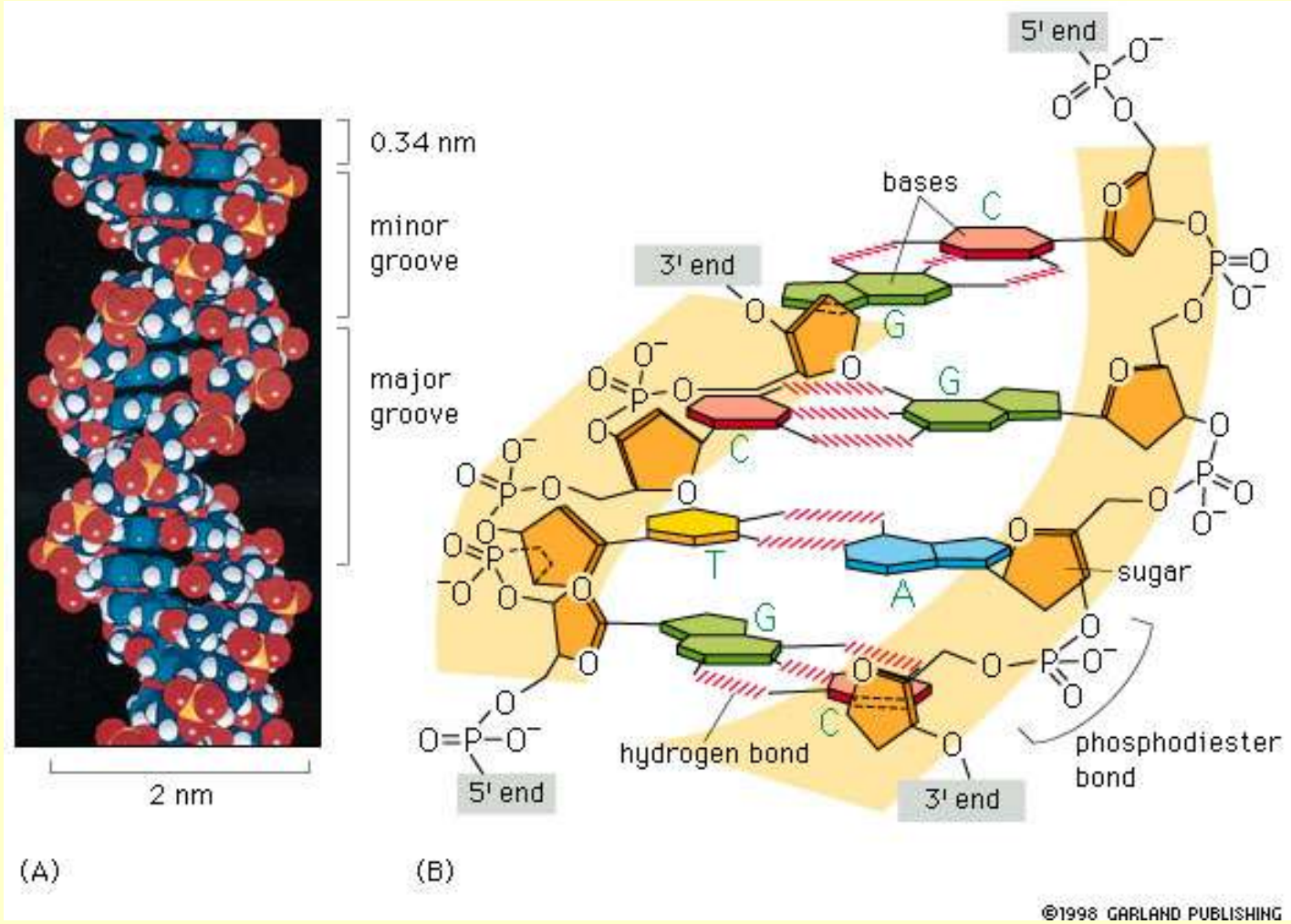
I. The Molecular Basis of Life

A. The Search for Genetic Material



1. Prior to the 1940s

- Proteins were believed to be the genetic material
  - 20 amino acids
  - More protein in chromosomes than DNA

b) Nucleic acids were believed to be too simple and too uniform

  – Only 4 nucleotide bases

B. Experiments lead to our understanding of the Structure and Function of DNA

1. Experiments of Frederick Griffith

a) Griffith was trying to find a vaccine against **Streptococcus pneumonie**, a bacterium that causes pneumonia in mammals. He knew:

- There are two strains of the bacteria: one produces smooth colonies, S, and one that produces rough colonies, R.
- Cells of the smooth strain are covered with a polysaccharide coat and the rough strains are not.
- The alternative phenotypes (S&R) are inherited

b) Griffith preformed 4 experiments

1) Griffith injected live S strain into mice.

Results:

Conclusion: The S strain is pathogenic

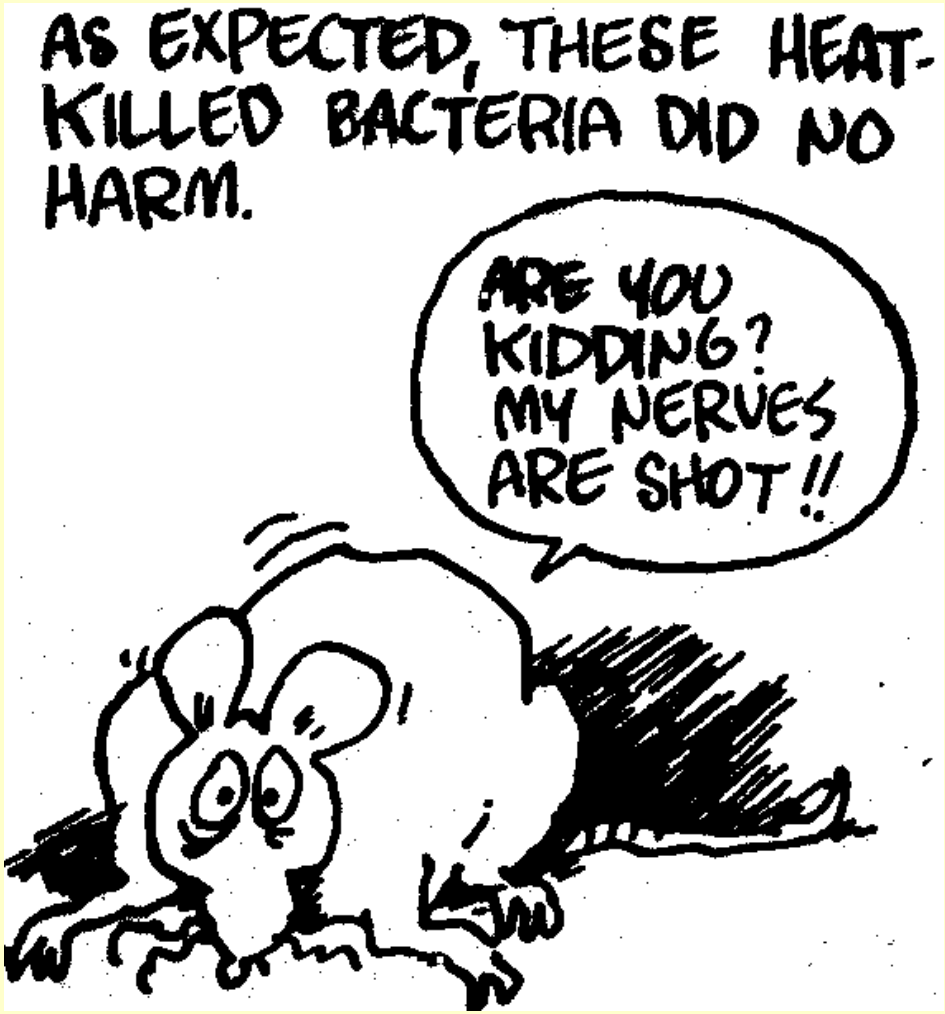
2) Griffith injected mice with live R strain.

Results:

Conclusions: R strain is non pathogenic

3) Mice were injected with heat killed S strain

Results:

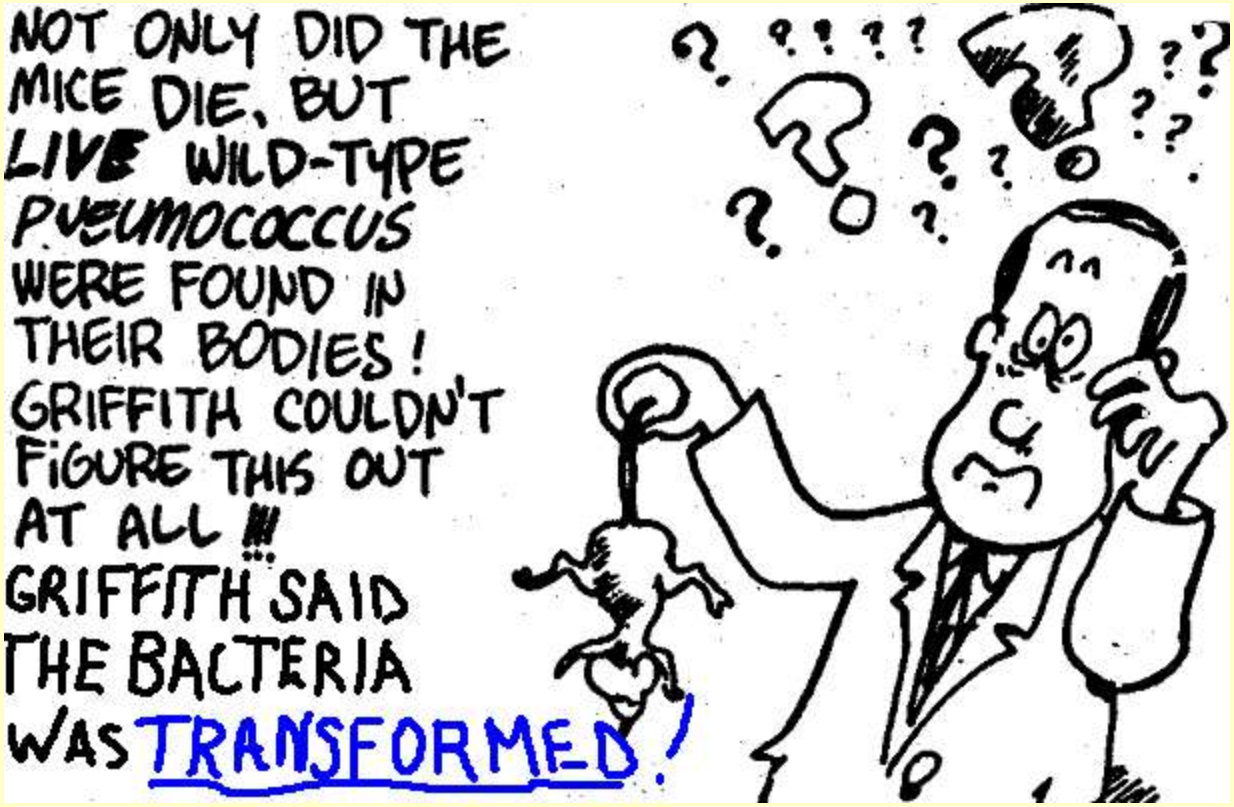


Conclusions: Heat killed bacteria are non pathogenic

4) Heat killed S and live R were injected into mice

Results:

Conclusion: The R strain acquired instructions to make the polysaccharide coat from the dead S strain



c) We now call the assimilation of external genetic material by another cell **Transformation**

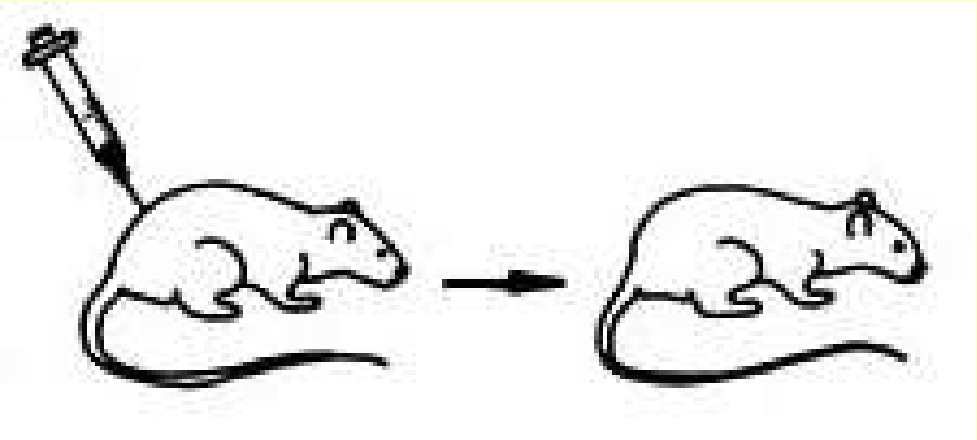
2. The Experiments of Oswald Avery

Avery was trying to determine what was causing the transformation. Keep in mind that he could separate the cell wall from the cytoplasm with a centrifuge but he could not separate the material in the cytoplasm.

3. Avery's 5 Experiments:

a) Heat killed S coat plus R bacteria injected into mice.

Results

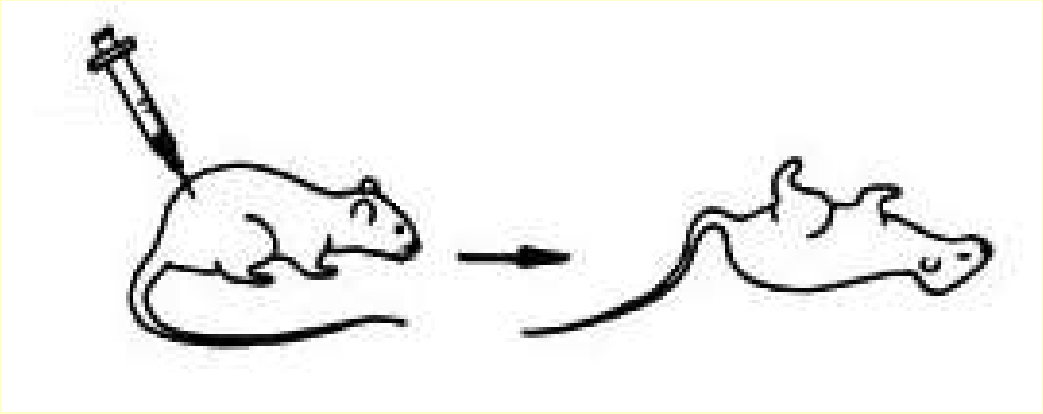


Conclusion: the coat is not the genetic material

Experiment #2

b) Heat killed S cytoplasm plus R bacteria injected

Results

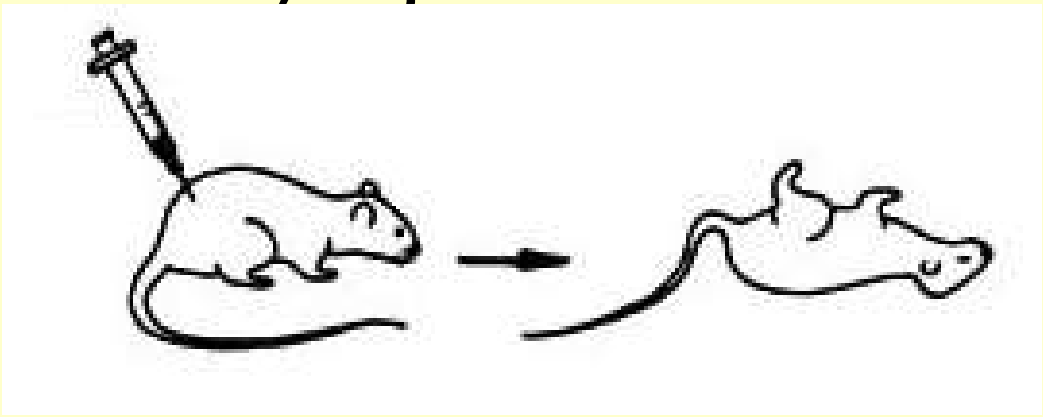


Conclusion: genetic material is in the cytoplasm

Experiment #3

c) S cytoplasm plus pepsin (breaks down proteins) injected with R strain into mic

Results:

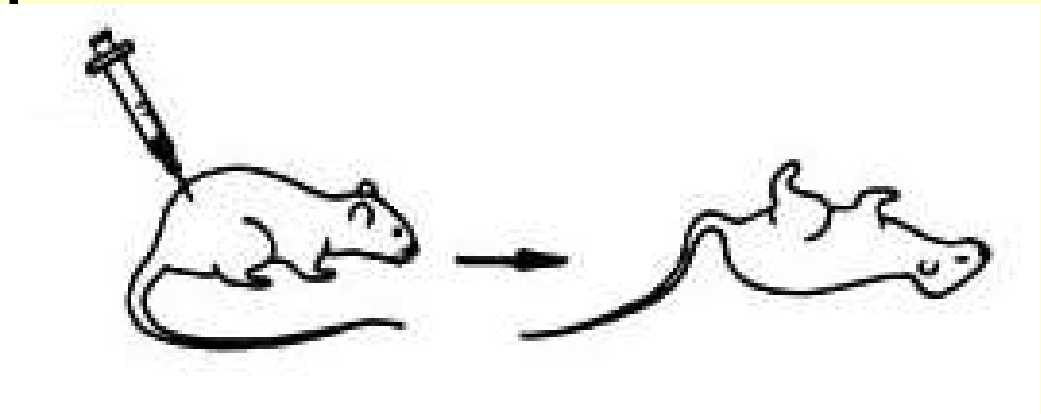


Conclusion: protein is NOT the genetic material

Experiment #4

d) Heat killed S cytoplasm plus R strain plus RNAase (breaks down RNA) injected into mice

Results

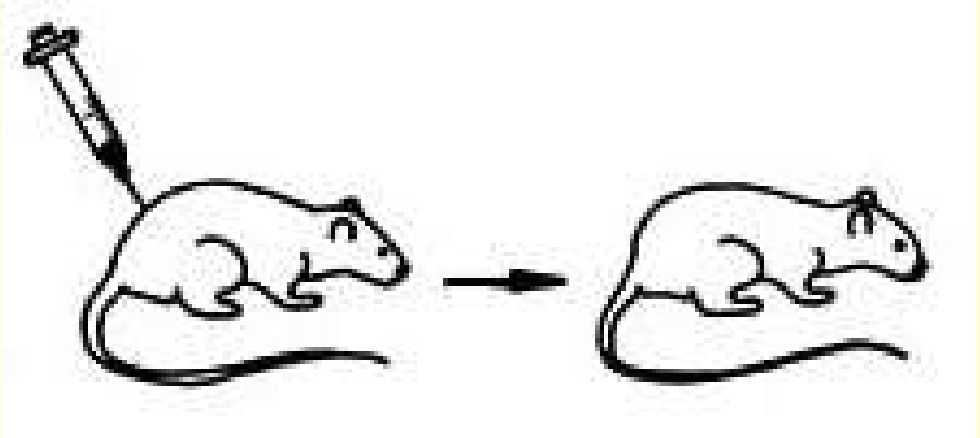


Conclusion: RNA is not the genetic material

Experiment #5

e) Heat killed S cytoplasm plus R strain bacteria plus DNAase is injected into mice.

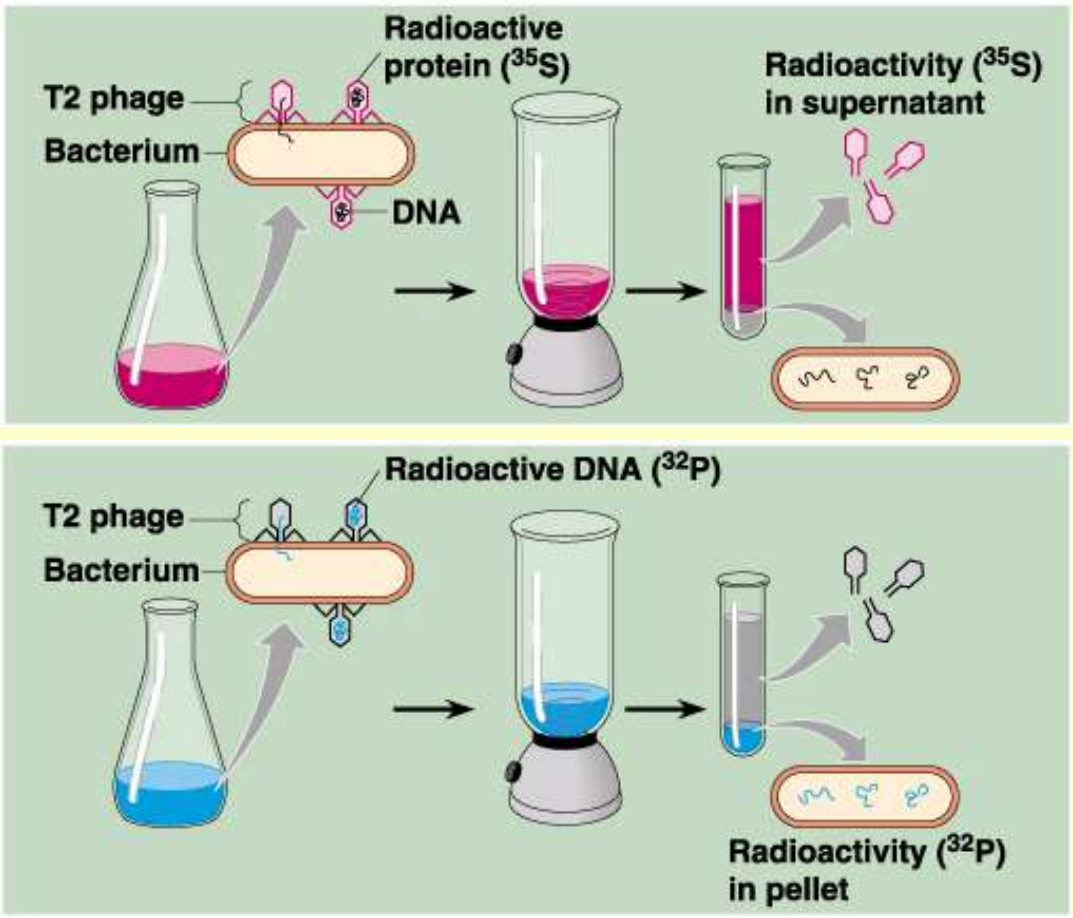
Results:



Conclusion: DNA is the genetic material

4. Alfred Hershey and Martha Chase confirm Avery's work

Hershey and Chase knew that viruses only contain DNA and protein. They conducted the following experiment to determine which compound entered the cell and was therefore the genetic material



Mix radioactively labeled phage with bacteria. The phage infects the bacterial cells.

Agitate in a blender to separate phage outside the bacteria from the cells and their contents.

Centrifuge and measure the radioactivity in the pellet and supernatant.

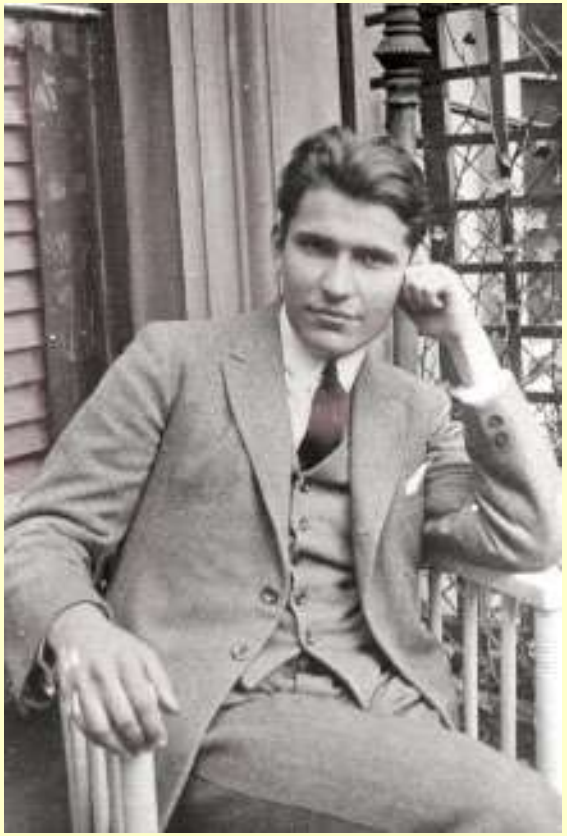
Results

- In cultures with tagged protein, the radioactivity was with the E. coli **cell walls**
- In cultures with tagged DNA, the radioactivity showed up in the **cytoplasm** of the E. coli

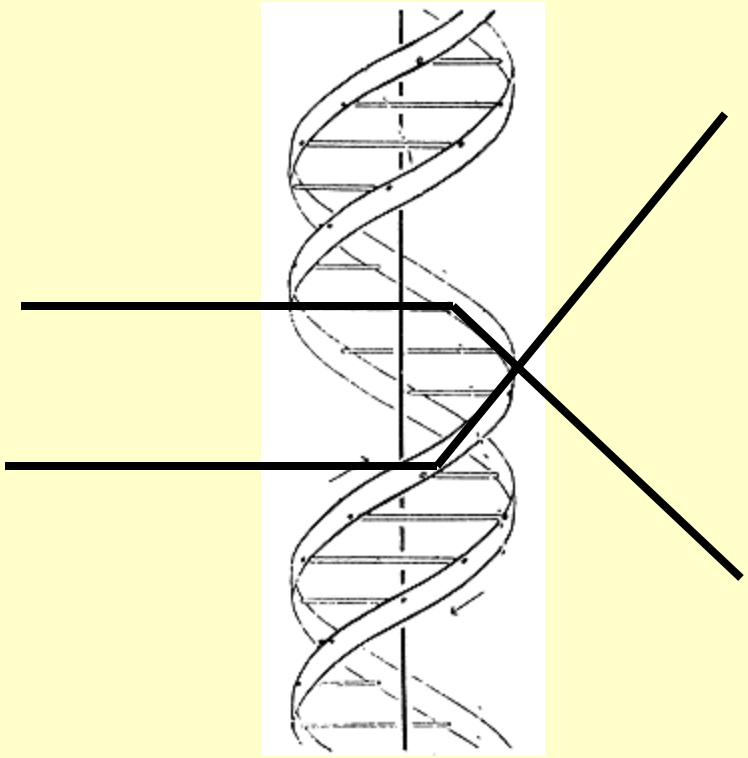
Conclusion:

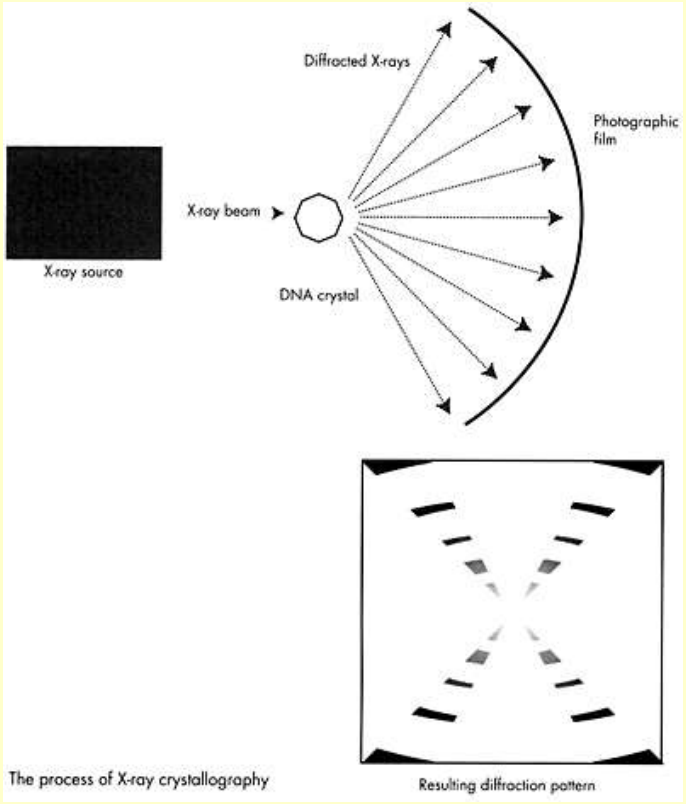
- **DNA** must be the genetic material

5. Additional Evidence is Supplied by Rosalind Franklin and Erwin Chargaff

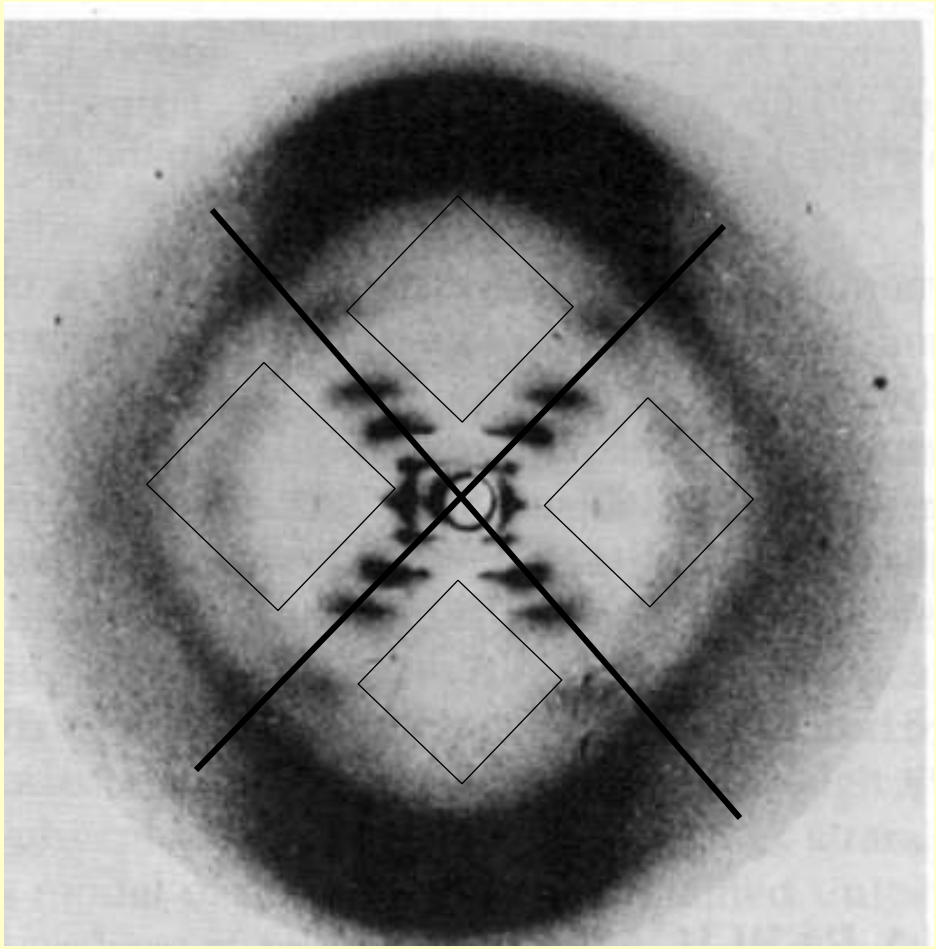


a) Franklin worked with **X-Ray Diffraction**





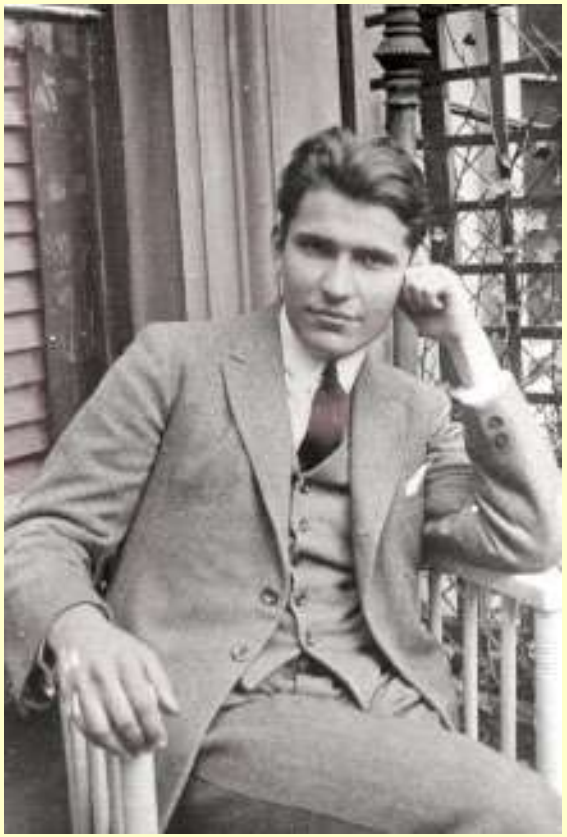
b) Photograph 51 reveled that:



1) The X indicates that the structure is a **helix**
2) The four diamond boxes show that a repeating series (sugar/phosphate backbone) lies to the **outside** of the molecule with the bases on the **inside**

.

Erwin Chargaff

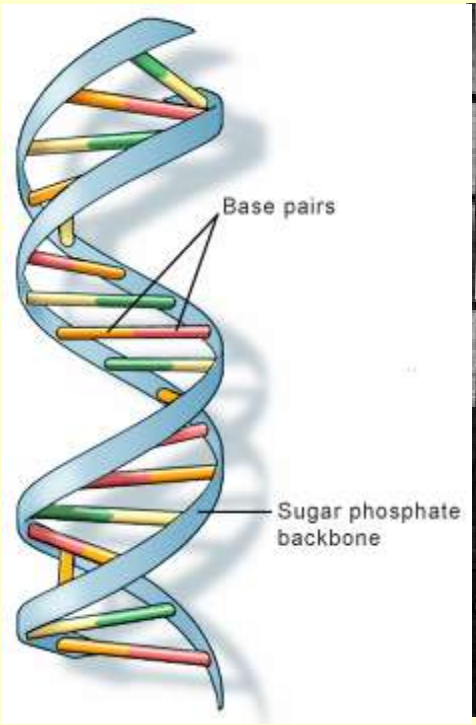


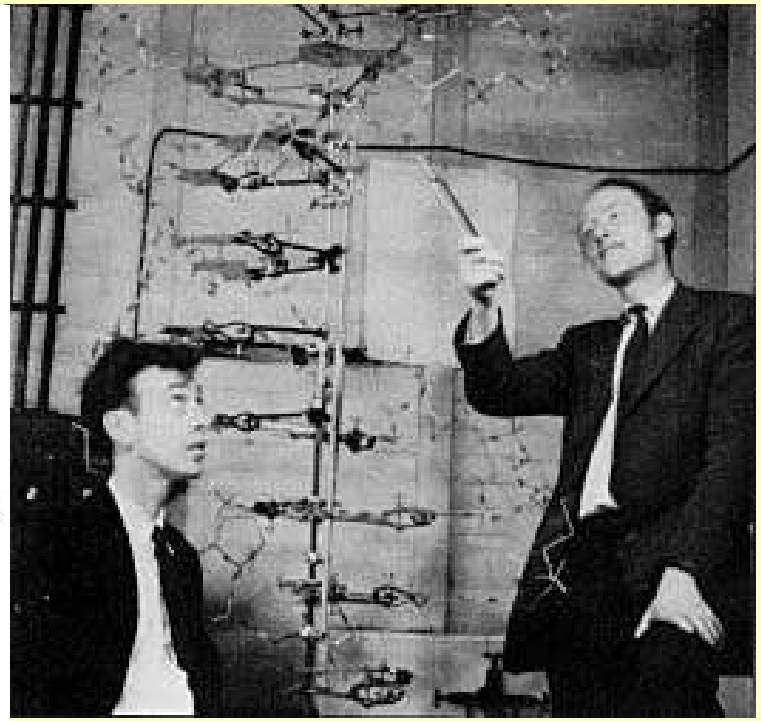
c) Determined that the amounts of **Adenine** & **thymine** are always the same and the amounts of **guanine** and **cytosine** are equal also

II. **James Watson** and **Francis Crick** determined the Structure of DNA by analyzing the work of other scientists

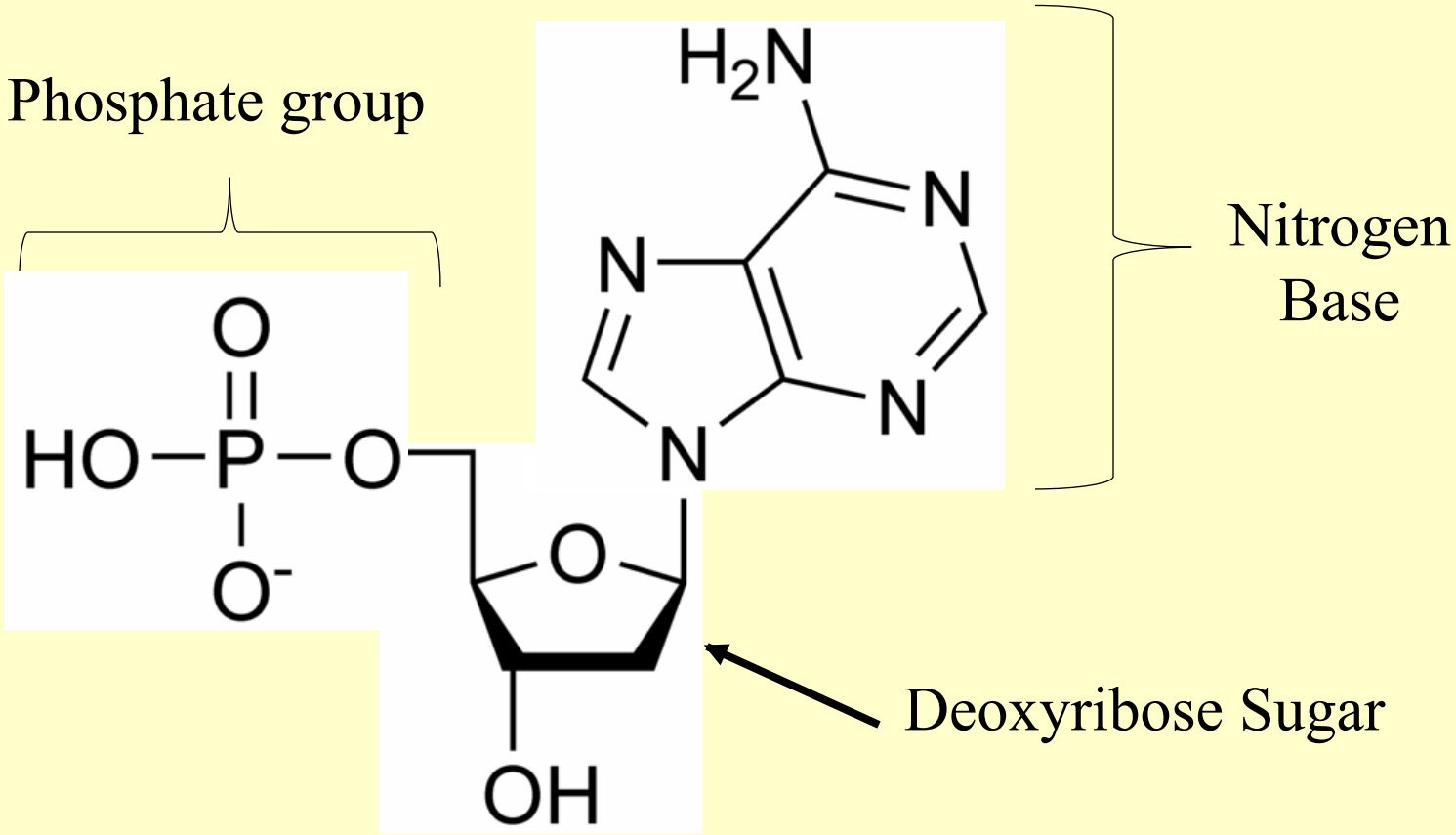
A. The Structure of DNA (Deoxyribonucleic Acid)

- DNA is double stranded and in the shape of a **double helix**



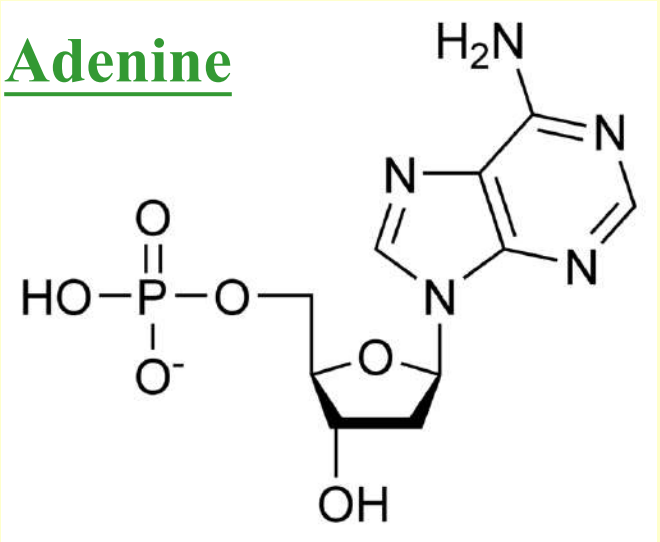


2. The monomers of DNA are called **nucleotides**: made of a **sugar** (deoxyribose), a **phosphate group** and a **nitrogen base**



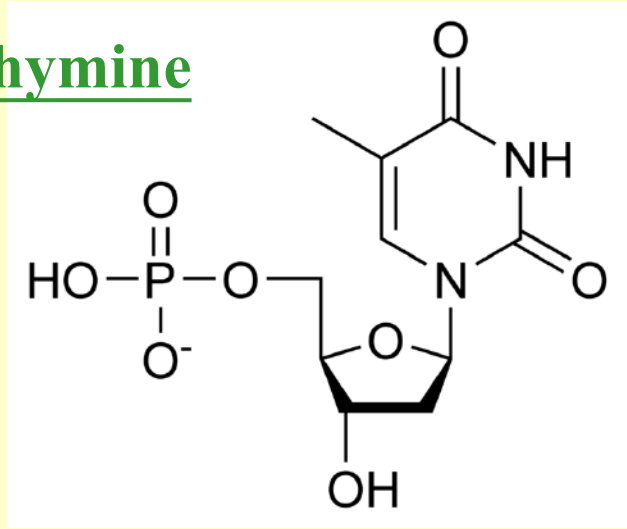
3. There are four types of DNA bases and they pair specifically:

Adenine

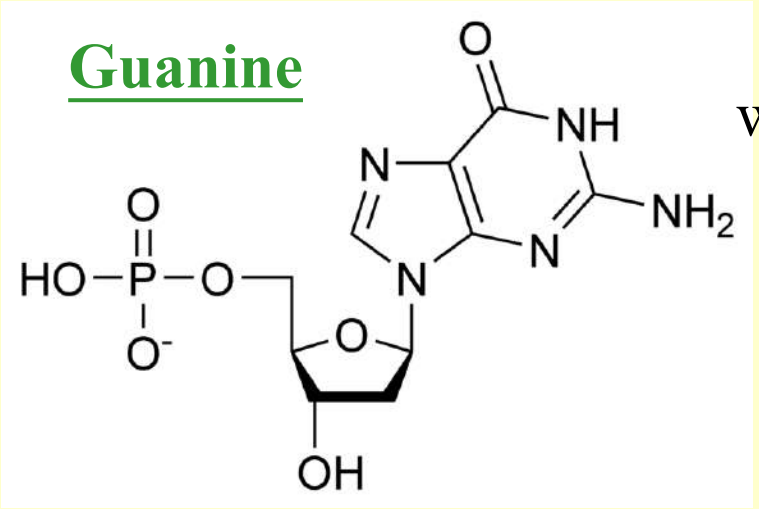


with

Thymine

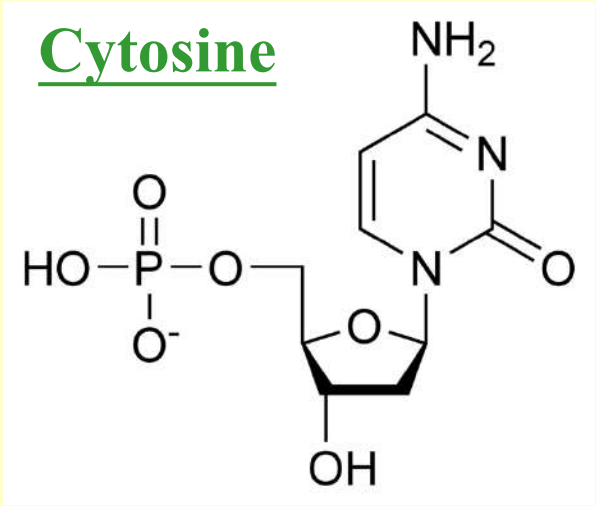


Guanine



with

Cytosine



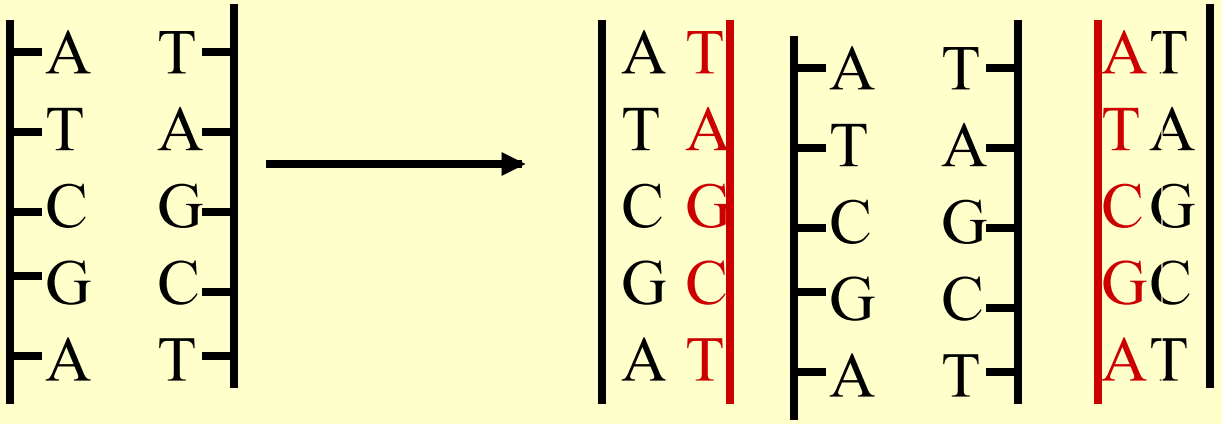
4. If the sequence of one strand is known, the other strand is known

B. DNA Replication: Watson & Crick hypothesize a semi-conservative model

1. The structure of DNA explains how it replicates

   a) DNA "unzips" down the middle

   b) **DNA polymerase** adds nucleotide to both sides of the DNA molecule producing an exact copy



Original

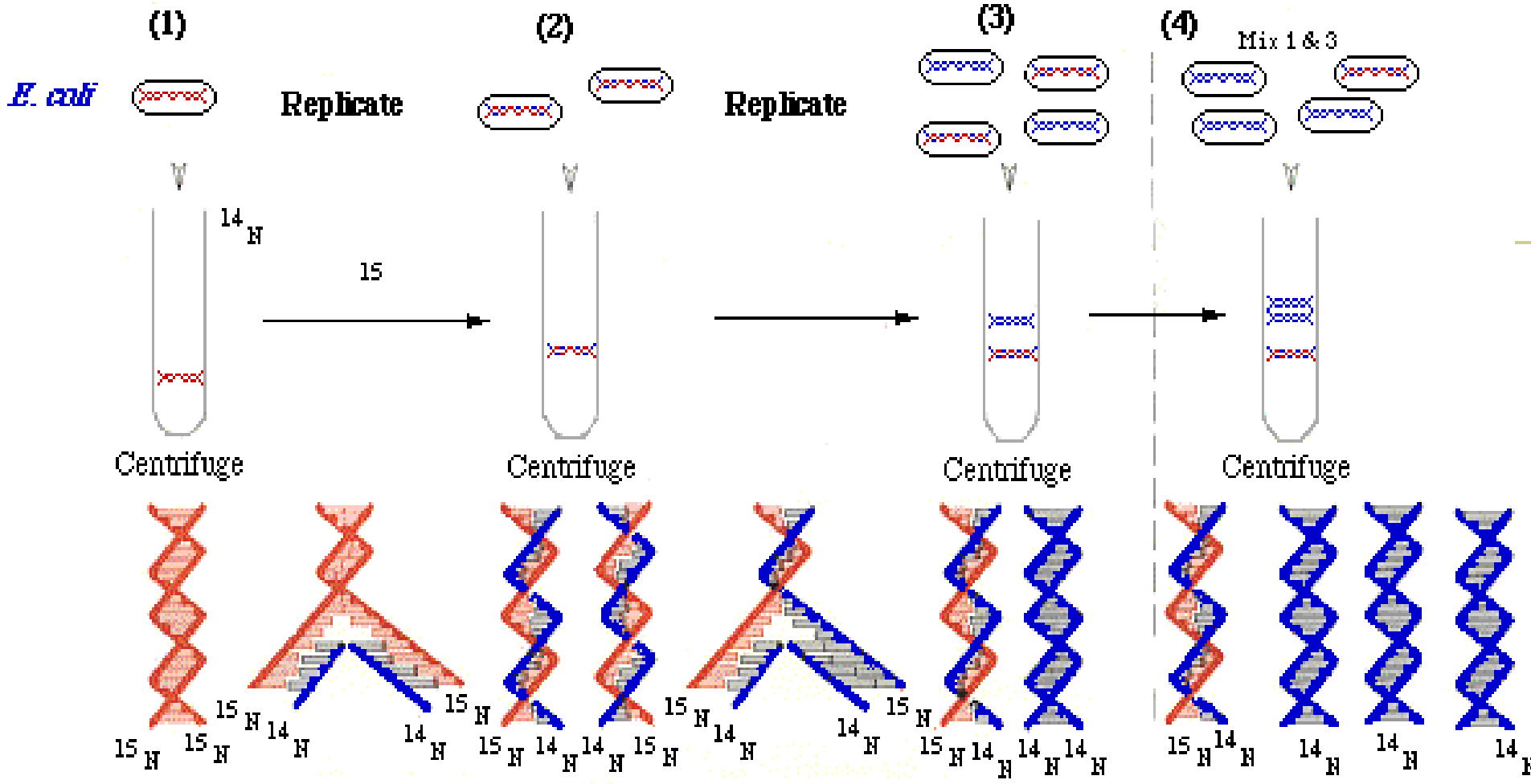
Exactly Copied DNA Strands

C. Matthew Meselson and Frank Stahl Confirm Semi-conservative Replication of DNA

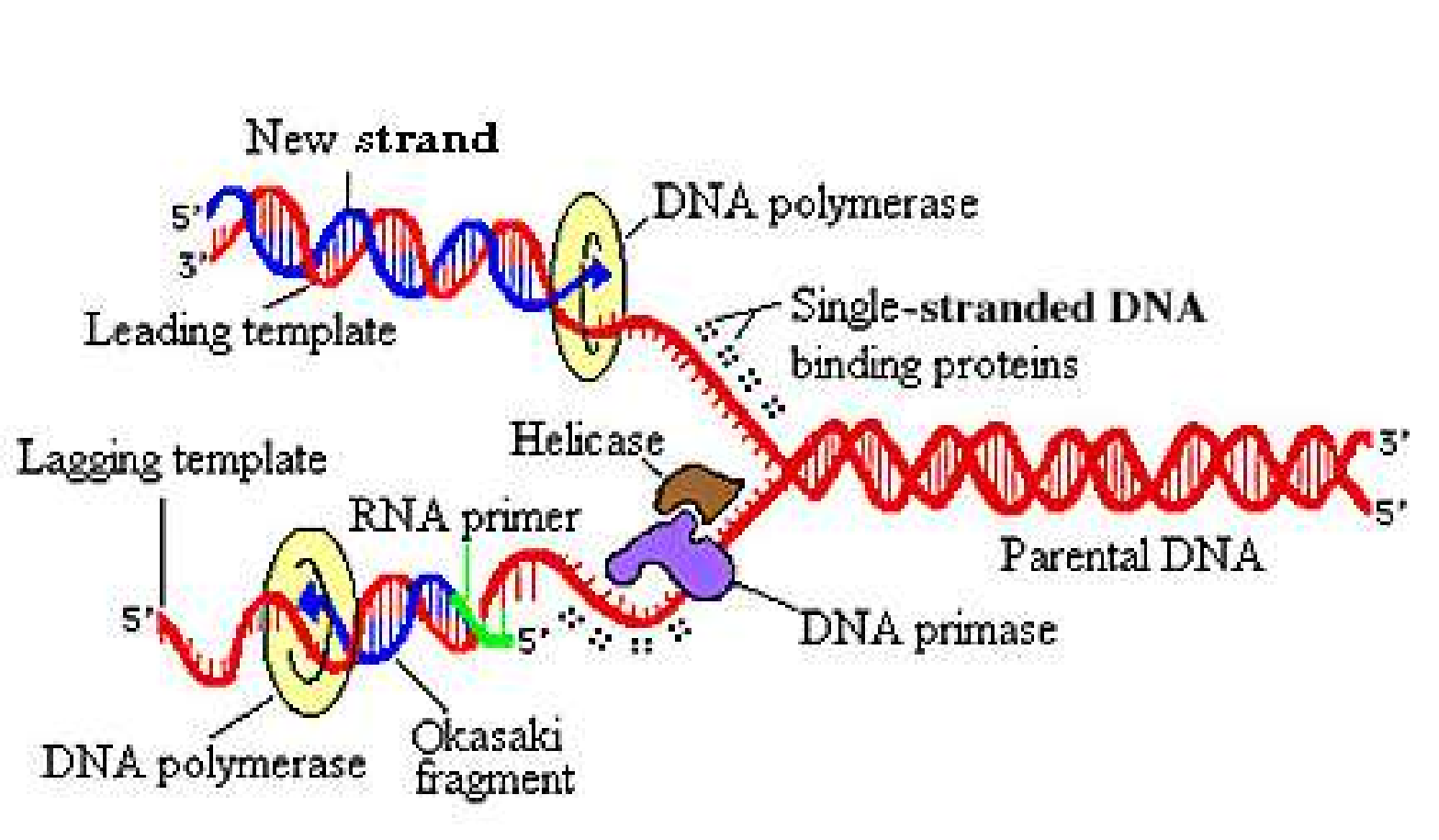
Courtesy of Dr. S. Chan, DNA Learning Center.
Noncommercial, educational use only.

Courtesy of Dr. J. Kruper, DNA Learning Center.
Noncommercial, educational use only.

Confirmation of Semi Conservative Replication of DNA



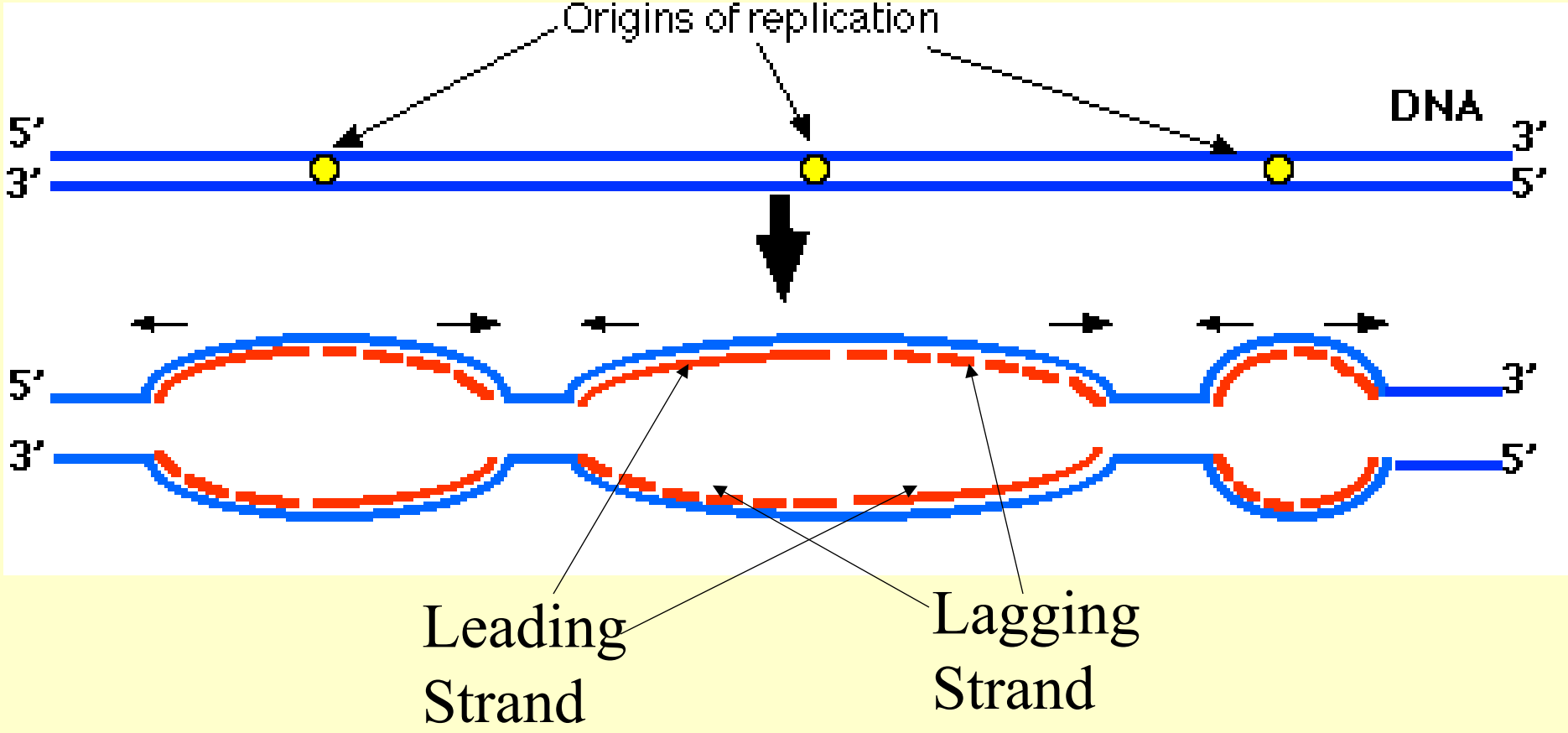
III. A Closer Look at Replication



A. Starting Replication

- **Origins of replication** -special sites that occur at many places along a DNA molecule
- DNA opens up in both directions forming a **replication bubble**.
- Two **replication forks** (where new strands are growing) form the replication bubble

B. Growing Replication Bubbles



C. DNA Replication: Reality on the leading strand

- **Helicases** -unwind the parental double helix.
- **Single Stranded binding proteins** -hold open the single strands.
- **Primase** -adds an RNA nucleotide primer to which DNA polymerase can attach
- **DNA Polymerase III** attaches to RNA primer and adds bases in **5'→3'** fashion

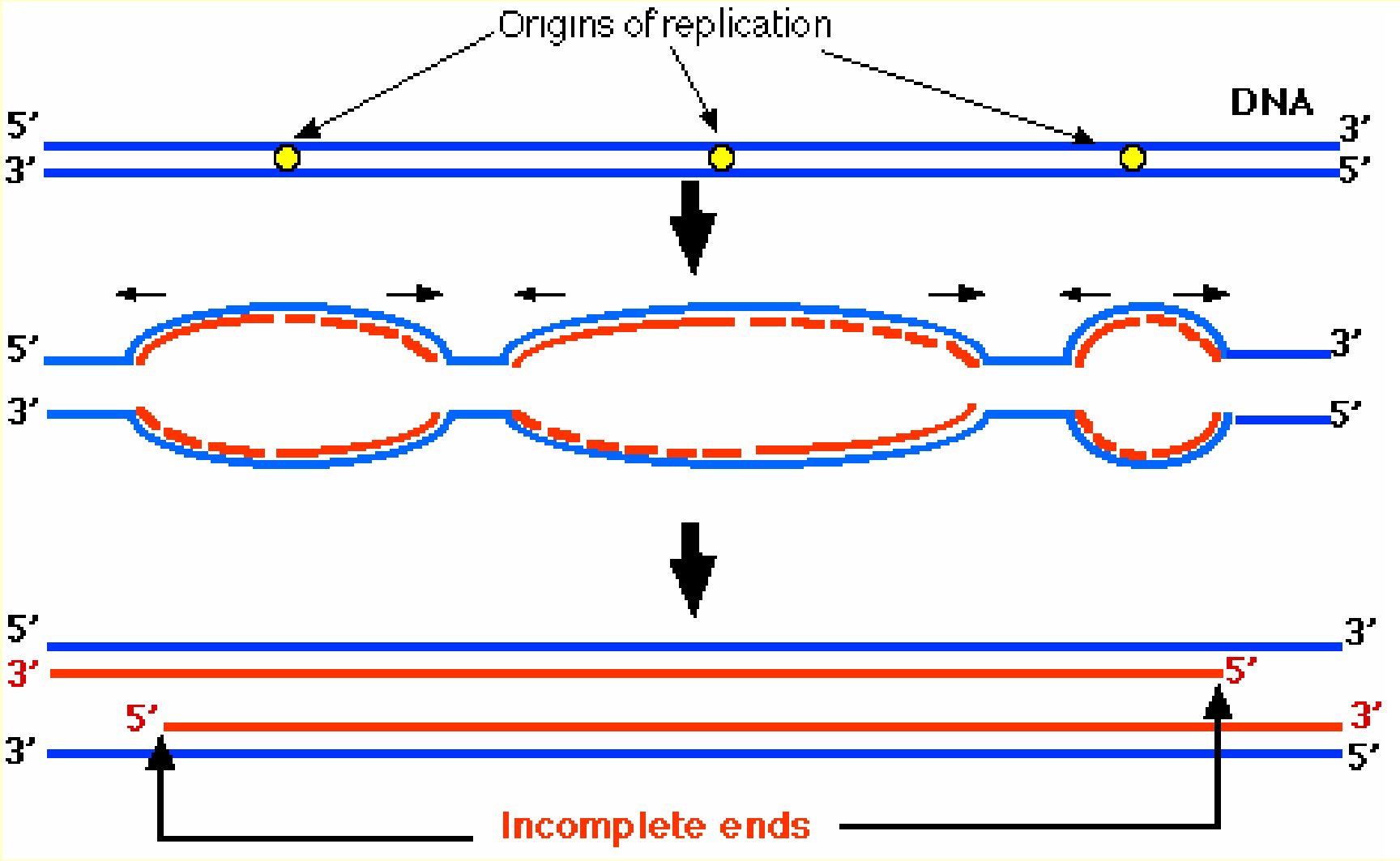
D. DNA Replication: Reality on the lagging strand

- **Primase** -adds an RNA nucleotide primer to which DNA polymerase can attach
- **DNA Polymerase III** attaches to RNA primer and adds bases in 5'→3' fashion
- As helicase unzips more DNA, primase adds another primer to which polymerase III adds bases in 5'→3' fashion until it encounters the previous primer.
- **DNA Polymerase I** removes RNA nucleotides and replaces them with DNA
- **DNA Ligase** joins DNA molecule formed by polymerase I and III

E. DNA Proofreading

- **Excision Repairs** occur when there are miss-matched bases.
- **Older DNA** strand is identified by the accumulation of ions.
- New strand is cut and polymerase adds the correct base.
- There are over 130 types of repair enzymes
- Initial pairing errors occur in about 1 in 10,000 bases, while only **1 in a billion** are not corrected

F. Problem in Replicating linear DNA



Problem in Replicating linear DNA

- DNA polymerase can only add nucleotides in a 5'→3'fashon to an existing nucleotide
- Therefore, small portions of the 5' end cannot be complete.
- This would result in the shortening of chromosomes every generation and the inevitable loss of important genes.
- The enzyme **telomerase** prevents this. Telomerase adds a non-coding sequence to the ends of chromosomes called a **telomere**