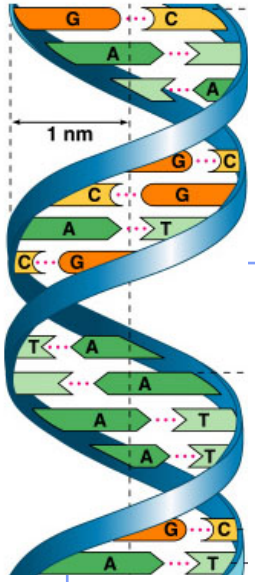
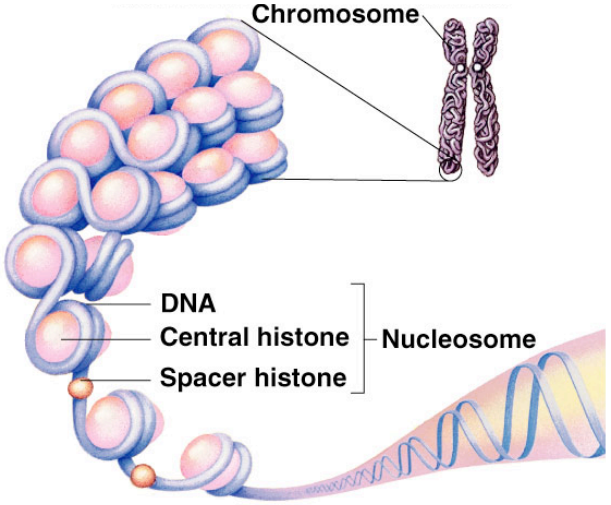




Ch.16

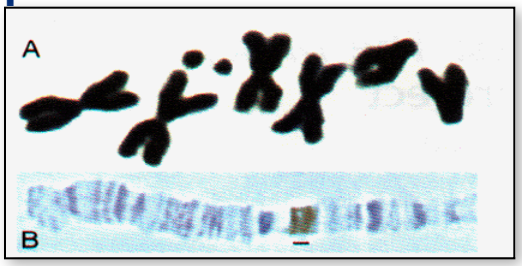
Research on the Genetic Material

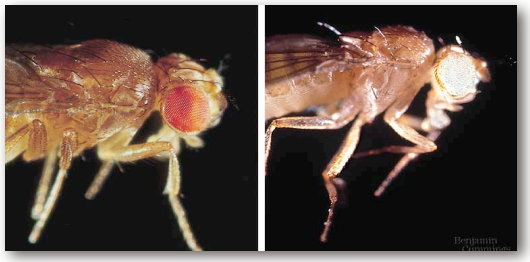
Scientific History

- **The march to understanding that DNA is the genetic material *(the molecule that stores the instructions cells use to form their characteristics)* in the cell involved many historical researchers:**
  - **T.H. Morgan (1908)**
  - **Frederick Griffith (1928)**
  - **Avery, McCarty & MacLeod (1944)**
  - **Erwin Chargaff (1947)**
  - **Hershey & Chase (1952)**
  - **Watson & Crick (1953)**
  - **Meselson & Stahl (1958)**

Chromosomes are Related to Phenotype

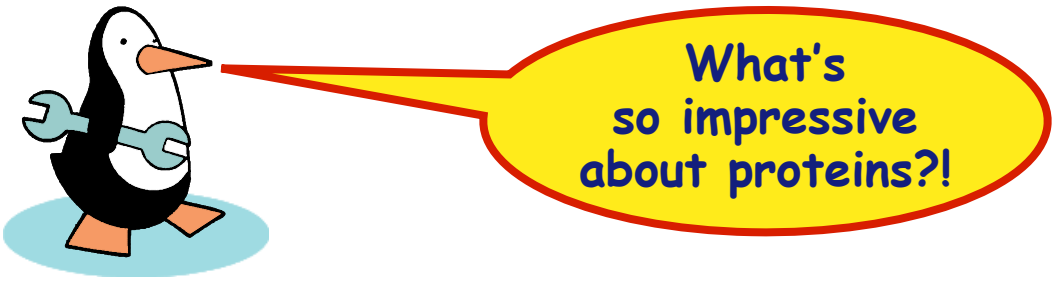
- **Thomas Hunt Morgan** **1908 | 1933**
  - Studied inheritance in *Drosophila* fruit flies
    - tried to figure out what determined an organism's phenotype *(= characteristics of organisms that result from the particular version of genes they inherit)*
  - **He was first to definitively associated an organism's phenotype with its chromosomes (DNA)**
    - He noted that the sex chromosomes (XX or XY) a fly inherited from its parents determined the anatomical sex of the fly
    - He realized that all white-eyed male fruit flies also inherited a specific X chromosome from the mother that must encode somehow the information for white eyes
      - He was the 1st person to link definitively the inheritance of a specific version (trait) of a characteristic with a particular chromosome.

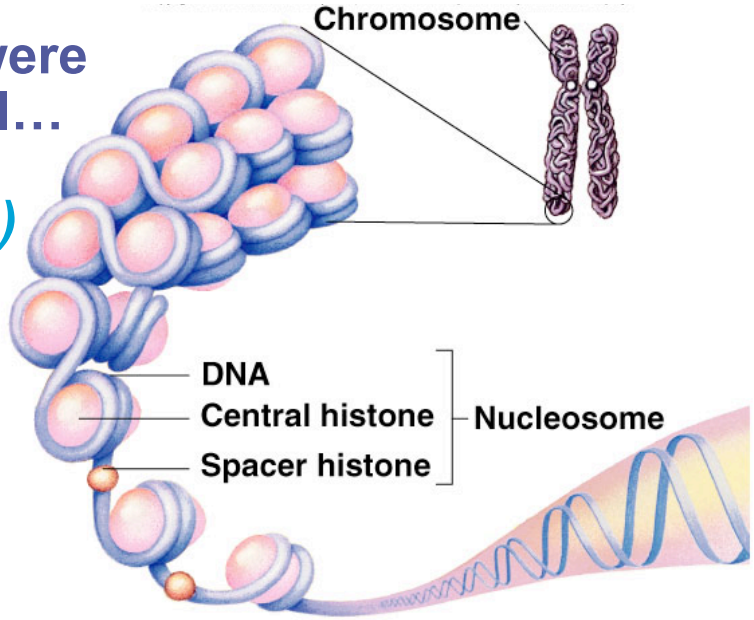




Genes are on Chromosomes

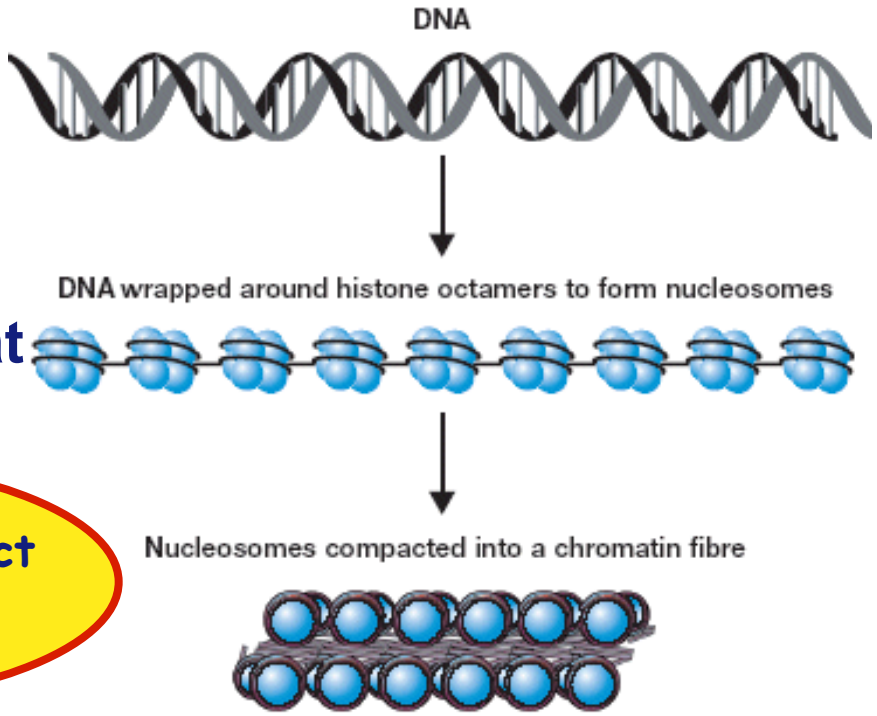
- **Morgan's conclusions:**
  - **genes** *("factors" that carry the information an organism uses to build its characteristics)* **are housed on chromosomes**
    - **Most scientists had doubts though still, and wondered if the genes were on the proteins or the DNA of the chromosomes.**
      - **initially (until 1940s) proteins were thought to be genetic material…** ***(with maybe then DNA only having an accessory function)***
        - ***Why did they think this?***





Proteins

- A great amount of heterogeneity (variation) is witnessed among different proteins
  - Proteins are made of 20 different building blocks (amino acids) that could be arranged in different orders and numbers to create a lot of variation in the final product
- All the different proteins each have highly specific functions
  - Nucleic Acids seemed too simple to code all the instructions a cell needs
    - Made of only 4 nitrogenous bases = A,C,G,T
      - Not like proteins with 20 different amino acids
- But it was DNA, not proteins, that carried the genetic information



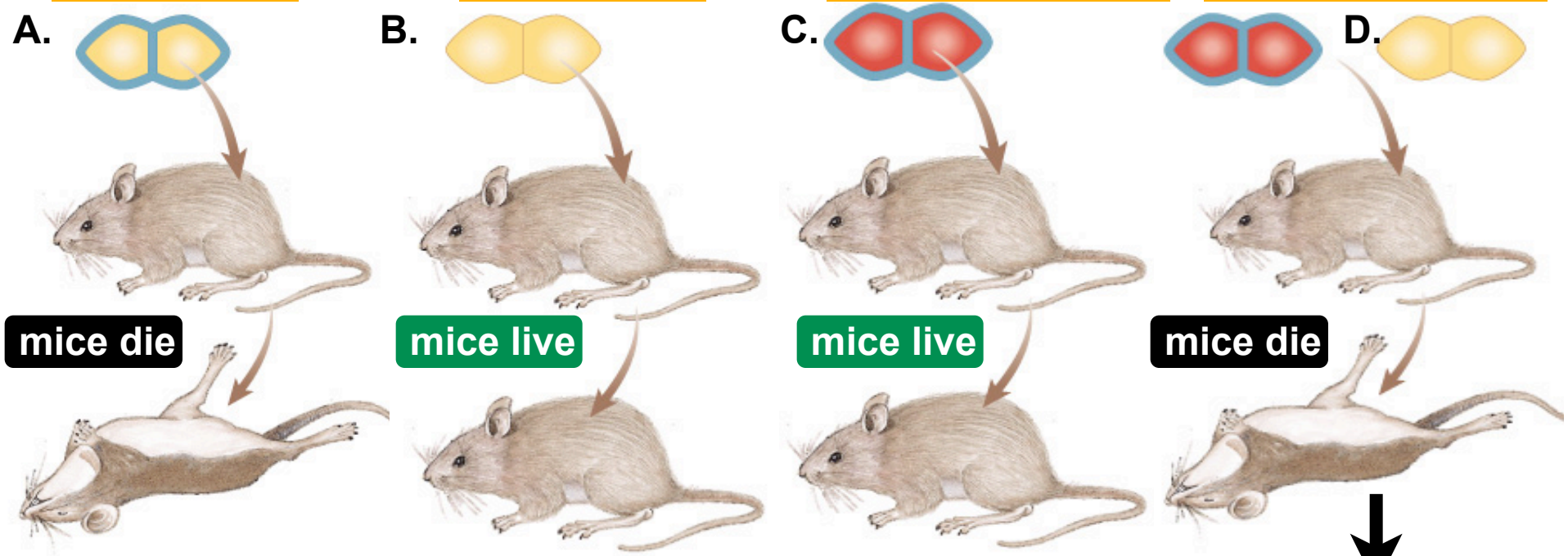
Can a genetic trait *(a version of a characteristics)* be transferred between different bacterial strains? 1928

- **Frederick Griffith**
  - **Studied *Streptococcus* pneumonia bacteria**
    - **He was working to find a vaccine against pneumonia (a type lung and/or blood infection)**
  - **He took live harmless (nonpathogenic) R strain bacteria, which did not kill mice *(R = "rough" because they had no smooth carbohydrate capsule outside the rougher cell wall)*, & mixed them with heat-killed pathogenic S strain bacteria, which caused the fatal disease in mice when alive *(S = "smooth" because they were able to make a capsule that protected them from detection by the mice immune systems)* and noticed that living, normally harmless R strain bacteria were able to transform themselves into harmful S strain types and kill the mice!!!**

    - **Through his research, he later hypothesized that an unknown substance passed from dead S bacteria to live R bacteria to change the live R bacteria's phenotype into a pathogenic one!**
  - **He called this the "Transforming Principle"**

Griffith's Experiment Involving Injecting Pneumonia Strains into Mice

A.	B.	C.	D.
live S pathogenic strain of *bacteria*	live R non-pathogenic strain of *bacteria*	heat-killed S pathogenic *bacteria*	mix heat-killed S pathogenic & R non-pathogenic *bacteria*
control group	control group	experimental group	experimental group



Transformation occurred in Group D = a change in genotype and, thus, phenotype due to an assimilation (internalization) of external DNA (carrying a new gene) by a cell.

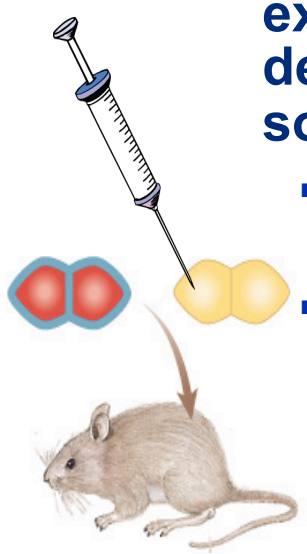
"Something" in the dead pathogenic bacteria could still transmit disease-causing properties, changing the non-lethal live ones into deadly ones, Griffith postulated.

When analyzing the blood of dead mice, he surprisingly found LIVING PATHOGENIC bacteria

Scientists now wondered: What is this transforming agent?

1944

- Avery, McCarty & MacLeod were determined to find out
  - Decided to extract both DNA (nucleic acid) & proteins from pathogenic S-strain *Streptococcus* pneumonia bacteria to characterize (determine) what the transforming agent was.
    - They wanted to answer the question: Which of the 2 molecules - Nucleic Acids or Proteins - in the cytoplasm of harmful bacteria can transform non-pathogenic bacteria (and their descendants)?
  - To make sure no intact DNA was present in the solution extracted from the harmful bacteria's cytoplasm, they destroyed all the harmful bacteria's DNA by treating the solution with nucleases first.
    - Then, they injected the solution, with its intact PROTEINS obtained from the harmful bacteria, into the harmless bacteria
    - Finally, they injected these treated harmless bacteria into live rodents
      - They saw NO EFFECT on the bacterial phenotype of the bacterial population reproducing inside the mice.
        - Harmless bacteria remained harmless and were killed by the mice's immune systems.
          - The mice lived!

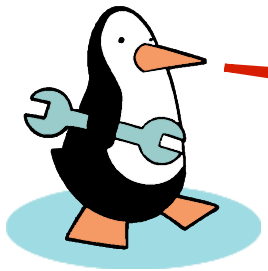


mice lived

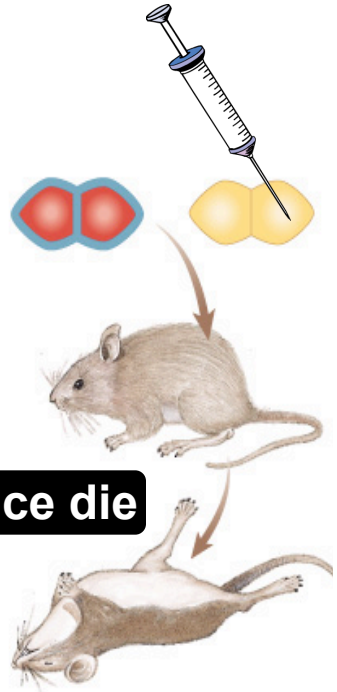
Scientists now wondered: What is this transforming agent?

1944

- Next, to make sure no intact protein was present in another batch of solution extracted from the harmful bacteria's cytoplasm, they destroyed all the harmful bacteria's protein by treating the solution with proteases this time.
  - Then, they injected the solution, with its intact DNA obtained from the harmful bacteria, into the harmless bacteria
  - Finally, they injected these treated harmless bacteria into live rodents
    - This time they DID see an EFFECT on the bacterial phenotype of the bacterial population reproducing inside the mice.
    - Harmless bacteria were TRANSFORMED into virulent (pathogenic/disease-causing) bacteria
      - Harmless bacteria became harmful and were no longer easily killed by the mice's immune systems.
        - The mice died!



What's the conclusion? Which molecule seems to control phenotype?



Avery, McCarty & MacLeod 1944 | No Nobel??!!

- **Conclusion:** Avery cautiously suggested that, indeed, DNA was the genetic material (not proteins).
  - **First experimental evidence that DNA was the genetic material but...**
- Scientists who had previously thought that the genetic material was protein still believed that the effects of the transforming principle were perhaps due to some undetected protein ASSOCIATED then with this DNA.

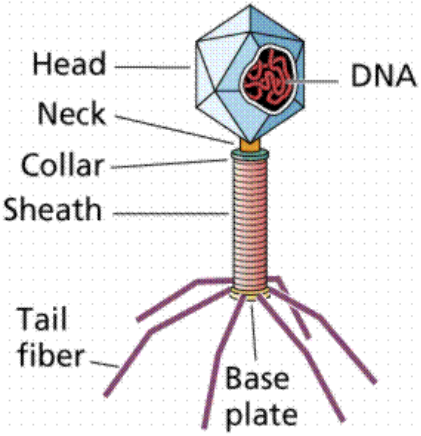
AP Oswald Avery

Maclyn McCarty

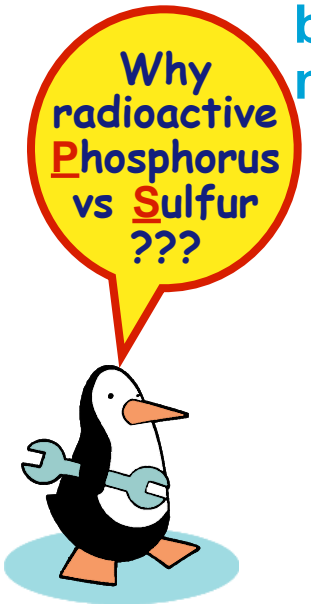
Colin MacLeod

Confirmation of DNA as hereditary material

1952 | 1969
Hershey



- Hershey & Chase decided to confirm that DNA was the genetic material
  - Know as the "blender" experiment
  - Worked with bacteriophages:
    - viruses that infect bacteria
      - Used the T2 phage that infects *E.coli*
  - They started by creating two types of radioactively-labeled versions of these bacterial viruses (phages) by infecting E. coli bacteria growing in 2 different types of media (mixtures of nutrients that you grow cells in or on) with bacteriophages

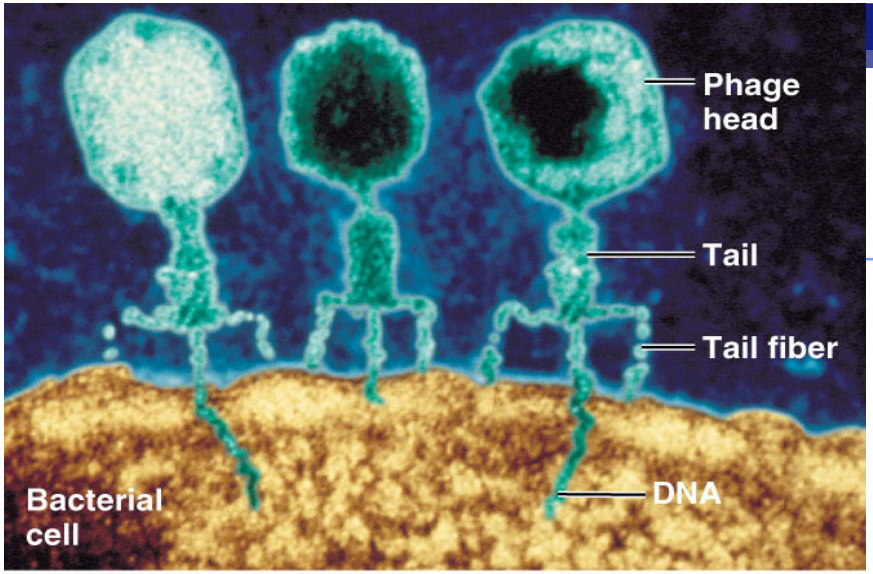


1. In one flask, they added bacteria and bacteriophages to a liquid broth medium containing the basic essential bacterial nutrients (necessary amino acids, glucose, ions, vitamins etc), all made from nonradioactive isotopes of elements, except that it did contain radioactive sulfur atoms, ^{35}S
2. In a second flask, they added bacteria and bacteriophages to a liquid medium containing the basic essential bacterial nutrients (necessary amino acids, glucose, ions, vitamins etc), all made from nonradioactive isotopes of elements, except that it did contain radioactive phosphorus atoms, ^{32}P

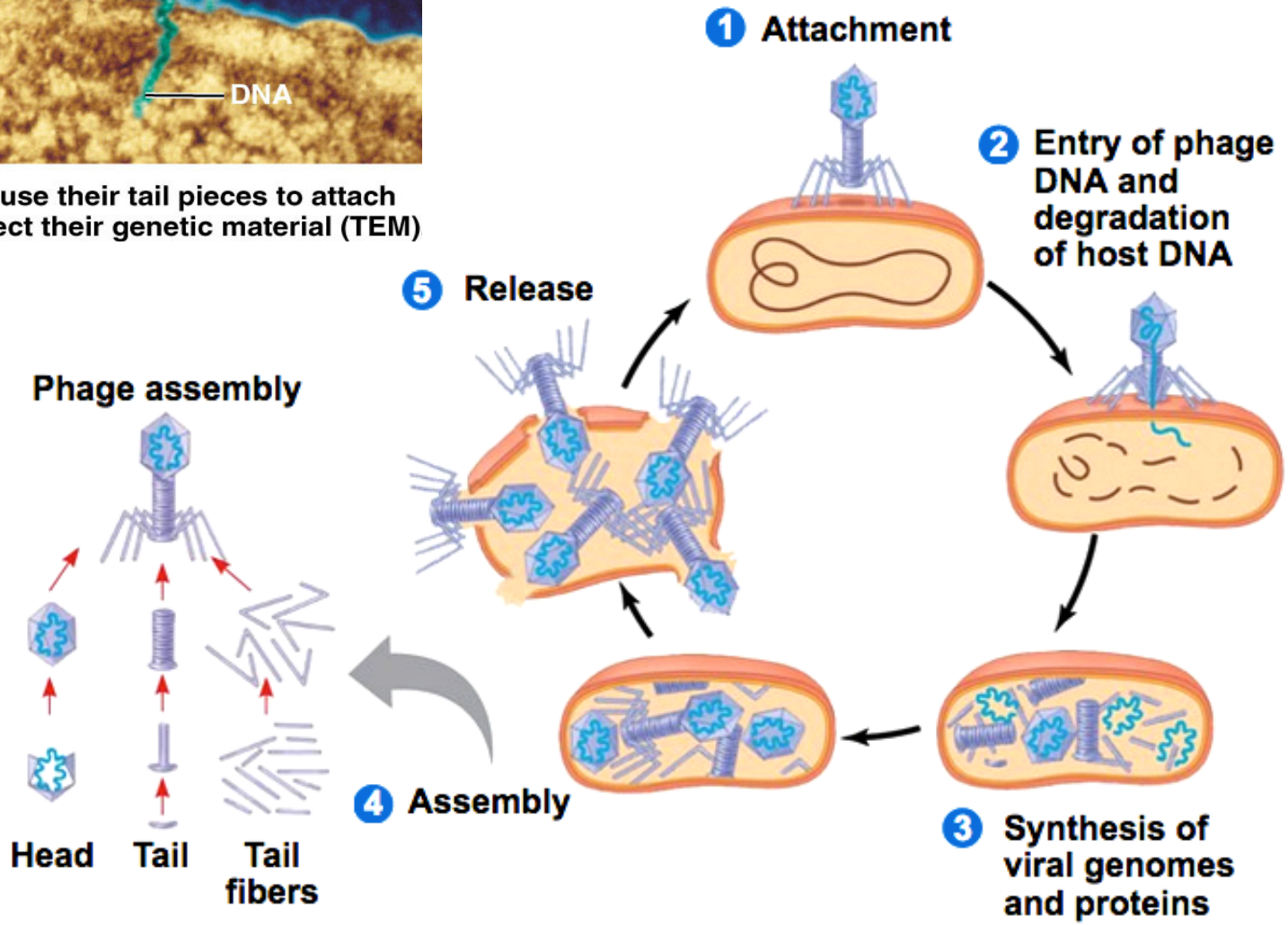
Virus Reproduction

- **Remember how bacteriophages replicate (Ch.19) when completing the Lytic Cycle:**
  - In the lytic cycle, phages attach to the plasma membrane of a bacterial cell when the phages' tail fibers of the capsid are attracted to certain plasma membrane proteins of the bacteria.
  - Once attachment is achieved, phages inject their dsDNA (their genome) into the bacterial cytoplasm.
  - Once the viral dsDNA (with all its genes for making viral proteins) is inside the bacteria, the bacteria's own DNA Polymerase makes many copies the entire viral genome.
  - At the same time, the bacteria's own RNA Polymerase starts transcribing the viral genes into mRNA, which are translated into viral proteins by the bacteria's own ribosomes.
  - Some viral proteins produced are nucleases, which cut the bacterial chromosome up into pieces, rendering it nonfunctional.
  - The viral proteins known as capsomeres attract each other forming new phage capsids surrounding copies of the viral dsDNA
  - After hundreds of new phages form, a viral protein causes the bacterial cell to lyse, releasing these hundreds of virions (virus copies) into the environment which can then go on to infect the next bacterial cells.

Lytic Cycle of Bacteriophages

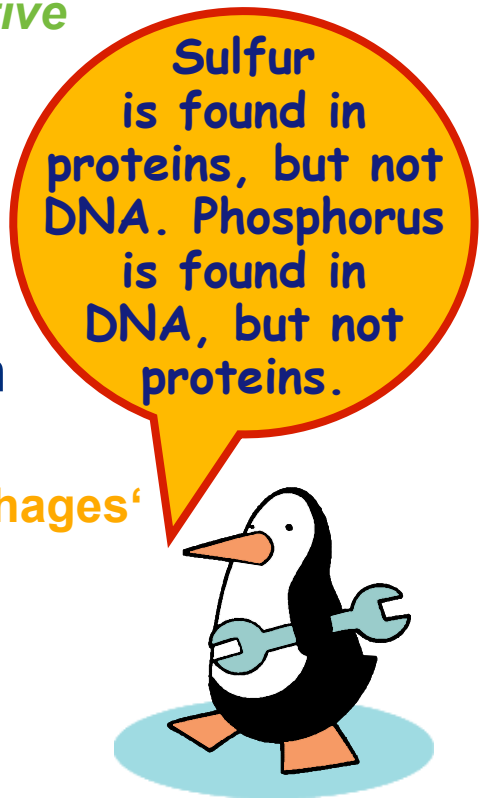


(a) T2 and related phages use their tail pieces to attach to the host cell and inject their genetic material (TEM)



Hershey-Chase "Blender" Experiment

- **Living cells use radioactive isotopes of elements in the exact same way chemically as nonradioactive isotopes of elements (Ch.2) since the number of valence electrons are the same in the various isotope atoms of an element.**
  - **As the phages infected the bacteria in each of the two flasks** ***(one with radioactive sulfur but nonradioactive phosphorus and the other with radioactive phosphorus but nonradioactive sulfur)*****, millions of new bacteriophages (made of viral capsid proteins and viral DNA) were made from the monomers that the bacteria had constructed from the nutrients available in the liquid broth medium inside their flask.**
    1. **This meant that in flasks with ^{35}S the bacteriophages' proteins were now labeling and traceable**
    2. **This meant that in flasks with ^{32}P the bacteriophages' DNA were now labeling and traceable**



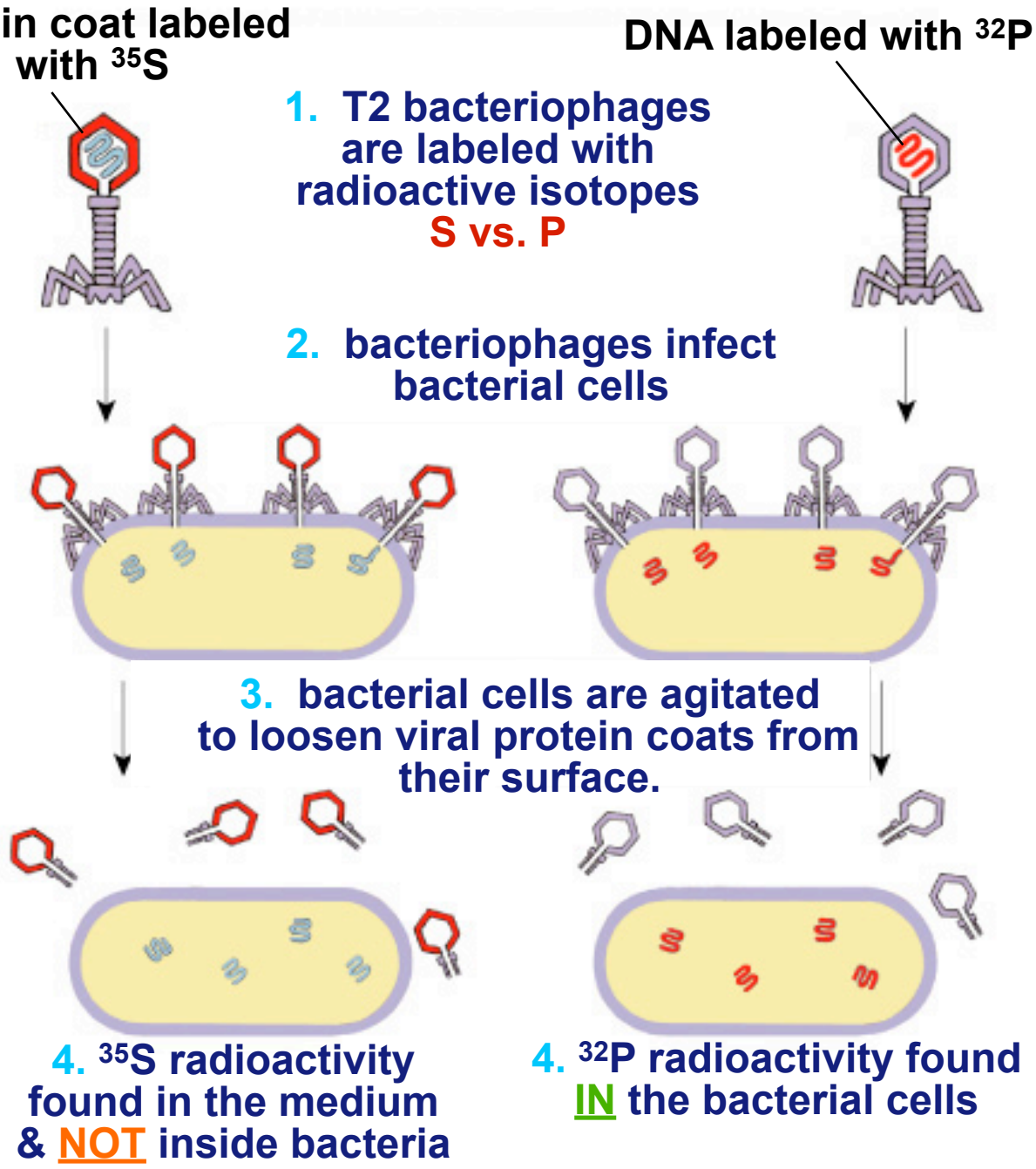
Hershey-Chase "Blender" Experiment

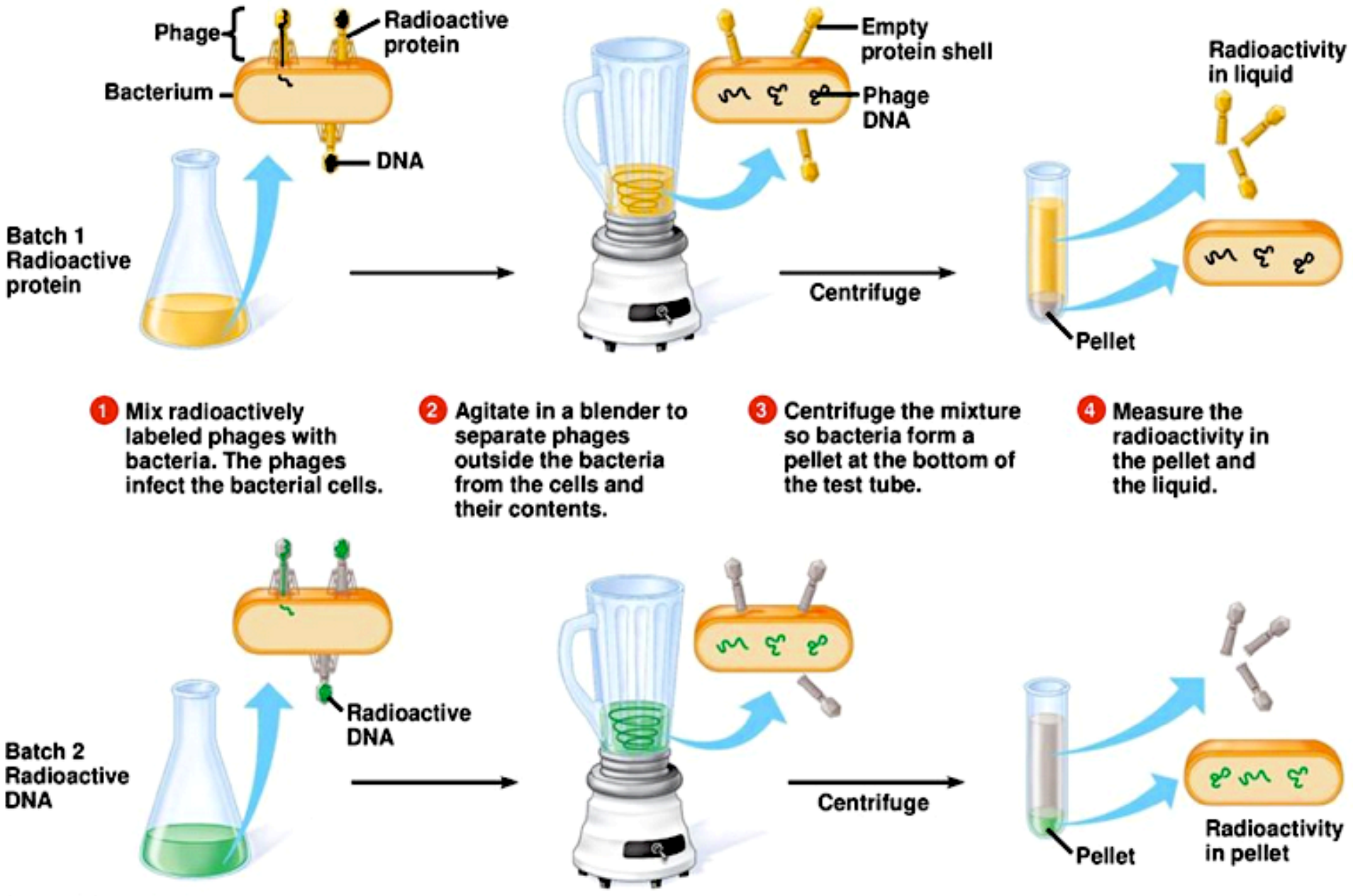
- **Hershey & Chase wanted to identify:**
  - **Which part of the phage enters the bacteria?**
    - **Which part of the phage is able to transform the phenotype of the bacteria and change it from an E. coli that doesn't produce new virions into an E. coli that starts making and releases new bacteriophages.**
      - **After building bacteriophages with their proteins radio-actively labeled, they transferred these viruses into a new flask with bacteria living in a liquid broth with nonradioactive nutrients in order to have these phages infect this new population of E. coli bacteria**
      - **Similarly, after building bacteriophages with their DNA radio-actively labeled, they transferred these viruses into a new flask with bacteria living in a liquid broth with nonradioactive nutrients in order to have these phages infect this new population of E. coli bacteria**
        - **Now they could trace where the proteins of the phages went during bacterial infection in one flask and where the DNA of the phages went during bacterial infection in the other flask.**
        - **Now they could find out which macromolecule is the transforming agent!**

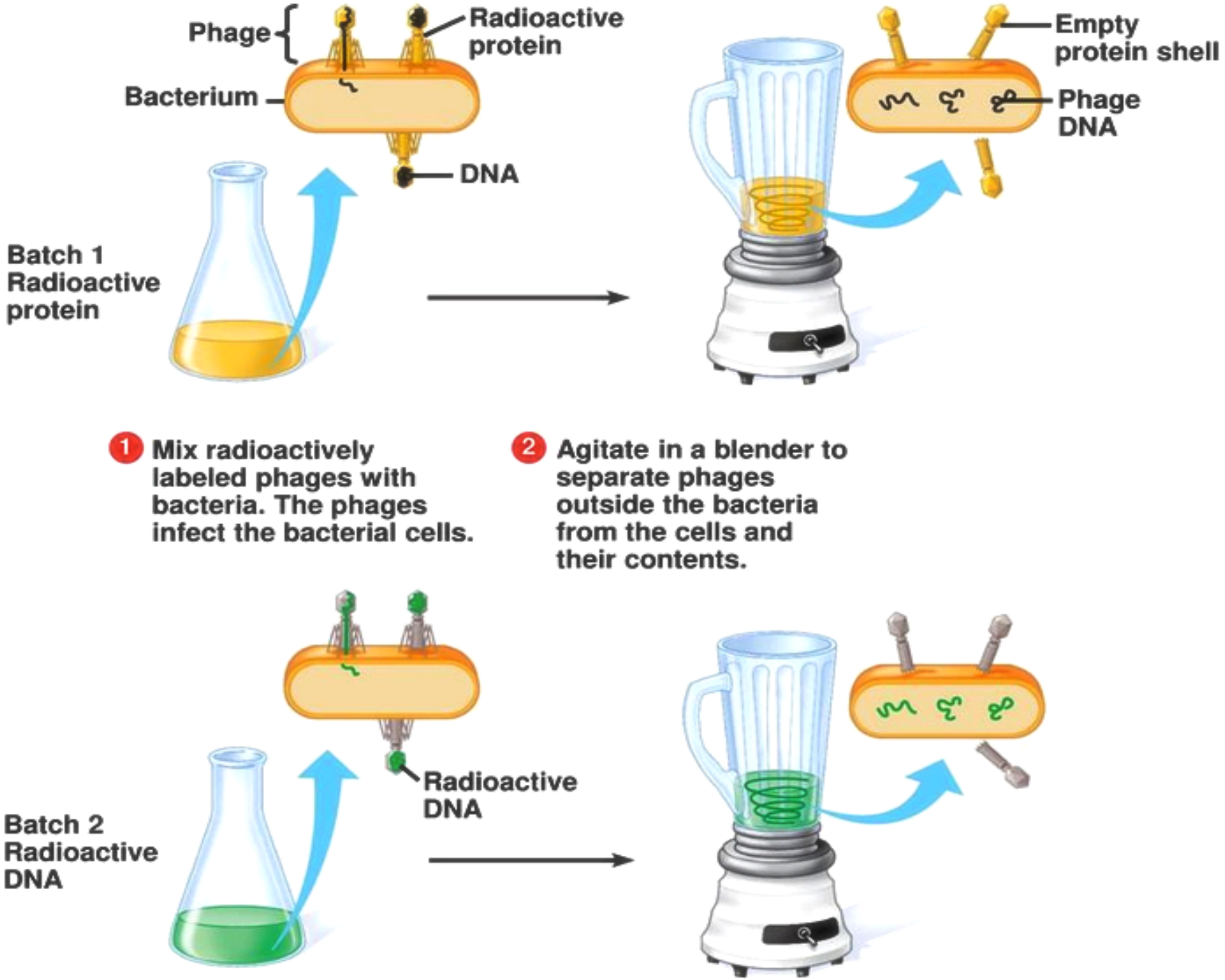
Hershey & Chase

Which radioactive marker is found inside the cell?

Which molecule (DNA or Proteins) carries viral genetic info and can transform the phenotype of the bacteria into one that is making new virus particles?




Phage
Radioactive protein
Bacterium
DNA
Batch 1 Radioactive protein
Empty protein shell
Phage DNA
Centrifuge
Radioactivity in liquid
Pellet
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.
Radioactive DNA
Batch 2 Radioactive DNA
Centrifuge
Pellet
Radioactivity in pellet


Phage
Radioactive protein
Bacterium
DNA
Batch 1 Radioactive protein
Empty protein shell
Phage DNA
1 Mix radioactively labeled phages with bacteria. The phages infect the bacterial cells.
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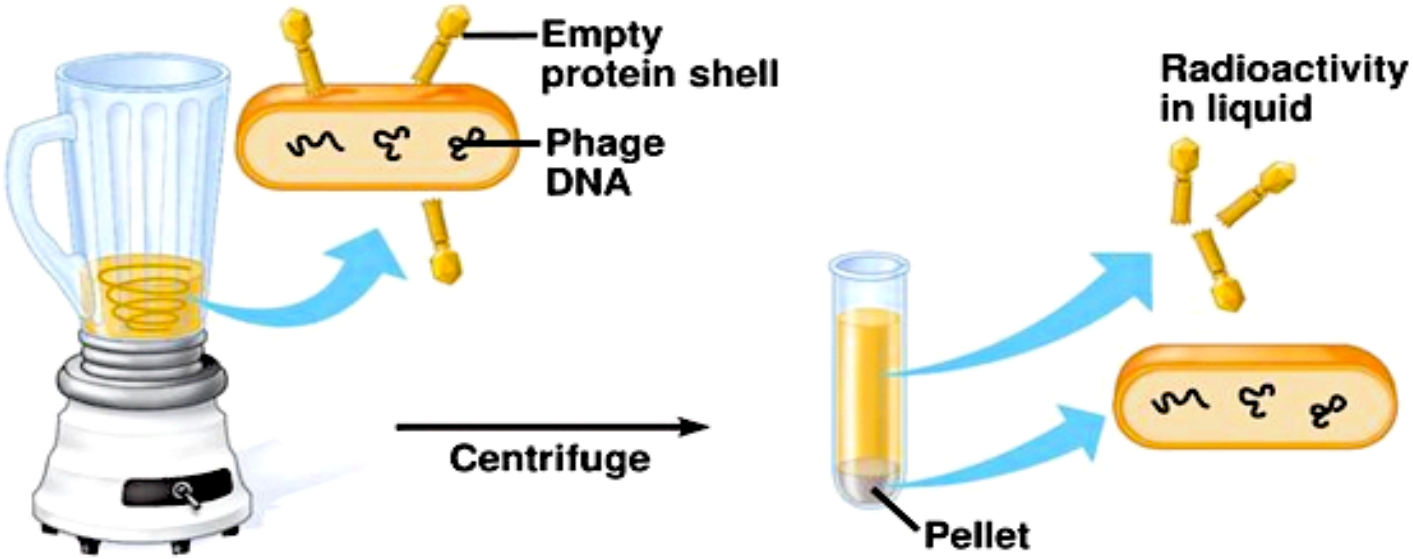
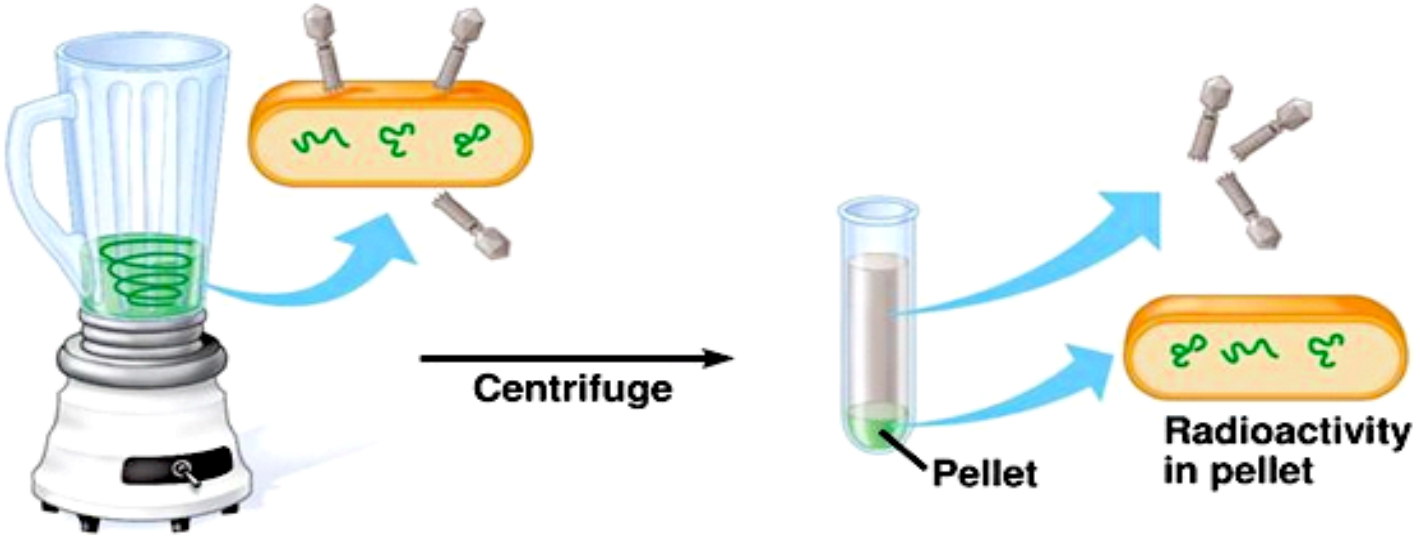
Radioactive DNA
Batch 2 Radioactive DNA



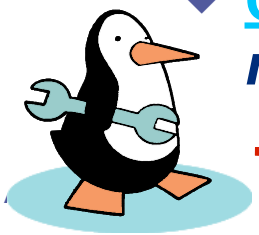
3 Centrifuge the mixture so bacteria form a pellet at the bottom of the test tube.

4 Measure the radioactivity in the pellet and the liquid.



The Hershey-Case Blender Experiment

- Following the addition of radioactively-labeled phages, the broth with the now infected bacteria and phages were put in a blender to loosen any parts of phages struck on the outer surface of the plasma membranes of the bacteria.
- The broth solution was then centrifuged to separate the bacteria fully from the rest of the broth solution and anything lighter left floating in the broth.
  - In the flask with added ^{35}S-labeled phages:
    - Radioactive proteins stayed in supernatant
    - Therefore, viral protein did NOT enter bacteria
  - In the flask with added ^{32}P-labeled phages:
    - Radioactive DNA stayed in pellet
    - Therefore, viral DNA DID enter bacteria
      - So DNA played an ongoing role in the infection process
  - Confirmed DNA is the "transforming factor" - *DNA is the molecule that can transform the phenotype of a cell!!!*



Taaa-Daaa!

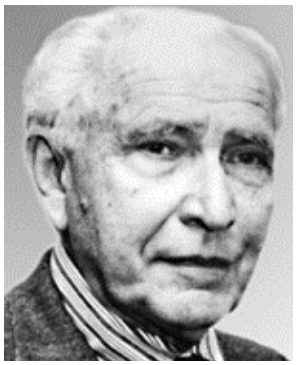
Hershey & Chase

1952 | 1969
Hershey

Martha Chase Alfred Hershey

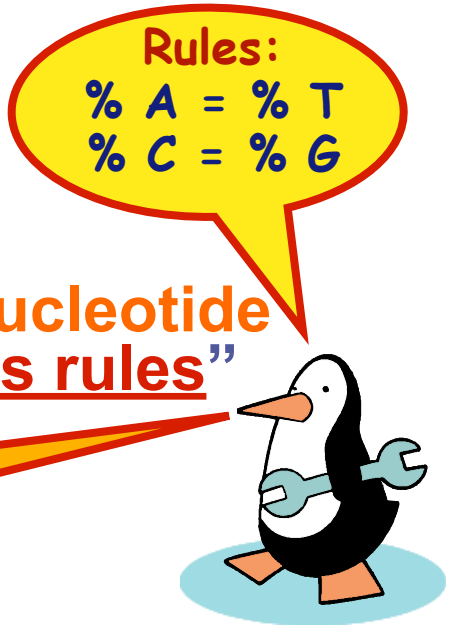
Chargaff Analyzed DNA Composition 1947

- **Concluded that the base composition of DNA varies from species to species *& that bases are present in characteristic ratios in different species.***
  - **Ex: Adenosine makes up 30% of human DNA but 26.0% in bacteria, for example.**
    - **Saw molecular diversity between species which helped make DNA much more credible as a genetic candidate**



Also noted that, within a species, all 4 bases are not present in equal quantities

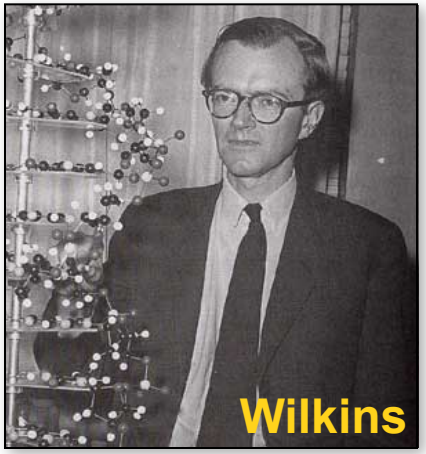
- **Ex: In humans:** A = 30.9%, T = 29.4%, G = 19.9%, C = 19.8%

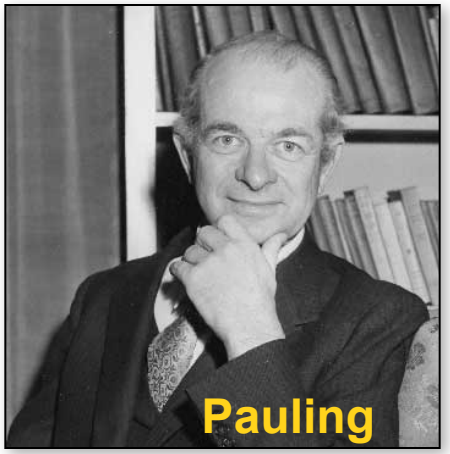


- **Equivalencies in percentages of nucleotide bases within a species: "Chargaff's rules"**

Determining DNA's Structure 1953 | 1962

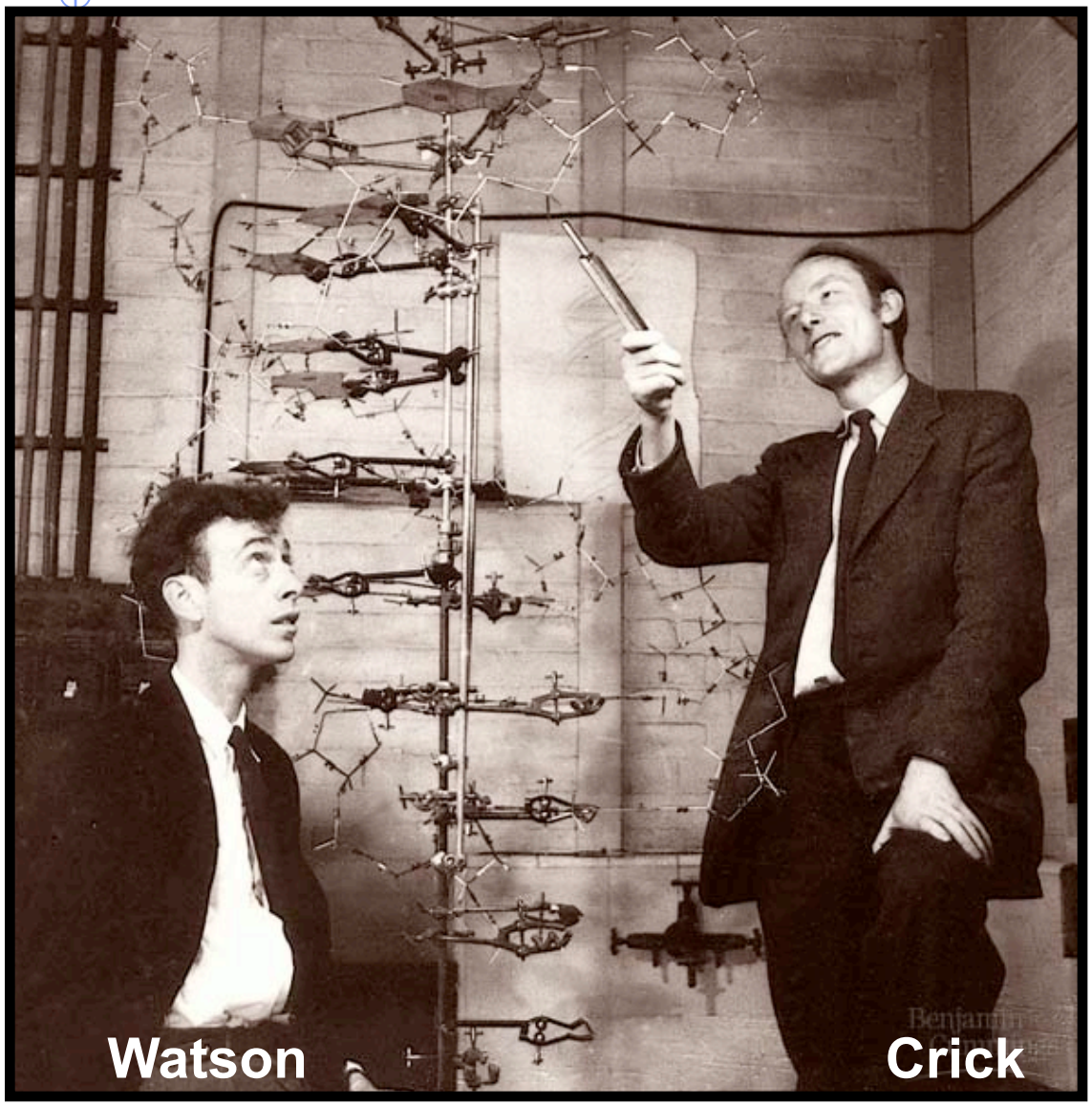
- Watson & Crick developed double helix model of DNA
  - other leading scientists had been working on this question too, including...
    - Rosalind Franklin
    - Maurice Wilkins
    - Linus Pauling
  - Watson & Crick's model was inspired by 3 recent discoveries:
    - Chargaff's rules
    - Pauling's alpha helical structure of a protein
    - X-ray crystallography DNA data from Franklin & Wilkins

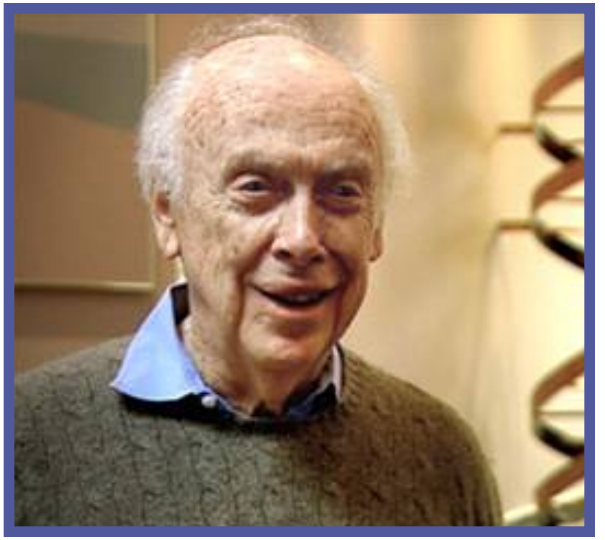


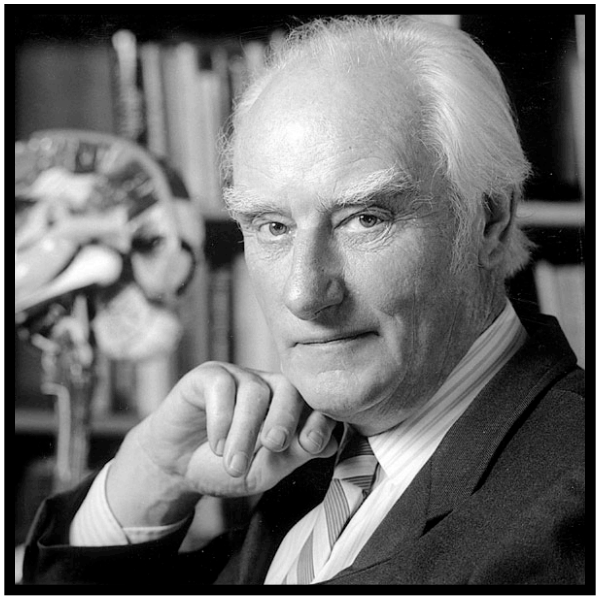


Watson and Crick

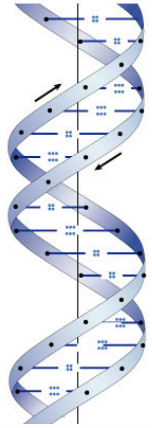
1953 article in *Nature*





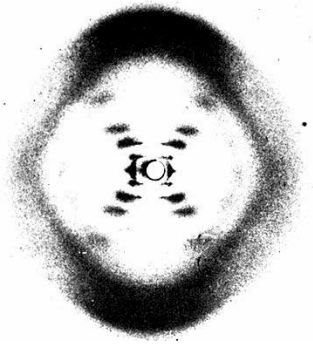


Rosalind Franklin (1920-1958)



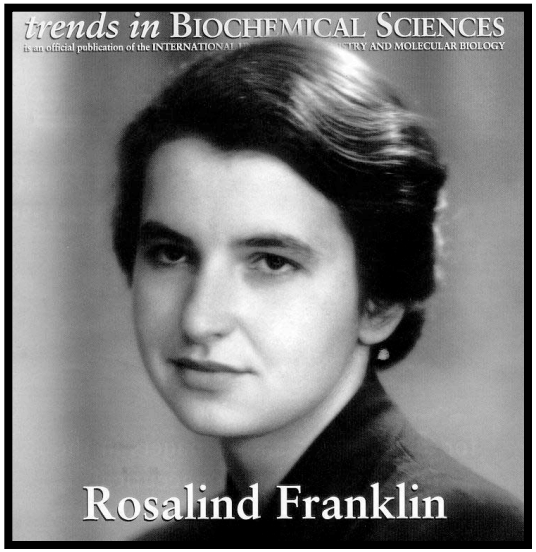
The technique with which Rosalind Franklin set out to elucidate the shape of DNA is called X-ray crystallography.

- 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.
  - 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.

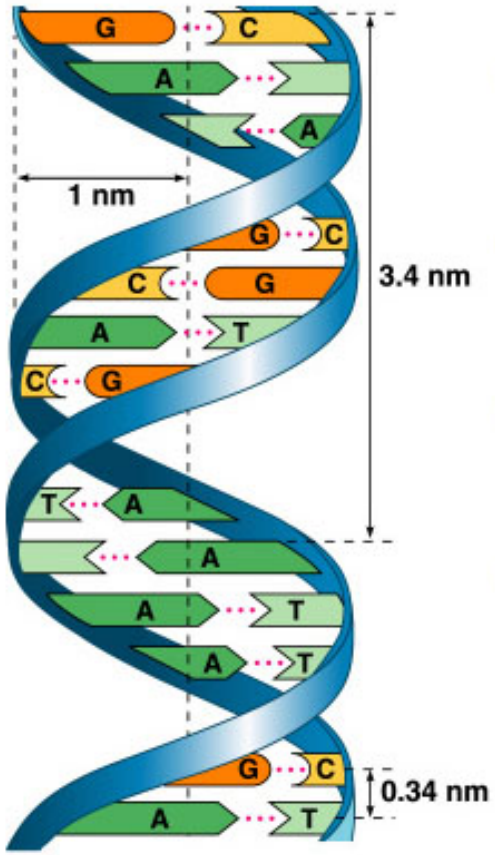


After Randall presented Franklin's data and her unpublished conclusions at a routine seminar, her work was provided - without Randall's knowledge - to her competitors at Cambridge University, Watson and Crick.

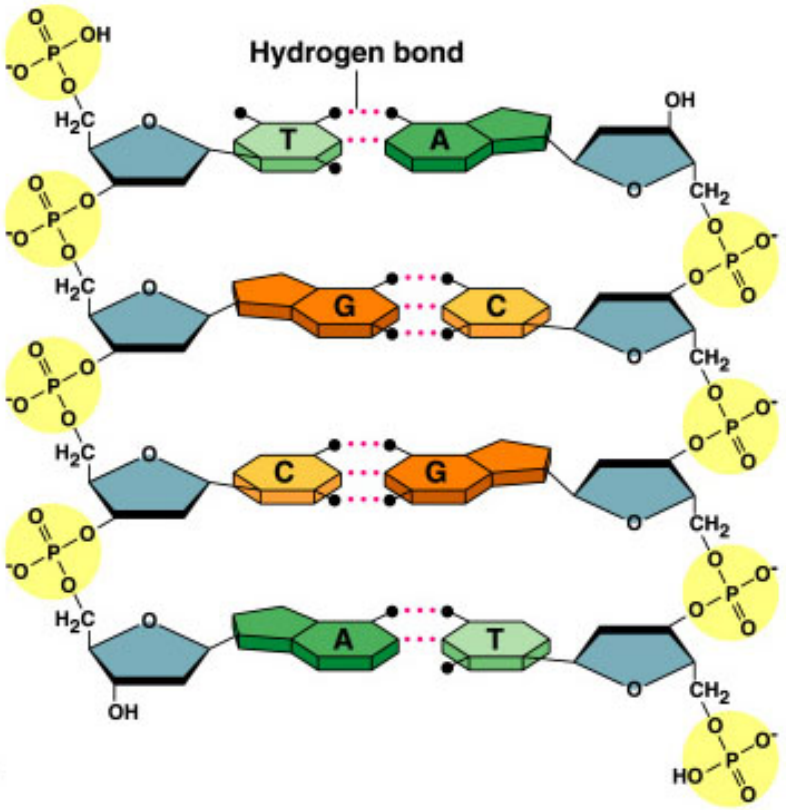
- These scientists used her data and that of other scientists to build their ultimately correct and detailed description of DNA's structure in 1953.



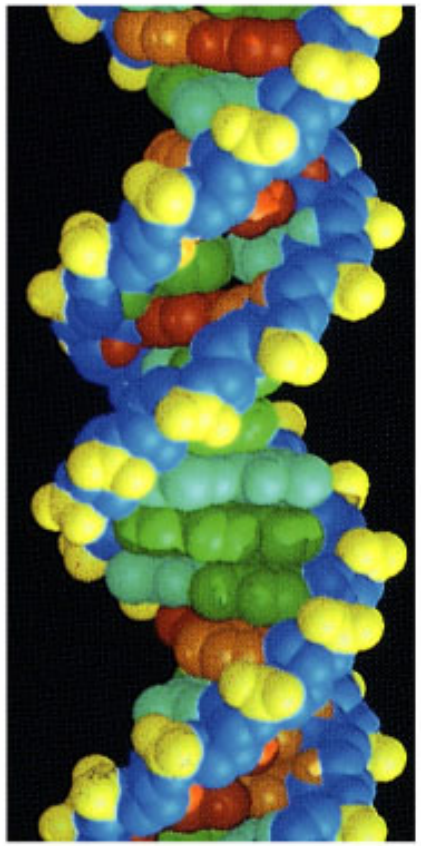
Double helix structure of DNA



(a) Key features of DNA structure



(b) Partial chemical structure

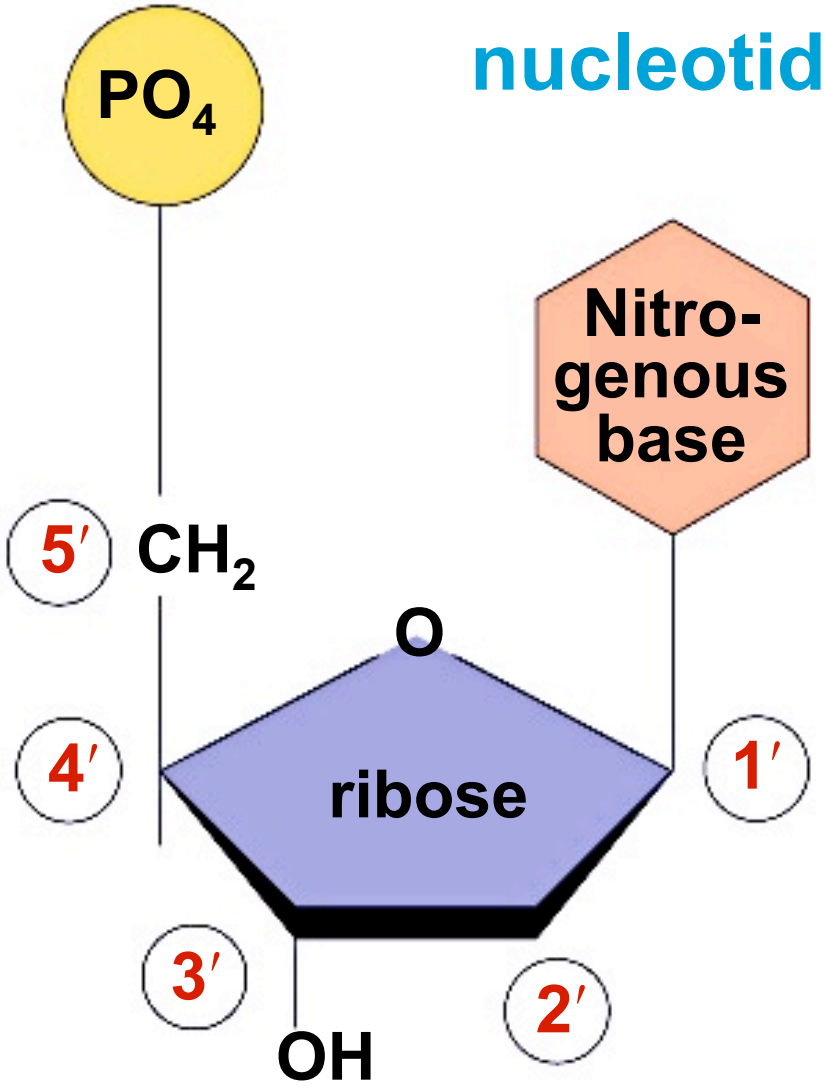


(c) Space-filling model

Directionality of DNA

- You need to number the carbons!
  - it matters!

nucleotide

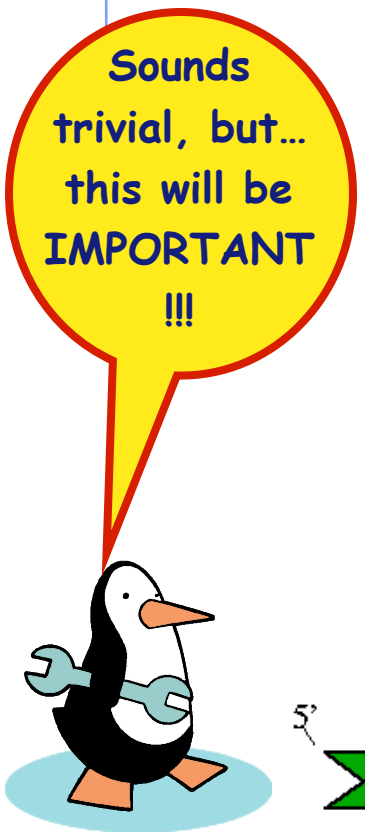


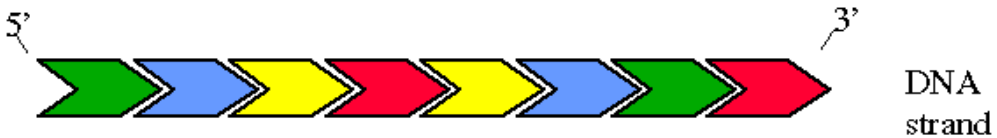
The DNA backbone

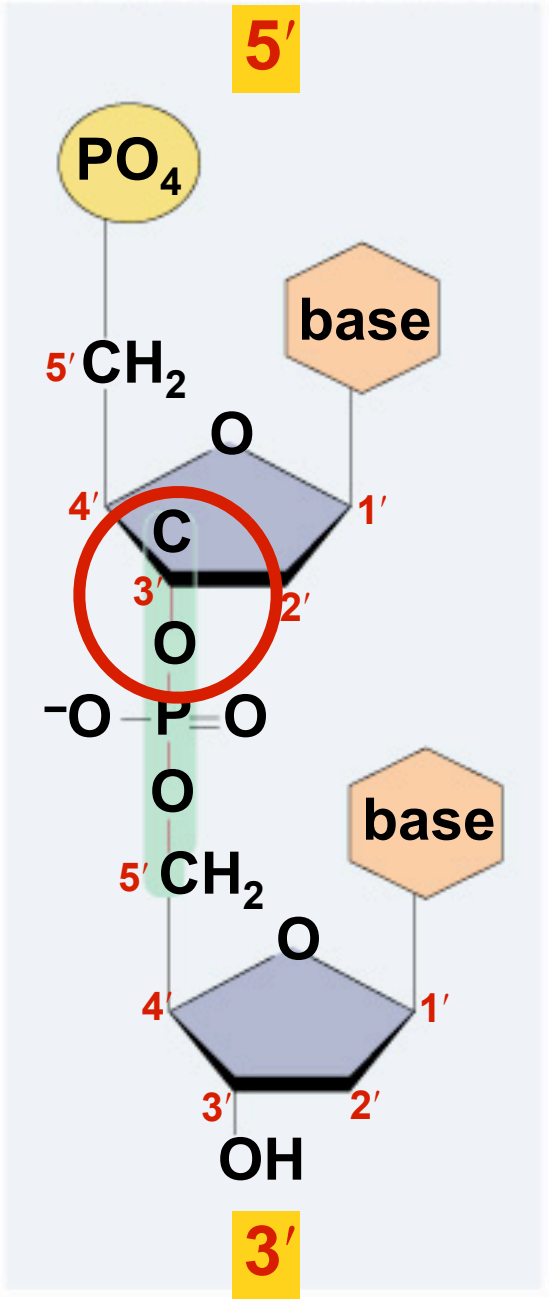
- A DNA backbone has been assembled
- Nucleotides are asymmetric.
  - They are linked into a DNA chain in a "head-to-tail" fashion.
    - Therefore, the entire DNA strand has a chemical polarity.

Scientists refer to the 3′ and 5′ ends of the DNA

- Look: hydroxyl group is sitting on the 3' carbon & phosphate group hangs off the 5' carbon

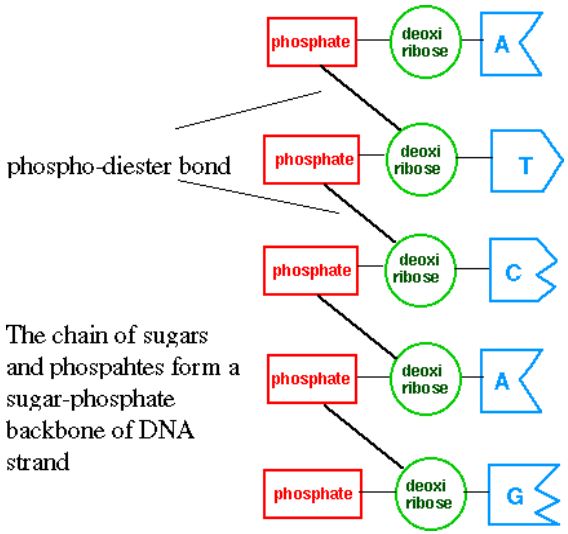


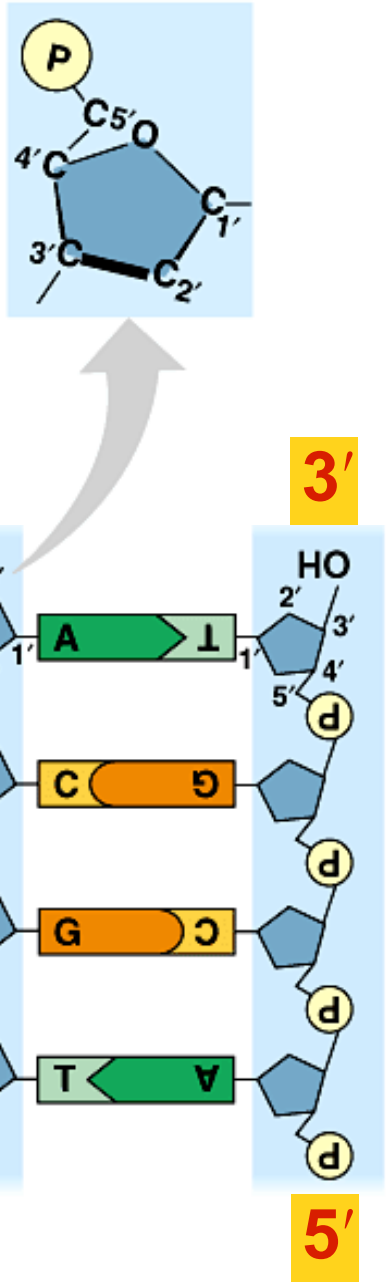




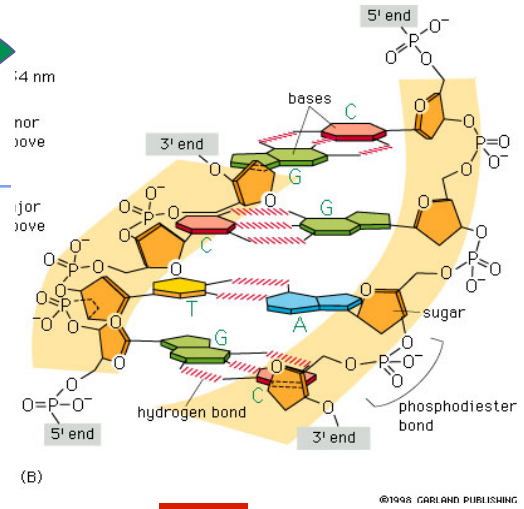
Anti-parallel strands

- Nucleotides in DNA backbone are bonded together between the sugar of one & the phosphate of the next
  - Phosphodiester bond
    - Nucleotides attach between 3′ & 5′ carbons of the ribose
      - DNA molecule has "directionality"
        - complementary strand runs in opposite direction
      - DNA is ANTIPARALLEL





Bonding in DNA



hydrogen bonds

5′

3′

covalent phosphodiester bonds

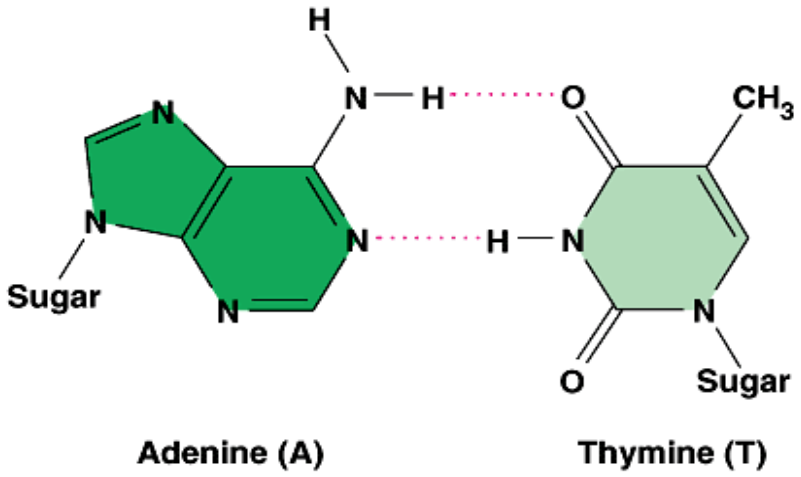
3′

5′

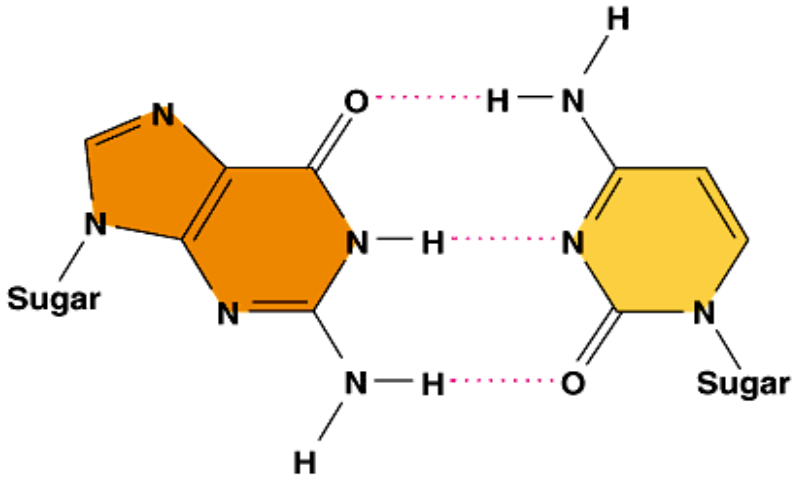
….which is a strong and which is a weak bond?
How do the bonds fit the mechanism for copying DNA?

DNA bases are complimentary!

- Purines
  - adenine (A)
  - guanine (G)
- Pyrimidines
  - thymine (T)
  - cytosine (C)
- Base Pairing
  - A : T
    - 2 H-bonds
  - C : G
    - 3 H-bonds
      - (slightly harder to pull apart DNA that is full of CG bonds versus AT bonds)



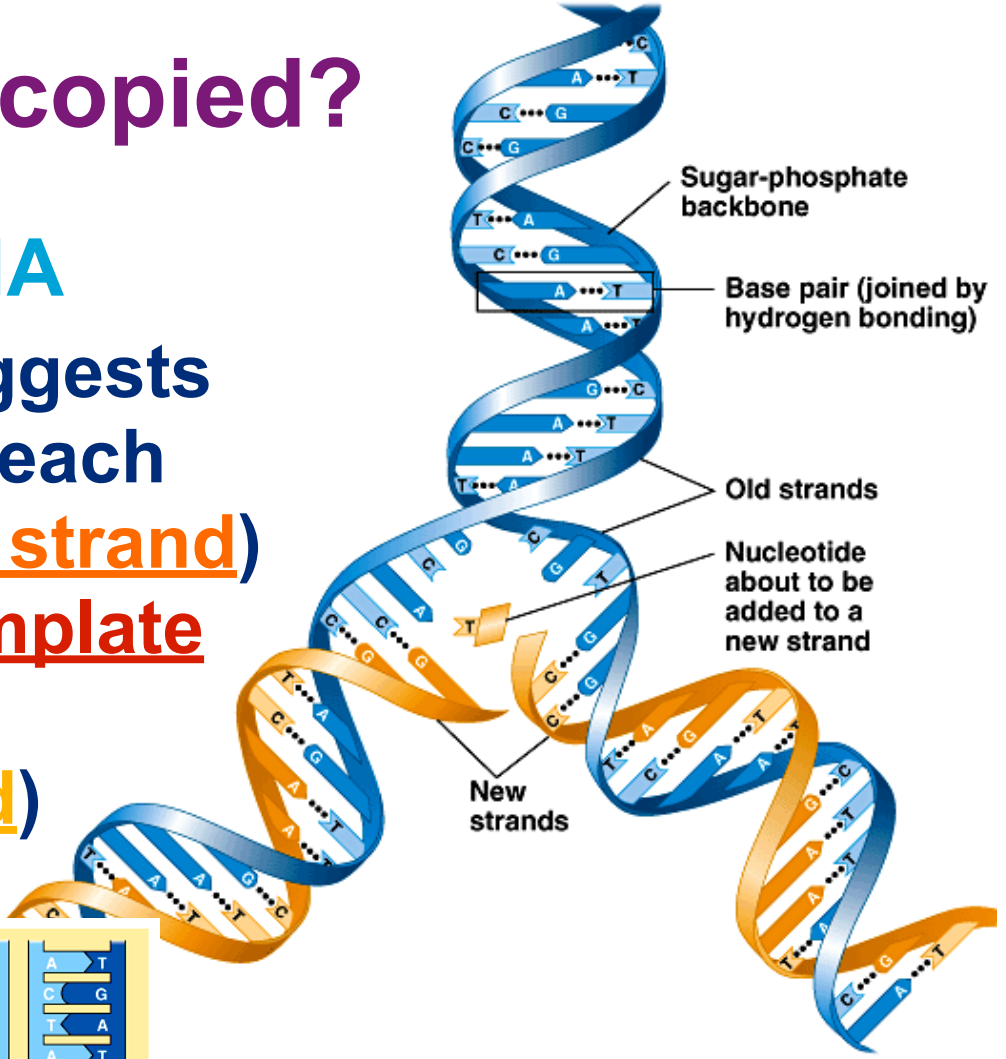
Adenine (A) Thymine (T)

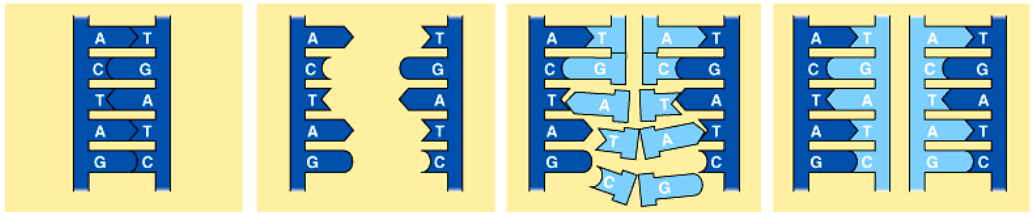


Guanine (G) Cytosine (C)

But how is DNA copied?

- Replication of DNA
  - base pairing suggests that it will allow each strand (parental strand) to serve as a template for a new strand (daughter strand)



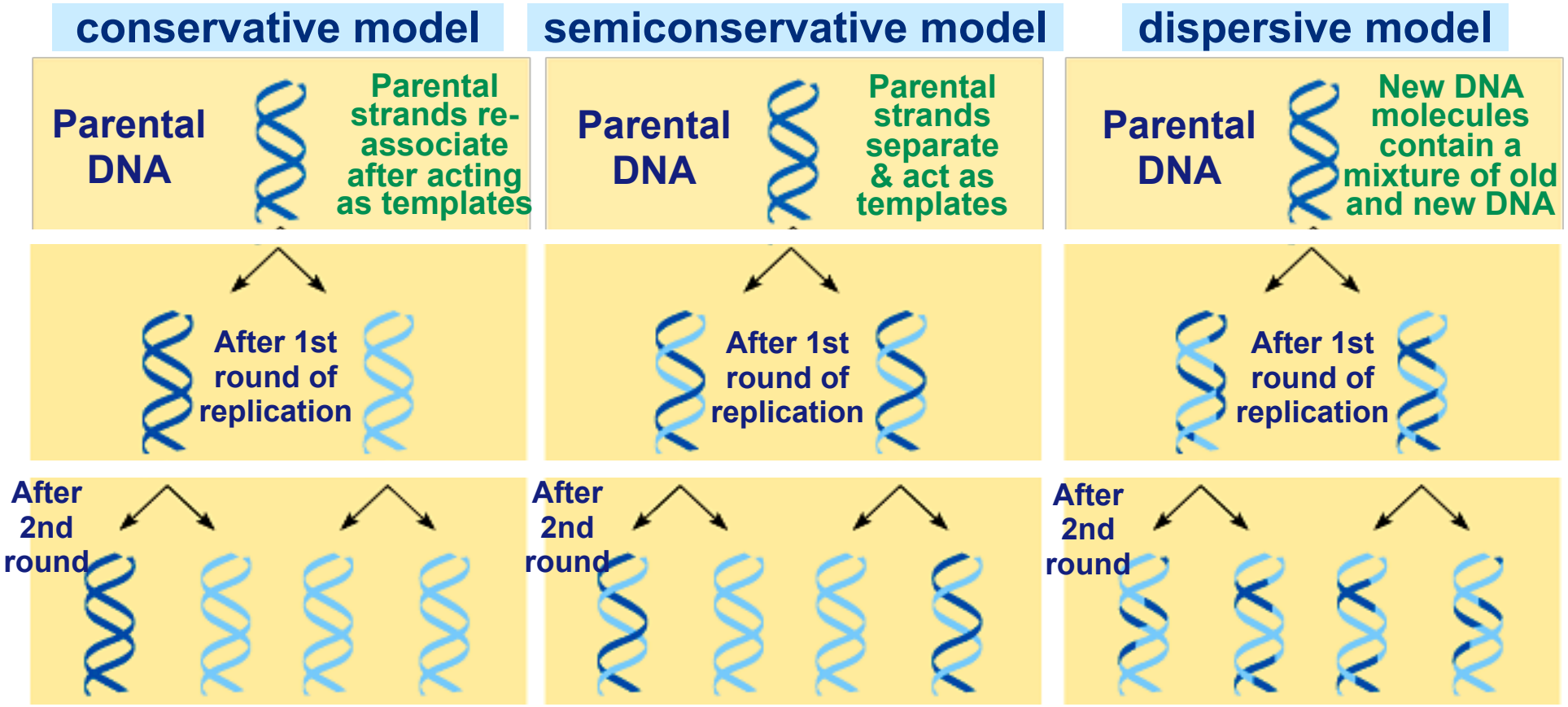


"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."
— Watson & Crick

How does DNA Replicate?

- Alternative models were proposed as to how DNA replicates
  - These become experimental predictions: if this is the replication method, then one expects to see the following happen after each round of replication *(see the three predictions below).*
    - Which one is occurs in reality?

Dark Blue = Original/Starting DNA in Parent Cell
Light Blue = Daughter/New DNA in Daughter & Granddaughter Cells

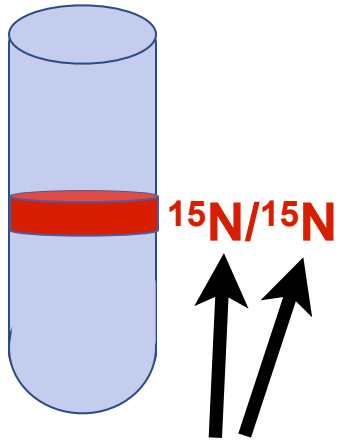


Finding Evidence for Semiconservative DNA Replication

1958

- Meselson & Stahl set out to determine which model of DNA Replication was the correct one
  - Grew bacteria in a medium containing nucleotide precursors labeled with heavy radioactive nitrogen isotopes
    - "Parent" nucleotides built by the bacteria and used in DNA strands were all labeled with heavy nitrogen = ^{15}N
      - Both strands of the circular dsDNA chromosome in the bacteria were made from nucleotides that weighed a lot because they contained in their nitrogenous bases of each nucleotide only ^{15}N isotope
      - If you took the DNA from many of these bacteria, suspended all of it in an aqueous solution, and then centrifuged the mixture, the "heavy" DNA would end up at a certain height in the test tube

"The Most Beautiful Experiment in Biology"



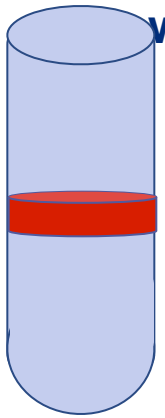
Two ^{15}N DNA strands

Finding Evidence for Semiconservative DNA Replication

1958

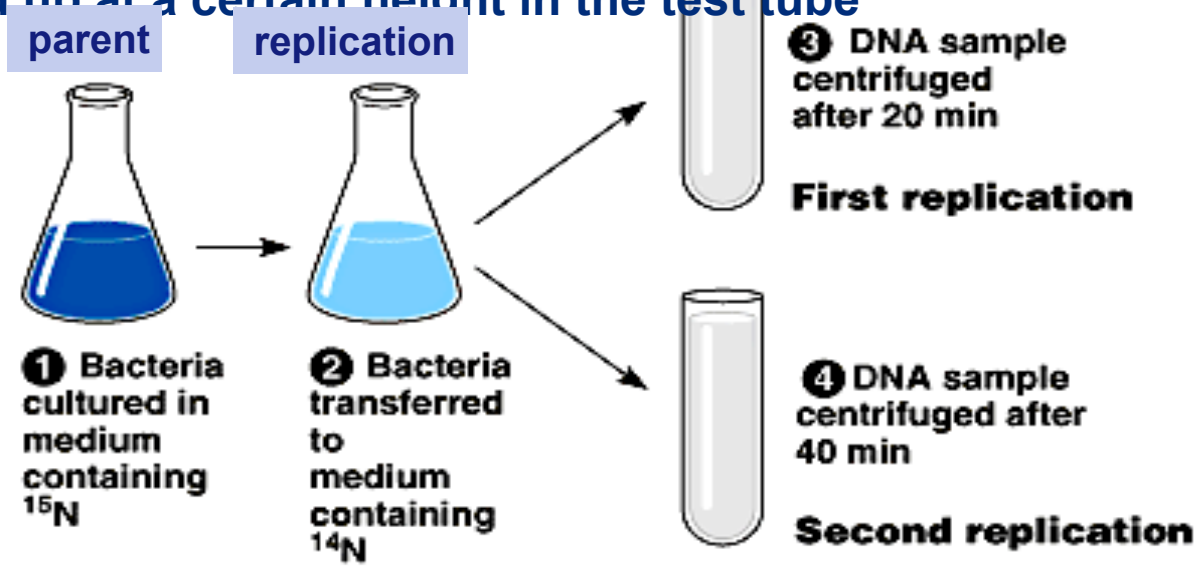
- **Meselson & Stahl**
  - **Grew bacteria in a medium containing nucleotide precursors labeled with heavy radioactive nitrogen isotopes**
    - **"parent" nucleotides built by the bacteria and used in DNA strands were all labeled with heavy nitrogen = ^{15}N**
      - **Both strands of the circular chromsome in the bacteria were made from nucleotides that weighed a lot because they contained in their nitrogenous bases of each nucleotide only ^{15}N isotope**
      - **If you took the DNA from many bacteria, suspended it an aqueous solution, and then centrifuged the mixture, the DNA would end up at a certain height in the test tube**

"The Most Beautiful Experiment in Biology"



$^{15}N/^{15}N$

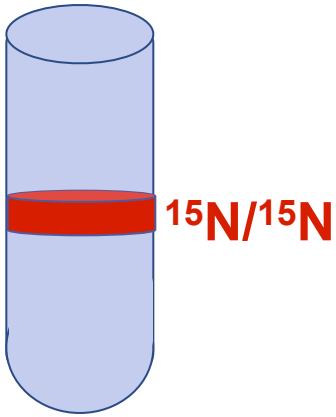
^{15}N parent strands



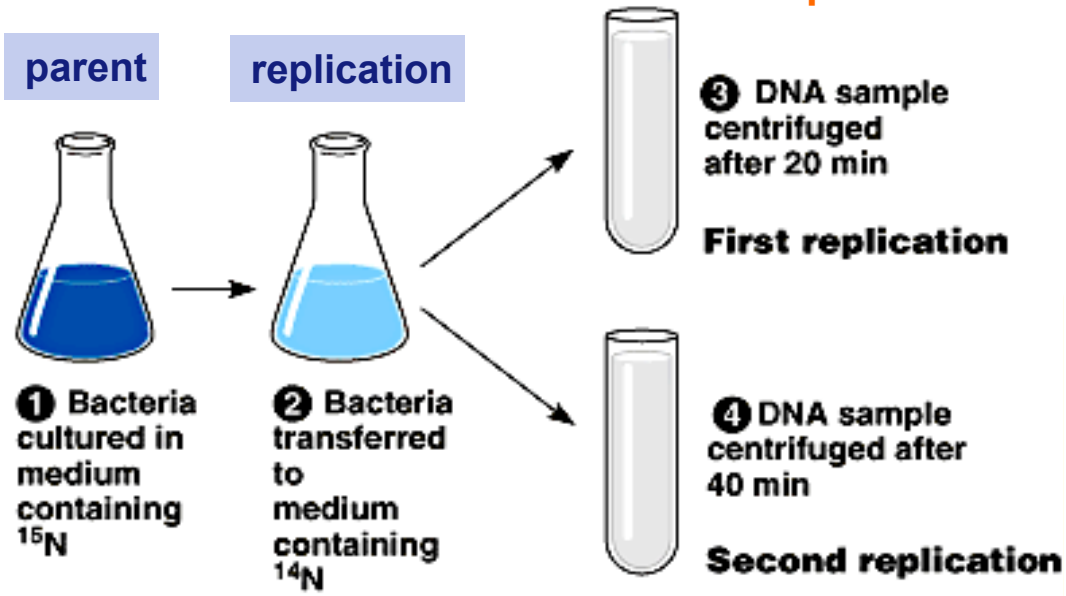
Finding Evidence for Semiconservative DNA Replication

1958

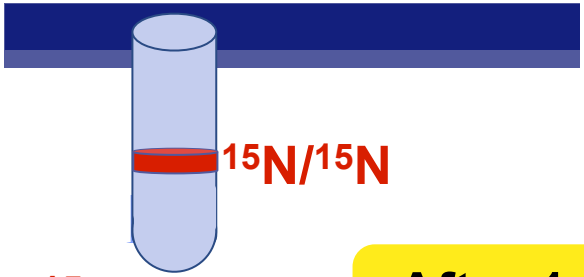
- Next, they transferred these bacteria (with heavy $^{15}N/^{15}N$ DNA) to a medium with only a lighter radioactive nitrogen isotope and waited for them to complete one, and later a second, round of binary fission.
  - Now, after every DNA replication, the nucleotides that are used to make the newly synthesized "daughter" DNA would be labeled with and made up of <u>lighter isotope</u> = ^{14}N
    - If you took the DNA from many of these second and third generation bacteria, suspended all of it in an aqueous solution, and then centrifuged the mixture, the height at which the bands of double-stranded DNA end up in the test tube (relative to the band from the original parent bacteria) will depend on composition of the two strands of DNA in the final duplicated chromosomes.



^{15}N parent strands

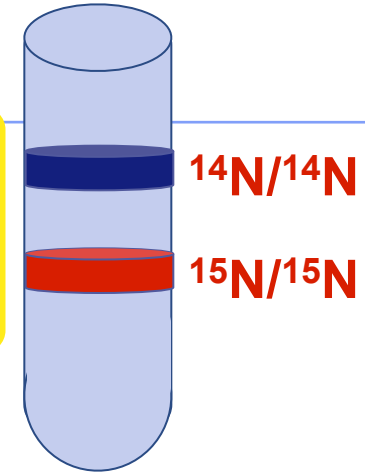


Predictions of DNA Band Location

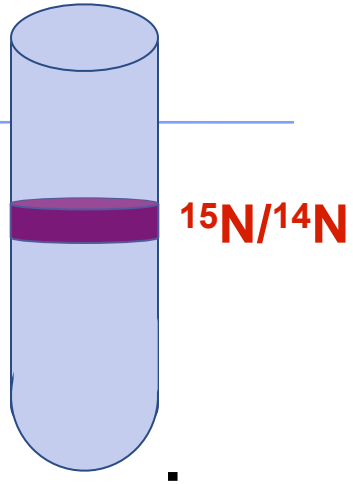


15N parent strands

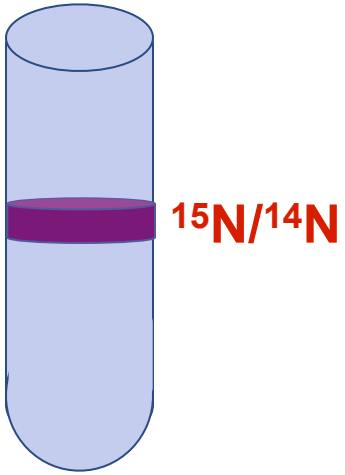
After 1st round of DNA replication



conservative



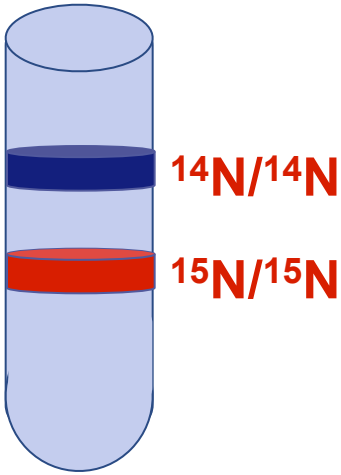
semi-conservative



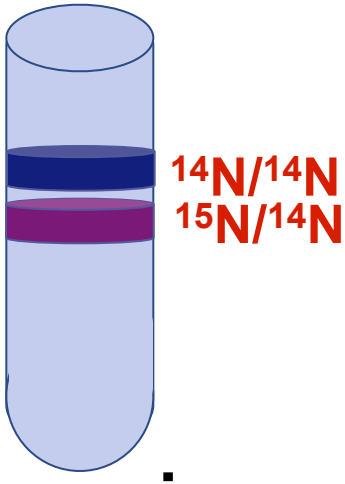
dispersive

Once they had predictions, they ran the experiment to observe where the actual bands end up!

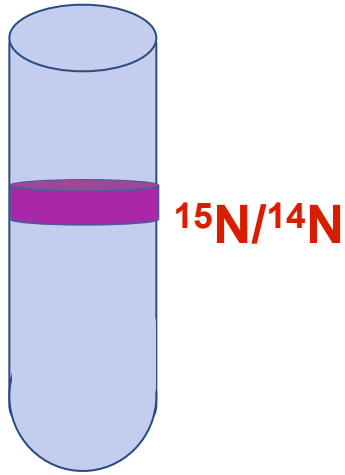
After 2nd round of DNA replication



conservative

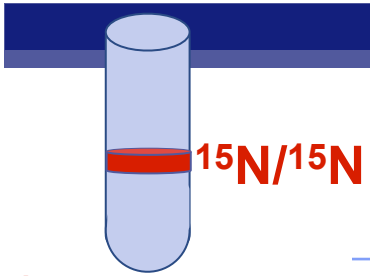


semi-conservative



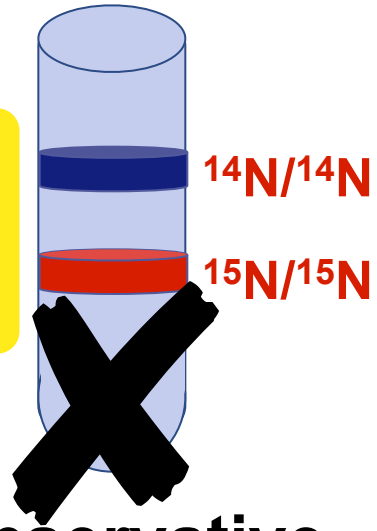
dispersive

Where was the DNA Band After the 1st Round of Cell Division?

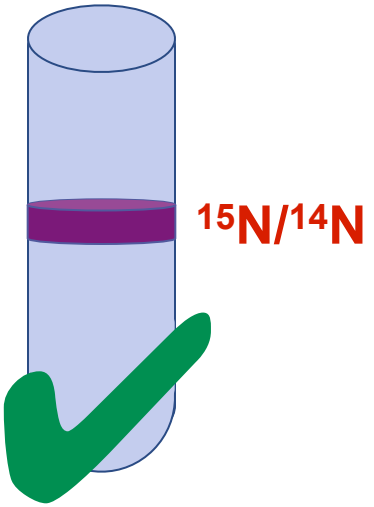


^{15}N parent strands

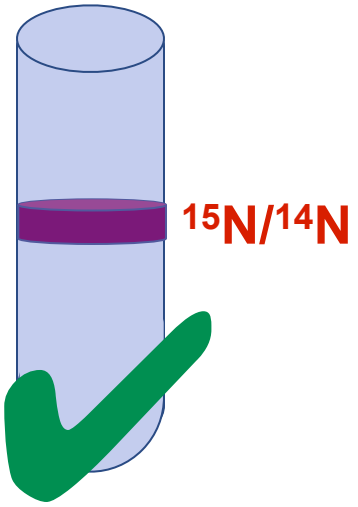
After 1st round of DNA replication



conservative

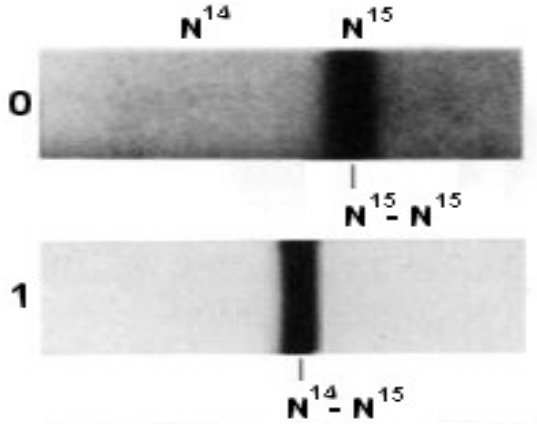


semi-conservative



dispersive

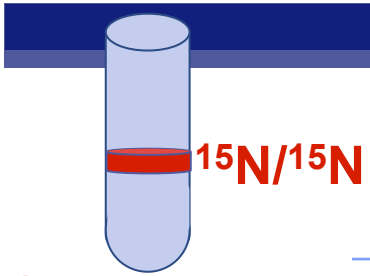
Parental DNA Band



DNA Band After One Round of Replication

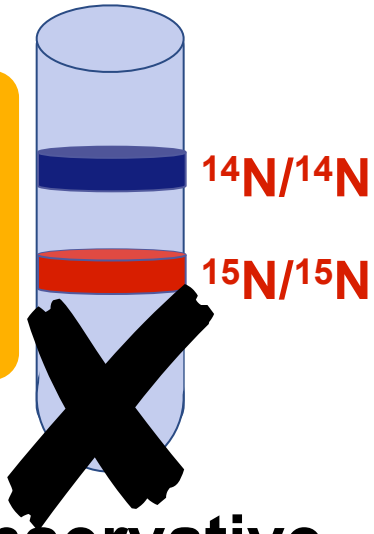
Conclusion: The Conservative Model is **NOT** accurate!

Where was the DNA Band After the 2nd Round of Cell Division?

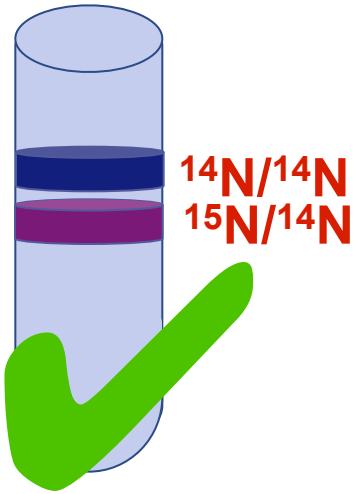


^{15}N parent strands

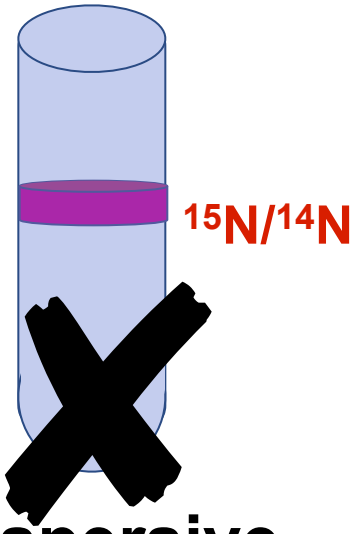
After 2nd round of DNA replication



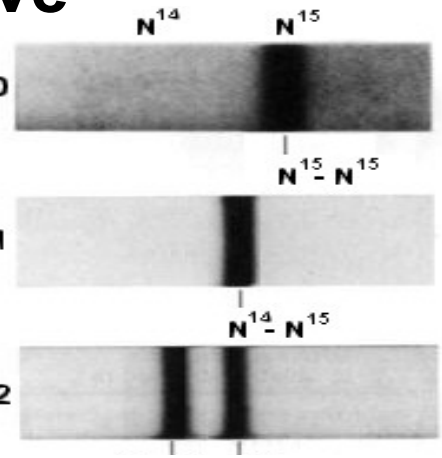
conservative



semi-conservative



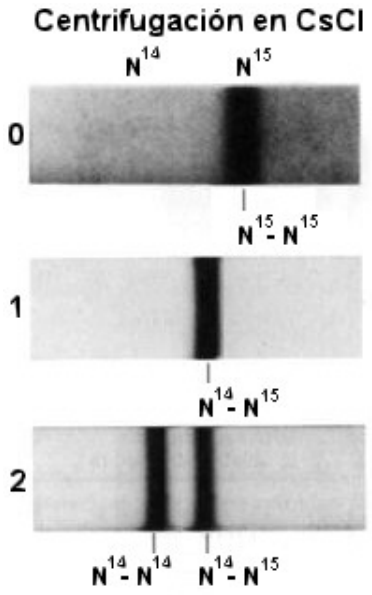
dispersive



Conclusion: The Dispersive Model is also **NOT** accurate!

Semiconservative Replication Occurs 1958

- **Make predictions…**
  - ^{15}N-containing strands replicated in ^{14}N-containing medium
  - 1st round of replication? **where should the bands be?**
  - 2nd round? **where should the bands be?**

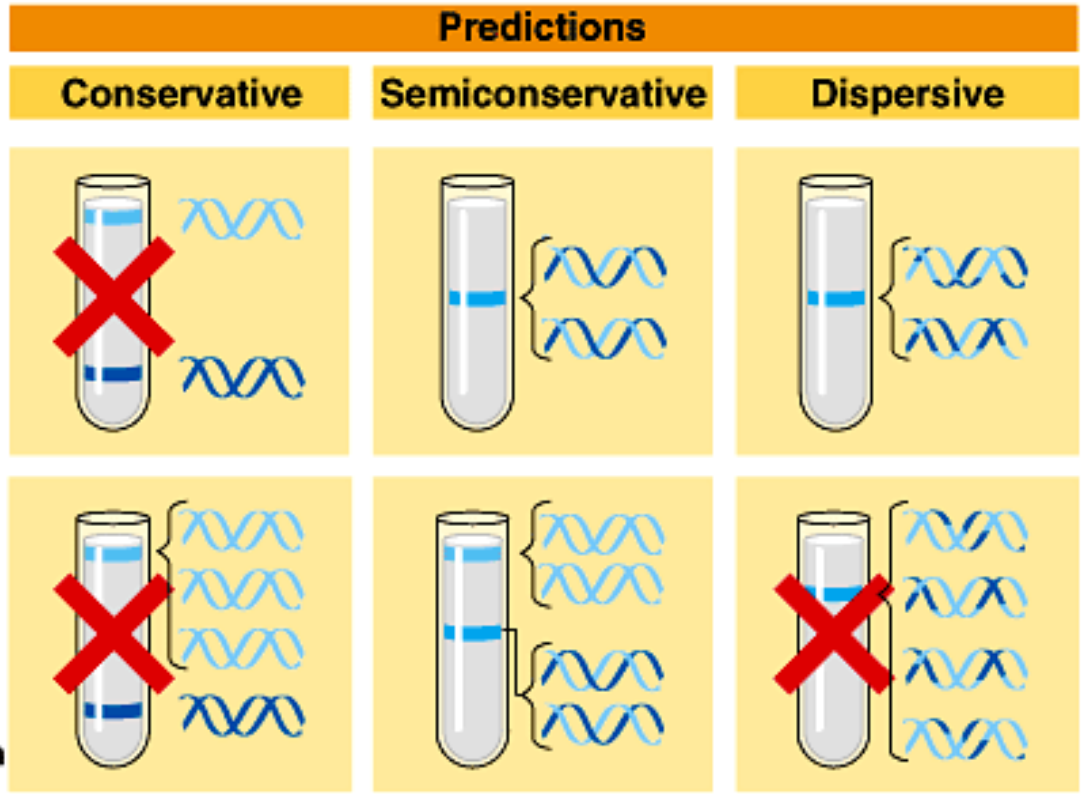


❸ DNA sample centrifuged after 20 min

First replication

❹ DNA sample centrifuged after 40 min

Second replication



Meselson & Stahl

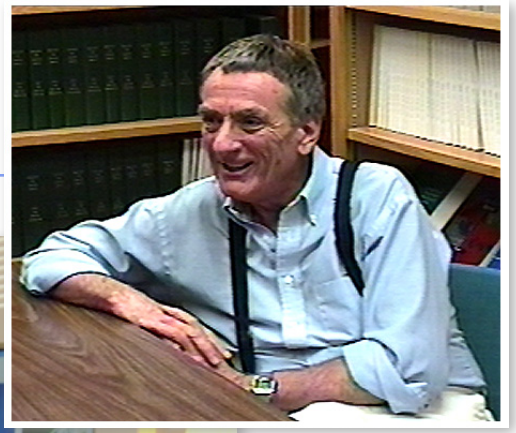
Matthew Meselson

Franklin Stahl

FIGURE 9-3. (Left) Matthew Meselso
[Courtesy of M. Meselson.]

Courtesy of Dr. M. Meselson, Harvard University.

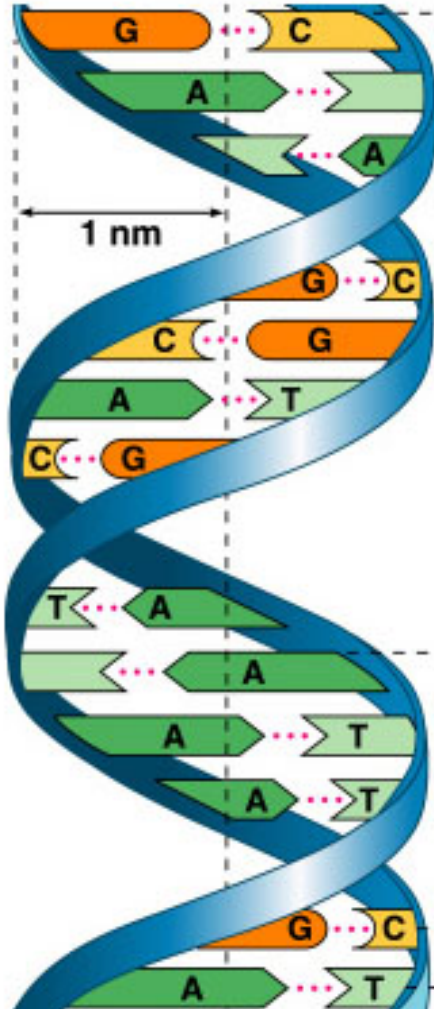
Matthew Meselson



Franklin Stahl

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"

- General Flow of genetic information in a cell: