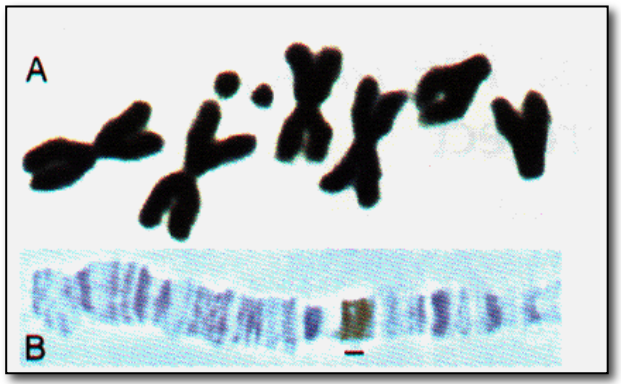
Molecular Genetics: The Early Experiments

BCH4027

1908 | 1933
Chromosomes related to phenotype

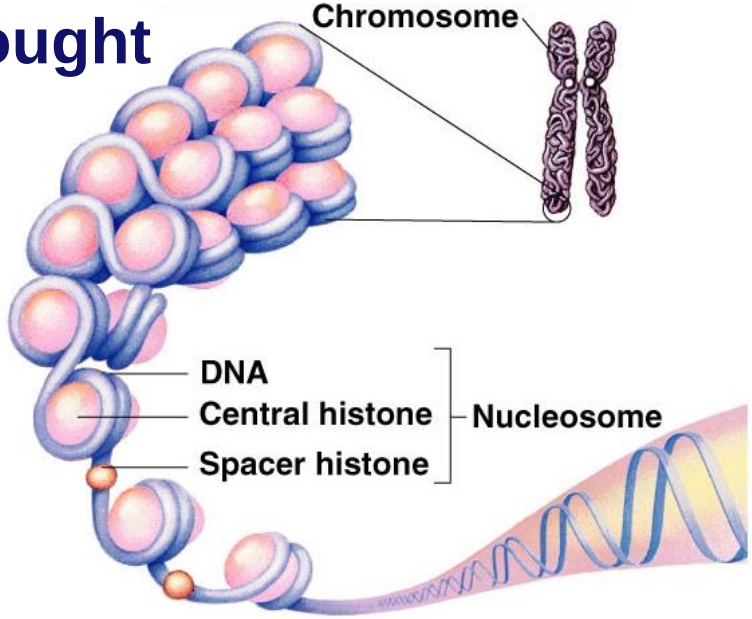
- **T.H. Morgan**
  - working with *Drosophila*
    - fruit flies
  - associated phenotype with specific chromosome
    - white-eyed male had specific X chromosome



1908 | 1933

Genes are on chromosomes

- Morgan's conclusions
  - genes are on chromosomes
  - but is it the protein or the DNA of the chromosomes that are the genes?
    - initially proteins were thought to be genetic material… Why?

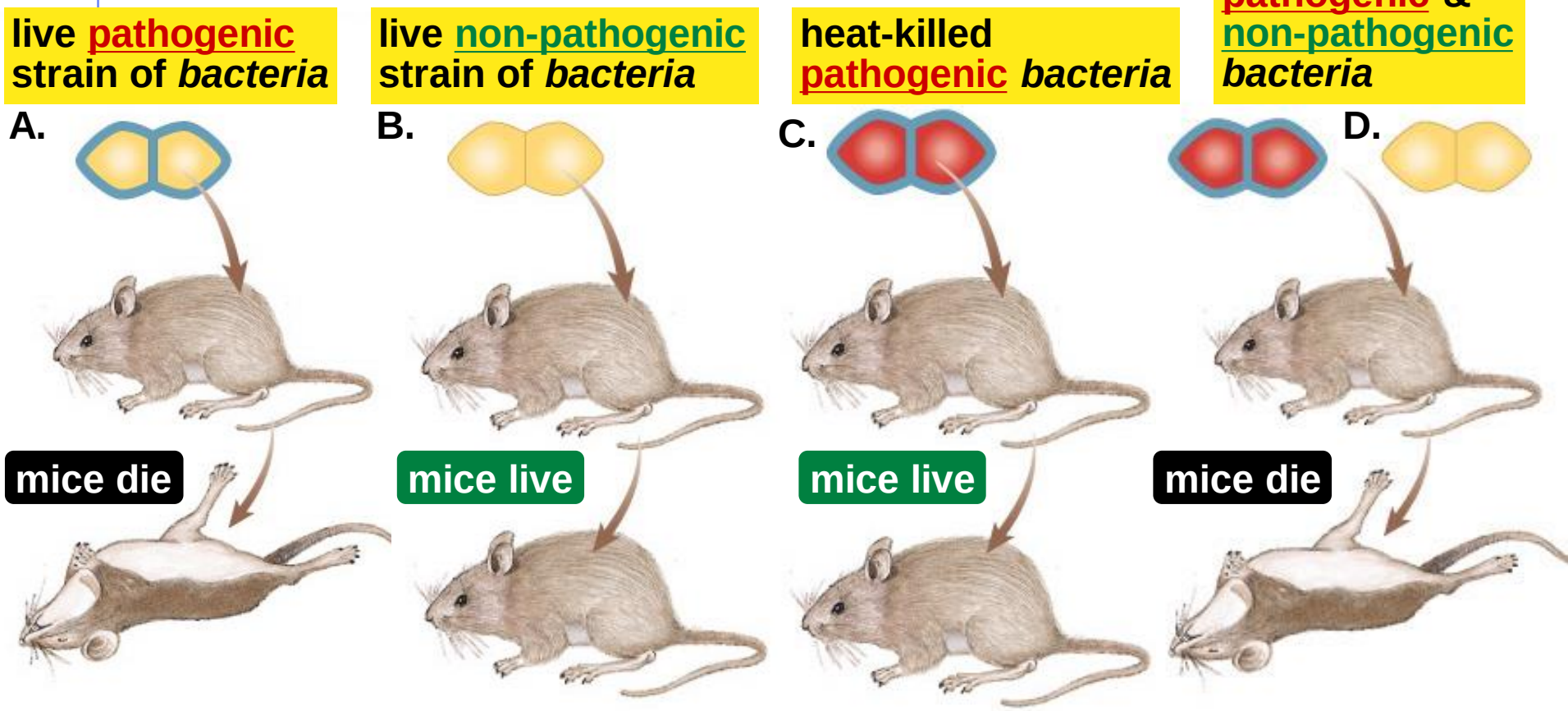


The "Transforming Principle"

1928

- Frederick Griffith
  - *Streptococcus* pneumonia bacteria
    - was working to find cure for pneumonia
  - harmless live bacteria ("rough") mixed with heat-killed pathogenic bacteria ("smooth") causes fatal disease in mice
  - a substance passed from dead bacteria to live bacteria to change their phenotype
    - "Transforming Principle"

The "Transforming Principle"



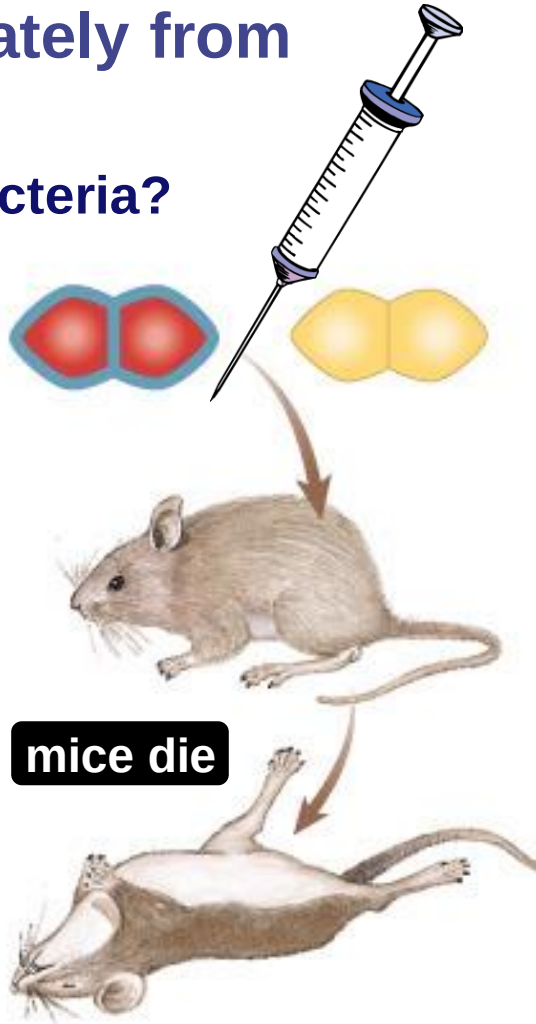
Transformation = change in phenotype

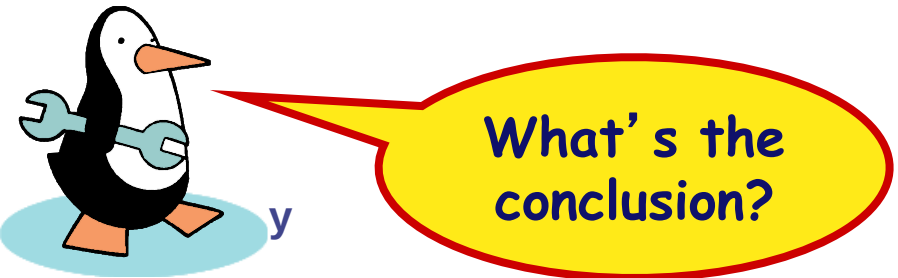
something in heat-killed bacteria could still transmit disease-causing properties

DNA is the "Transforming Principle"

1944

- Avery, McCarty & MacLeod
  - purified both DNA & proteins separately from *Streptococcus* pneumonia bacteria
    - which will transform non-pathogenic bacteria?
  - injected protein into bacteria
    - no effect
  - injected DNA into bacteria
    - transformed harmless bacteria into virulent bacteria

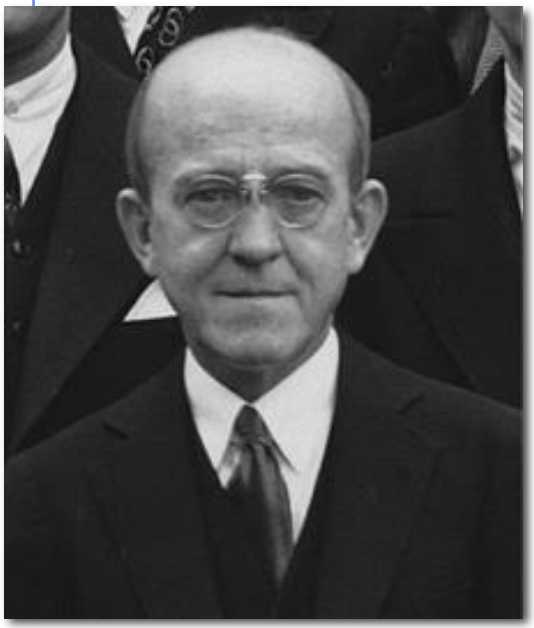




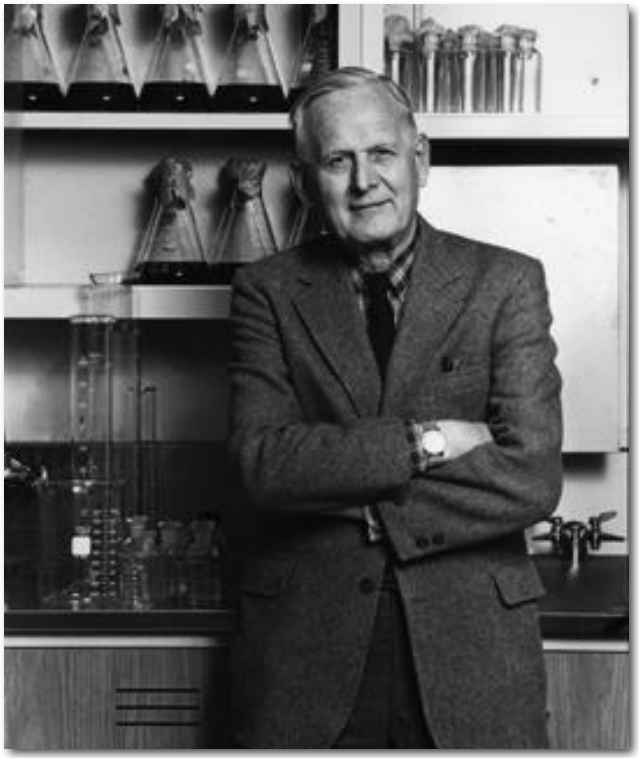
1944 | ??!!

Avery, McCarty & MacLeod

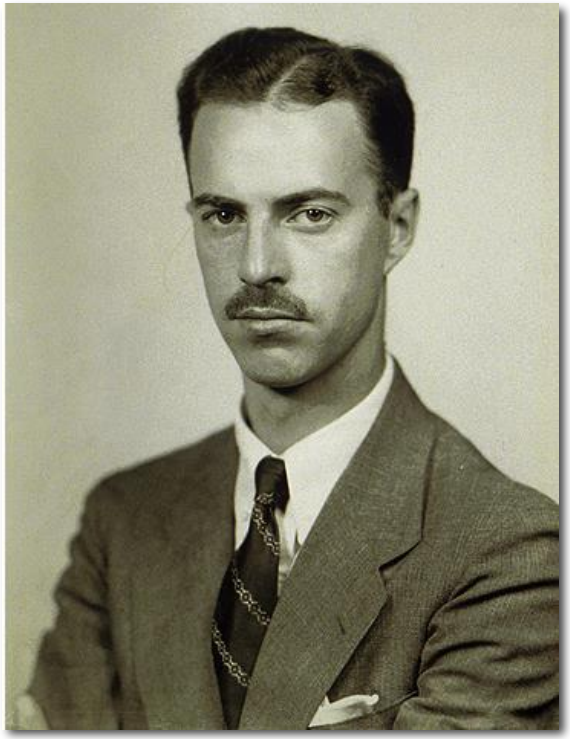
- Conclusion
  - first experimental evidence that DNA was the genetic material



Oswald Avery



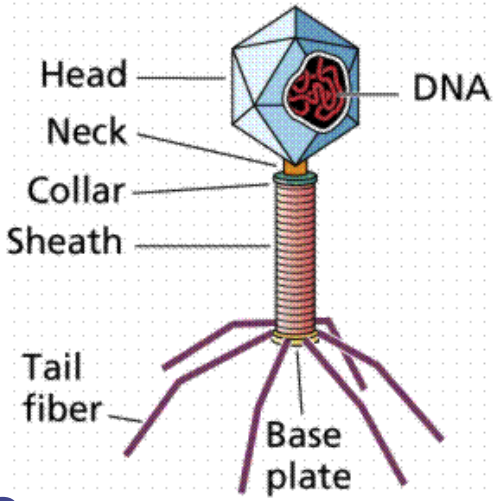
Maclyn McCarty



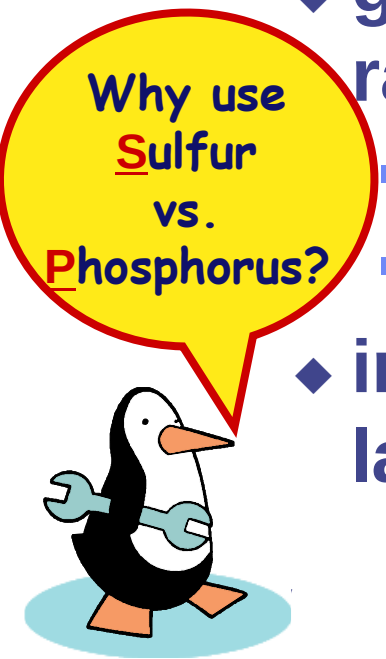
Colin MacLeod

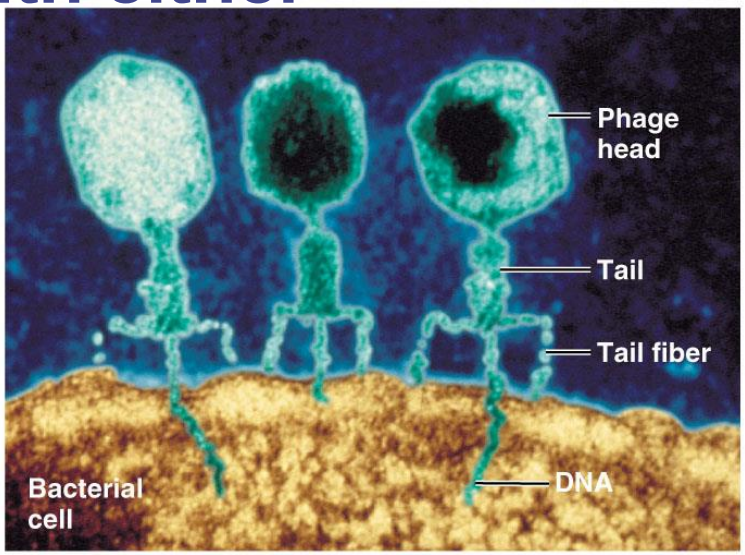
Confirmation of DNA

1952 | 1969
Hershey

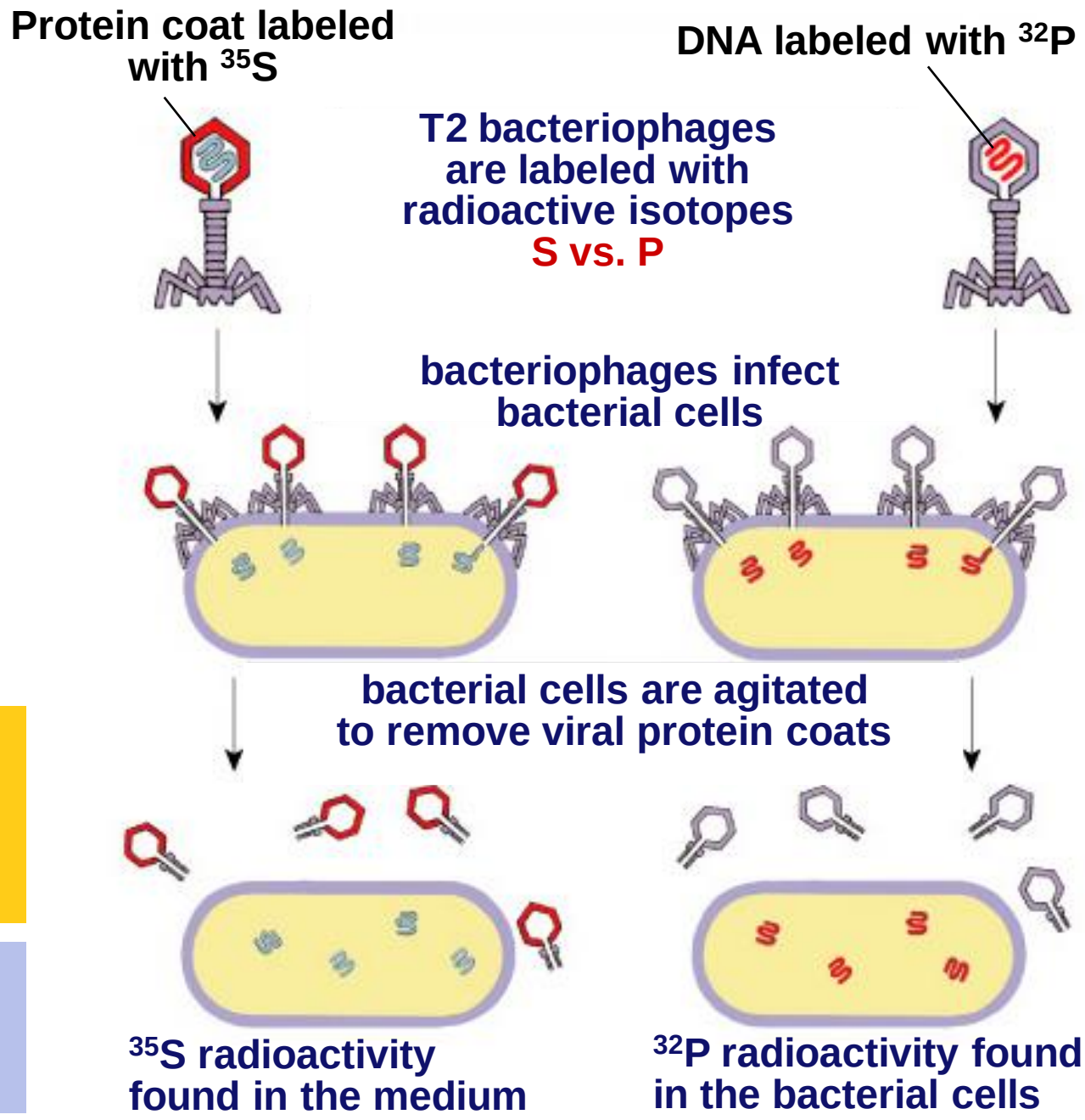


- Hershey & Chase
  - classic "blender" experiment
  - worked with bacteriophage
    - viruses that infect bacteria
  - grew phage viruses in 2 media, radioactively labeled with either
    - ^{35}S in their proteins
    - ^{32}P in their DNA
  - infected bacteria with labeled phages



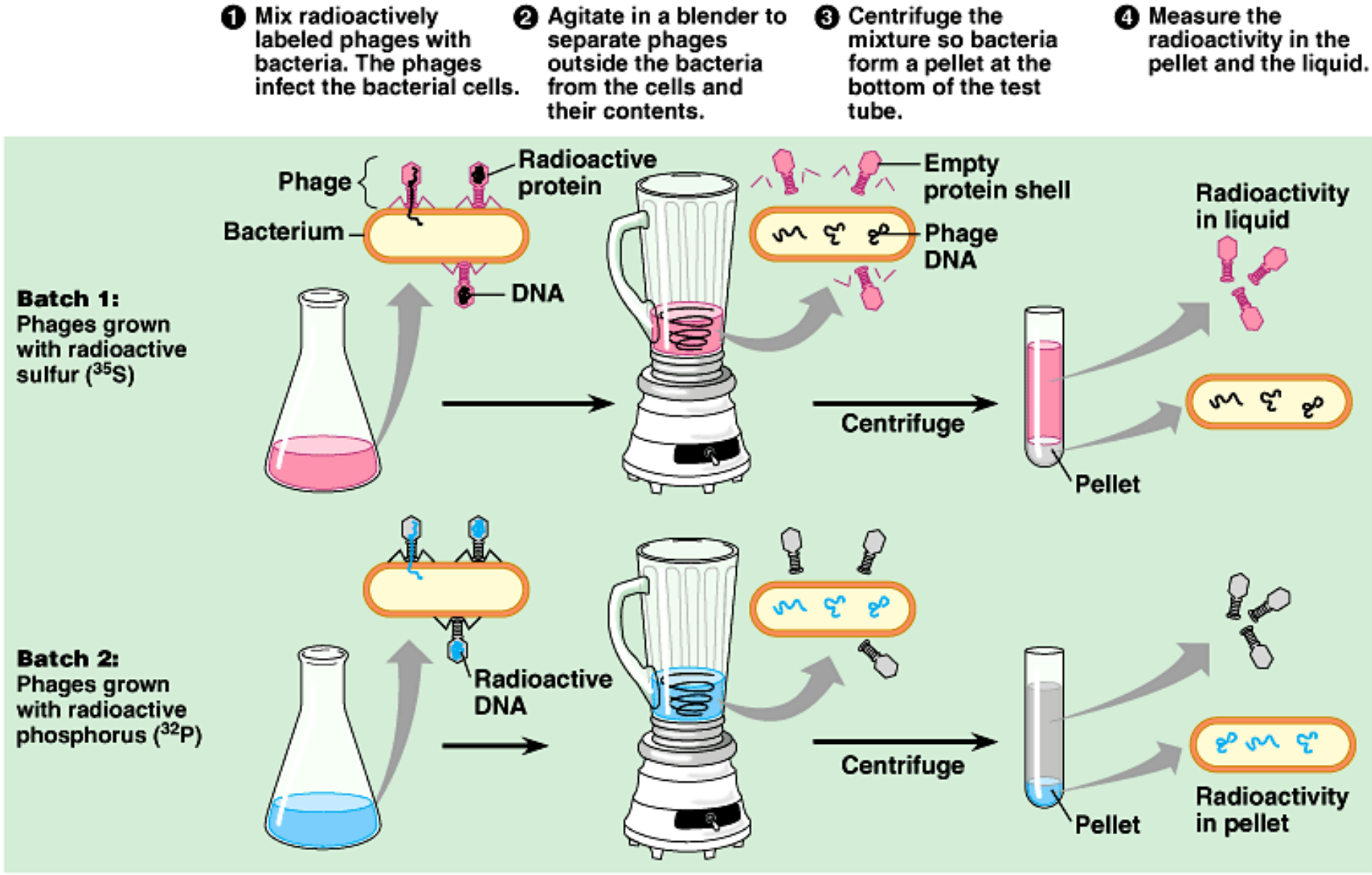


Hershey & Chase



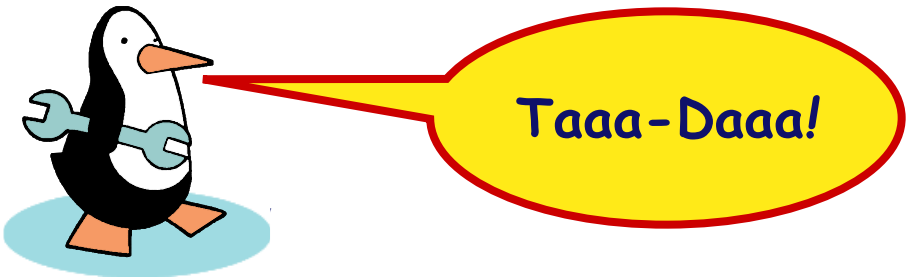
Which radioactive marker is found inside the cell?

Which molecule carries viral genetic info?


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.
Batch 1:
Phages grown with radioactive sulfur (^{35}S)
Phage
Radioactive protein
Bacterium
DNA
Empty protein shell
Phage DNA
Radioactivity in liquid
Centrifuge
Pellet
Batch 2:
Phages grown with radioactive phosphorus (^{32}P)
Radioactive DNA
Centrifuge
Pellet
Radioactivity in pellet

Blender experiment

- Radioactive phage & bacteria in blender
  - ^{35}S phage
    - radioactive proteins stayed in supernatant
    - therefore viral protein did NOT enter bacteria
  - ^{32}P phage
    - radioactive DNA stayed in pellet
    - therefore viral DNA did enter bacteria
  - Confirmed DNA is "transforming factor"



1952 | 1969
Hershey

Hershey & Chase

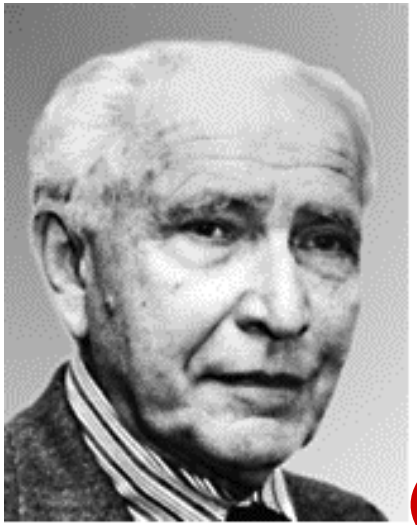
AP Biology

Martha Chase Alfred Hershey

1947

Chargaff

- **DNA composition: "Chargaff's rules"**
  - varies from species to species
  - all 4 bases not in equal quantity
  - bases present in characteristic ratio
    - humans:



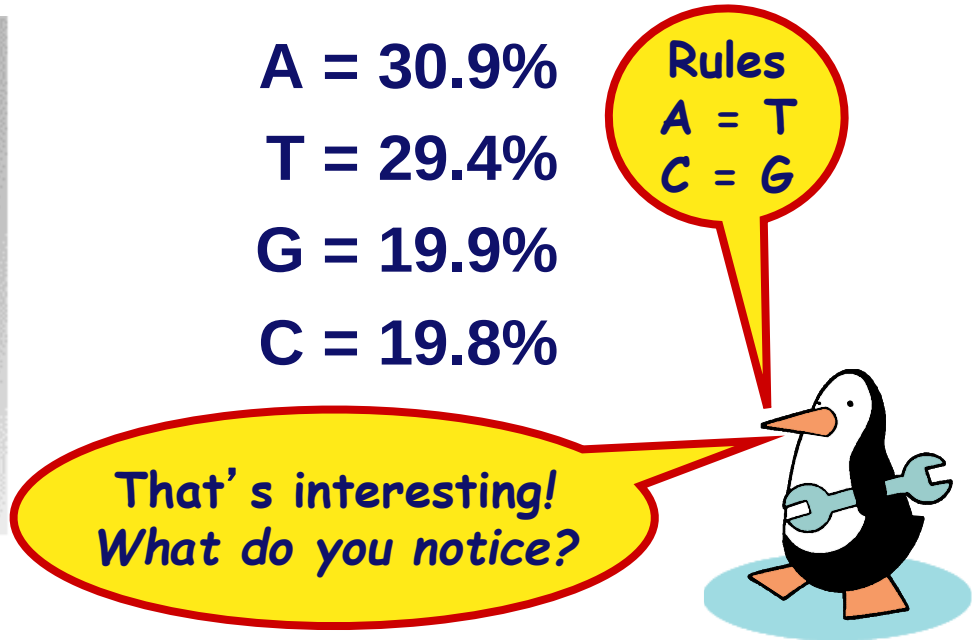
Erwin Chargaff

A = 30.9%

T = 29.4%

G = 19.9%

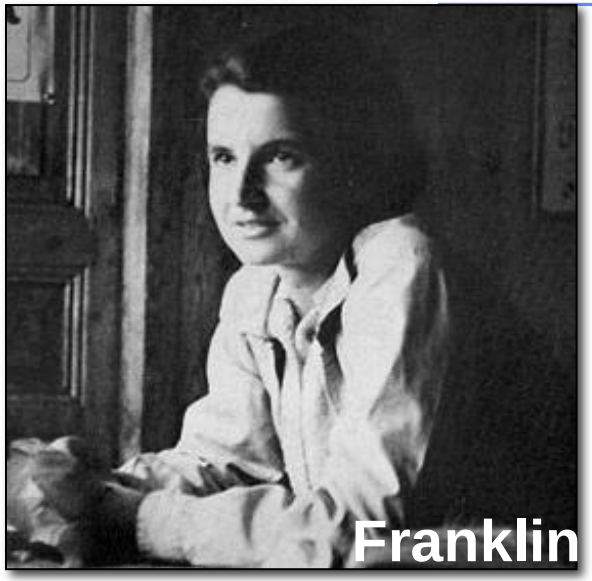
C = 19.8%

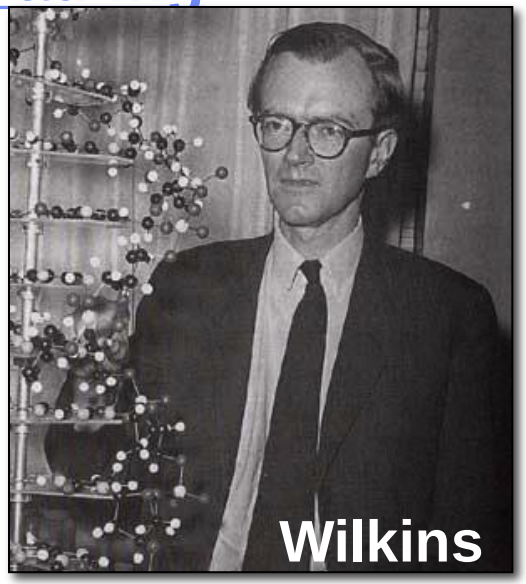


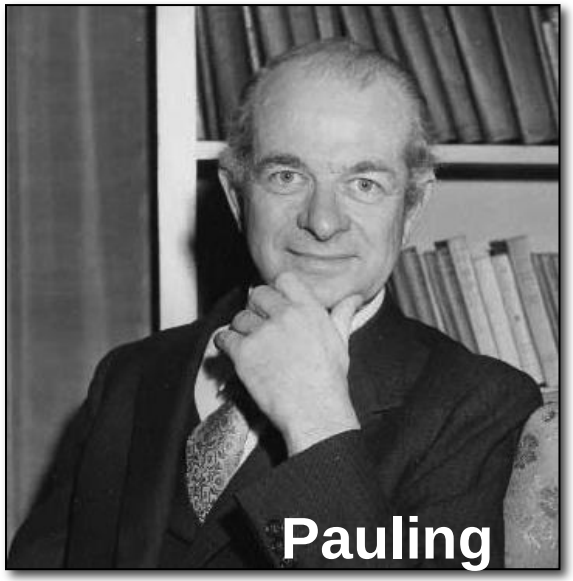
1953 | 1962

Structure of DNA

- **Watson & Crick**
  - **developed double helix model of DNA**
    - **other leading scientists working on question:**
      - Rosalind Franklin
      - Maurice Wilkins
      - Linus Pauling

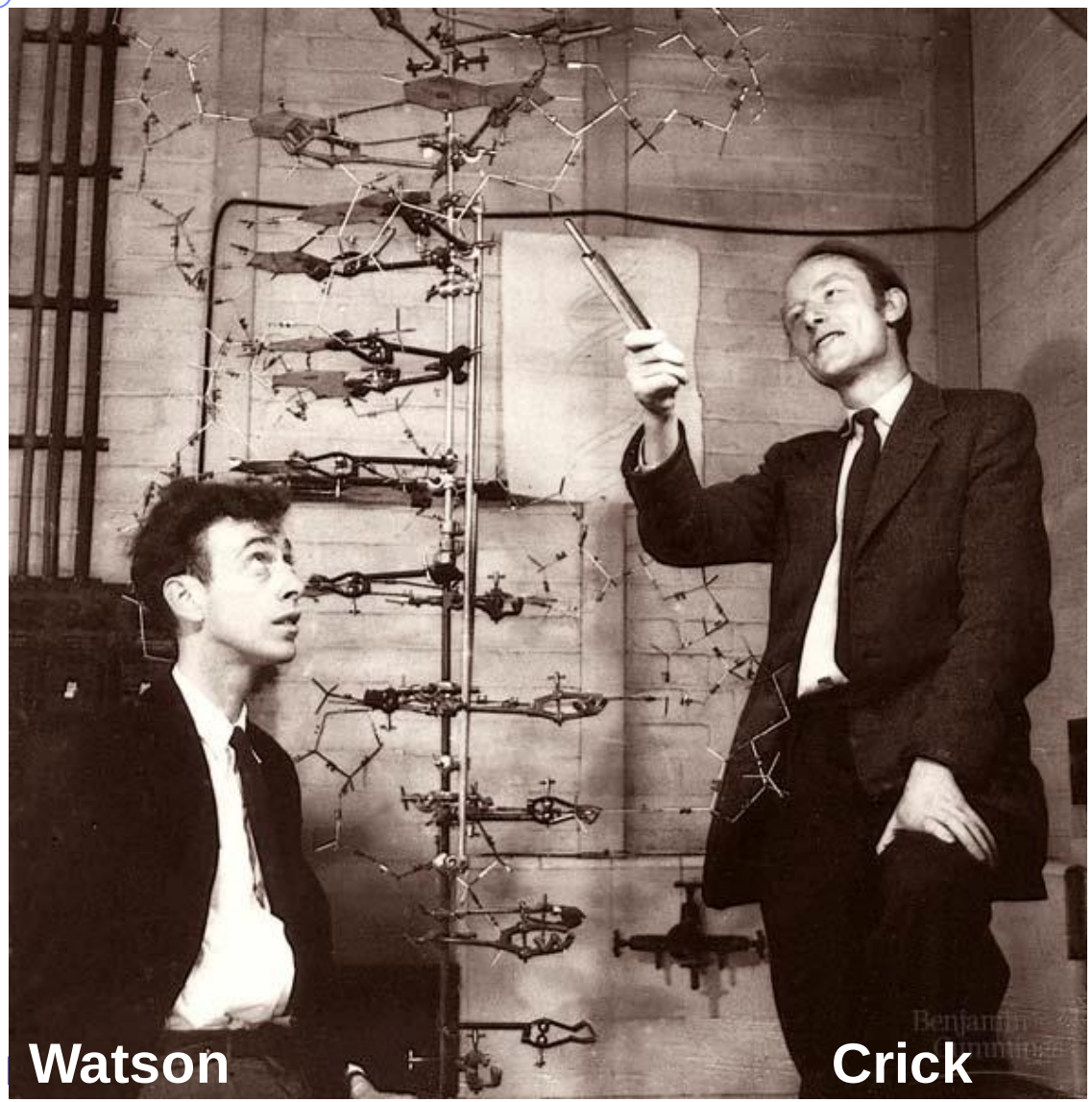

Franklin

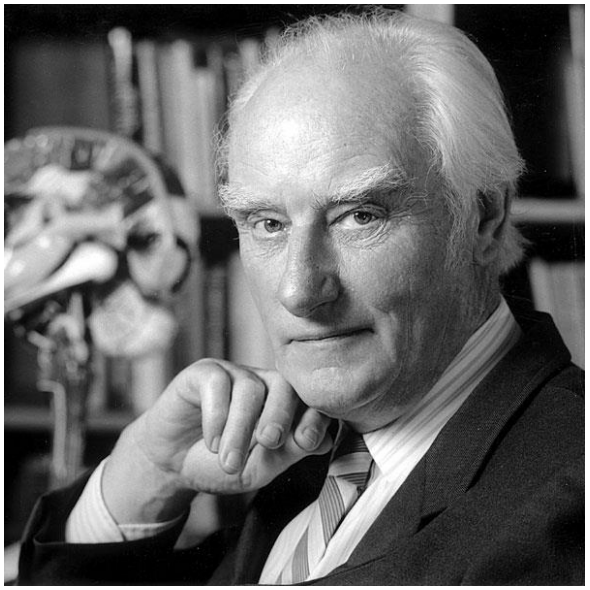

Wilkins


Pauling

Watson and Crick

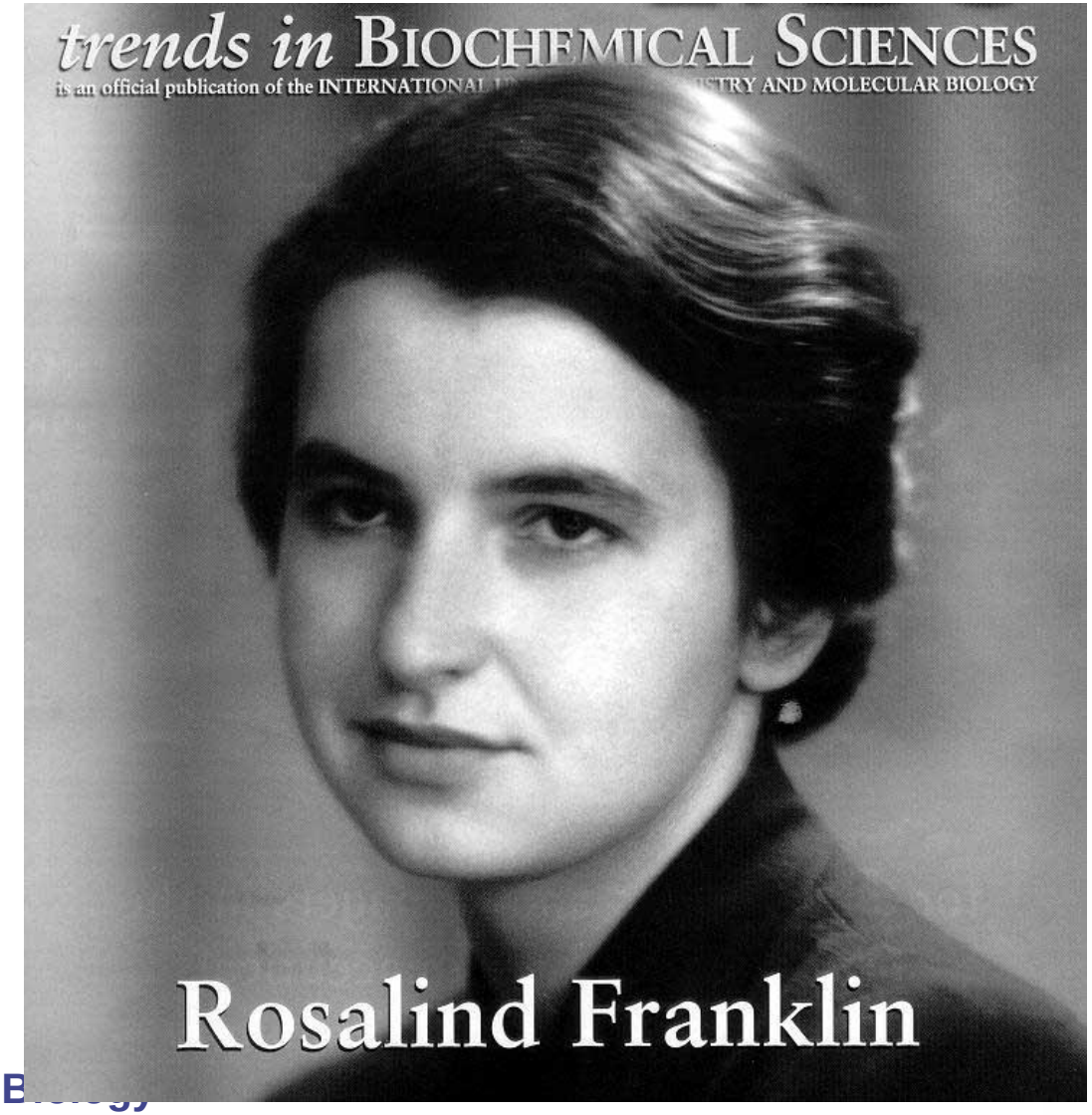
1953 article in Nature

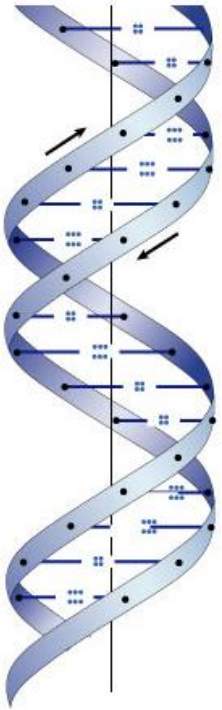


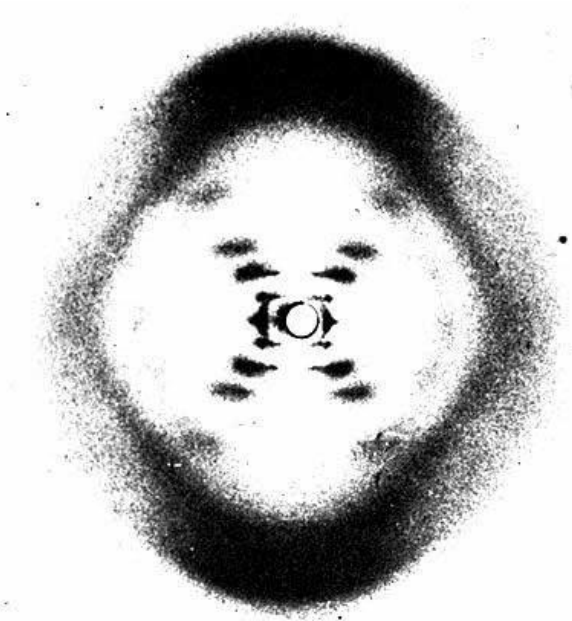


AP

Rosalind Franklin (1920-1958)



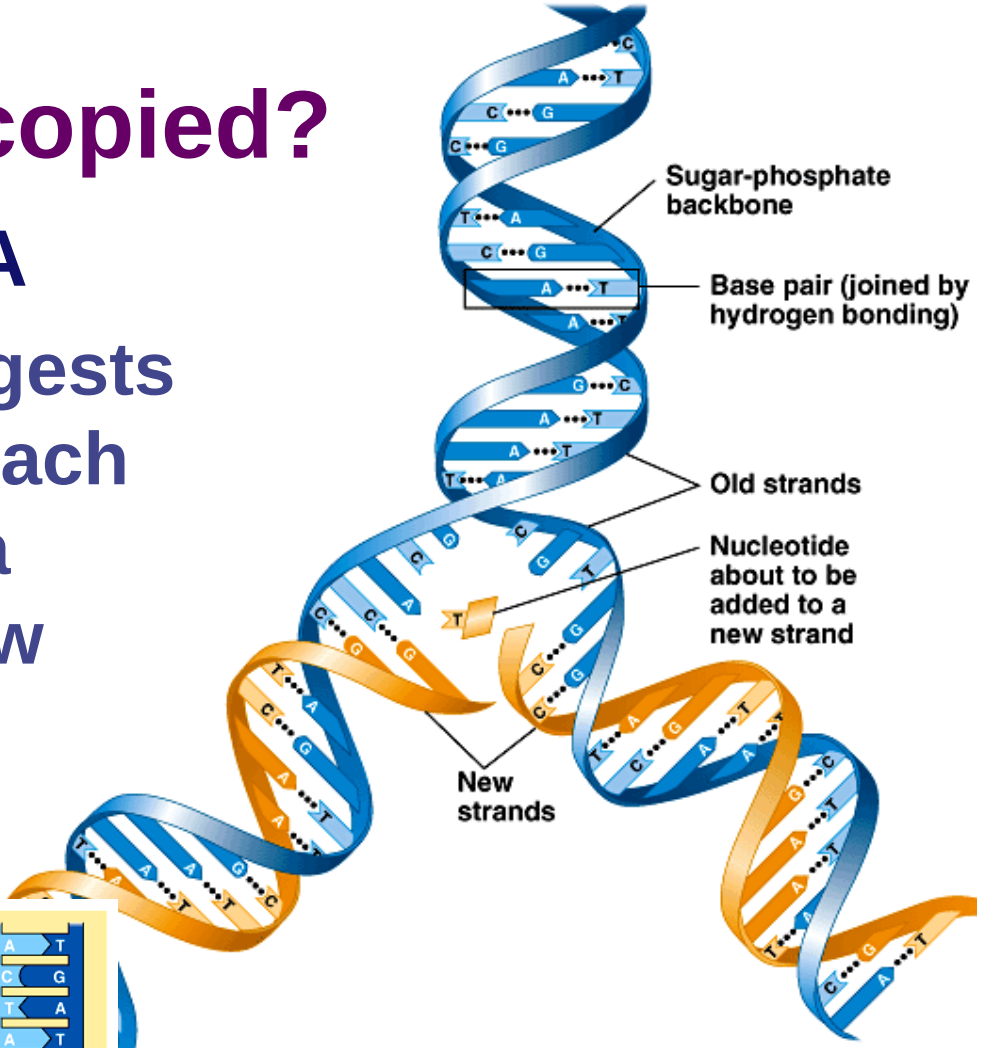


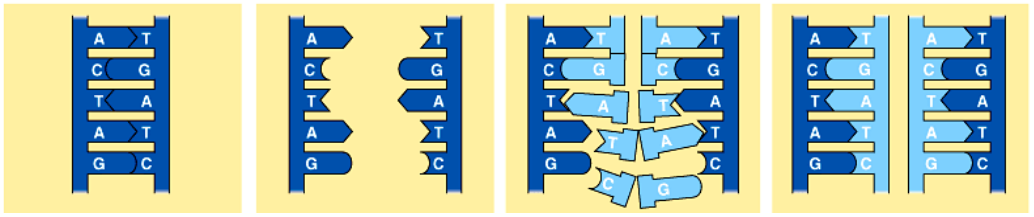


A chemist by training, Franklin had made original and essential contributions to the understanding of the structure of graphite and other carbon compounds even before her appointment to King's College. Unfortunately, her reputation did not precede her. James Watson's unflattering portrayal of Franklin in his account of the discovery of DNA's structure, entitled "The Double Helix," depicts Franklin as an underling of Maurice Wilkins, when in fact Wilkins and Franklin were peers in the Randall laboratory. And it was Franklin alone whom Randall had given the task of elucidating DNA's structure. The technique with which Rosalind Franklin set out to do this is called X-ray crystallography. With this technique, the locations of atoms in any crystal can be precisely mapped by looking at the image of the crystal under an X-ray beam. By the early 1950s, scientists were just learning how to use this technique to study biological molecules. Rosalind Franklin applied her chemist's expertise to the unwieldy DNA molecule. After complicated analysis, she discovered (and was the first to state) that the sugar-phosphate backbone of DNA lies on the outside of the molecule. She also elucidated the basic helical structure of the molecule. After Randall presented Franklin's data and her unpublished conclusions at a routine seminar, her work was provided - without Randall's knowledge - to her competitors at Cambridge University, Watson and Crick. The scientists used her data and that of other scientists to build their ultimately correct and detailed description of DNA's structure in 1953. Franklin was not bitter, but pleased, and set out to publish a corroborating report of the Watson-Crick model. Her career was eventually cut short by illness. It is a tremendous shame that Franklin did not receive due credit for her essential role in this discovery, either during her lifetime or after her untimely death at age 37 due to cancer.

But how is DNA copied?

- **Replication of DNA**
  - base pairing suggests that it will allow each side to serve as a **template** for a new strand





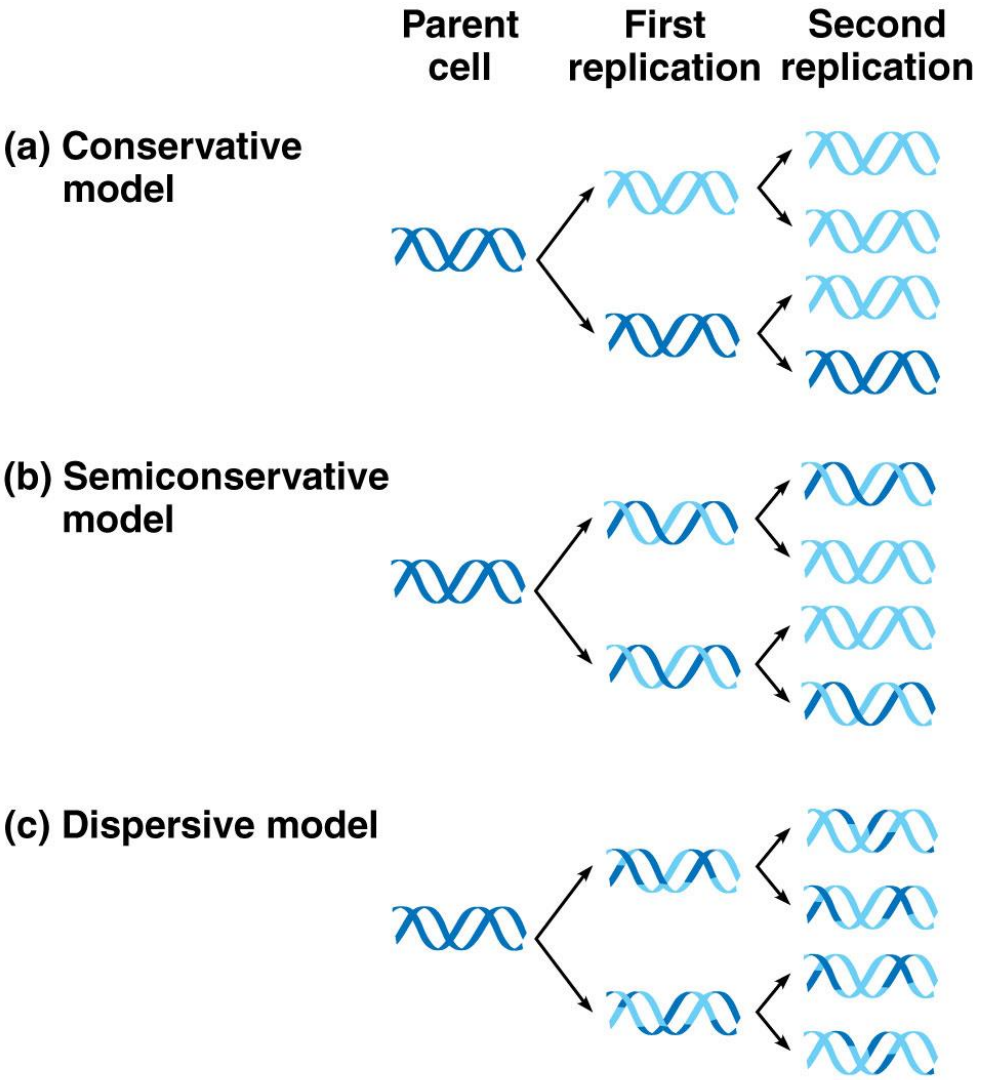
"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

— Watson & Crick

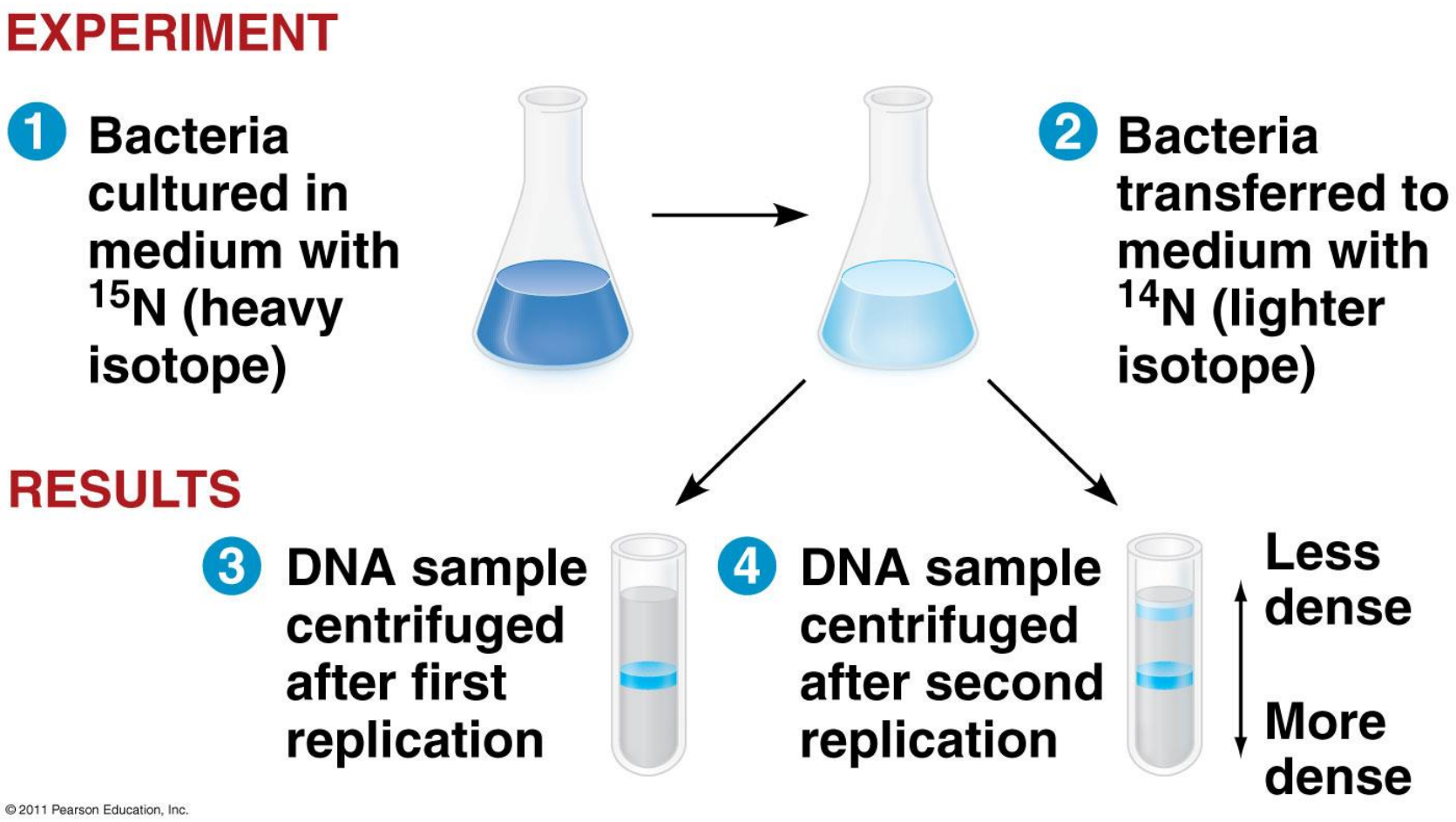
How does DNA replicate?

Replication: Making DNA from existing DNA

3 alternative models of DNA replication



Meselson & Stahl

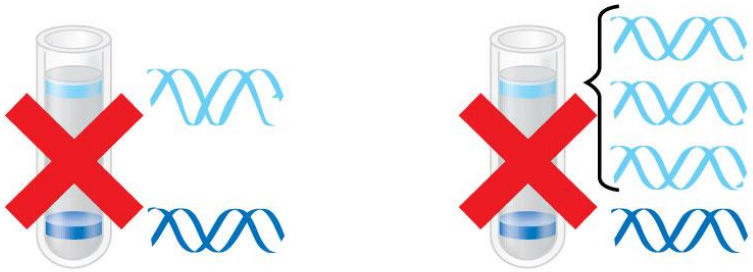


Meselson & Stahl

CONCLUSION

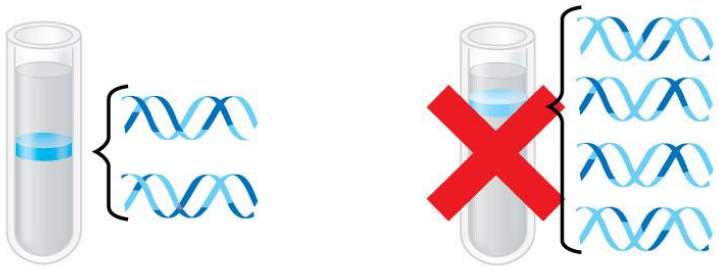
Predictions: **First replication** **Second replication**

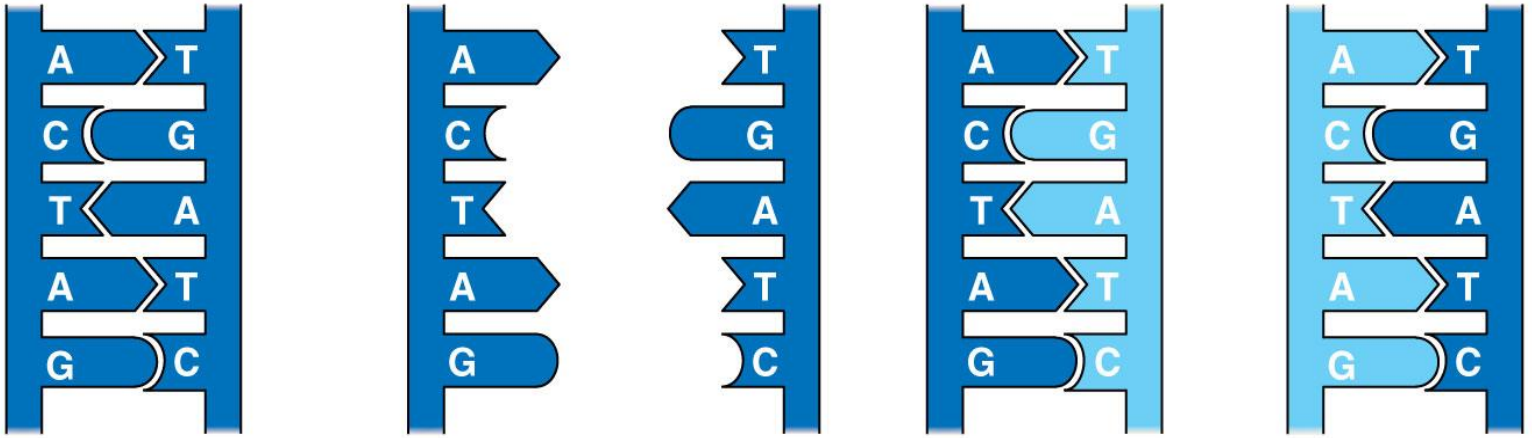
Conservative model



Semiconservative model

Dispersive model





(a) Parent molecule

(b) Separation of strands

(c) "Daughter" DNA molecules, each consisting of one parental strand and one new strand

Scientific History

- March to understanding that DNA is the genetic material
  - T.H. Morgan (1908)
    - genes are on chromosomes
  - Frederick Griffith (1928)
    - a transforming factor can change phenotype
  - Avery, McCarty & MacLeod (1944)
    - transforming factor is DNA
  - Erwin Chargaff (1947)
    - Chargaff rules: A = T, C = G
  - Hershey & Chase (1952)
    - confirmation that DNA is genetic material
  - Watson & Crick (1953)
    - determined double helix structure of DNA
  - Meselson & Stahl (1958)
    - semi-conservative replication

The "Central Dogma"

- Flow of genetic information in a cell