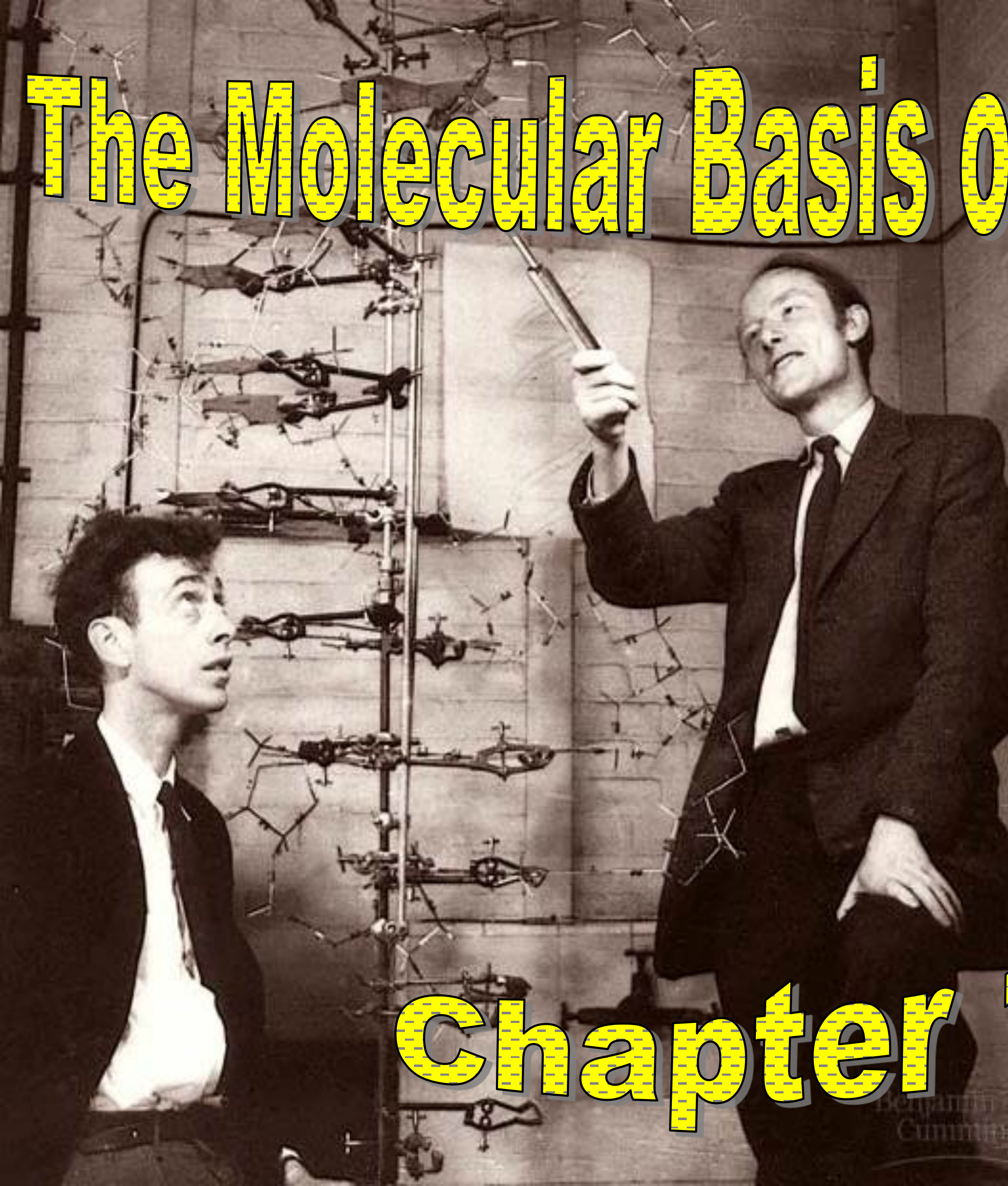
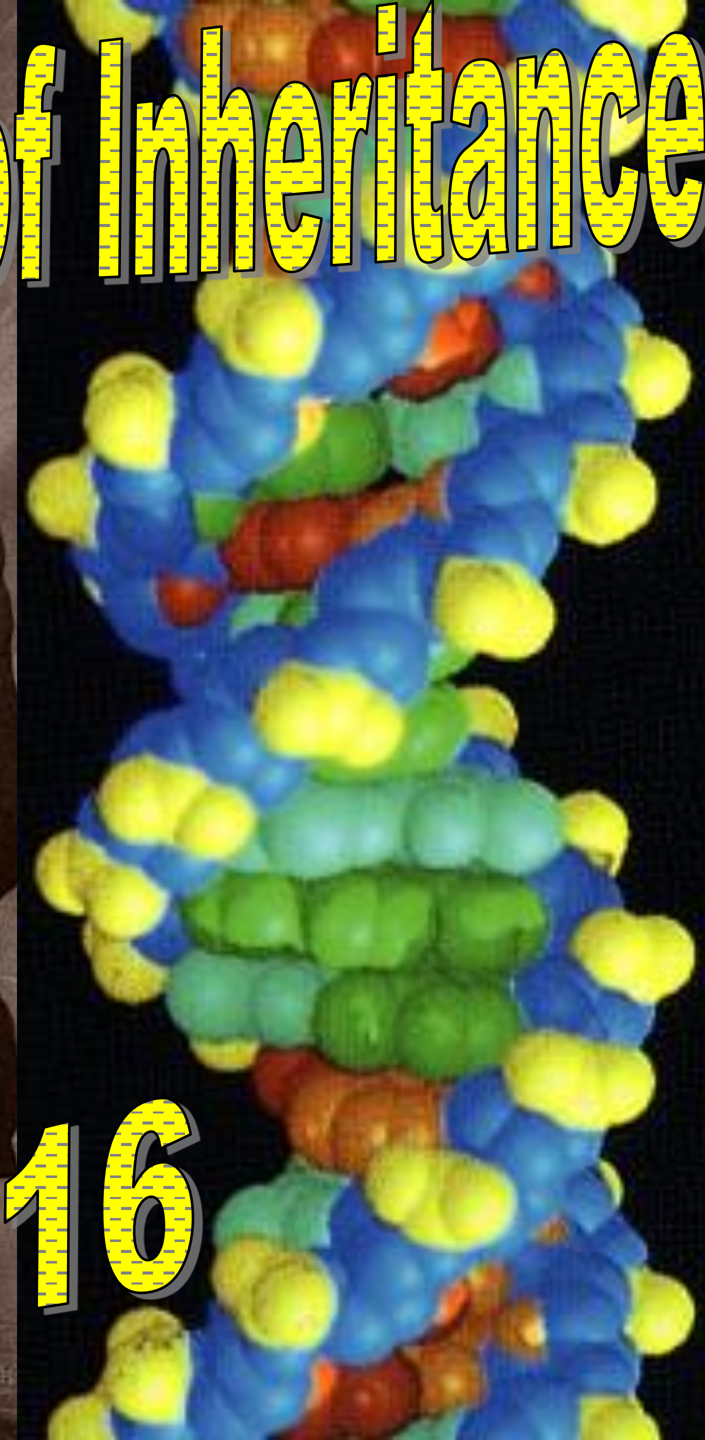
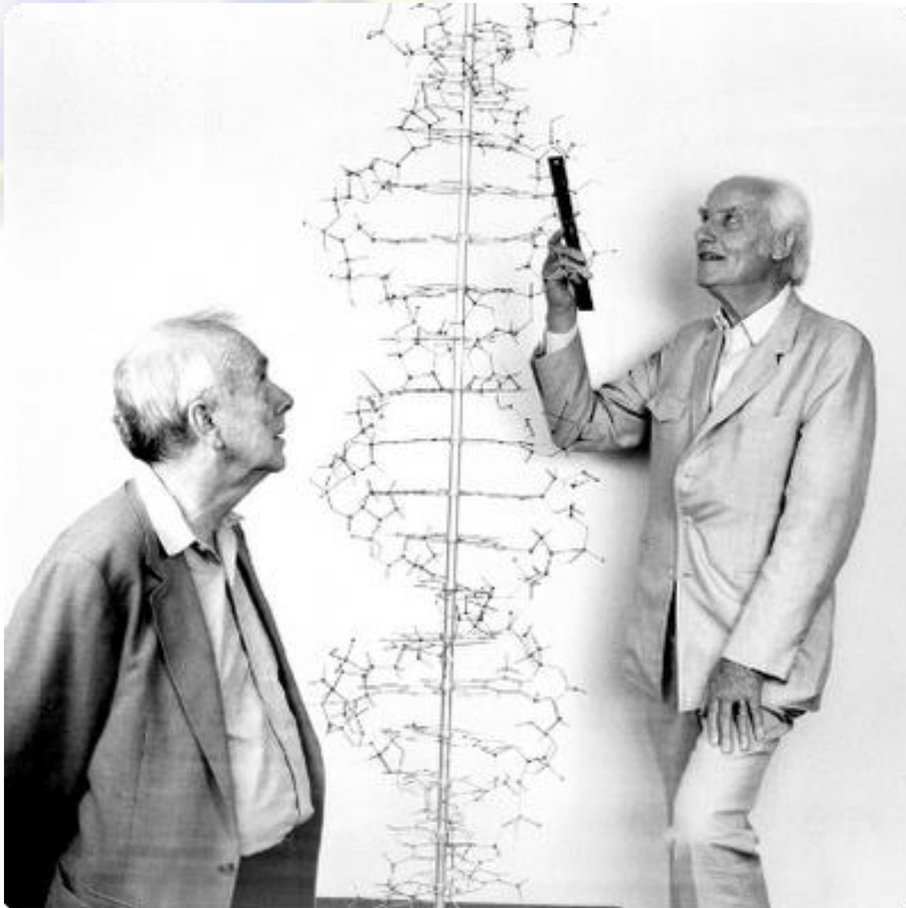
The Molecular Basis of Inheritance





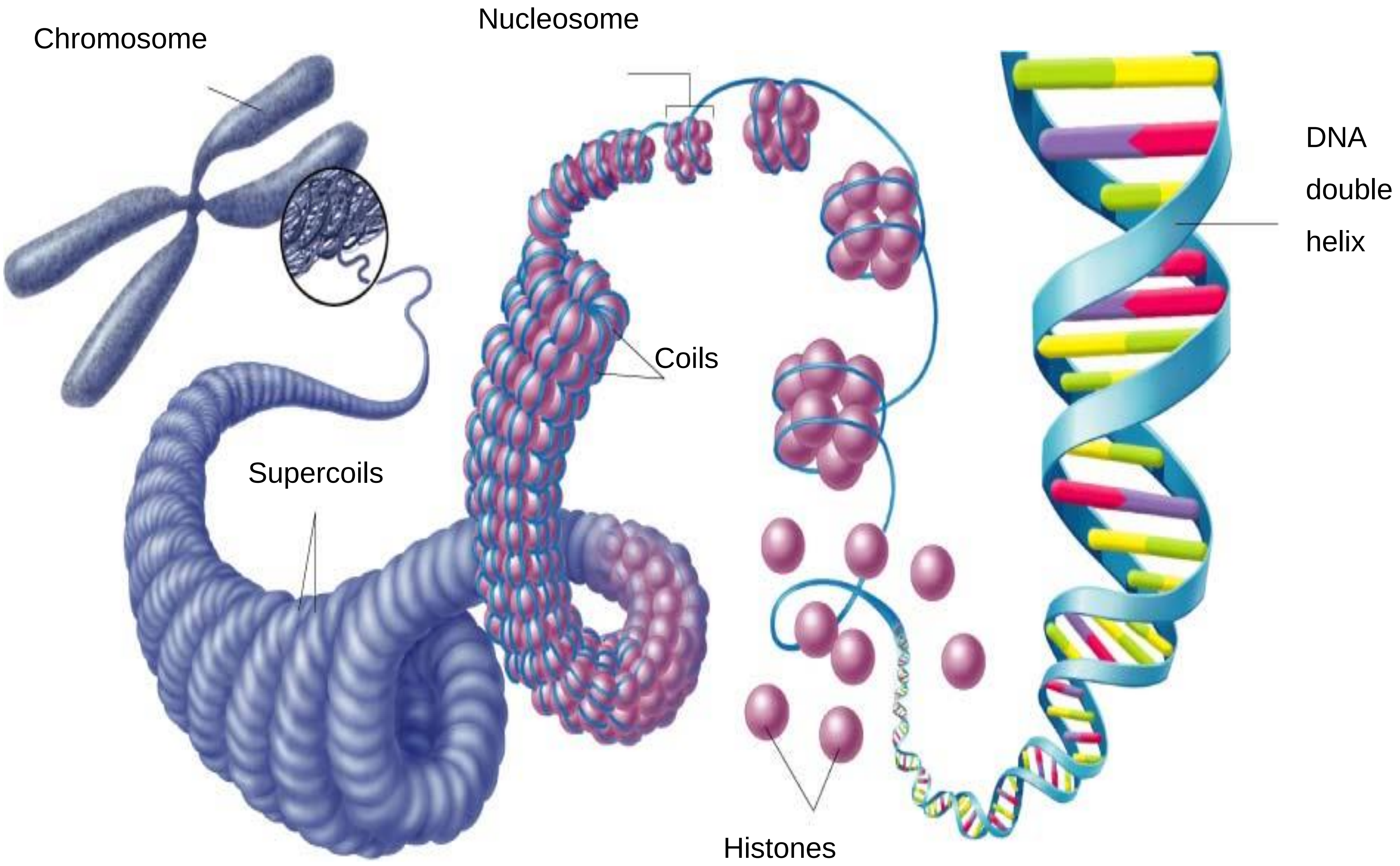
Chapter 16



James D. Watson

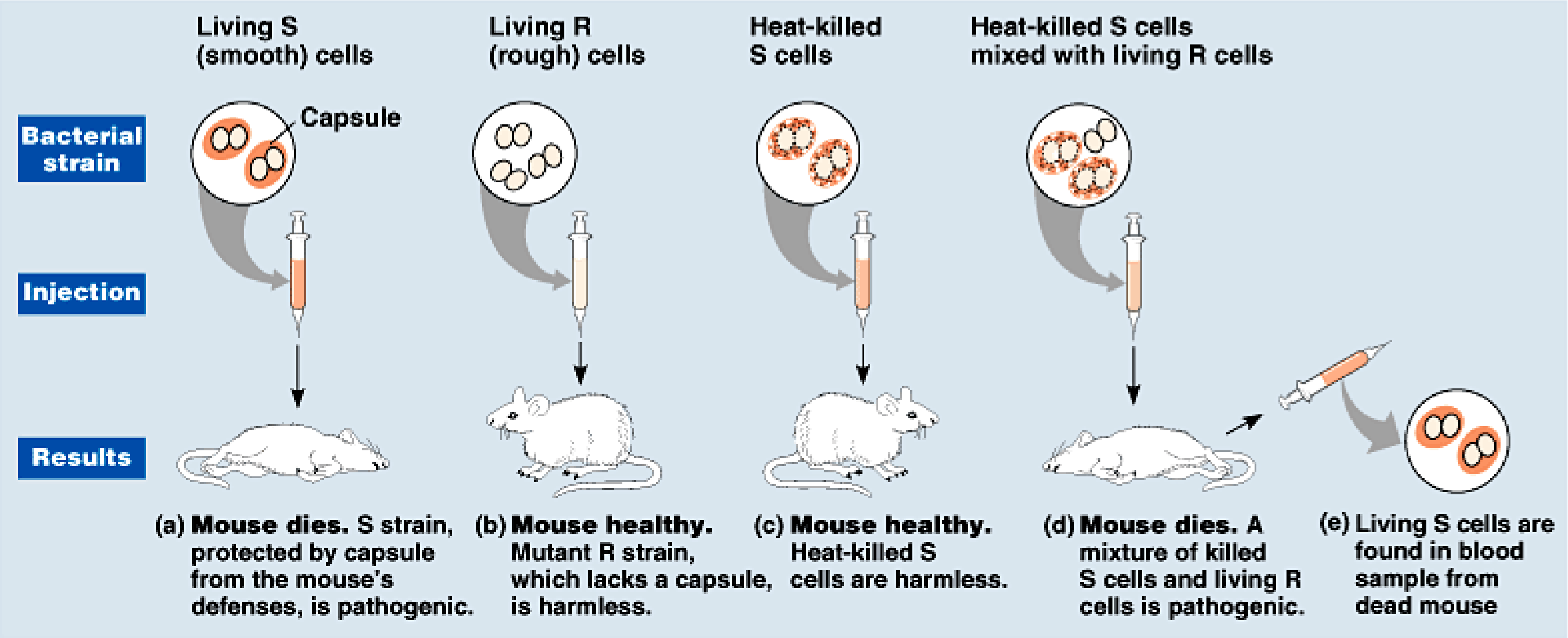
Francis Crick, 1916–2004

Chromosomes



Evidence That DNA Can Transform Bacteria

- Frederick Griffith's experiment 1928
- Griffith called the phenomenon *transformation* – a change in genotype and phenotype due to the assimilation of external DNA by a cell.



Conclusion:

Of all the molecules tested, only DNA changed the nonvirulent bacteria to become virulent; DNA can change a bacteria's genotype, thus changing it's phenotype

transformation

Evidence That DNA Can Transform Bacteria

- Avery, MacLeod, and McCarty
- Used a similar experiment to Griffith's
- One extra step was to take isolated DNA from the heat killed virulent bacteria and mixed it with non-encapsulated bacteria, which killed the mouse
- This confirmed that DNA was the material that transformed the avirulent bacteria

Procedures:

1. The encapsulated, virulent bacteria is injected into the mouse. The mouse dies.

2. The non-encapsulated, avirulent bacteria is injected into the mouse. The mouse lives.

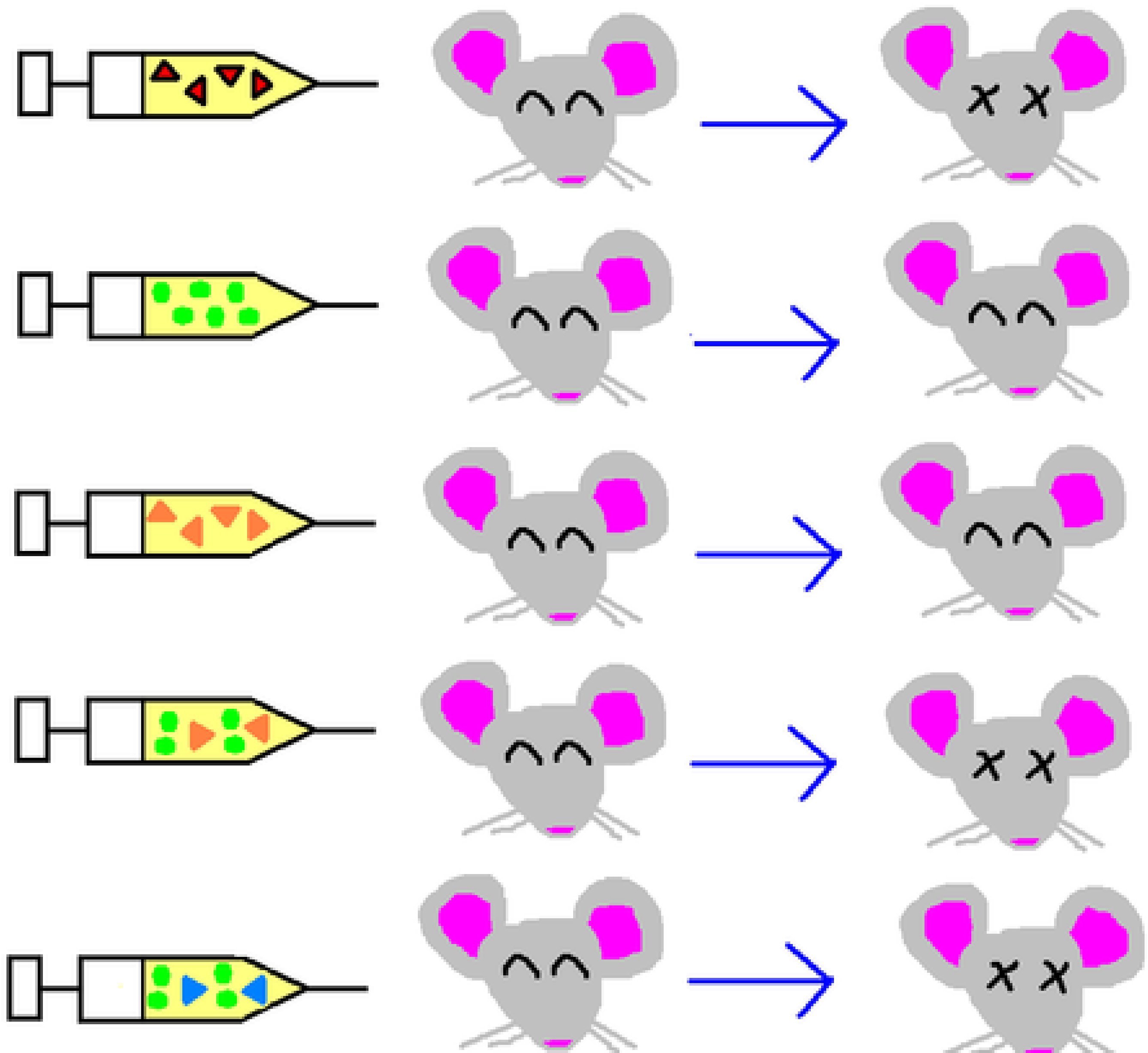
3. The heat-killed, virulent bacteria is injected into the mouse. The mouse lives.

4. The heat-killed, virulent bacteria is mixed with the non-encapslated avirulent bacteria and injected into the mouse. The mouse dies.

5. The DNA extracted from the heat-killed, virulent bacteria is mixed with the non-encapslated avirulent bacteria and injected into the mouse. The mouse dies.

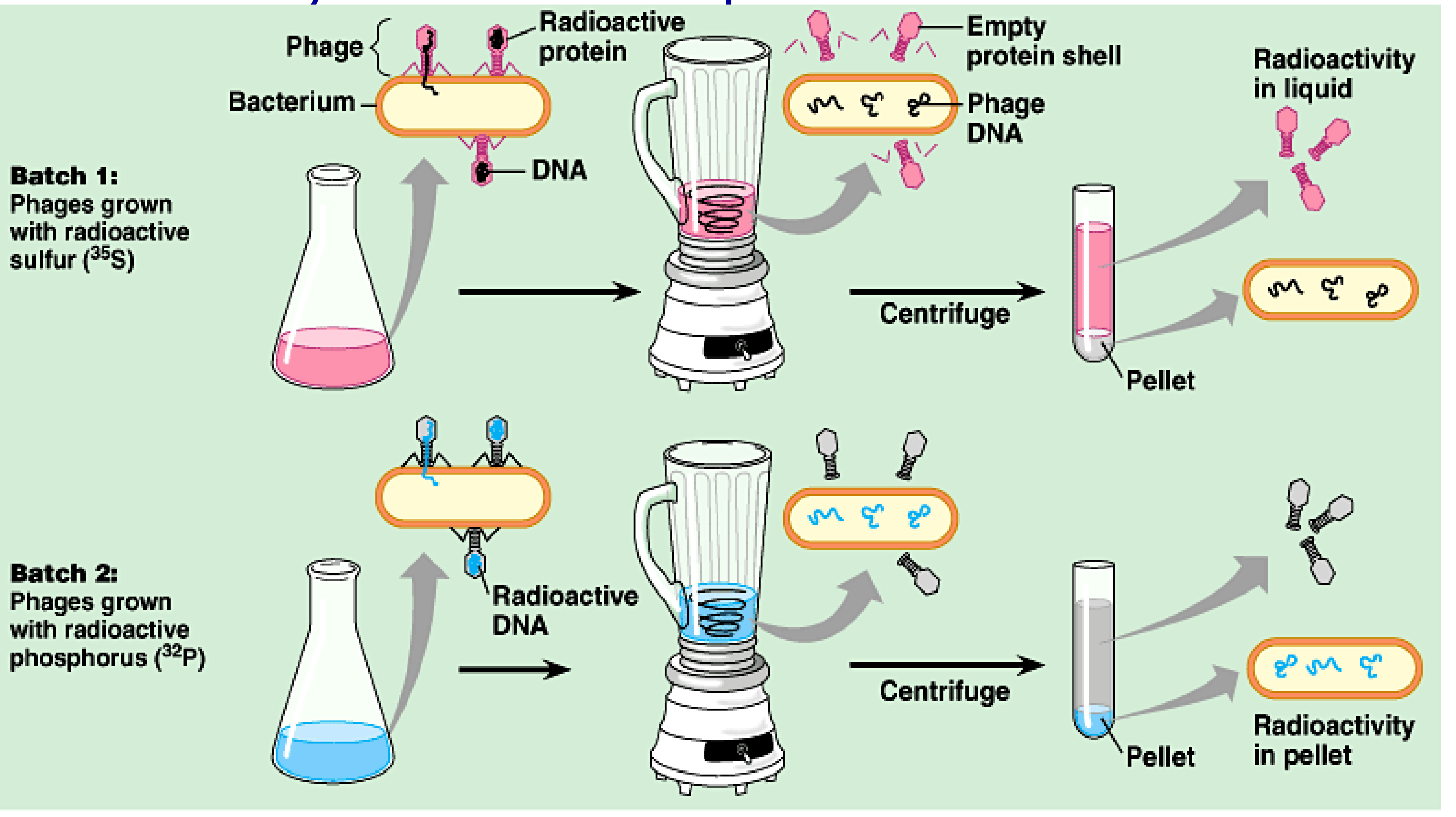
Legend:

- encapsulated, virulent bacteria
- heat-killed, virulent bacteria
- DNA extracted from
- non-encapsulated, avirulent bacteria



Evidence That DNA Can Transform Bacteria

- Hershey and Chase experiment 1952



Conclusion:

Injected viral DNA provides genetic information that makes the cells produce more viruses

Evidence that nucleic acids, not proteins were the genetic material

Chargaff’s Experiment

(Gonick & Whellis 1991)

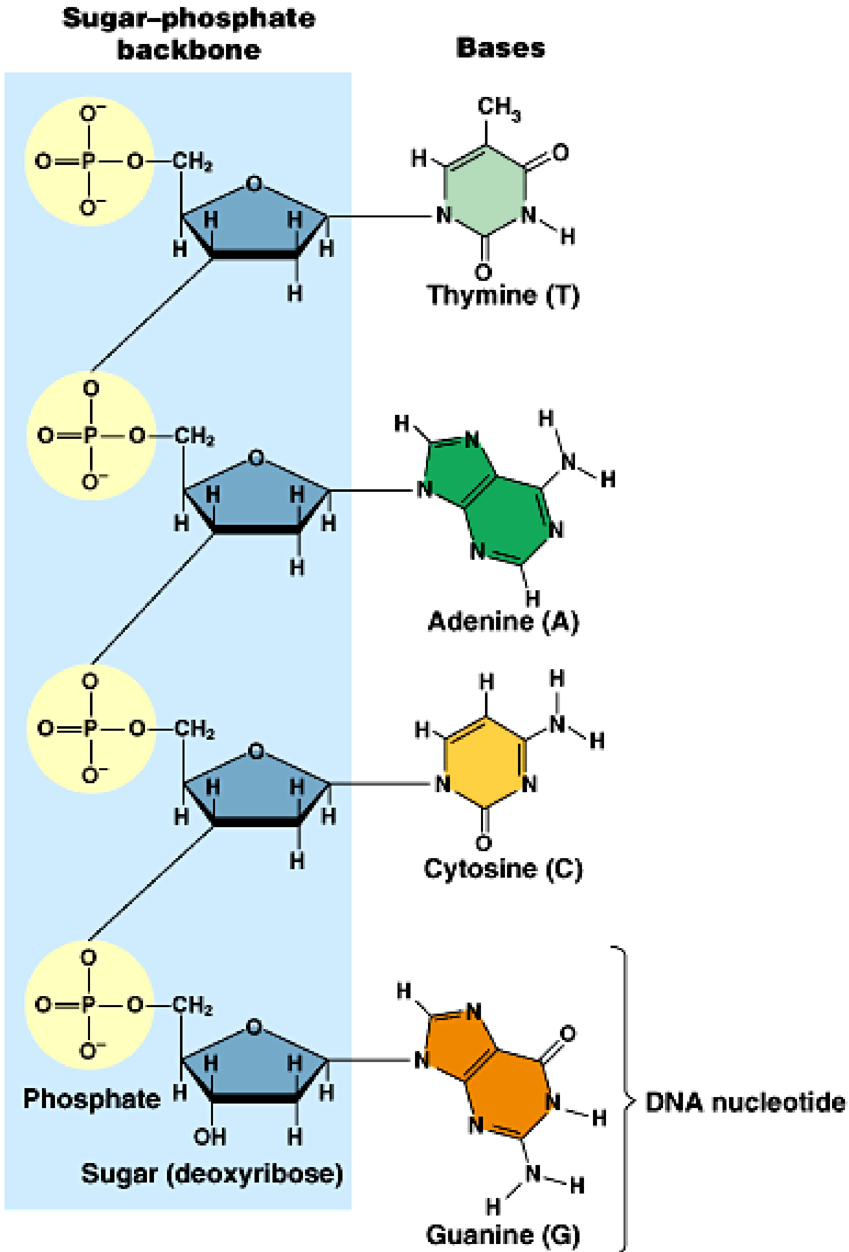
- The composition of DNA varied from one species to another, in particular in the relative amounts of the bases A, C, T, G
- In any DNA, the number of A’s was the same as the number of T’s; number of C’s was equal to the number of G’s
- What did this mean? Chargaff couldn’t say

Additional Evidence

- Diploid sets of chromosomes have twice as much DNA as the haploid sets found in the gametes of the same organism
- Ratio of DNA bases varies from species to species but are all present in the same amount

  A = T

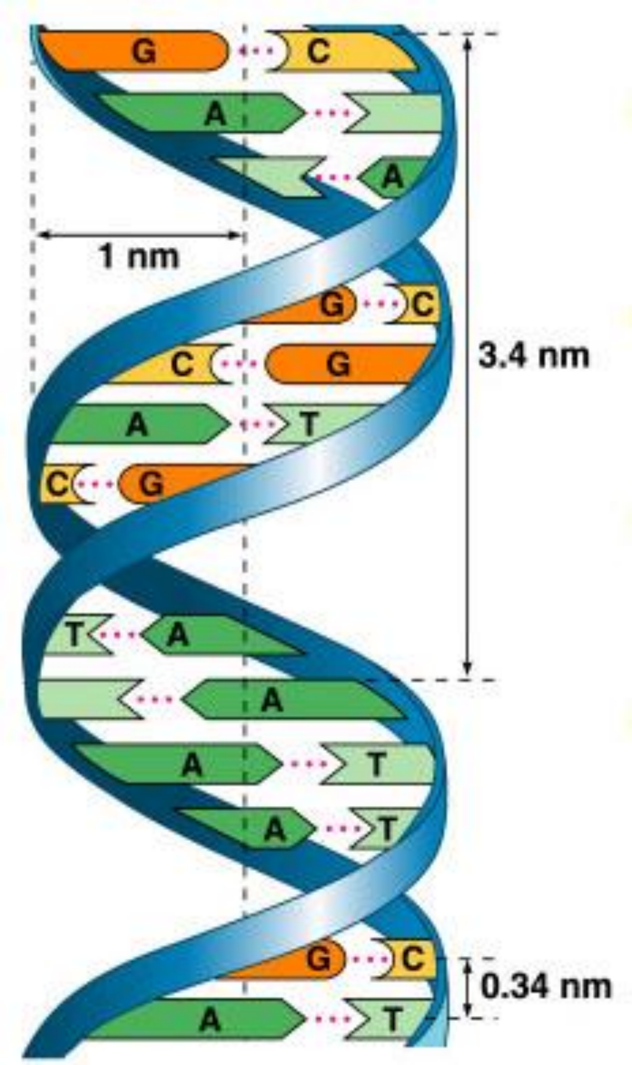
  C = G



James Watson and Francis Crick

Using Rosalind Franklin's X ray diffraction image of DNA, they deduced:

- Helical shape – double stranded
- 10 layers of base pairs per turn
- One full turn every 3.4 nm
- Width of the helix (suggest 2 strands)
- Spacing of the nitrogenous bases

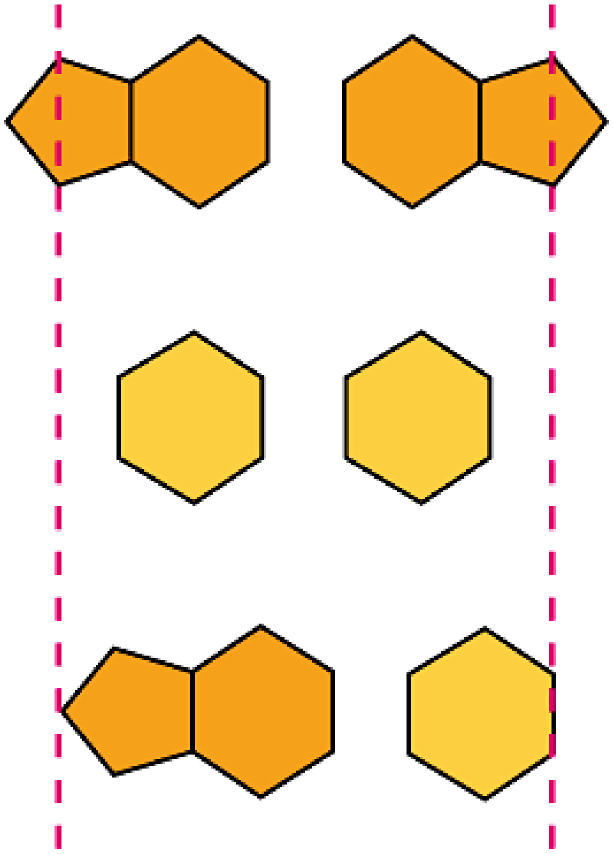


James Watson and Francis Crick

Purine + purine: too wide

Pyrimidine + pyrimidine: too narrow

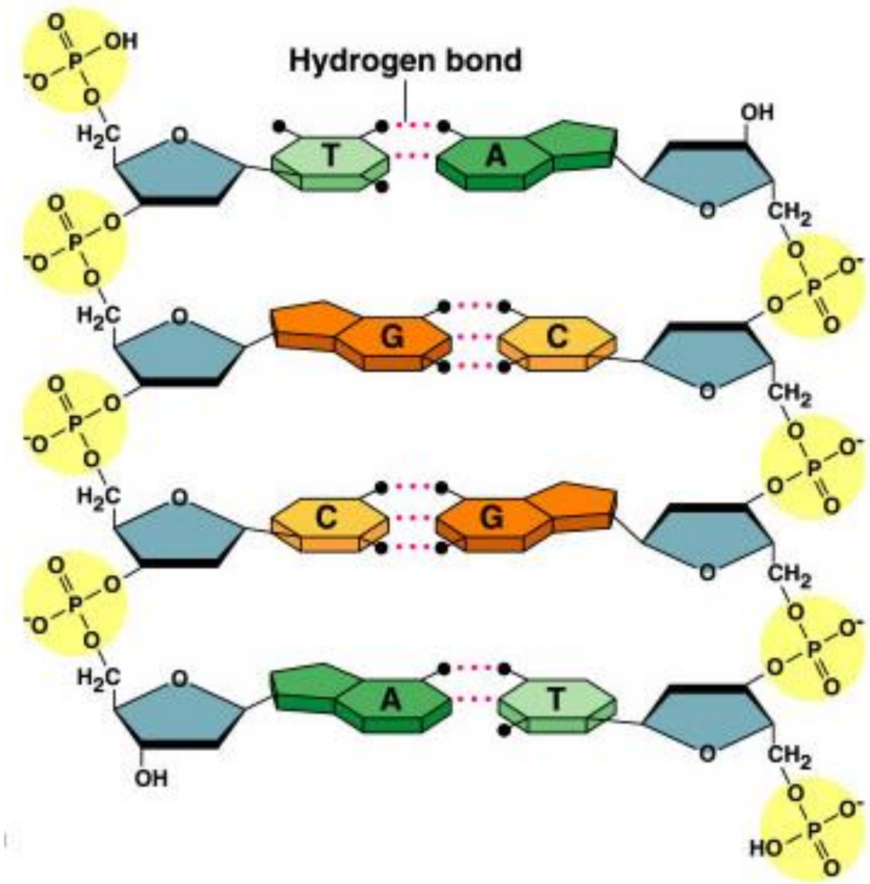
Purine + pyrimidine: width consistent with X-ray data



James Watson and Francis Crick

Structure of DNA

- Purines (Adenine & Guanine) always bond with pyrimidines (Thymine & Cytosine)
- Adenine forms 2 hydrogen bonds with thymine
- G forms 3 hydrogen bonds with cytosine

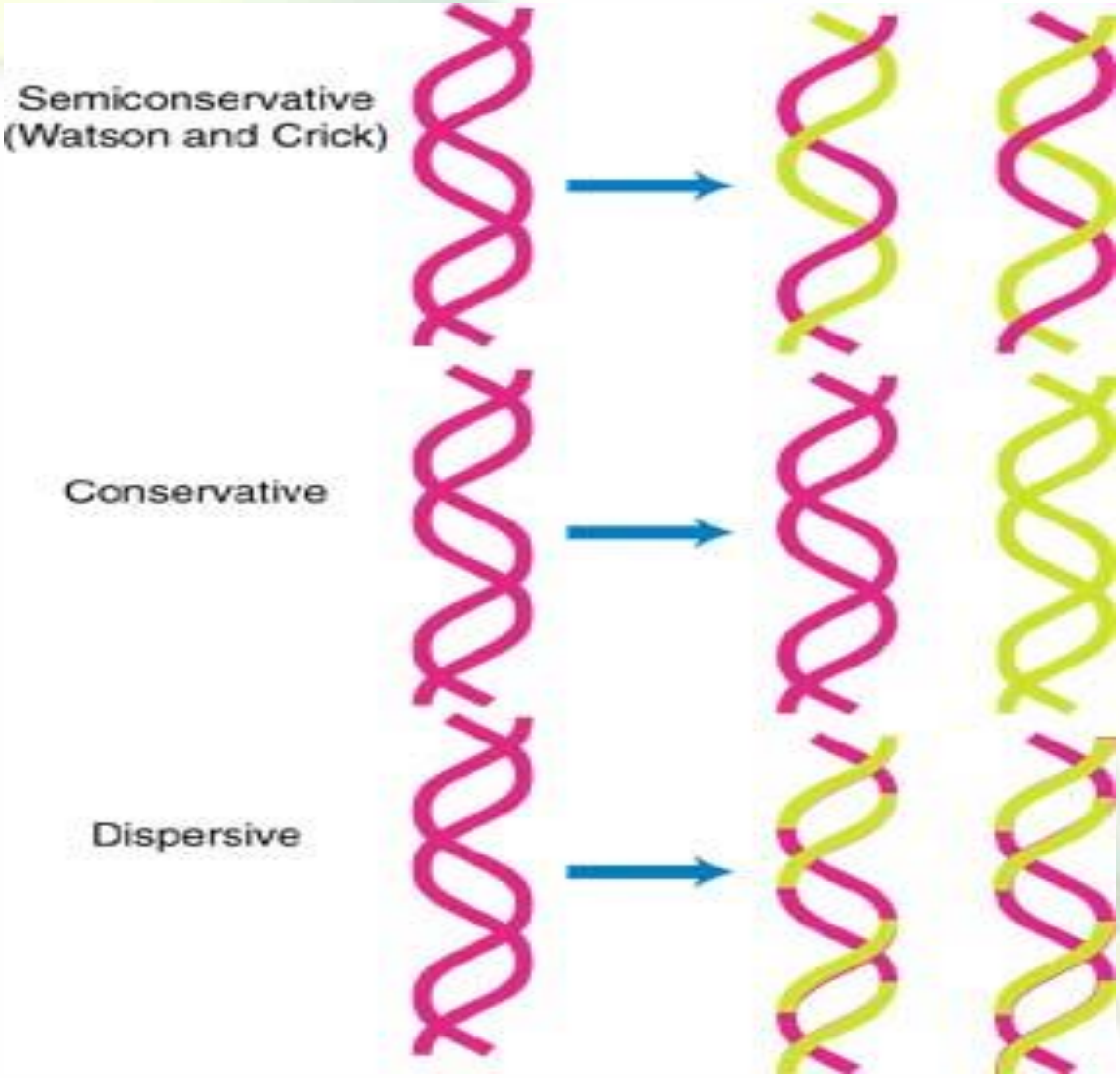


How is DNA Replicated?

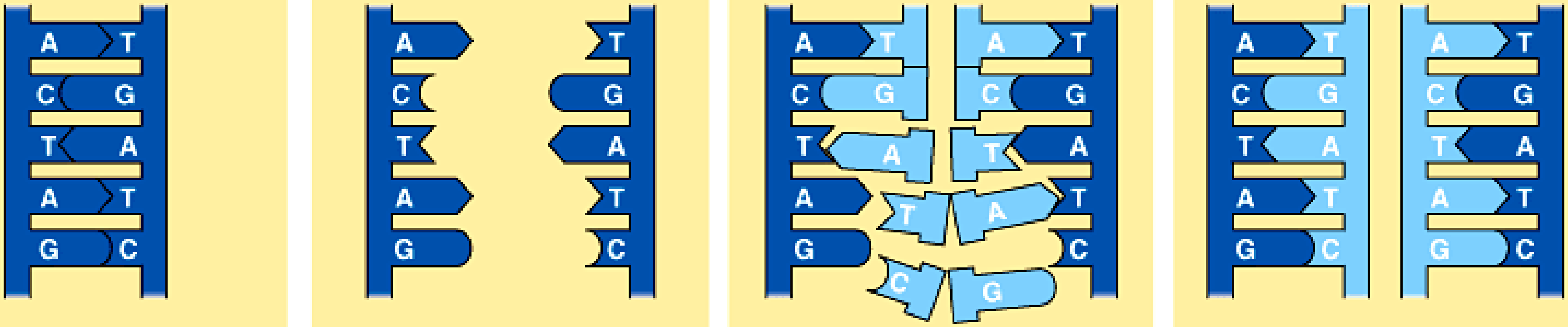
Conservative,

Semiconservative or Dispersive?

3 Hypotheses for Replication



Semi-conservative DNA Replication



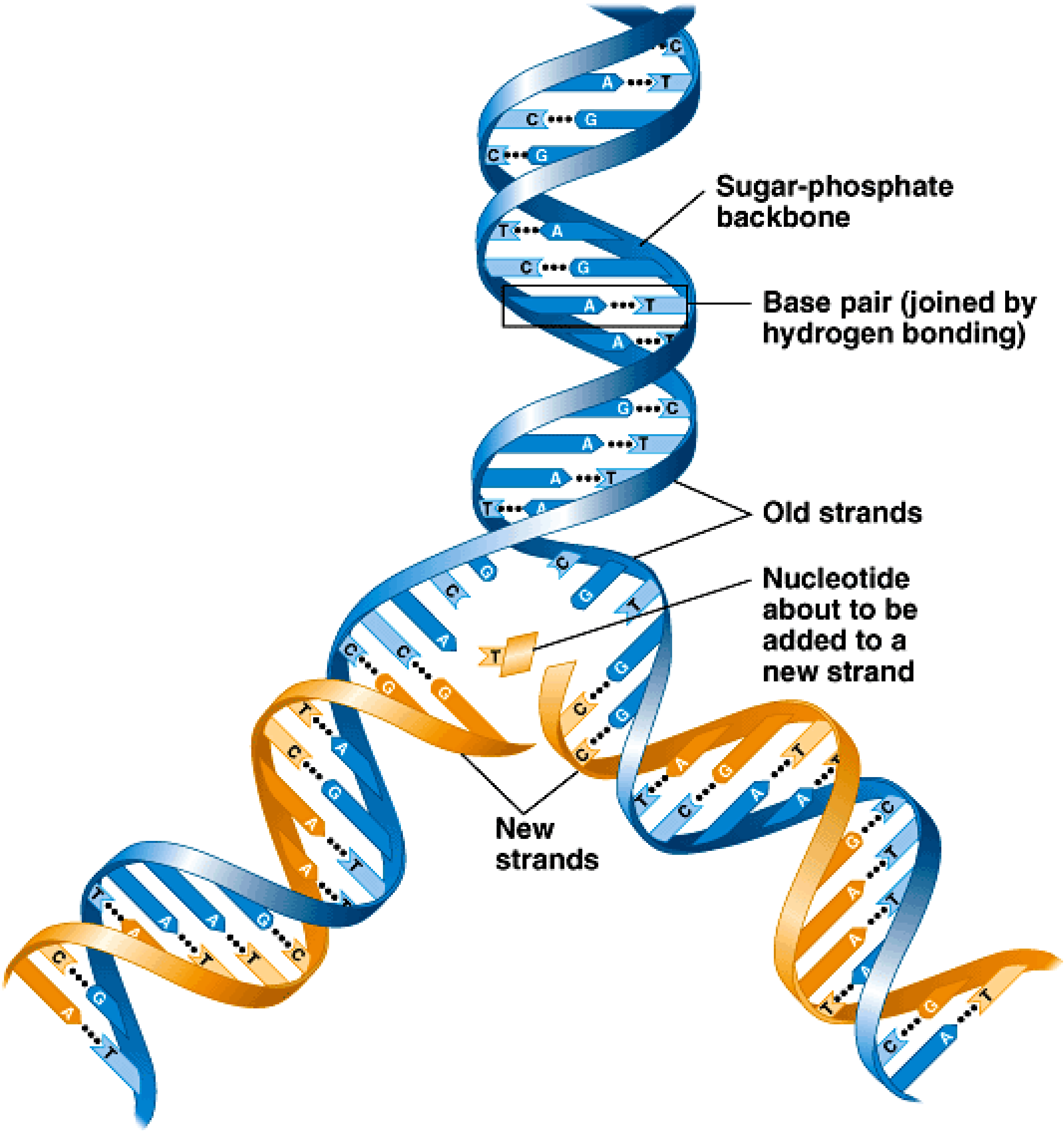
(a) The parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.

(b) The first step in replication is separation of the two DNA strands.

(c) Each parental strand now serves as a template that determines the order of nucleotides along a new complementary strand.

(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each "daughter" DNA molecule consists of one parental strand and one new strand.

DNA Replication

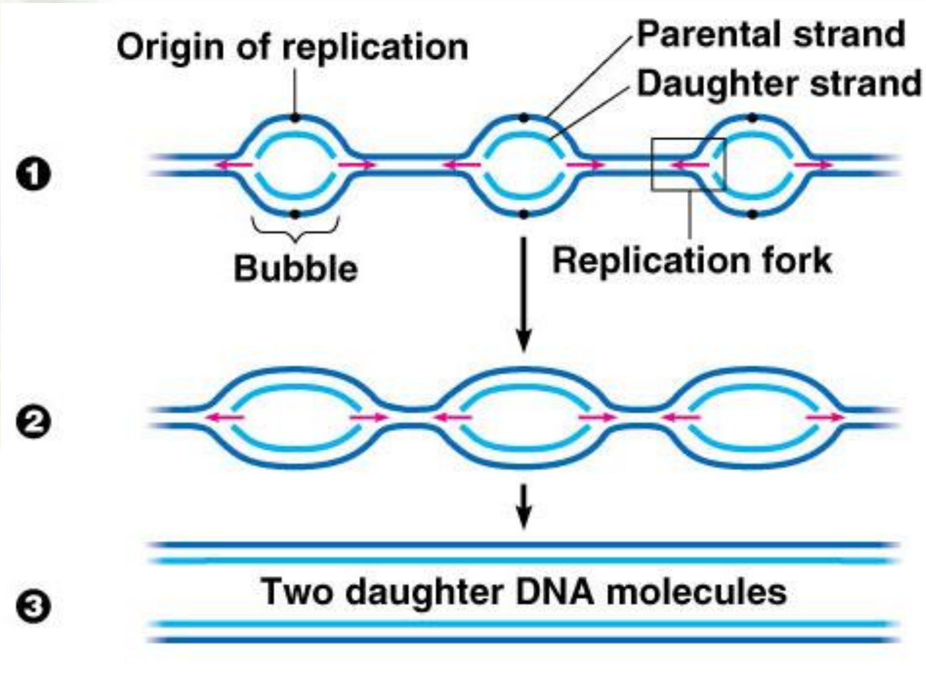


DNA Replication

- A human cell has 6 billion base pairs on 46 chromosomes
- There is one DNA molecule per chromosome
- DNA replication is so exact that there is 1 error per 1 billion nucleotides
- DNA replication utilizes more than a dozen enzymes and other proteins

Origin of Replication

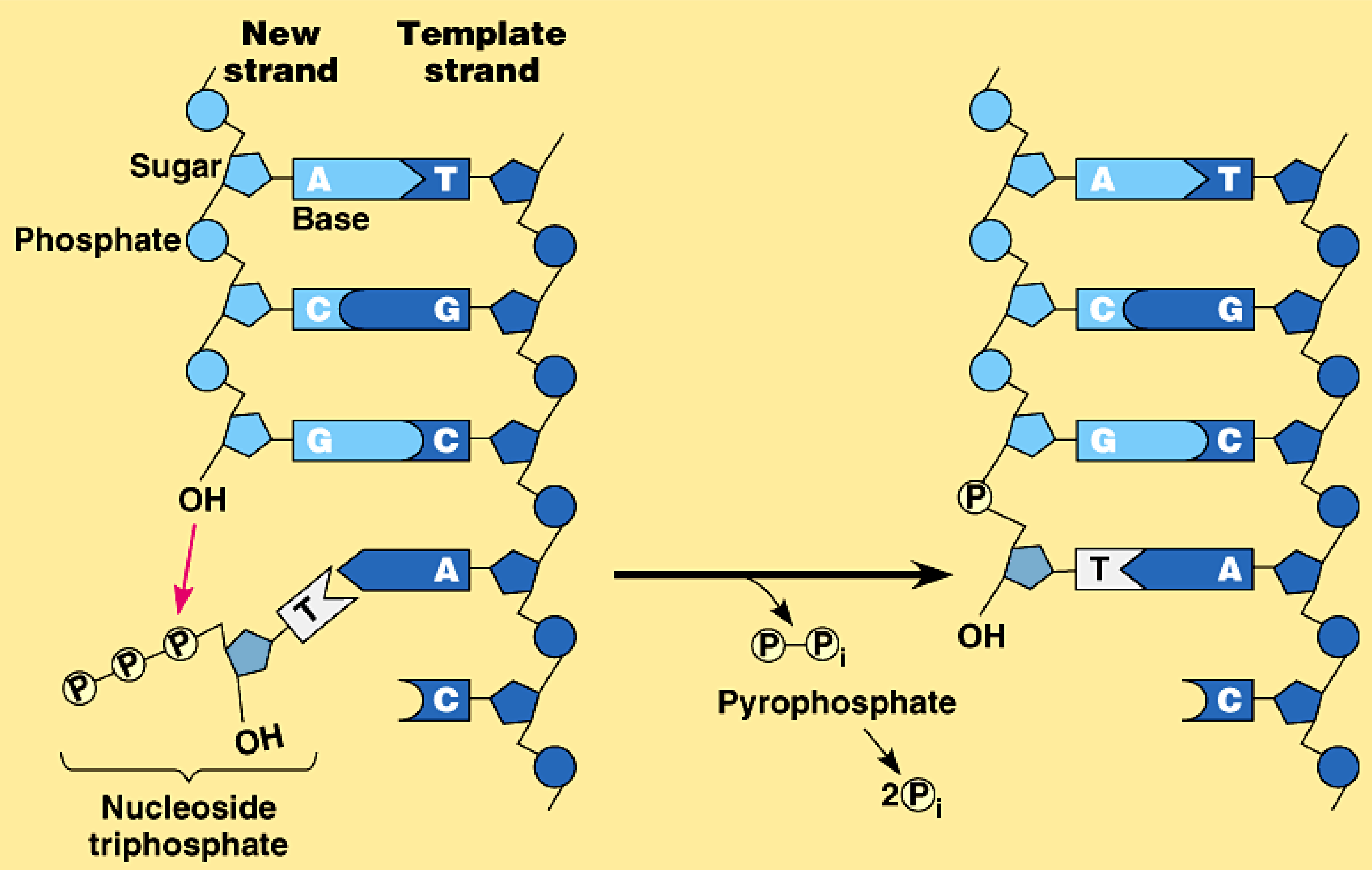
- Proteins recognize the sequence and attach to the DNA
- Replication occurs in both directions and at each end produces a replication fork where the new strand of DNA is elongated



Elongating a New DNA Strand

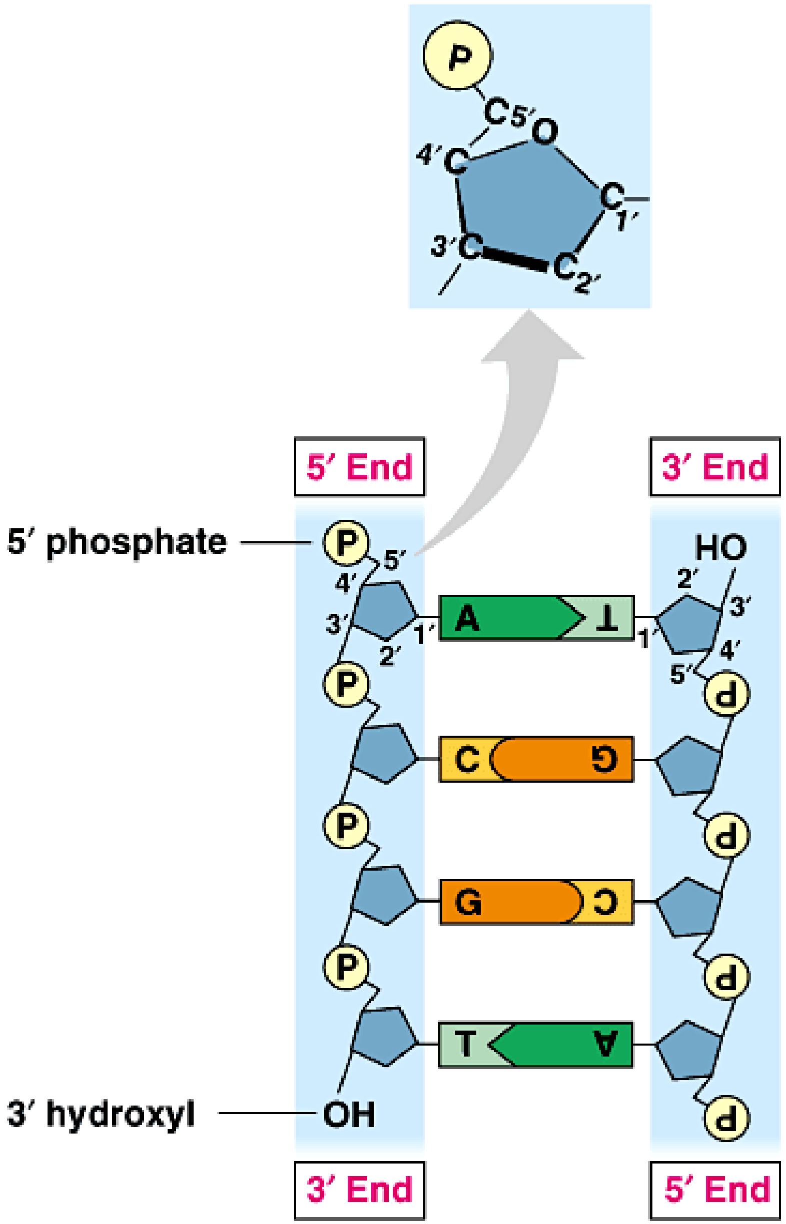
- The enzyme called DNA polymerase catalyses nucleotide attachment
- The rate of elongation is about 50 nucleotides per second in human cells
- Nucleotides that serve as substrates for DNA polymerase are triphosphates (tails have an unstable cluster of negative charges) as monomers join the DNA strand
  – 2 phosphate groups (called pyrophosphates) hydrolysis to 2 P_i and provides energy

Elongating a New DNA Strand



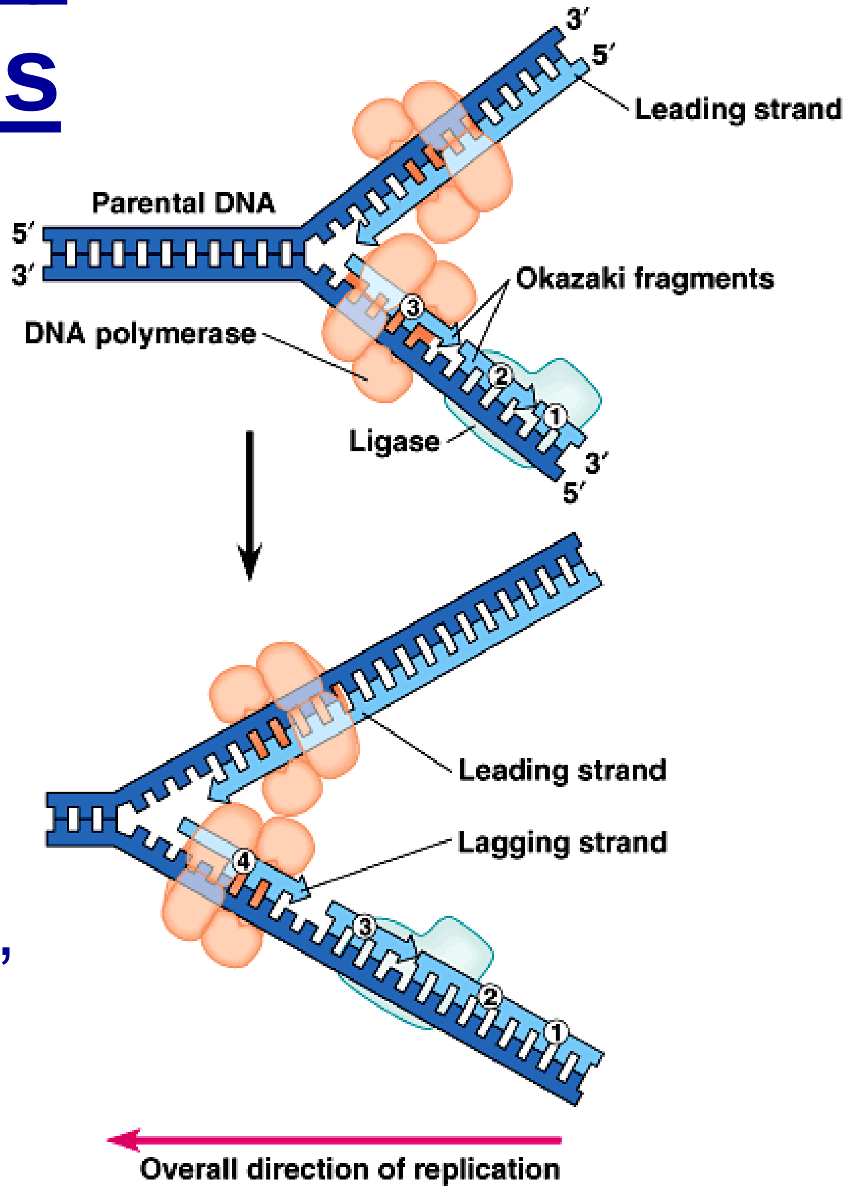
Antiparallel Arrangement

- DNA polymerase adds nucleotides only to the free 3’ end
- Elongation occurs 5’ → 3’



Synthesis of Leading and Lagging Strands

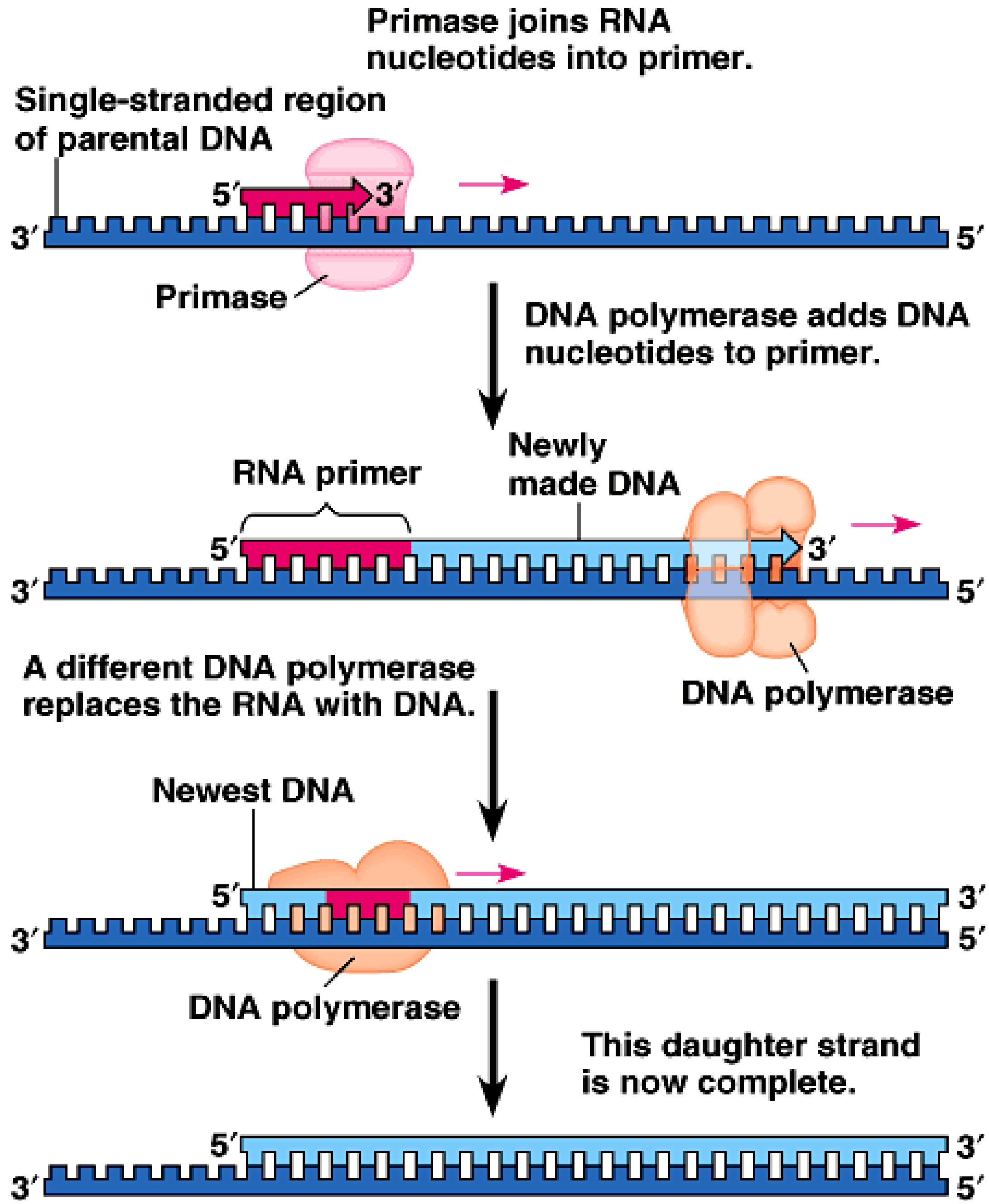
- *Leading Strand* – DNA copied in the 5’ → 3’ direction
- *Lagging Strand* – DNA copied in the opposite directions away from the replication fork
  - *Okazaki fragments* – short fragments that are synthesized in the 3’ → 5’ direction
  - Joined together by DNA ligase



Priming DNA Synthesis

- The start of a new chain of DNA is a short stretch of RNA called a *primer*
- The enzyme *primase* joins RNA nucleotides to make the primer
- A different DNA polymerase replaces the RNA primer with DNA

Priming DNA Synthesis



Proteins in DNA Replication

Initiation of replication

Double helix unwinds, providing single-stranded DNA templates	**Helicases and single-strand binding proteins**

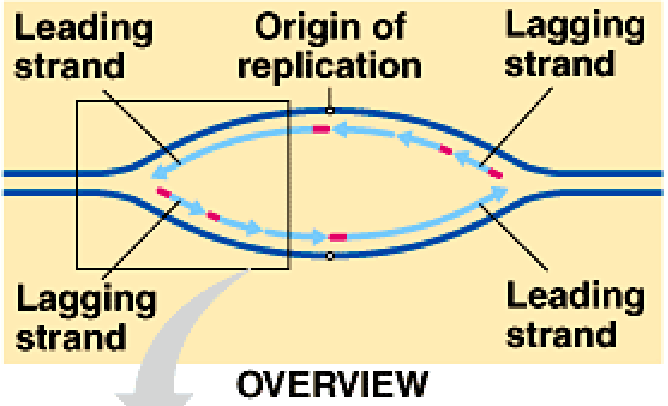
Synthesis of leading strand

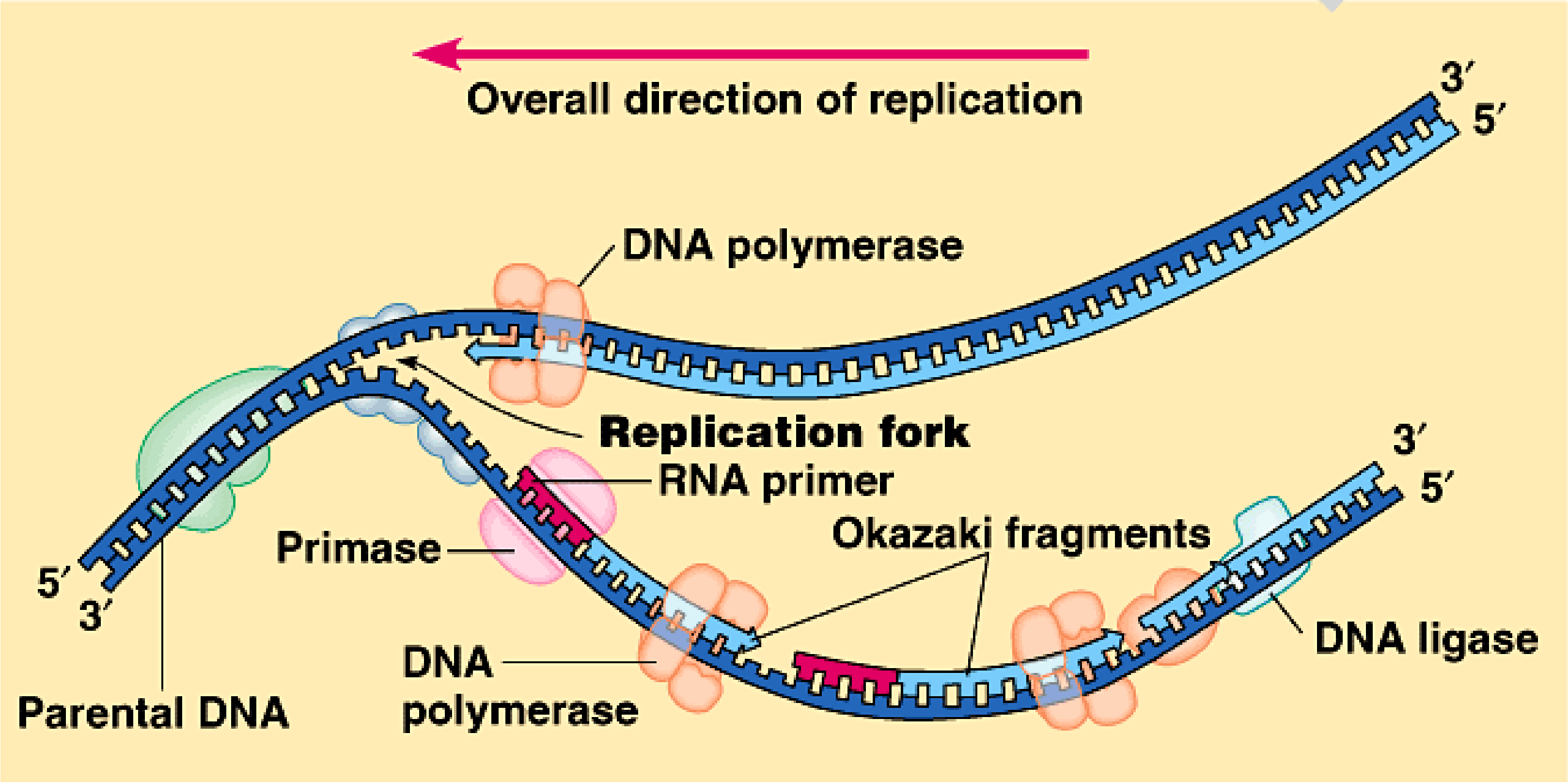
Priming	**Primase**
Elongation	**DNA polymerase**
Replacement of RNA primer by DNA	**DNA polymerase**

Synthesis of lagging strand

Priming for Okazaki fragment	**Primase**
Elongation of fragment	**DNA polymerase**
Replacement of RNA primer by DNA	**DNA polymerase**
Joining of fragments	**Ligase**

A Summary of DNA Replication



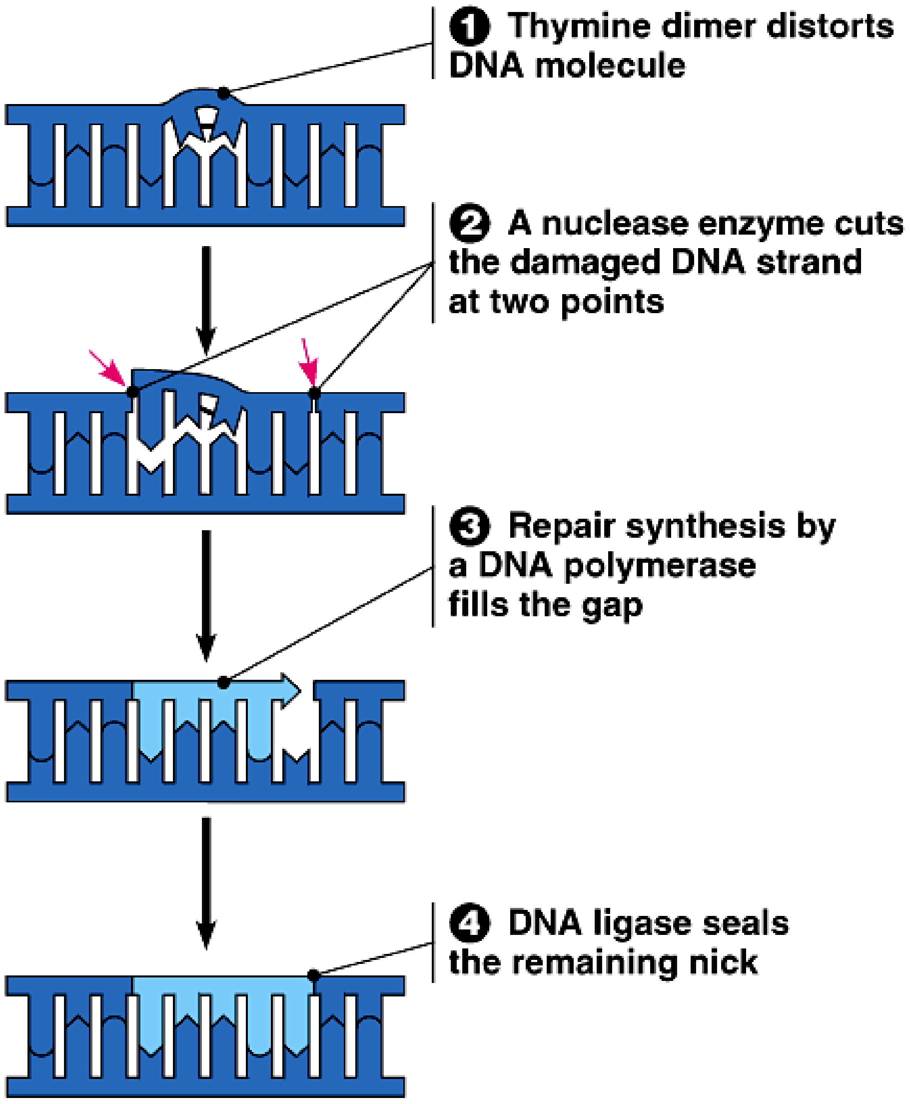


Enzymes Proofread DNA

- DNA polymerase proofreads each nucleotide against its template – if incorrect, it removes and replaces it.
- *Mismatch repairs* – cells use special enzymes to fix incorrectly paired nucleotides.
- DNA requires frequent repair due to environmental factors:
  - Radioactive emissions
  - X-rays
  - UV light
  - Spontaneous chemical changes
- Cells continually monitor and repair its genetic material using 130 enzymes

Enzymes Proofread DNA

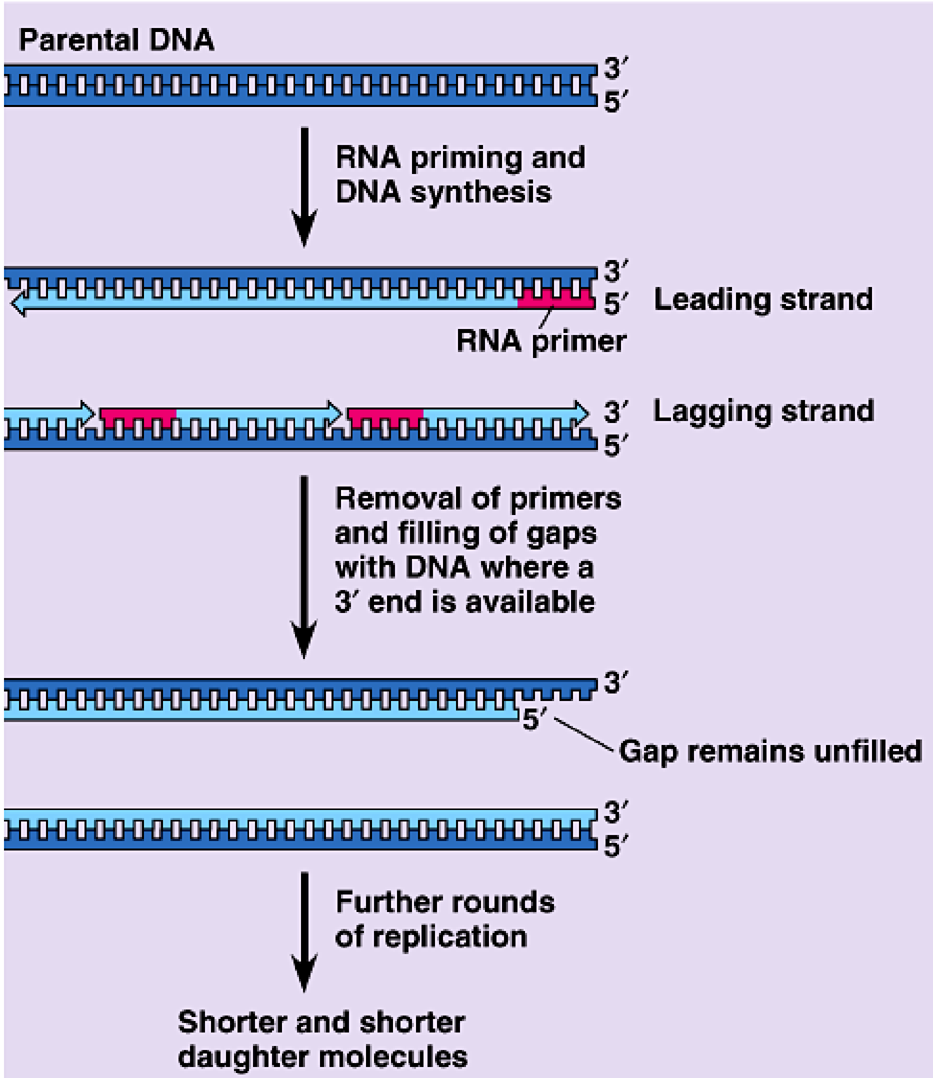
- *A Nuclease* (DNA polymerase and/or ligase) – may cut out a strand of damaged DNA called *nucleotide excision repair*

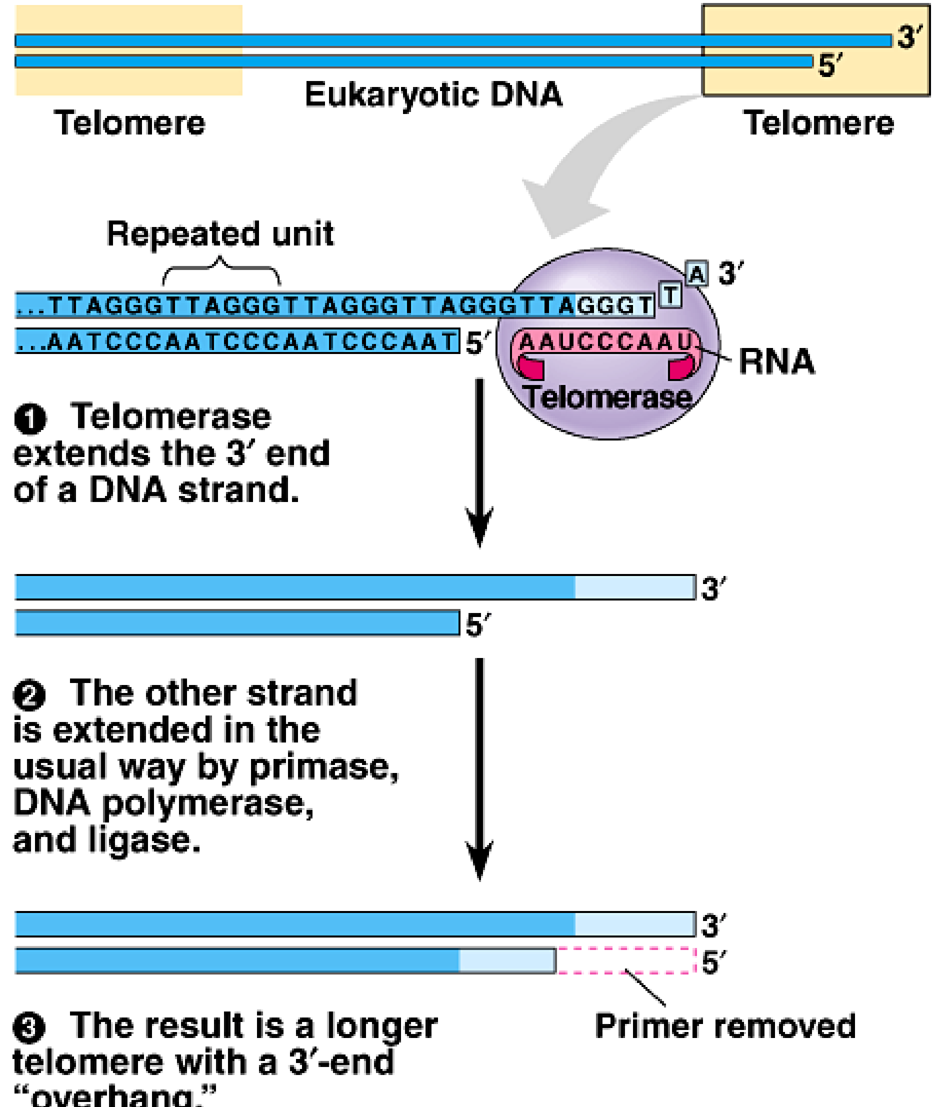


Ends Are Replicated By a Special Mechanism

- Since there is no way to complete the 5' ends of daughter DNA, replication could produce shorter and shorter strands
- Special sequences called *telomeres* at ends contain multiple repetitions of one short sequence
- Telomeres are not present in most multicellular organisms.
- *Telomerase* – catalyzes lengthening of telomeres: may be a limiting factor in the life span of certain tissues

Ends Are Replicated By Special Mechanism





Telomeres