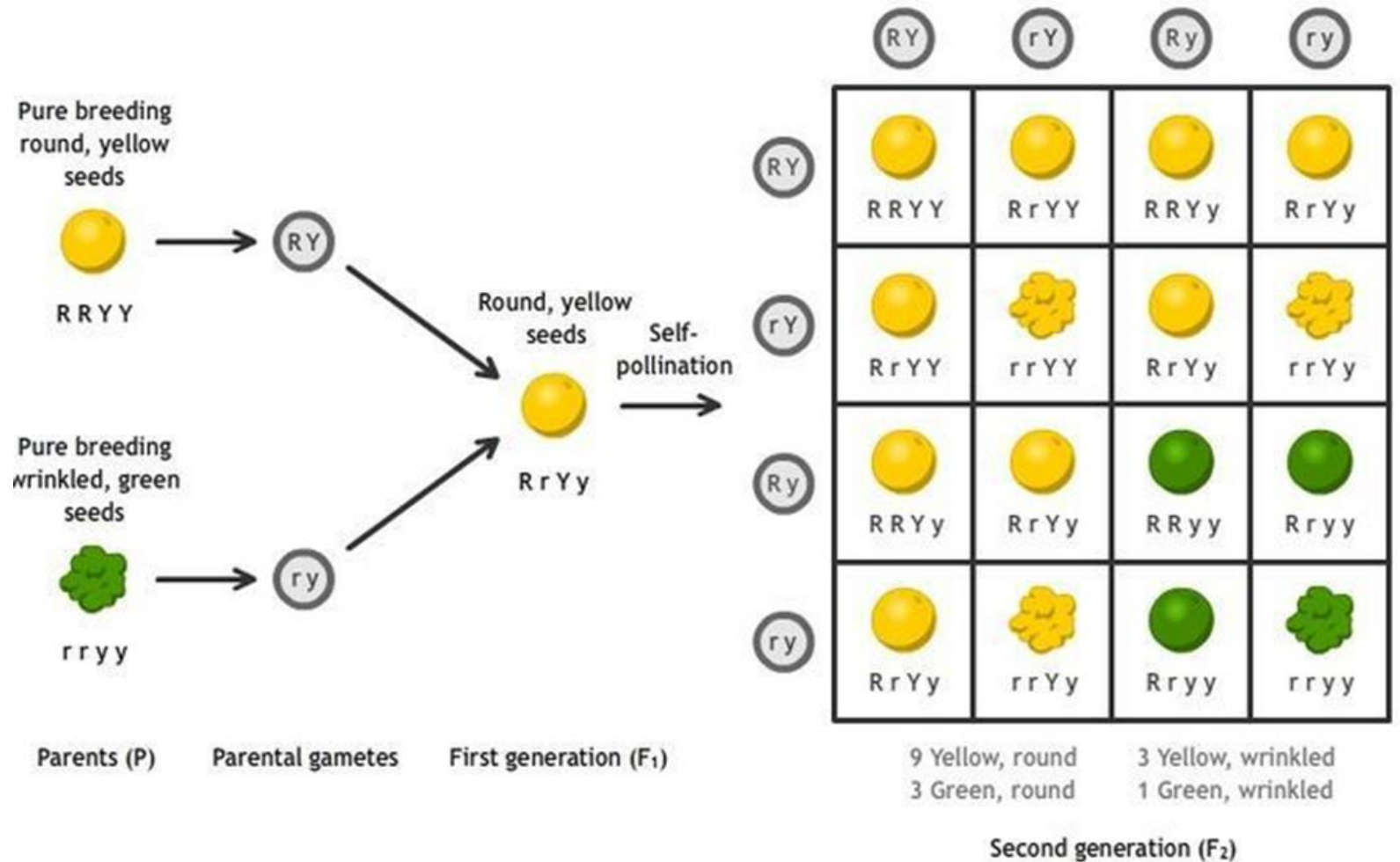
William Astbury
(1898 – 1961)



- 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.

Johann Gregor Mendel principles

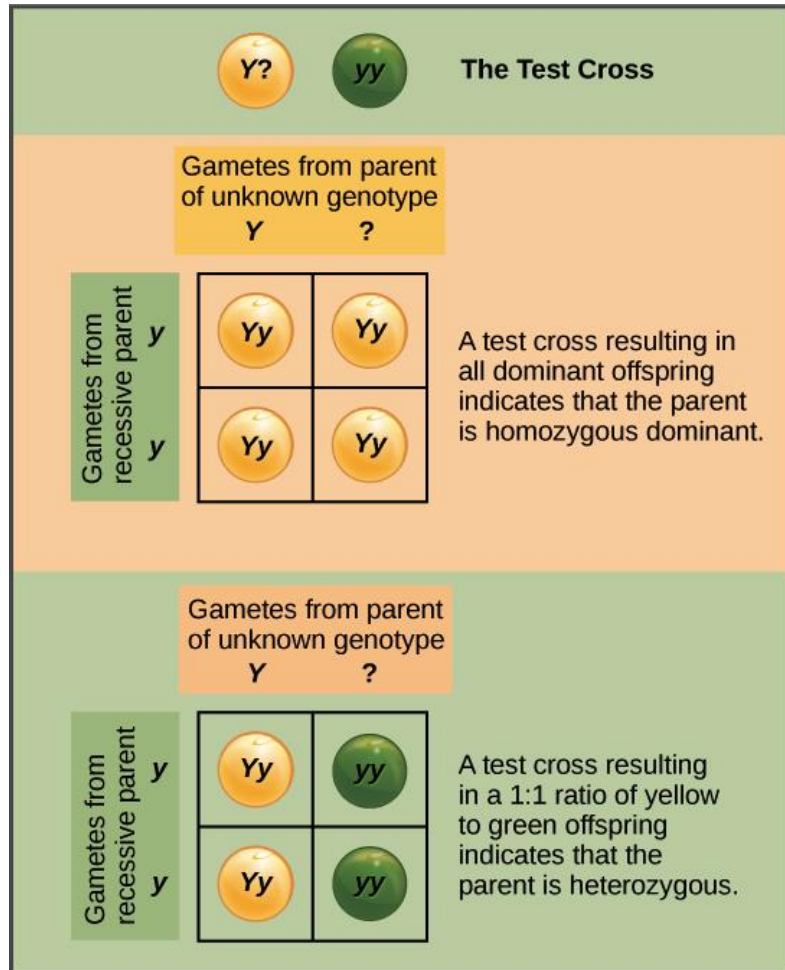
- 1. Dominance
- 2. Segregation
- 3. Independent assortment



Johann Gregor Mendel principles



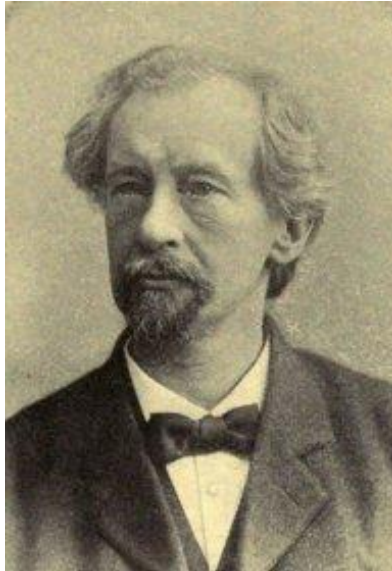
Johann Gregor Mendel
(1822 – 1884)



- 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.

Rediscovery of Mendel's principles

- 1890 – Hugo de Vries, Carl Correns, Erich von Tschermak rediscovered Mendel's principles of heredity.
- 1901 – Hugo de Vries – introduce term „Mutation“



H. De Vries
(1848 – 1935)



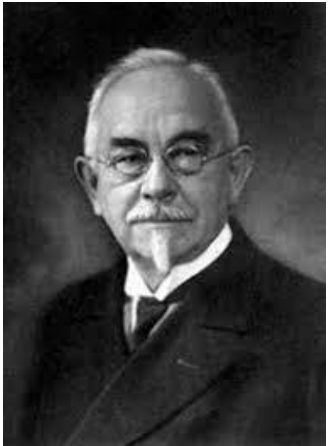
C. Correns
(1864-1933)



E. Von Tschermak
(1871 – 1968)

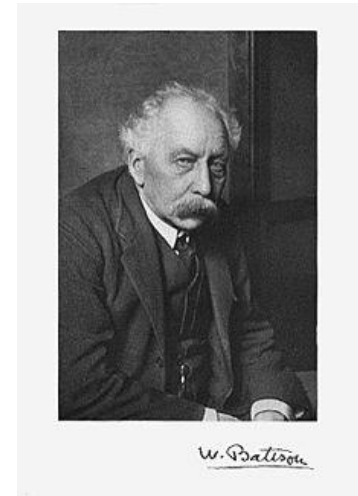
Rediscovery of Mendel's principles

- 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.



Wilhelm Johansen
(1857 – 1927)

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



William Bateson
(1861 – 1926)

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".



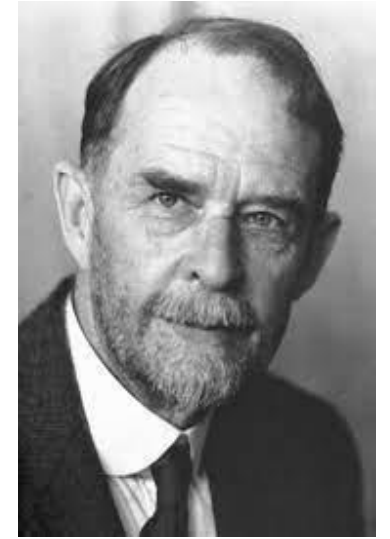
Walter Sutton
(1877 – 1916)



Theodor Boveri
(1862 – 1915)

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.

Discovery of bacterial Transformation

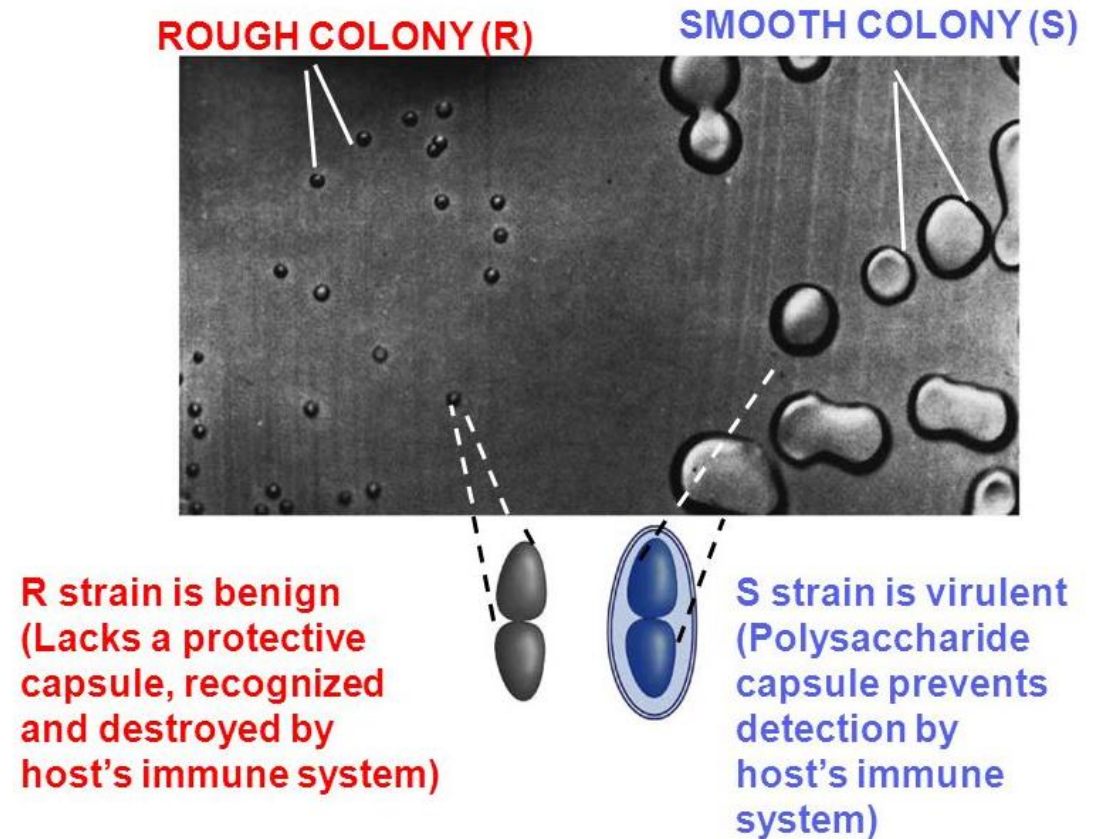
- Frederick Griffith - English bacteriologist.
- In the 20s of the 20th century, he examines the bacterium *Streptococcus pneumoniae*, as a consequence of the Spanish flu in 1918, often accompanied by pneumonia caused by this bacterium.
- The Ministry of Health requires research on *S. pneumoniae* and 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 bacterium distinctly changes its form and function.



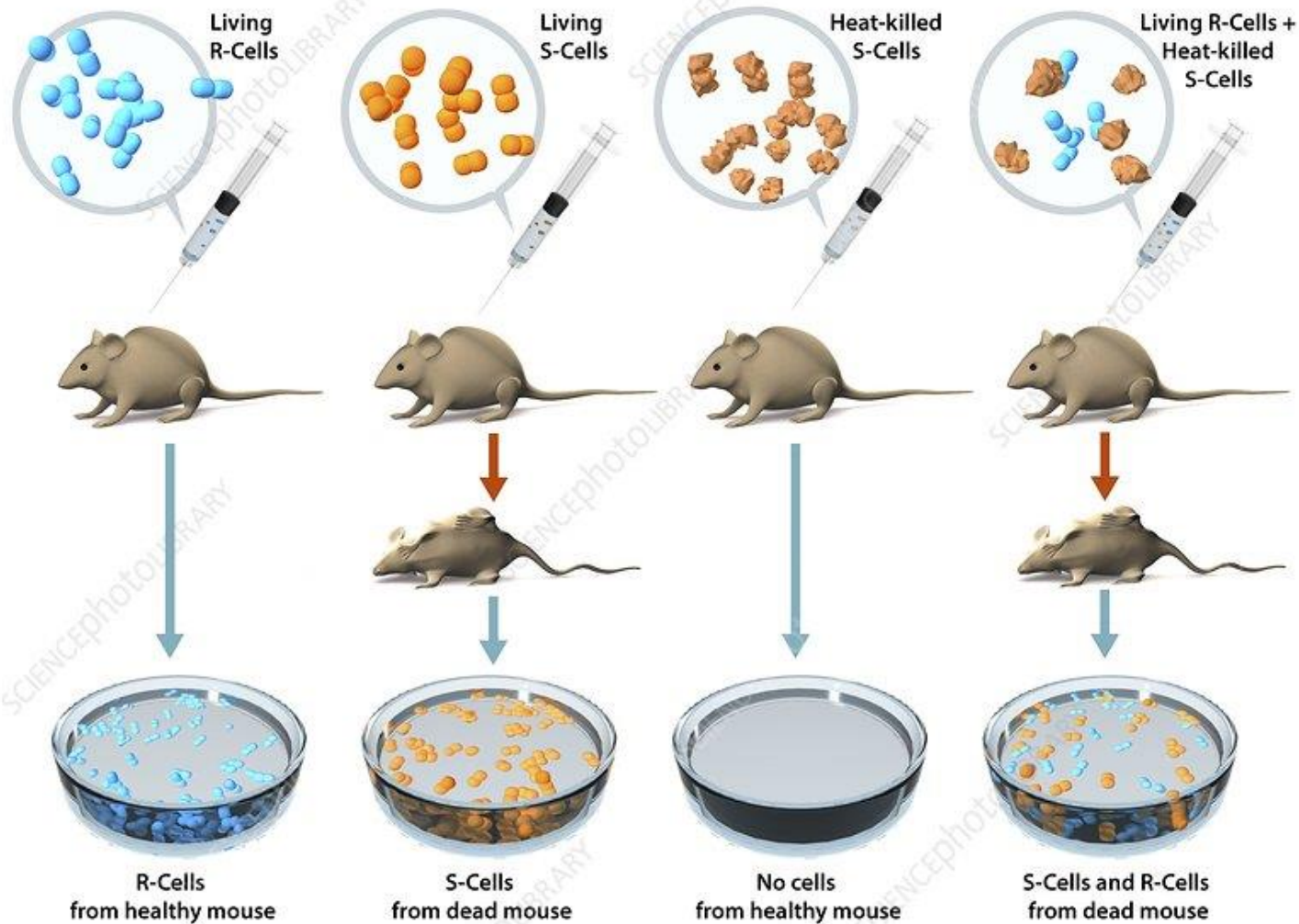
Frederick Griffith
(1877 – 1941)

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.



Discovery of bacterial Transformation



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

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.)

CONTENTS.

	PAGE
I. OBSERVATIONS ON CLINICAL MATERIAL	113
Types in Lobar Pneumonia	114
Variety of Types in Sputum from the same Case	114
A Rough Virulent Strain	117
A Strain agglutinating specifically with two different Group IV Sera	119
II. EXPERIMENTAL MODIFICATION	120
Attenuation in Culture	120
(1) Growth in Immune Serum	120
(2) Growth on Solid Media	121
(3) Differences between Individual R and S colonies	122
Reversion from Rough to Smooth	125
A. Origin of the R Strains used	125
B. Passage of R II Strains	126
C. Massive Dosage with R II	129
Inoculation of living R and killed S cultures	129
Preliminary Experiments	129
Group IV S culture + R I and II	132
Type I S culture + R II and I	134
Type III S culture + R I and II	141
Type II S culture + R I	144
Types I and II S cultures + R Group IV	146
Inoculation of living and dead R cultures	147
III. DISCUSSION	148
IV. SUMMARY	157

I. OBSERVATIONS ON CLINICAL MATERIAL.

SINCE communicating my report¹ 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 accumulated².

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.

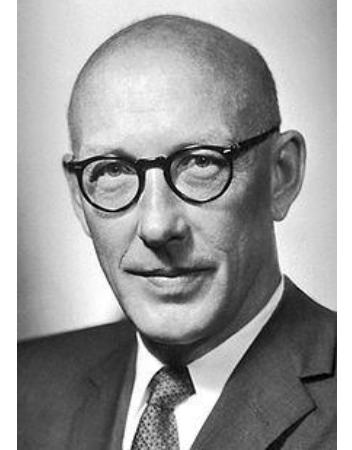
² I owe many thanks to Dr. J. Bell Ferguson, formerly Medical Officer of Health for Smethwick, for sending me many specimens from cases of lobar pneumonia.

Genes and enzymatic activity

- They were using mold *Neurospora crassa* model, new to the molecular biologists.
- They *x-rays* *Neurospora crassa* 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)

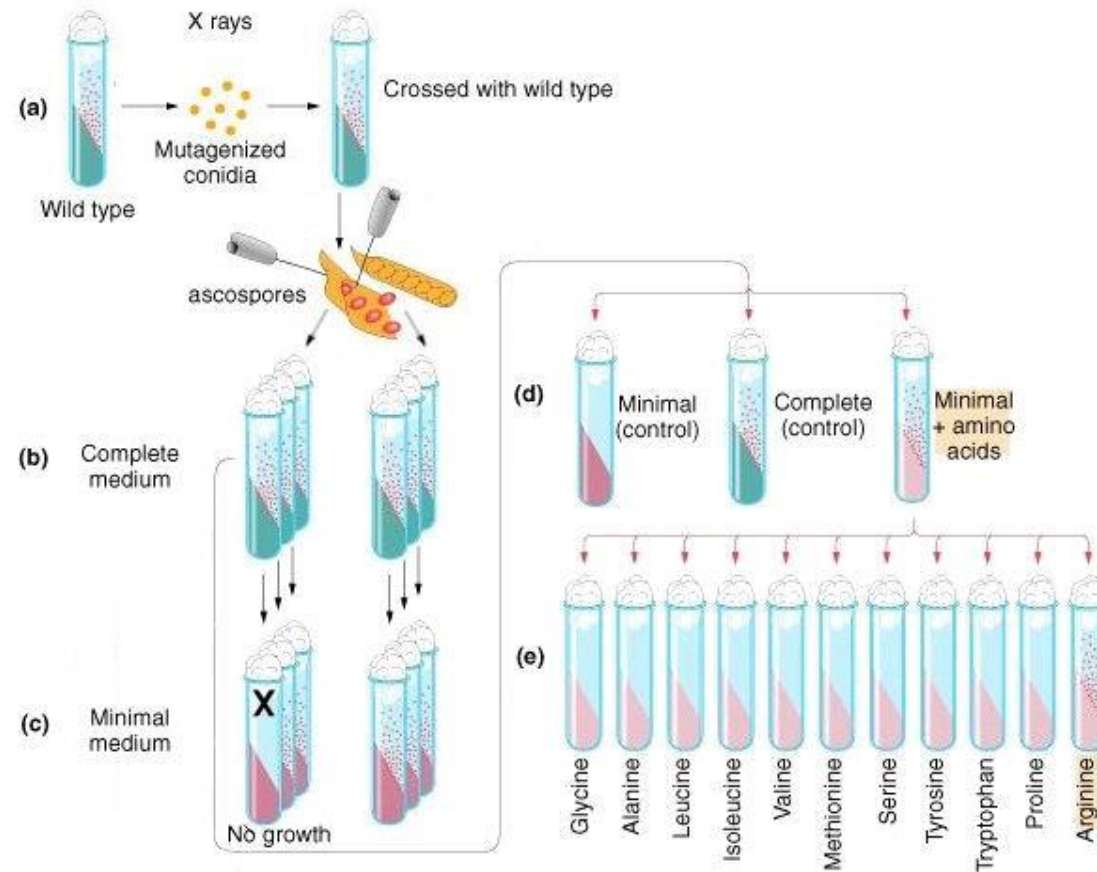


Edward Tatum
(1909 – 1975)

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**.



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.

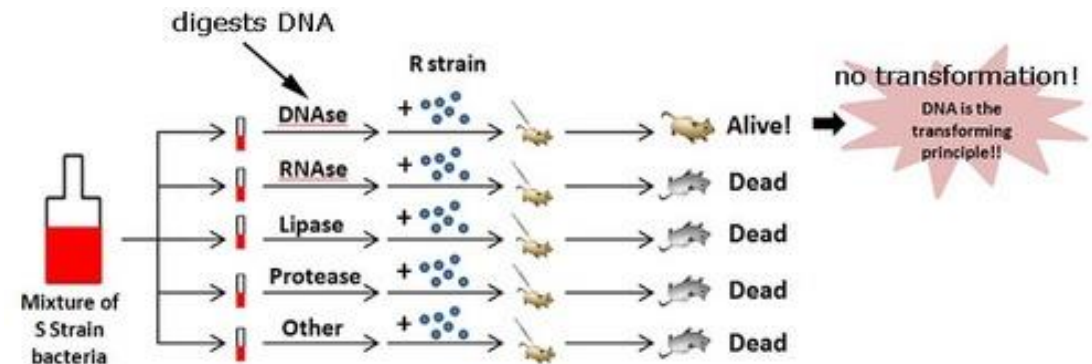
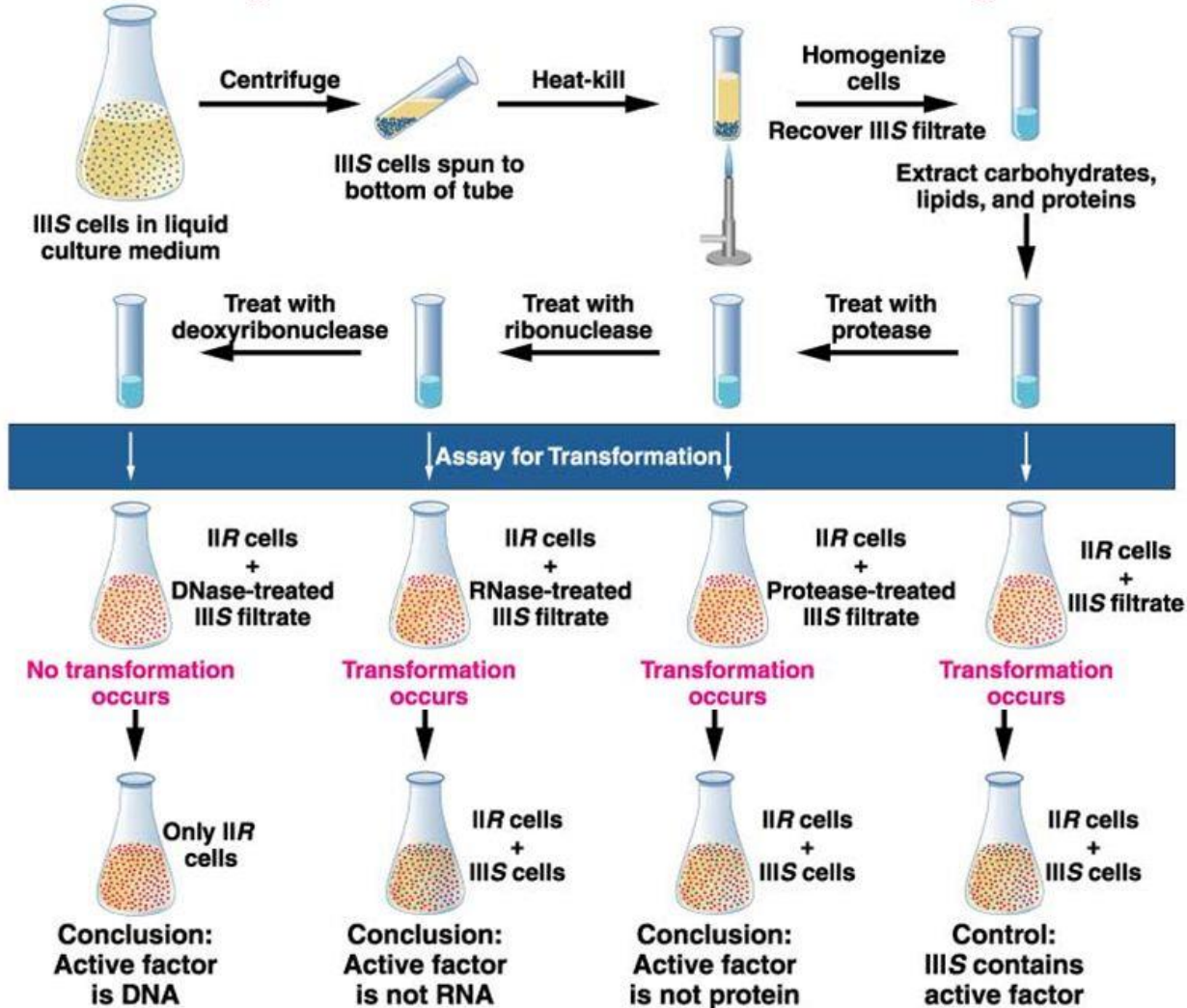
(From the Hospital of The Rockefeller Institute for Medical Research)

PLATE 1

(Received for publication, November 1, 1943)

DNA harbors the genetic information

Oswald Avery's Isolation of the Transforming Substance

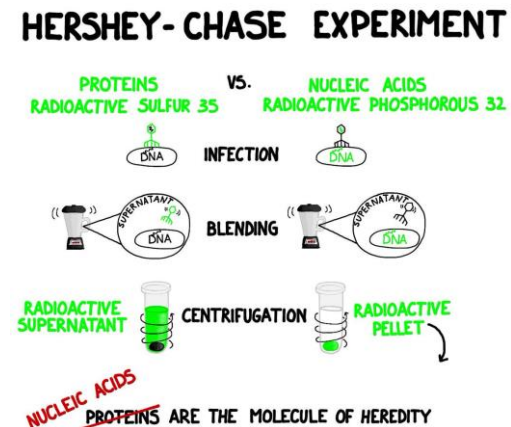


Hereditary genetic information is carried by DNA

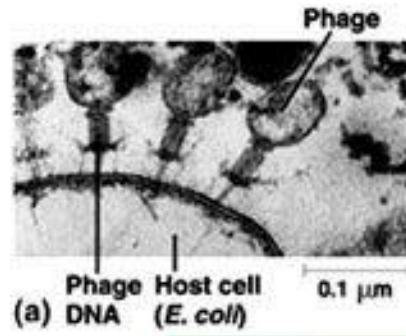
- Phage DNA and proteins are separable.
- The phages inject their DNA into the host bacteria.
- The phages inject their DNA into the bacteria and then the DNA serves as as replicating element genetic element of phages.



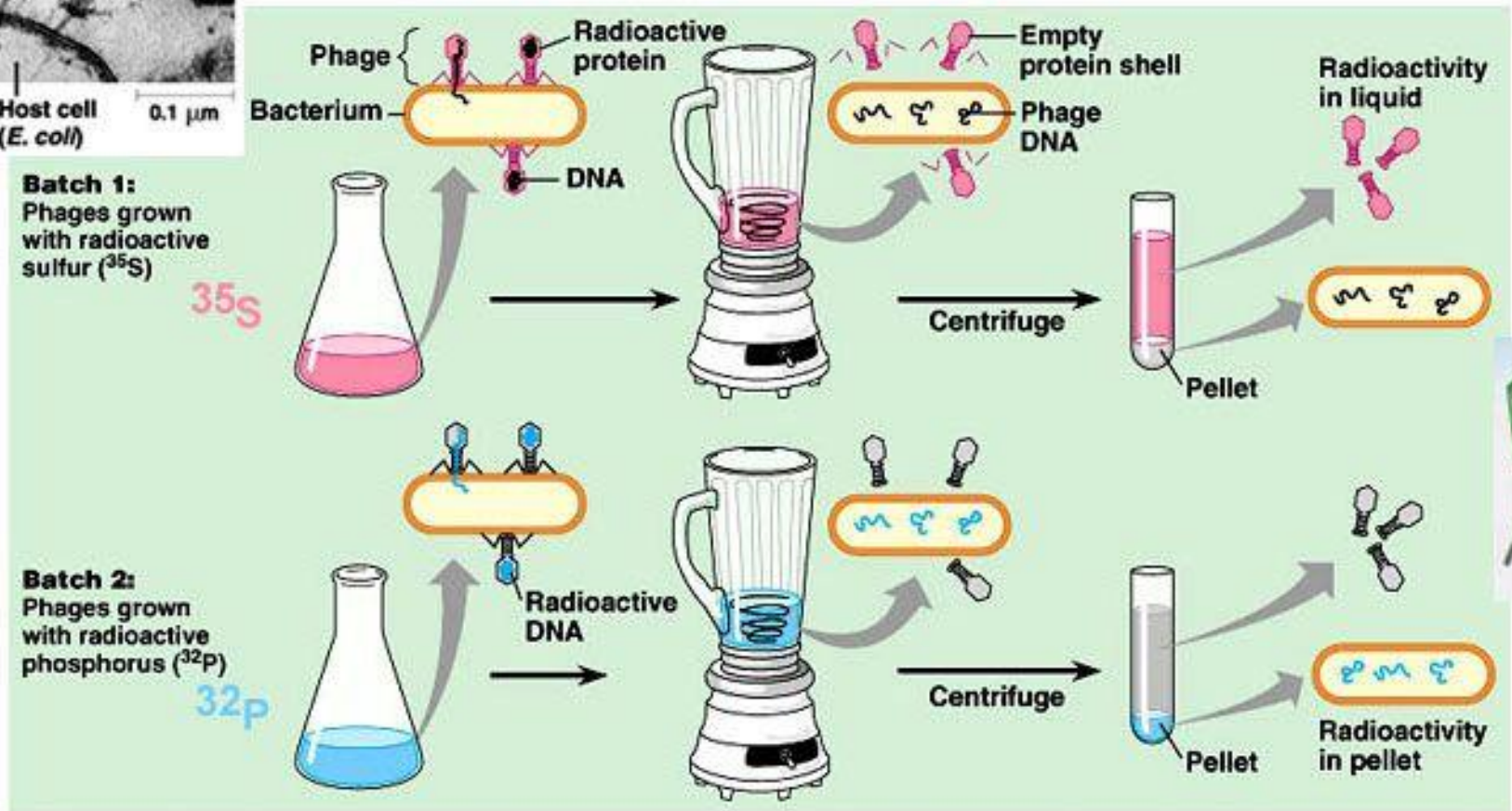
Martha Chase and Alfred Hersey
1952 CSHL USA



Hereditary genetic information is carried by DNA



- 1 Mix radioactively labeled phages with bacteria. The phages infect the bacterial cells.
- 2 Agitate in a blender to separate phages outside the bacteria from the cells and their contents.
- 3 Centrifuge the mixture so bacteria form a pellet at the bottom of the test tube.
- 4 Measure the radioactivity in the pellet and the liquid.



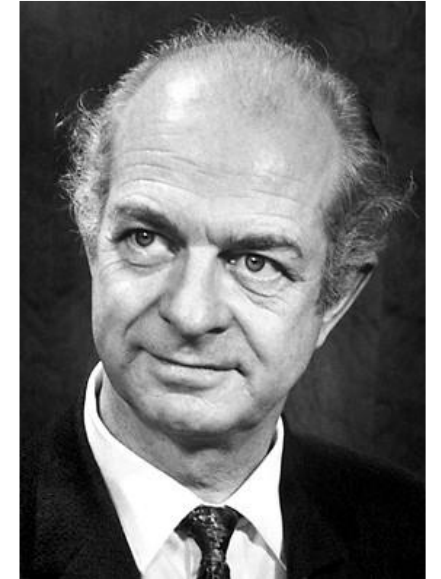
(b) The experiment showed that T2 proteins remain outside the host cell during infection, while T2 DNA enters the cell.

- Waring blender experiment.



Mutation in the DNA causes disease

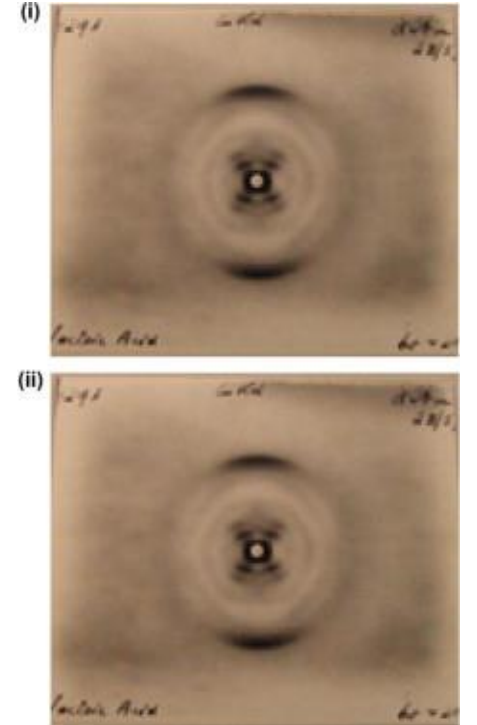
- He applied principles of quantum mechanics in chemistry and also participates in on the study of the spatial structure of proteins.
- He formulates a hypothesis, that the cause of sickle cell anemia could be abnormal form of hemoglobin.
- The hypothesis successfully verified by electrophoretic techniques, 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)

Hunt for the structure of DNA

- In the 1950s, three groups 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**.
- 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.

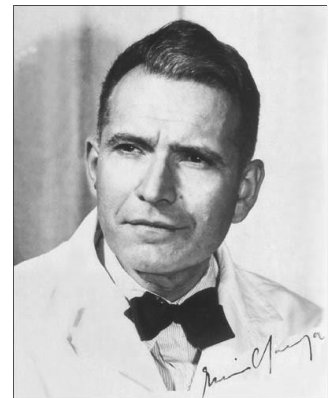
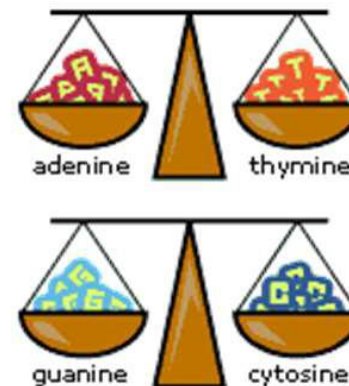
Hunt for the structure of DNA

- 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 and Francis Crick derive the structure of DNA on the basis of the following data:
- Chemical data: Erwin Chargaff principles:
 - the concentration of thymine and adenine is the same
 - the concentration of cytosine and guanine is the same
- Physical data: Maurice Wilkins and Rosalind Franklin after exposure of purified DNA molecules to X-rays, there is a characteristic scattering of rays that signal method of arranging DNA components into a helix.



Discovery of DNA structure

Rosalinda Franklin

James Watson a Francis Crick

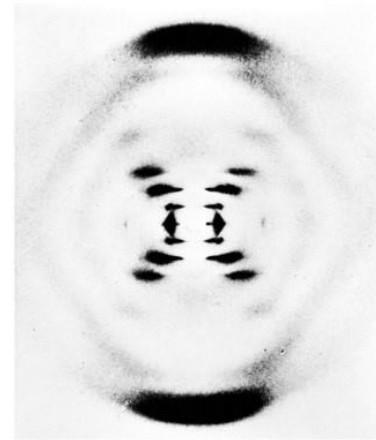
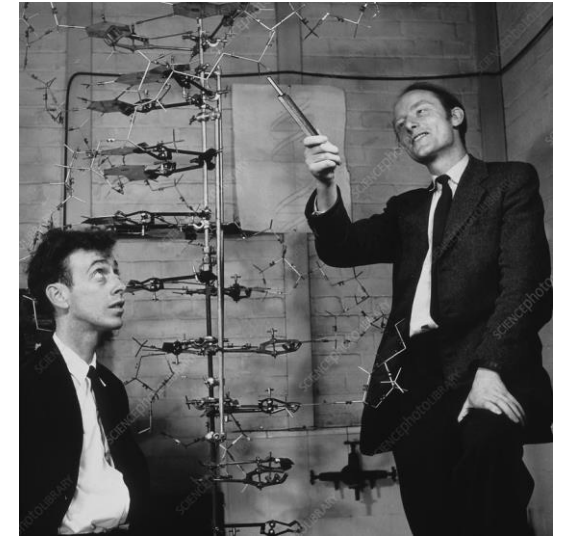
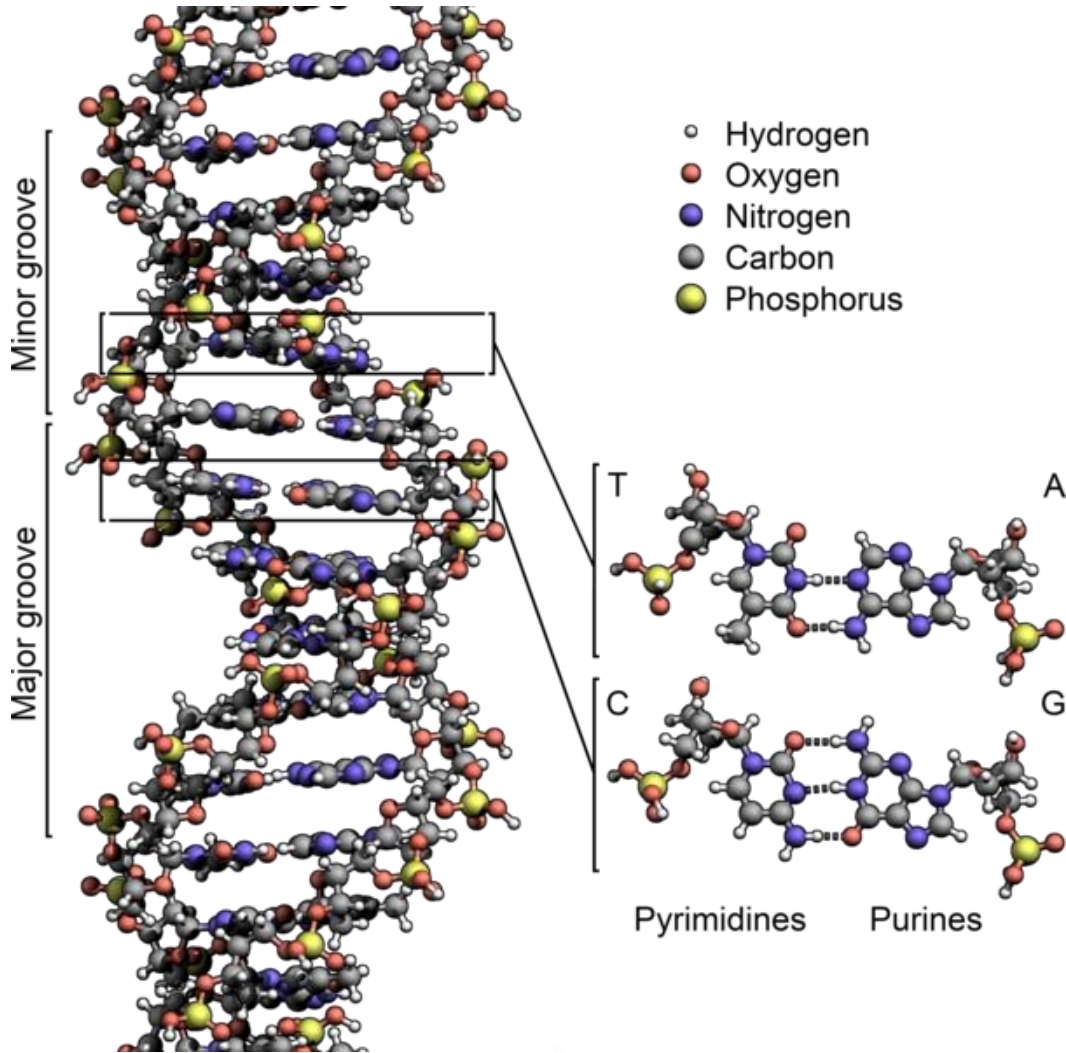
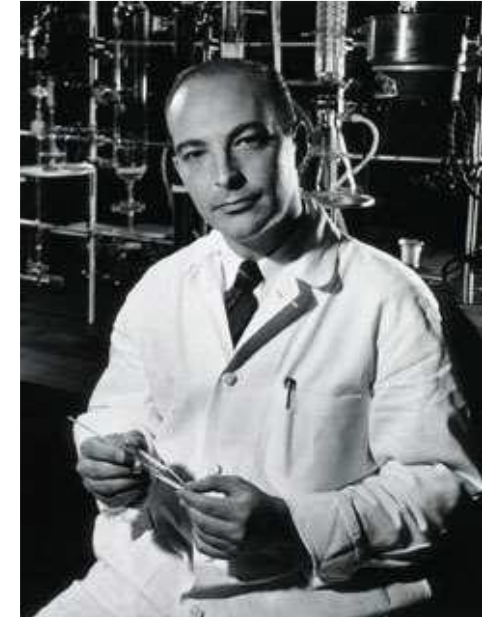


Photo 51

Discovery of DNA polymerase

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



Arthur Kornberg
(1918 – 2007)

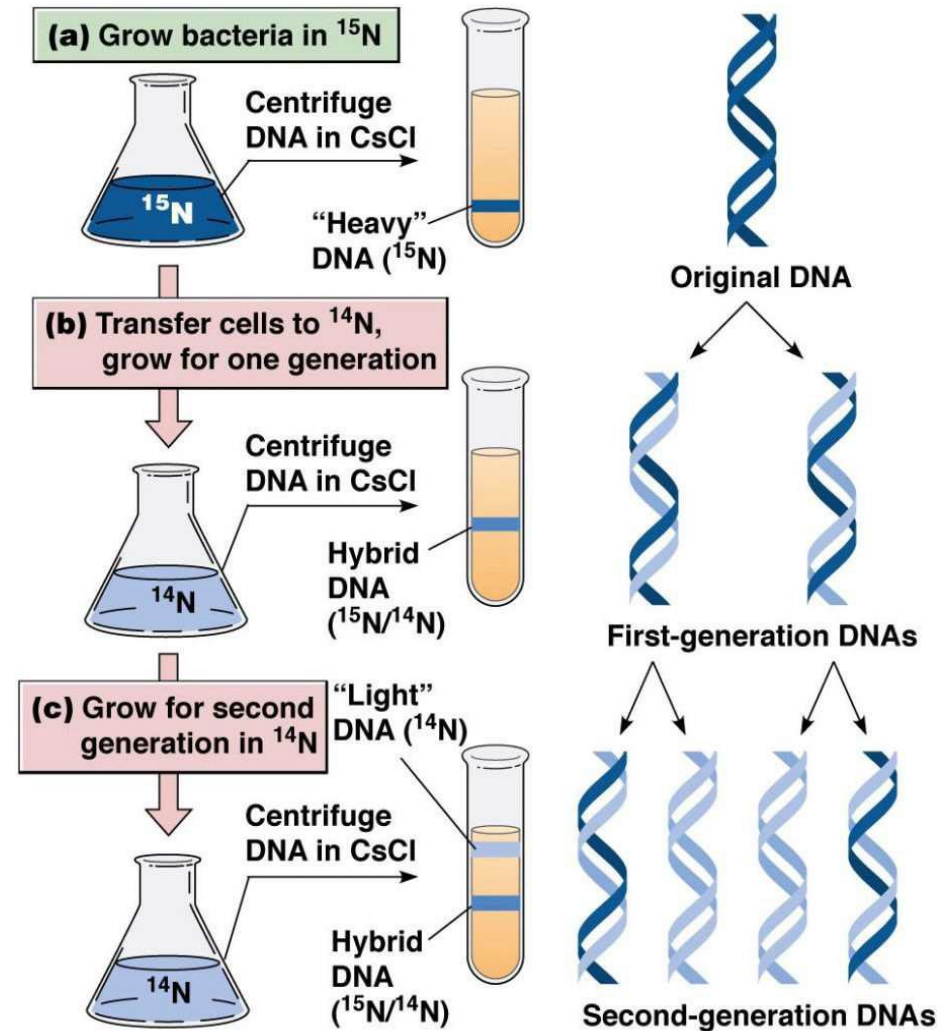
DNA replication is semiconservative process

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



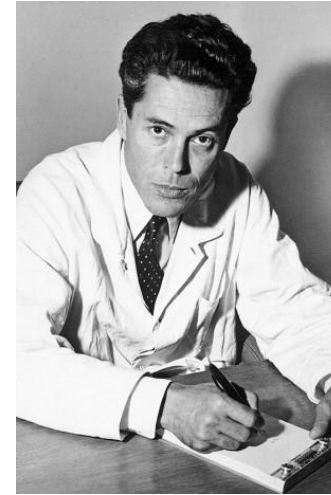
Matthew Meselson
(1930 -

Franklin Stahl
(1929 -



There is intermediate between DNA and protein

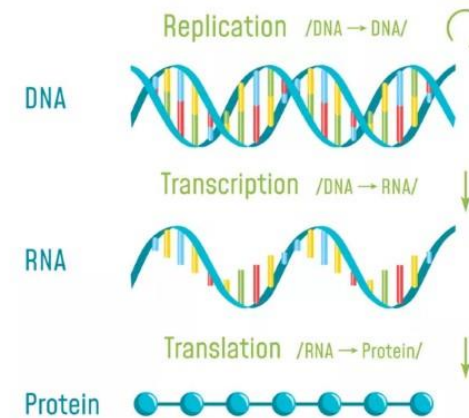
- At the beginning of 1960, Jacob and Monod observed regulatory proteins at the edges of the genes and control the transcription of these genes into messenger RNA, in other words they direct transition of these genes.
- Two concepts of utmost importance came out of those experiments in 1961:
 - that of messenger RNA
 - the operon.



Jacques Monod
(1910 – 1976)



Francois Jacob
(1920 – 2013)



Cracking the genetic code

- Work by Crick and coworkers 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 1968 **H.G. Khorana R. Holley M. Nirenberg** were awarded by the Nobel Prize in Physiology or Medicine.



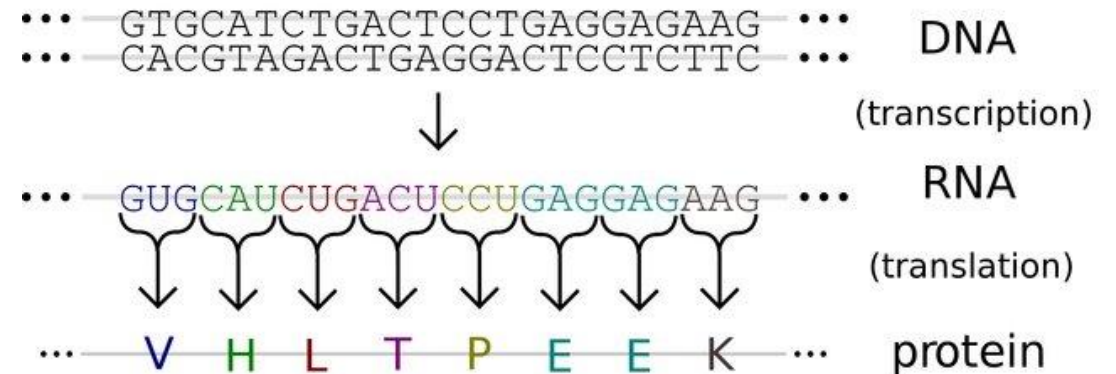
H. G. Khorana



R. Holley



M. Nirenberg



Cracking the DNA code

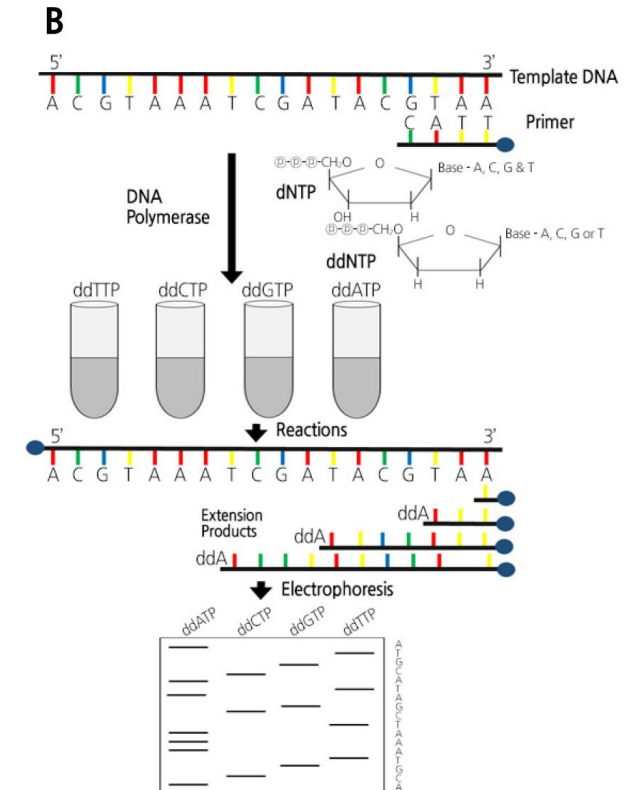
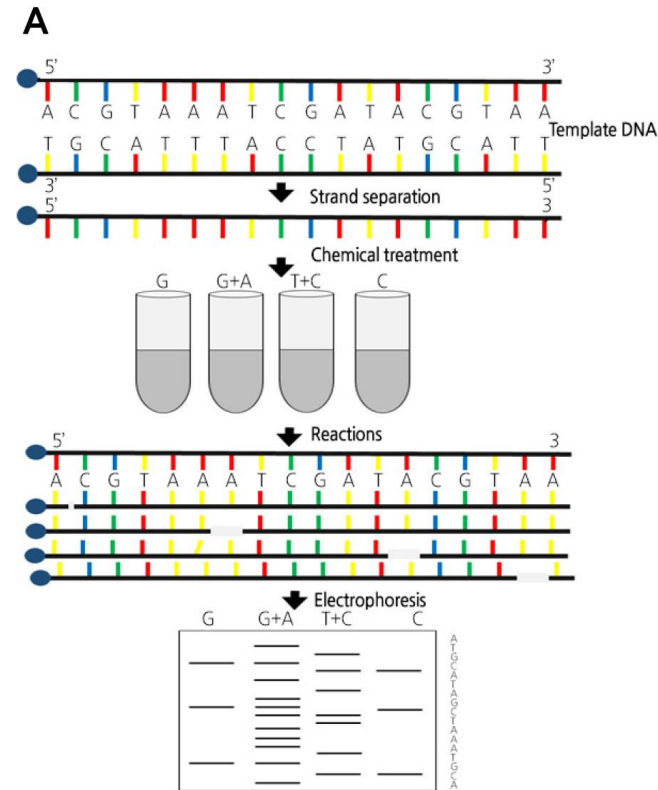
- Khorana synthesis of polynucleotides with defined sequence of nucleotides, repeated mostly → in vitro transcription → in vitro translation → **polypeptide analysis**. The technique **works** only for **DNA**, not for **RNA**.
- 1961 - Nirenberg and Matthaei discovered that **poly-U RNA** nucleotide makes **phenylalanine** polypeptide **chain** *in vitro*.
- Team of researchers worked to identify meaning for all 64 codons.
- 1966 – The complete Genetic 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.

DNA sequencing

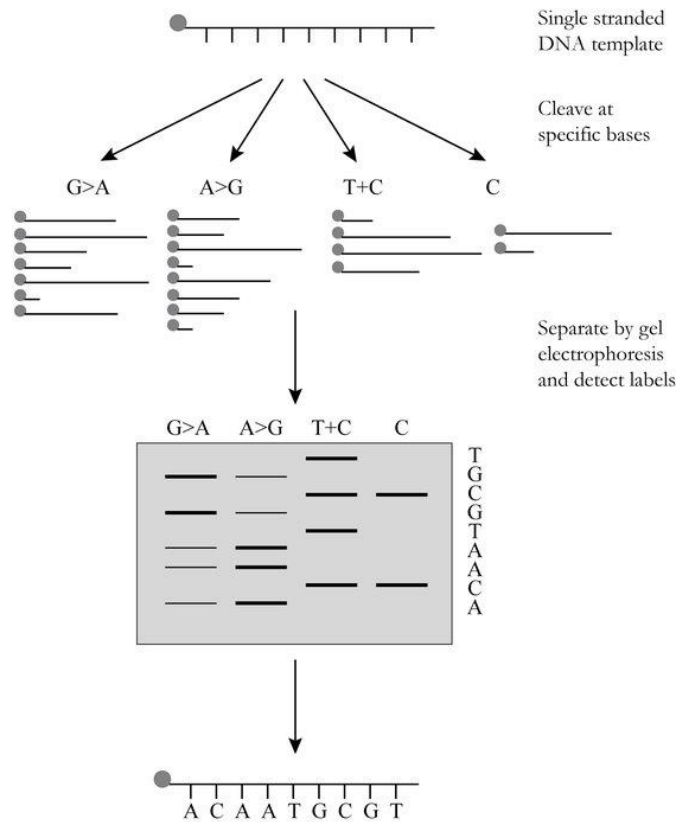
- Maxam–Gilbert technique depends on the relative chemical liability of different nucleotide bonds, 1977.
- Sanger method interrupts elongation of DNA sequences by incorporating dideoxynucleotides into the sequences, 1975.
- Gilbert and Sanger were awarded by the Nobel Prize in Chemistry in 1980.



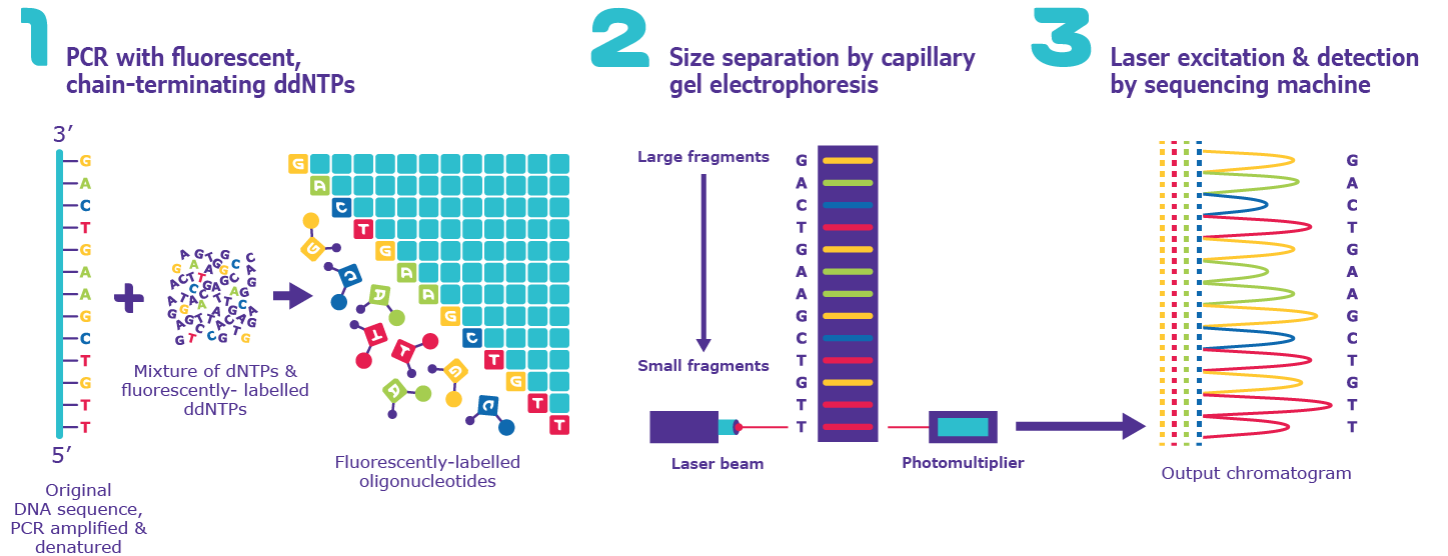
DNA sequencing

- Maxam–Gilbert technique

● label



- Sanger method

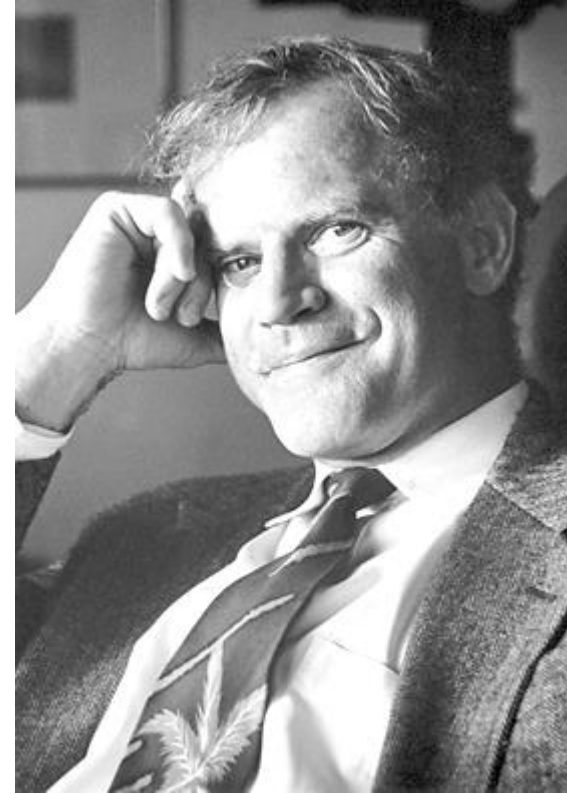


https://www.researchgate.net/publication/268048875_Strategies_for_de_novo_DNA_sequencing/figures?lo=1

<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).

RNA interference – non-coding RNA

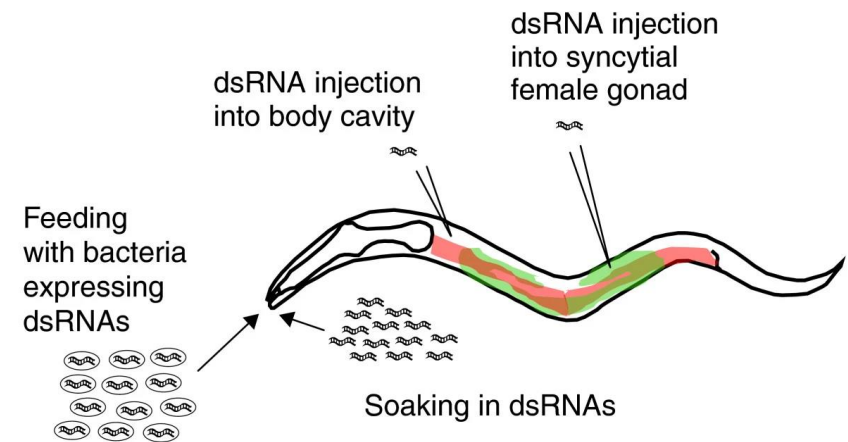
- In 1998, **Fire** and **Mello** demonstrated that they could efficiently and selectively **dial down the expression** of various **genes** in the worm *Caenorhabditis elegans* by injecting small quantities of **short interfering RNA** (siRNA) molecules, which comprise paired strands of RNA.
- Discovery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



Andrew Z. Fire



Craig C. Mello

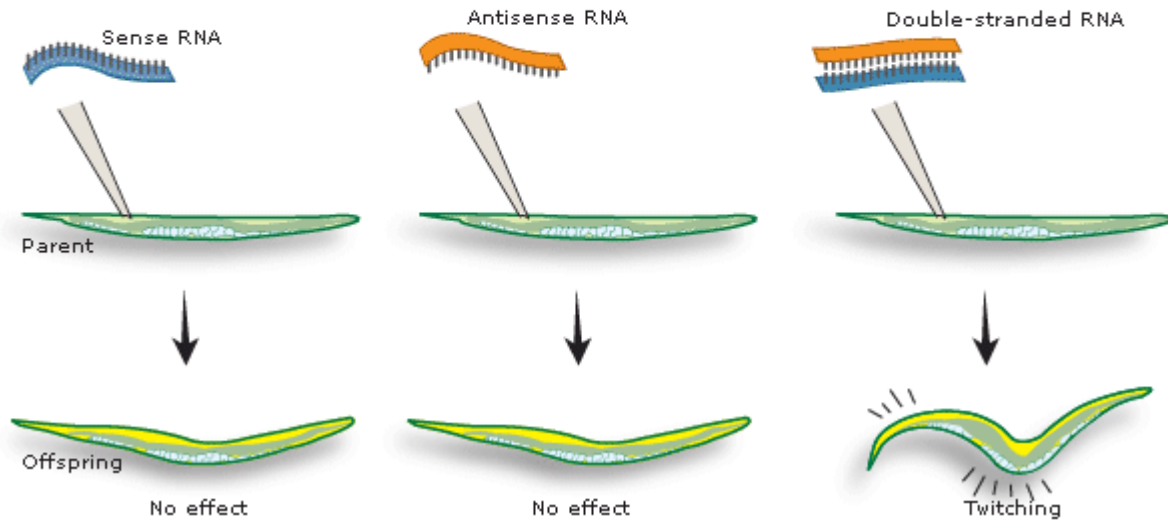


RNA interference – non-coding RNA

Gene silencing

Fire and Mello injected RNA corresponding to a gene important for muscle function in the worm *C. elegans*.

Single-stranded RNA (sense or antisense) had no effect. But double-stranded RNA caused the worm to twitch in a similar way to worms that lack a functional gene for the muscle protein.



Loss of target mRNA

Fire and Mello injected RNA (*mex-3* RNA) into the gonads of the worm *C. elegans* and studied the effect on the corresponding mRNA.

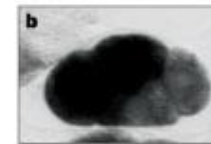
They found that double-stranded RNA, but not single-stranded RNA, eliminated the target mRNA.

A four-cell embryo from *C. elegans*.



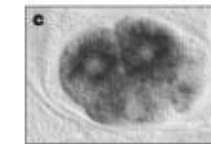
NATURE FEB 1998

Uninjected



mex-3 RNA (stained black) is abundant in the early embryo.

Antisense RNA



Injection of antisense RNA reduced the content of mRNA to some extent.

Double-stranded RNA



The target mRNA was eliminated after injection of double-stranded RNA.

CRISPR method for genomic DNA editing

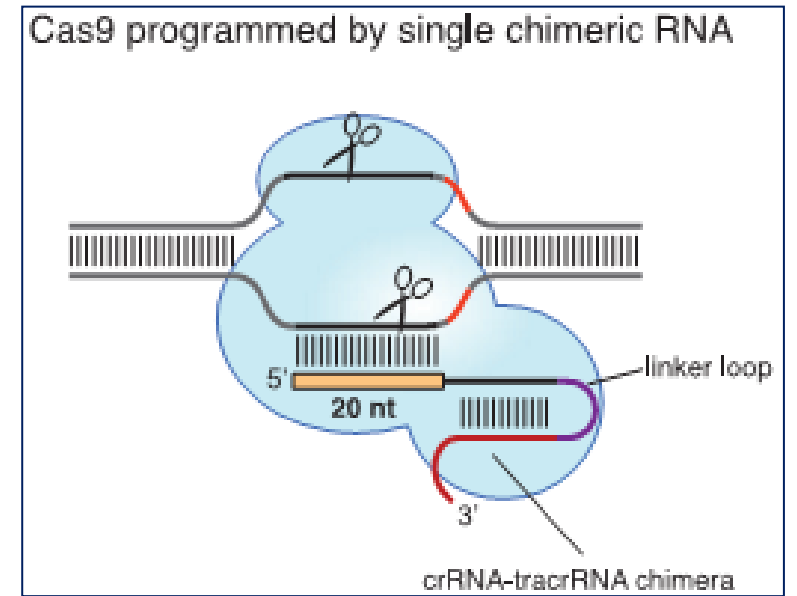
- Nobel Prize for Chemistry in 2020.



Emmanuelle Charpentier



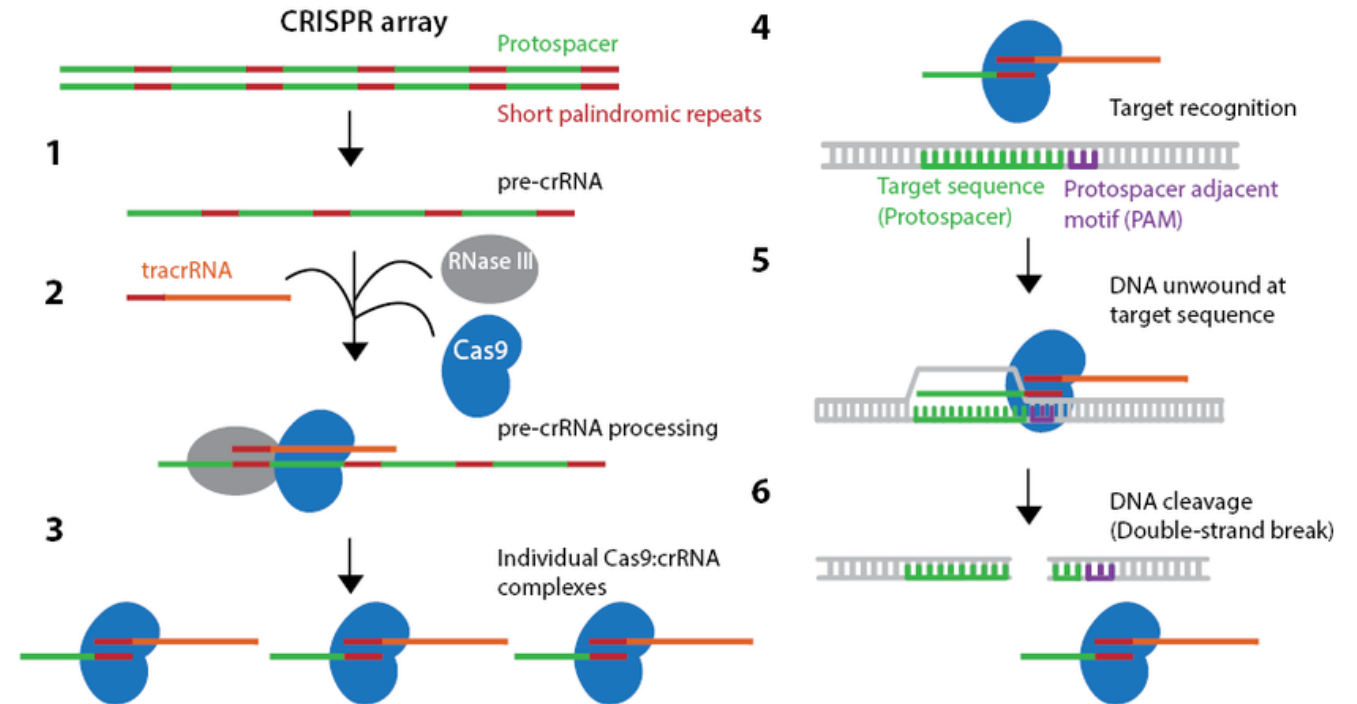
Jennifer A. Doudna



17 AUGUST 2012 VOL 337 SCIENCE

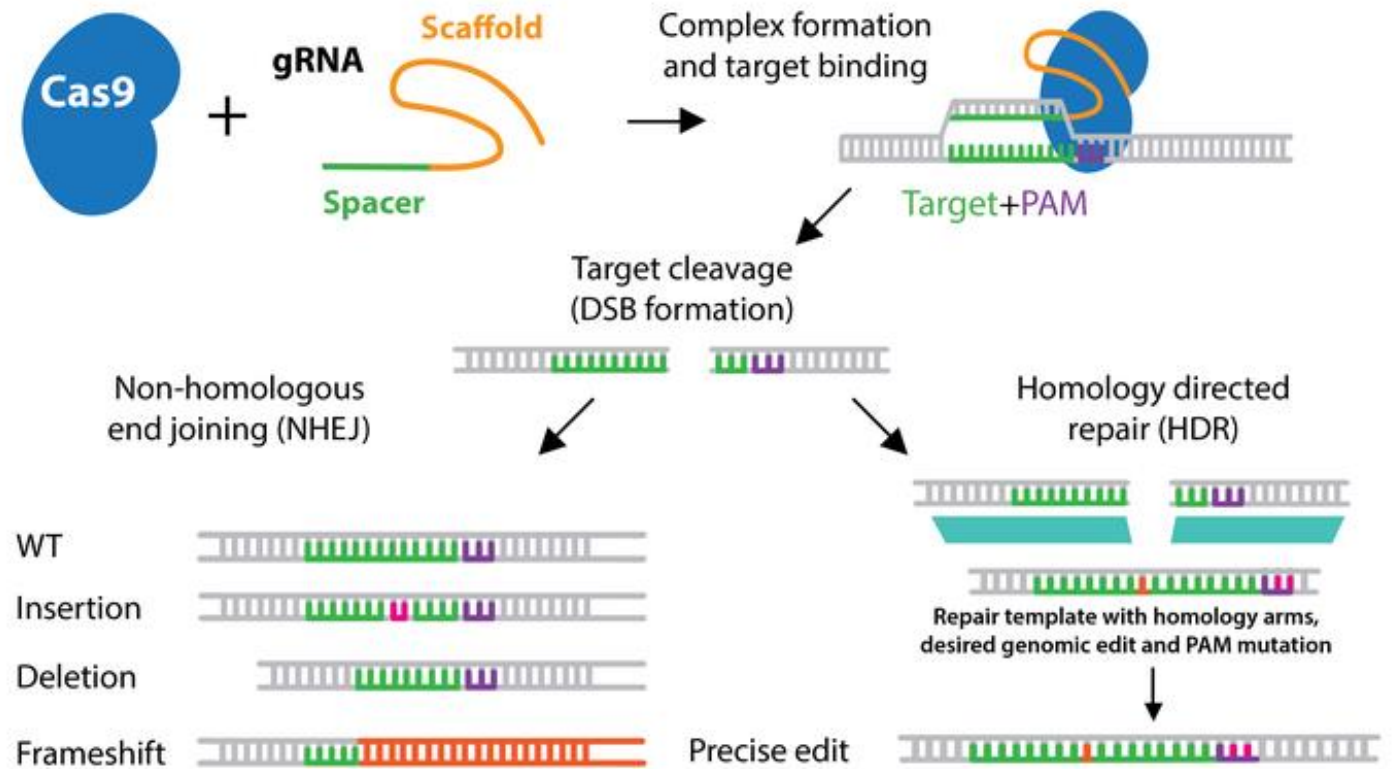
CRISPR method for genomic DNA editing

- 2011 — Emmanuelle Charpentier, showed that **tracrRNA** forms a **duplex** with **crRNA**, and that it is this duplex that guides Cas9 to its targets.
- 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.



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*) demonstrated targeted genome cleavage in human and mouse cells.
 - (i) could be programmed to target multiple genomic loci
 - (ii) could drive homology-directed repair.



Current age of Molecular biology

- Research area
- 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.

Current age of Molecular 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 replacement.
- 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).

