Chapter 16:
DNA Structure & Replication

1. DNA Structure

2. DNA Replication

1. DNA Structure

Chapter Reading – pp. 313-318

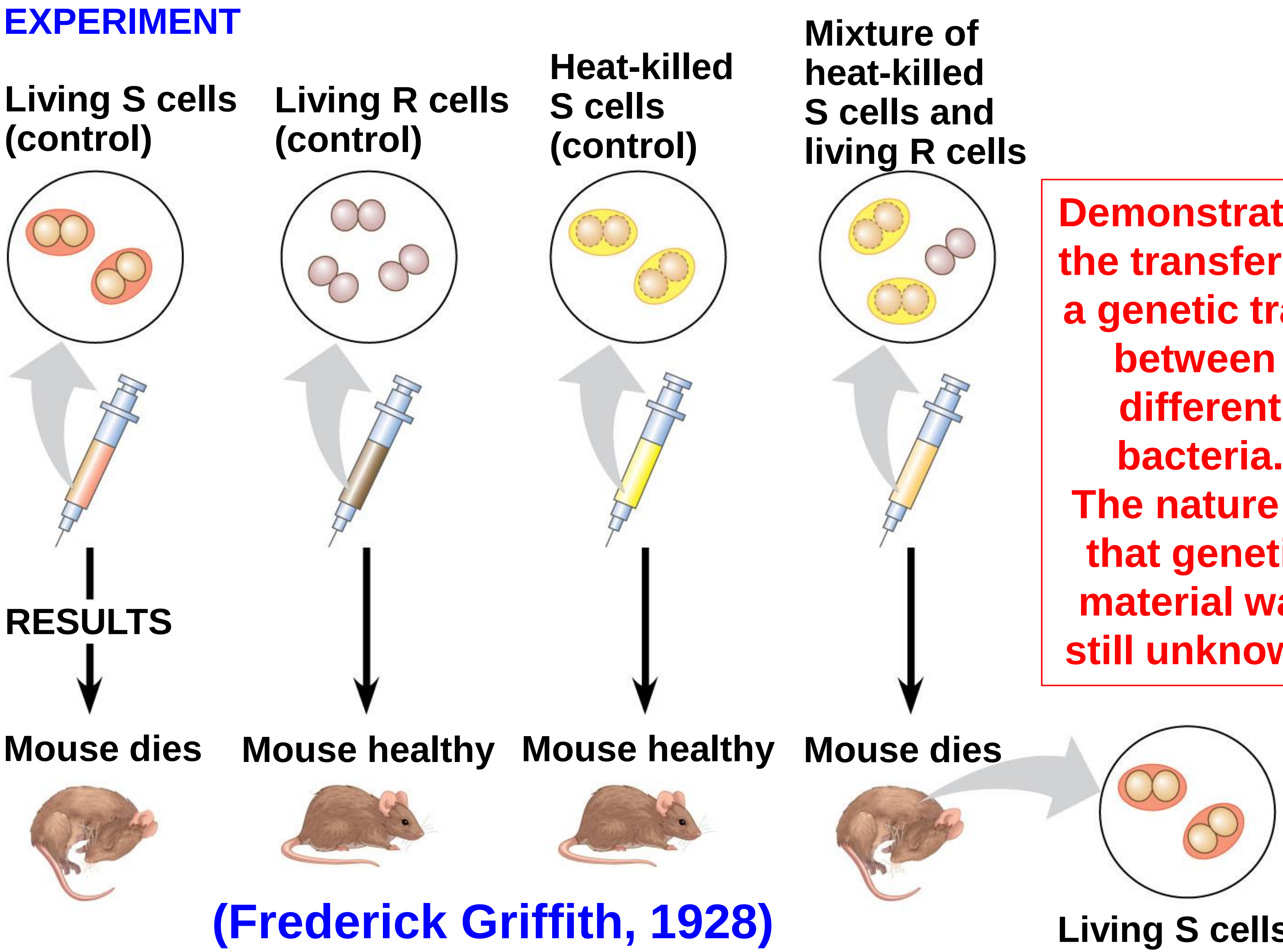
Genetic Material: Protein or DNA?

Until the early 1950's no one knew for sure, but it was generally thought that protein was the genetic material. Why?

- **protein is made of 20 different amino acids**
- **DNA is made of only 4 different nucleotides**
- **protein could theoretically store more info**
  - **a "20 letter alphabet" vs a "4 letter alphabet"**
  - **it was assumed that life was so complex, therefore a "bigger alphabet" was necessary to somehow encode it!**

Some classic experiments would prove otherwise…

Transformation of Bacteria



Demonstrated the transfer of a genetic trait between different bacteria.
The nature of that genetic material was still unknown.

(Frederick Griffith, 1928)

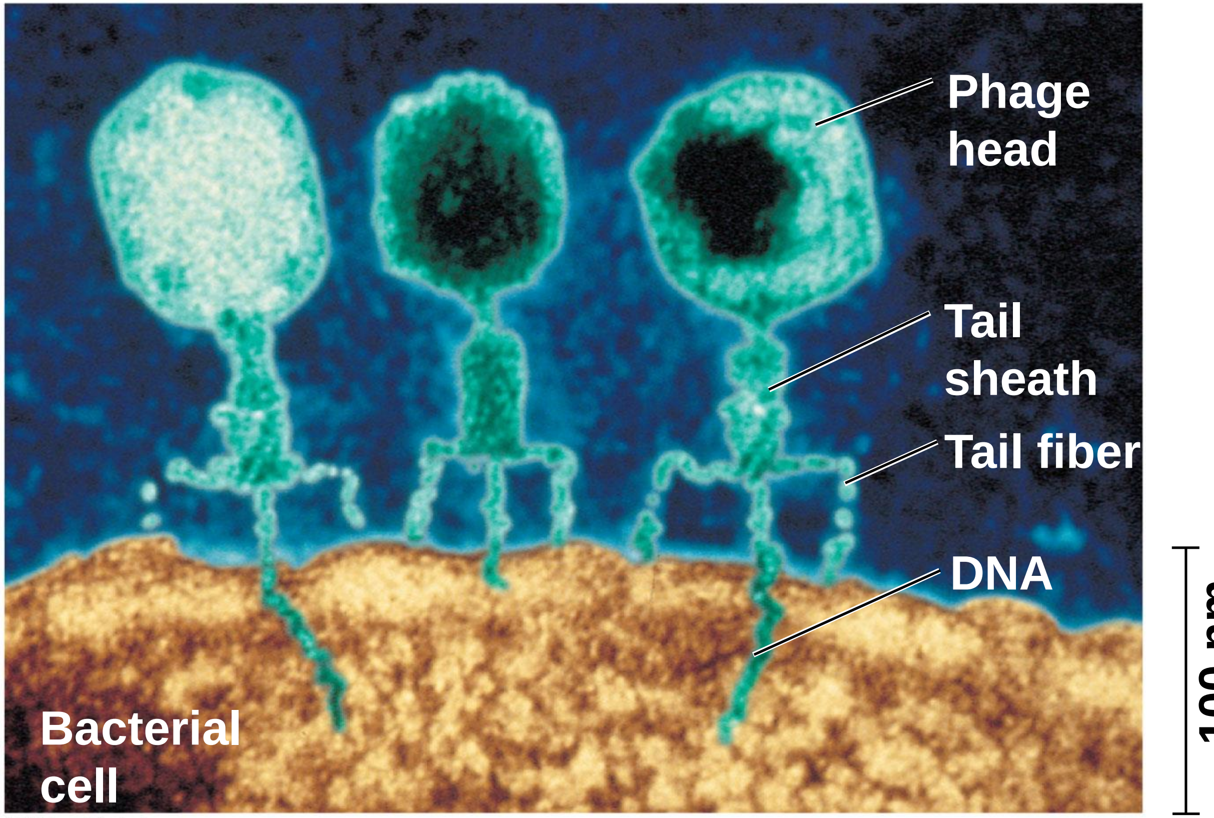
What is the Genetic Material of Bacteriophages?

Bacteriophages are viruses that infect bacteria.

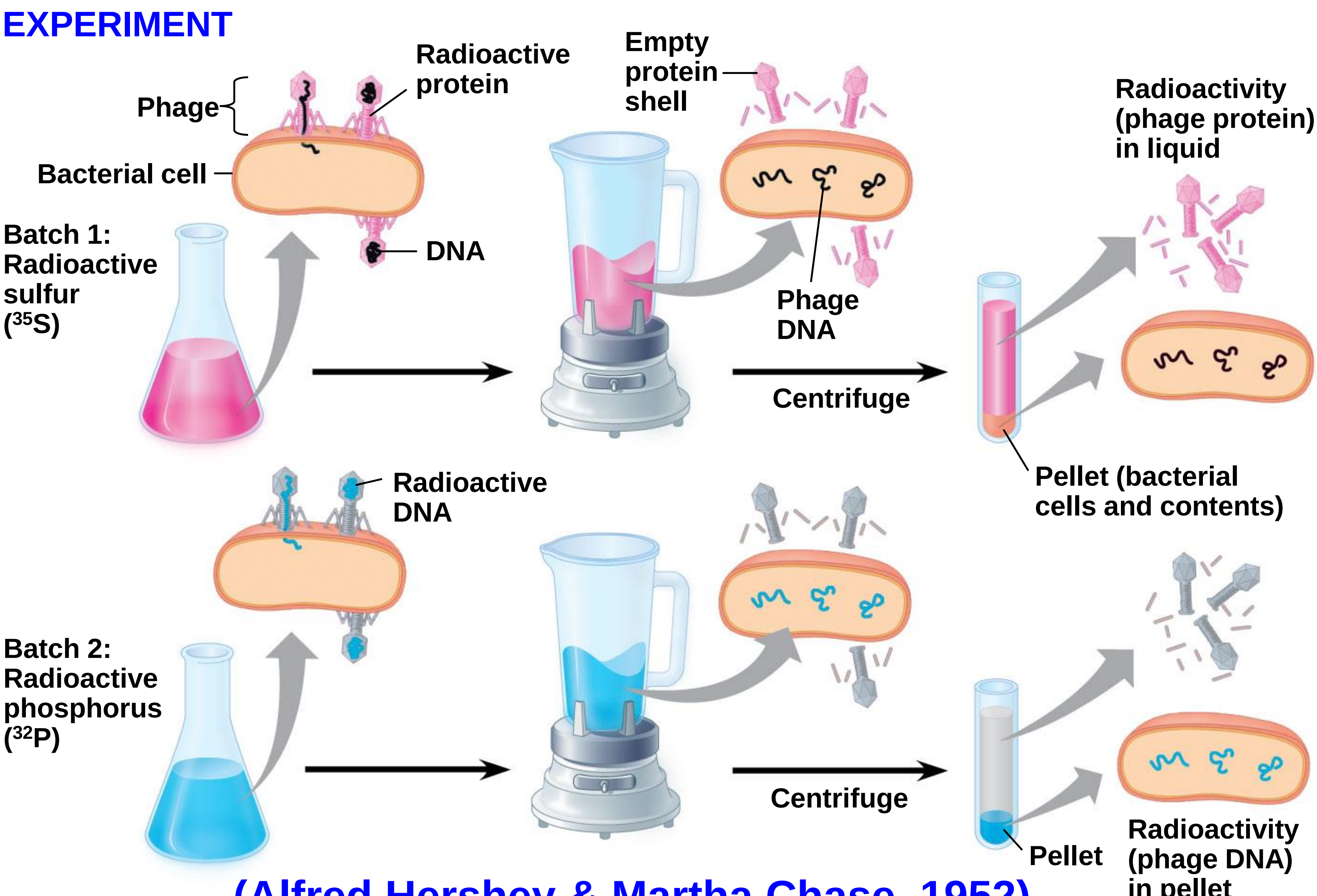
- consist of a protein capsid which contains DNA

What enters the bacterial host cell, viral protein, DNA, or both?

- whatever enters the host cell should be the genetic material



Bacteriophage Genetic Material is DNA

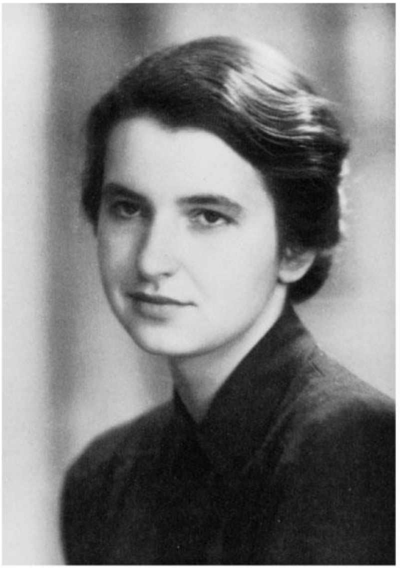


(Alfred Hershey & Martha Chase, 1952)

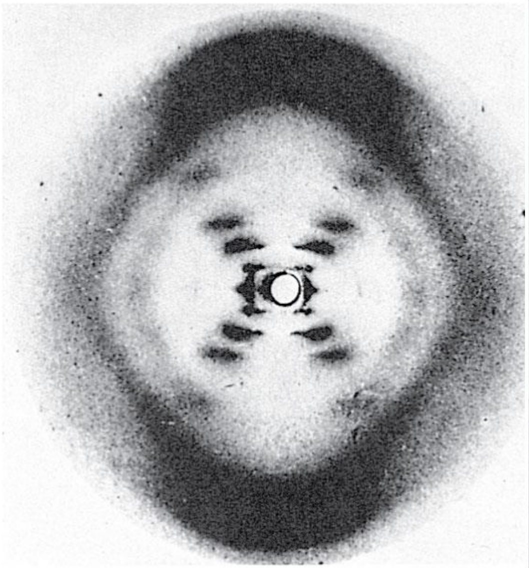
The Discovery of DNA Structure

Using the technique of x-ray crystallography, Rosalind Franklin, James Watson & Francis Crick figured out the structure of DNA

- **Watson & Crick used the X-ray diffraction data of Rosalind Franklin to deduce the structure of DNA**



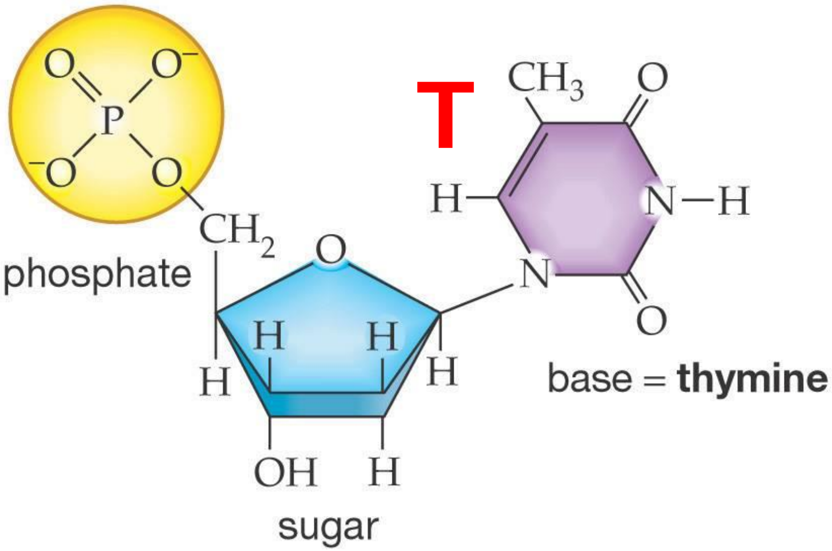
(a) Rosalind Franklin

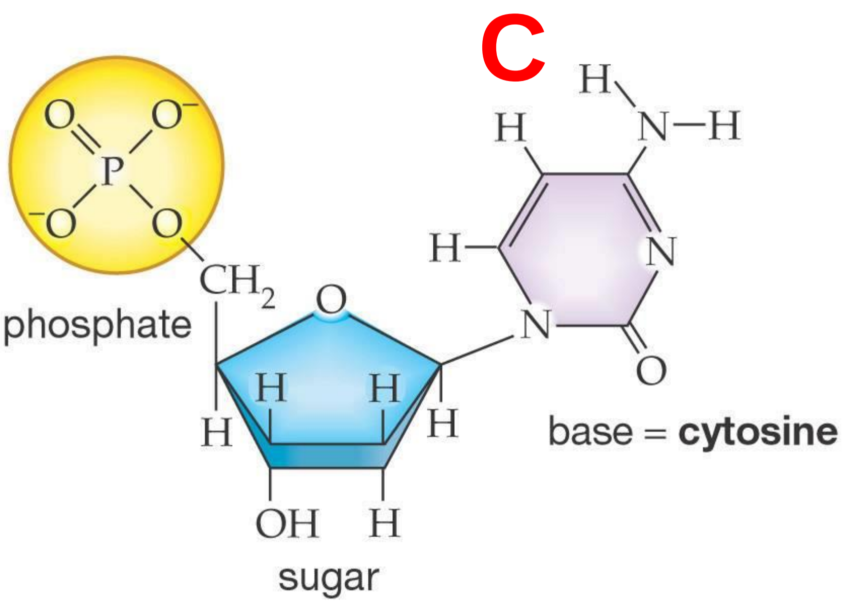


(b) Franklin's X-ray diffraction photograph of DNA

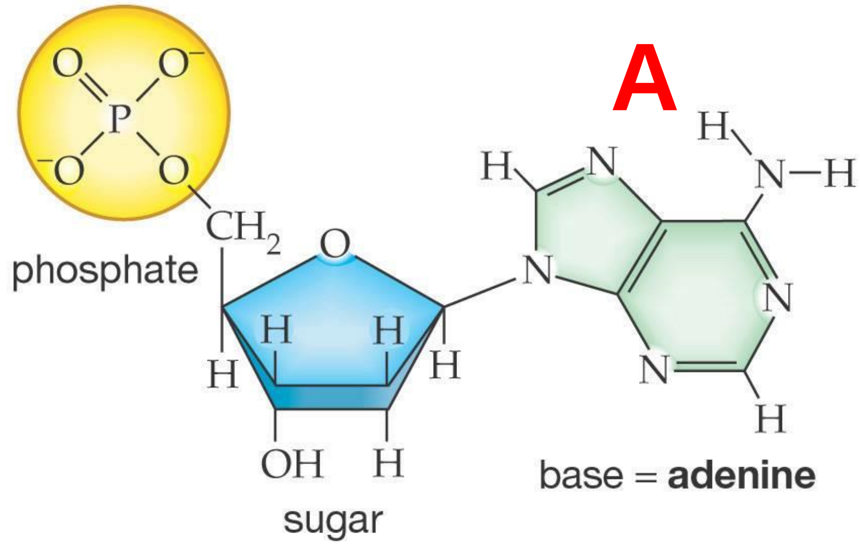
DNA: a Polymer of 4 Nucleotides

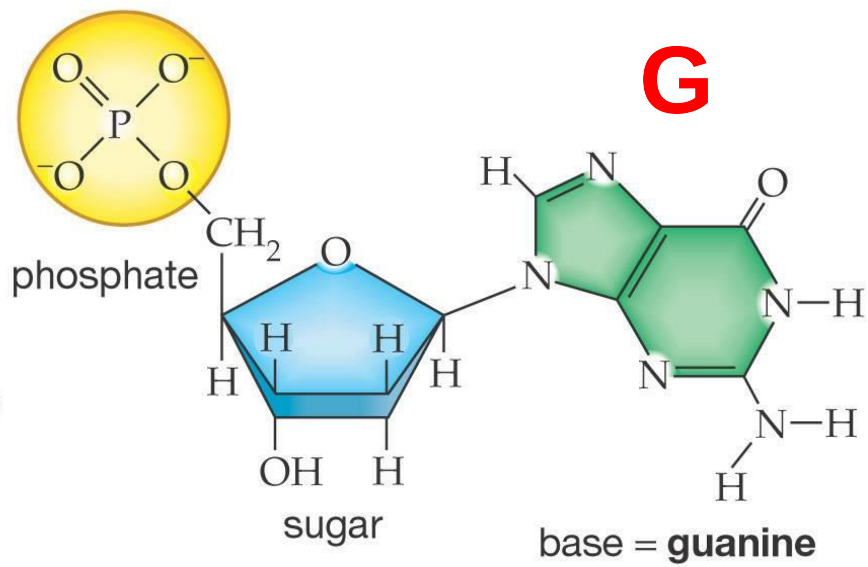
pyrimidines

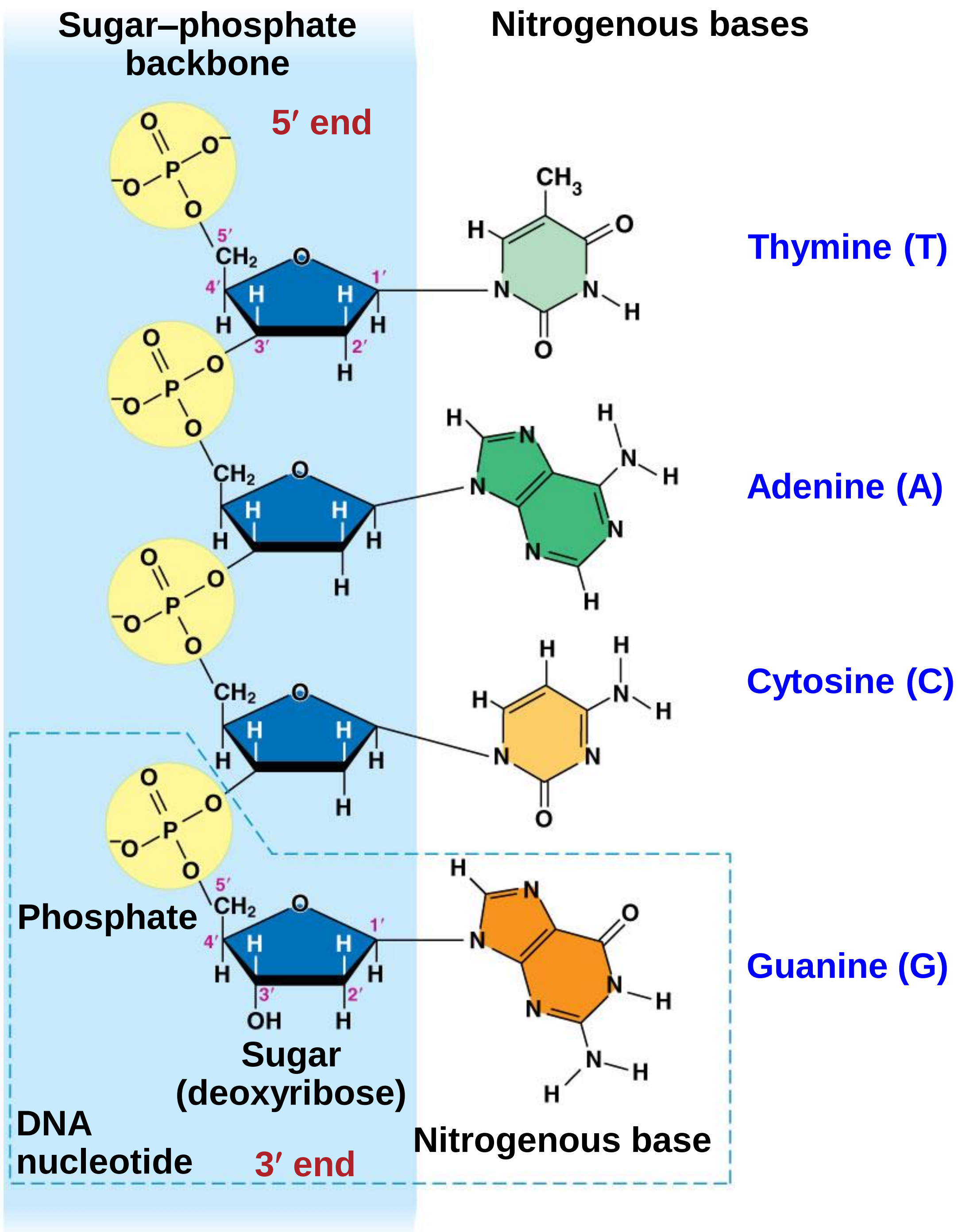




purines







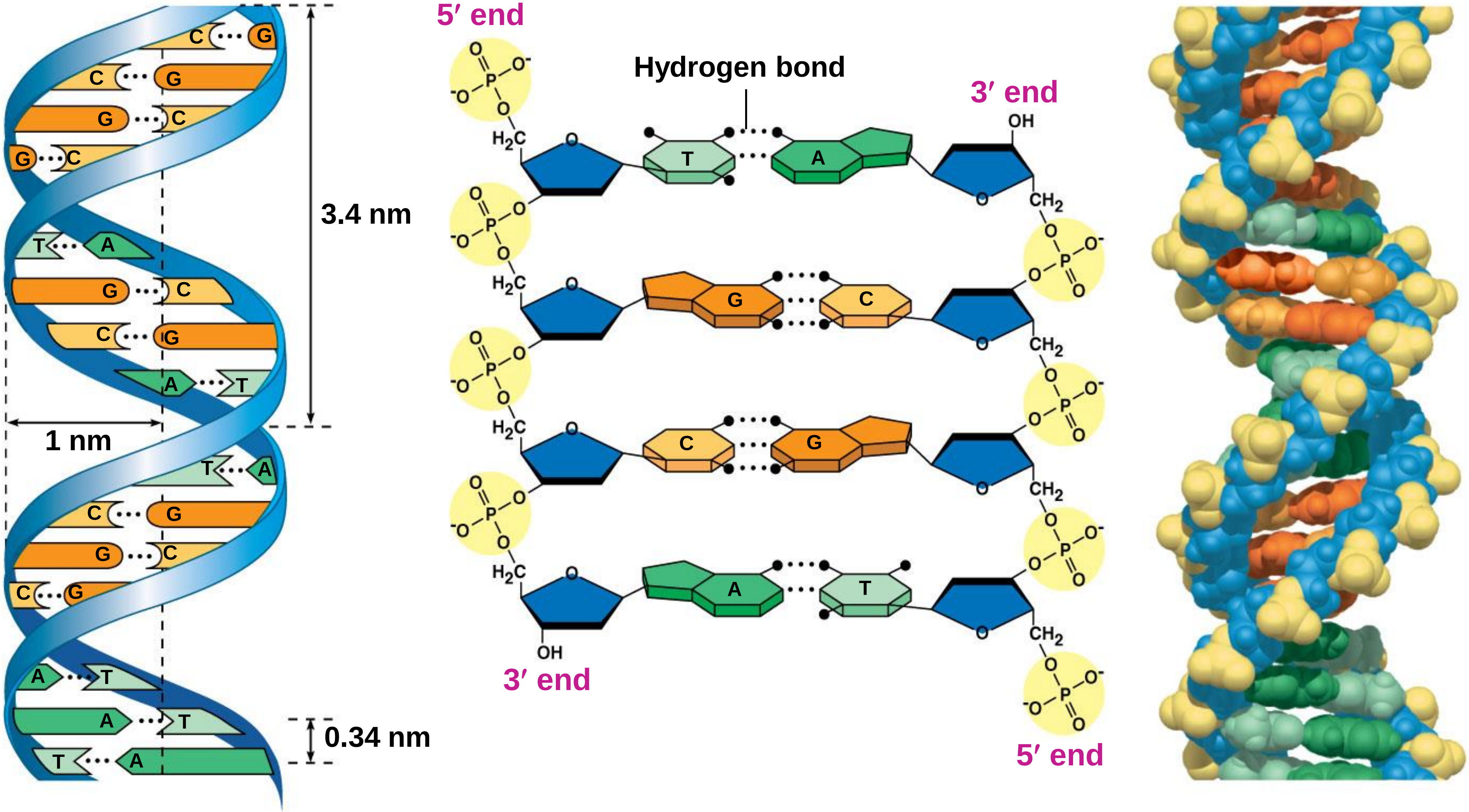
Structure of a DNA Strand

A single DNA polymer or "strand" consists of a <u>sugar-phosphate backbone</u> with the bases project out.

The ends of a DNA strand are different, with one end having a free <u>5' phosphate</u>, and the other having a free <u>3' hydroxyl group</u>

Structure of Double-stranded DNA

- the 2 strands are <u>anti-parallel</u> and interact via <u>base pairs</u>



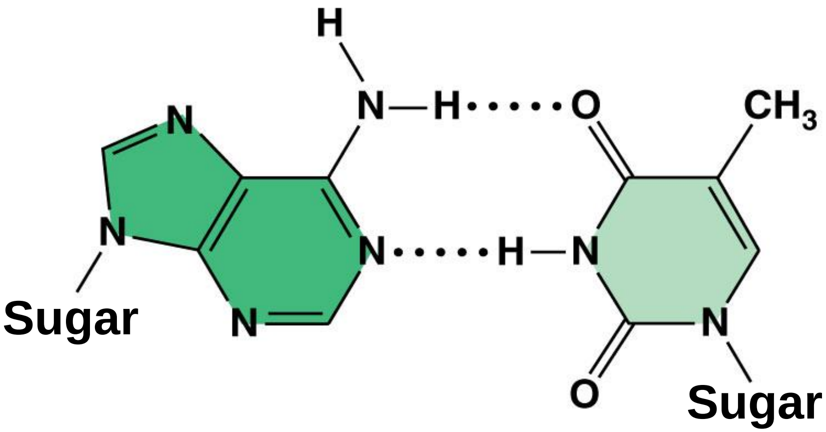
(a) Key features of DNA structure

(b) Partial chemical structure

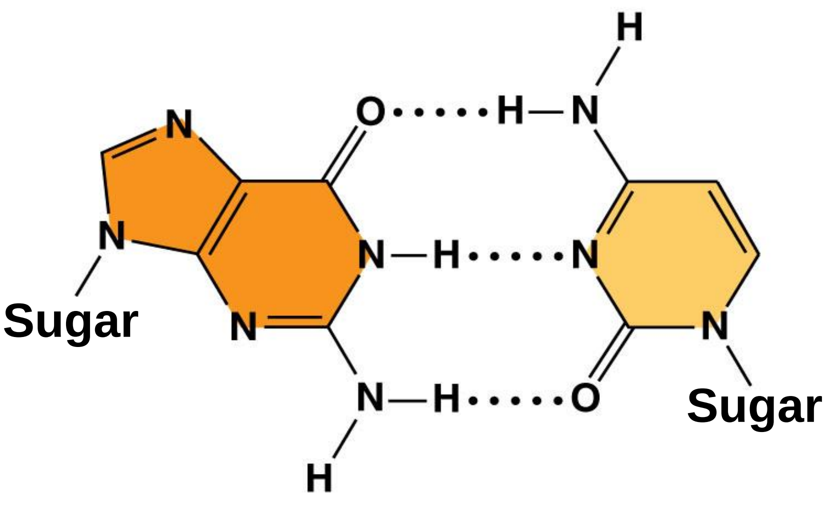
(c) Space-filling model

DNA “Base-Pairing”

Base pairs are held together by hydrogen bonds.



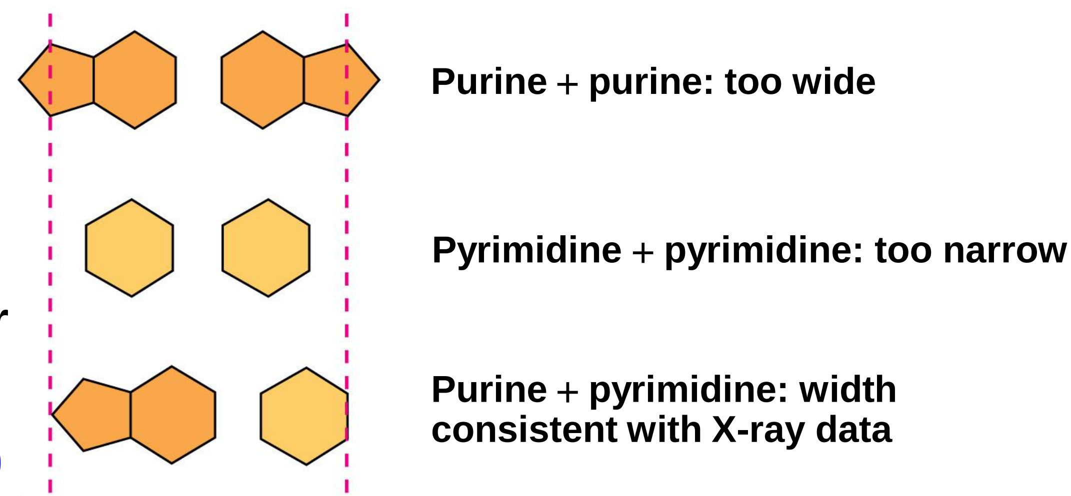
Adenine (A) Thymine (T)



Guanine (G) Cytosine (C)

Why only A:T and C:G?

- the position of chemical groups involved in H-Bonds
- the size of the bases (purine & pyrimidine)



The DNA “Sequence”

The DNA sequence is the linear order of nucleotides in a DNA strand:

- **each DNA strand in the double helix has its own sequence**
- **the sequences in each strand are considered as <u>complementary</u> to each other**
    - **they differ, but “fit just right” with each other**
    - **ea strand will “fit” with only 1 complementary strand**

e.g.

```
5′  A–C–A–C–A–C–A–C–A–C  3′
3′  T–G–T–G–T–G–T–G–T–G  5′
```

2. <u>DNA Replication</u>

Chapter Reading – pp. 318-330

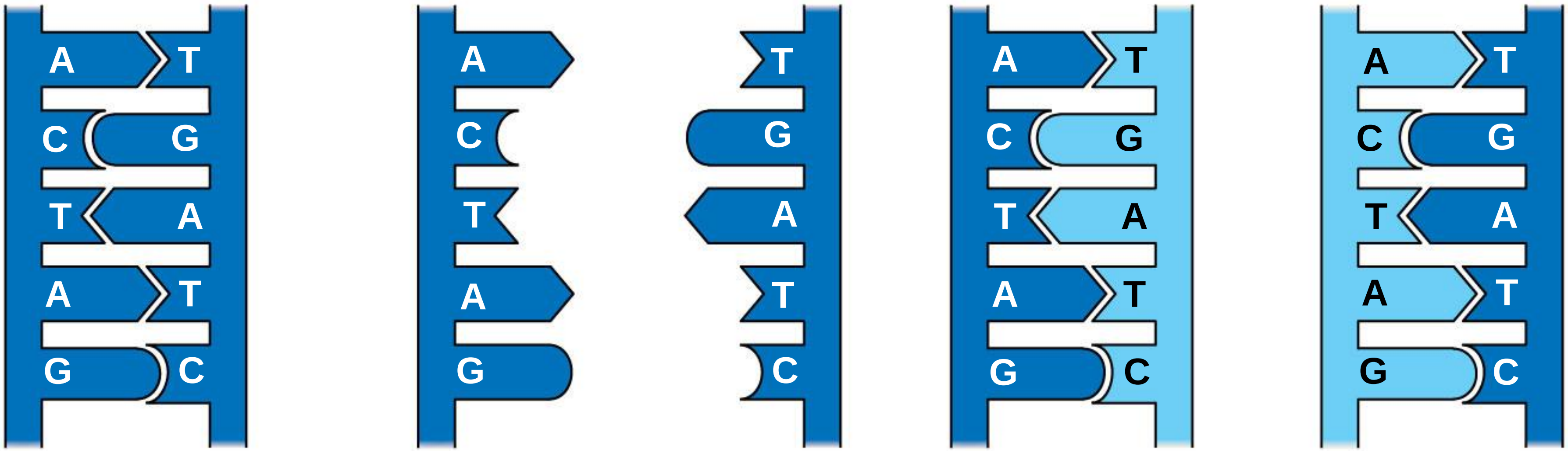
How is DNA Replicated?

Every time a cell reproduces (i.e., divides) it must replicate its chromosomes (DNA) during S phase.

The process of DNA replication was originally proposed to depend on the rules of base pairing:

- **A:T & T:A , C:G & G:C**
- **the sequence of one strand dictates the sequence of the other**
- **each strand of the double helix could serve as a <u>template</u> to make a complementary strand**

Model for DNA Replication



(a) Parent molecule

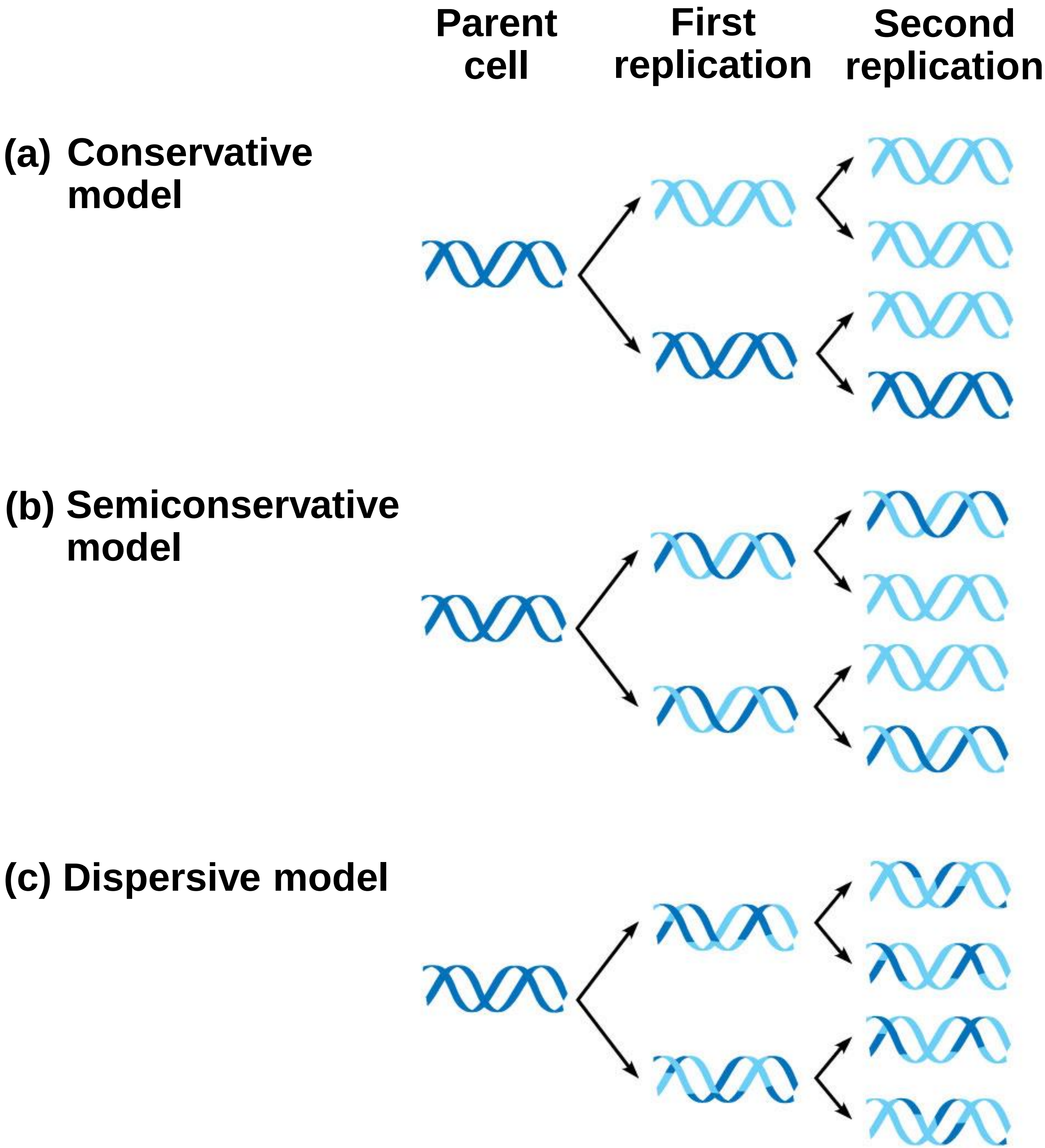
(b) Separation of strands

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

The <u>semiconservative model</u> of DNA replication proposed that each original strand serves as a template to produce a new complementary strand.

- **note that ea original strand ends up in a different molecule**

Other Models



CONSERVATIVE
The original DNA strands stay together

SEMICONSERVATIVE
The original DNA strands remain intact in separate molecules

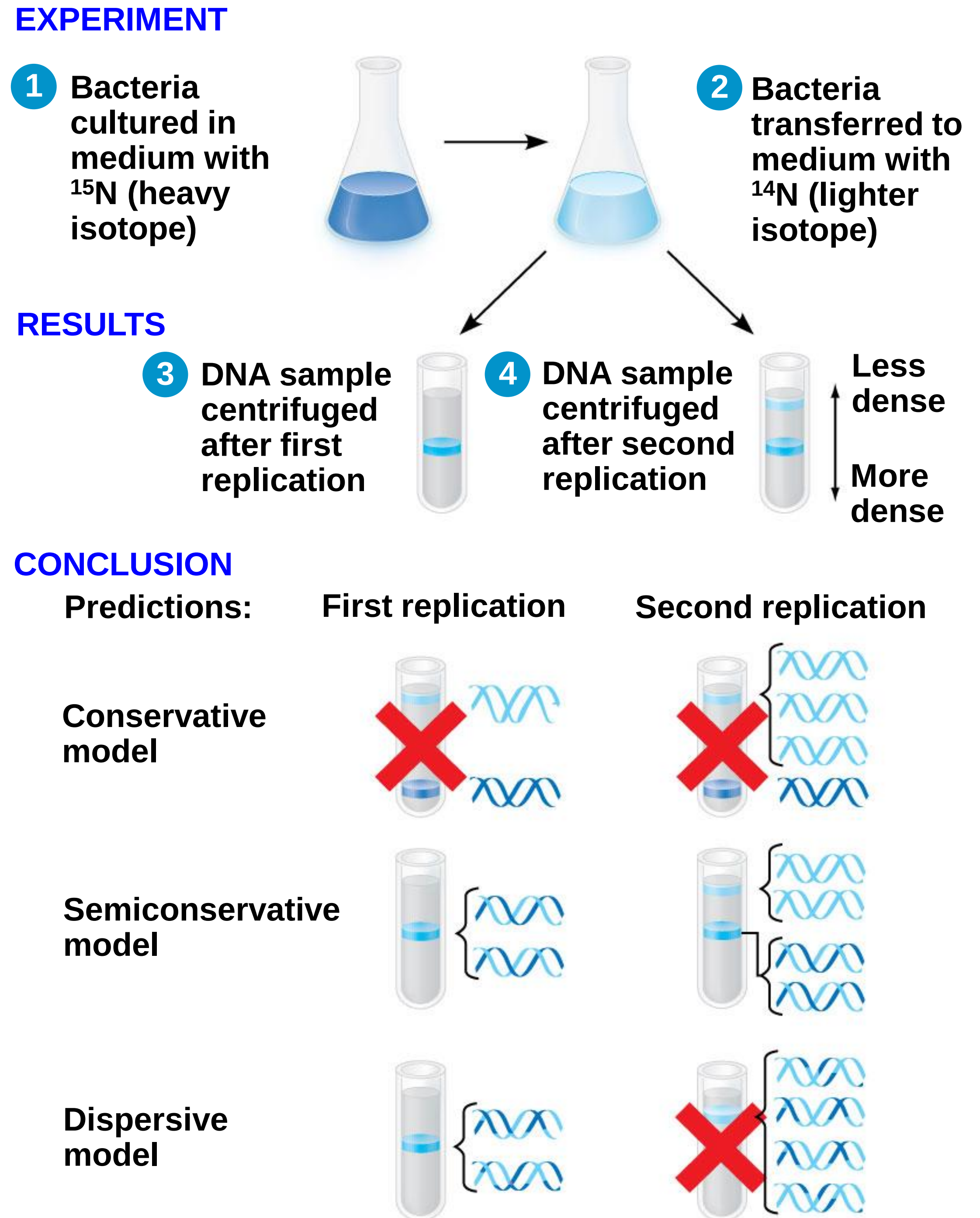
DISPERSIVE
The original DNA strands are dispersed among the all daughter strands

Testing the Models

In this experiment, bacteria with DNA containing the "heavy" isotope ^{15}N were allowed to reproduce in medium containing lighter ^{14}N.

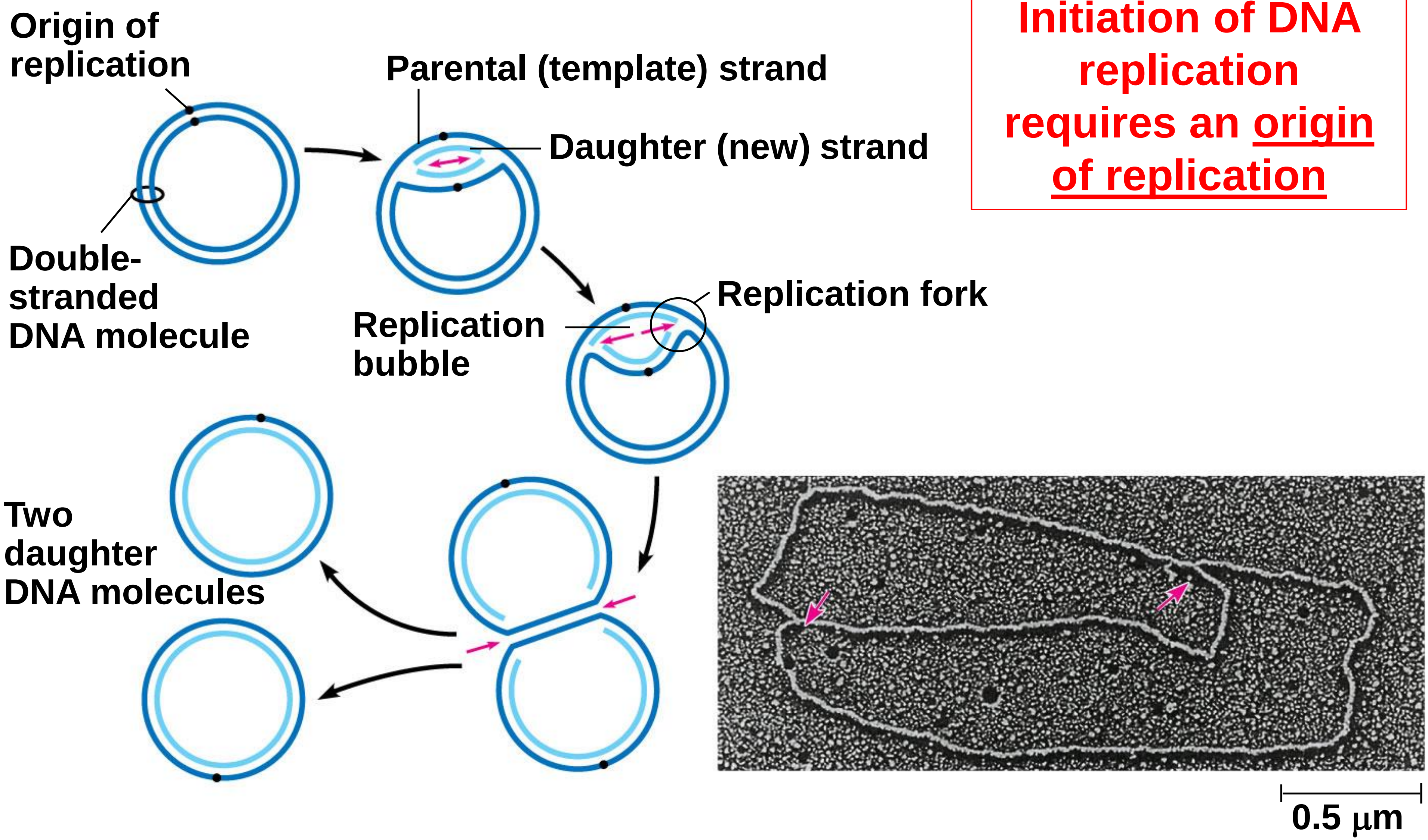
Density-gradient centrifugation revealed that DNA replication is <u>semiconservative.</u>

Matthew Meselson & Franklin Stahl, 1958



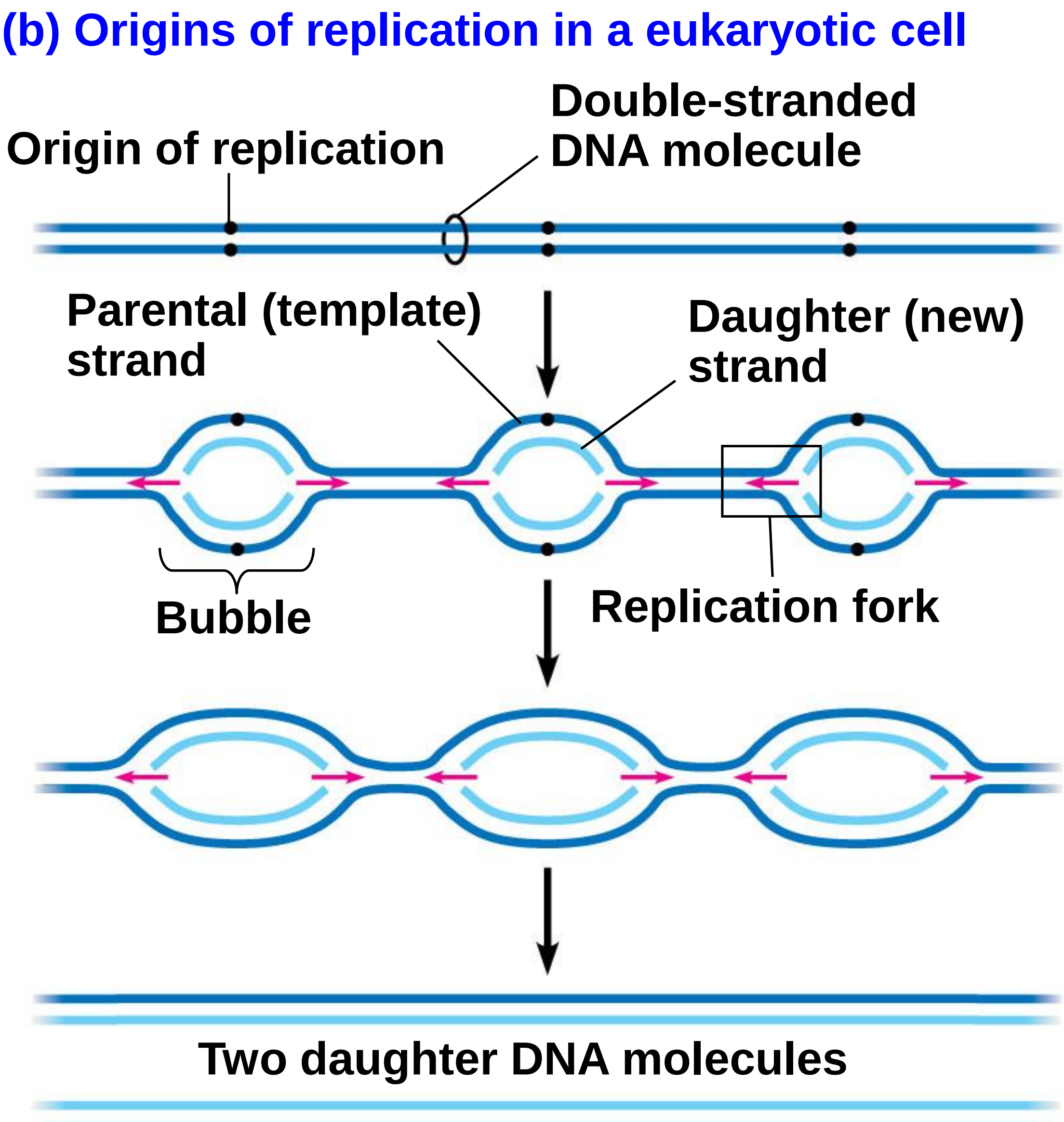
DNA Replication in Bacteria

(a) Origin of replication in an *E. coli* cell

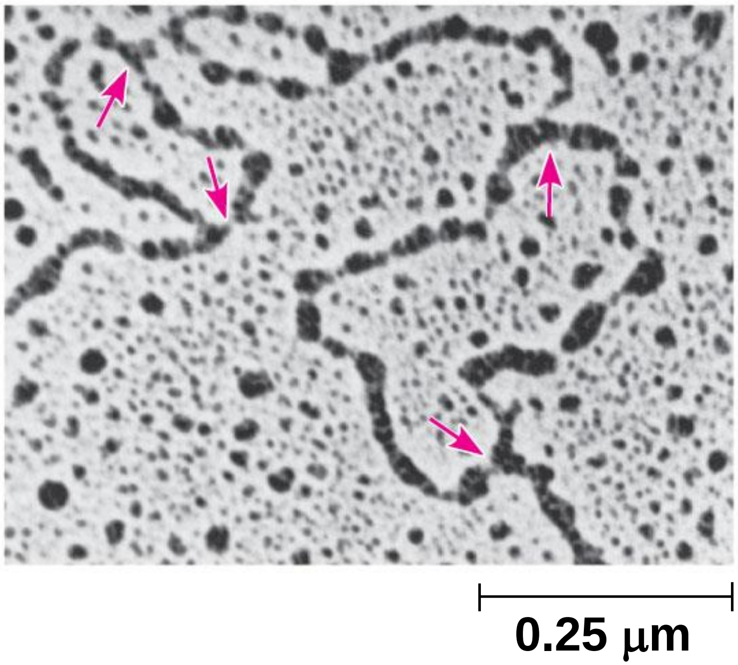


Initiation of DNA replication requires an <u>origin of replication</u>

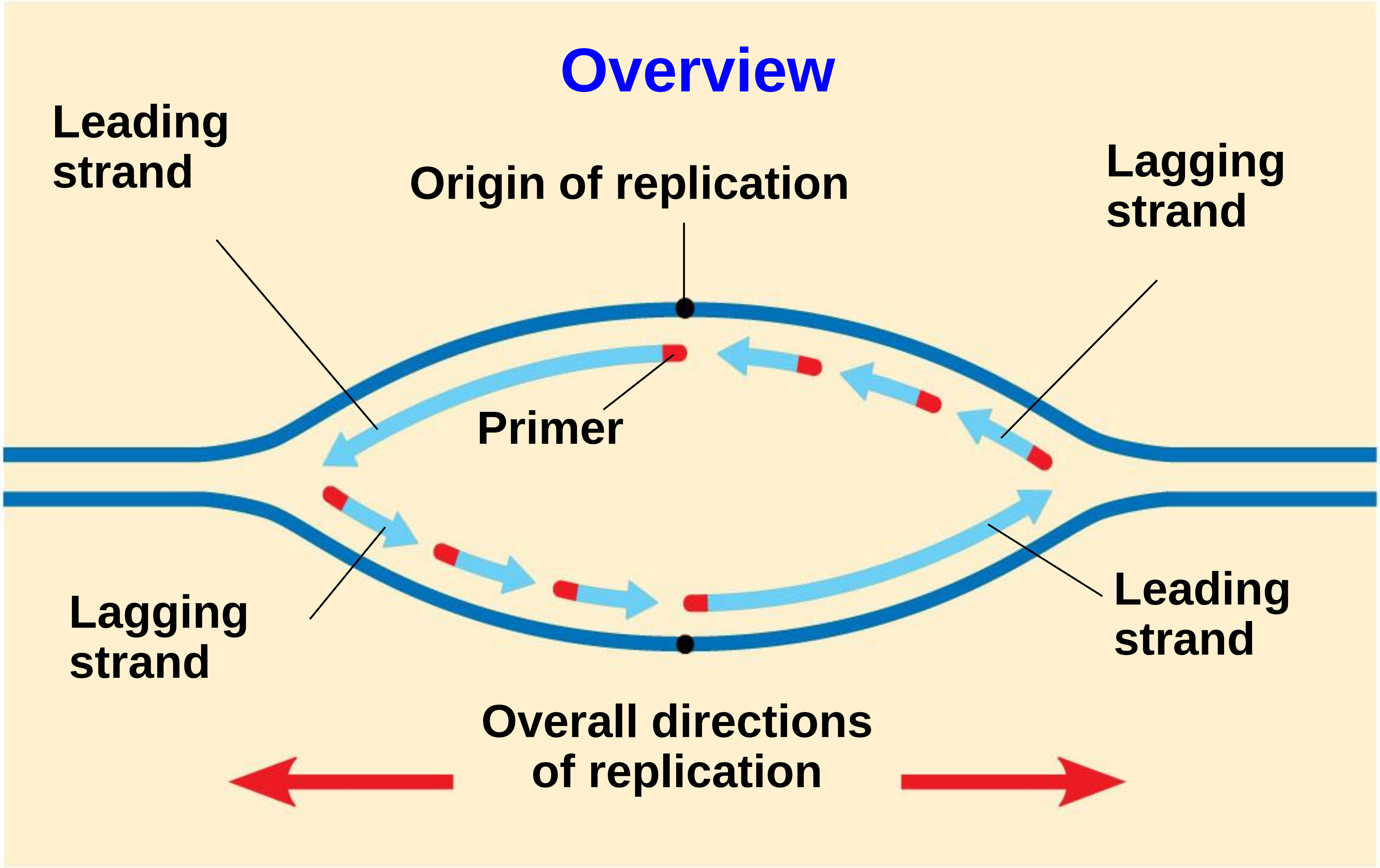
DNA Replication in Eukaryotes



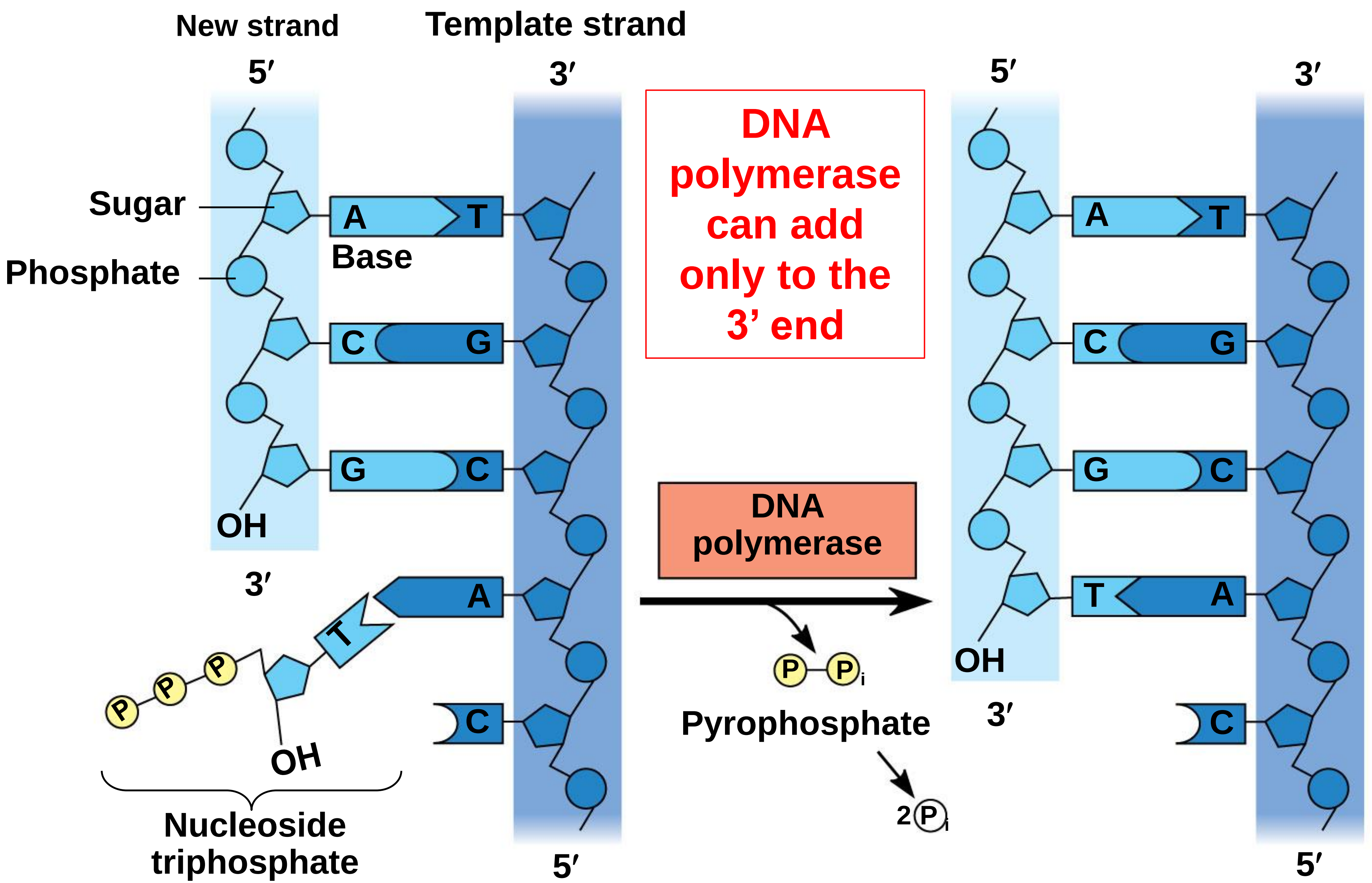
Eukaryotic DNA replication requires multiple <u>origins of replication</u>



Overview of DNA Replication



DNA Replication proceeds 5' to 3'

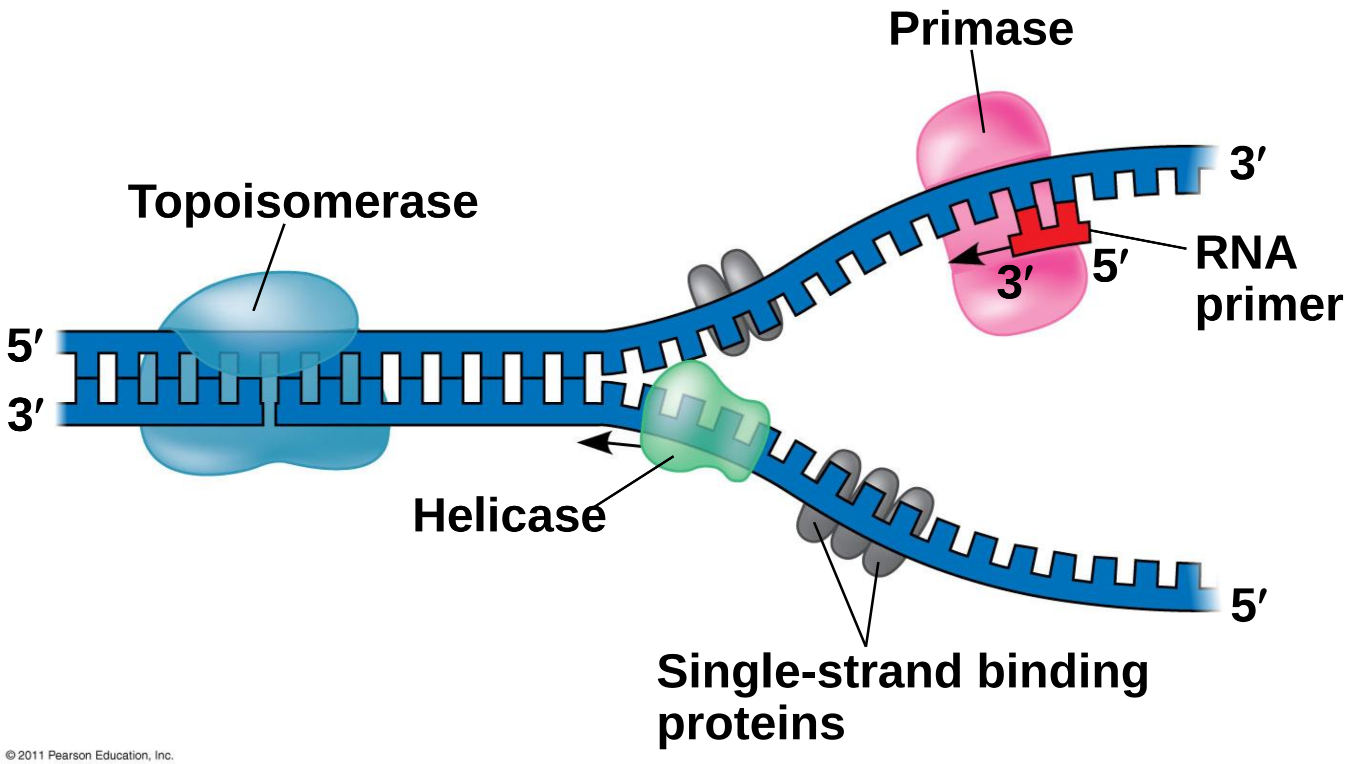


Enzymes involved in DNA Replication

DNA Polymerase – synthesizes new DNA

Helicase – unwinds DNA double helix

Topoisomerase – relieves tension due to DNA unwinding

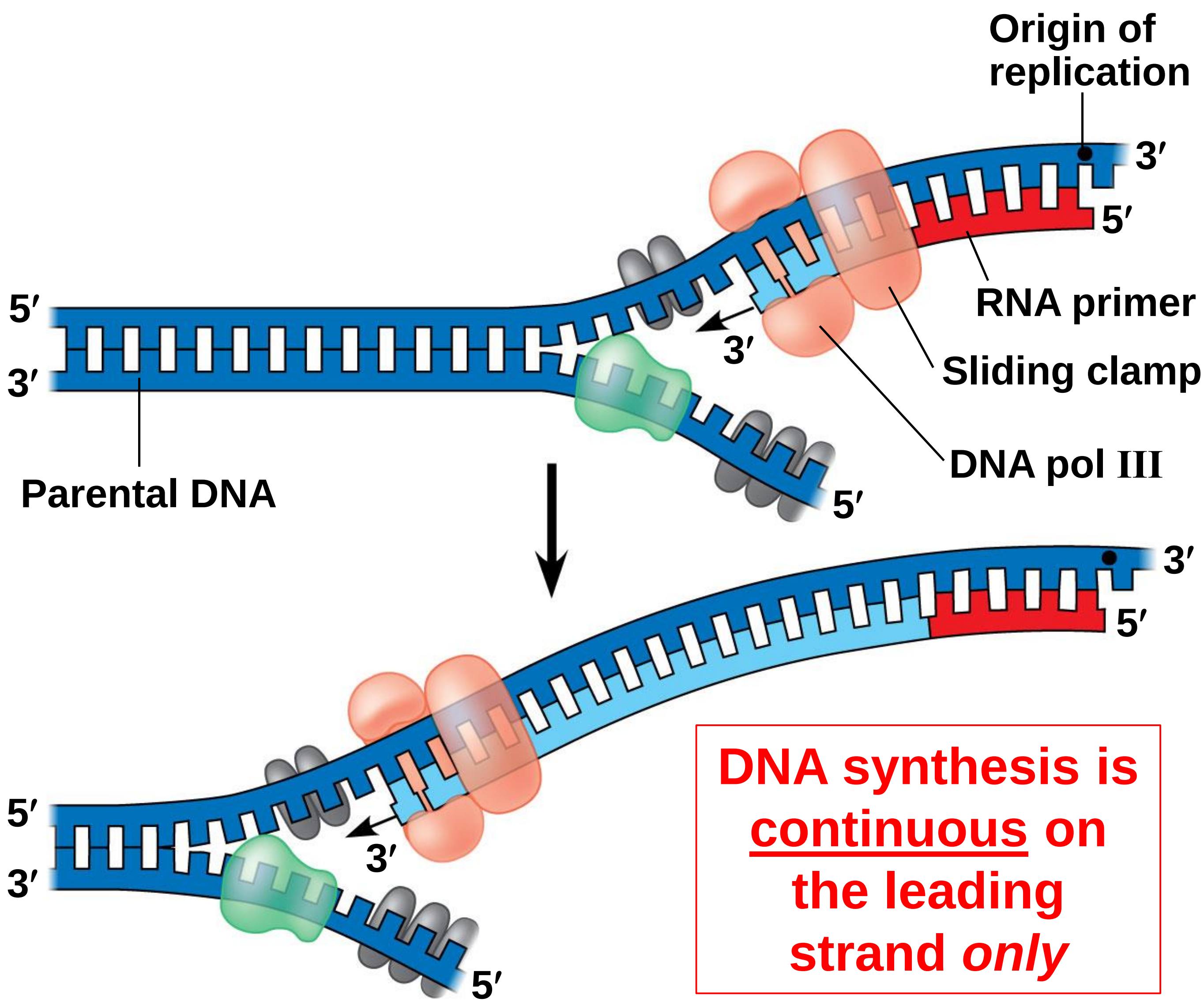


Primase – makes short RNA primers

DNA Ligase – connects DNA fragments

Leading Strand DNA Synthesis

Proceeds toward unwinding <u>replication fork</u>:

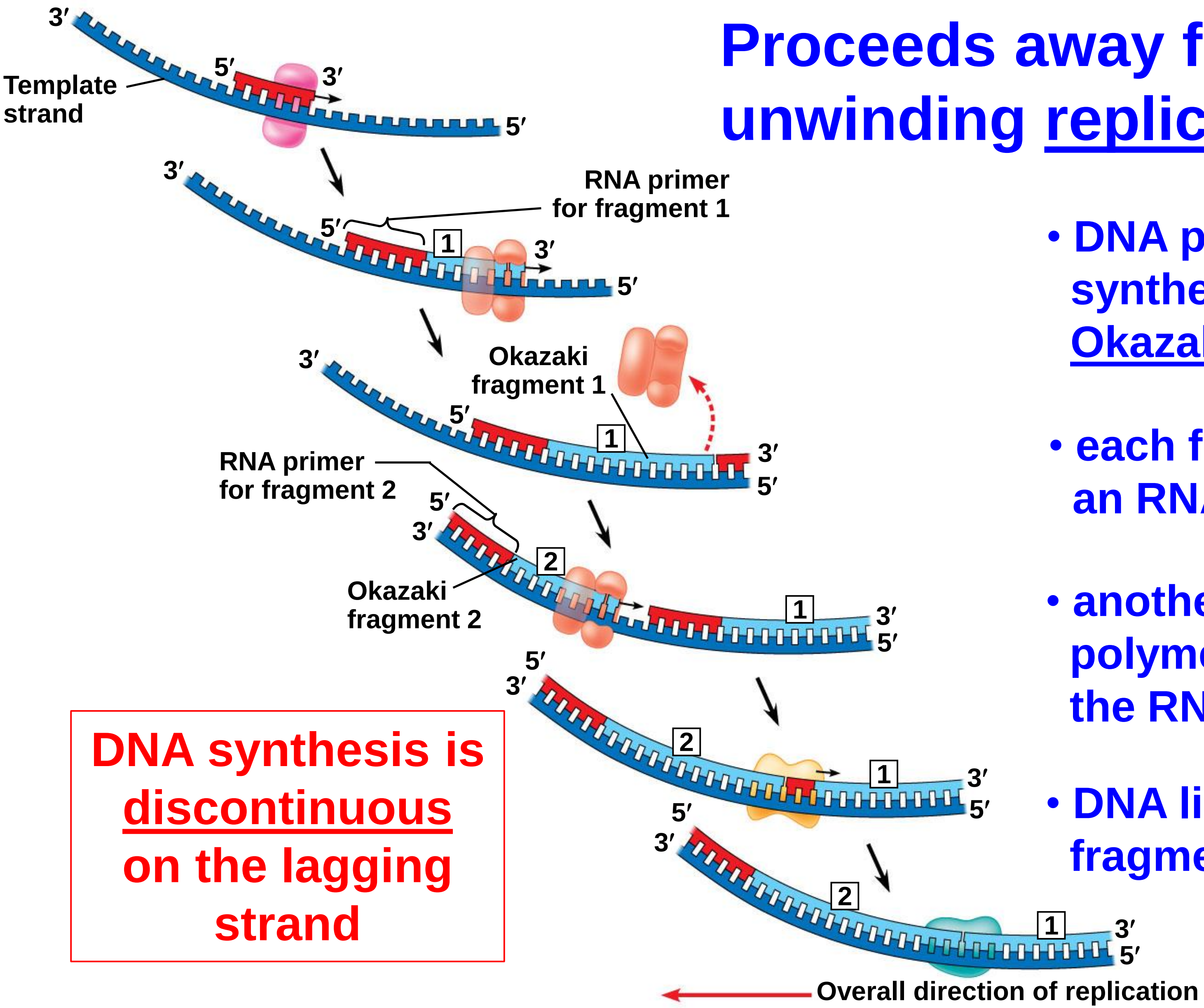


DNA synthesis is <u>continuous</u> on the leading strand *only*

<u>DNA polymerase</u> can only synthesize DNA in a 5' to 3' direction.

<u>DNA polymerase</u> requires an RNA primer which it can extend in a continuous manner toward the unwinding replication fork

Lagging Strand DNA Synthesis

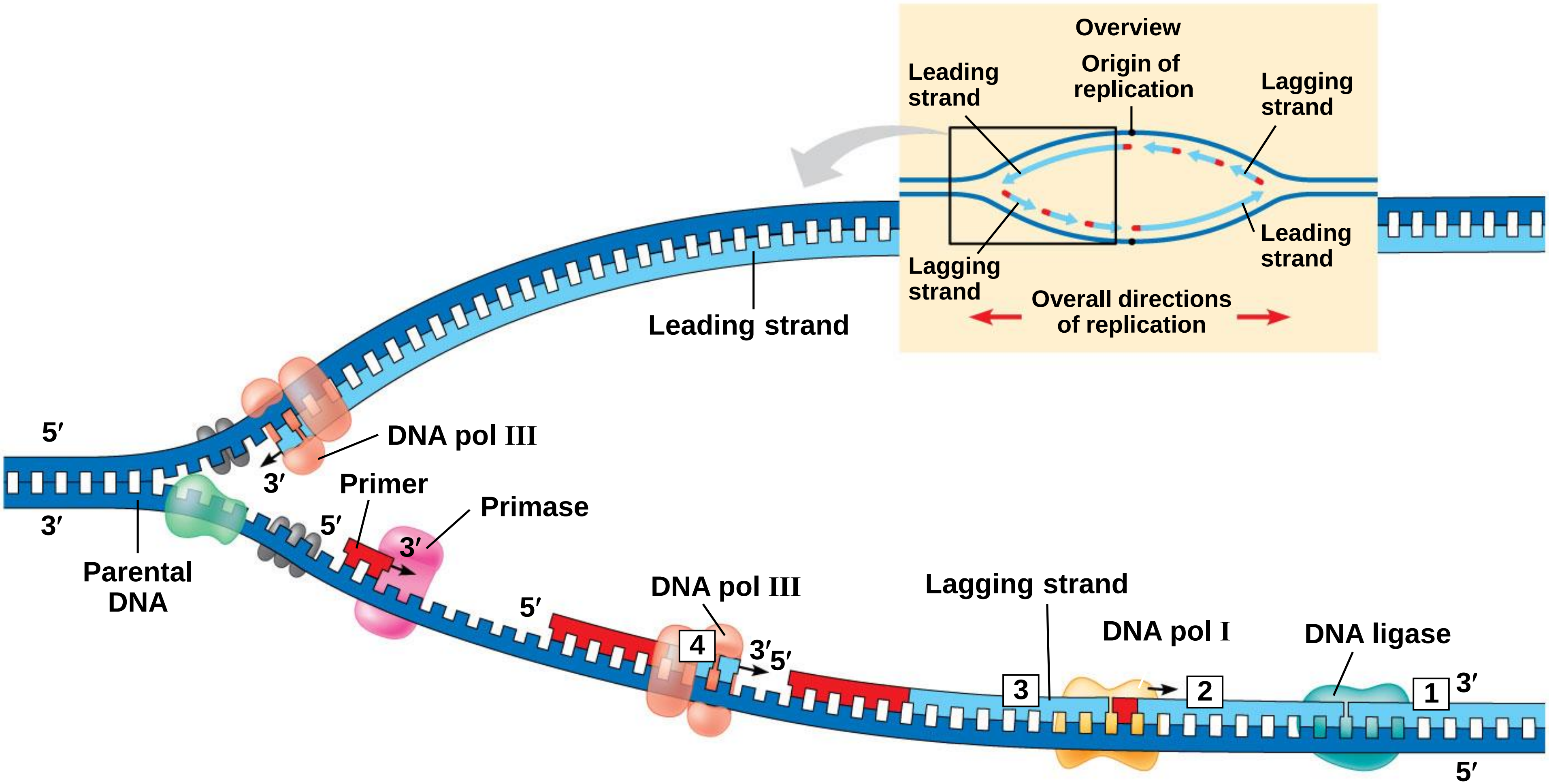


Proceeds away from the unwinding <u>replication fork</u>:

- DNA polymerase synthesizes DNA in <u>Okazaki fragments</u>
- each fragment requires an RNA primer
- another DNA polymerase will replace the RNA with DNA
- DNA ligase will link the fragments together

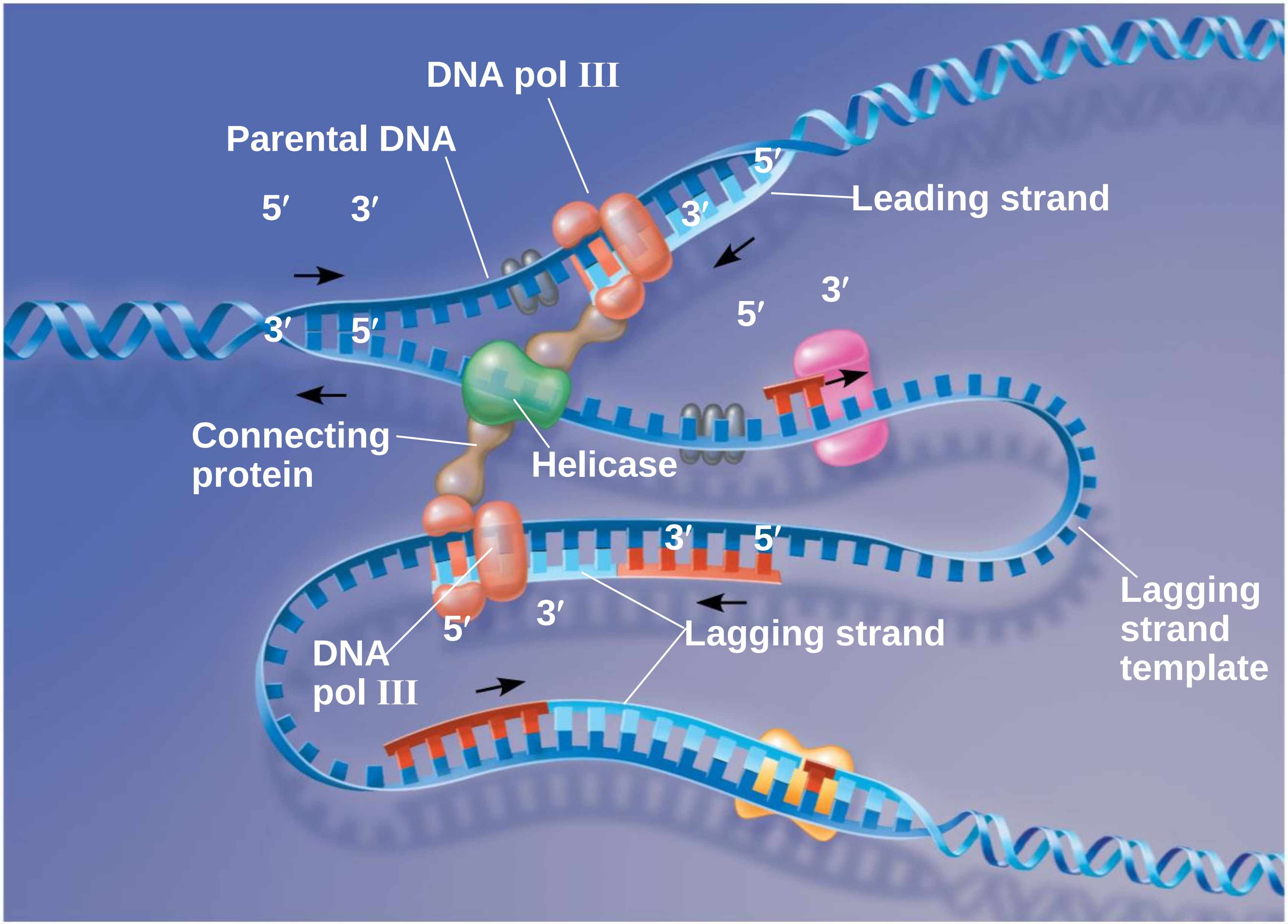
DNA synthesis is <u>discontinuous</u> on the lagging strand

Summary of DNA Replication

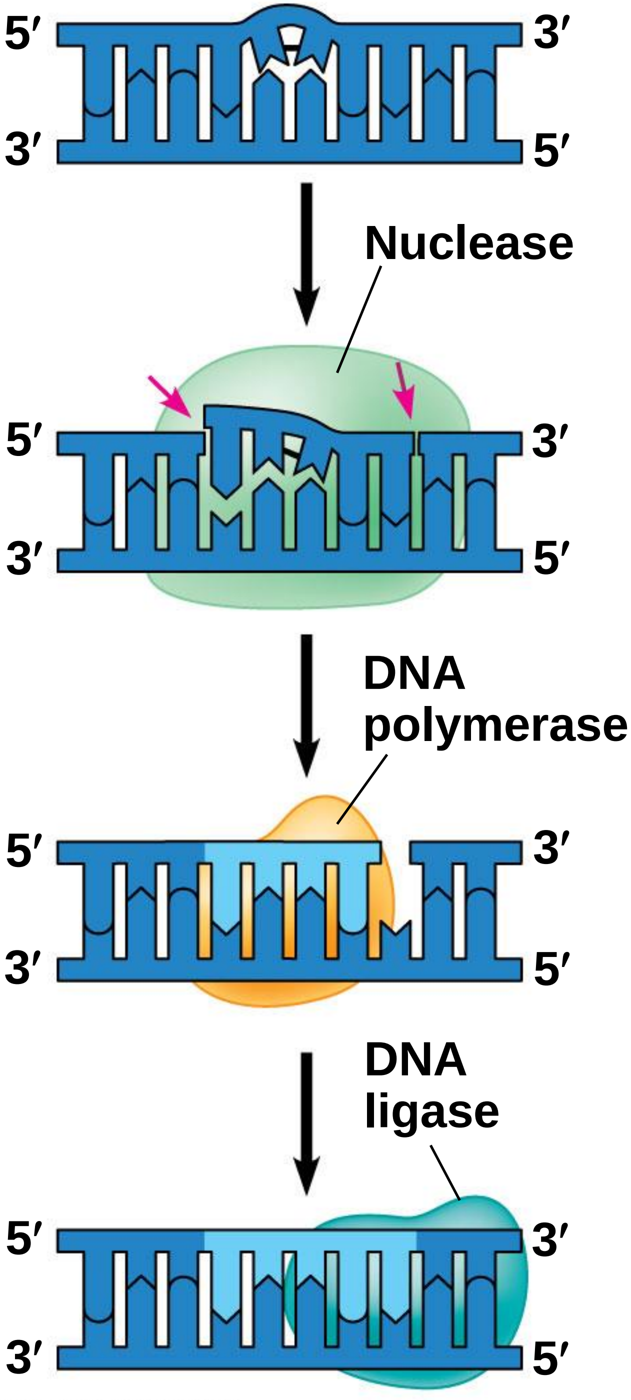


DNA replication proceeds in this manner in ALL living organisms.

Current Model of DNA Replication



DNA Repair



When DNA is damaged it is essential that the DNA is repaired so it can be replicated and expressed properly.

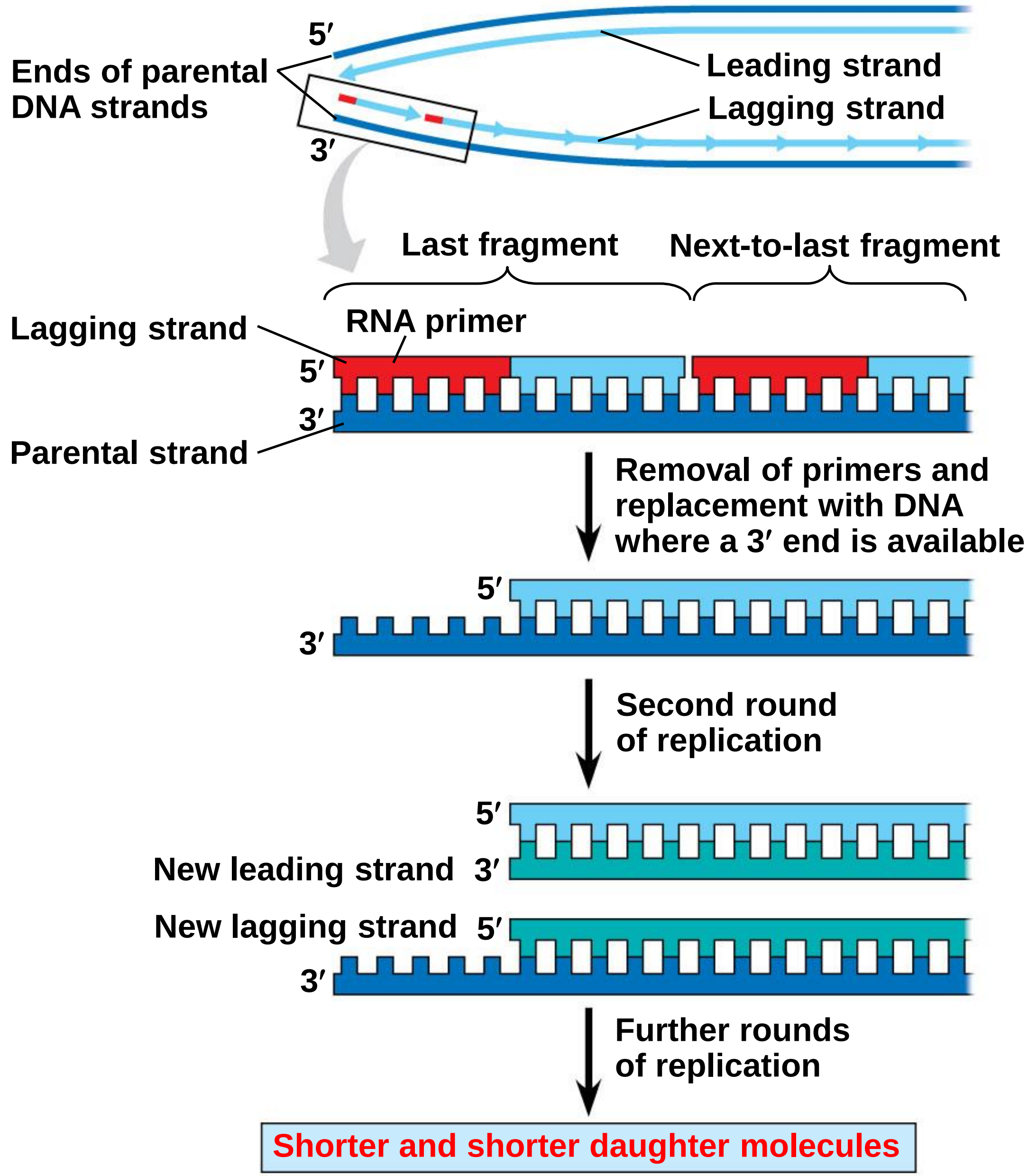
- **special enzymes recognize and remove the damaged portion of DNA**
- **a DNA polymerase will fill in the gap**
- **DNA ligase will then connect the newly made DNA to the adjacent strand**

The Problem with Telomeres

The ends of linear chromosomes, the <u>telomeres</u>, cannot be completely copied on the lagging strand.

This results in progressive shortening of the chromosome every time it is replicated.

<u>Telomerase</u> will solve this problem in certain cell types



…more on Chromatin

Chromatin refers to the complex of DNA and histone proteins in eukaryotic nuclei:

- **chromosomal DNA wraps around histone proteins to form structures called nucleosomes that look like "beads on a string"**
- **different parts of a chromosome can be in various states of "packing"**

EUCHROMATIN – loosely packed DNA

HETEROCHROMATIN – tightly packed DNA

Key Terms for Chapter 16

- bacterial transformation, bacteriophage
- x-ray crystallography
- pyrimidine, purine, base-pair, complementary
- double helix, anti-parallel, sugar-phosphate backbone
- DNA replication, template
- conservative, semiconservative, dispersive
- DNA polymerase, helicase, topoisomerase, primase, DNA ligase
- leading, lagging strand; continuous, discontinuous
- Okazaki fragment, telomere, telomerase
- chromatin, nucleosome, euchromatin, heterochromatin

Relevant Chapter Questions 1-7, 9