SAPIENZA
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Molecular Biology

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SAPIENZA
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All functions of a living organism rely on its genetic material

genome

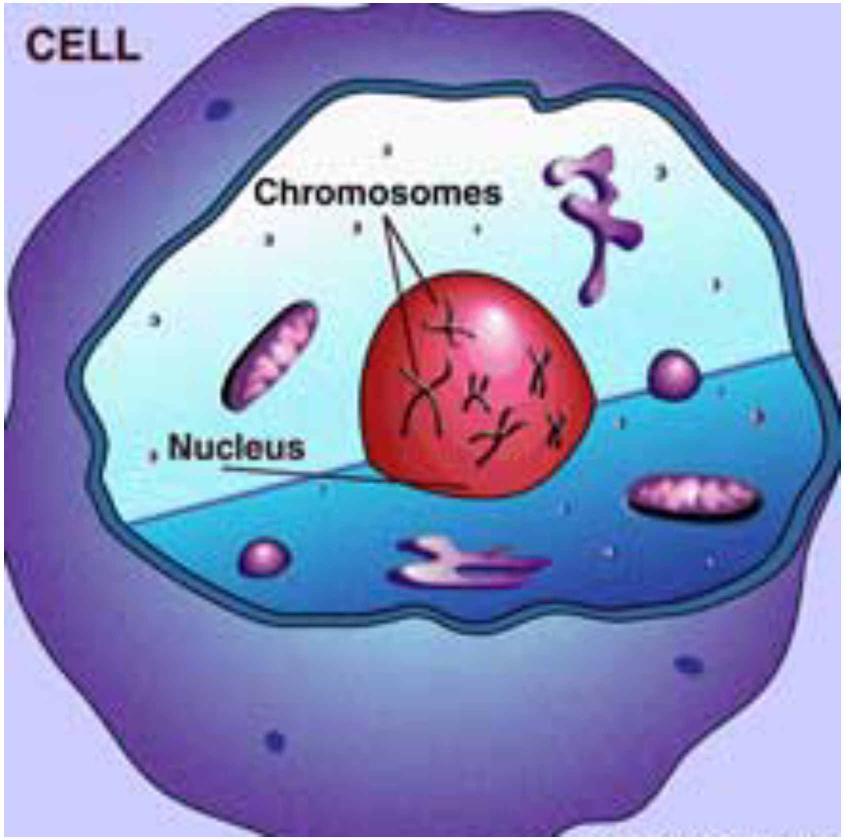
- All features of a person depend on his genetic material
- Even mental abilities and personal attitudes are inherited.
- Many diseases and predisposition are also inherited

Many diseases are acquired from the environment (infections, allergies…), but many derive from our genome and can be transmitted to next generations

INHERITED DISORDERS

GENETICS and MOLECULAR BIOLOGY

study the molecular mechanisms at the basis of these pathologies



What is a **genome?**

What is a **chromosome?**

What is a **gene?**

What is **DNA?**

How works DNA?

the genome is made of chromosomes

chromosomes contain genes

genes are made of DNA

DNA is made of four units nucleotides

G A T C

genome

↓

chromosomes

↓

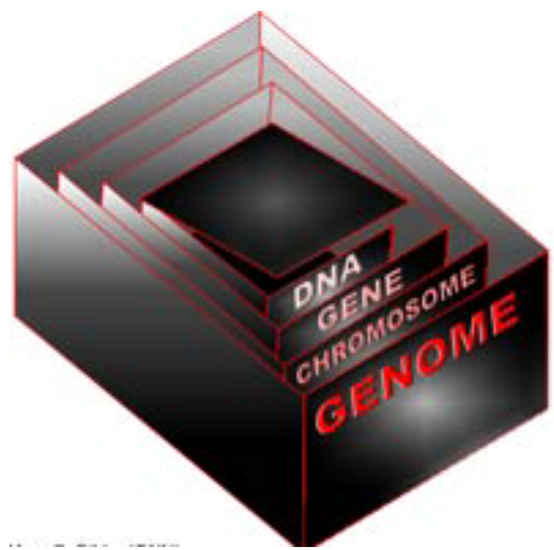
genes

↓

DNA

↓

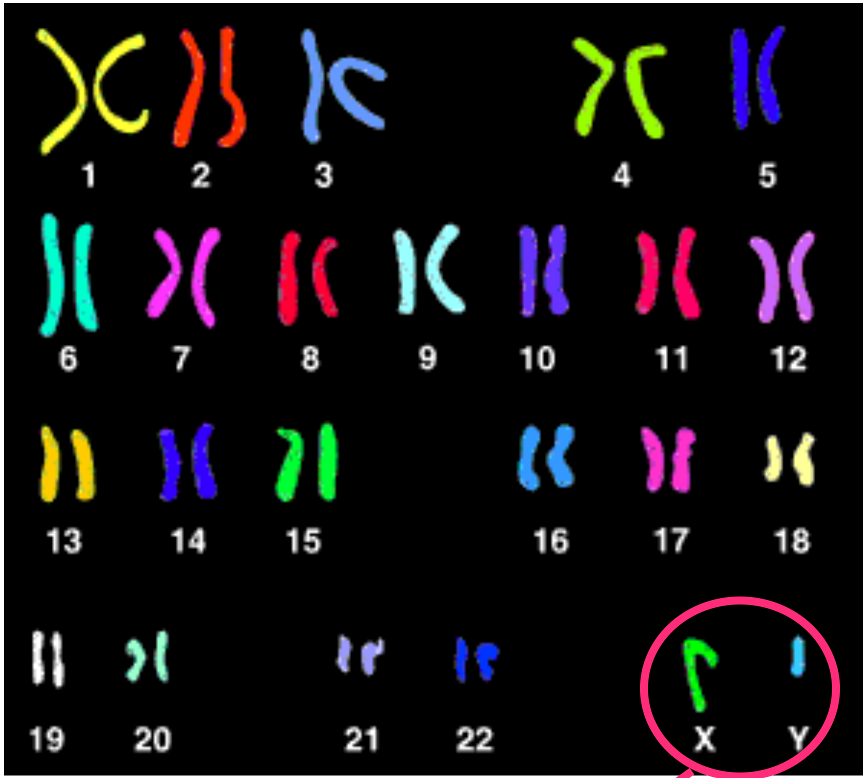
AGTCCGCGAATACAGGCTCGGT



Ogni specie ha il suo genoma "distintivo"

Organismo	n° cromosomi
Pisello	14
Girasole	34
Gatto	38
Pesce	42
Uomo	46
Cane	78

Chromosomes are present in couples

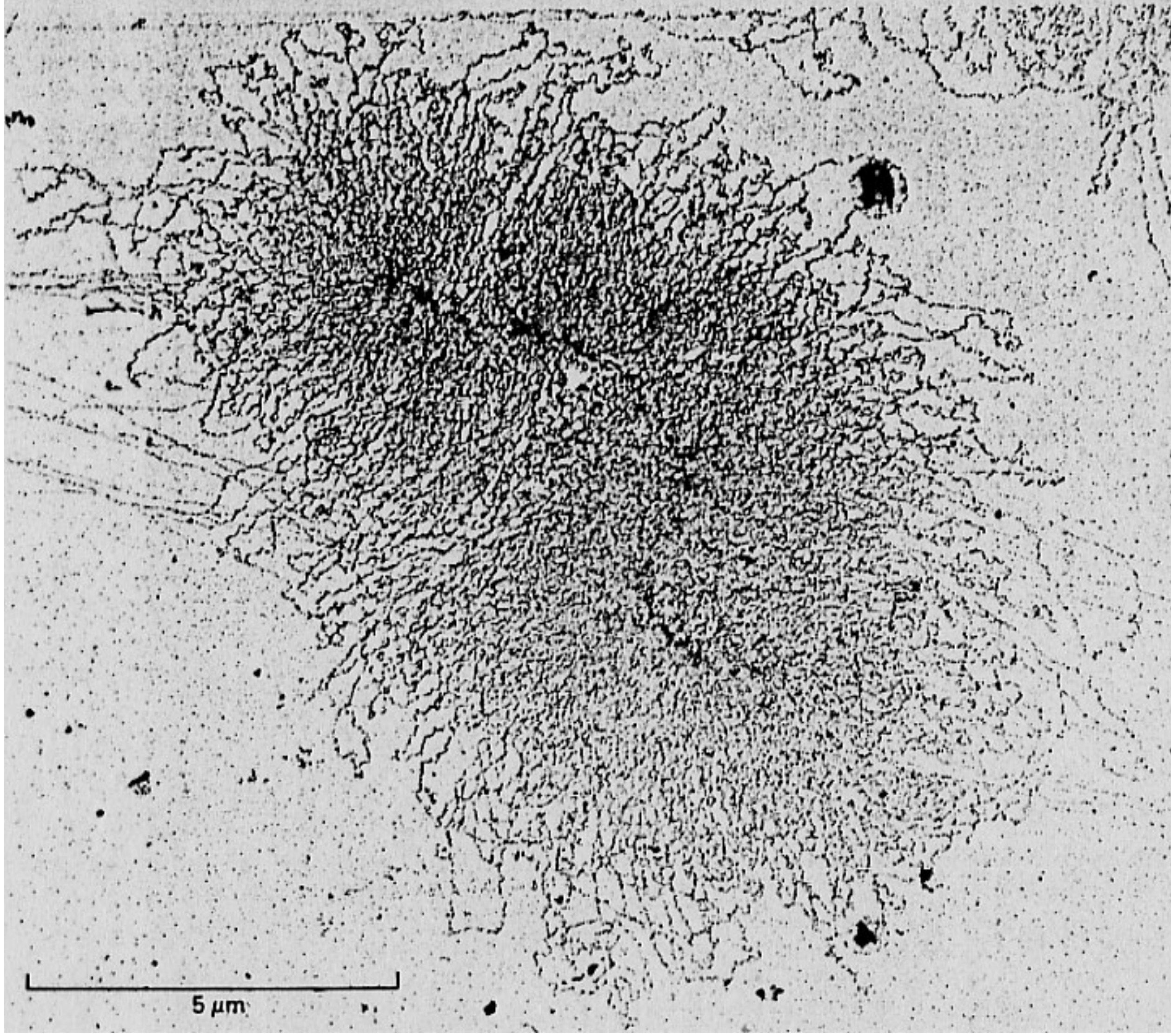


Sexual chromosomes are different

Men have 44 Chromosomes plus XY

Women have 44 chromosomes plus XX

Several diseases are characterized by alterations in the number or Structure of specific chromosomes – **Down Syndrome**



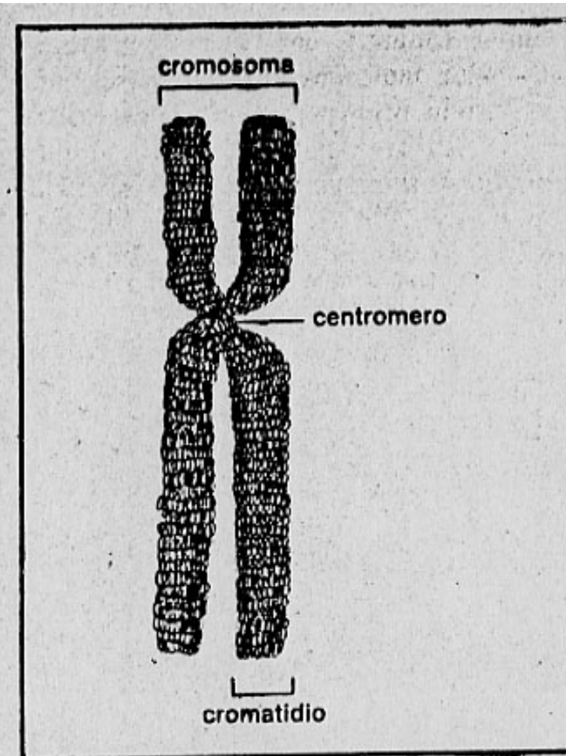
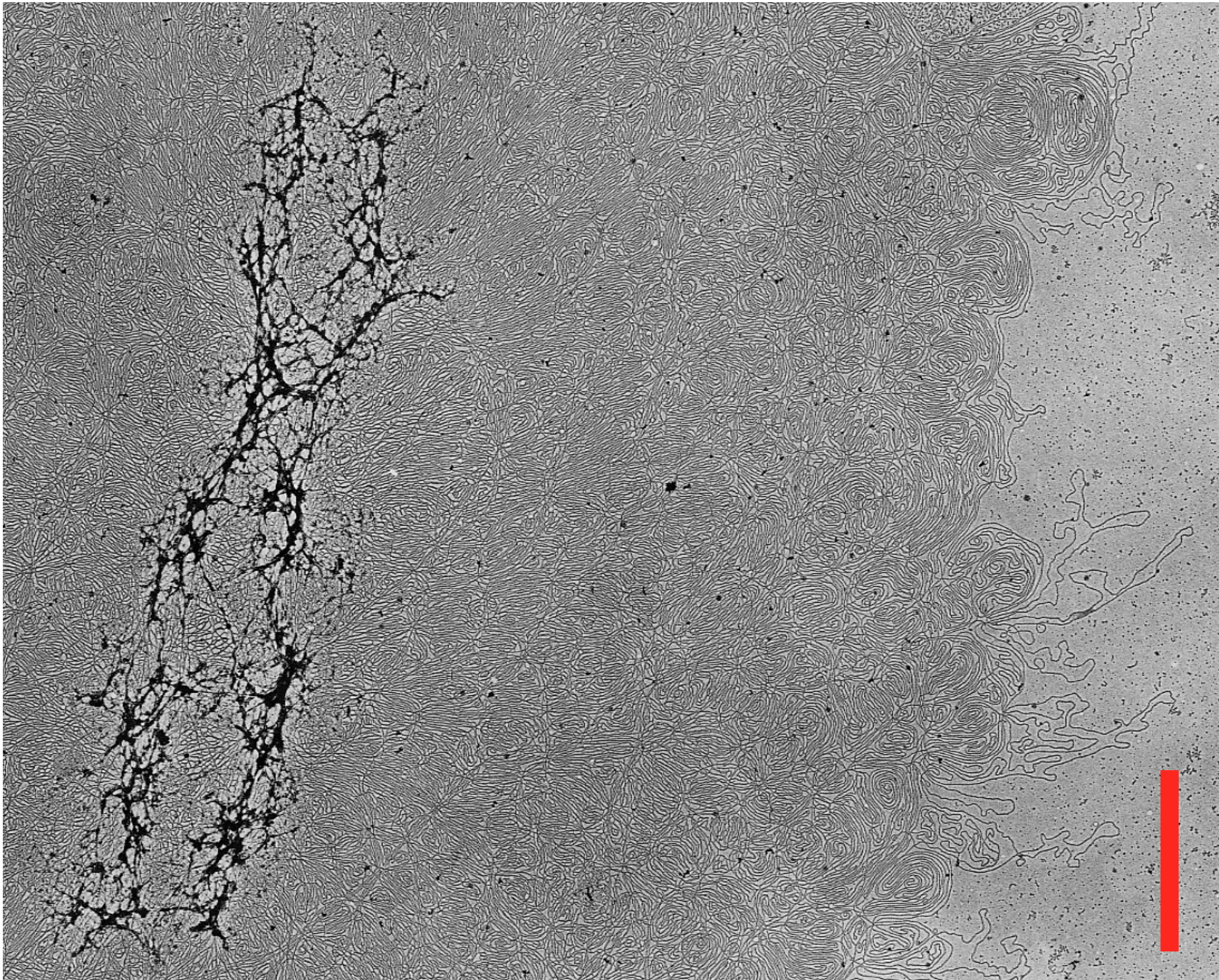
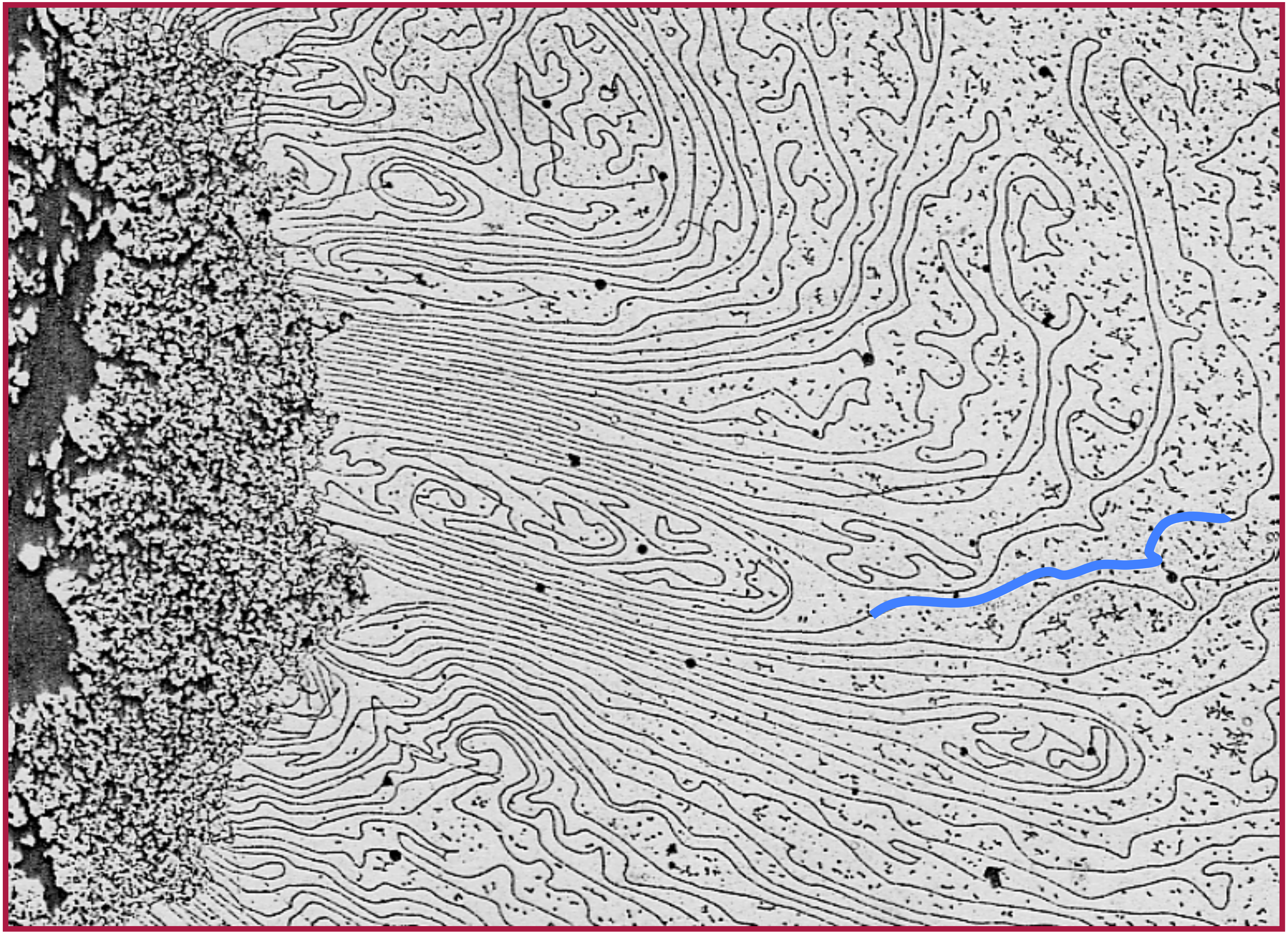


Figura 8.22. *Schema di un tipico cromosoma metafasico. Ogni cromatidio è formato da una delle due molecole figlie identiche di DNA (una di esse nello schema è colorata) che si sono formate durante una fase precedente del ciclo cellulare per duplicazione del DNA.*





HISTORY OF THE GENE, 1860 TO JUST BEFORE ENCODE

Definition 1860s–1900s: Gene as a discrete unit of heredity

In particular, the word *gene* was first used by Wilhelm Johannsen in 1909, based on the concept developed by Gregor Mendel in 1866 (Mendel 1866).
The etymology of the term derives from the Greek *genesis* ("birth") or *genos* ("origin").

Definition 1910s: Gene as a distinct locus

Thomas Hunt Morgan and his students were studying the segregation of mutations in *Drosophila melanogaster*. They were able to explain their data with a model that genes are arranged linearly, and their ability to cross-over is proportional to the distance that separated them.

Definition 1940s: Gene as a blueprint for a protein

Beadle and Tatum (1941), who studied *Neurospora* metabolism, discovered that mutations in genes could cause defects in steps in metabolic pathways.

Definition 1950s: Gene as a physical molecule

The fact that heredity has a physical, molecular basis was demonstrated by the observation that X rays could cause mutations (Muller 1927).

Definition 1960s: Gene as transcribed code

The solution of the three-dimensional structure of DNA by Watson and Crick in 1953 (Watson and Crick 1953) that ex-plained how DNA could function as the molecule of heredity.

Definition 1970s–1980s: Gene as open reading frame (ORF) sequence pattern

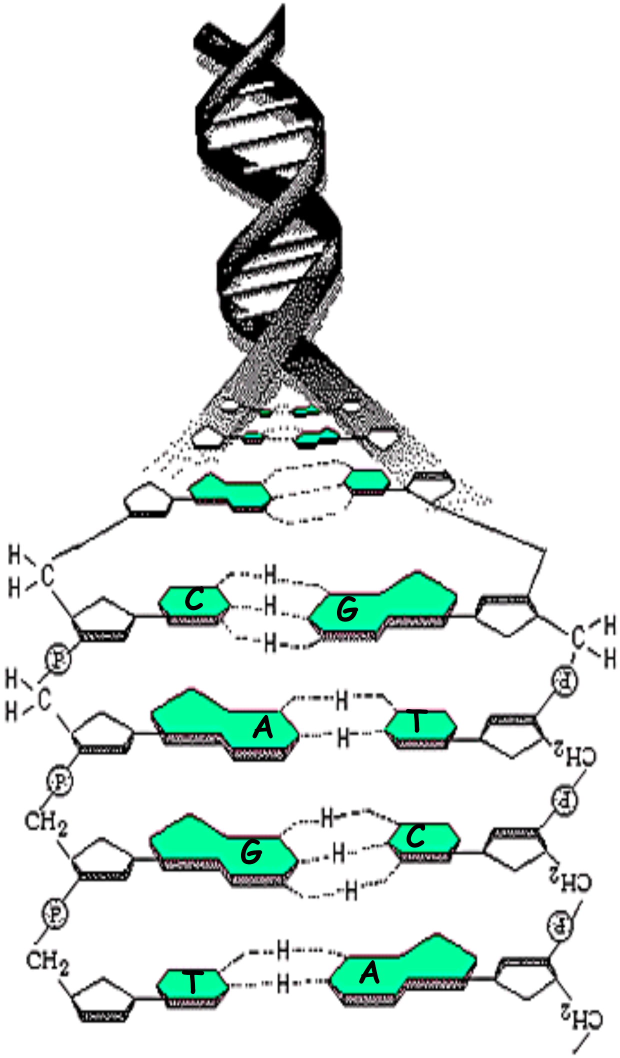
The development of cloning and sequencing techniques in the 1970s, combined with knowledge of the genetic code, revolu-tionized the field of molecular biology by providing a wealth of information on how genes are organized and expressed.

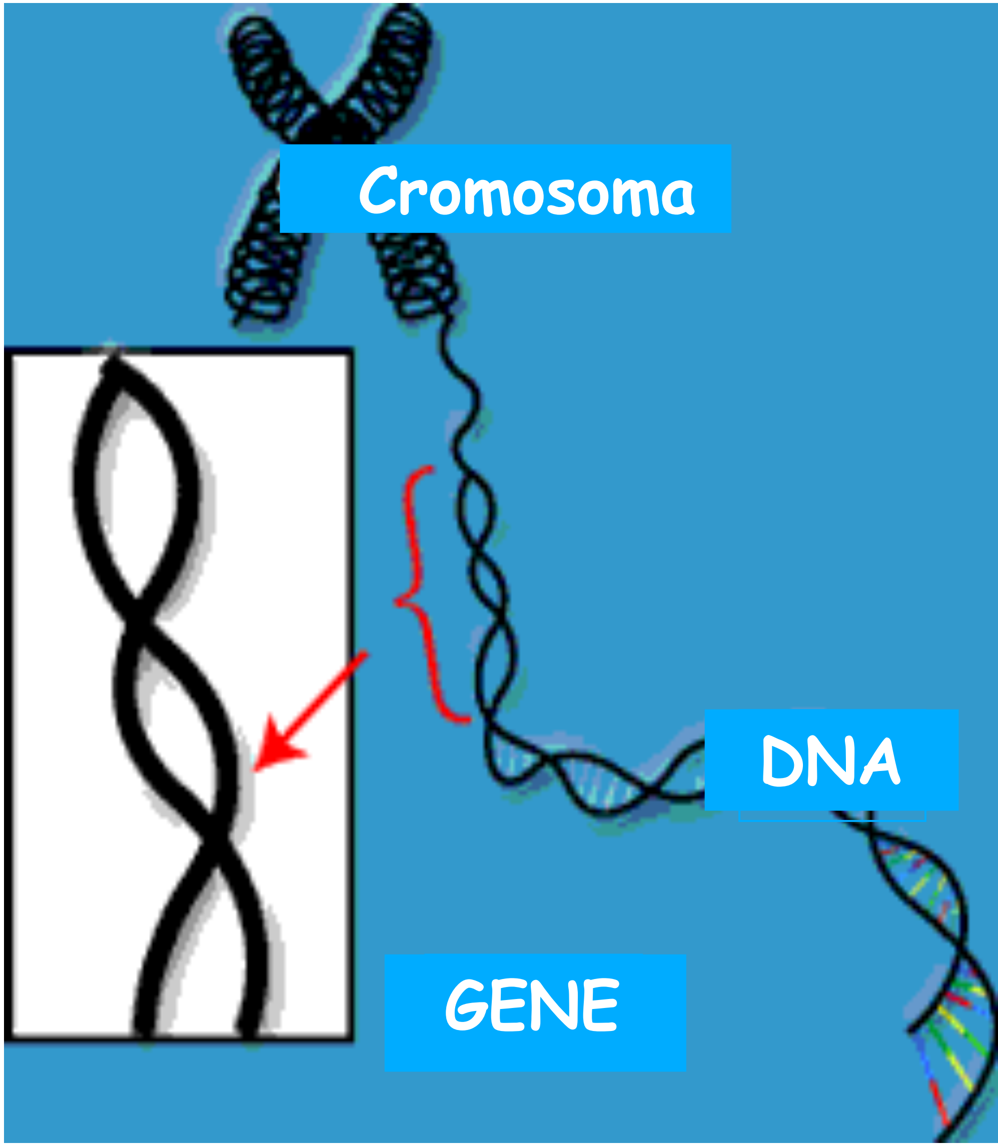
Definition 1990s–2000s: Annotated genomic entity, enumerated in the databanks (current view, re-ENCODE)

The current definition of a gene used by scientific organizations that annotate genomes still relies on the sequence view. Thus, a gene was defined by the Human Genome Nomenclature Organi-zation as "a DNA segment that contributes to phenotype/ function.

A current computational metaphor: Genes as "subroutines" in the genomic operating system

Given that counting genes in the genome is such a large-scale computational endeavor and that genes fundamentally deal with information processing, the lexicon of computer science natu-rally has been increasingly applied to describing them.


C
G
A
T
G
C
T
A


Cromosoma
DNA
GENE

HUMAN GENOME (3 billions of nucleotides)

1 meter of DNA in each cell

One gene (1 micron – 10^{-6} m)

Roma **Paris**

1500 Km

1,5 m

Library of 300 volumes,
1000 pages/volume
10.000 characters/page

→ One gene = 1 page

Extremely small

How to study it?

Not only identify functions, but also
integrate them in the context of all the other informations
contained in the genome

Three major revolutions in Molecular Biology

1950	**Resolution of DNA structure** Implications regarding the mechanisms of DNA replication and gene expression
1970	**DNA cloning** Definition of gene structure - molecular definition of several pathologies
1990	**Genome sequencing** Identification of complex functions and analysis of multifactorial diseases

1953
the birth of Molecular Biology

1859

1869

1944

1953

2008

The origin of species
Darwin

Hereditary laws
Mendel

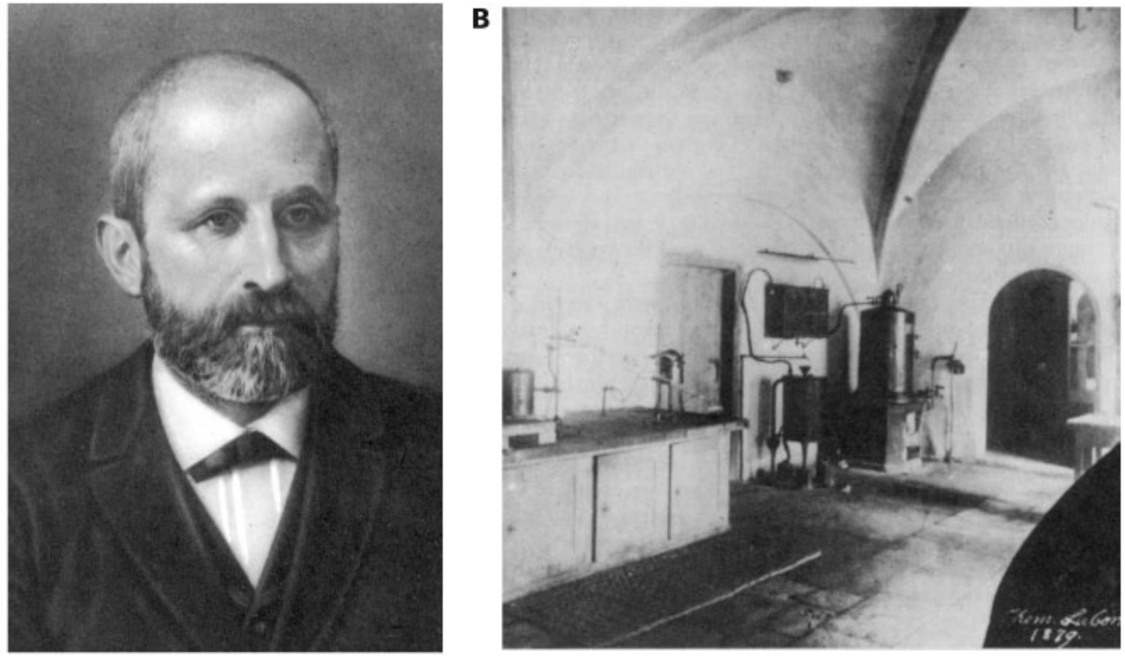
Nuclein
Miescher

DNA
Hereditary material

Resolution of DNA structure
Watson/Crick

Figura 1.4 **Friedrich Miescher** (1844-1895) **e (B) il laboratorio** situato in un vecchio castello dove, nel 1879, isolò la "nucleina".

A B



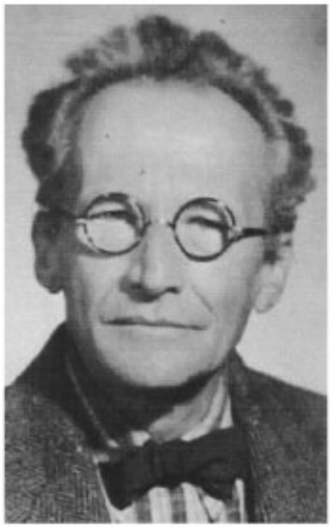
In late August **1869**, **Miescher** reported finding this same material not only in pus cells but also in yeast, kidney, liver, testicular , and nucleated red blood cells. He concluded that this material did not behaved like any of the known classes of proteins. If it was not a protein, then what else could it be?

He termed this new substance nuclein, which would later be identified as DNA

To distinguish nuclein chemically from other known cell substances, he undertook to determine its elementary composition. This involved determining the relative proportions of hydrogen, carbon, oxygen, and nitrogen present in the substance

1944

A



B

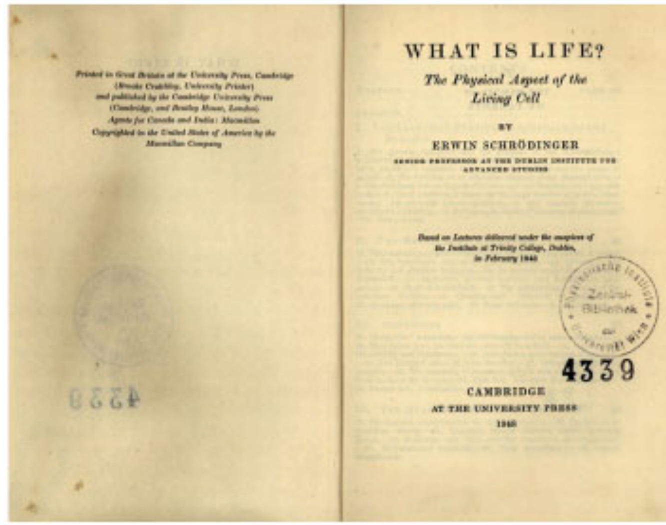


Figura 1.1 (**A**) **Erwin R.J.A. Schrödinger** (1887-1961) e (**B**) **frontespizio del suo libro** ***Che cos'è la vita?***, pubblicato nel 1944.

LA BIOLOGIA MOLECOLARE

In1945 John Atsbury proposed the term Molecular Biology for a universitary department in Leeds – later called Dept of biomolecular Structure

1947 – The **Molecular Biology Unit at** MRC of Cambridge was funded

two molecular biology schools

Informationalists (USA) – to understand the processes through which genetic information is decoded (Delbruck)

Structuralists (UK) – to apply physico-chemical methods to unravel the structure of biological molecules (Perutz Kendrew – UK, Pauling – USA)

INFORMATIONALISTS

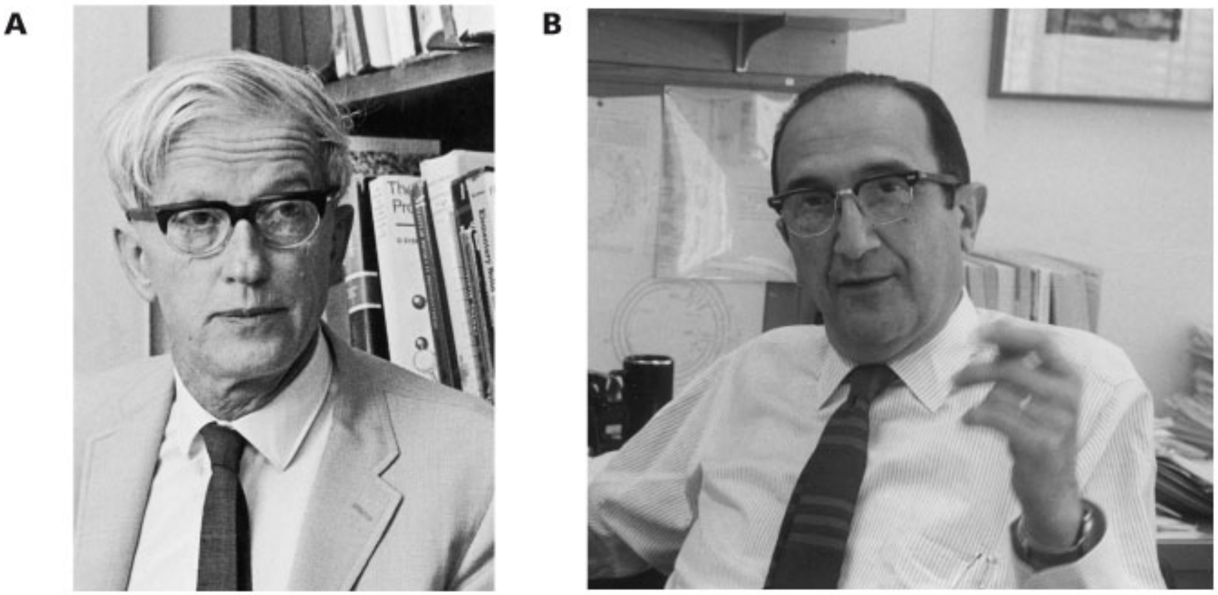
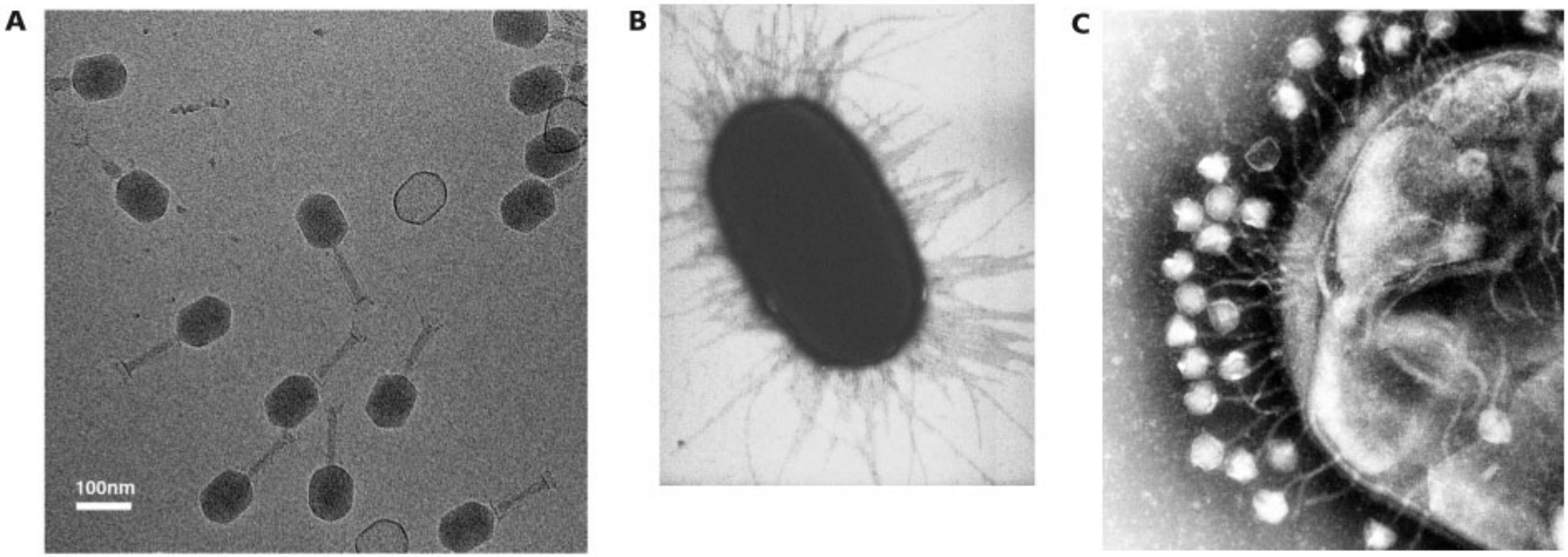
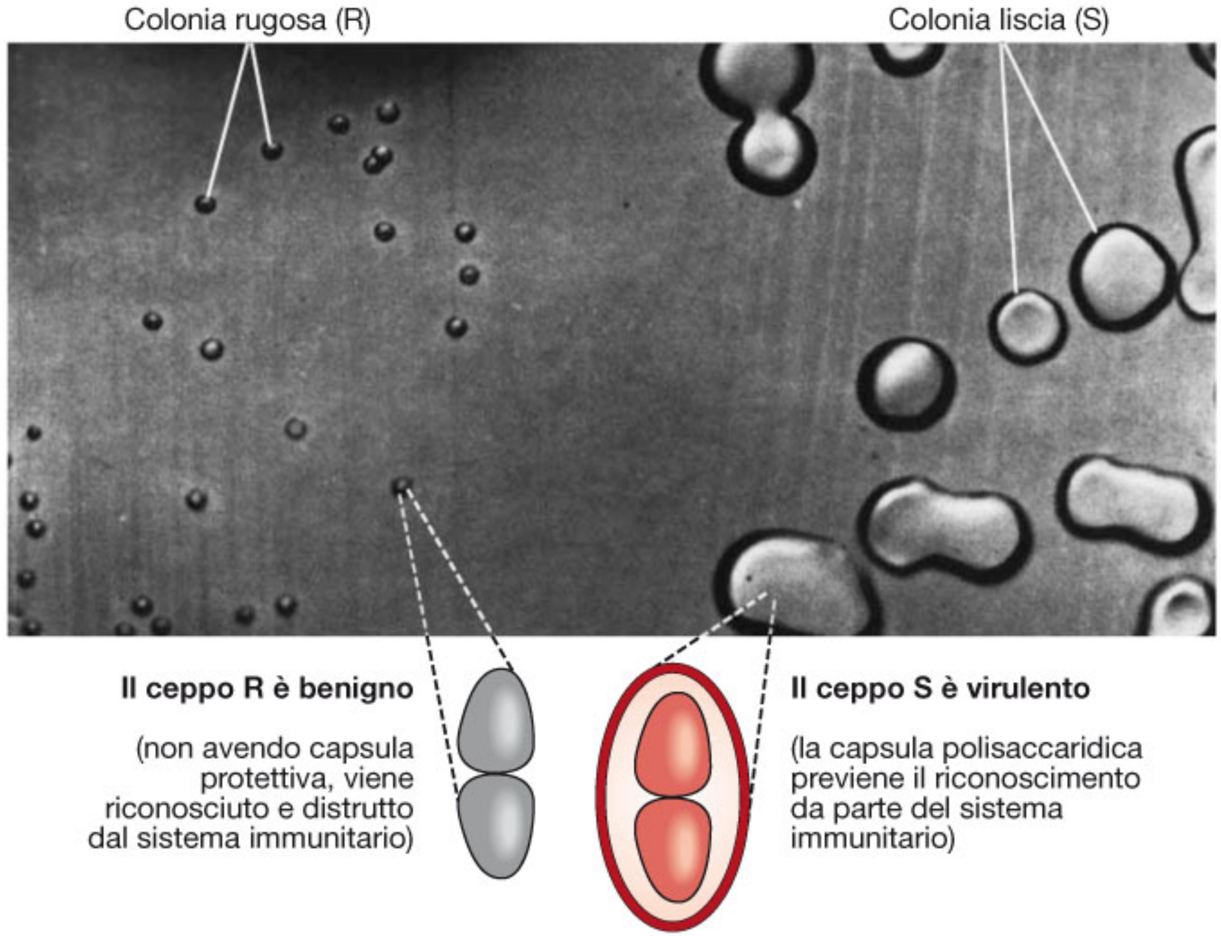


Figura 1.2 (A) Max Delbruck (1906-1981) e (B) Salvador Luria (1912-1991).



Figfigura 1.3 (**A**) **Il fago T4 e** (**B**) **il suo ospite *Escherichia coli* visti al microscopio elettronico.** (**C**) Particelle del fago mentre infettano una cellula di *E. Coli*.

Figura 1.7 **I due ceppi di *Streptococcus pneumoniae*: il ceppo R (*rough*, rugoso) e il ceppo S (*smooth*, liscio).**



1928 - Griffith

Demonstration that a non-virulent strain could be converted into a virulent one providing extracts from virulent strains and that the substance was heat-resistant

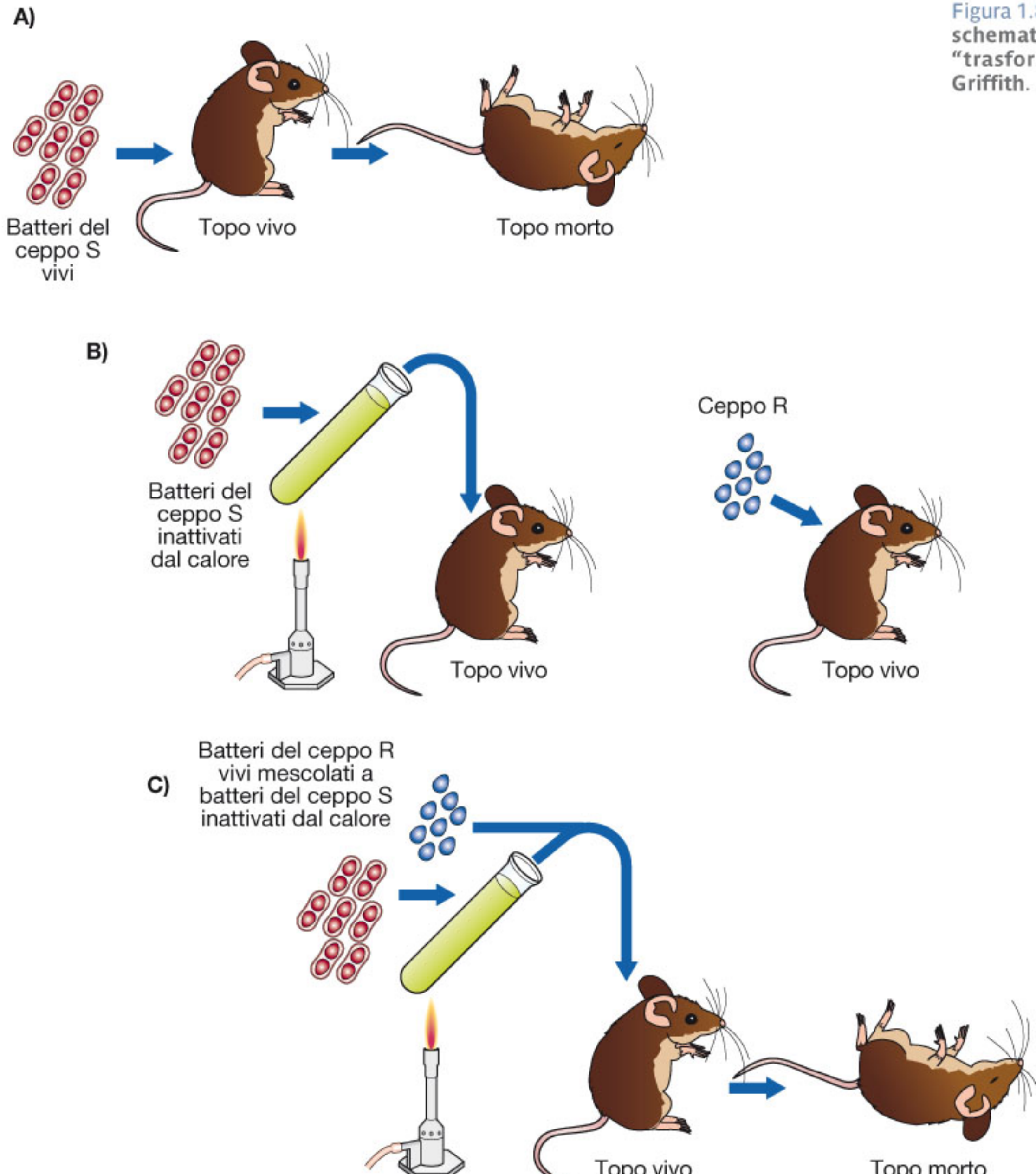


Figura 1.8 **Rappresentazione schematica dell'esperimento di "trasformazione" batterica di Griffith.**

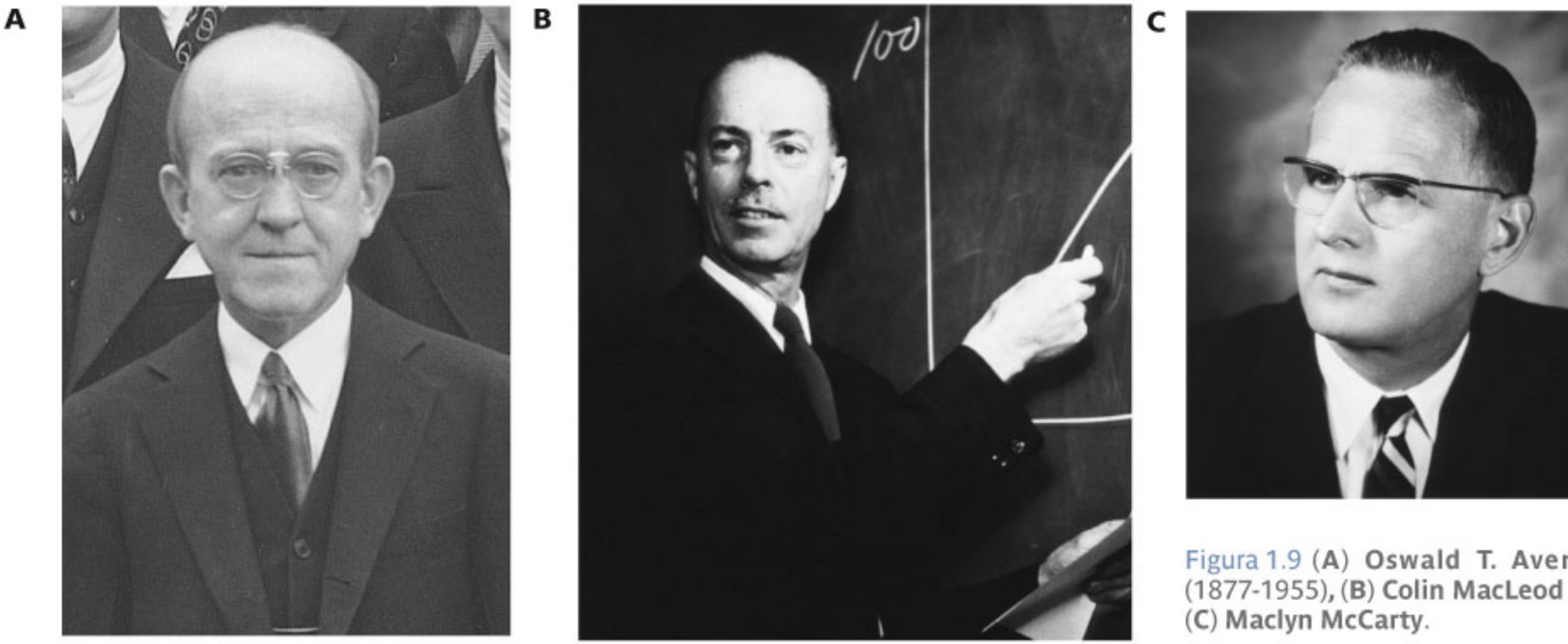
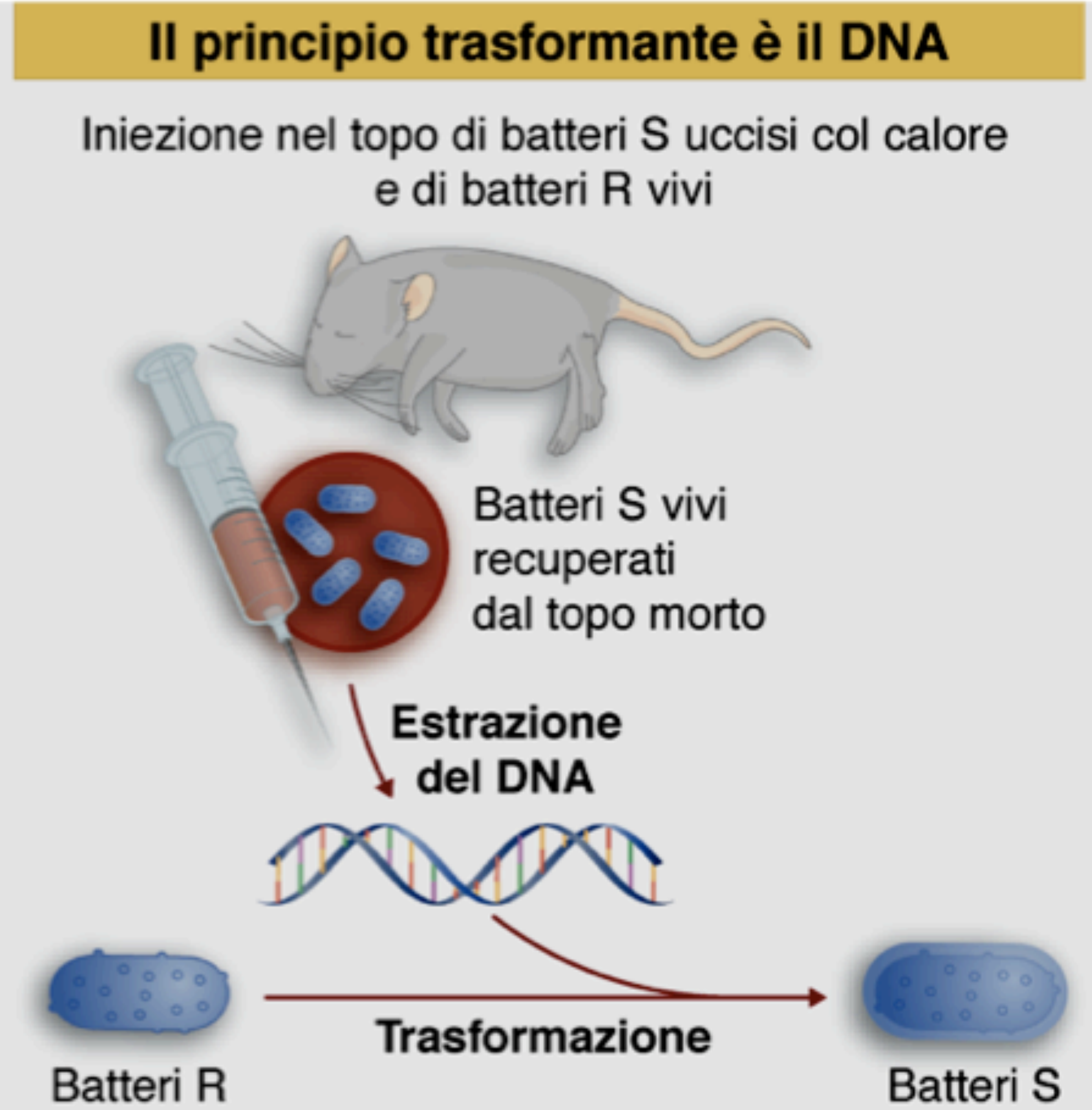


Figura 1.9 (**A**) **Oswald T. Avery** (1877-1955), (**B**) **Colin MacLeod** e (**C**) **Maclyn McCarty**.

1944 – Avery

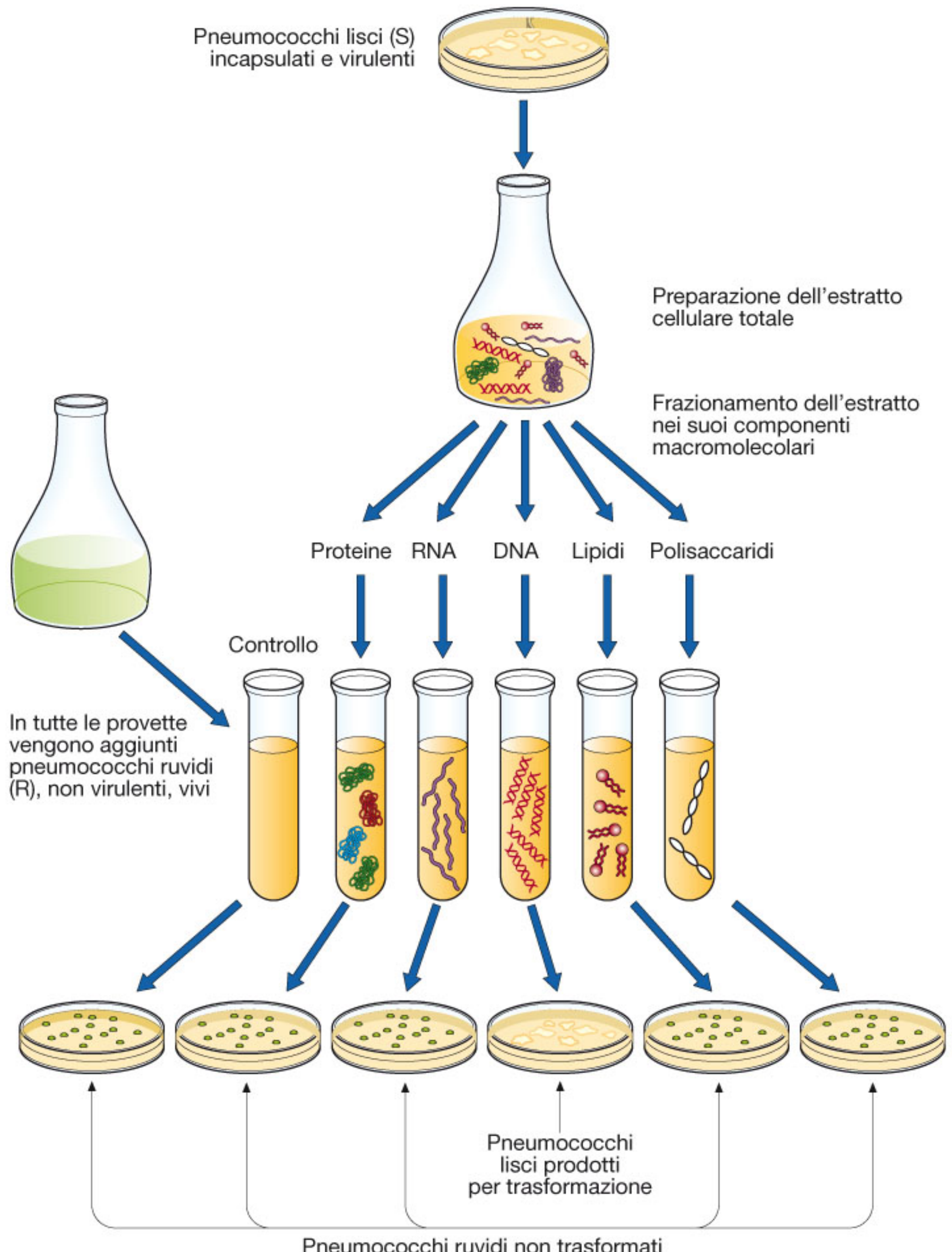
The transforming principle was sensible to DNase and not to protease

Osvald Avery: 1944



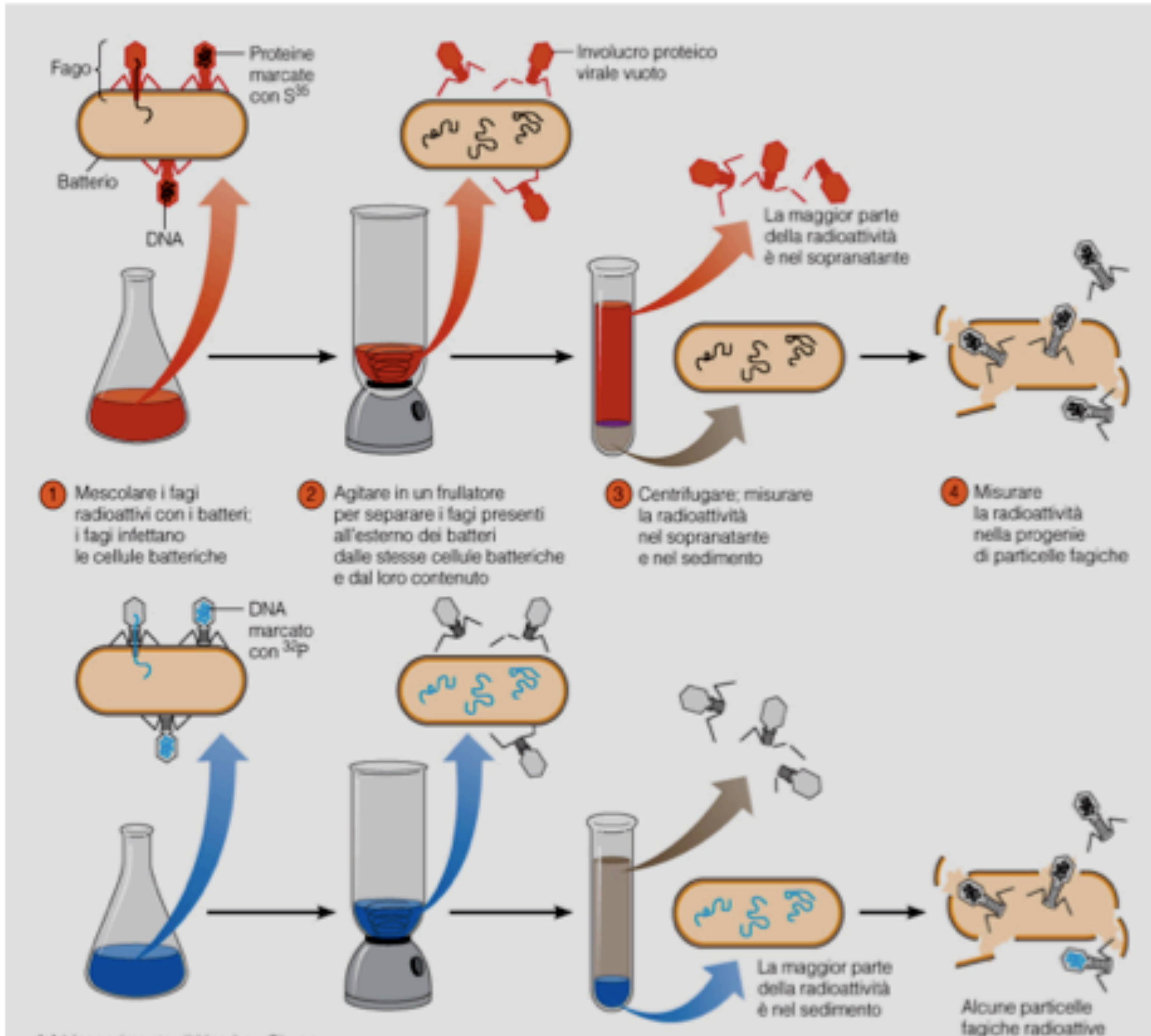
La comunità scientifica, tranne Watson e Crick, fu riluttante ad accettare i risultati di Avery e continuò a ritenere che le proteine fossero i geni. Per molti anni fino al 1952, il lavoro di Avery fu ignorato.

Figura 1.10 La "bomba di Avery". Avery e colleghi, a partire dai Pneumococchi lisci e virulenti, fecero un estratto cellulare che era in grado di trasformare il ceppo ruvido. In seguito purificarono tutte le componenti dell'estratto, che divisero in tubi diversi. Solo il DNA era in grado di trasformare il ceppo di Pneumococco ruvido nel ceppo liscio e patogeno, dimostrando che solo il DNA era il principio trasformante.



Hershey and Chase 1952: Il DNA del fago T2 trasporta l'informazione genetica.

ly P^{32} labelled DNA,
l not S^{35} labelled
teins, enters the
ected cells



1953
the birth of Molecular Biology

1895 — Discovery of X rays

1912 — Cristal Irradiation

1920 — Irradiation *Biological fibers*

1944 — DNA Hereditary material

1953 — Resolution of DNA Structure *Watson/Crick*

2001 — Sequencing of human genome

Structural studies **– X RAY DIFFRACTION**

X rays were discovered in **1895** Wurzburg by Wilhelm **Roentgen**
Nobel 1901

Irradiation of cristals with X rays -**1912** – Max **von Laue**
Nobel 1914

1920 – **Herzog** – diffraction of natural fibers (cellulose)

In**1936** Max **Perutz** and John **Kendrew** in Cambridge started to study mioglobin and hemoglobin

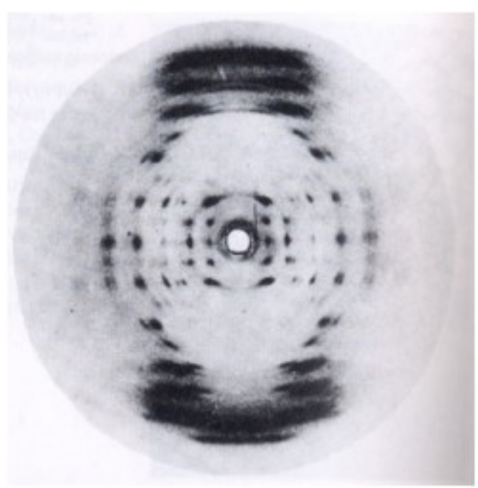
Linus **Pauling** at Caltech defined α-helix of proteins – Nobel **1954**
Nobel price for peace in 1962

Verso la definizione della struttura del DNA

1938 - **Atsbury** worked on dry fibers of DNA – periodic structure

1951- Maurice **Wilkins** and Rosalind **Franklin** (King's College, Londra) – used wet fibers – good diffraction patterns, helical structure and diameter

Wilkins – single filament
Franklin – described the phosphate outside and the bases inside



Francis **Crick** (fisico) – graduated nel 1937. Nel mezzo del suo PhD scoppiò la guerra
Cominciò a lavorare con Perutz all'MRC su struttura di proteine

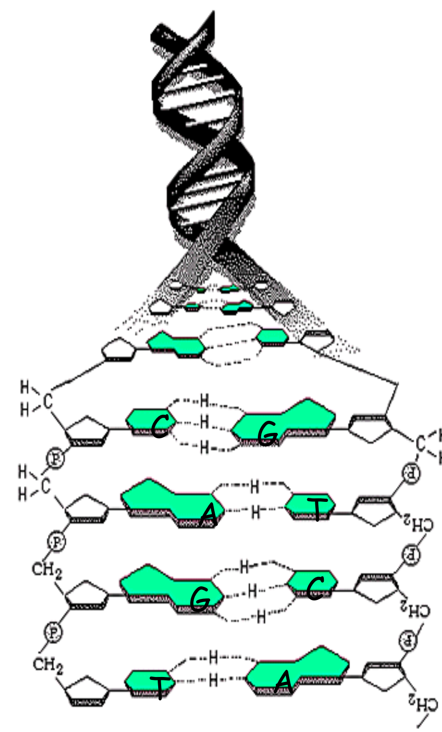
James **Watson** (biologo USA) – incontrò Wilkins a Napoli e rimase affascinato dagli studi strutturali sul DNA essendo sensibilizzato dalle conoscenze sugli esperimenti di Avery– decise di andare a Cambridge

Nel 1951 Crick ancora non aveva preso il PhD (aveva 35 anni), mentre Watson aveva appena 23 anni

Il **6 febbraio 1953** Watson visitò a Londra la Franklin…2-eliche, fosfati esterni
Di ritorno a Cambridge Jerry **Donohue** indicò l'esatta forma tautomerica della basi..keto e non enolo………si potevano finalmente formare i legami idrogeno tra le basi………………

Discoveries that led to the definition of DNA structure

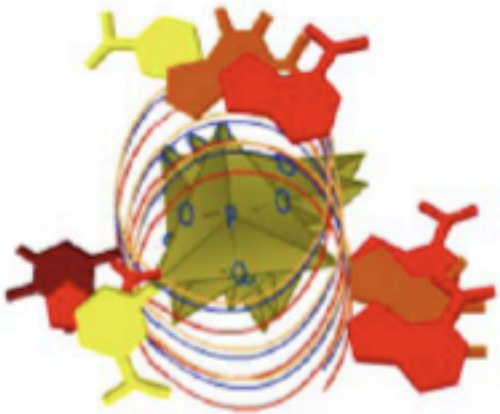
Chemistry of nucleotides (Levene)
DNAhuge molecule (Caspersson)
G=C A=T content (Chargaff)
Genetic function of DNA (Avery)
X diffraction on biological fibers (Atsbury)
α-helix of proteins (Pauling, Perutz, Kendrew)
Periodic structure of DNA (Atsbury)
External phosphate, bases inside, 2/3 helices
(Wilkins e Franklin)
Tautomeric form of the bases (Donohue)



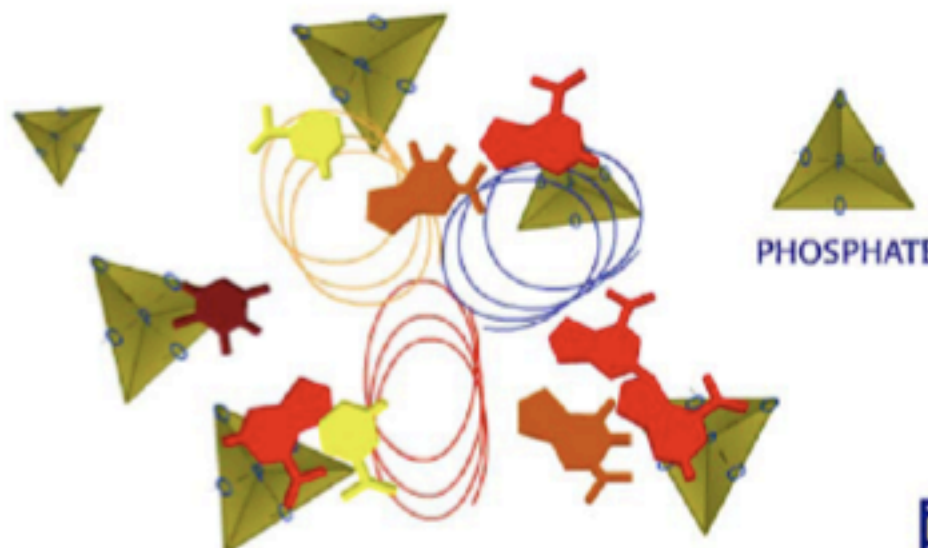
17 Marzo 1953 – Nature
The discovery of the double helix

Il modello a tripla elica di Pauling

Nel 1953 Linus Pauling annunciato di avere scoperto la struttura del DNA, presentando in un lavoro un modello a tripla elica con i gruppi fosfati all'interno.

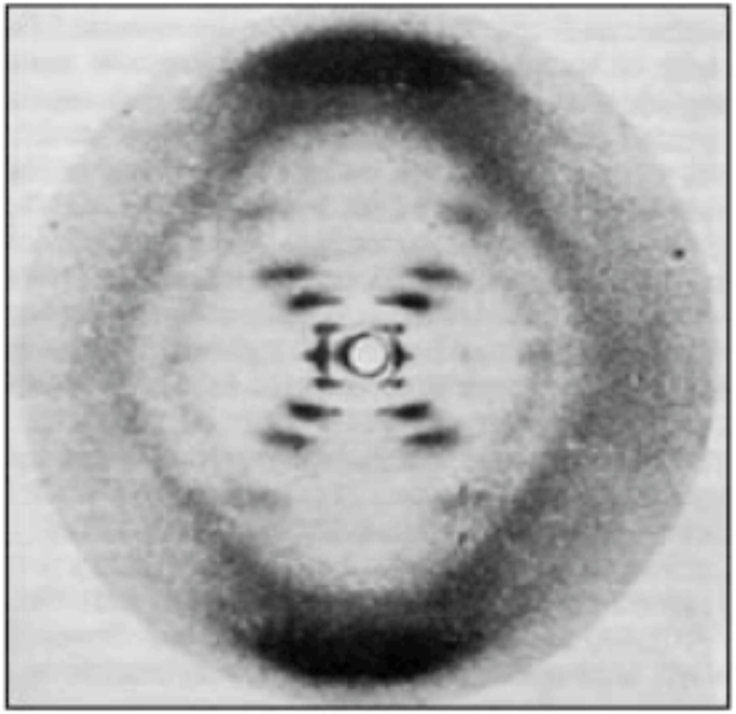


Incredilbilmente lo scienziato che ha scritto un libro sulla natura del legame chimico non ha considerato che le forze repulsive generate dalle cariche negative dei gruppi fosfati avrebbero fatto collassare la struttura.



Watson e Crick si resero conto dell'errore di Pauling

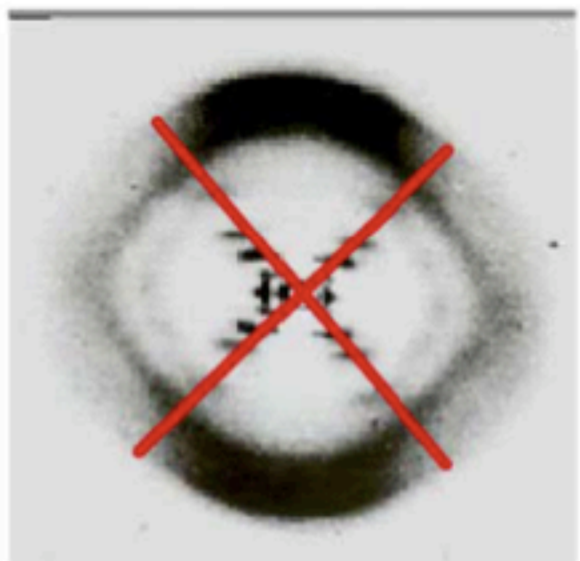
(lo stesso commesso da loro stessi nel loro primo modello).



Franklin's X-ray photograph shows
DNA's 'B'-form (1952)

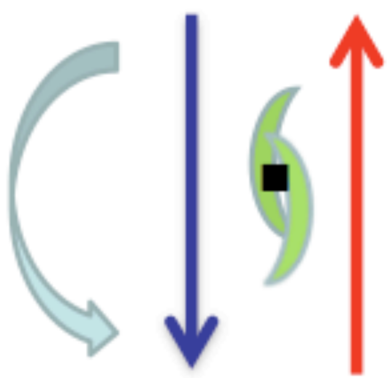
La fotografia della Franklin, disegnata da Watson, fu utilizzata da Crick per calcolare alcuni parametri dell'elica.

La X dell'immagine di diffrazione dimostra un profilo ad elica



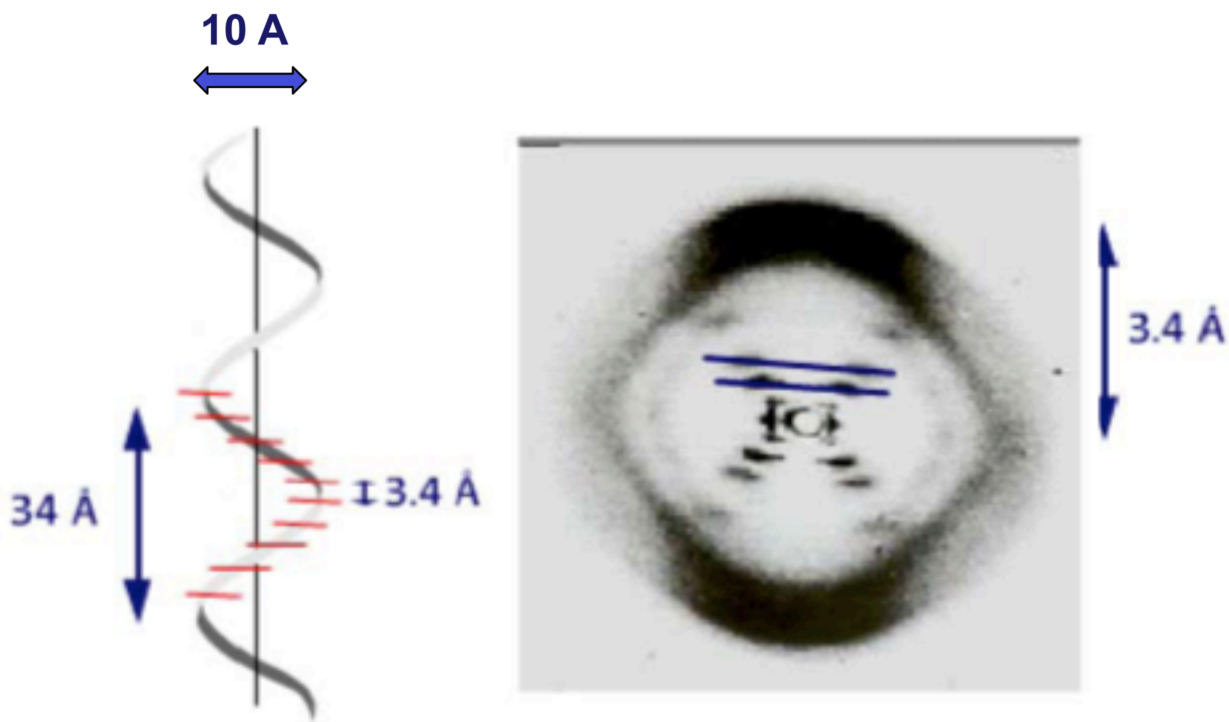
Il profilo regolare dei raggi X dimostra che il diametro dell'elica è costante per tutta la sua lunghezza
Rotazione di 180°

Rotazione di 180°



La distribuzione ad X indica una simmetria rotazionale doppia, quindi i due filamenti sono antiparalleli

Nella diffrazione ai raggi X , più vicini sono i segnali più lontani sono i punti di diffrazione nella molecola.



La distanza delle barre orizzontali di 3,4 nm corrisponde al passo dell'elica, mentre la distanza tra il punto centrale (Equatore) del diffrattrogramma e la riflessione al meridiano (bordo superiore), di 0,34 nm, corrisponde alla distanza tra le basi impilate.

Inizio della Biologia Molecolare:
1953 - scoperta della struttura del DNA

Implicazioni riguardo:

i meccanismi di trasmissione del materiale ereditario -

concetto di stampo

concetto molecolare di mutazione

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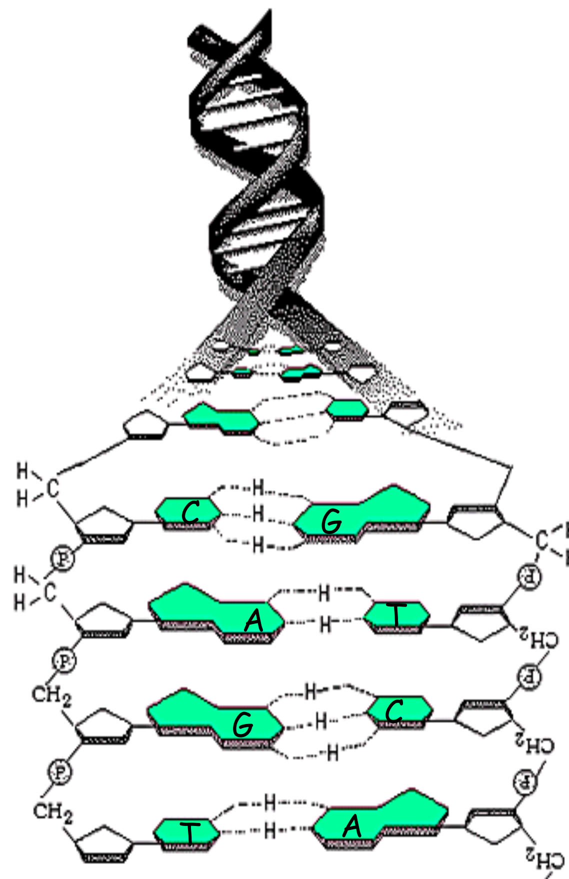

A Structure for Deoxyribose Nucleic Acid
J. D. Watson and F. H. C. Crick
April 25, 1953, *Nature, 171, 737-738*

Figure 1. This figure is purely diagrammatic. The two ribbons symbolize the two phophate-sugar chains, and the horizonal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in **the two chains run in opposite directions**

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Anni '50 – '60

- Meccanismi di replicazione del DNA (DNA polimerasi)
- Scoperta dell'RNA e dell'RNA messaggero
- Meccanismi di trascrizione del DNA e RNA polimerasi
- Definizione del codice genetico

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Three major revolutions in Molecular Biology

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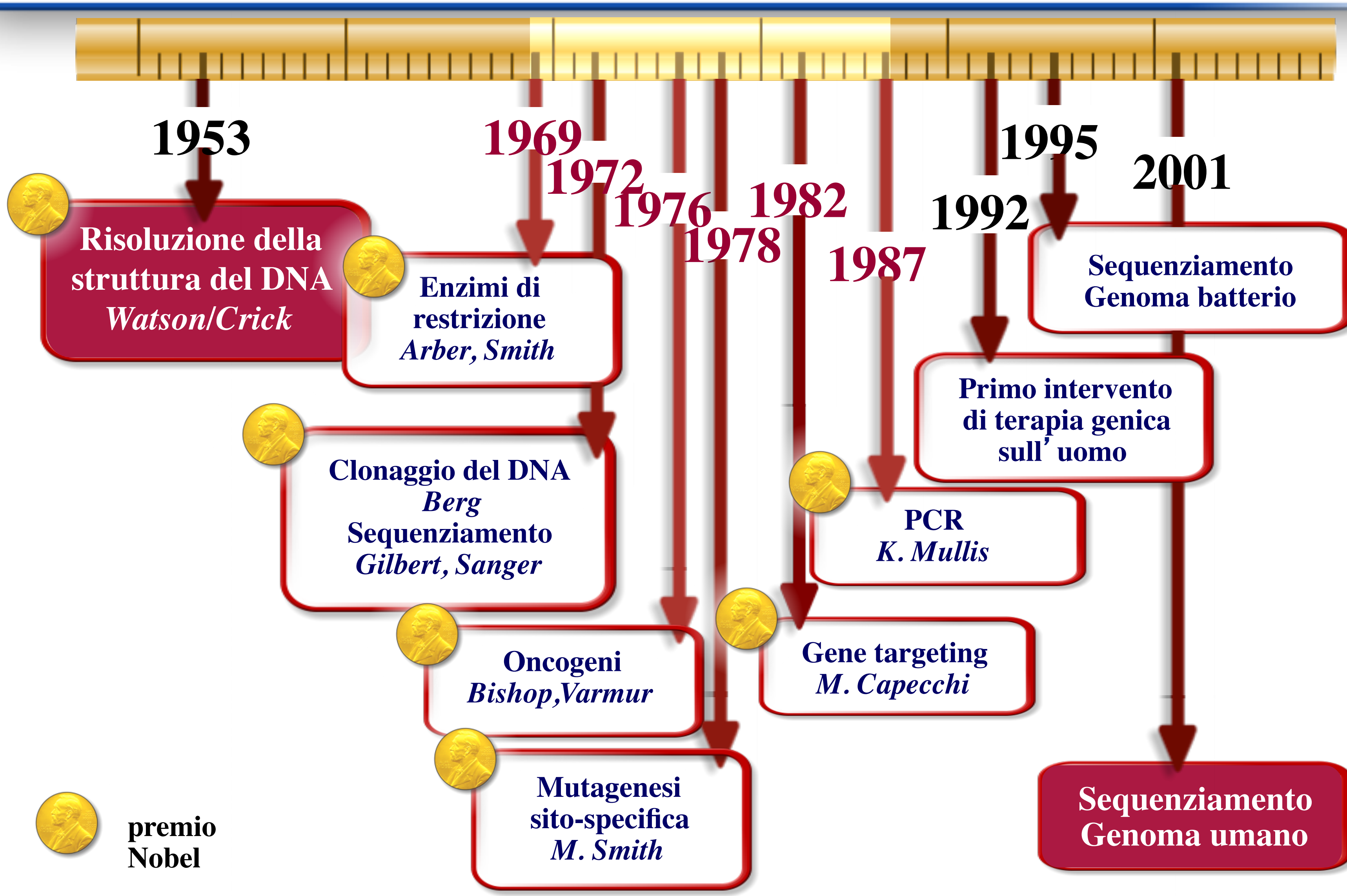
1970	**DNA cloning** Definition of gene structure - molecular definition of several pathologies
1990	**Genome sequencing** Identification of complex functions and analysis of multifactorial diseases

Funzione catalizzatrice della scoperta del DNA: scoperte rivoluzionarie in pochissimi anni



F. Griffth 1928: ha scoperto la trasformazione

Ha dimostrato che ceppi di Streptococcus peneumoniae uccisi al calore possono Trasformare un ceppo avirulento in virulento