THE MOLECULAR BASIS OF INHERITANCE

Chapter 13

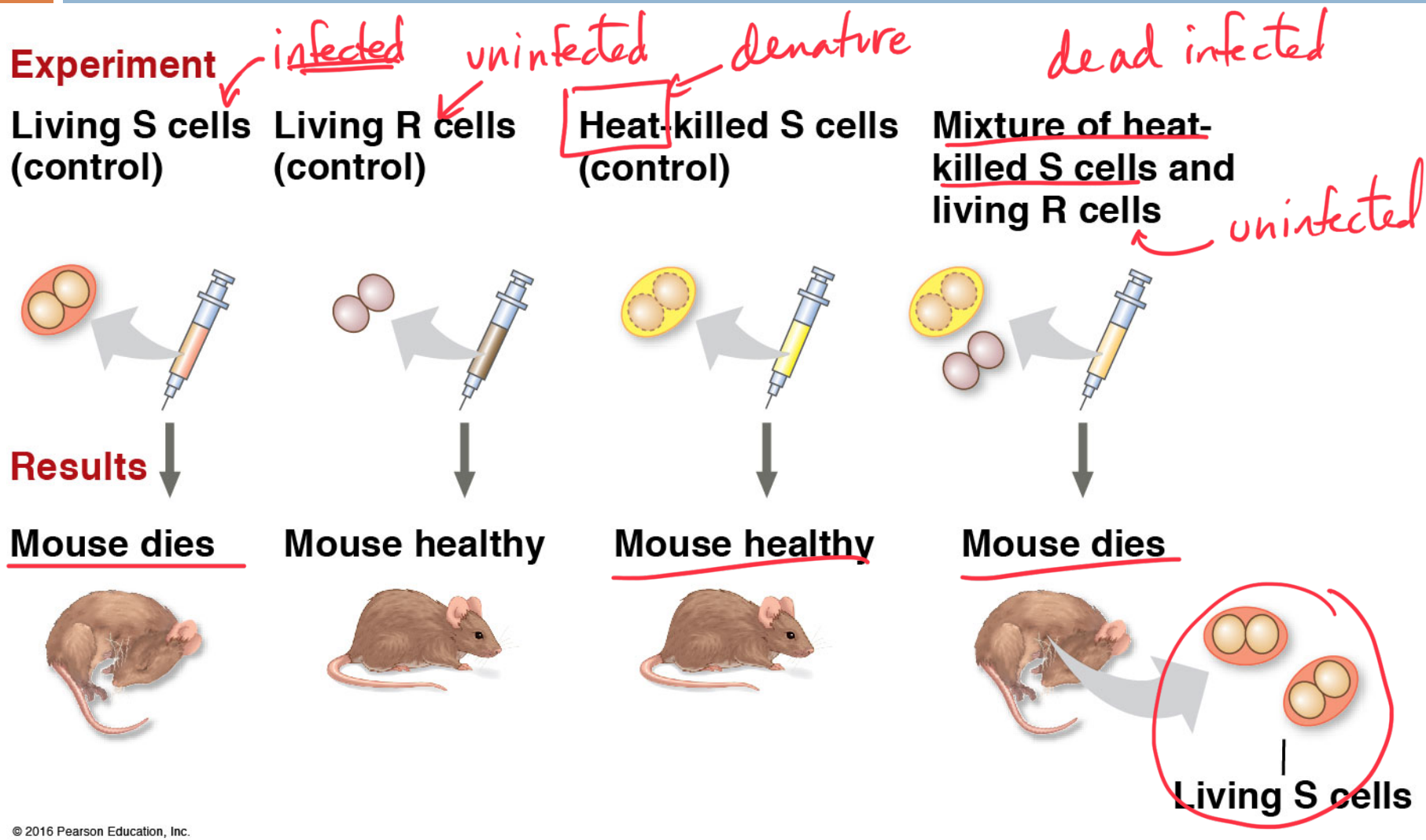
What you must know

- The structure of DNA.
- The knowledge about DNA gained from the work of Griffith; Avery, MacLeod, and McCarty; Hershey and Chase; Wilkins and Franklin; and Watson and Crick.
- That replication is semiconservative and occurs 5' to 3'.
- The roles of DNA polymerase, ligase, helicase, and topoisomerase in replication.
- The general differences between bacterial chromosomes and eukaryotic chromosomes.
- How DNA is packaged can affect gene expression.

Problem:

Is the genetic material of organisms made of DNA or proteins?

Frederick Griffith (1928)



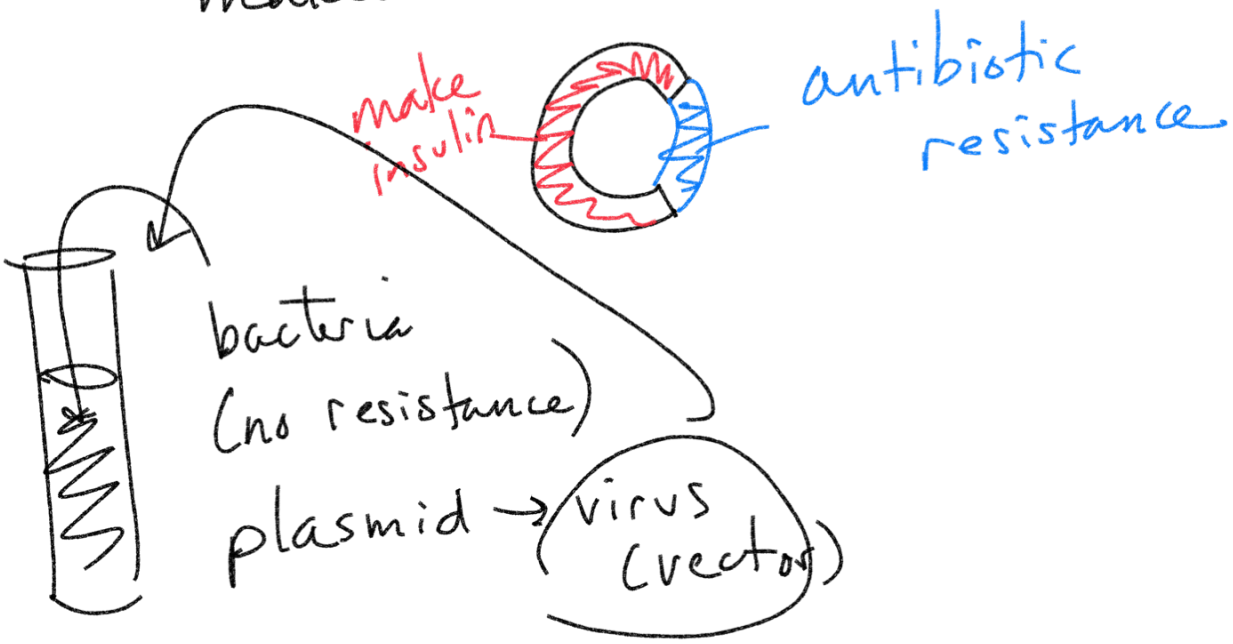
W-GB General Biology Week 27 4/12

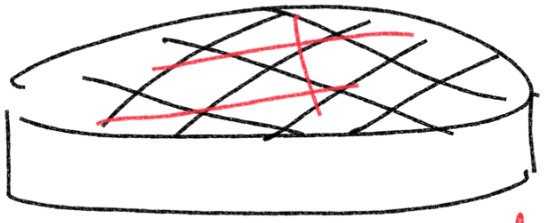
Plasmid → polymer of DNA that we use to introduce some functionality to bacteria

"Vector"

plasmid

Plasmid that contains the code to create the enzyme that makes insulin.

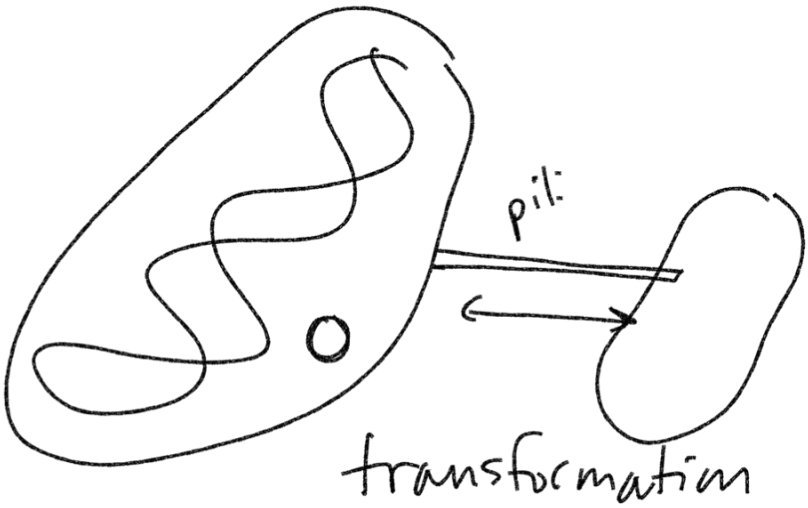




nutrients + antibiotics

original bacteria → die

original bacteria + plasmid → live


pil:
transformation

Frederick Griffith (1928)

Conclusion: living R bacteria transformed into deadly S bacteria by unknown, heritable substance

Avery, McCarty, MacLeod (1944)

- Tested DNA, RNA, & proteins in heat-killed pathogenic bacteria
- Discovered that the transforming agent was DNA

Hershey and Chase (1952)

- Bacteriophages: virus that infects bacteria; composed of DNA and protein

Protein = radiolabel S

DNA = radiolabel P

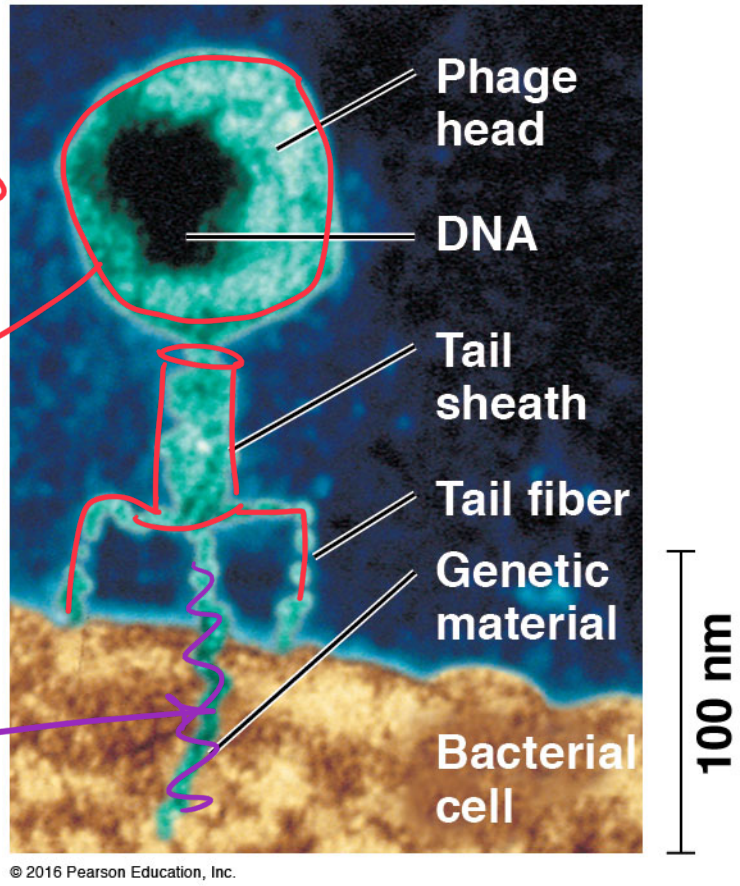
only found in nucleic acids

protein

C, H, O, N, P, S

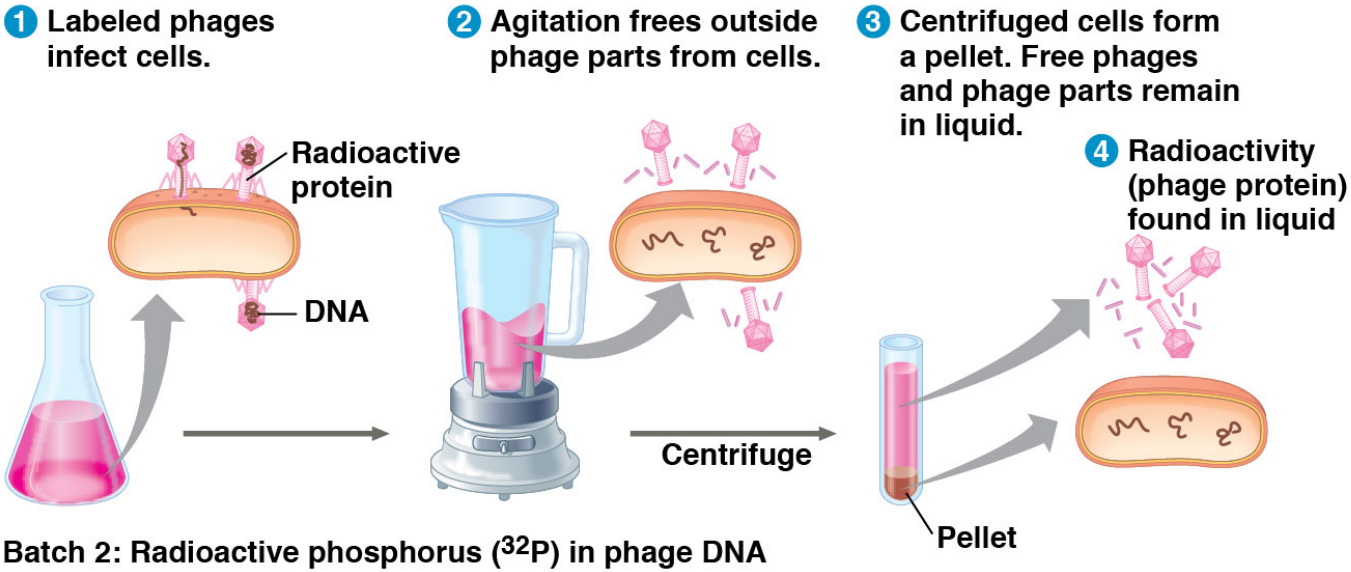
only found macro in protein molecules

DNA

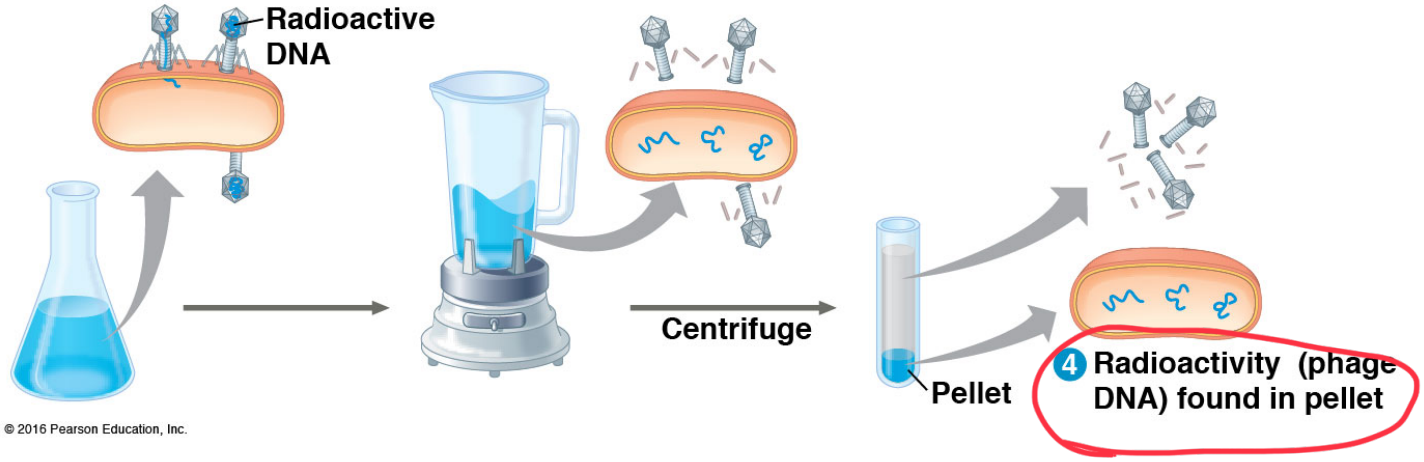


Experiment

Batch 1: Radioactive sulfur (^{35}S) in phage protein



Batch 2: Radioactive phosphorus (^{32}P) in phage DNA



Conclusion: DNA entered infected bacteria → DNA must be the genetic material!

Problem:

What is the structure of DNA?

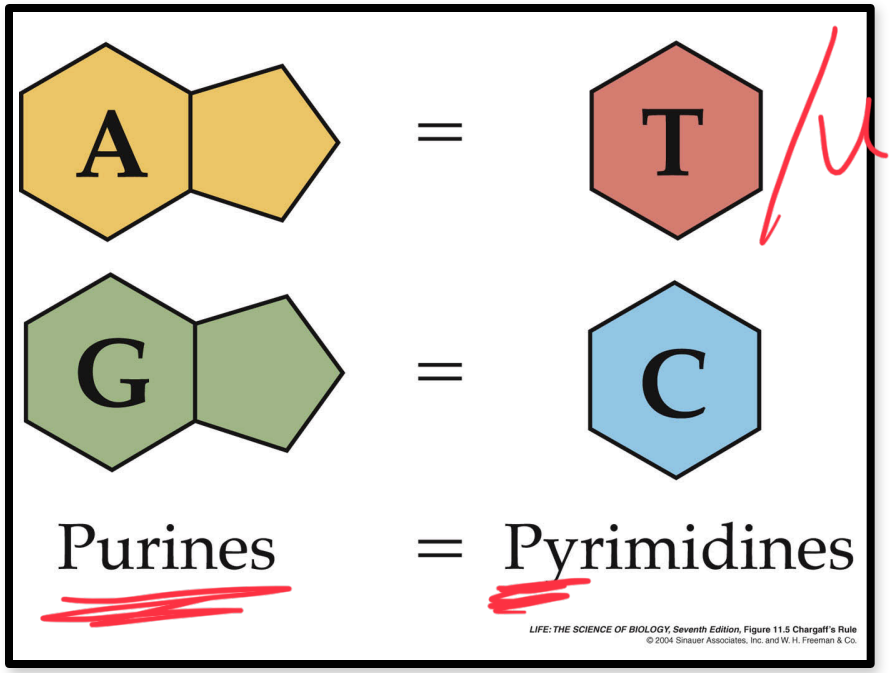
Edwin Chargaff (1947)

Chargaff's Rules:

- DNA composition varies between species
- Ratios: %A = %T and %G = %C

CUT the Py

Pure As Gold

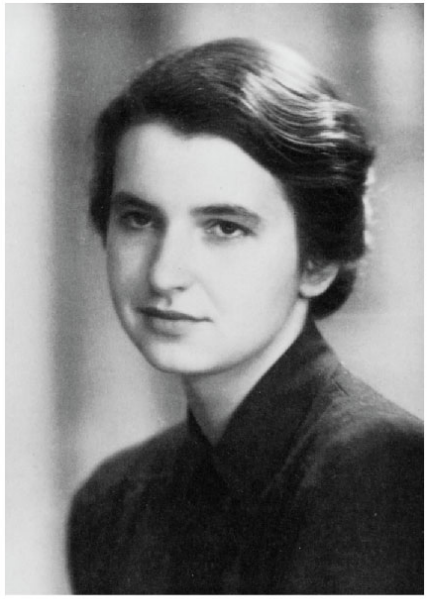


A-T

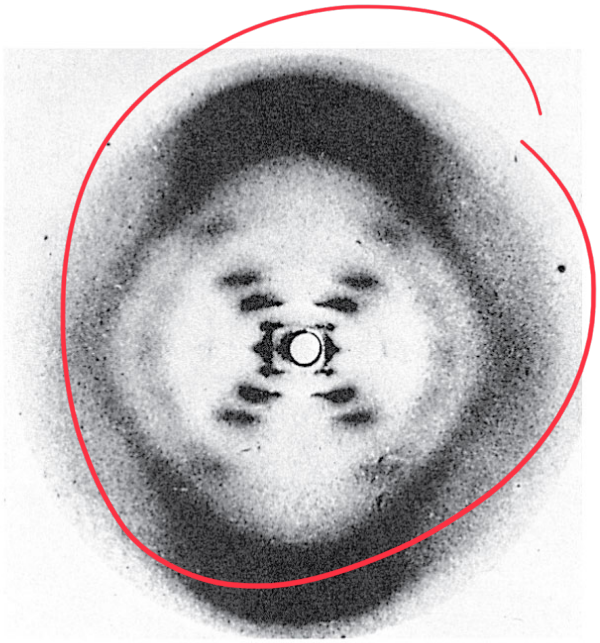
G-C

Rosalind Franklin (1950's)

- Worked with Maurice Wilkins
- X-ray crystallography = images of DNA
- Provided measurements on chemistry of DNA



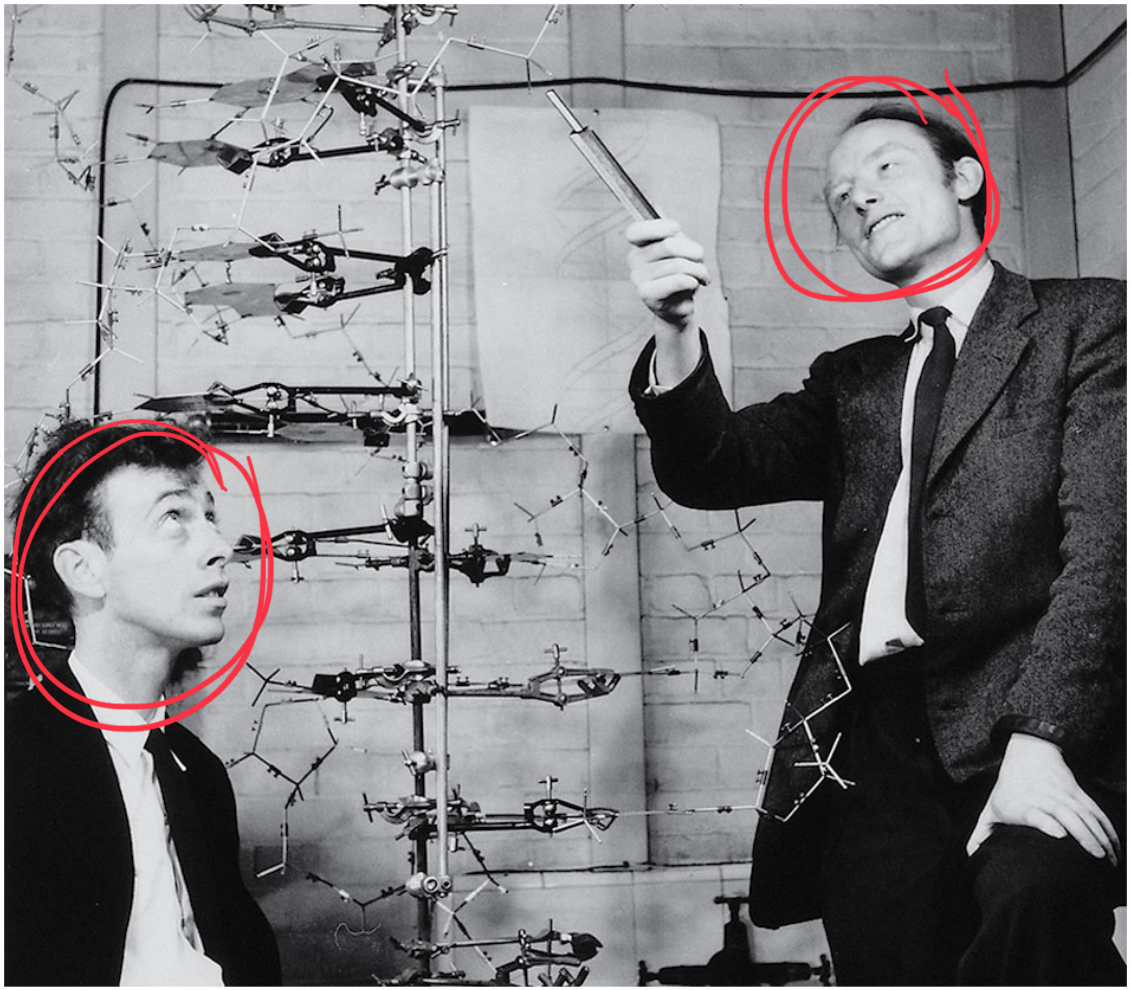
(a) Rosalind Franklin



(b) Franklin's X-ray diffraction photograph of DNA

James Watson & Francis Crick (1953)

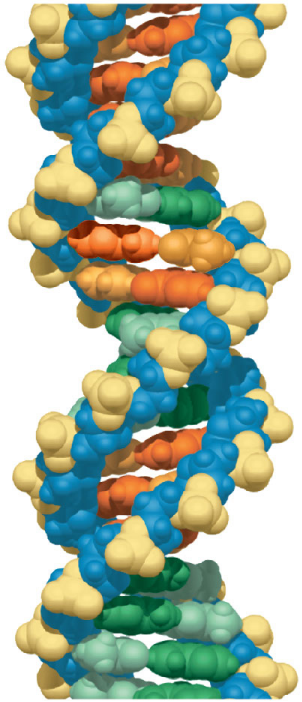
- Discovered the double helix by building models to conform to Franklin's X-ray data and Chargaff's Rules.



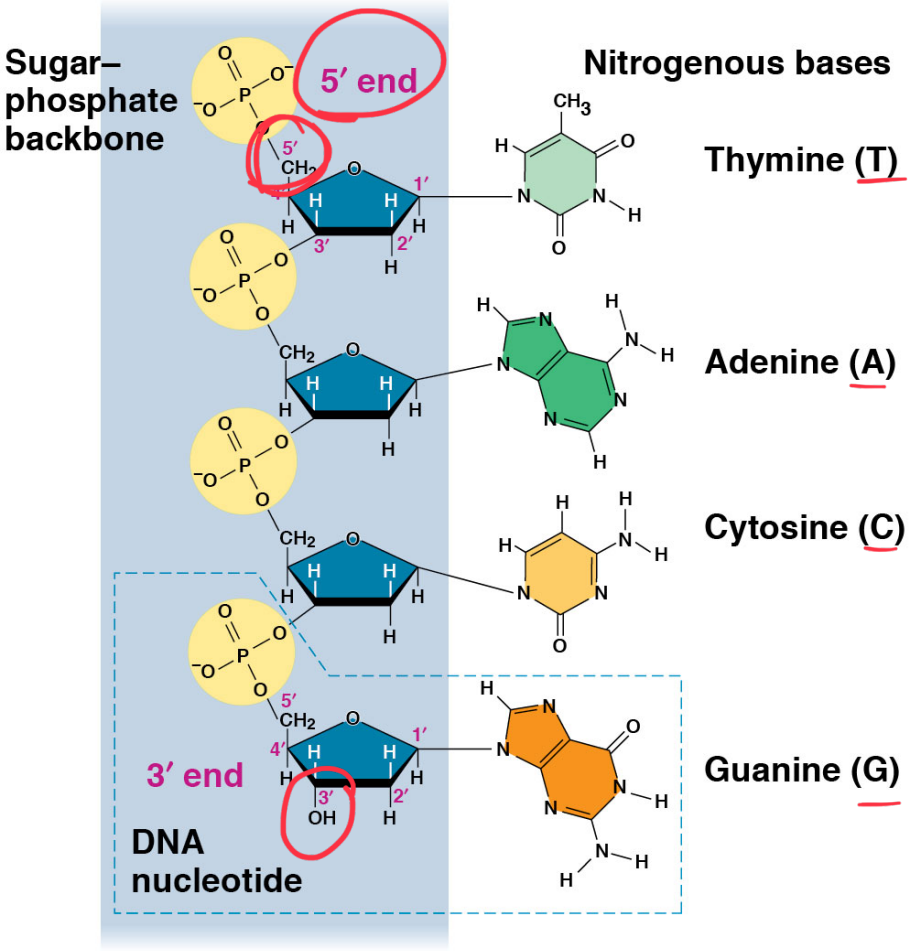
DNA = Double Helix

"Backbone" = sugar + phosphate

"Rungs" = nitrogenous bases



(c) Space-filling model



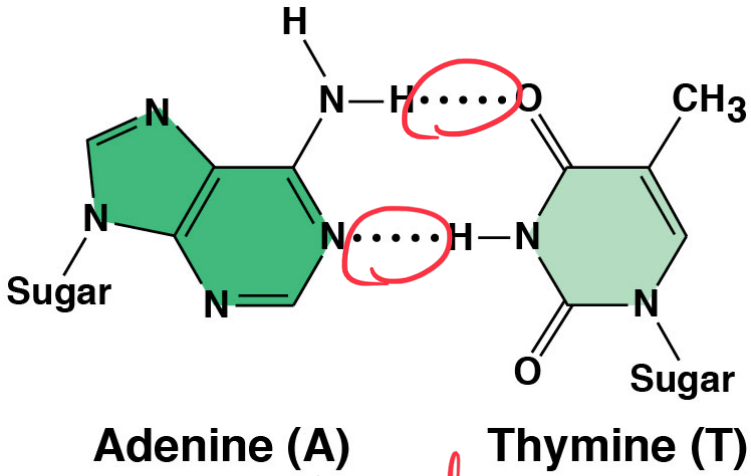
Nitrogenous Bases

- Adenine (A)
- Guanine (G)

purine

- Thymine (T)
- Cytosine (C)

pyrimidine

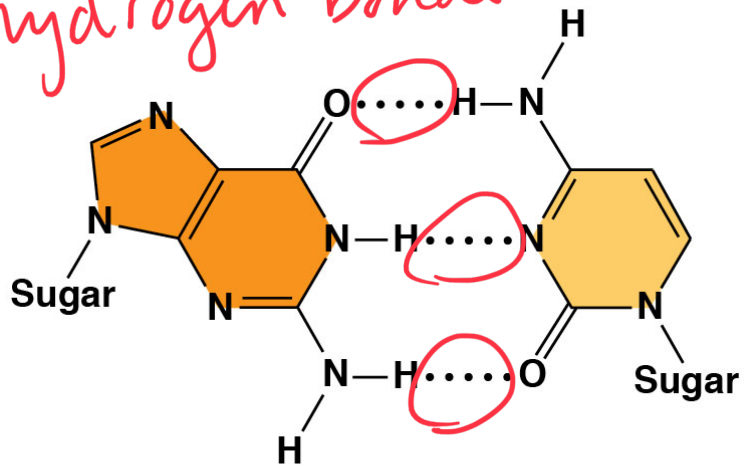


Adenine (A) Thymine (T)

hydrogen bonds

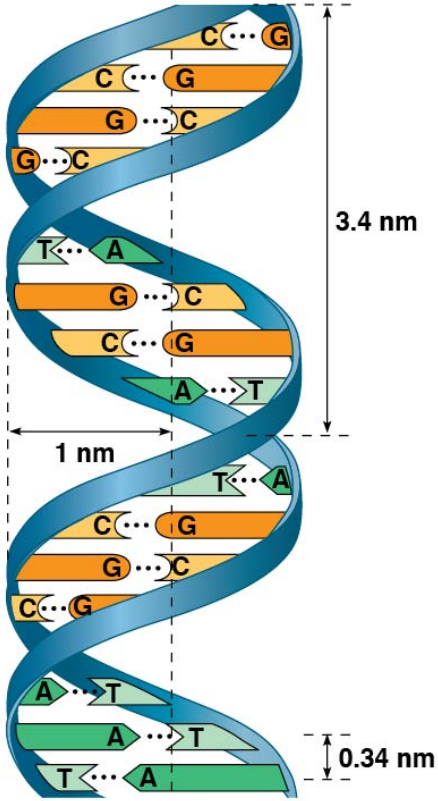
Pairing:

- Purine + Pyrimidine
  - A = T 2HBs
  - G ≡ C 3HBs

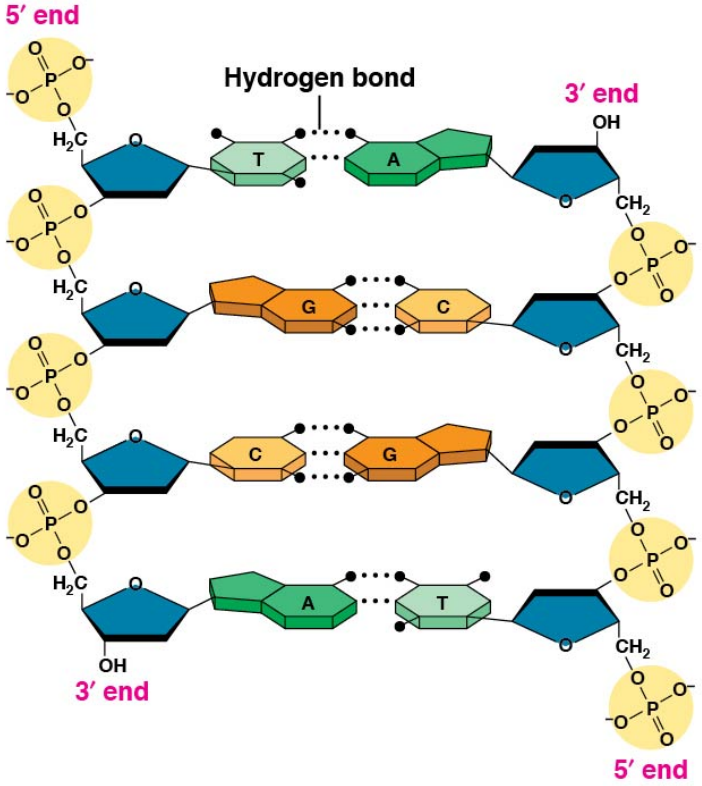


Guanine (G) Cytosine (C)

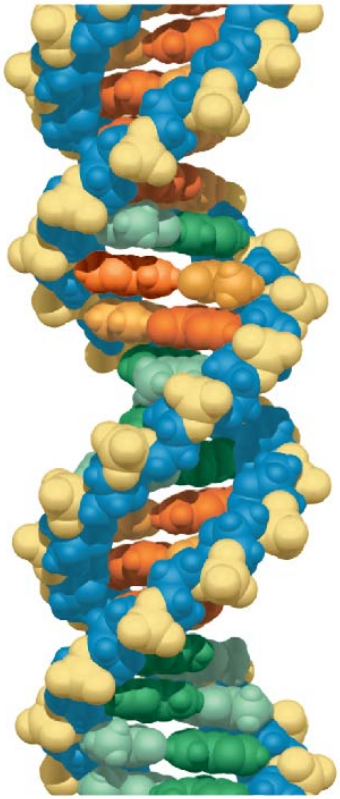
Hydrogen Bonds



(a) Key features of DNA structure



(b) Partial chemical structure

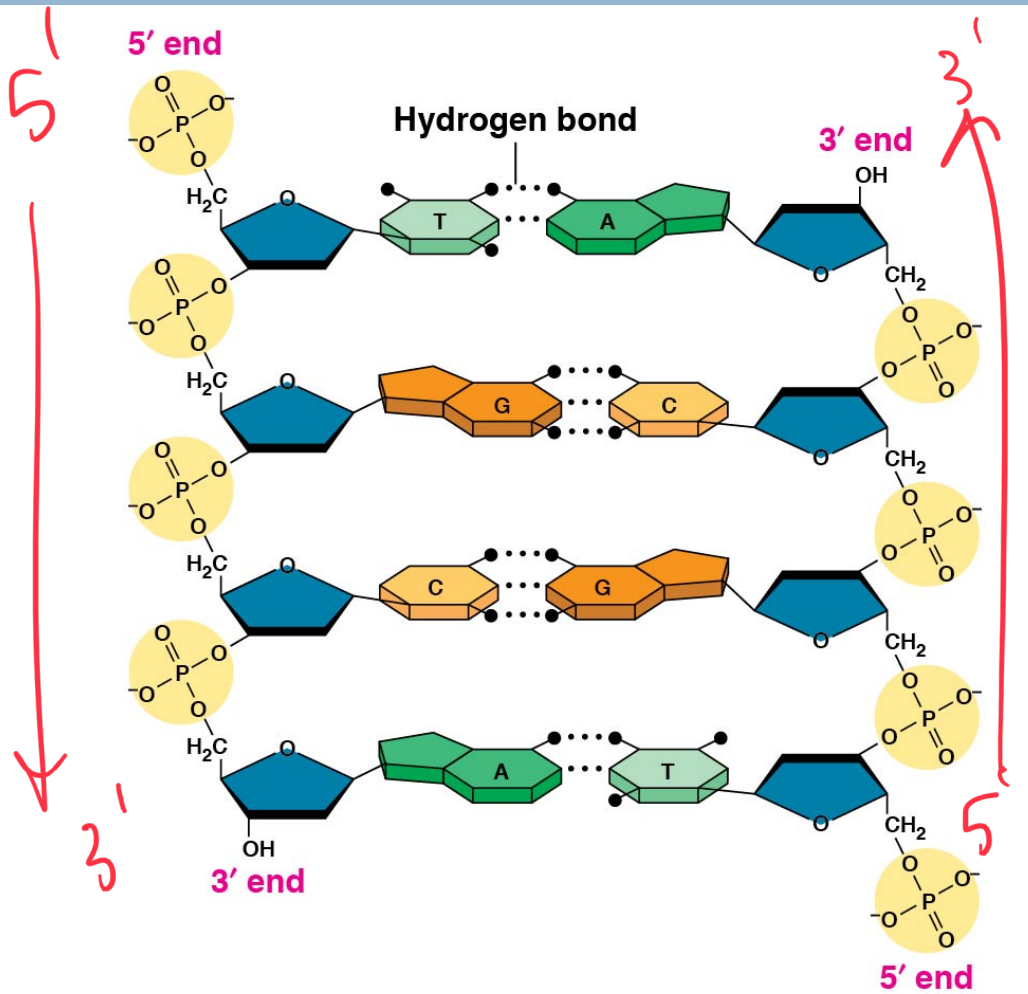


(c) Space-filling model

Hydrogen bonds between base pairs of the two strands hold the molecule together like a zipper.

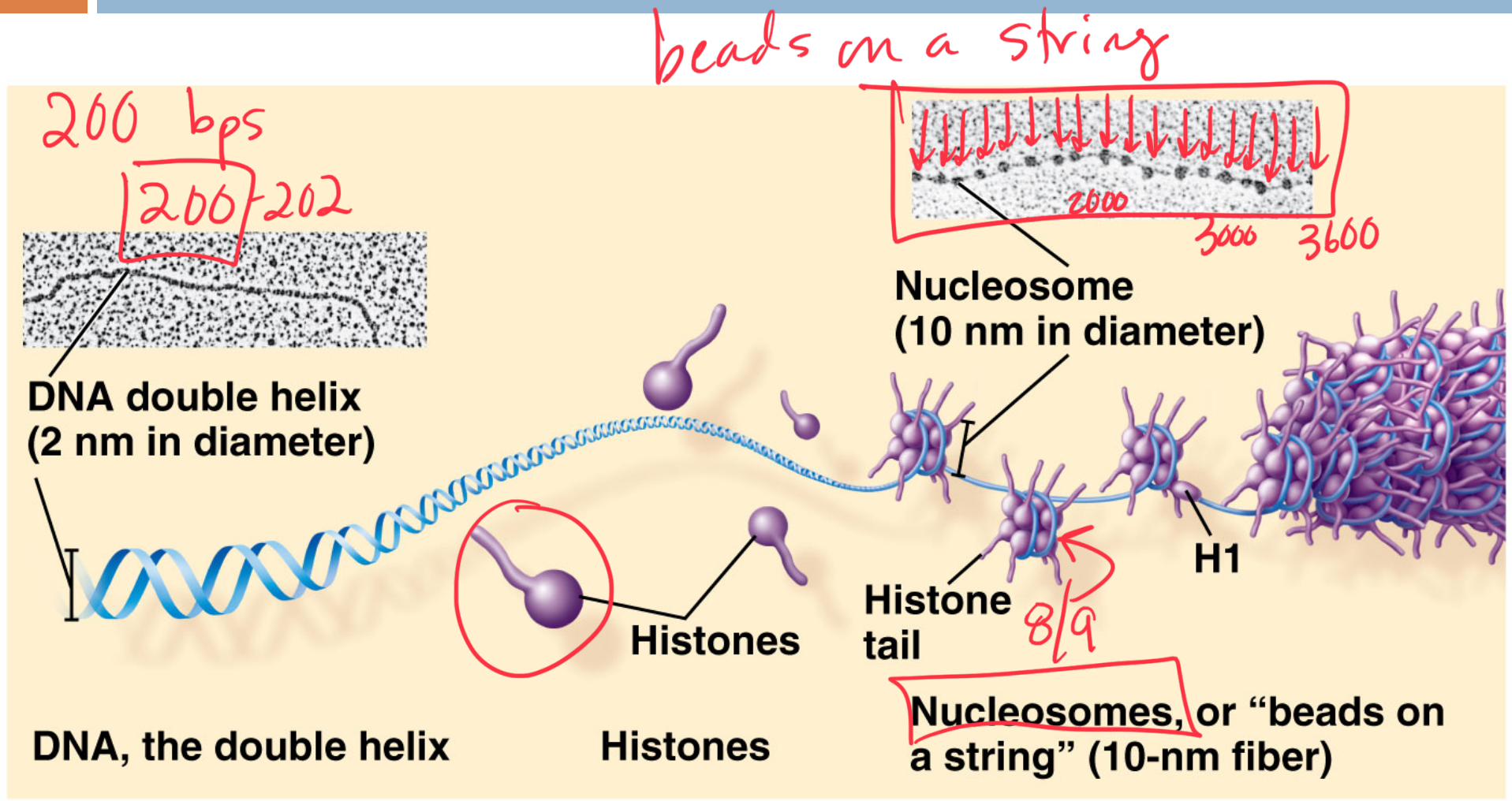
DNA strands are **Antiparallel**

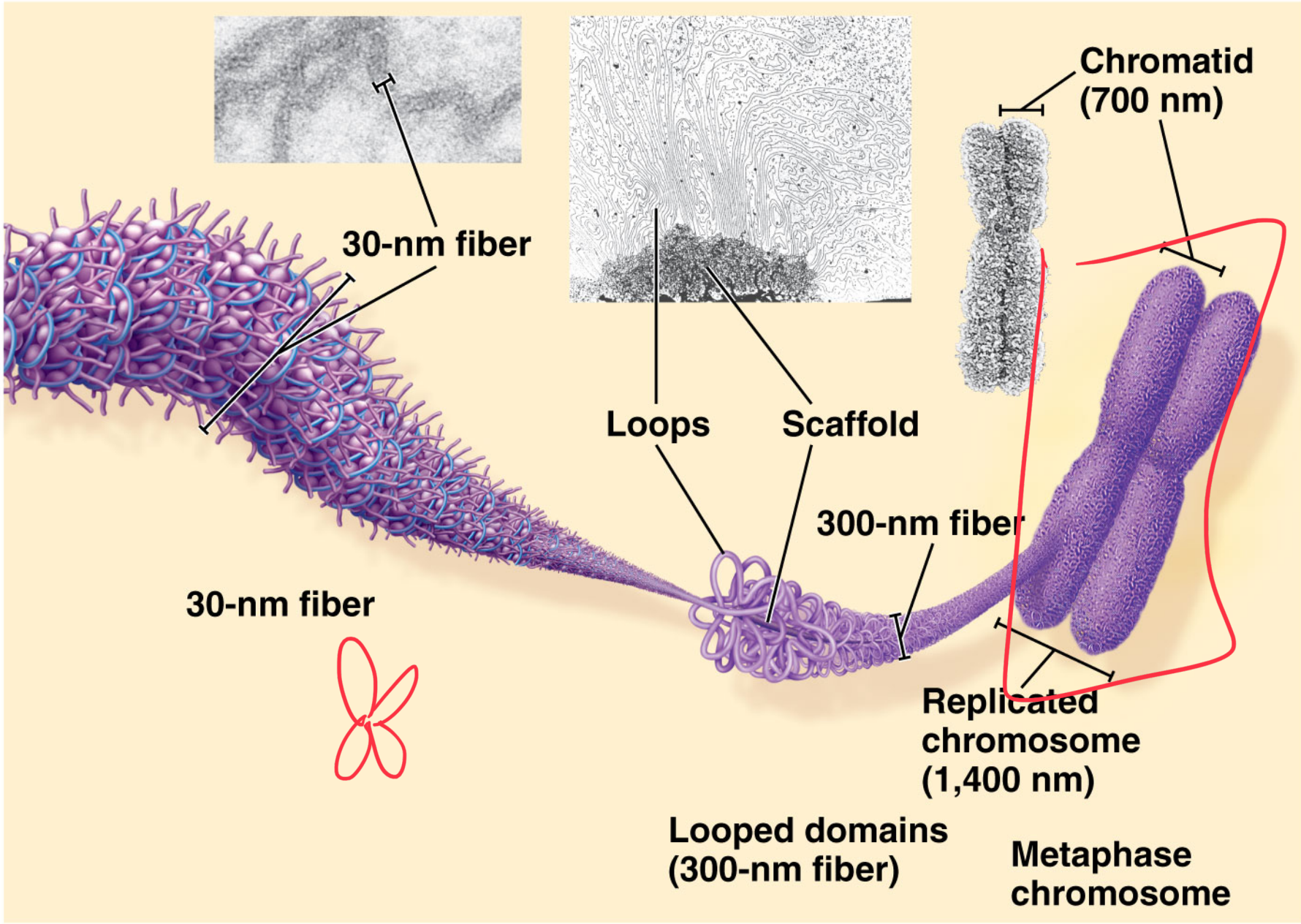
One strand (5'→ 3'), other strand runs in opposite, upside-down direction (3' → 5')



(b) Partial chemical structure

How DNA is packaged





DNA Comparison

Prokaryotic DNA

- Double-stranded
- Circular
- One chromosome
- In cytoplasm
- Supercoiled DNA (nucleoid)
- No histones

Eukaryotic DNA

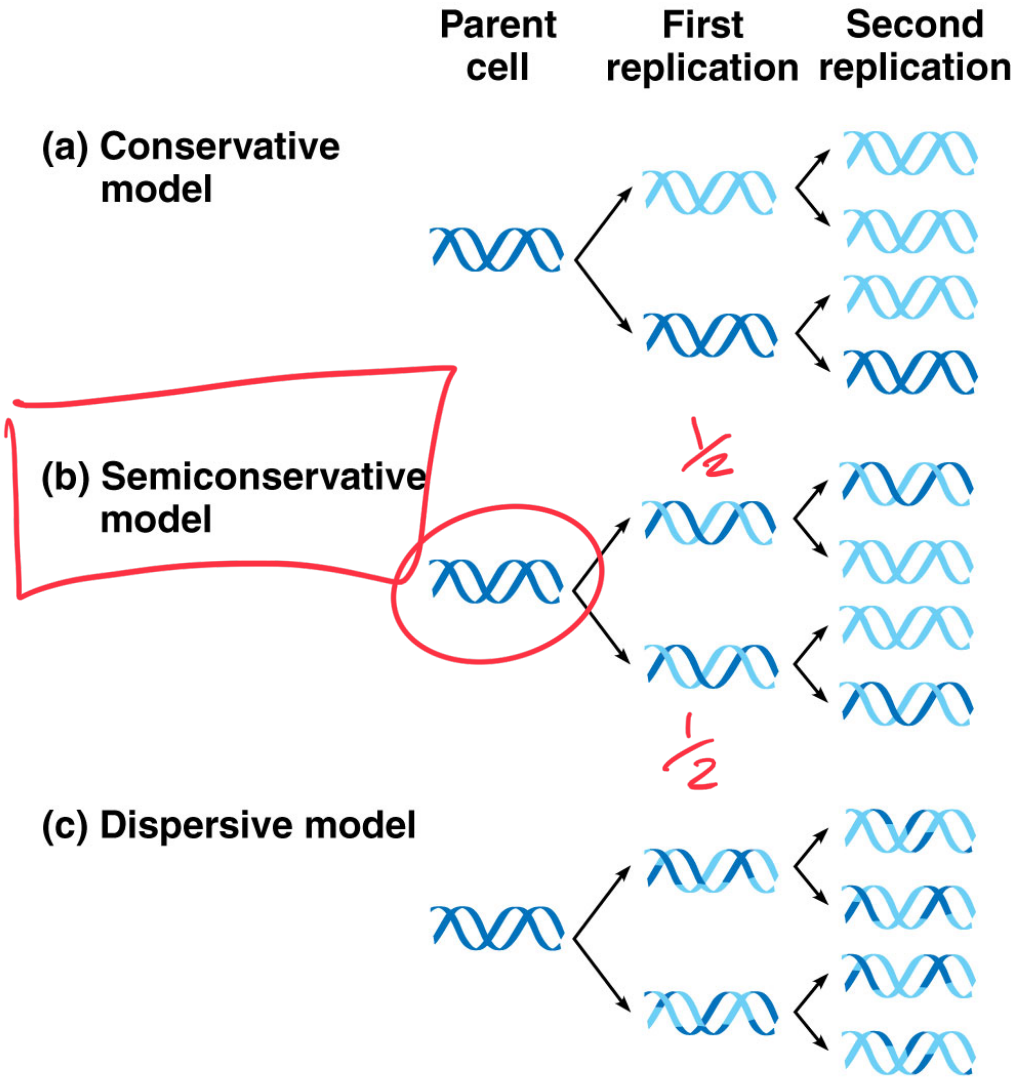
- Double-stranded
- Linear
- Usually 1+ chromosomes
- In nucleus
- Chromatin = DNA wrapped around histones (proteins)

Problem:

How does DNA replicate?

Replication: Making DNA from existing DNA

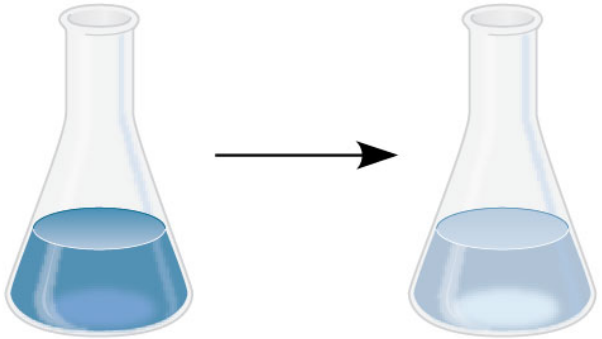
3 alternative models of DNA replication



Meselson & Stahl

Experiment

1. Bacteria cultured in medium with ^{15}N (heavy isotope)



2. Bacteria transferred to medium with ^{14}N (lighter isotope)

Results

3. DNA sample centrifuged after first replication

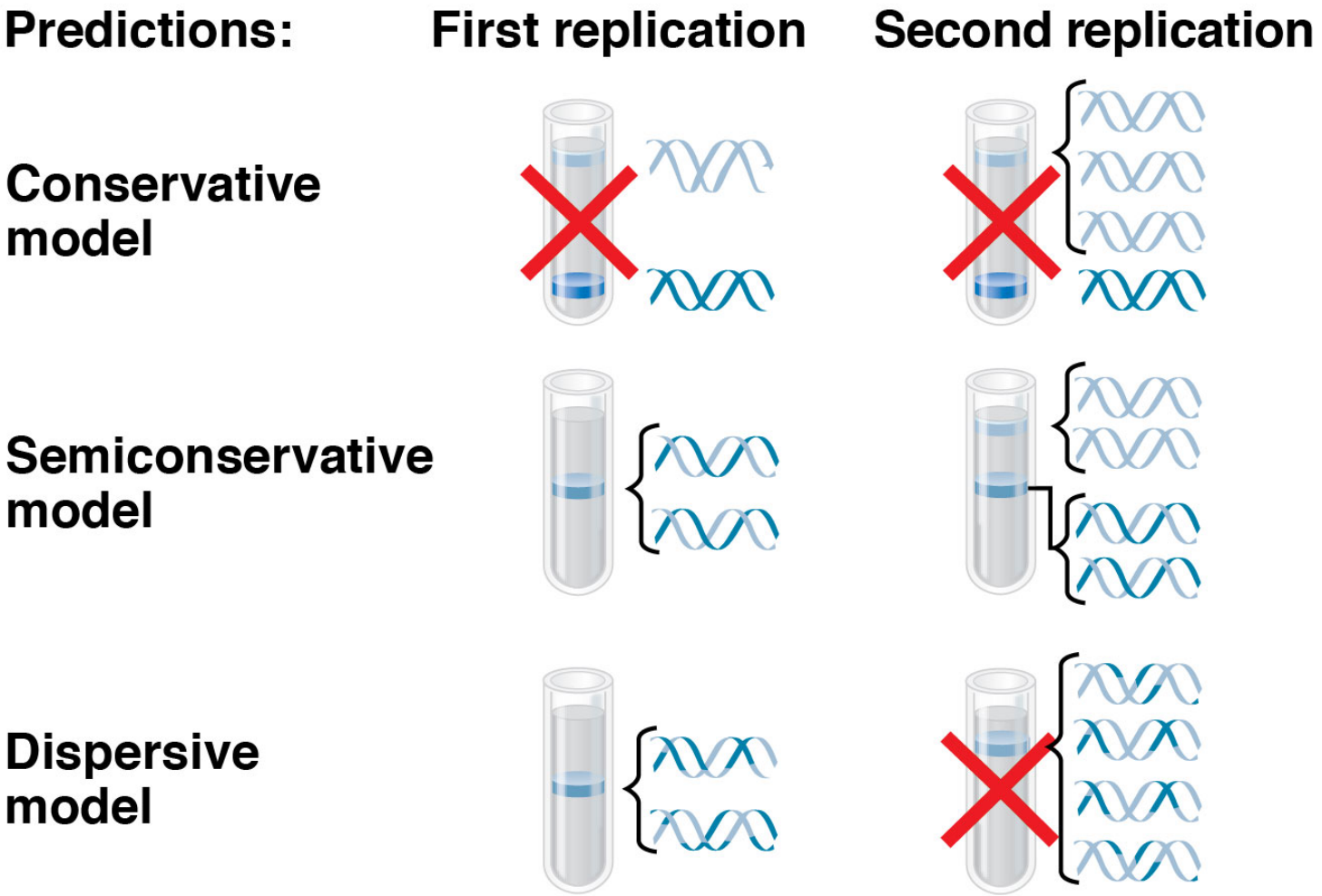
4. DNA sample centrifuged after second replication

Less dense

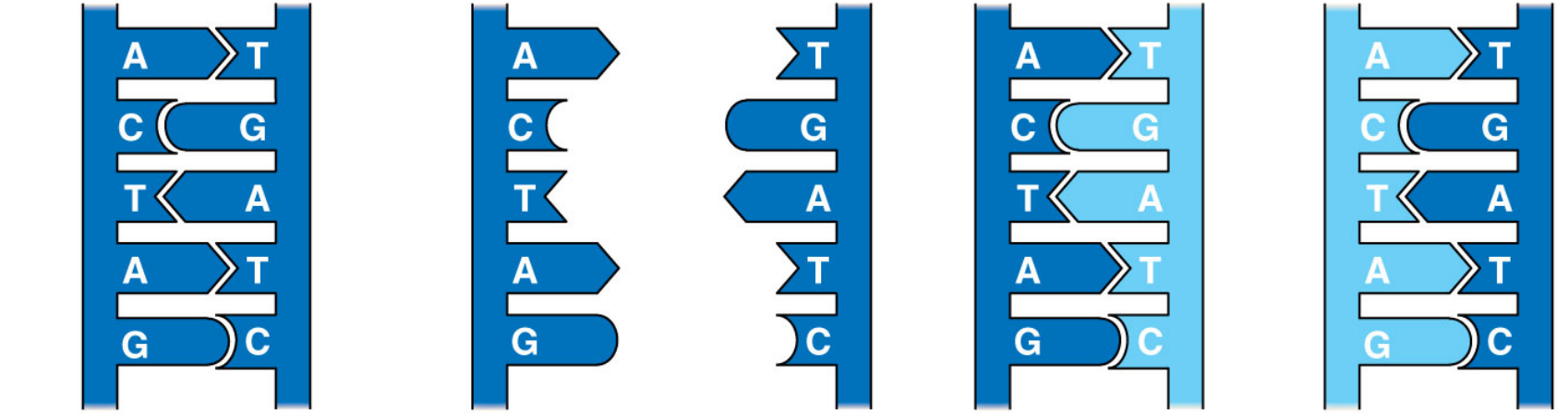
More dense

Meselson & Stahl

Conclusion



Replication is semiconservative



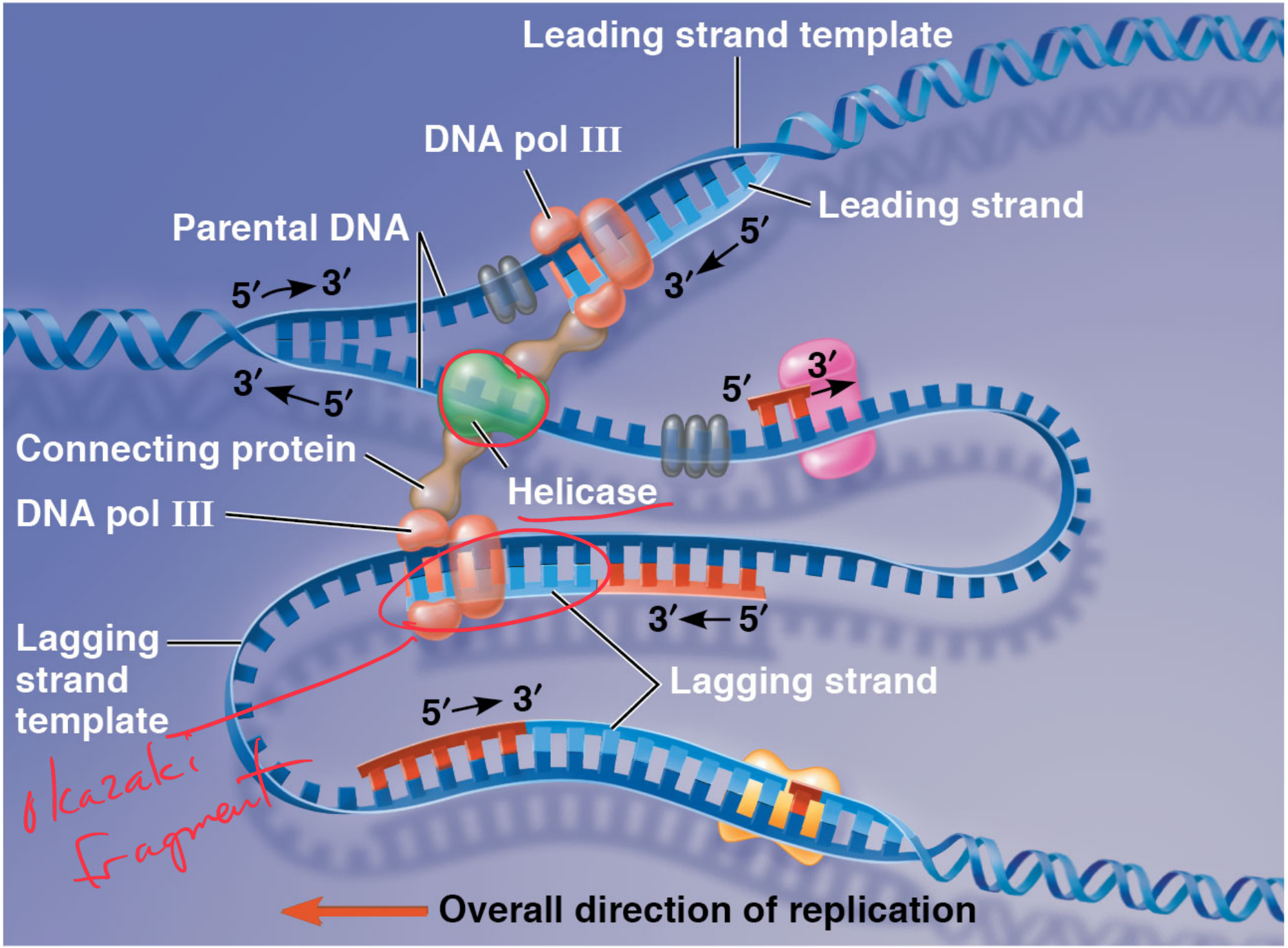
(a) Parent molecule

(b) Separation of strands

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

DNA Replication Video

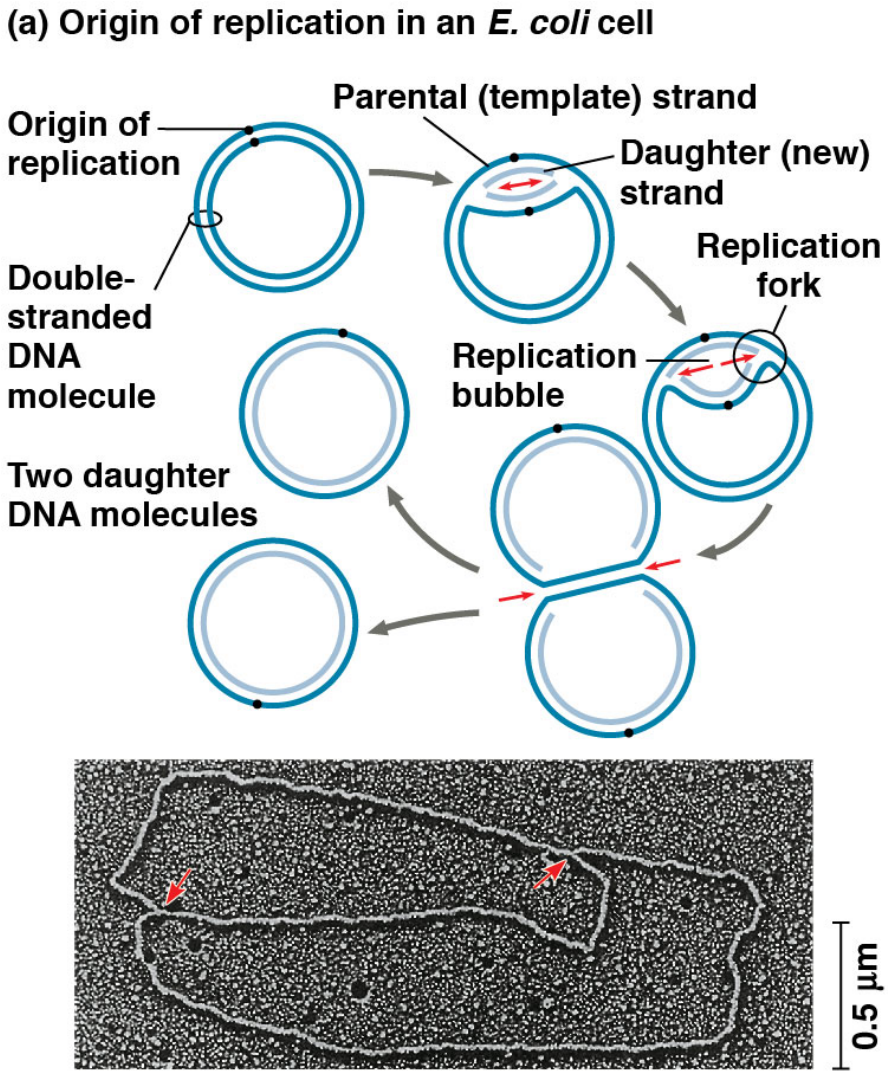
http://www.youtube.com/watch?v=4jtmOZalvS0&feature=related



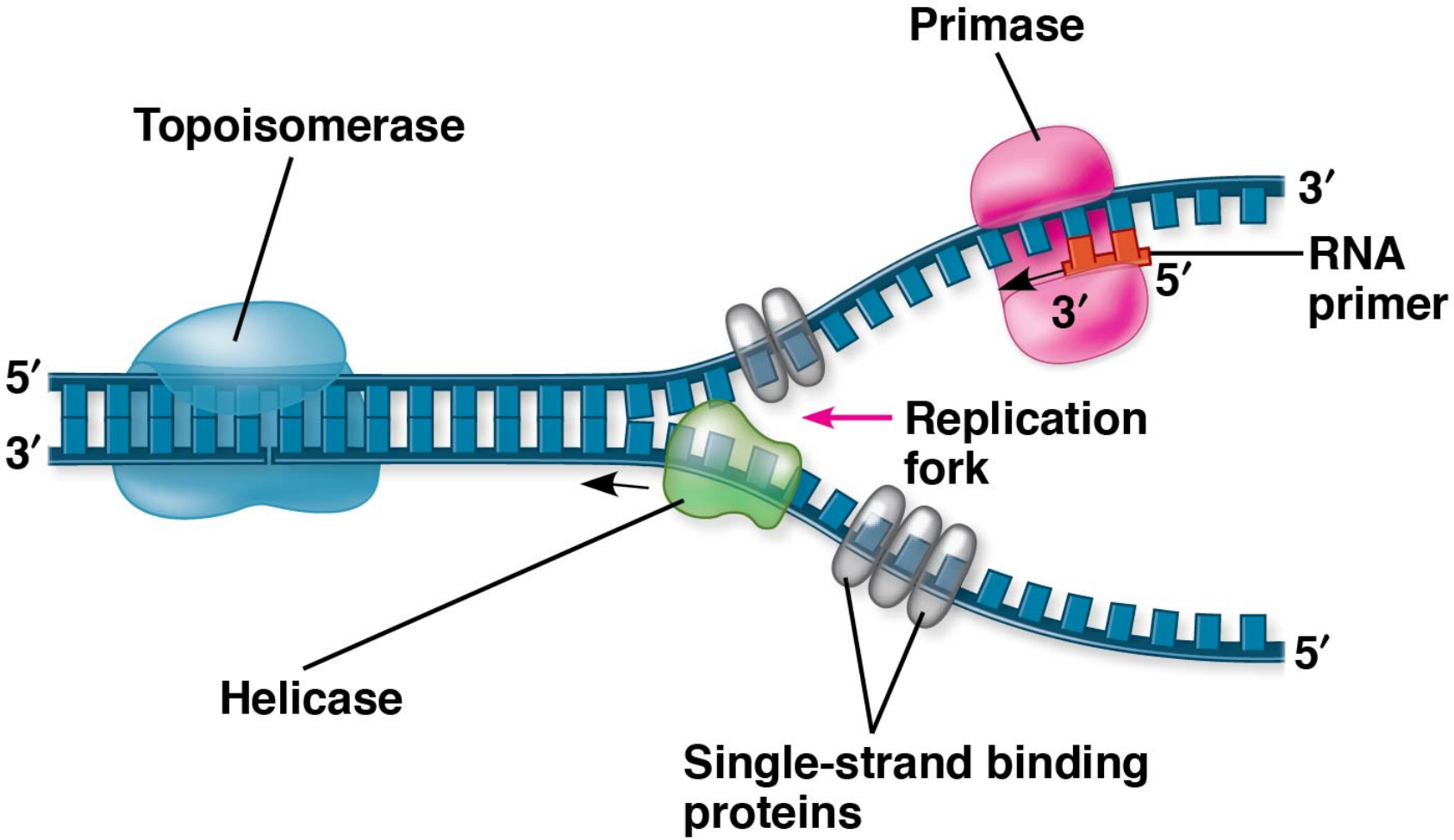
Major Steps of Replication:

1. **Helicase:** unwinds DNA at *origins of replication*
2. Initiation proteins separate 2 strands → forms *replication bubble*
3. **Topoisomerase:** relieves overwinding strain ahead of replication forks by breaking, swiveling, rejoining DNA strands
4. **Primase:** puts down RNA primer to start replication
5. **DNA polymerase III:** adds complimentary bases to *leading strand* (new DNA is made 5' → 3')
6. *Lagging strand* grows in 3'→5' direction by the addition of *Okazaki fragments*
7. **DNA polymerase I:** replaces RNA primers with DNA
8. **DNA ligase:** seals fragments together

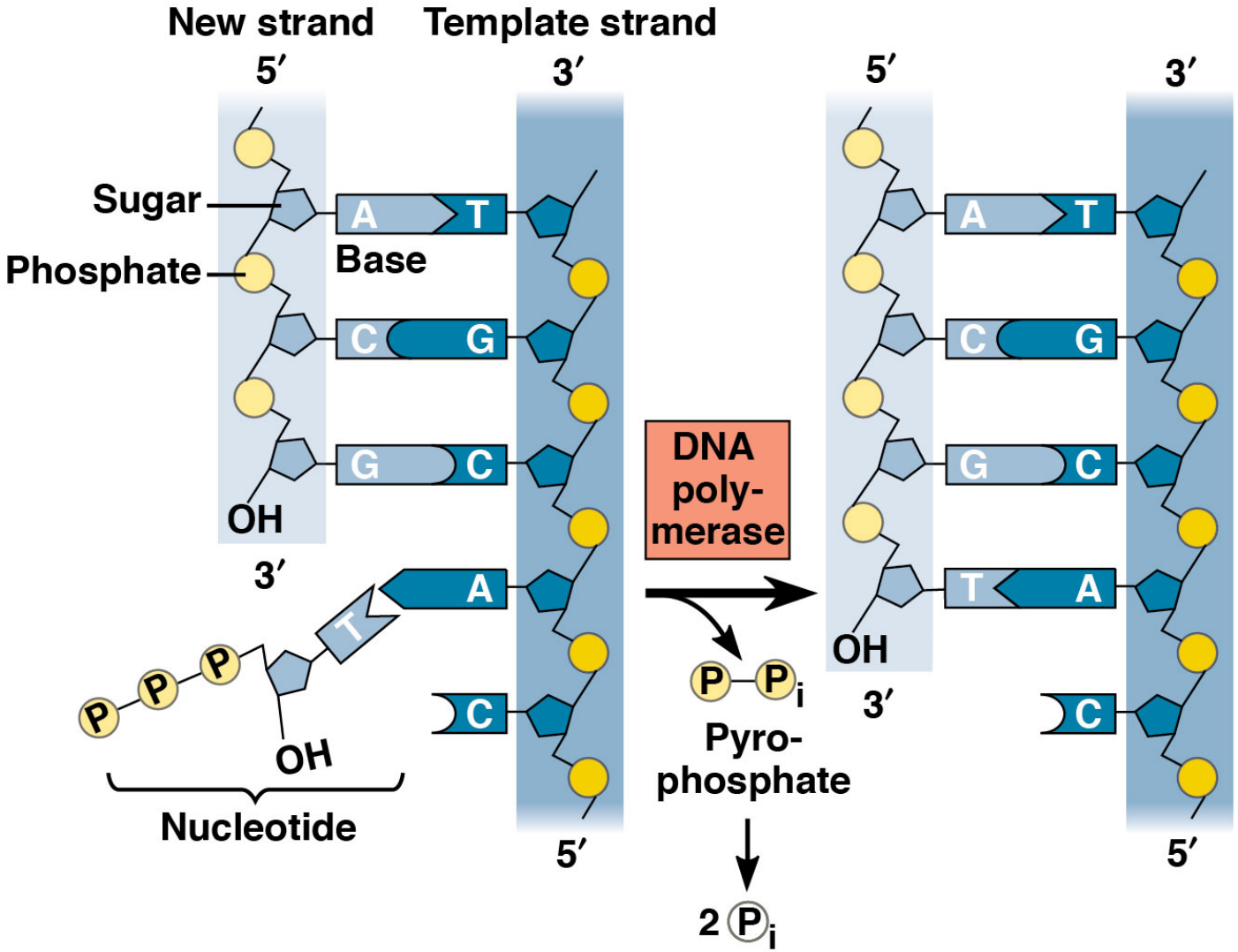
1. Helicase unwinds DNA at *origins of replication* and creates *replication forks*



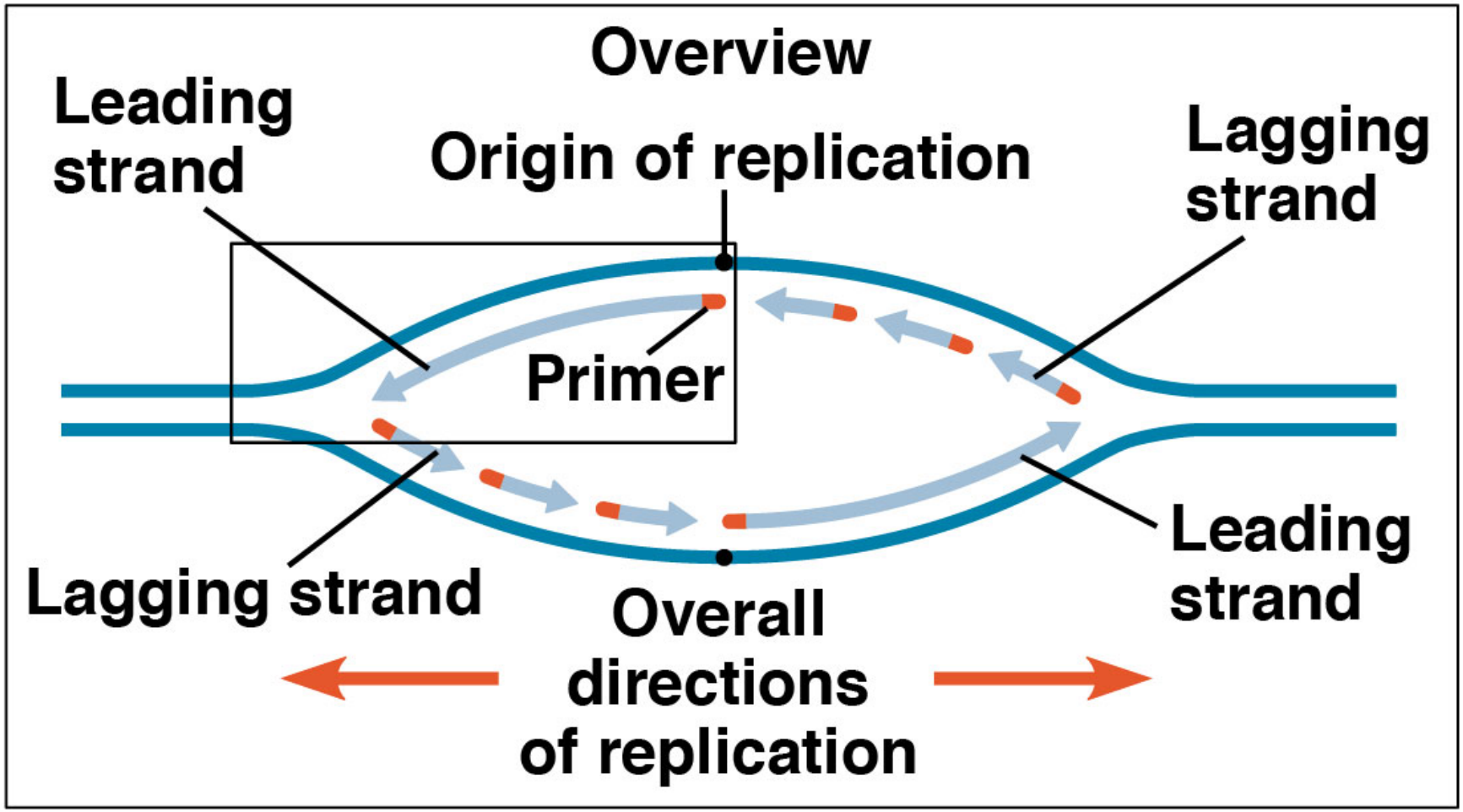
4. Primase adds *RNA primer*



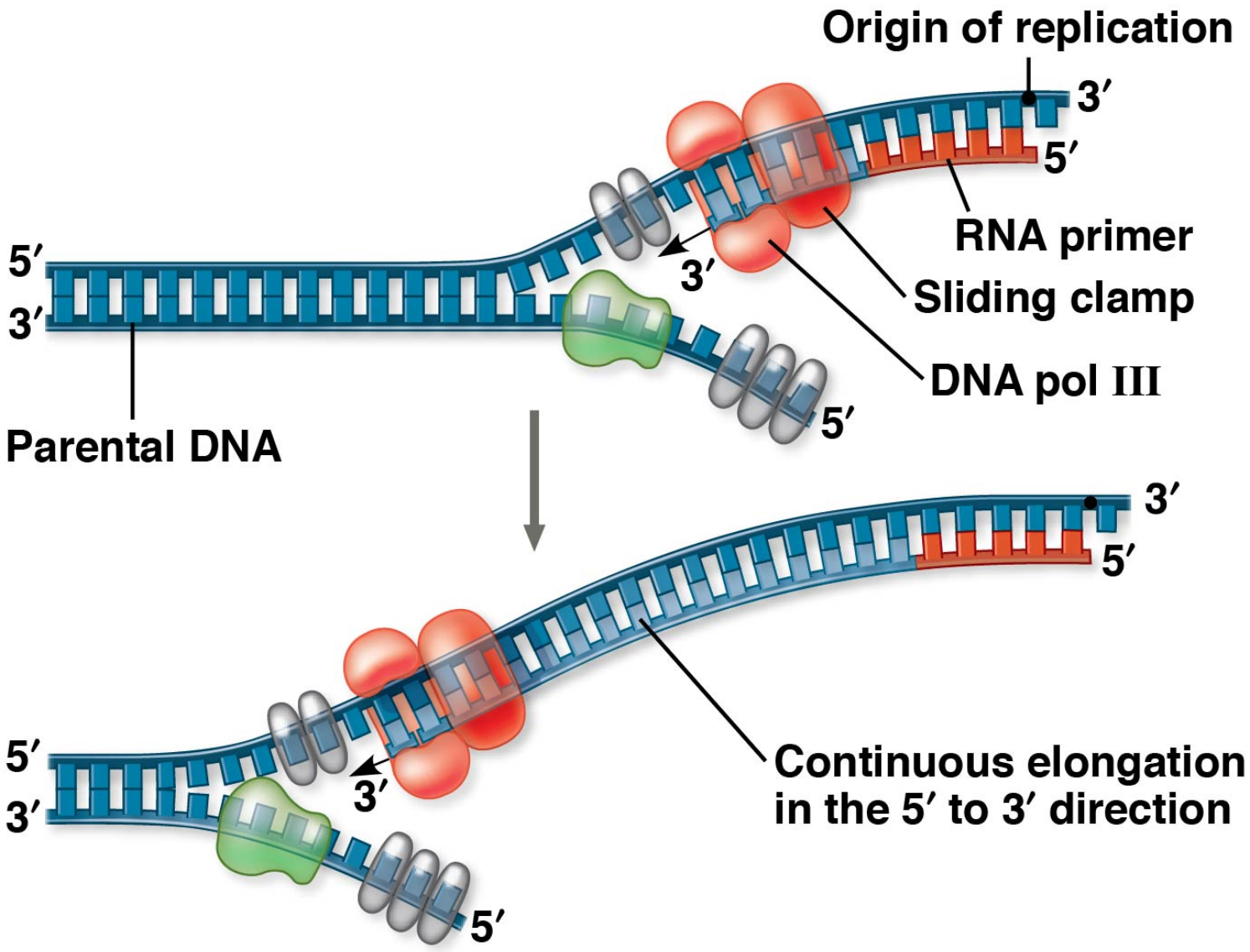
5. DNA polymerase III adds nucleotides in 5'→3' direction on *leading strand*



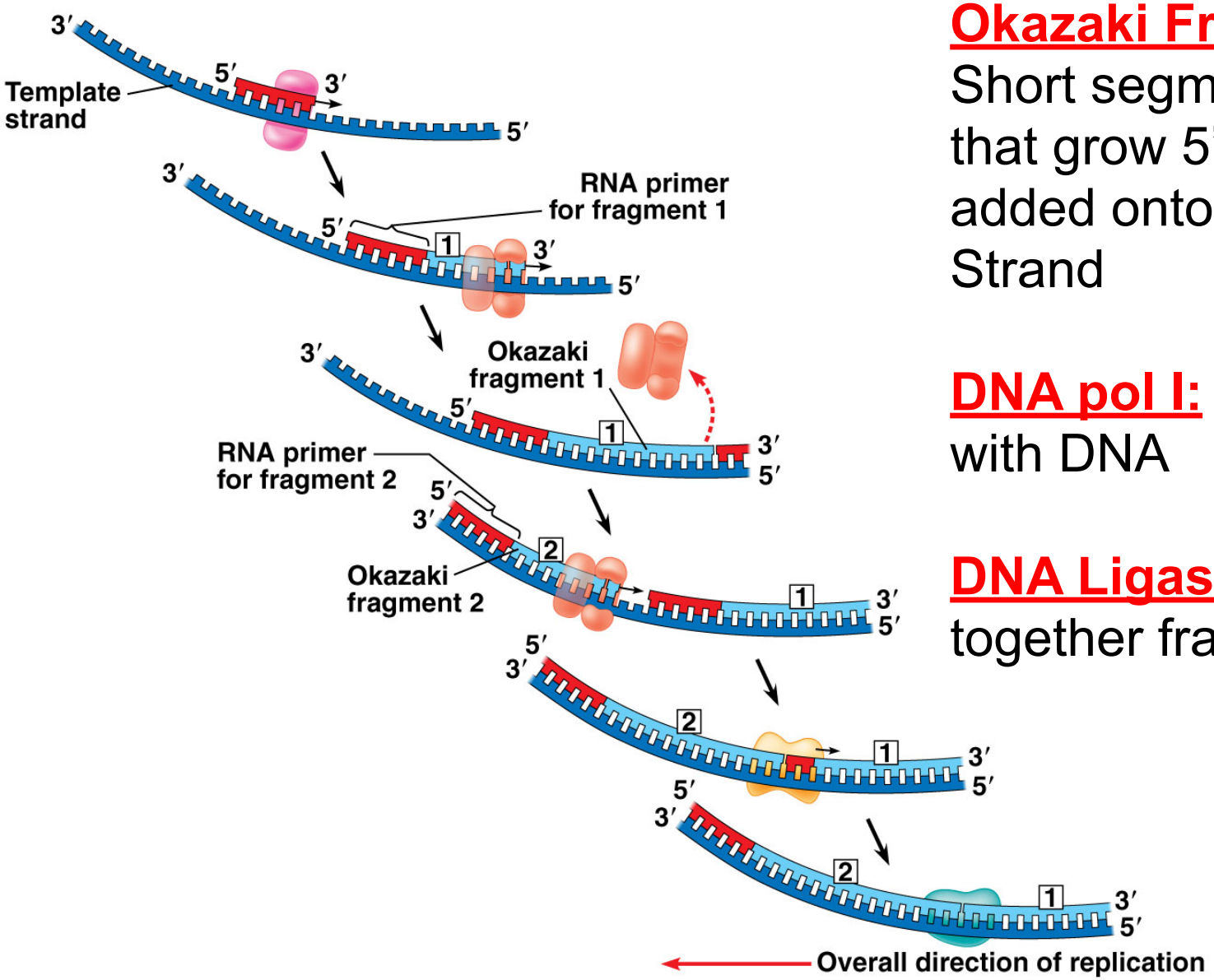
Leading strand vs. *Lagging strand*



Replication on leading strand



Replication on lagging strand

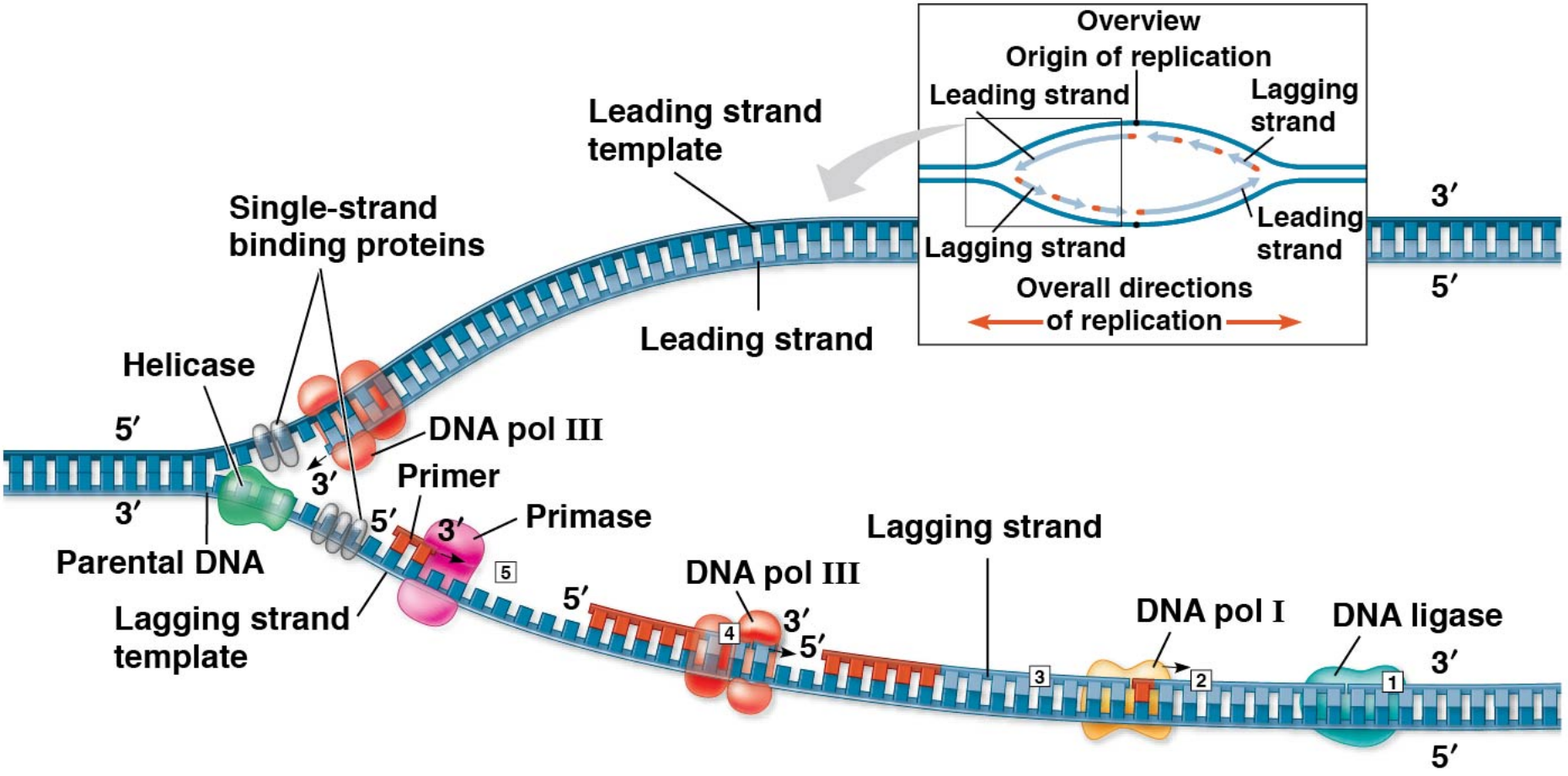


Okazaki Fragments: Short segments of DNA that grow 5'→3' that are added onto the Lagging Strand

DNA pol I: replace RNA with DNA

DNA Ligase: seals together fragments

Summary of DNA Replication



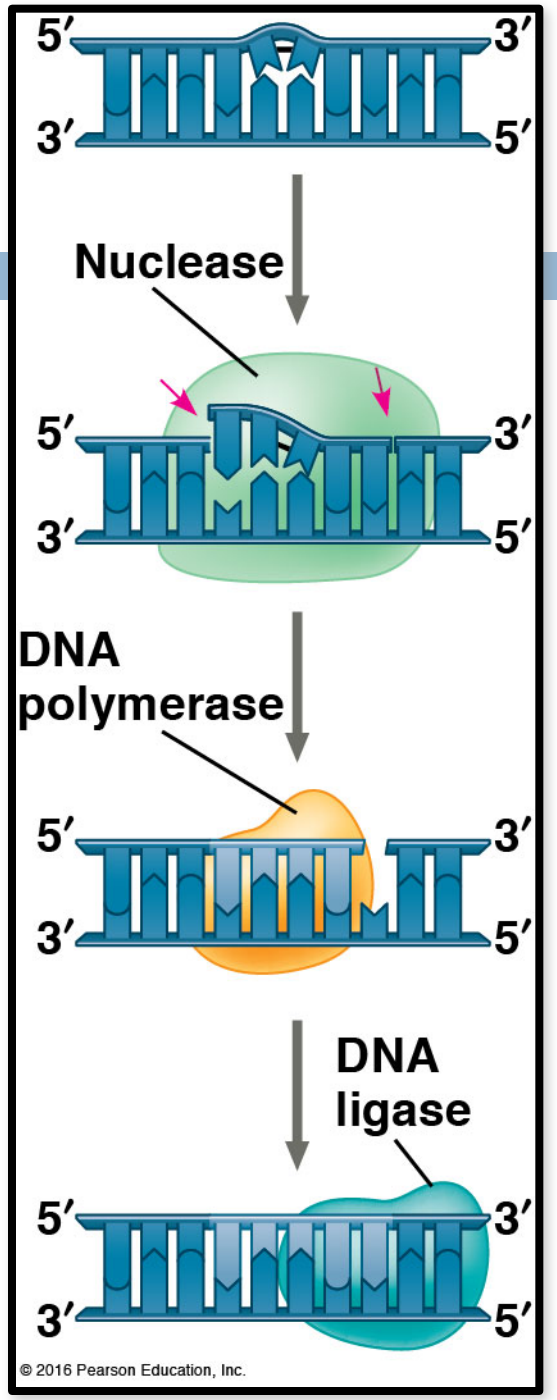
Proofreading and Repair

- DNA polymerases proofread as bases added
- Errors:
  - Pairing errors: 1 in 100,000 nucleotides
  - Complete DNA: 1 in 10 billion nucleotides

- Mismatch repair: special enzymes fix incorrect pairings

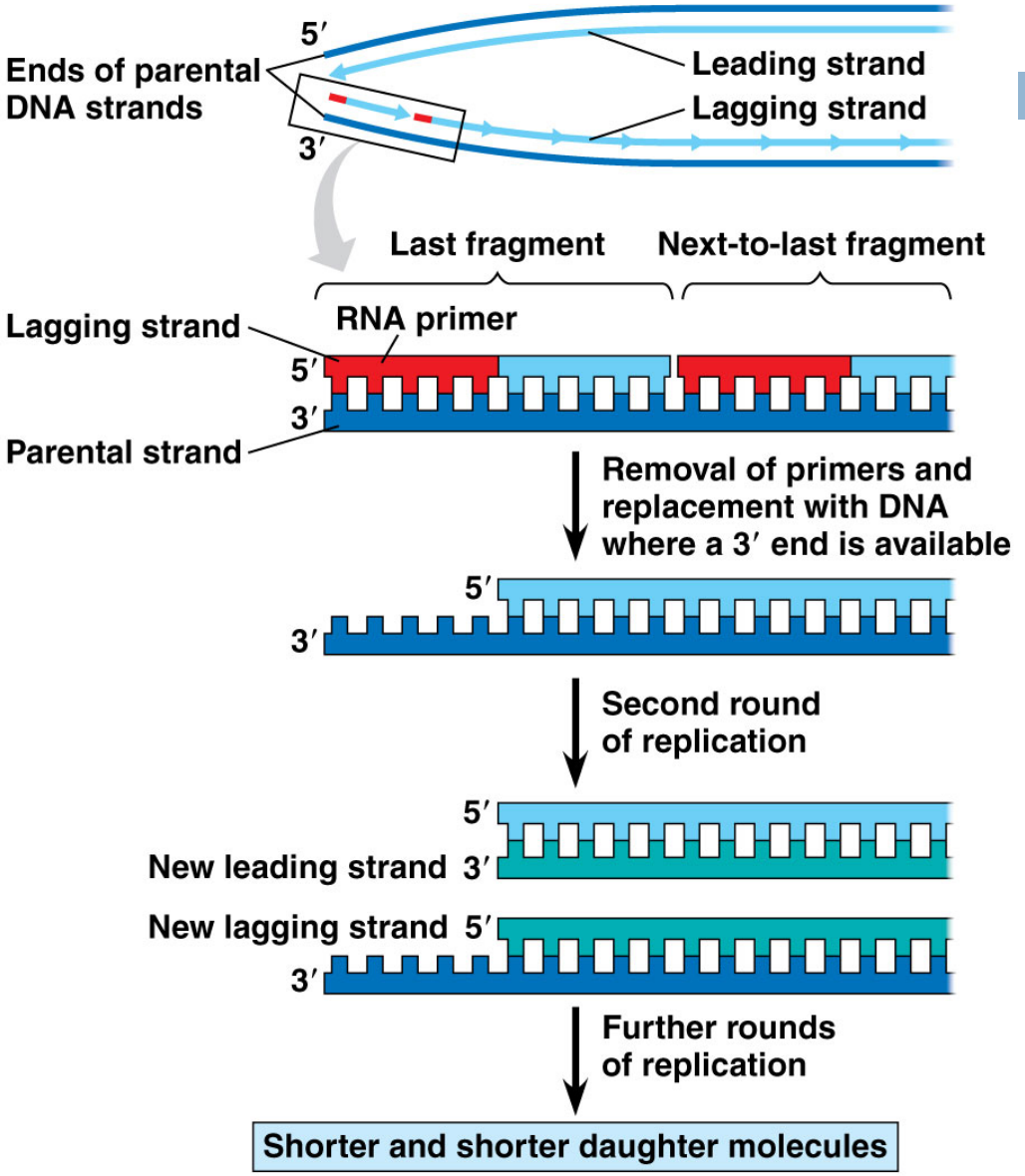
Nucleotide Excision Repair

- **Nucleases** cut damaged DNA
- DNA poly and ligase fill in gaps



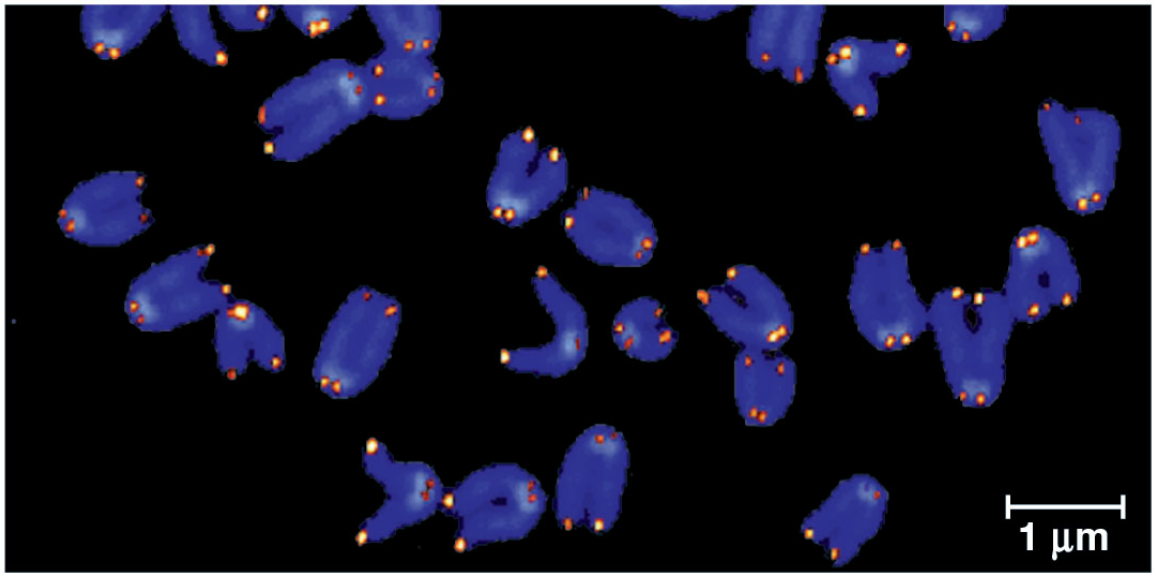
Problem at the 5’ End

- DNA poly only adds nucleotides to 3’ end
- No way to complete 5’ ends of daughter strands
- Over many replications, DNA strands will grow shorter and shorter



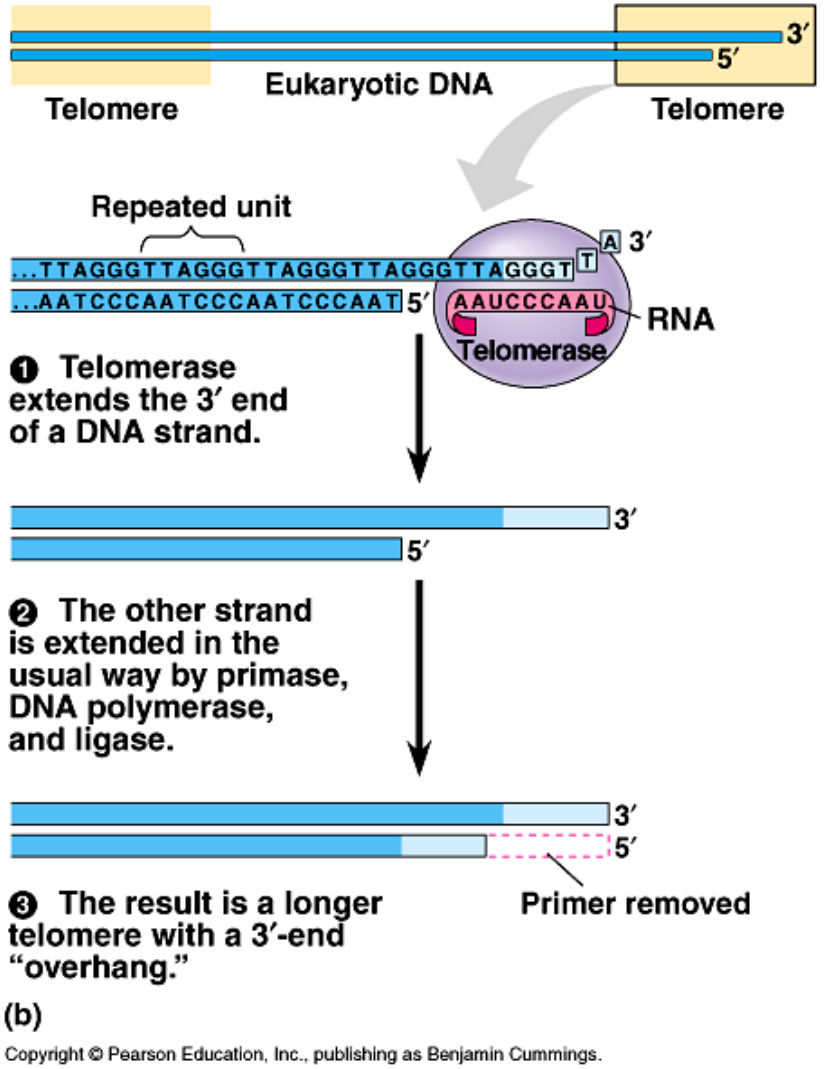
Telomeres: repeated units of short nucleotide sequences (TTAGGG) at ends of DNA

- Telomeres "cap" ends of DNA to postpone erosion of genes at ends (TTAGGG)
- **Telomerase**: enzyme that adds to telomeres
  - Eukaryotic germ cells, cancer cells



Telomeres stained orange at the ends of mouse chromosomes

Telomeres & Telomerase



BioFlix: DNA Replication

http://media.pearsoncmg.com/bc/bc_0media_bio/bioflix/bioflix.htm?8apdnarep