Ch. 16 Warm-Up

1. Draw and label a nucleotide.

2. Why is DNA a double helix?

3. What is the complementary DNA strand to:

   DNA: A T C C G T A T G A A C

Ch. 16 Warm-Up

1. What was the contribution made to science by these people:
   A. Hershey and Chase
   B. Franklin
   C. Watson and Crick
2. Chargaff's Rules: If cytosine makes up 22% of the nucleotides, then adenine would make up ___ % ?
3. Explain the semiconservative model of DNA replication.

Ch. 16 Warm-Up

1. What is the function of the following:
   A. Helicase
   B. DNA Ligase
   C. DNA Polymerase (I and III)
   D. Primase
   E. Nuclease
2. How does DNA solve the problem of slow replication on the lagging strand?
3. Code the complementary DNA strand: 3' T A G C T A A G C T A C 5'
4. What is the function of telomeres?

THE MOLECULAR BASIS OF INHERITANCE

Chapter 16

What you must know

- The structure of **DNA.**
- The major steps to **replication.**
- The difference between **replication, transcription,** and **translation.**
- The general **differences** between the **bacterial chromosome** and **eukaryotic chromosomes.**
- How DNA is packaged into a **chromosome.**

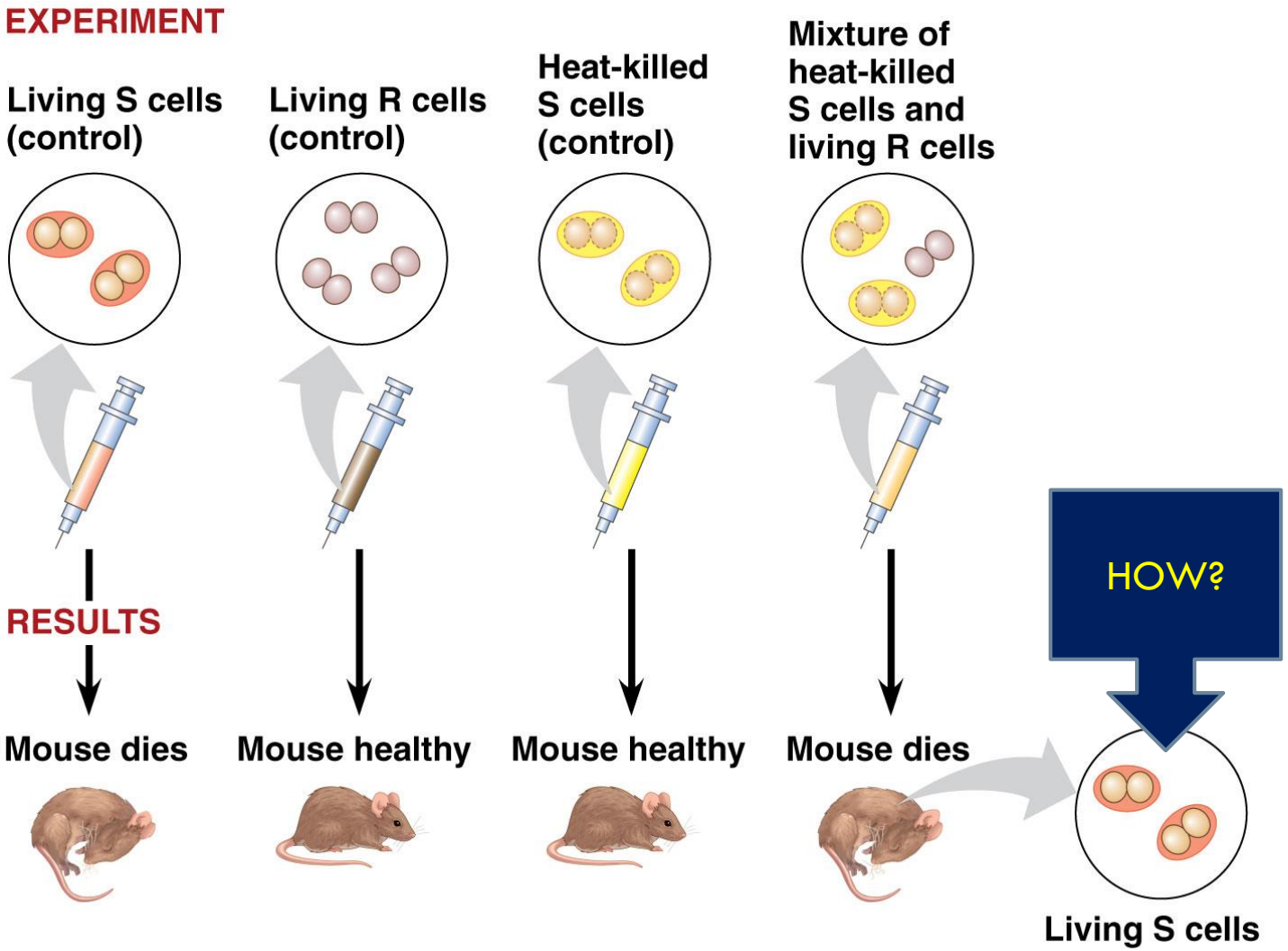
Problem:

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

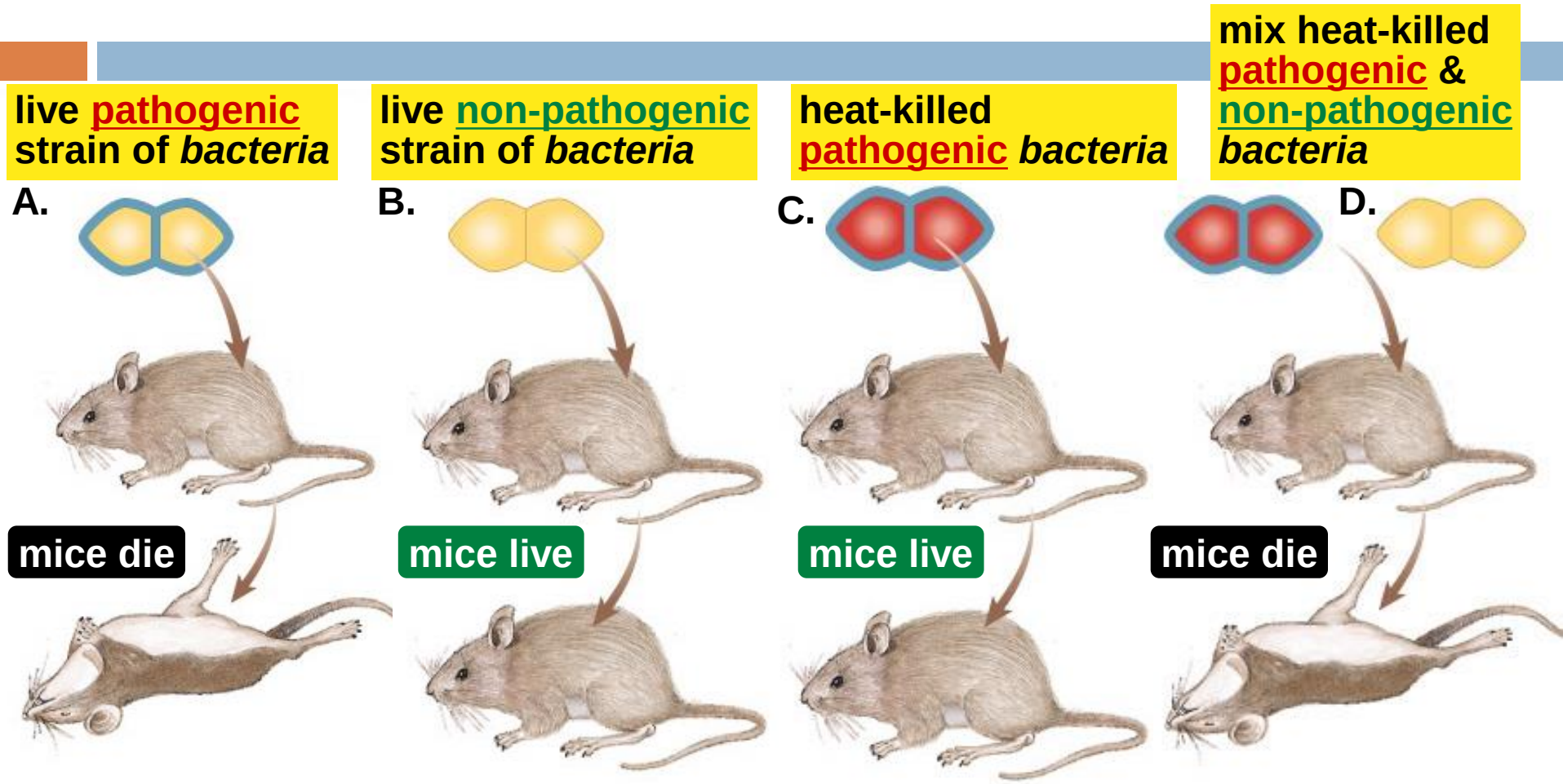
Frederick Griffith (1928)

S strain of bacteria
= pathogenic
(causes pneumonia in mice)

R strain of bacteria
= non-pathogenic



The "Transforming Principle"



Transformation = change in phenotype

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

Frederick Griffith (1928)

Conclusion: living R bacteria transformed into deadly S bacteria by an unknown, heritable substance *(R bacteria must have acquired something from the S bacteria)*

- *Still unclear WHAT the transforming, heritable substance was…until…*

Oswald Avery, *et al.* (1944)

- Discovered that the transforming agent was DNA… But still skepticism from scientific community

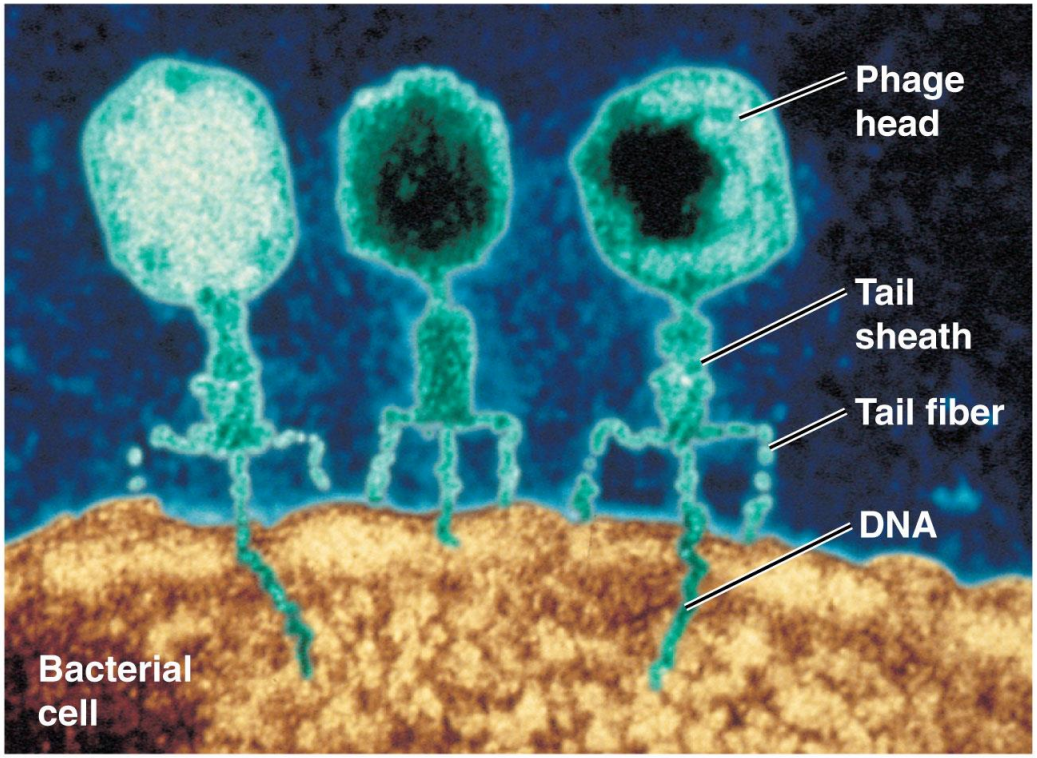
Hershey and Chase (1952)

To answer the question: Is DNA or PROTEIN the heritable substance that determines traits?

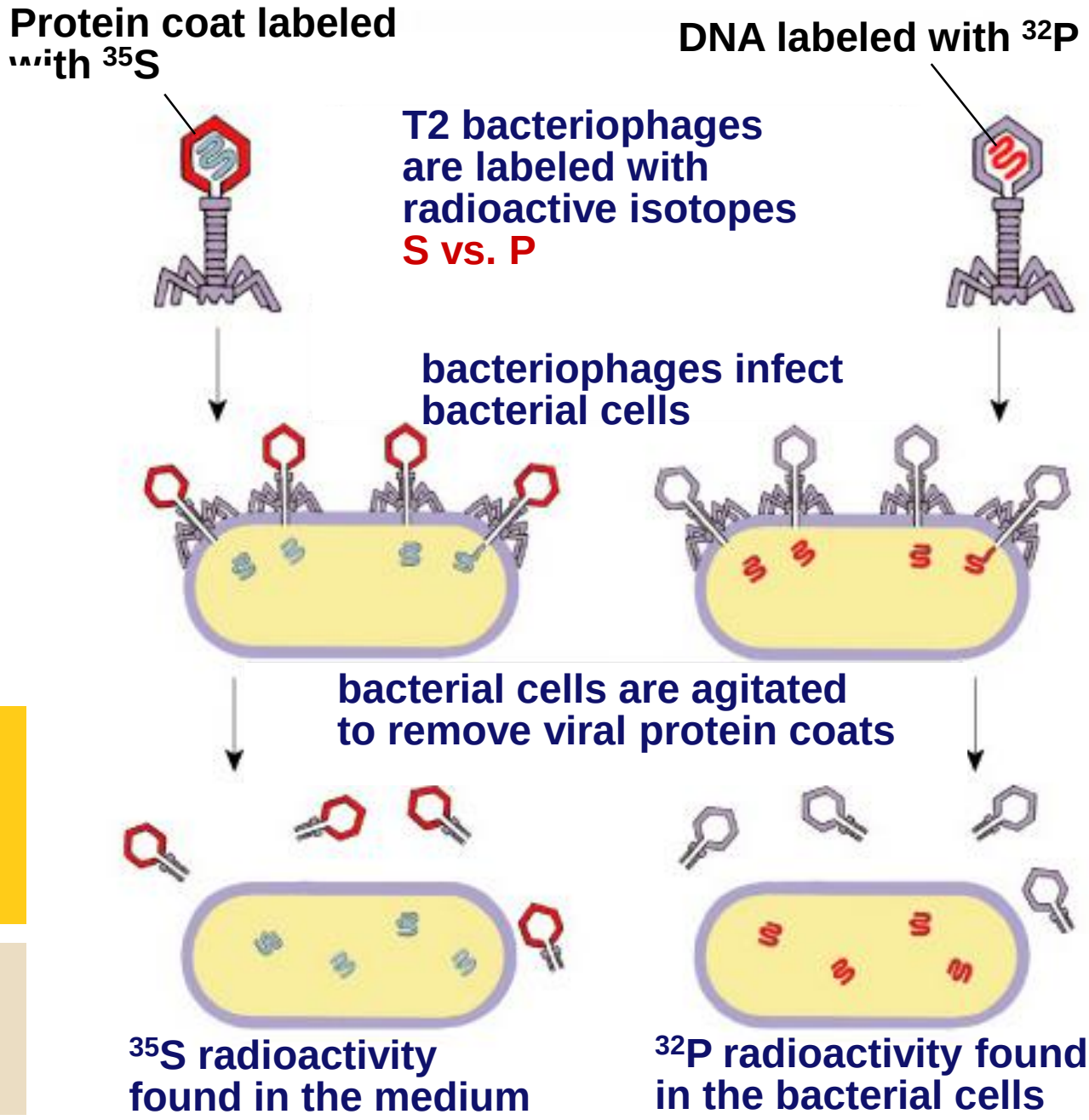
- Bacteriophages: virus that infects bacteria; composed of DNA and protein

Protein = radiolabel S
(radioactive sulfur label)

DNA = radiolabel P
(radioactive phosphorus label)



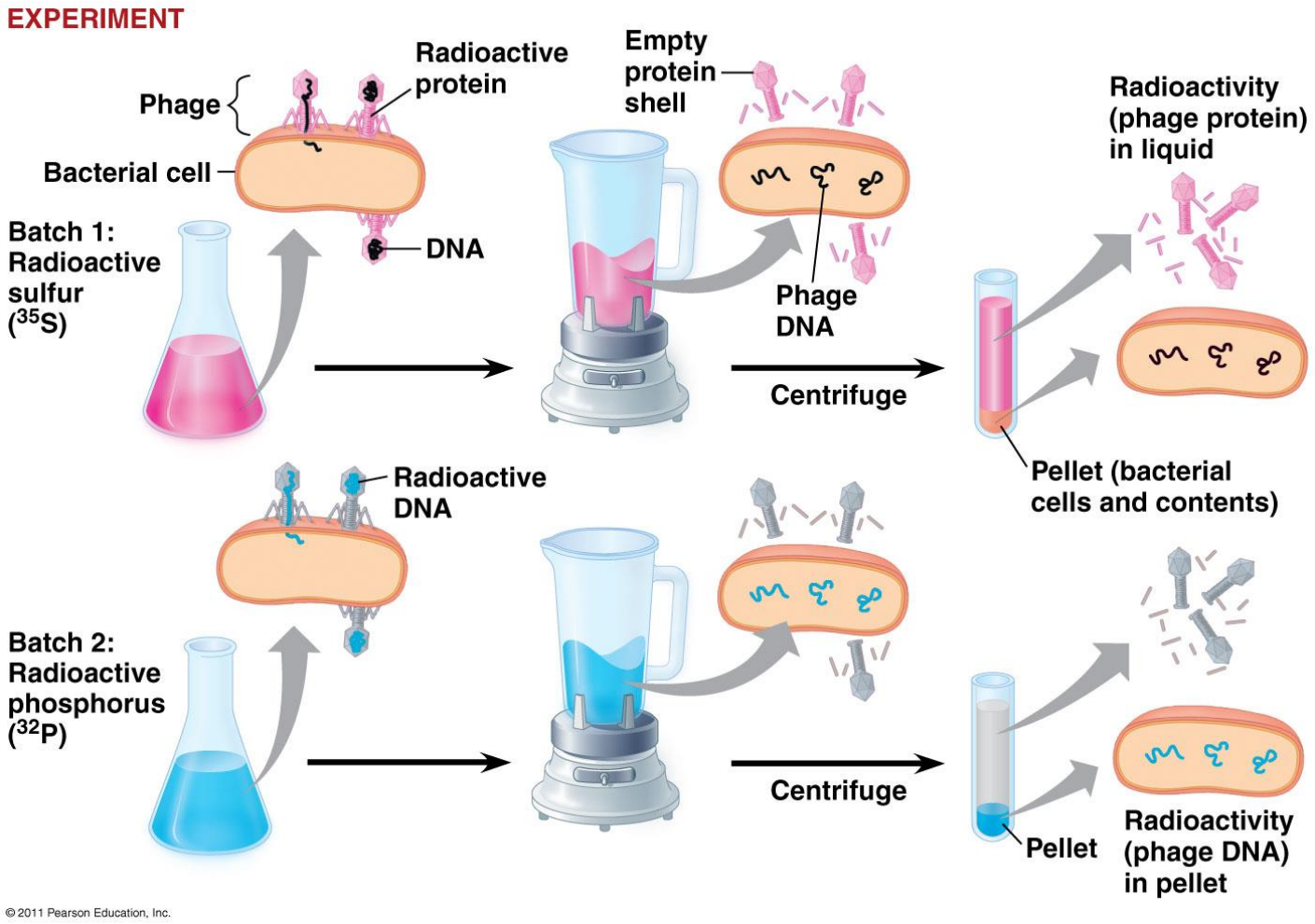
Hershey & Chase



Which radioactive marker is found inside the cell?

Which molecule carries viral genetic info?

Hershey and Chase (1952)



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

Hershey & Chase

1952 | 1969
Hershey

Martha Chase **Alfred Hershey**

Review and Reflect

1. What question **Frederick Griffith** investigating?

2. Describe his experiment to a peer.

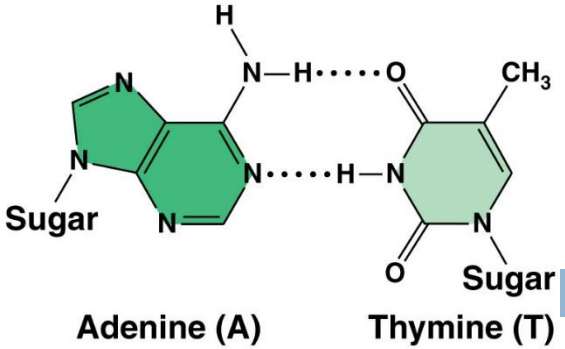
3. What did he discover?

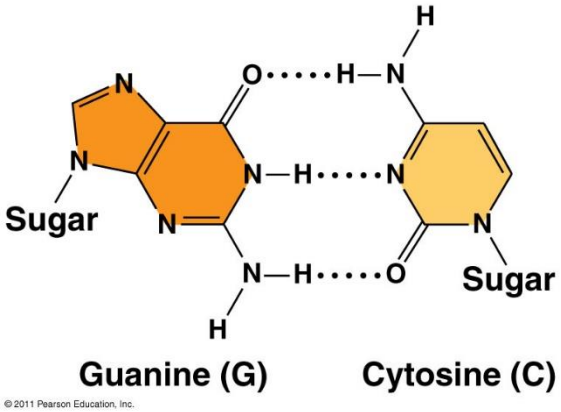
4. How did he make his conclusion?

Review and Reflect

1. What question was Hershey and Chase investigating?
2. Describe their experiment to a peer.
3. What was their final conclusion?
4. What was their evidence to support this conclusion?

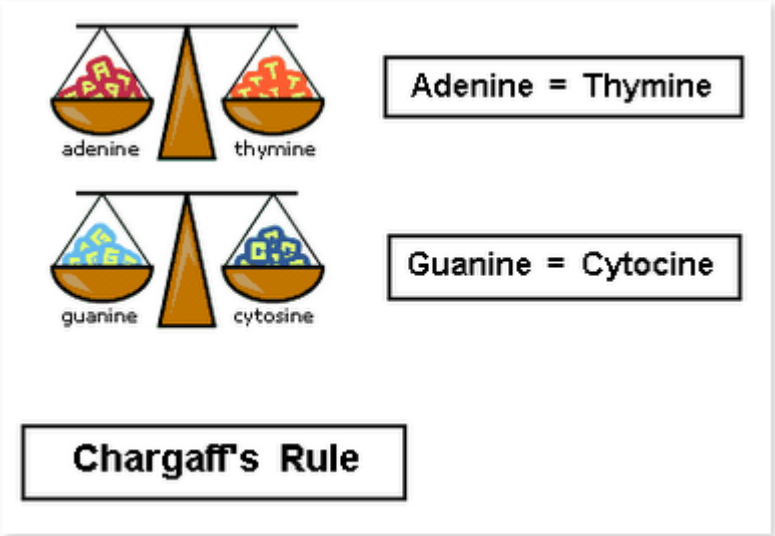
Edwin Chargaff (1947)





Chargaff's Rules:

- DNA composition varies between species



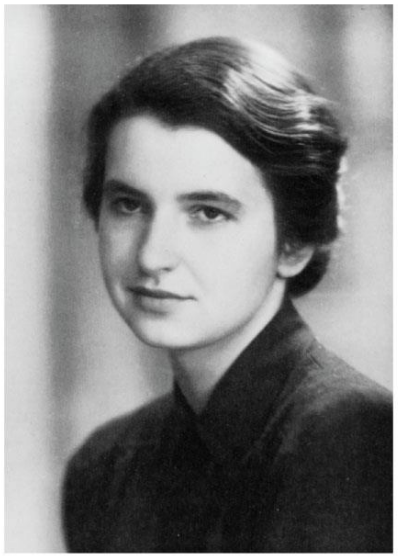
Ratios:

%A = %T and

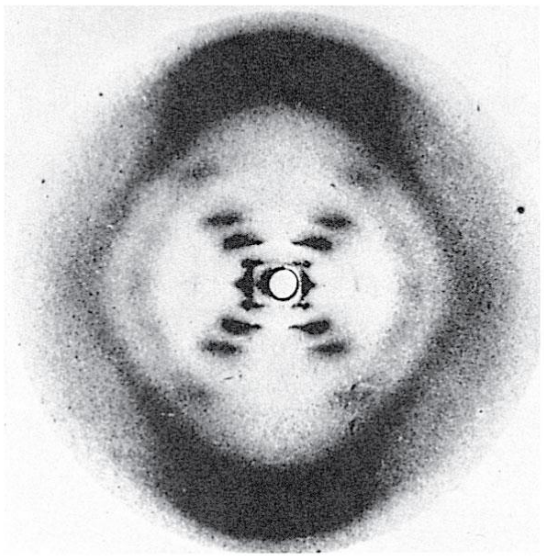
%G = %C

Rosalind Franklin (1950's)

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



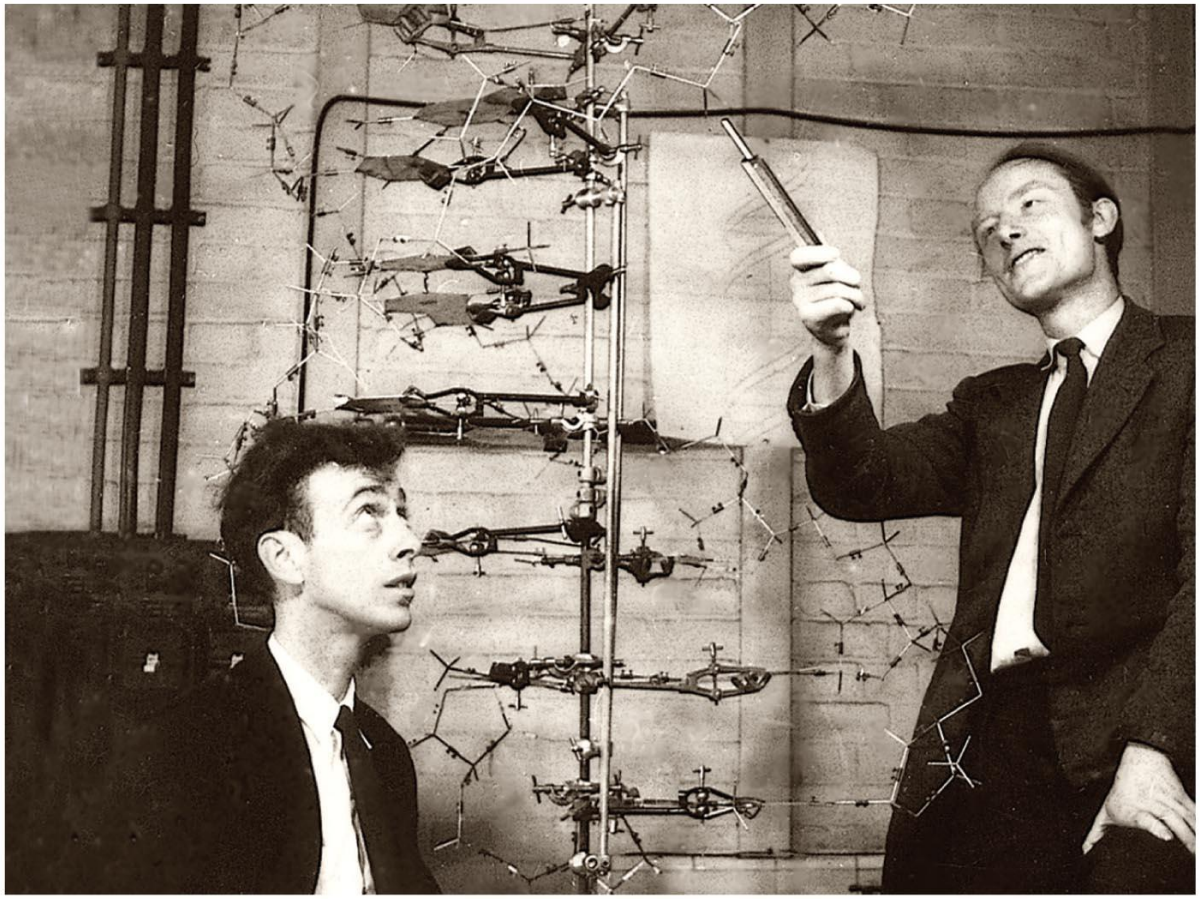
(a) Rosalind Franklin



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

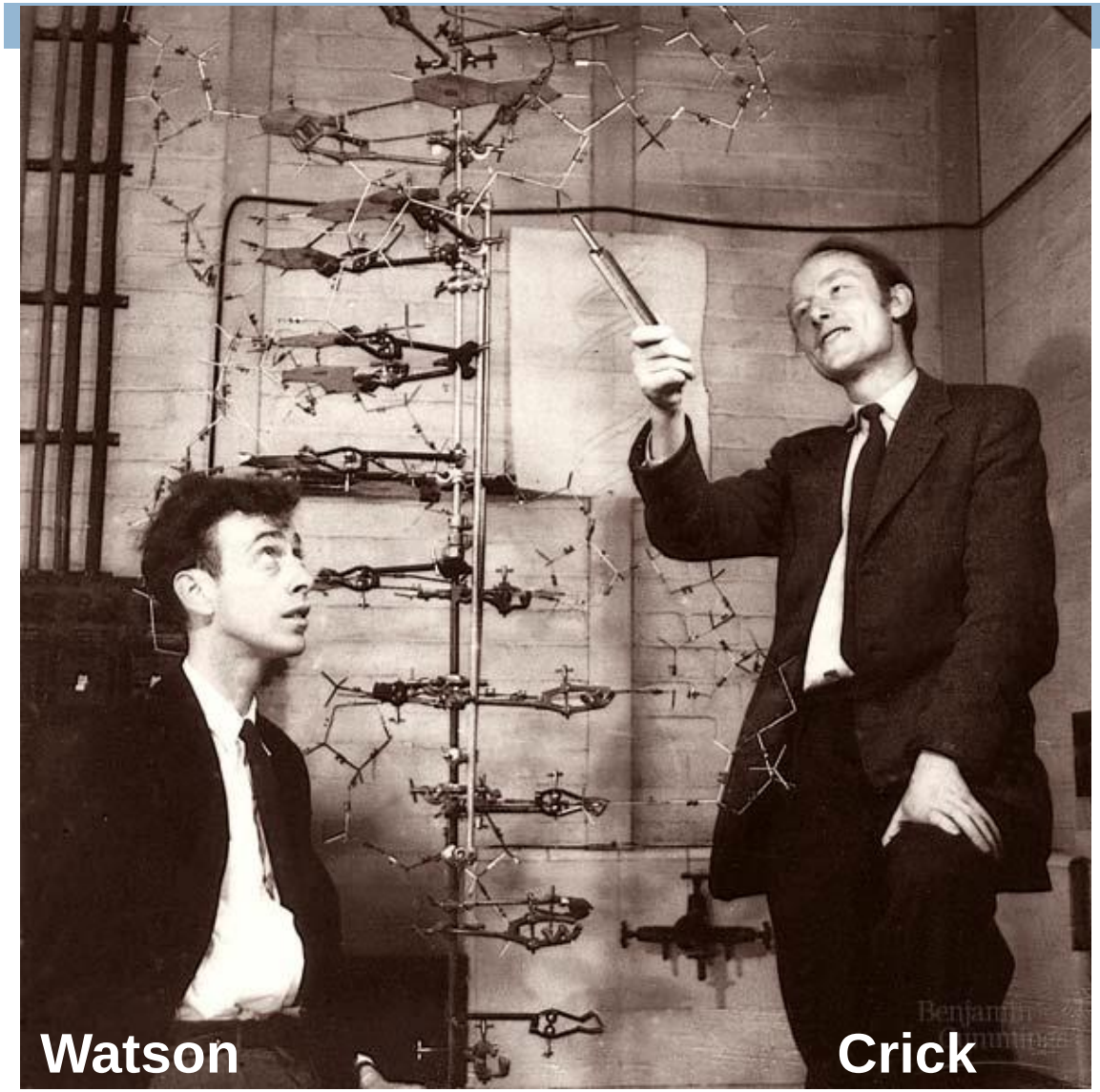
James Watson & Francis Crick (1953)

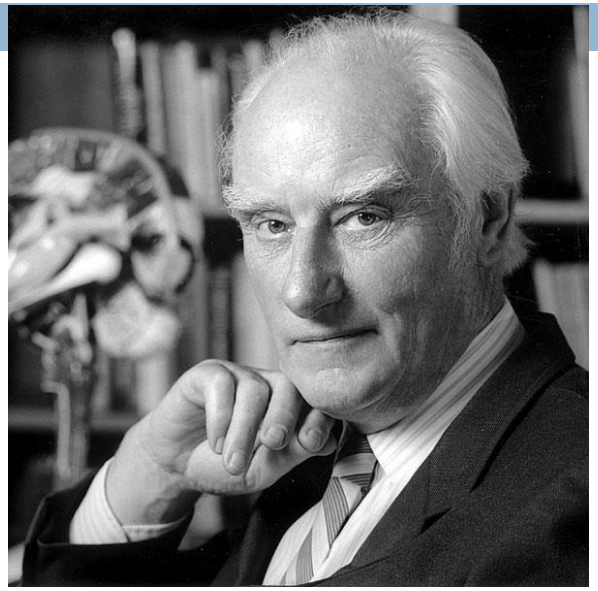
- Discovered the double helix by building models to conform to Franklin's X-ray data and Chargaff's Rules.



1953 article in Nature

Watson and Crick



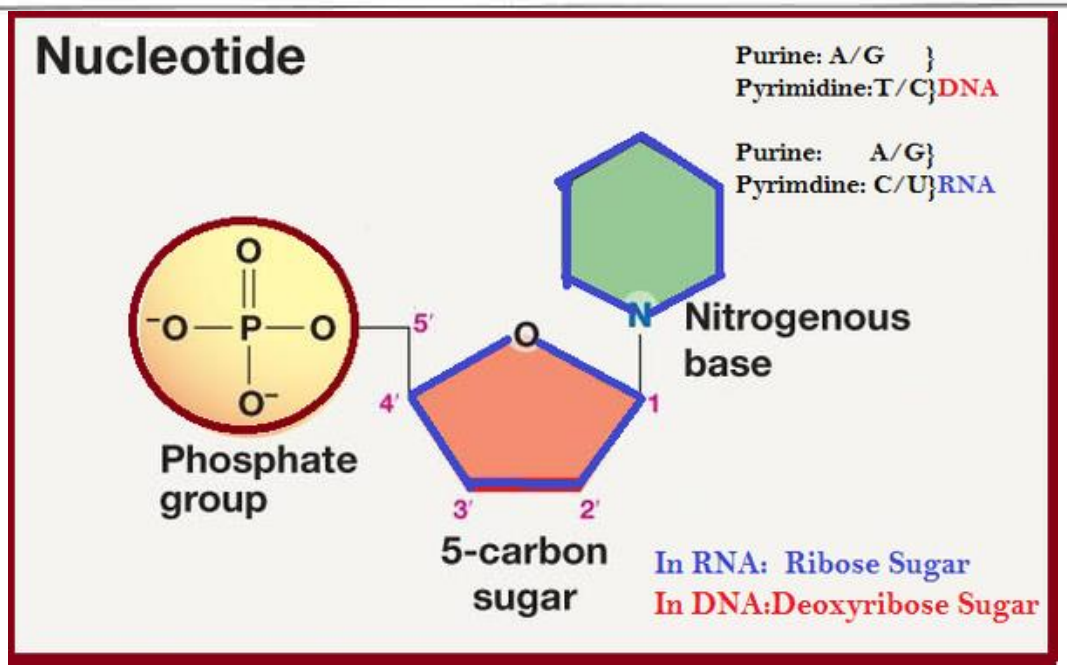


Structure of DNA

BUILDING BLOCKS OF DNA:

Nucleotides:

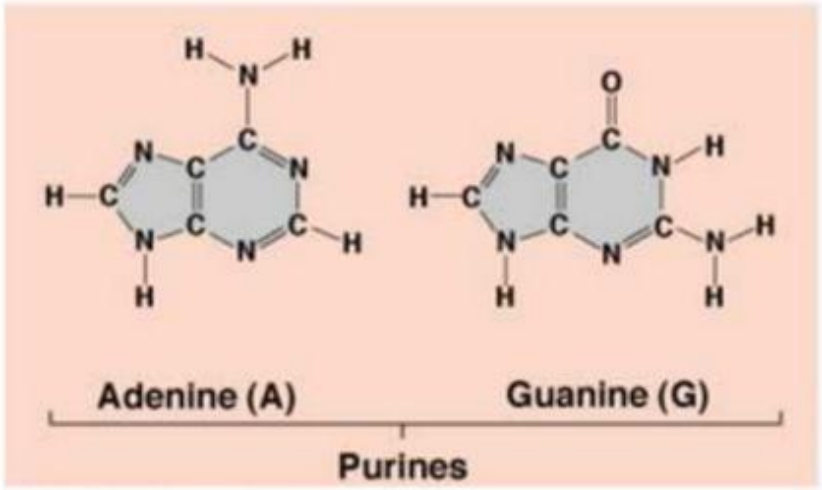
1. 5 carbon sugar (deoxyribose)
2. Nitrogenous base (A, T, C, or G)
3. Phosphate group

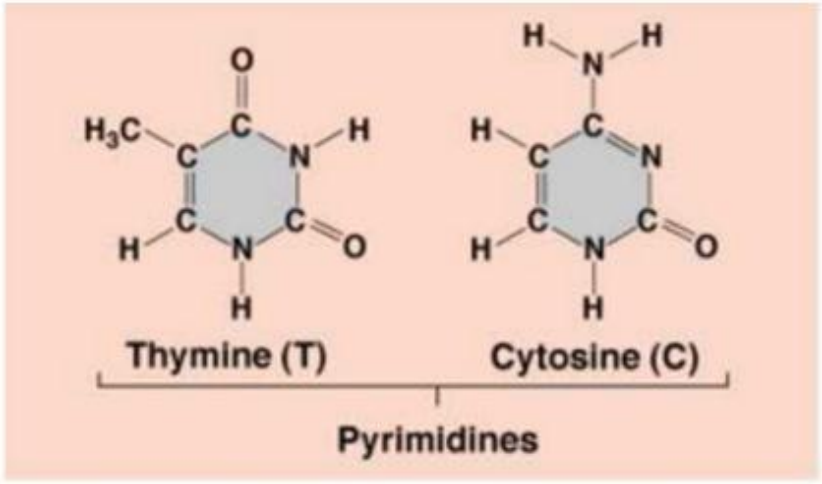


Purines vs. Pyrimidines

- **Purines**: 2 rings
  - Adenine (A)
  - Guanine (G)
- **Pyrimidines**: 1 ring
  - Cytosine (C)
  - Thymine (T)

"The kids at "*AG*" are pure."





Structure of DNA

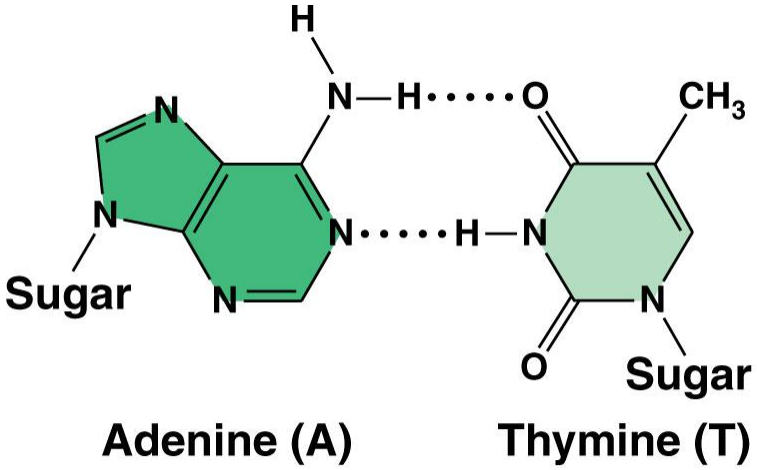
Nitrogenous Bases

- Adenine (A)
- Guanine (G)

purine

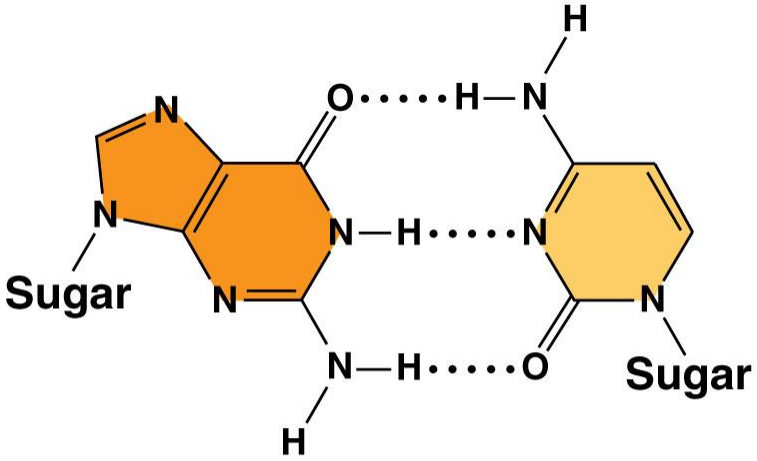
- Thymine (T)
- Cytosine (C)

pyrimidine



Adenine (A) Thymine (T)

- Pairing:
  - purine + pyrimidine
  - A = T
  - G ≡ C

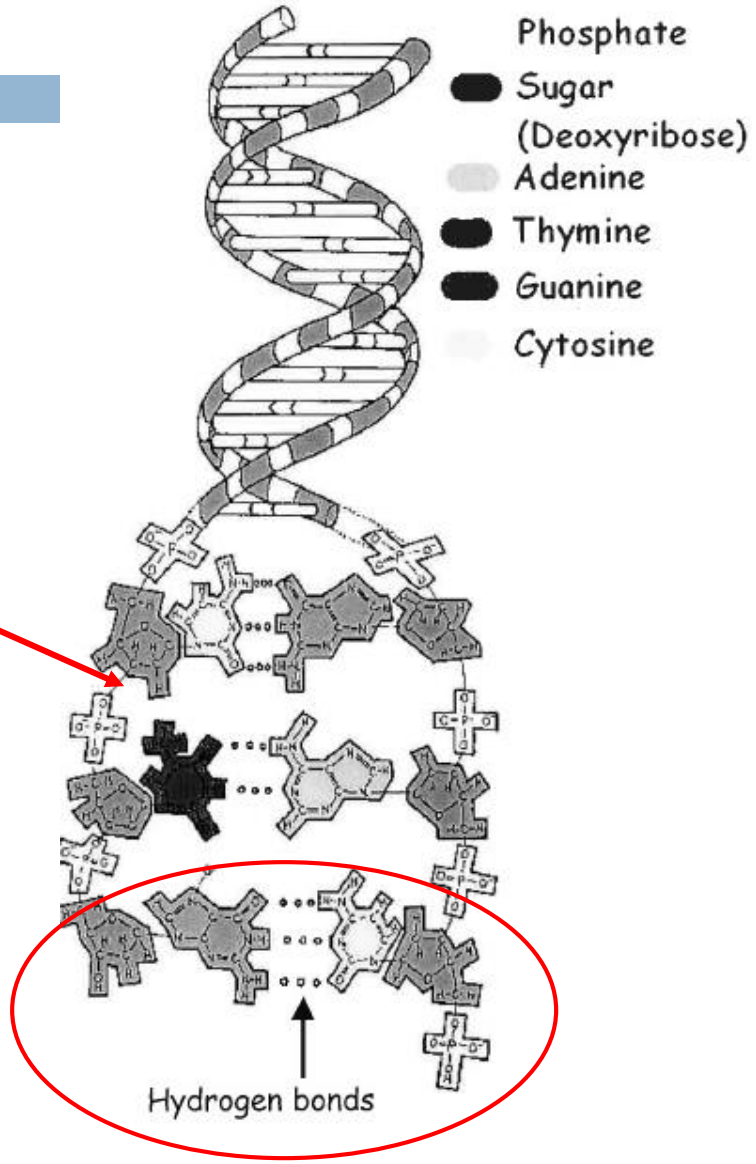


Guanine (G) Cytosine (C)

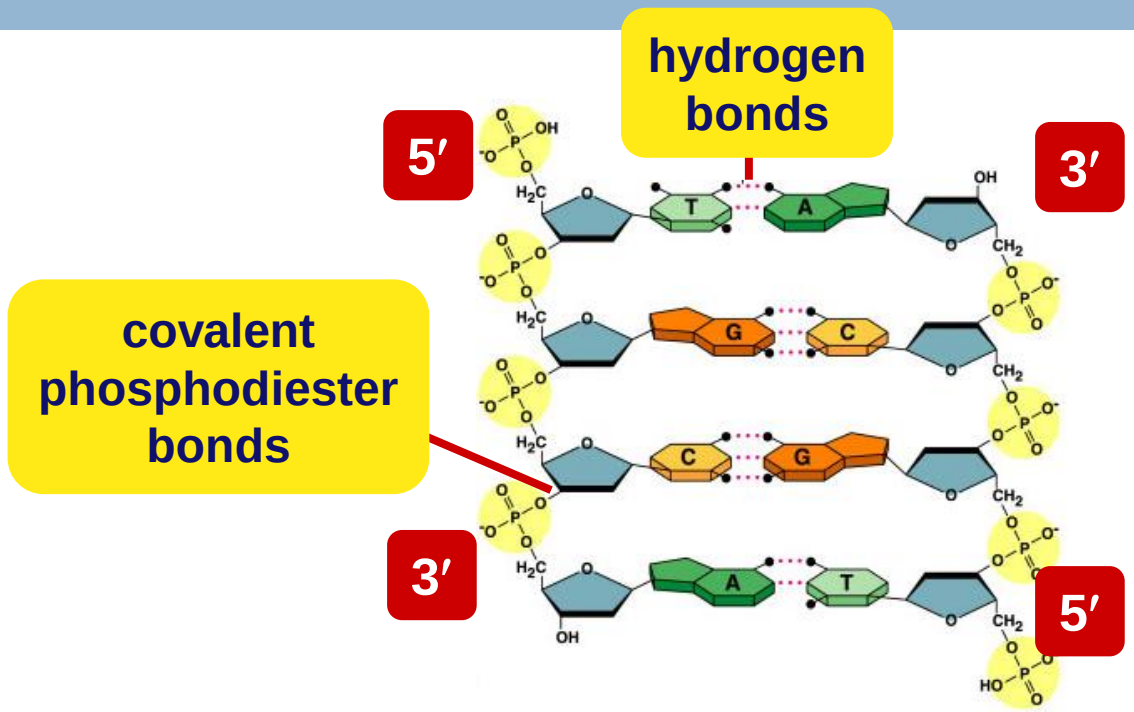
Structure of DNA

DNA = double helix

- "Backbone" = sugar + phosphate
  - Held together by strong phosphodiester bonds
- "Rungs" = nitrogenous bases (purine + pyrimidine)



Bonding in DNA

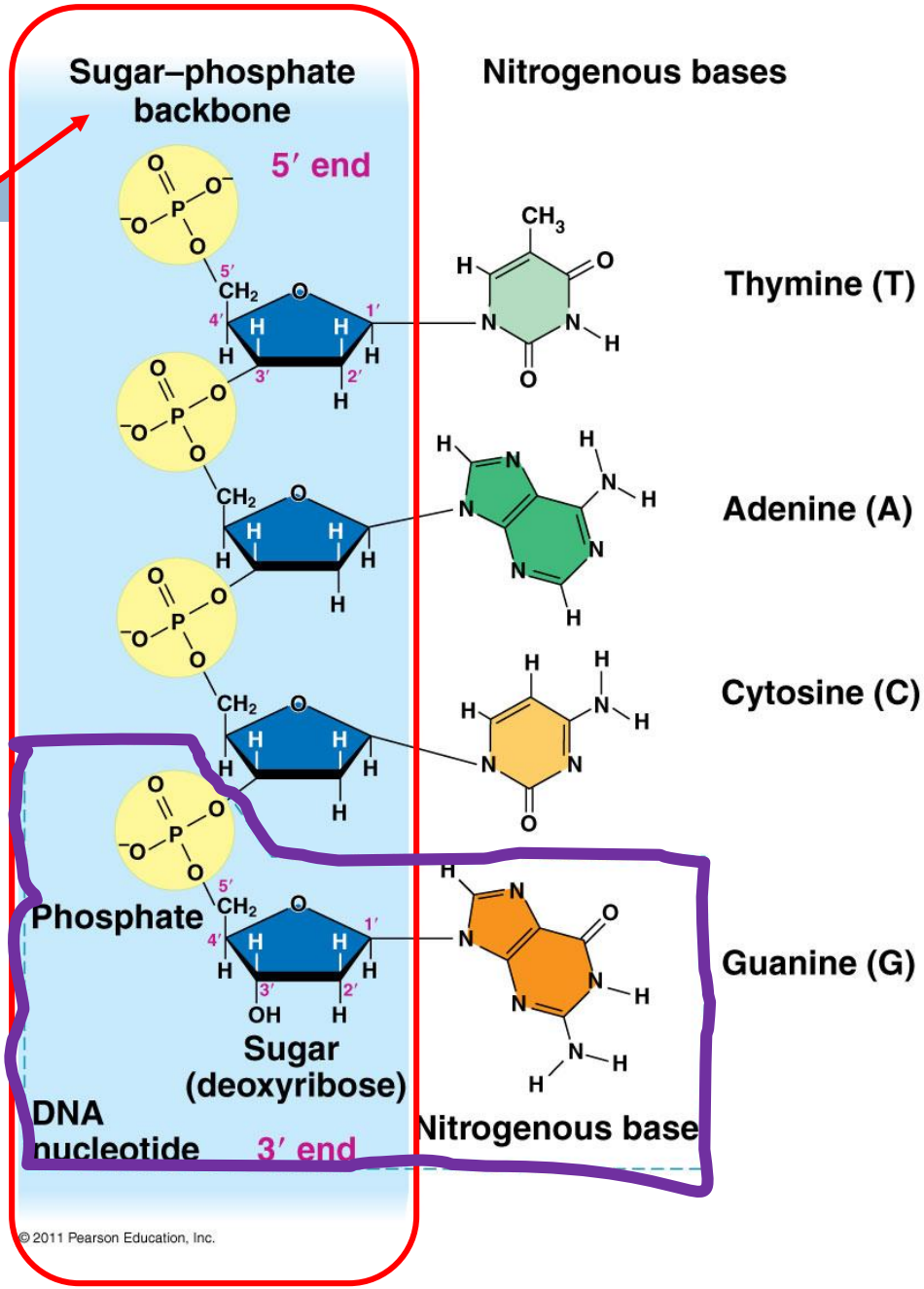


….strong or weak bonds?
How do the bonds fit the mechanism for copying DNA?

Structure of DNA

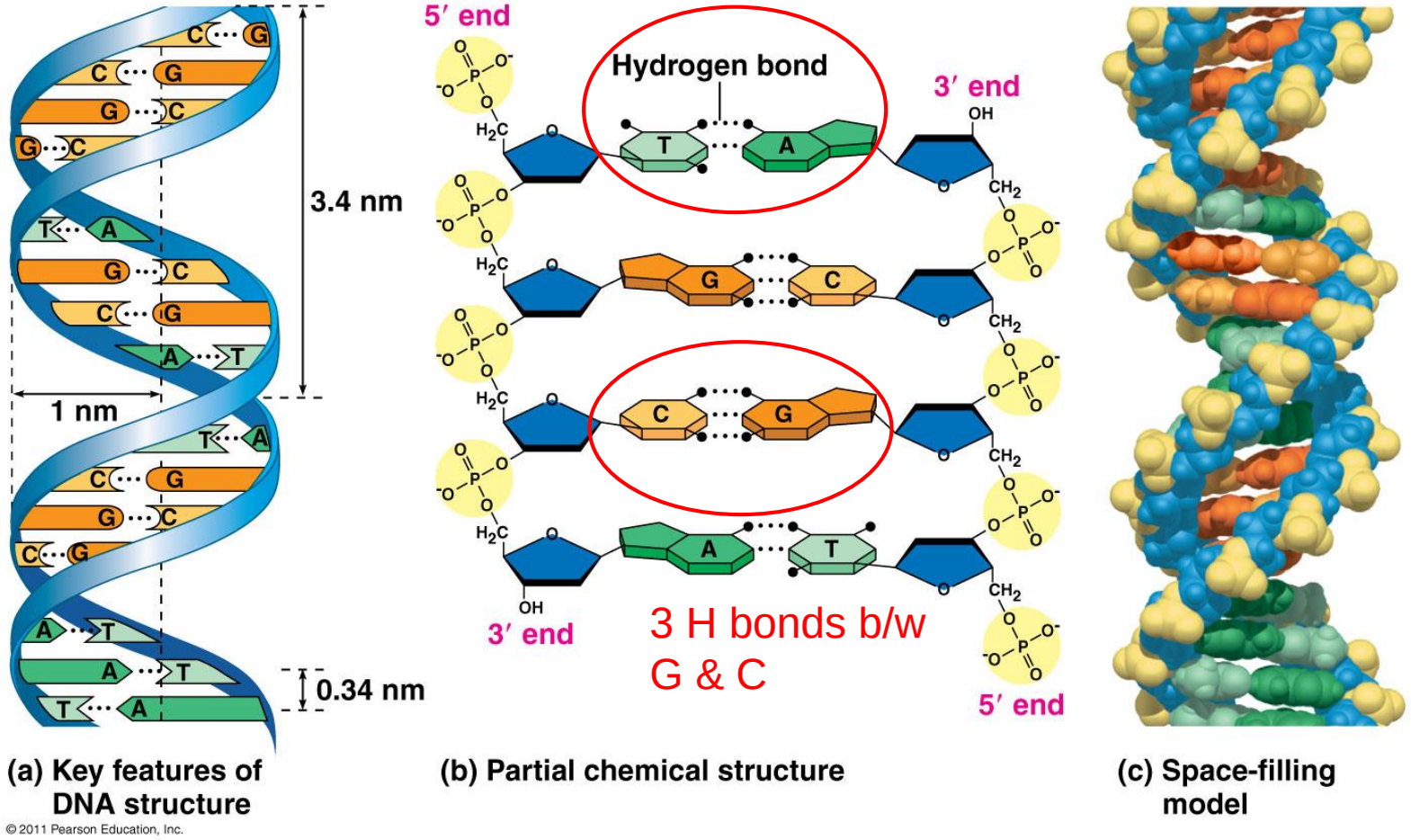
DNA = double helix

- "Backbone" = sugar + phosphate
- "Rungs" = nitrogenous bases (purine = pyrimidine)



Structure of DNA

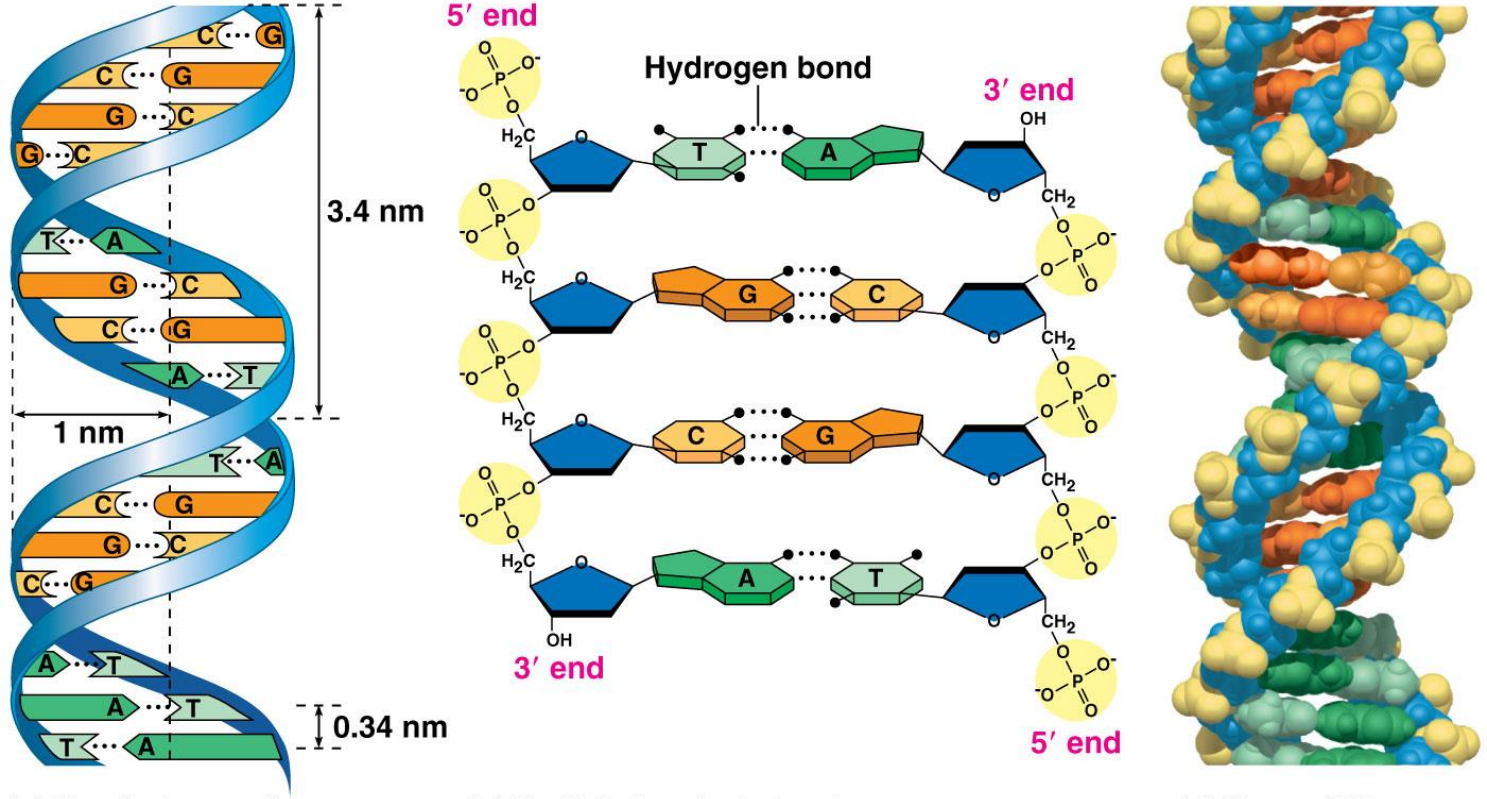
2 H bonds b/w A and T



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

Structure of DNA

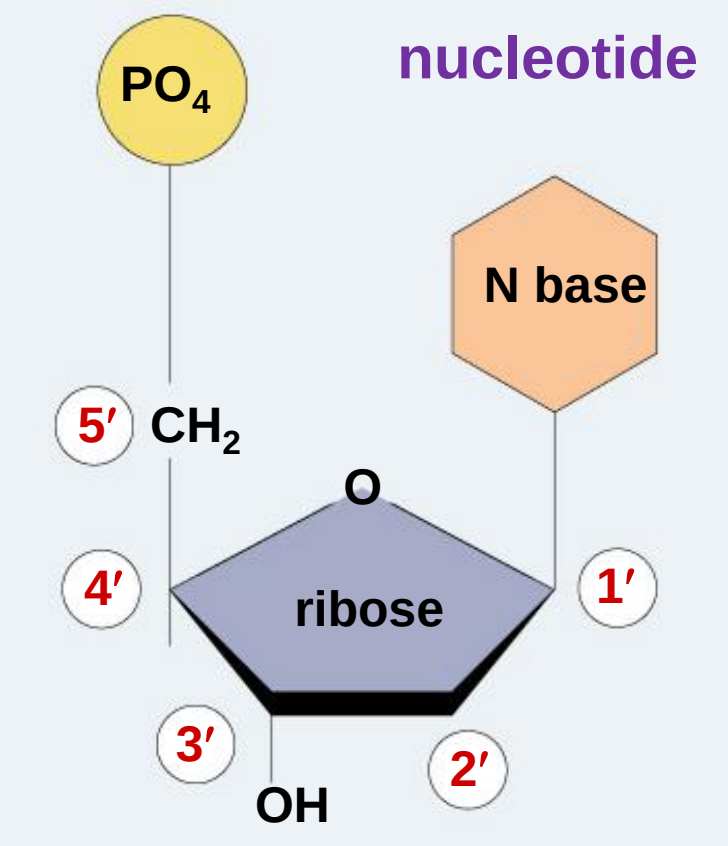
Antiparallel: one strand (5'→ 3'), other strand runs in opposite, upside-down direction (3' → 5')



Directionality of DNA

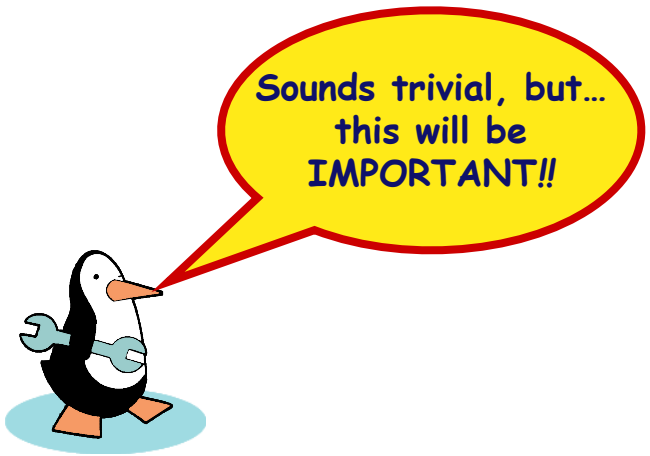
- Notice how the Carbons are numbered

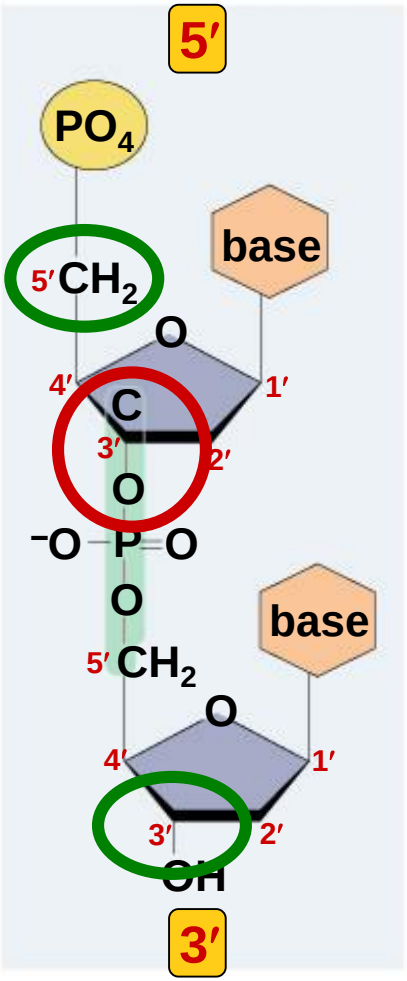
This will be IMPORTANT!!



The DNA backbone

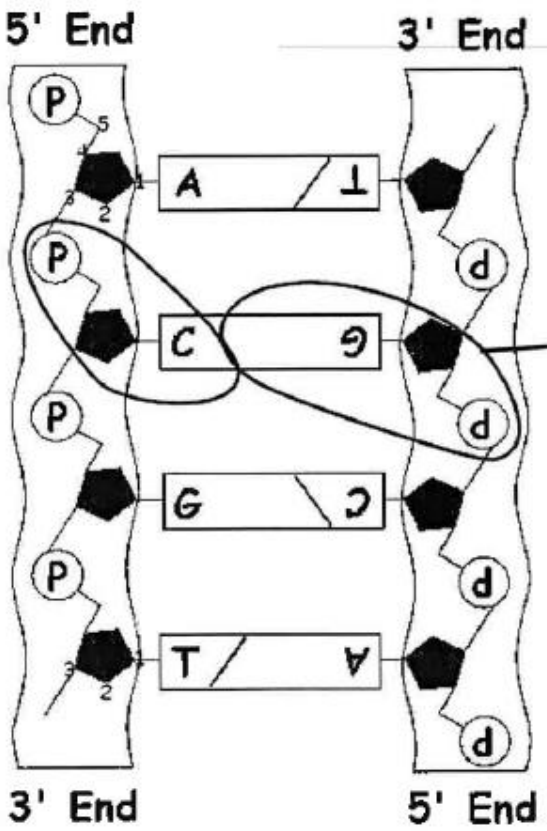
- Putting the DNA backbone together
  - Refer to the 3' and 5' ends of the DNA





Antiparallel Strands

5' end
5th carbon in deoxyribose



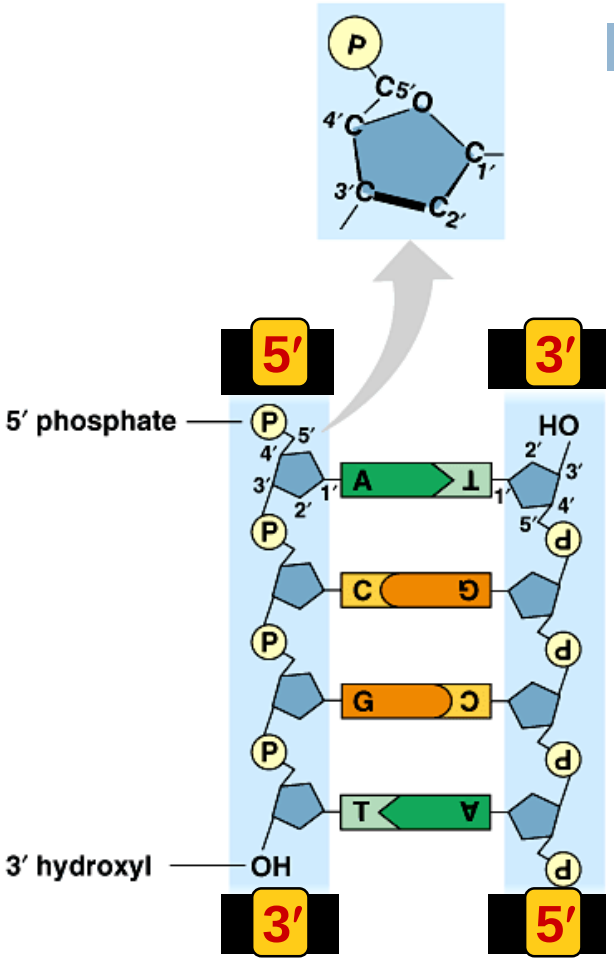
One strand 5' at top & 3' at bottom
Other strand: 5' at bottom & 3' at top

Nucleotide

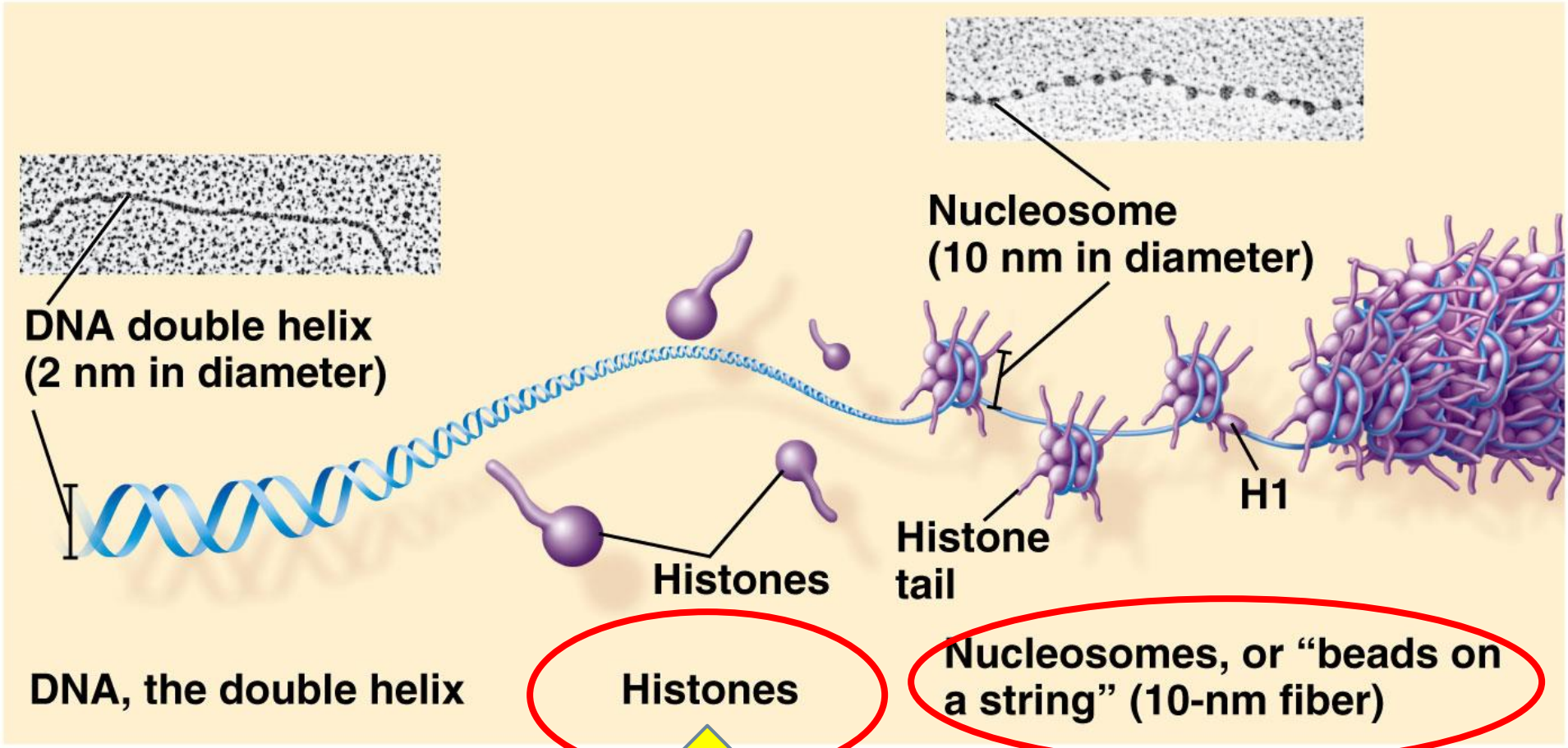
3' end
3rd carbon in deoxyribose

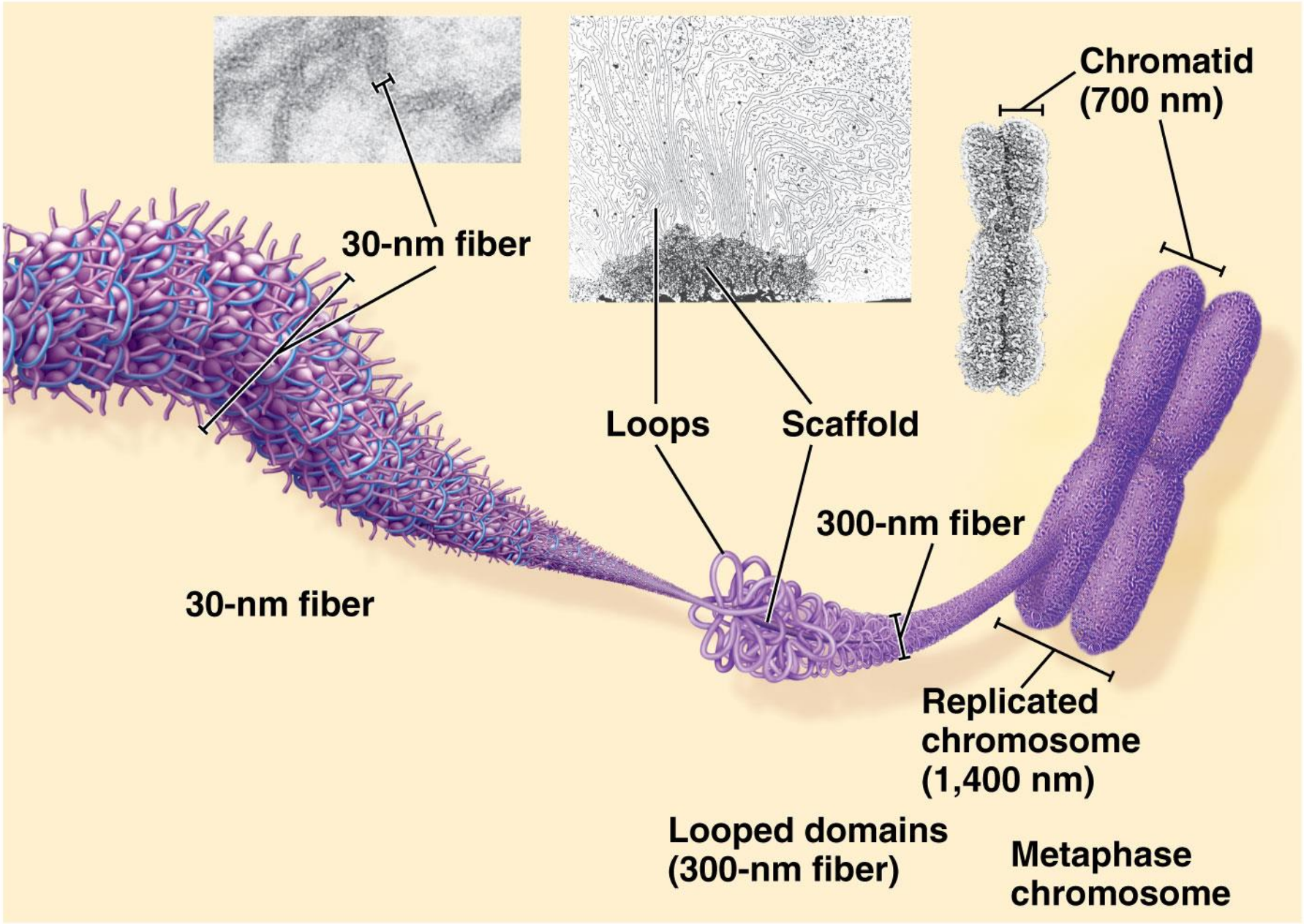
Anti-parallel strands

- Nucleotides in DNA backbone are bonded from phosphate to sugar between 3’ and 5’ carbons
  - DNA molecule has “direction”
  - Complementary strand runs in opposite direction
  - Called “anti-parallel strands”



Eukaryotic Chromosome





DNA Comparison

Prokaryotic DNA

- Double-stranded
- Circular
- One chromosome
- In cytoplasm
- **No histones**
- Supercoiled DNA

Eukaryotic DNA

- Double-stranded
- Linear
- Usually 1+ chromosomes
- In nucleus
- **DNA wrapped around histones (proteins)**
- Forms chromatin

Review and Reflect

1. How did the work of Edwin Chargaff and Rosalind Franklin help Watson and Crick to make their discovery of the structure of DNA?

2. Why do we say that DNA is anti-parallel?

3. Thoroughly describe the structure of DNA to a peer. Include the monomer unit (and its parts), appropriate bonds, and orientation.

4. Besides location, list 3 differences between eukaryotic and prokaryotic DNA/chromosomes.

The Double Helix

- Watch the HHMI video clip here: http://www.hhmi.org/biointeractive/double-helix

1. Go to Canvas → AP Biology
2. Click on the Assignment "The Double Helix – Quiz"
3. Open the PDF.
4. Type in answers as you watch, or after viewing if you prefer.
5. Save the document as "Last name, First name_DNA"
6. Submit the assignment on Canvas.

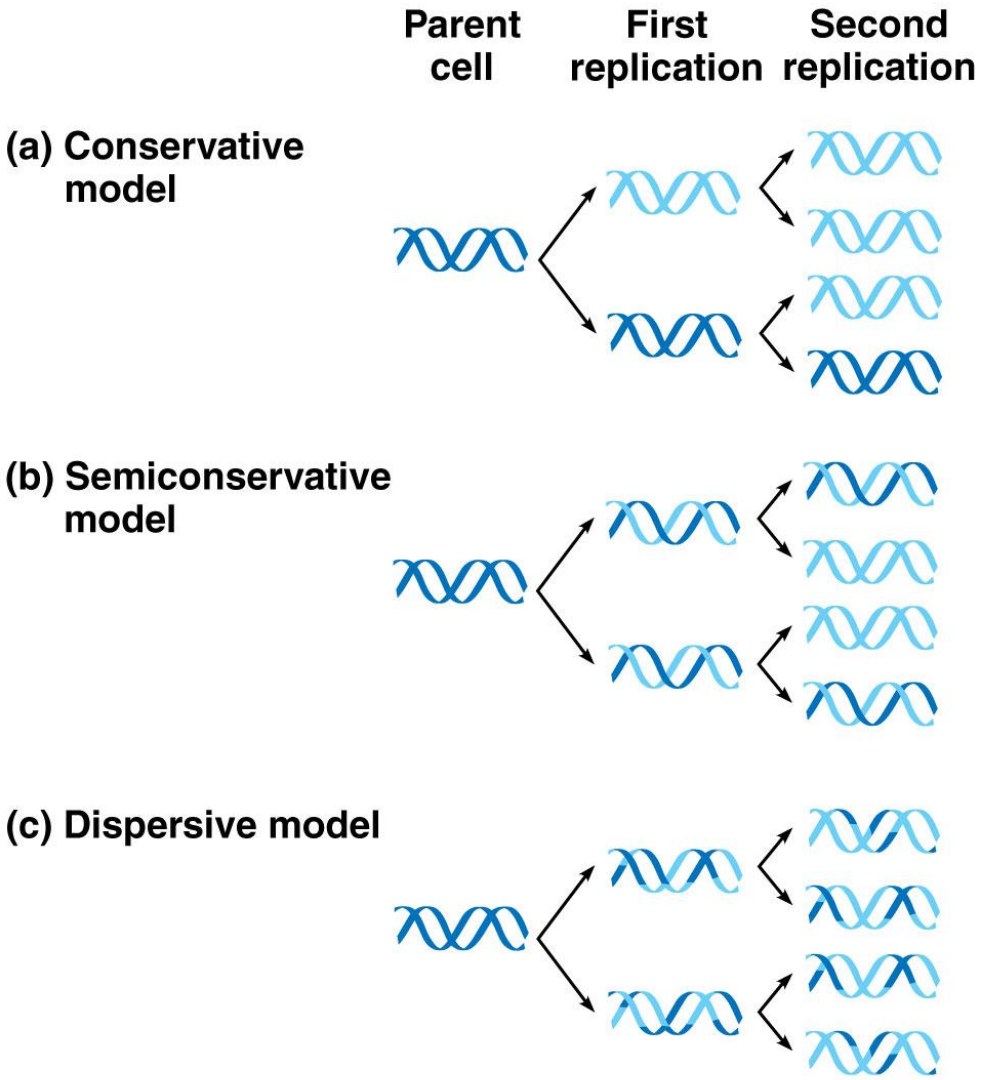
Problem:

How does DNA replicate?

Replication: Making DNA from existing DNA

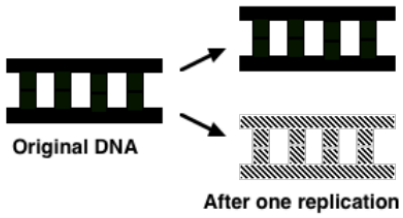
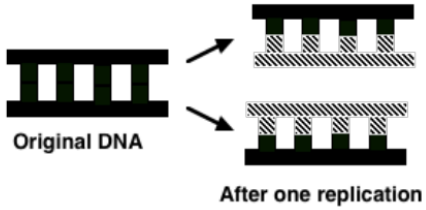
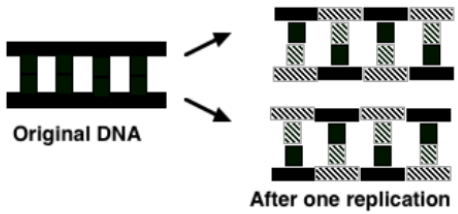
3 alternative models of DNA replication

Newly made DNA is light blue



Pulse-Chase Primer: The Meselson-Stahl Experiment

- Read the Introduction

Type of Replication	Composition of DNA Molecules Before and After
(a) Conservative Replication – the original DNA molecule remains intact and a new DNA molecule is synthesized that contains no part of the original. It is a completely new molecule.	
(b) Semiconservative Replication – each of the two DNA molecules is composed of one strand from the original molecule and one newly synthesized strand.	
(c) Dispersive Replication – each of the two DNA molecules is composed of sections of the original DNA and newly synthesized DNA randomly interspersed along each strand. Note that even though the illustrations look like there is a pattern, in dispersive replication, the original and newly synthesized nucleotides would be randomly interspersed.	

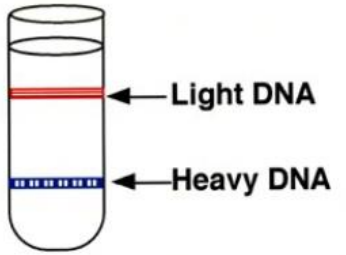


Figure 1: Result of the centrifugation of light and heavy DNA

THE PULSE PHASE

Using this technique, Meselson and Stahl grew *E. coli* on a medium containing ^{15}N for many generations. This ensured that all of the DNA would be labeled with ^{15}N. As the bacteria grew and reproduced, they incorporated the ^{15}N isotope. This is referred to as the **pulse** phase of the experiment (i.e., the pulse is exposing the cells to a particular version of a compound). Meselson and Stahl next took some of these bacteria, prepared the DNA as before, and centrifuged it. Because all DNA was labeled with ^{15}N, a single band was formed. This is "Generation Zero."

1. Choose one colored pencil. Using **Figure 1** as a reference, indicate the location of the band for heavy DNA (^{15}N) in Generation Zero in the centrifuge tube represented to the right.

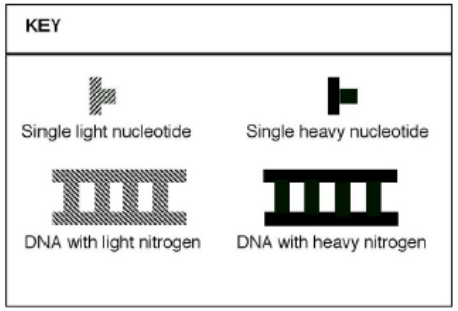
Generation Zero

THE CHASE PHASE

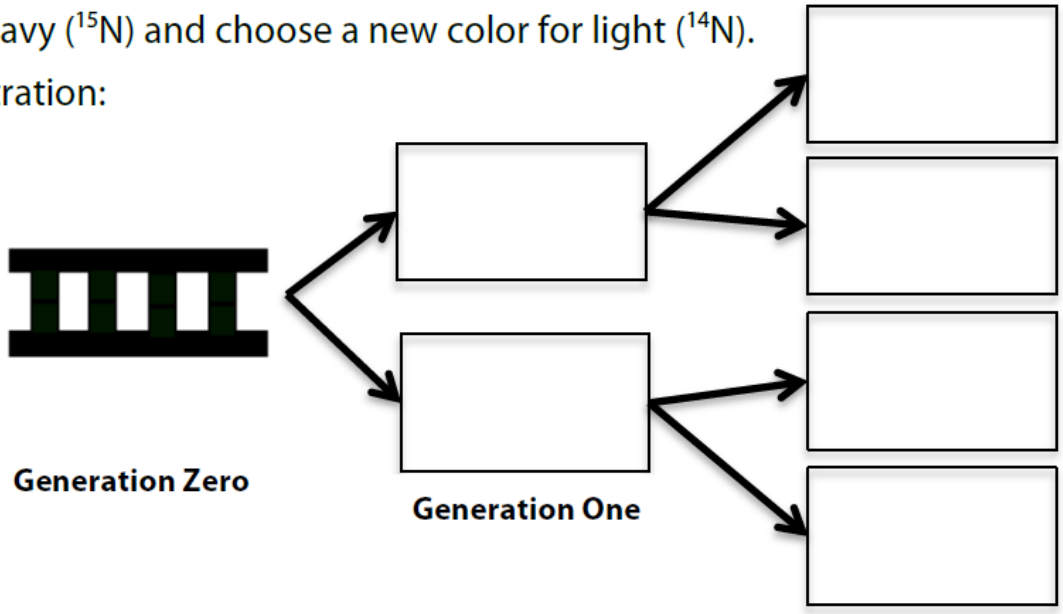
Next, the bacteria with heavy DNA were moved to a culture medium containing ^{14}N. This step marks the beginning of the **chase** phase of the experiment (i.e, exposing cells to a different version of the same compound). After 20 minutes (the time it takes for *E. coli* to grow and produce the next generation), a sample was prepared for centrifugation. This was identified as "Generation One." Another sample was taken after another 20 minutes had passed. This was "Generation Two," and so on.

QUESTIONS

2. If DNA replication is **semiconservative**, use the key provided to illustrate the arrangement of light and heavy isotopes of nitrogen in the DNA molecules formed in Generation One and in Generation Two. Assume that each bacterium divided exactly once per generation. Use the same color as earlier for heavy (^{15}N) and choose a new color for light (^{14}N).



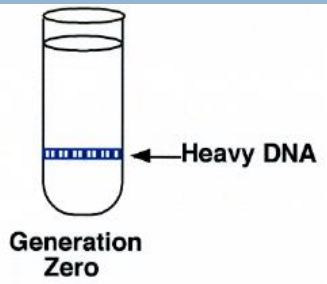
   a. Illustration:



   b. Explanation:

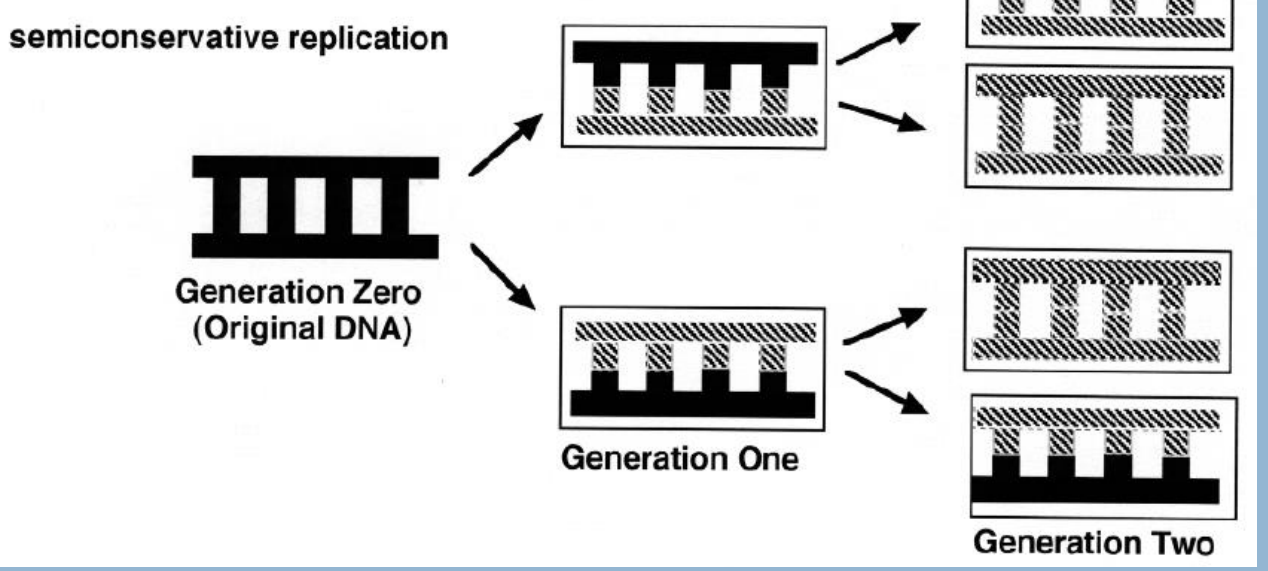
ANSWERS TO QUESTIONS

1. Using Figure 1 as a reference, indicate the location of the band for heavy DNA in Generation Zero in the centrifuge tube represented to the right.



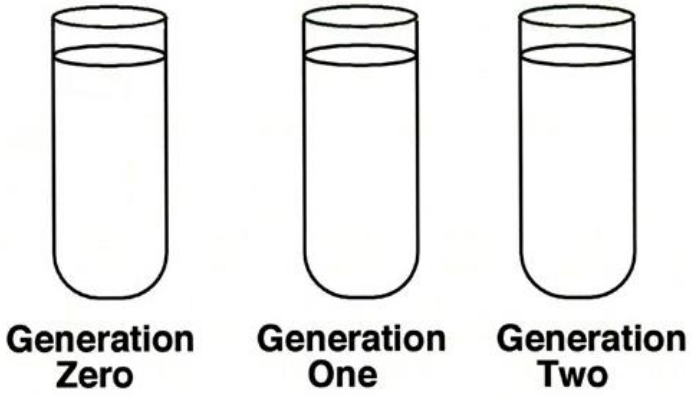
2. If DNA replication is **semiconservative**, use the key provided to illustrate the arrangement of light and heavy isotopes of nitrogen in the DNA molecules formed in Generation One and in Generation Two. Assume that each bacterium divided exactly once per generation. Use the same color as earlier for heavy (^{15}N) and choose a new color for light (^{14}N).

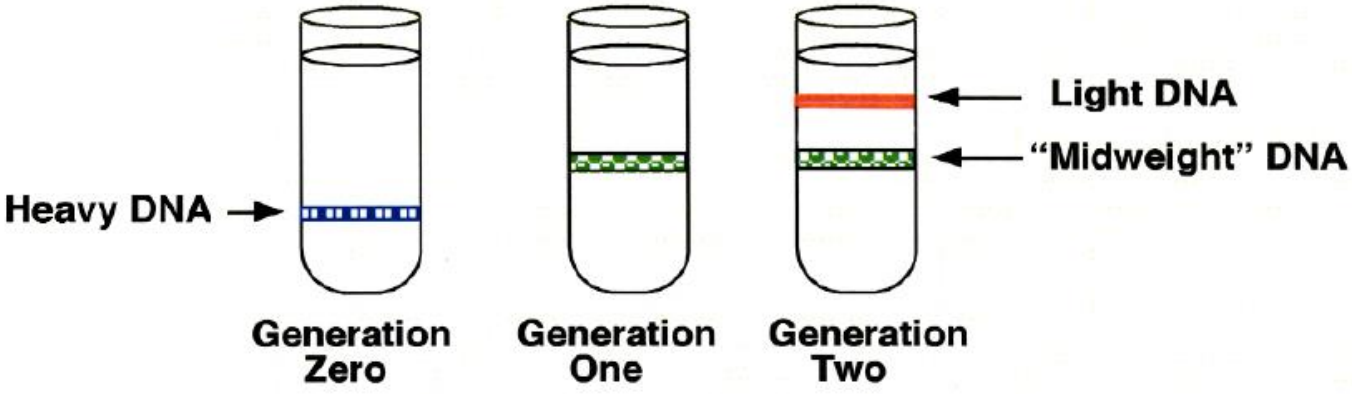
a. Illustration:



b. Explanation: **The original strand contains only heavy ^{15}N. If replication is semiconservative, in Generation One, each of the two DNA molecules will consist of one strand of the original DNA and one strand of complementary DNA made using ^{14}N available in the media. The original DNA serves as a template for replication. The DNA separates between the nitrogen bases. E. coli synthesizes new nucleotides. These nucleotides are what bond to the original strands that have separated. In Generation Two, when the DNA molecules separate during replication, two of the new DNA molecules receive an original heavy strand of DNA. The other two DNA molecules consist entirely of DNA strands synthesized using ^{14}N available in the media.**

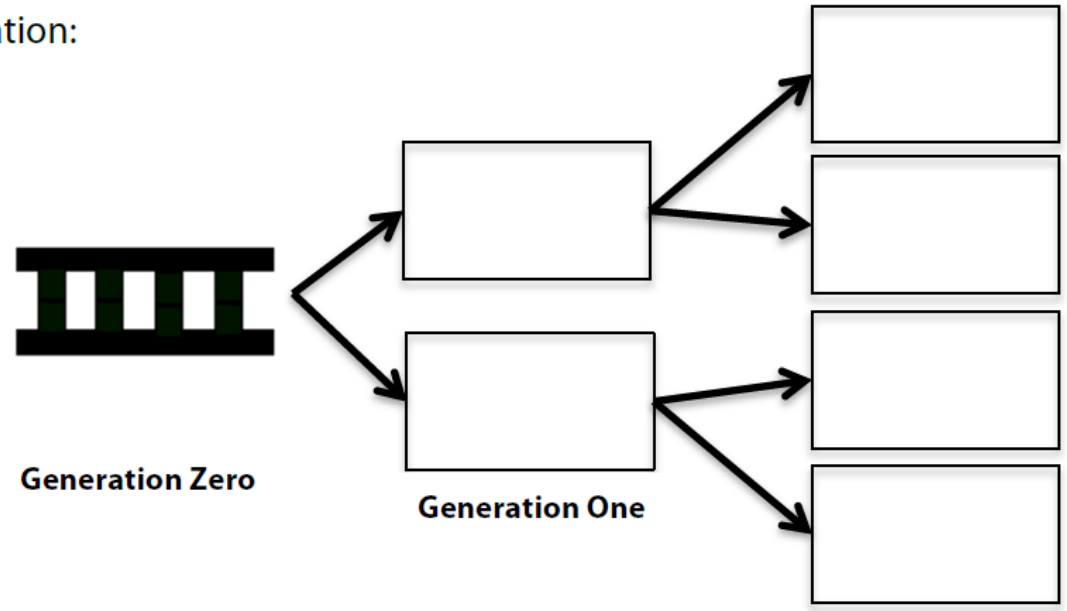
c. Using **Figure 1** as the standard, sketch where the bands of DNA would collect in the tubes for Generations Zero, One, and Two if DNA replication is **semiconservative**.





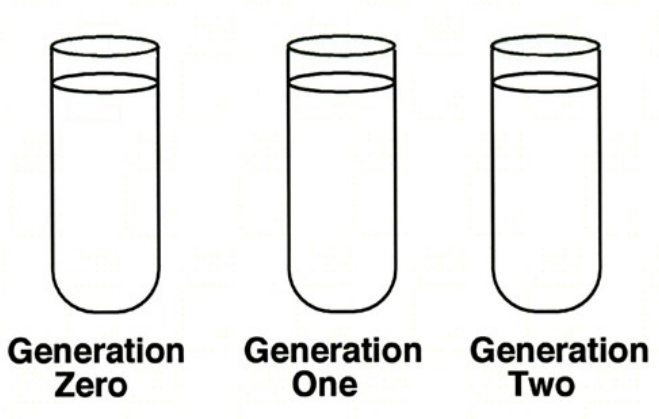
3. Using the key provided in question 2, illustrate the location of light and heavy isotopes of nitrogen in the strands of DNA in Generations Zero, One, and Two if DNA replication is **conservative**.

a. Illustration:

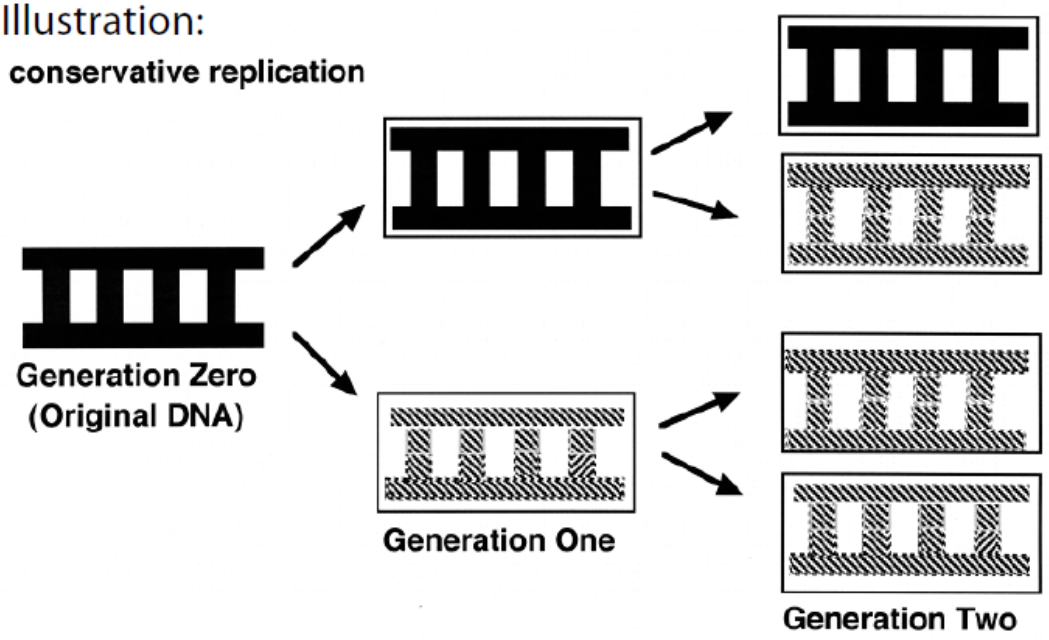


b. Explanation:

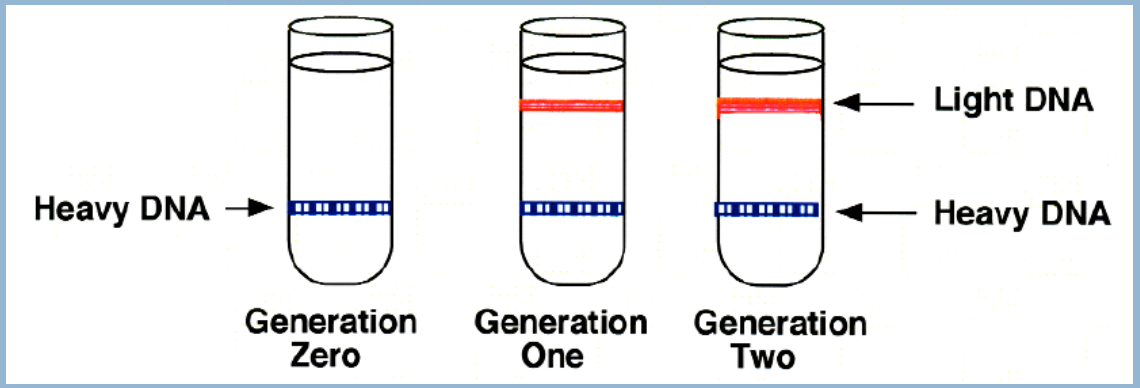
c. In the tubes to the right, illustrate the banding patterns Meselson and Stahl would have observed if the results of their experiment supported the **conservative** model of DNA replication.



a. Illustration:

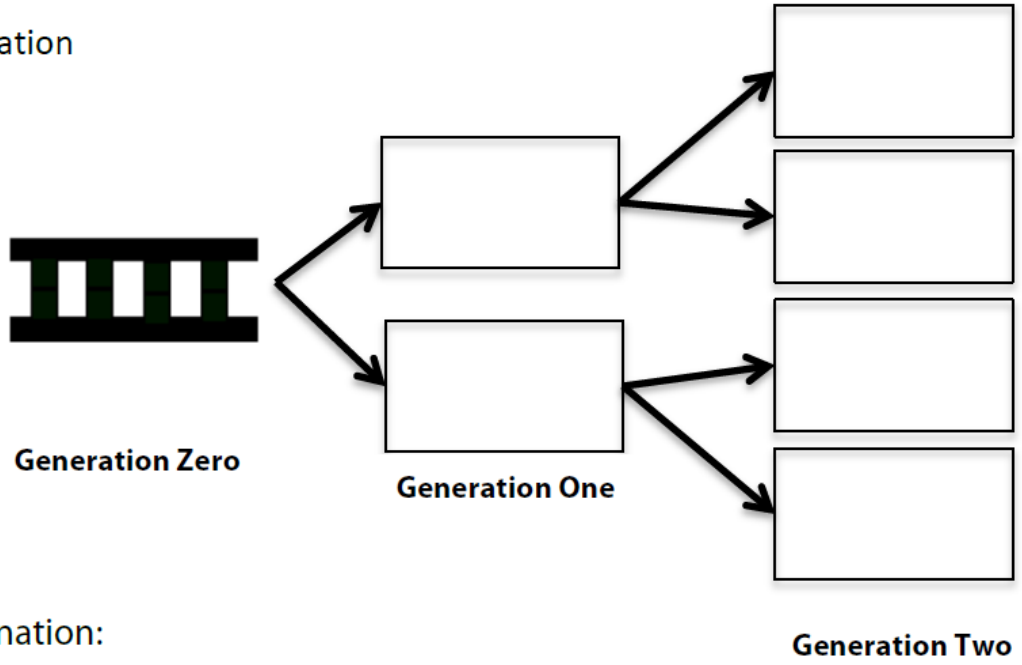


b. Explanation: **If replication is conservative, the original molecule serves as the template. During the chase, the only nitrogen available for the synthesis of new DNA molecules is light. Therefore, all of the newly synthesized molecules will be light DNA. The original DNA molecule will remain intact and composed entirely of heavy DNA. Analysis will show an increasing amount of light DNA from one generation to the next.**



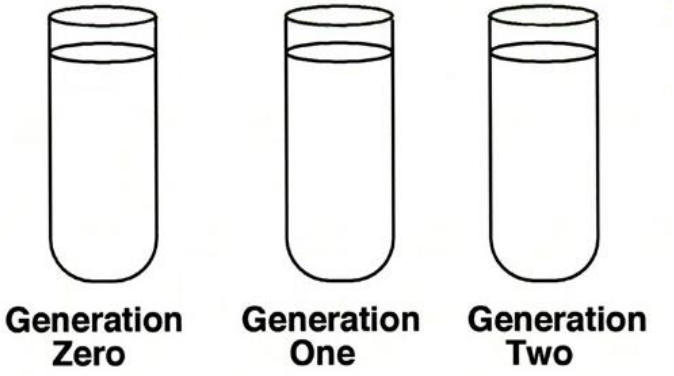
4. Using the key provided in question 2, illustrate the location of light and heavy isotopes of nitrogen in the strands of DNA in Generations Zero, One, and Two if DNA replication is **dispersive**.

   a. Illustration



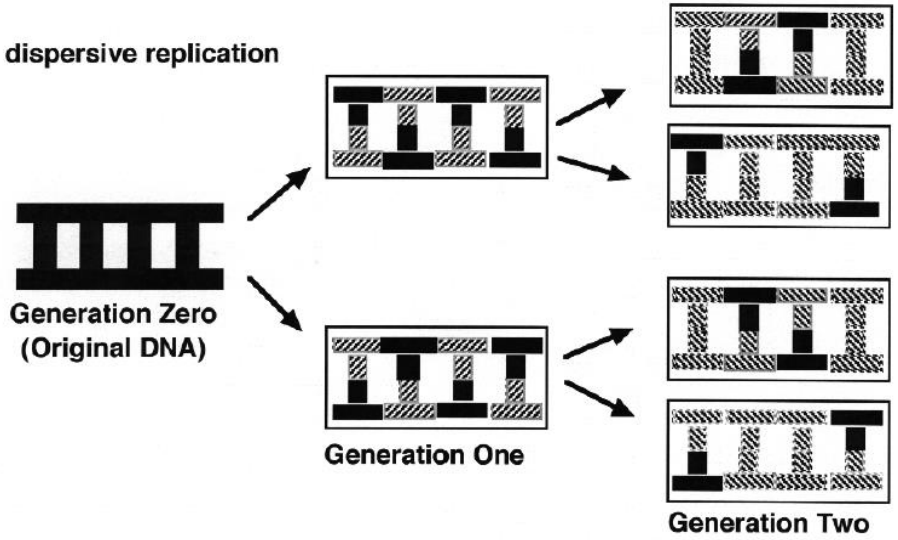
   b. Explanation:

   c. In the tubes to the right, illustrate the banding patterns Meselson and Stahl would have observed if the results of their experiment supported the **dispersive** model of DNA replication.



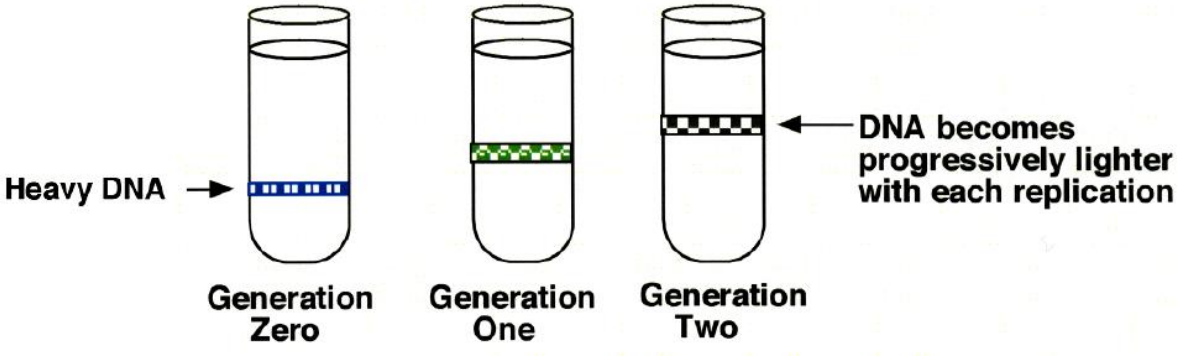
4. Using the key provided in question 2, illustrate the location of light and heavy isotopes of nitrogen in the strands of DNA in Generations Zero, One, and Two if DNA replication is **dispersive**.

   a. Illustration

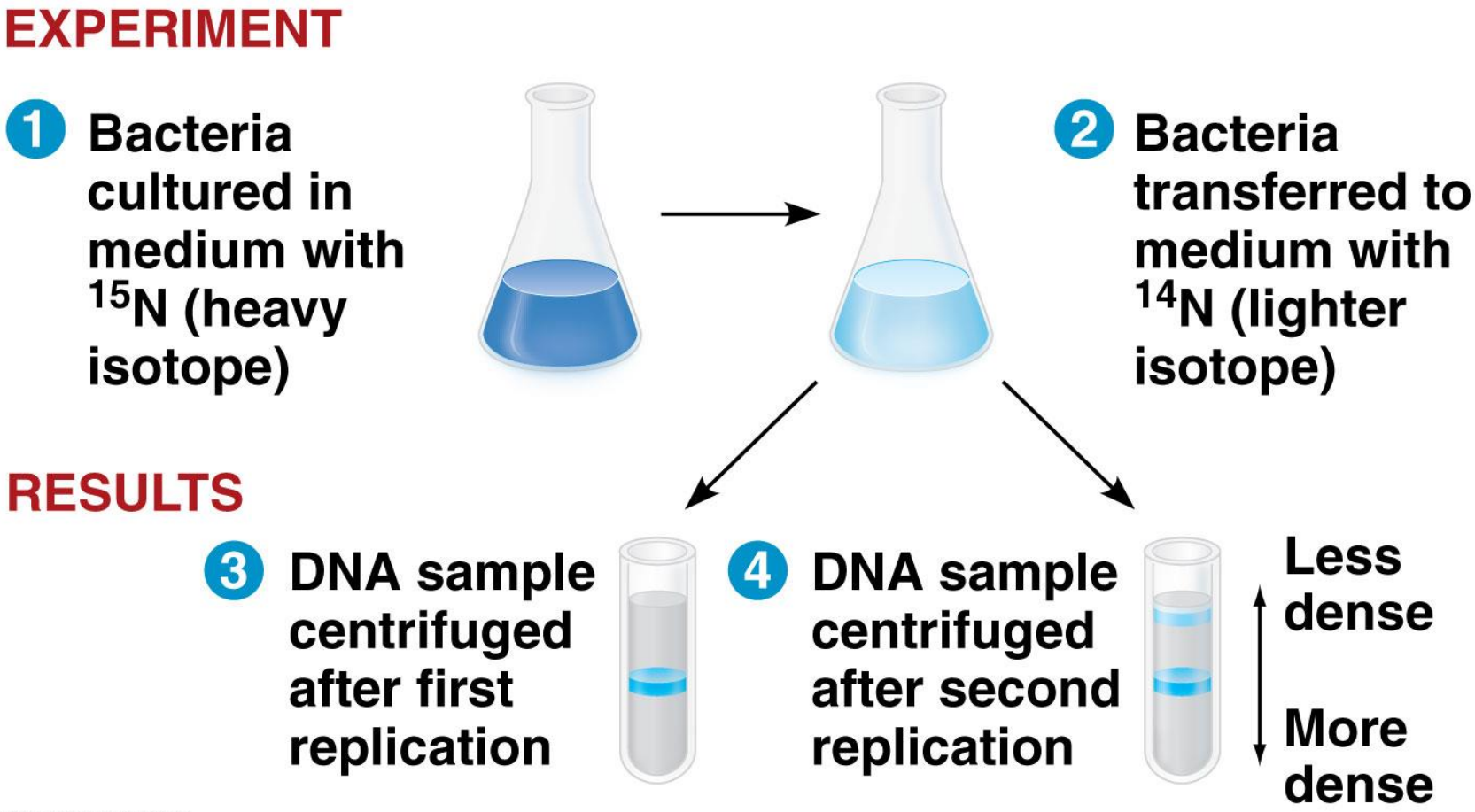


   b. Explanation: **Answers will vary. The distribution of heavy and light nucleotides in the newly synthesized DNA will be random. However, the number of heavy DNA nucleotides cannot increase from one generation to the next. The number of light DNA nucleotides will increase.**

   c. In the tubes, illustrate the banding patterns Meselson and Stahl would have observed if the results of their experiment supported the dispersive model of DNA replication.

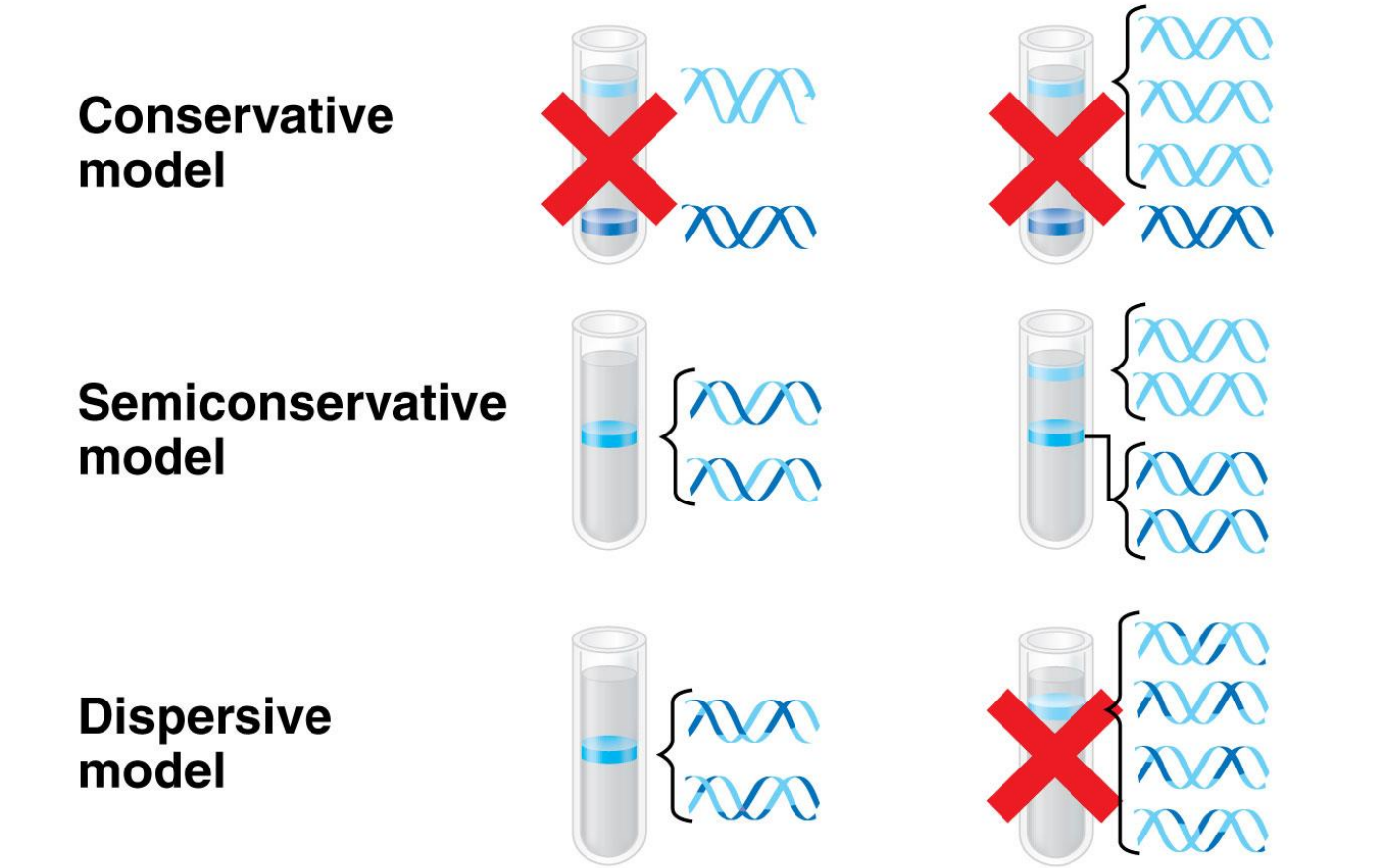


Meselson & Stahl

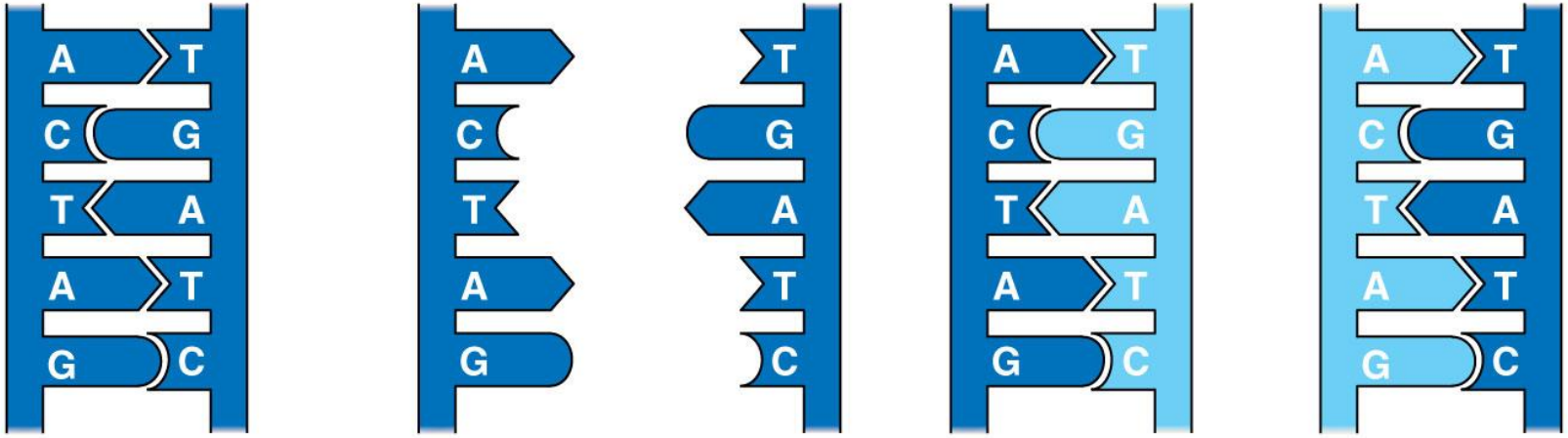


Meselson & Stahl

CONCLUSION



Replication is semiconservative



(a) Parent molecule

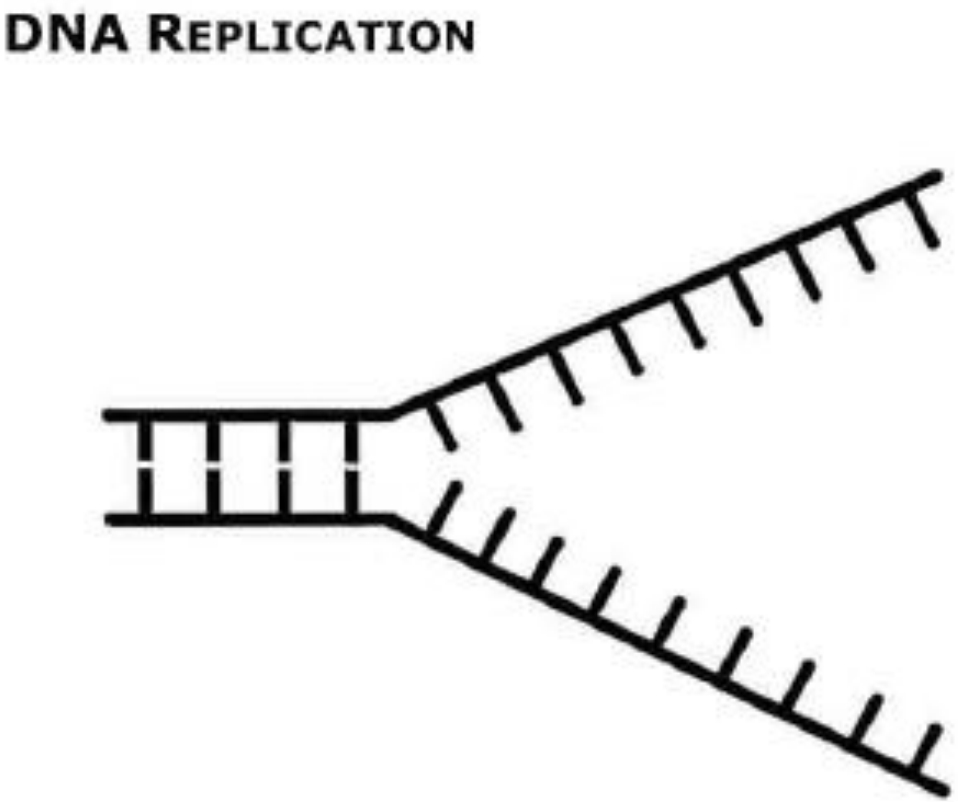
(b) Separation of strands

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

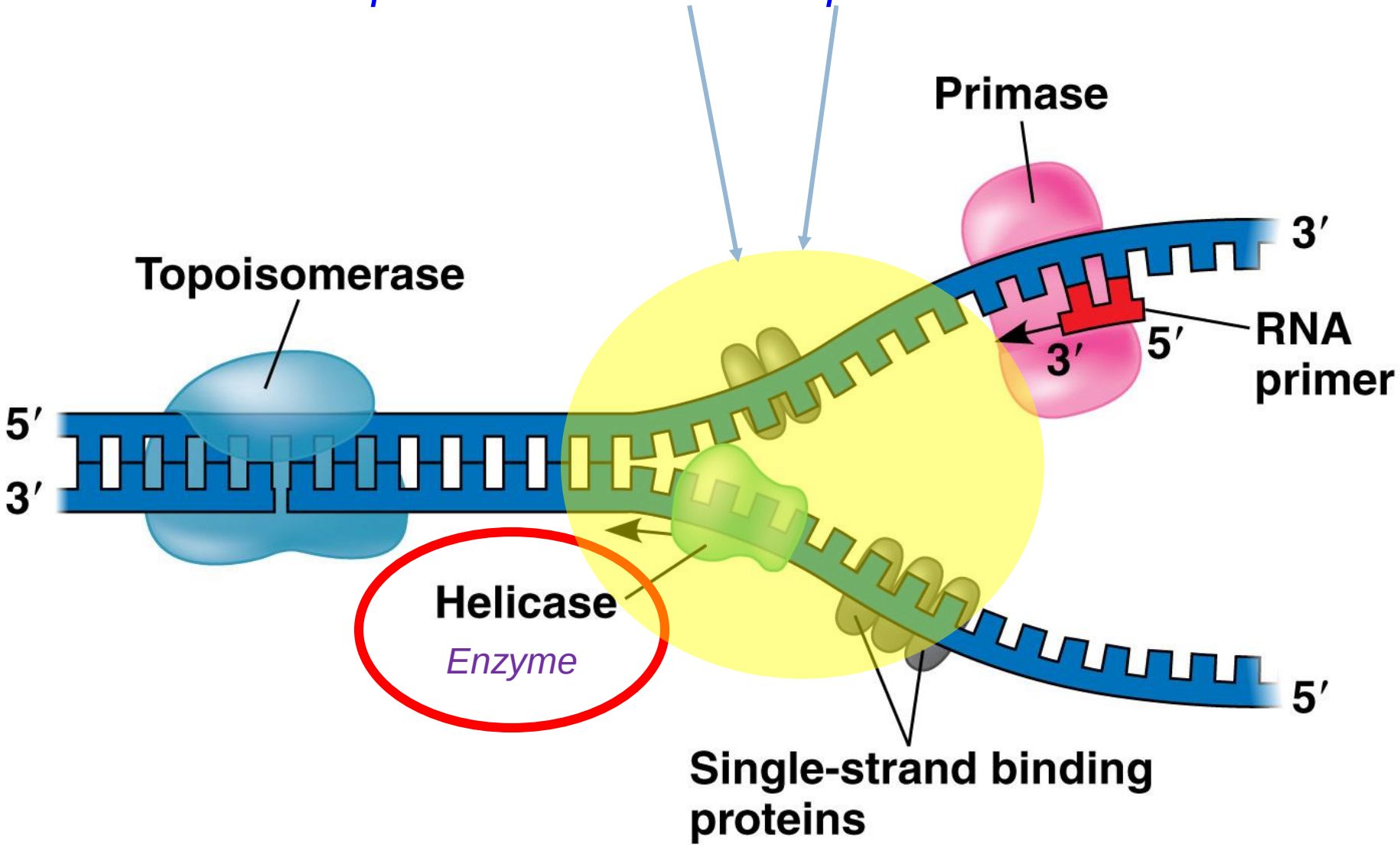
DNA Replication Video

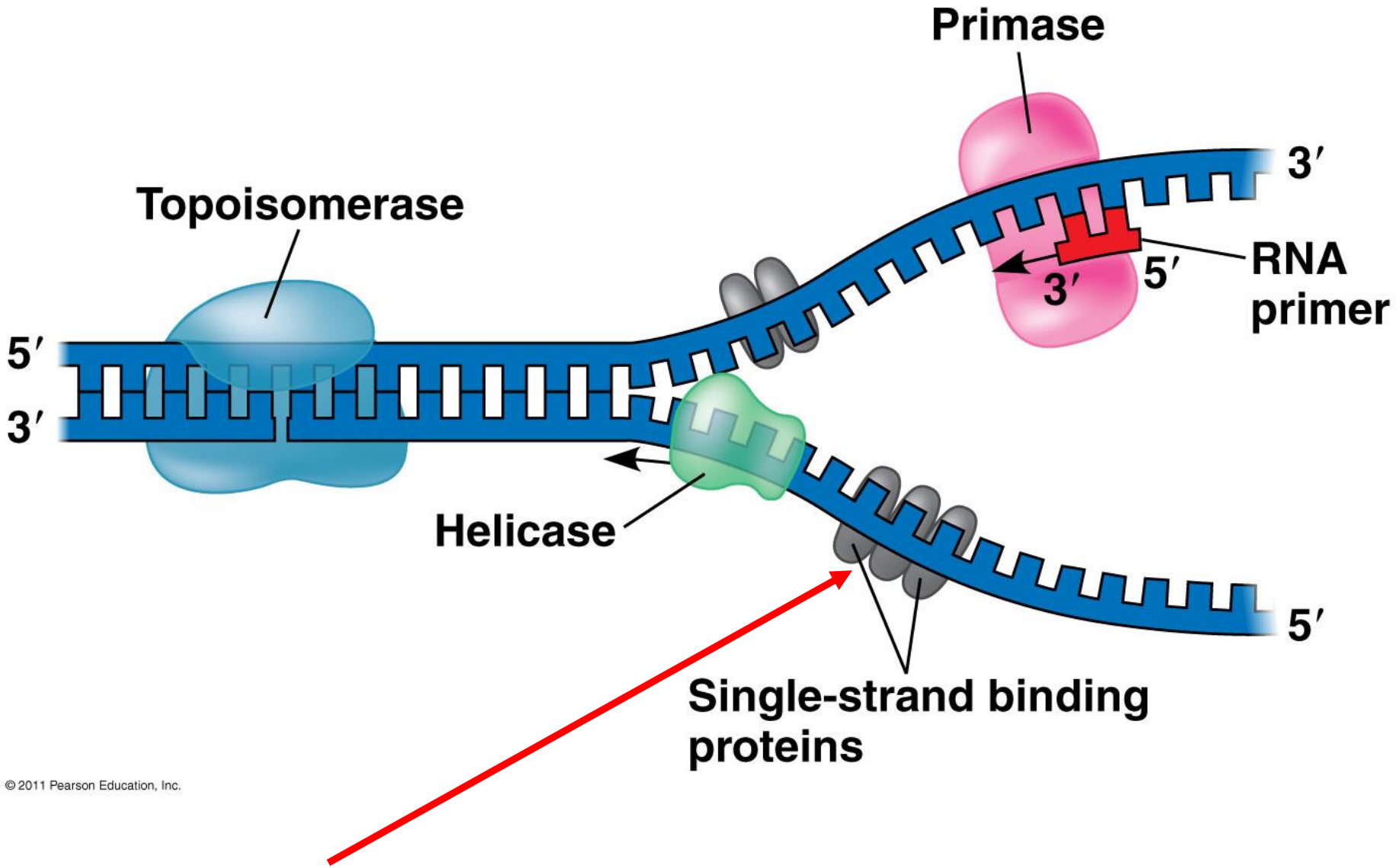
Amoeba Sisters – with handout

Draw, Label, annotate as we go



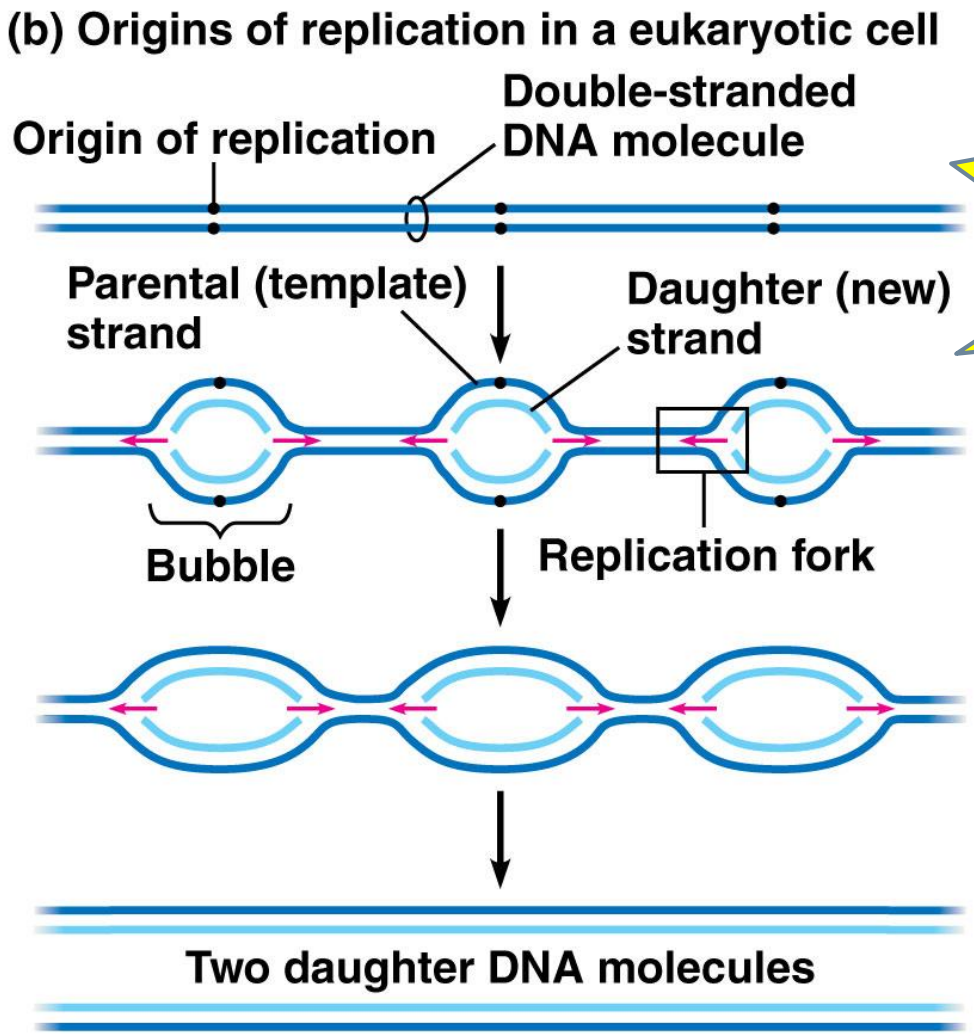
1. **Helicase:** unwinds DNA at *origins of replication* and creates a *replication bubble* or *replication fork*



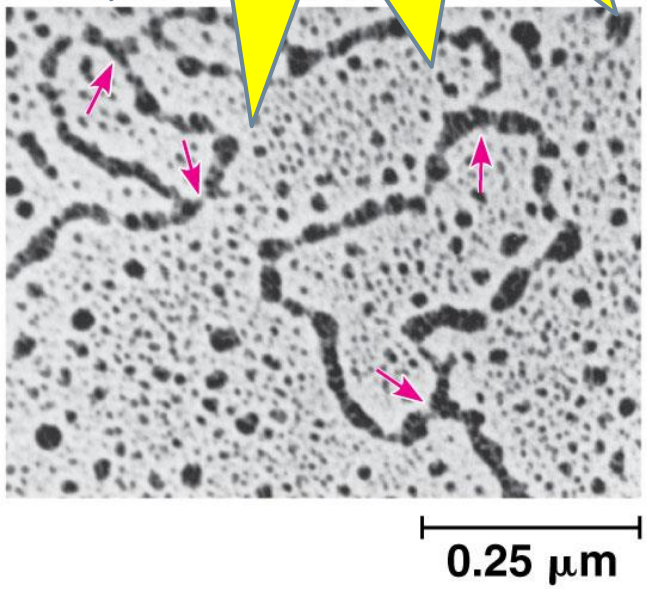


2. Initiation proteins called single-strand binding proteins (ssbp) separate 2 strands → forms *replication bubble* → stabilizes the bubble to hold it open

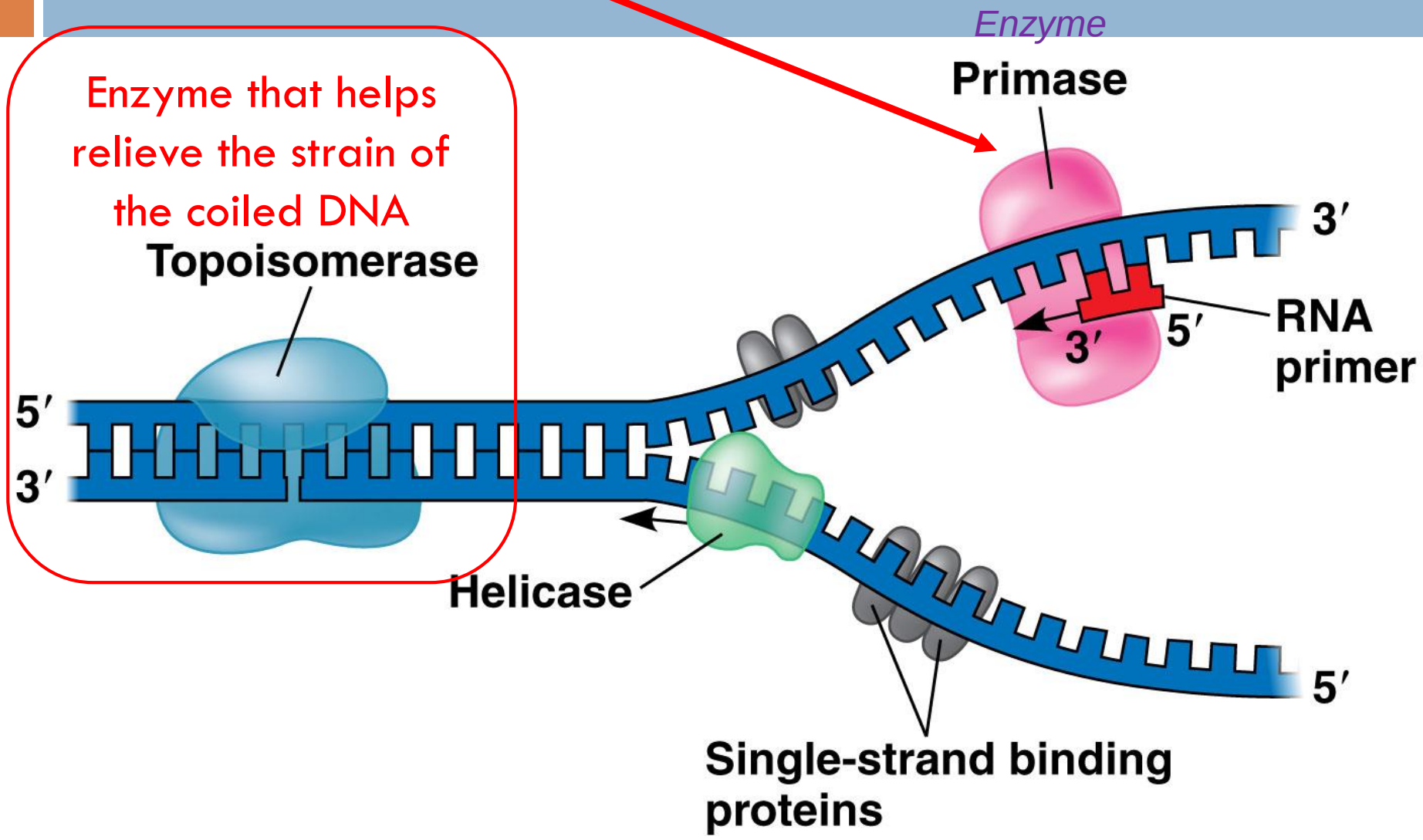
Helicase unwinds DNA at *origins of replication* and creates *replication forks*



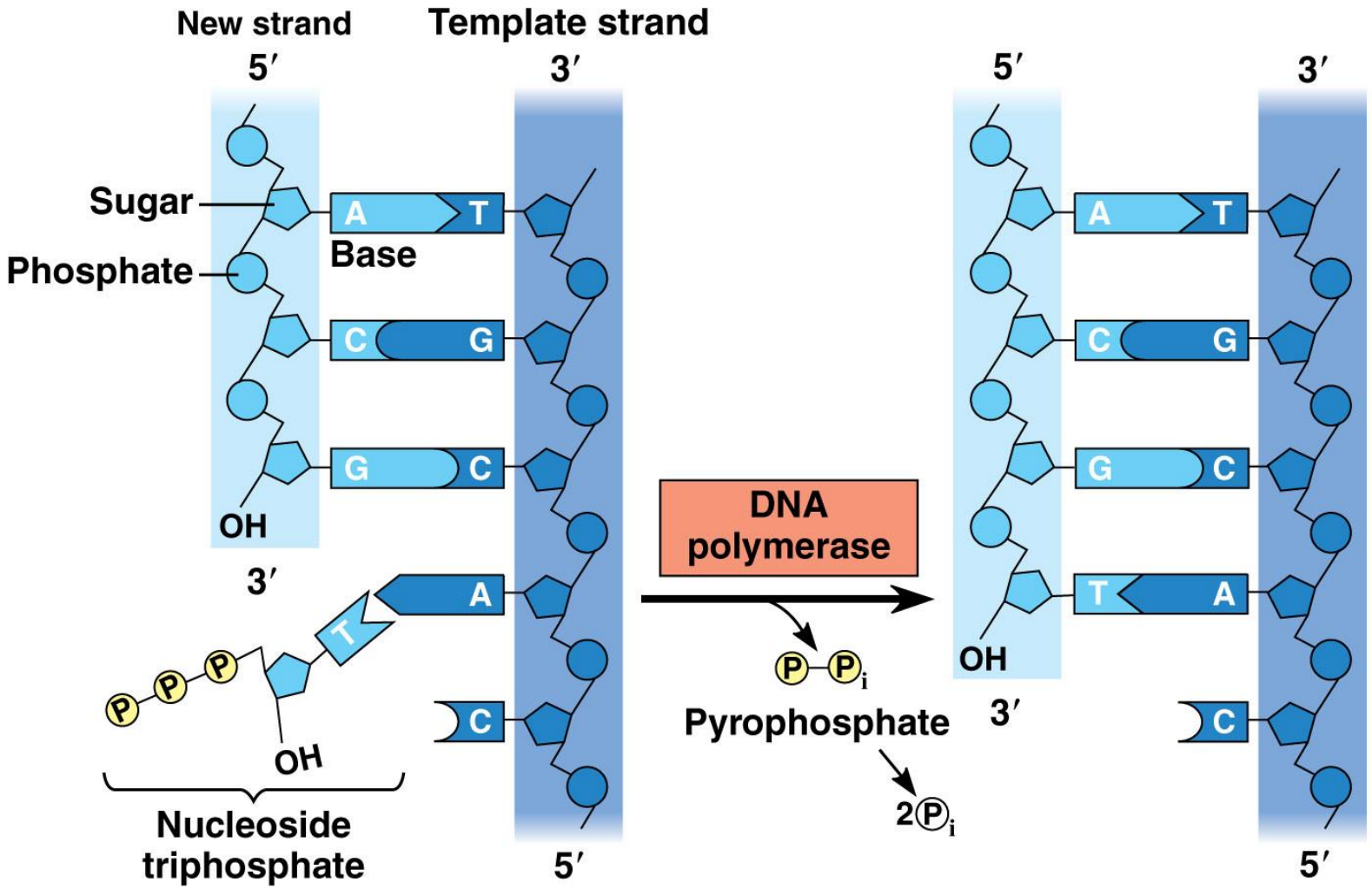
Can have several replication bubbles at the same time along the DNA strand



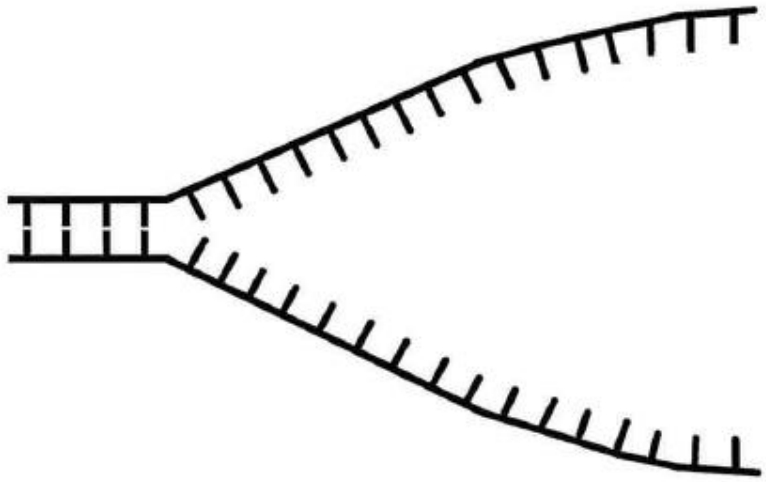
3. Primase: puts down RNA primer to start replication



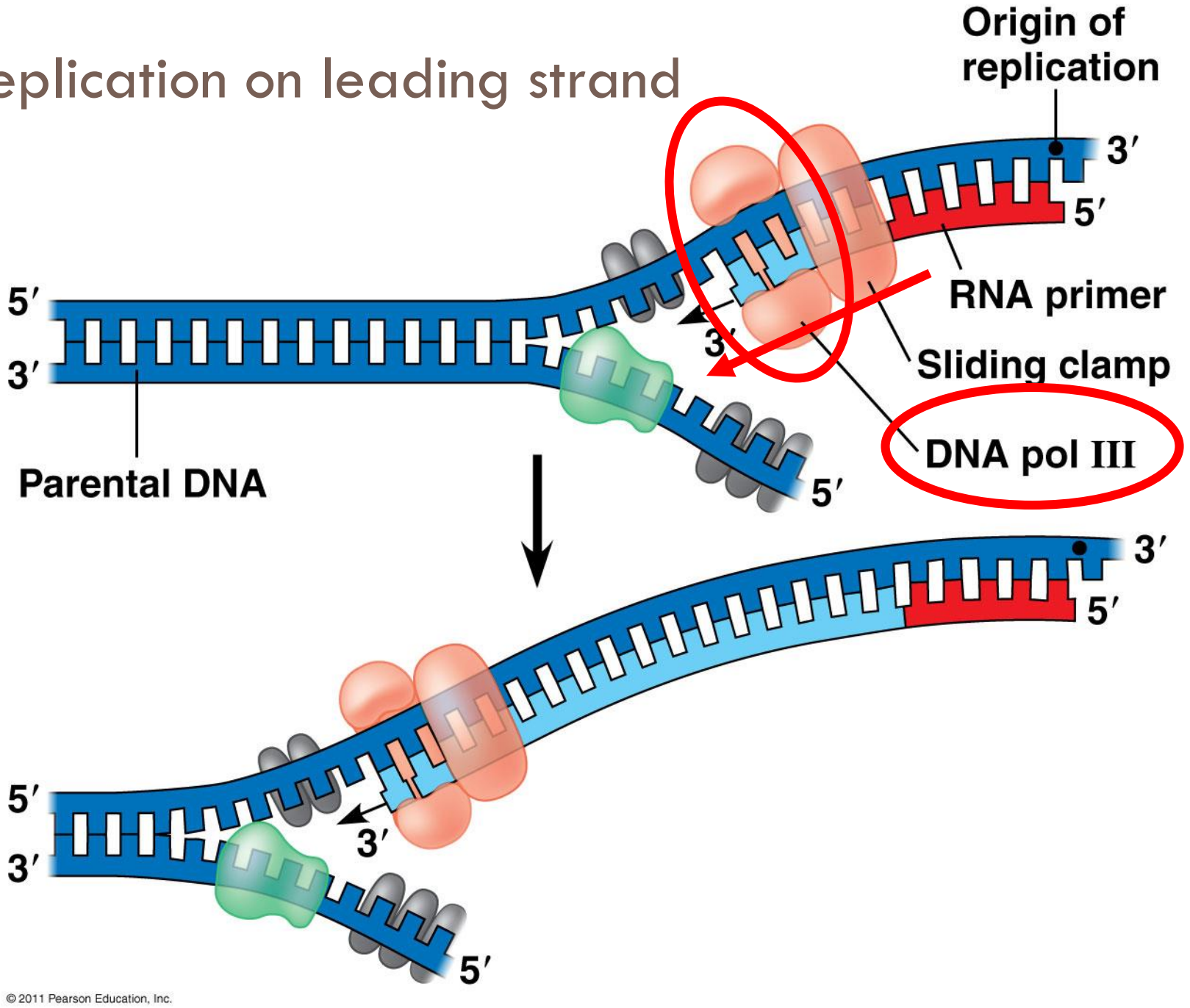
4. DNA polymerase III adds complementary nucleotides in 5'→3' direction on *leading strand*



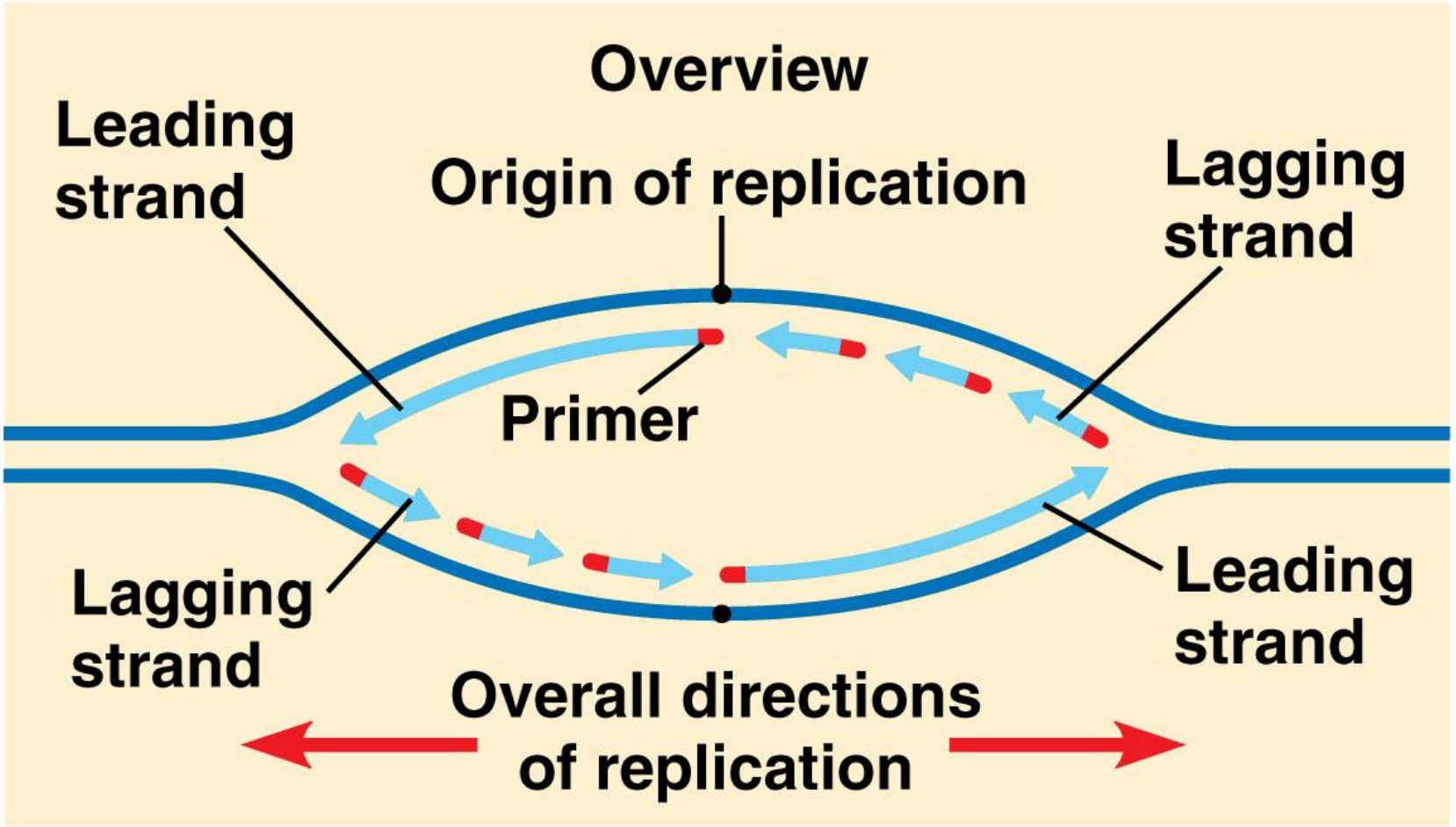
Draw, Label, annotate as we continue



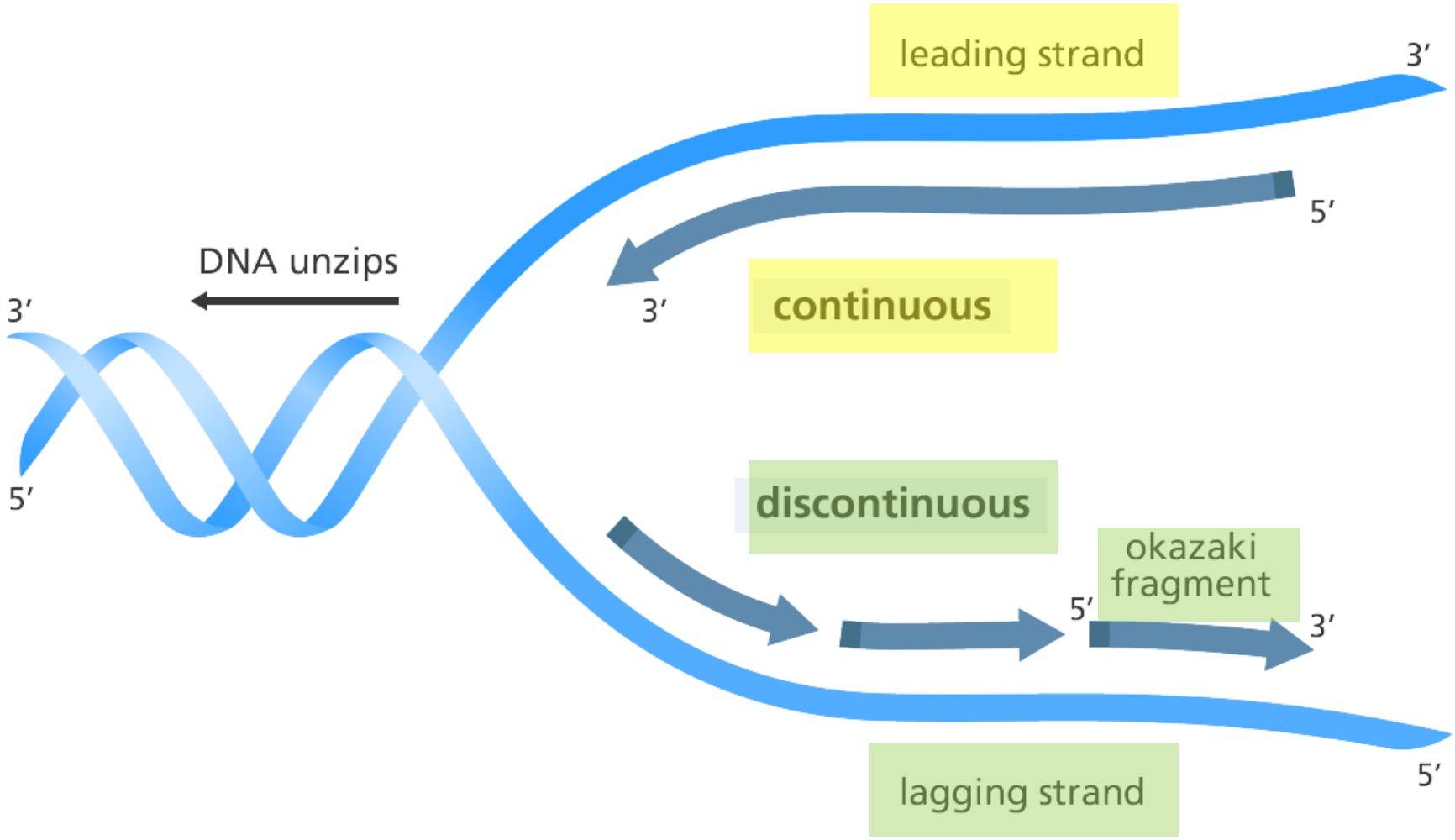
Replication on leading strand



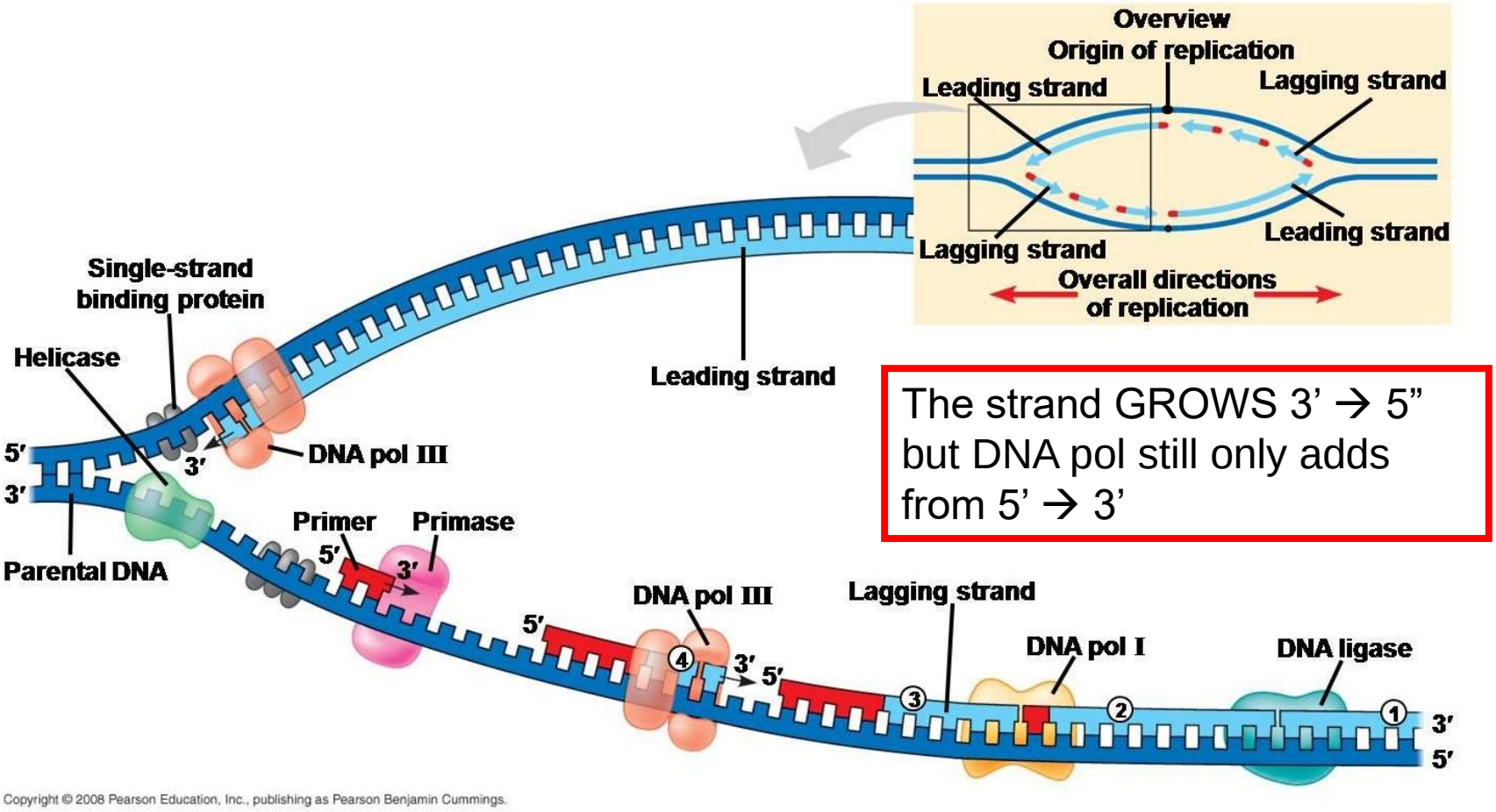
Leading strand vs. *Lagging strand*



DNA replication fork



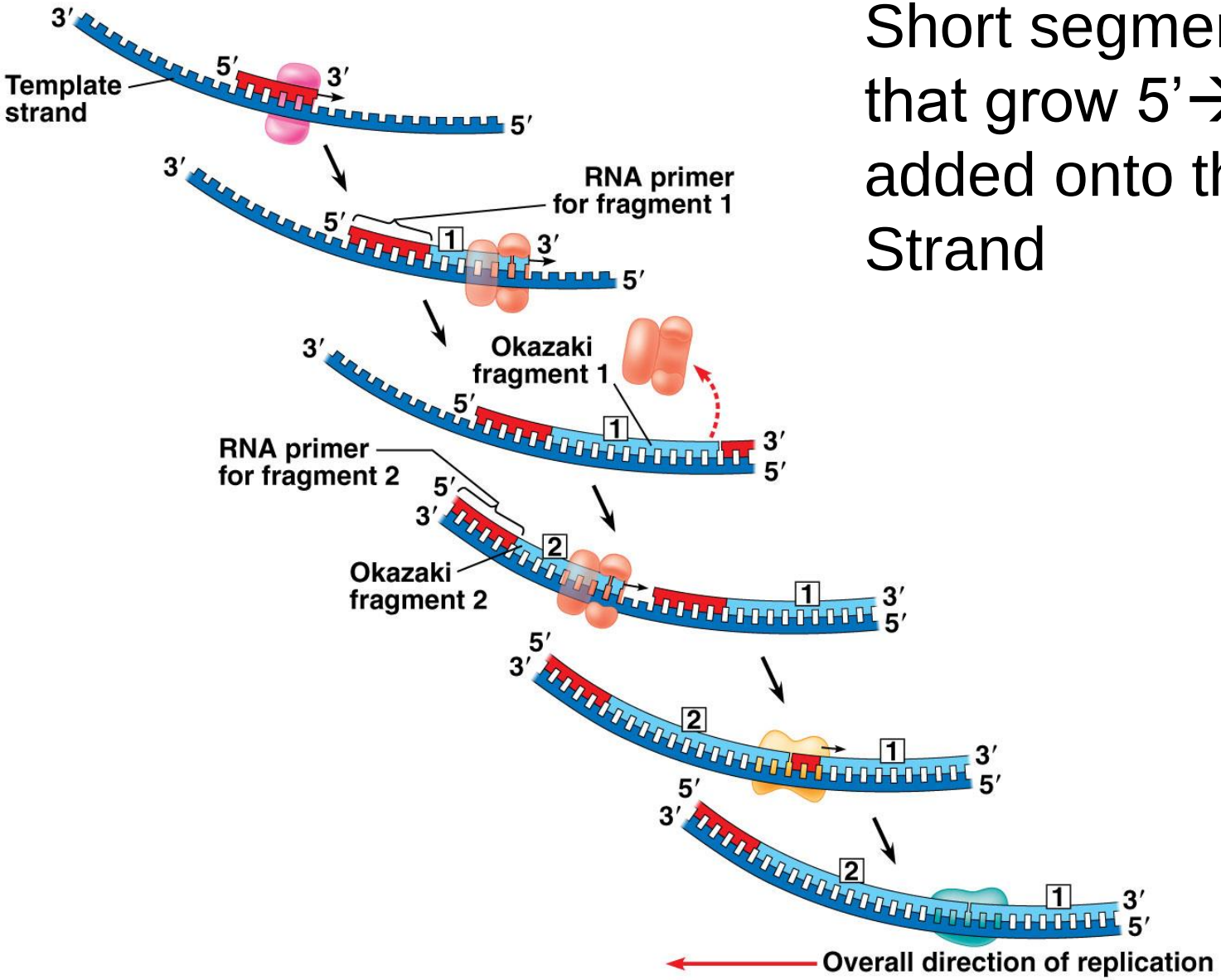
5. Lagging strand grows in 3'→5' direction by the addition of *Okazaki fragments*



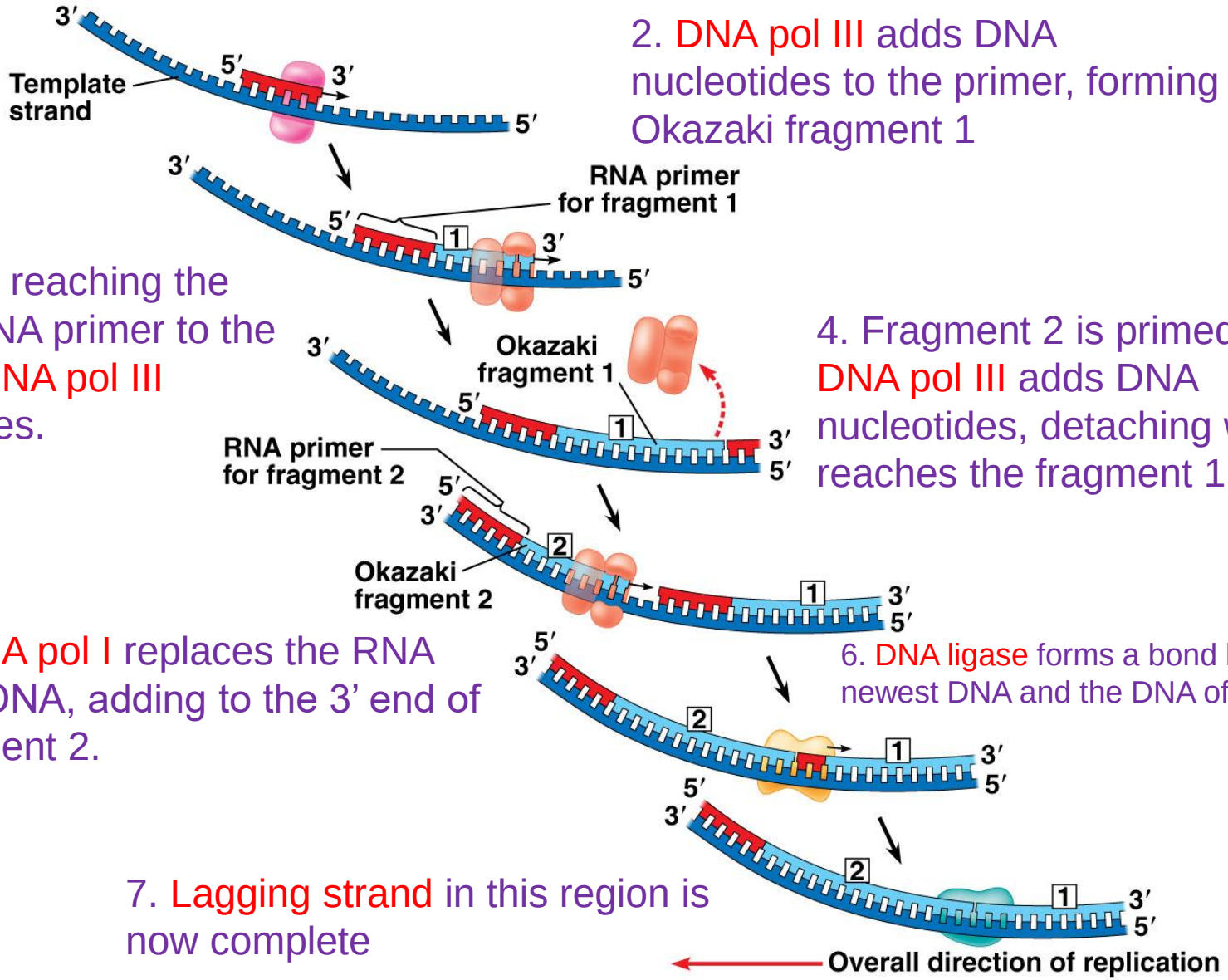
The strand GROWS 3' → 5" but DNA pol still only adds from 5' → 3'

Okazaki Fragments:

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



1. Primase joins RNA nucleotides into a primer.



2. DNA pol III adds DNA nucleotides to the primer, forming Okazaki fragment 1

3. After reaching the next RNA primer to the right, DNA pol III detaches.

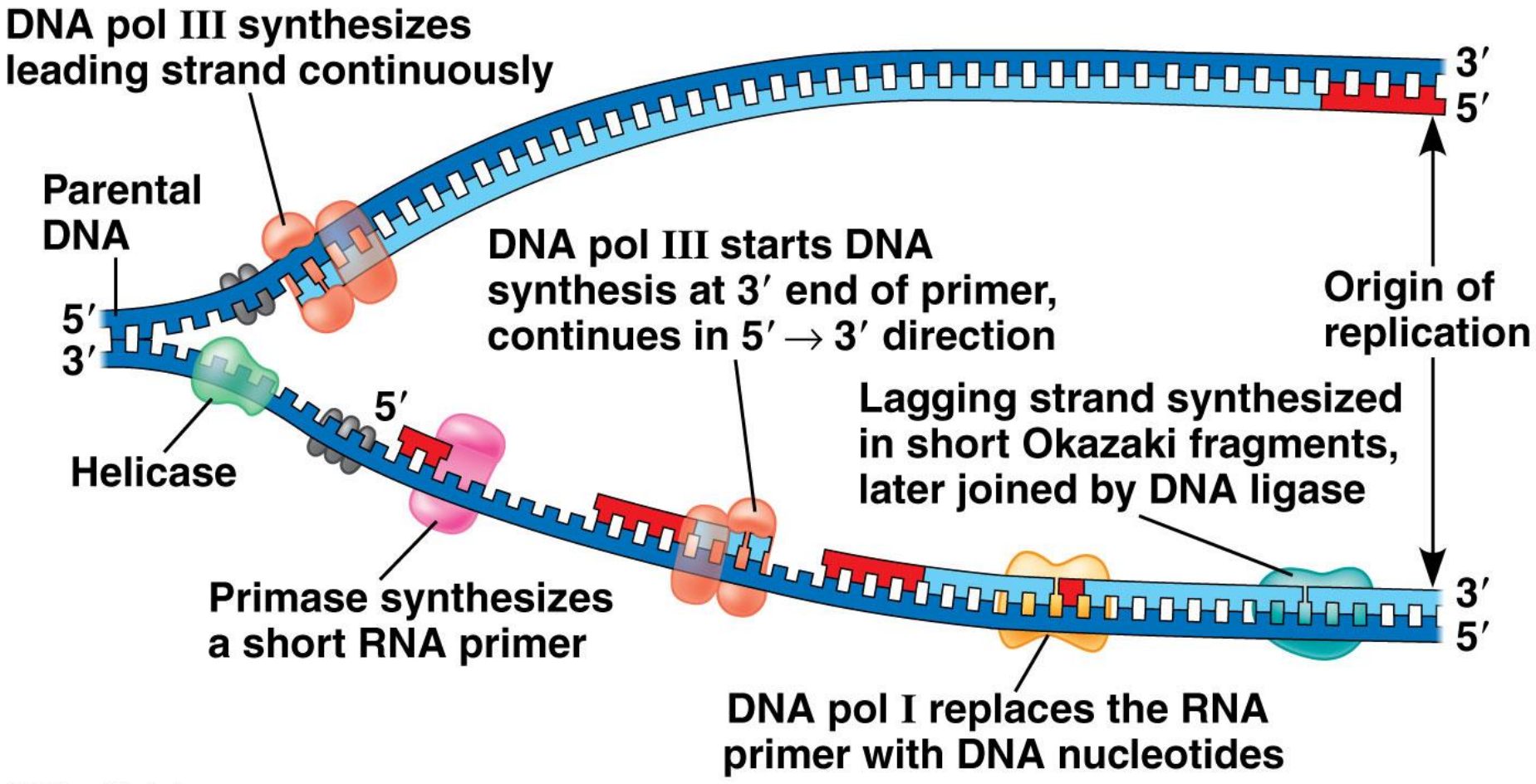
4. Fragment 2 is primed. The DNA pol III adds DNA nucleotides, detaching when it reaches the fragment 1 primer.

5. DNA pol I replaces the RNA with DNA, adding to the 3' end of fragment 2.

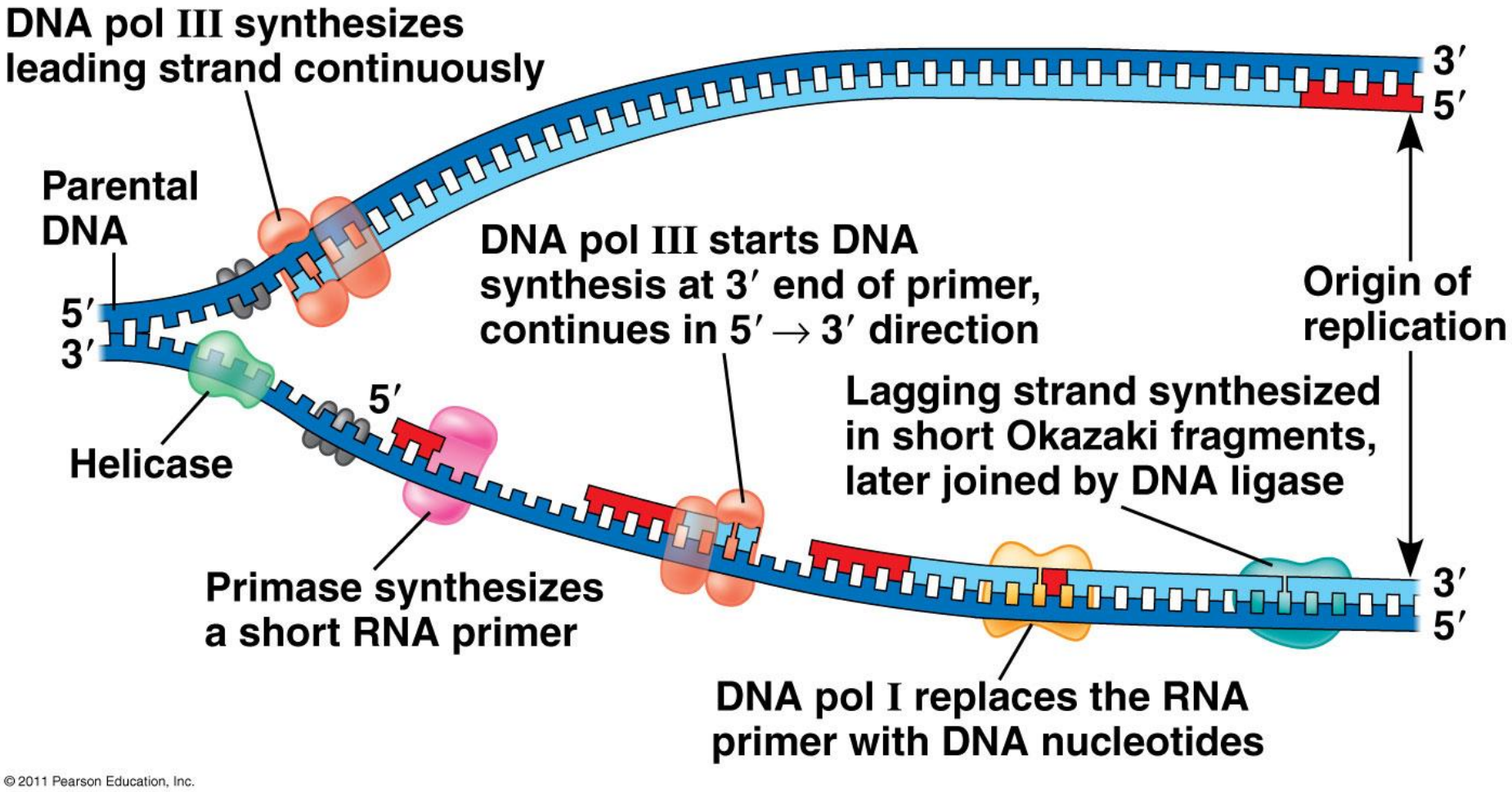
6. DNA ligase forms a bond b/w the newest DNA and the DNA of fragment 1.

7. Lagging strand in this region is now complete

6. **DNA polymerase I:** replaces RNA primers with DNA on lagging strand



7. DNA ligase: seals fragments together



DNA Replication – McGraw Hill

Table 16.1 Bacterial DNA Replication Proteins and Their Functions

Protein	Function
Helicase	Unwinds parental double helix at replication forks
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template
Topoisomerase	Relieves "overwinding" strain ahead of replication forks by breaking, swiveling, and rejoining DNA strands
Primase	Synthesizes an RNA primer at 5′ end of leading strand and of each Okazaki fragment ofl agging strand
DNA pol III	Using parental DNA as a template, synthesizes new DNA strand by covalently adding nucleotides to the 3′ end of a pre-existing DNA strand or RNA primer
DNA pol I	Removes RNA nucleotides of primer from 5′ end and replaces them with DNA nucleotides
DNA ligase	Joins 3′ end of DNA that replaces primer to rest of leading strand and joins Okazaki fragments of lagging strand

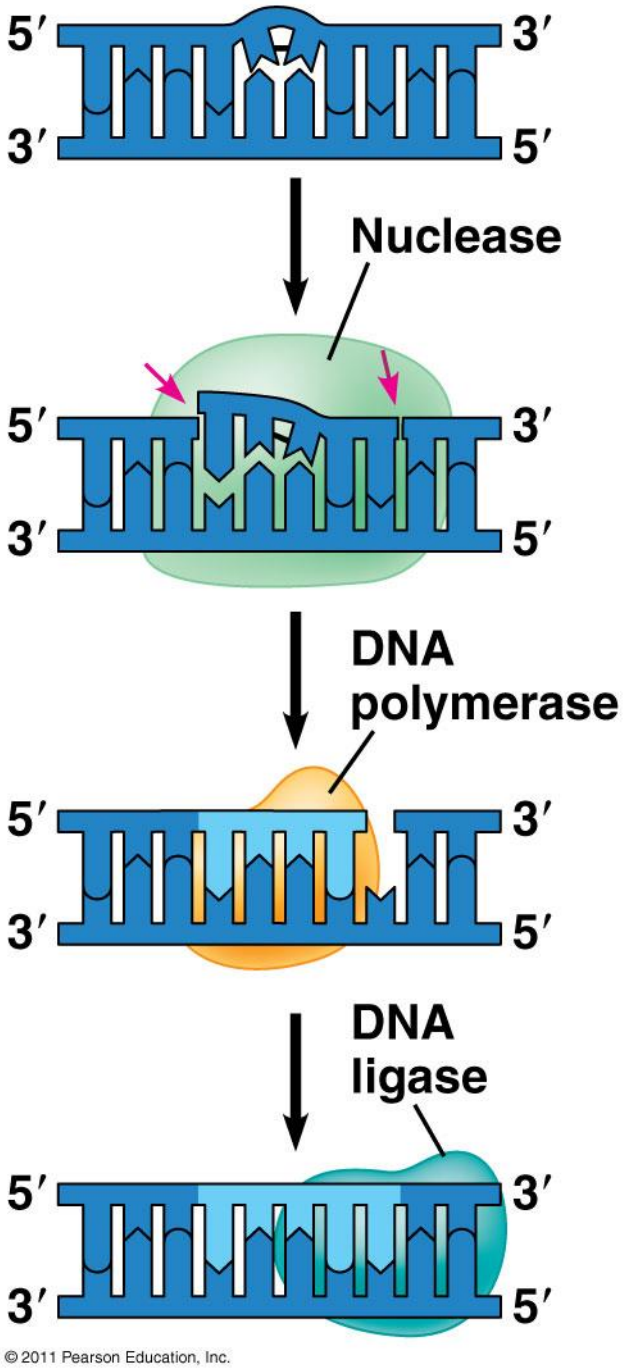
Proofreading and Repair

- DNA polymerases proofread as bases added
- Mismatch repair: special enzymes fix incorrect pairings
- Nucleotide excision repair: Repairs damaged DNA (due to chemicals or UV radiation)
  - **Nucleases** cut damaged DNA
  - DNA poly and ligase fill in gaps

Nucleotide Excision Repair

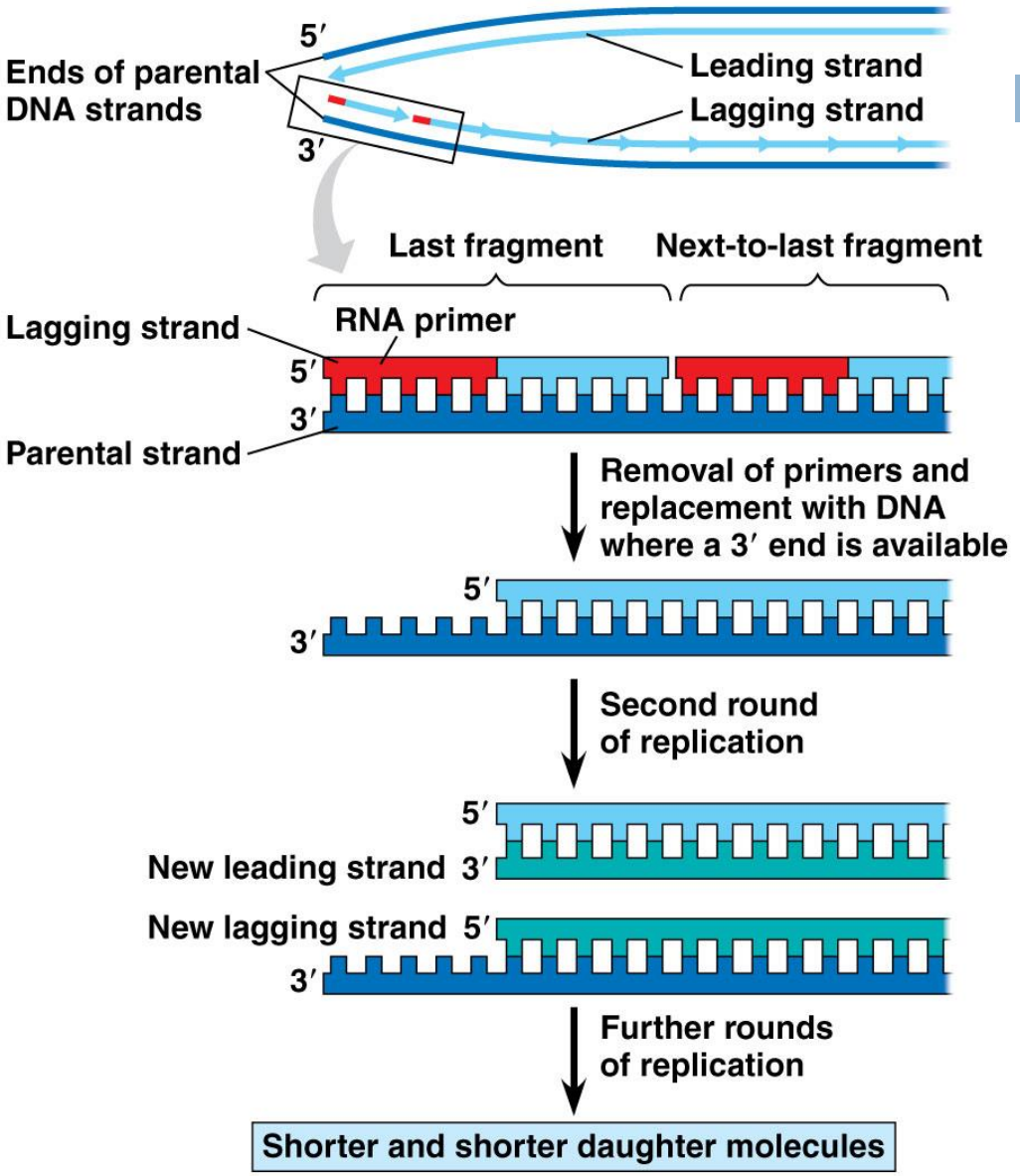
Error Rates:

- Pairing errors: 1 in 100,000 nucleotides
- Complete DNA: 1 in 10 billion nucleotides



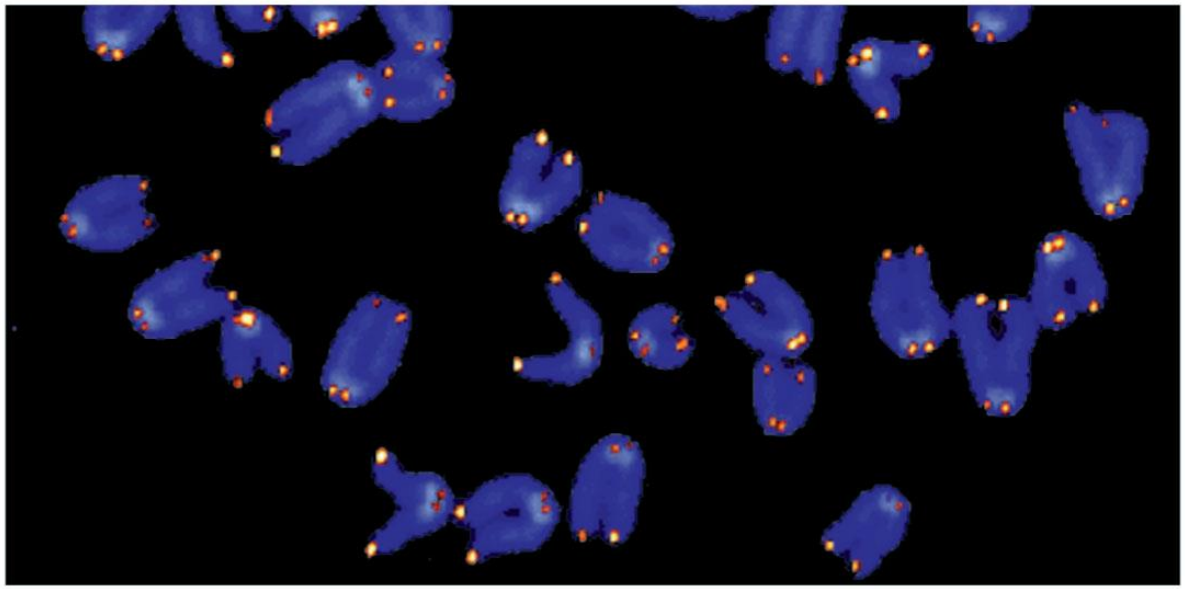
Problem at the 5' End

- 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



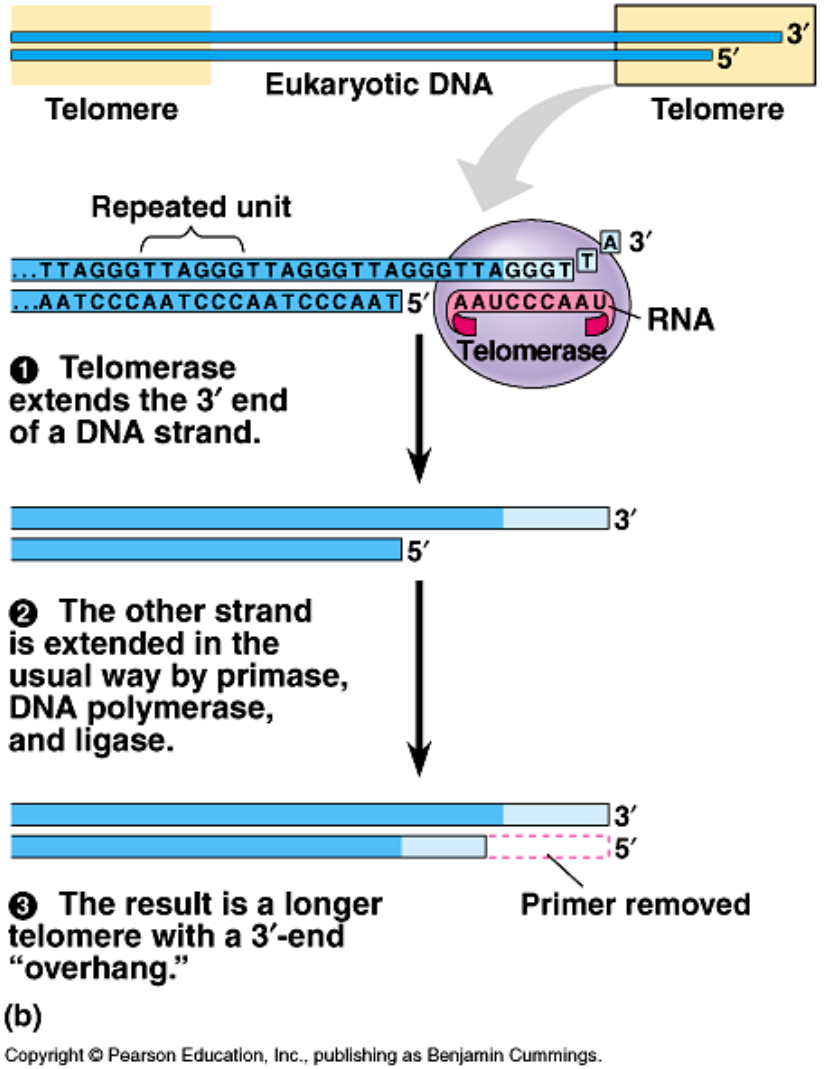
Telomeres: repeated units of short nucleotide sequences (TTAGGG) at ends of DNA

- 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

DNA Replication Video

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