Lecture # 9

Exam next week!

Chapters 10, 11 & 12 - Cell division, Genetics & DNA Replication

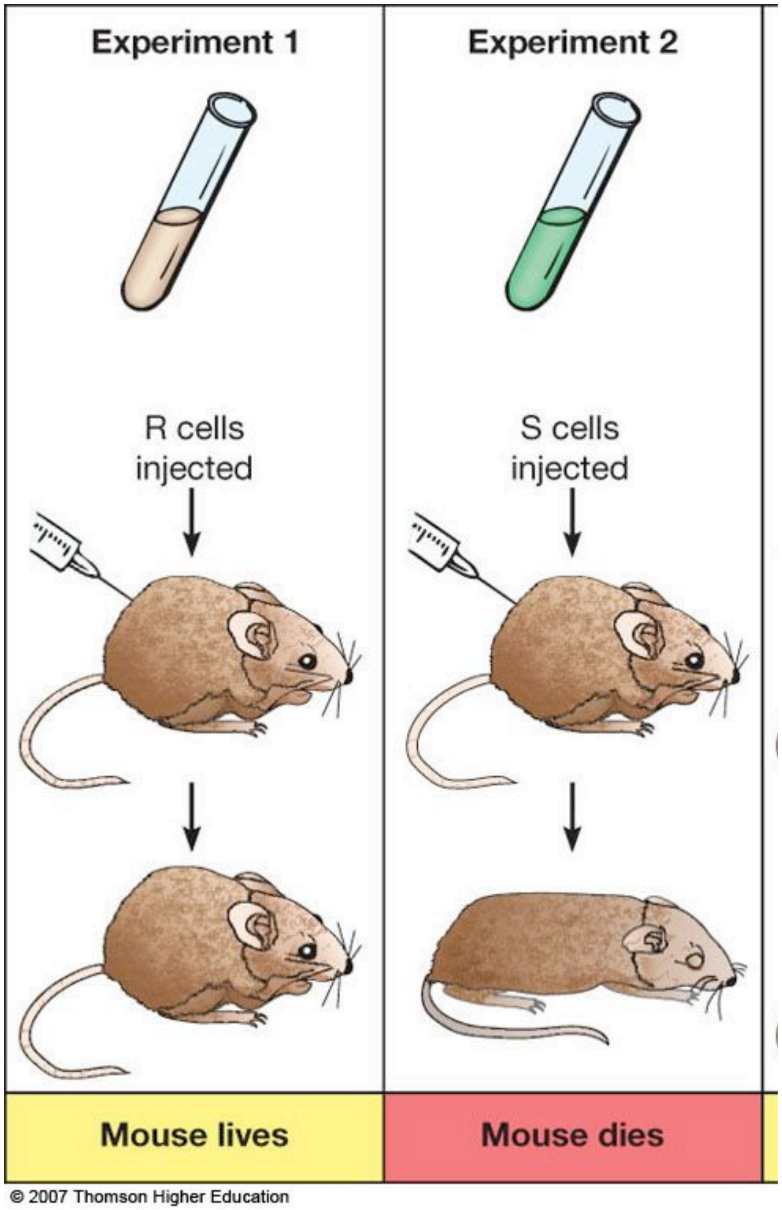
This week:

Why we believe in DNA, DNA Replication, PCR & DNA sequencing

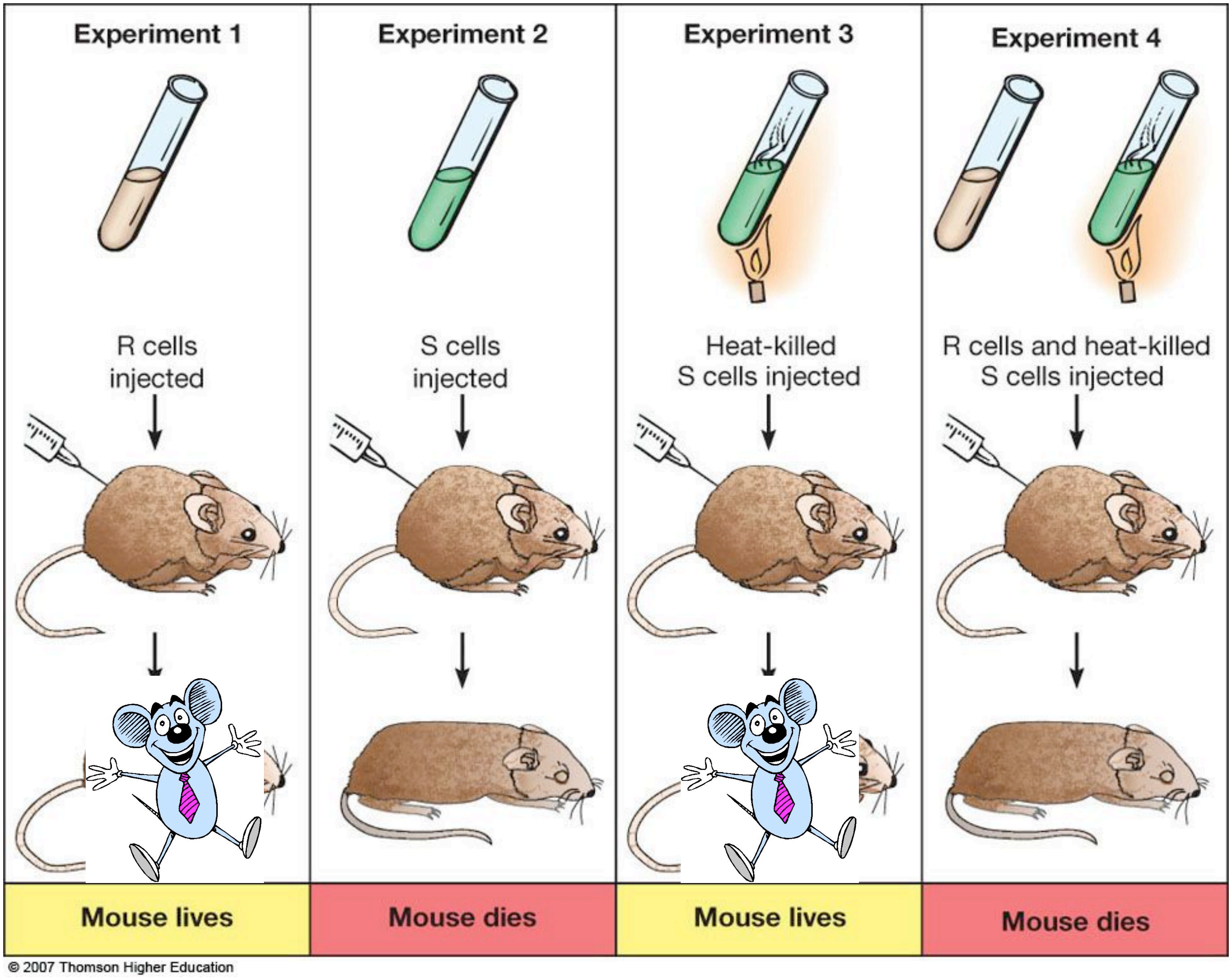

3232
DNA
TESTING LAB INC
PATERNITY TESTING
Who's the Daddy?
Monday - Friday
Saturday - Sunday
Phone Number


DNA
TESTING LAB INC
PATERNITY TESTING
Who's the Daddy?
1 (800) R-U-MY KID
Who's the Daddy?
1 (800) R-U-MY KID

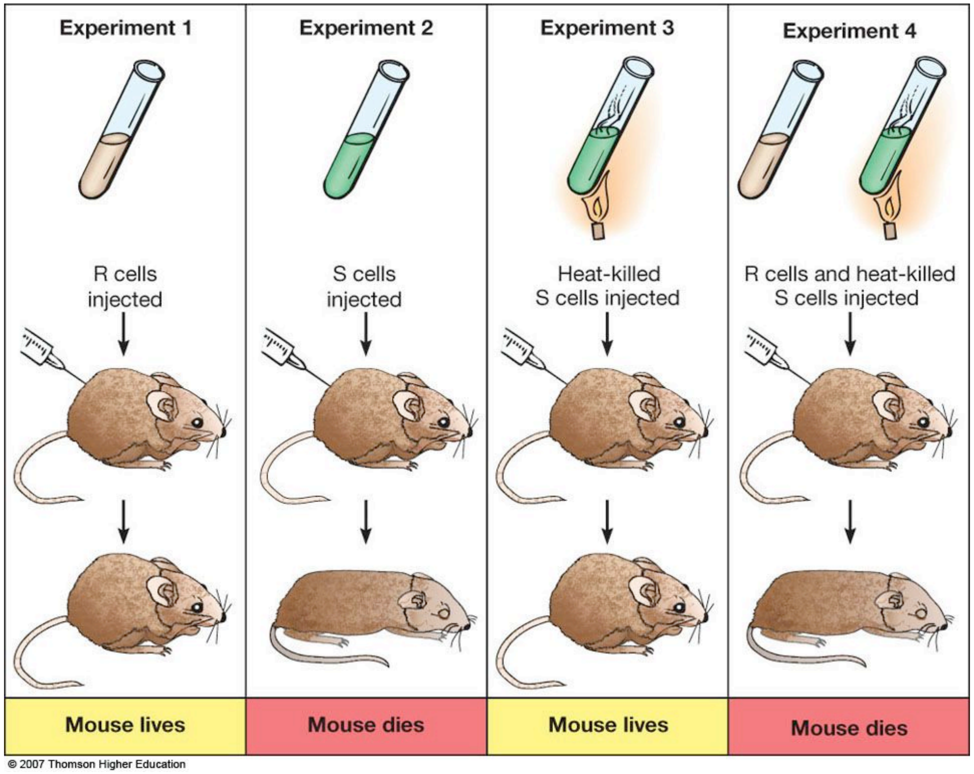
Frederick Griffith (1928) - bacterial transformation



Frederick Griffith - bacterial transformation

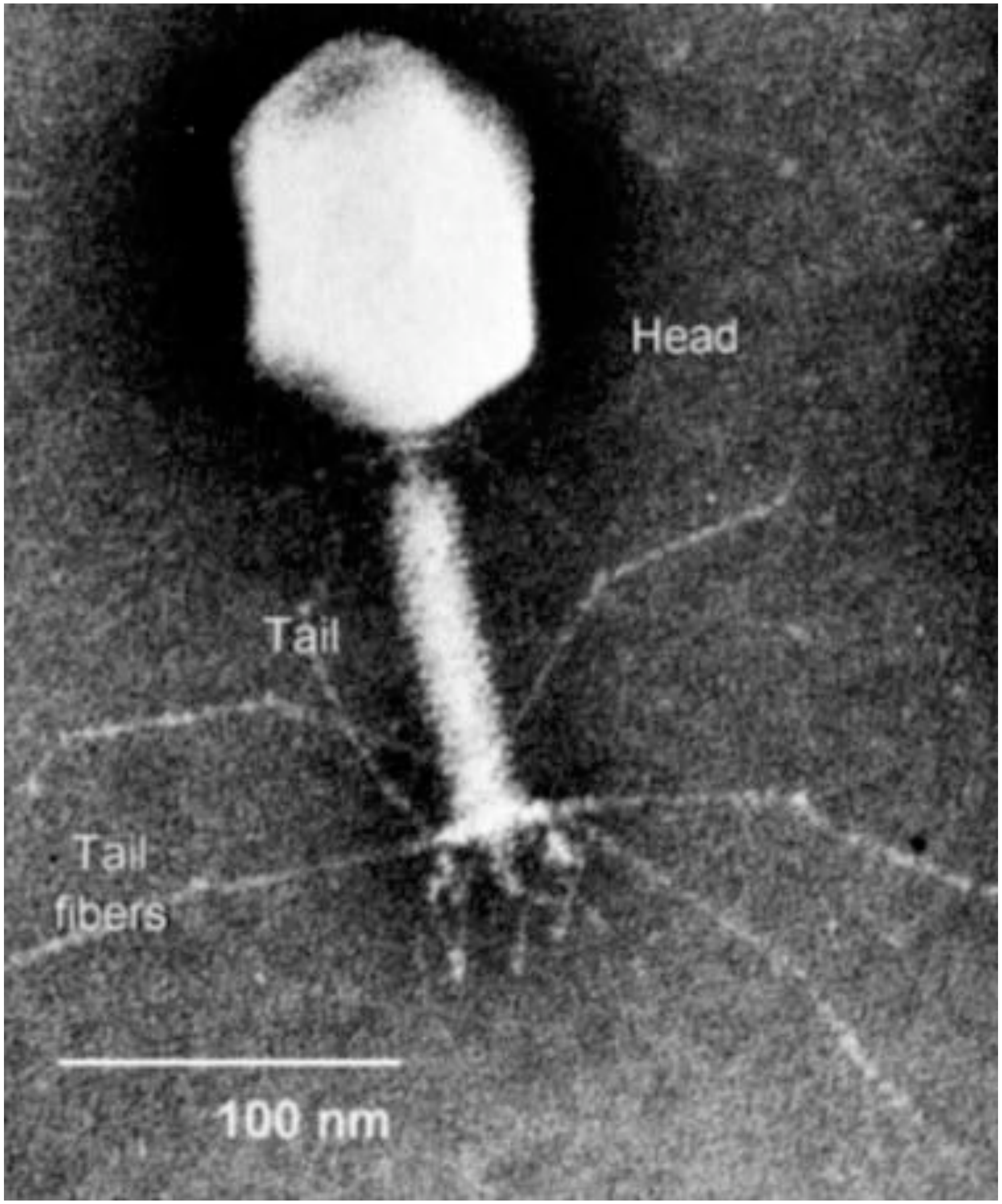


Frederick Griffith - bacterial transformation



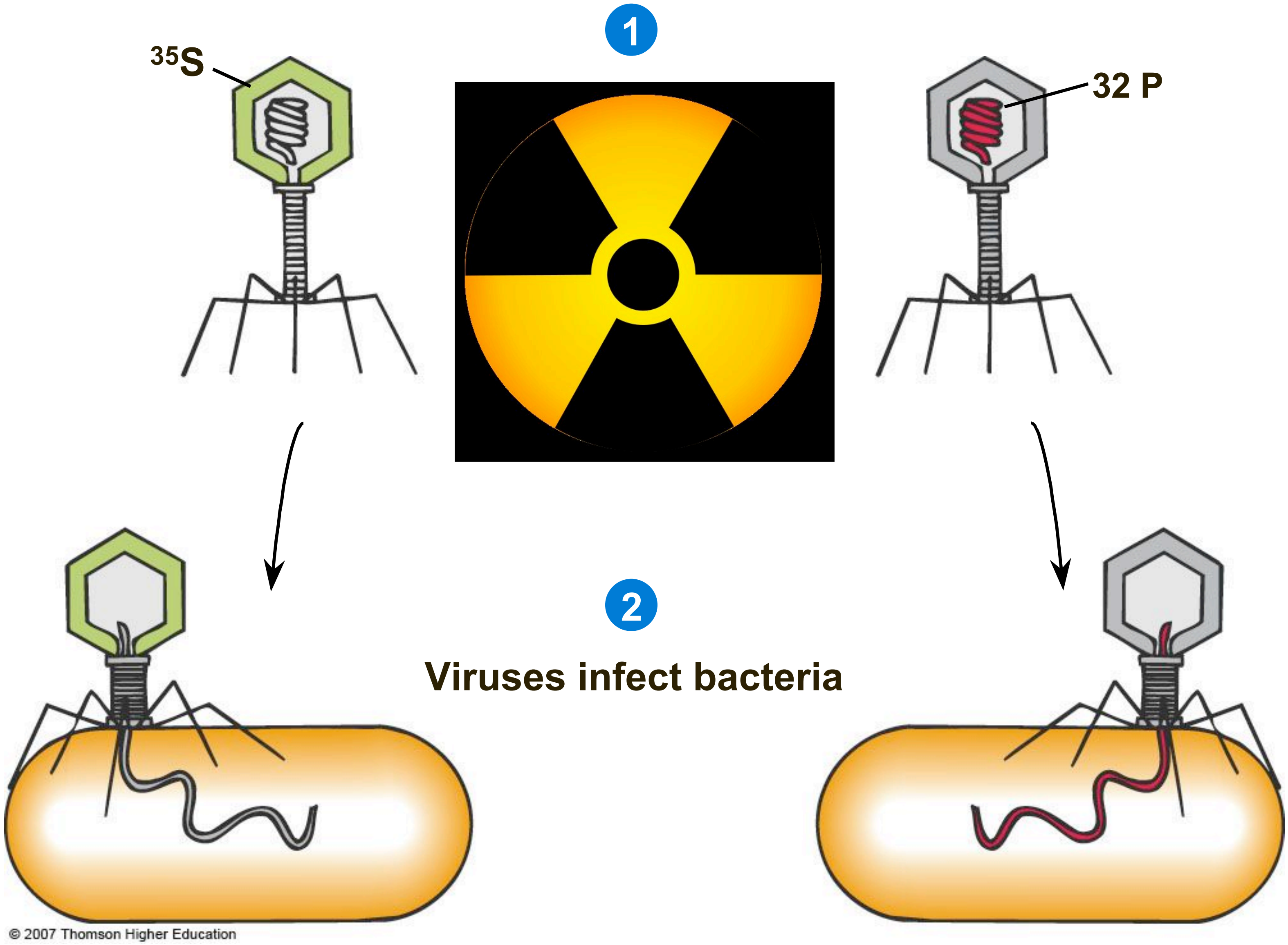
Conclusion: Something travels from the dead S cells to the R cells
(NB, proteins tend to get destroyed by heat)

Further support by Oswald Avery (1940s)



T4
Bacteriophage

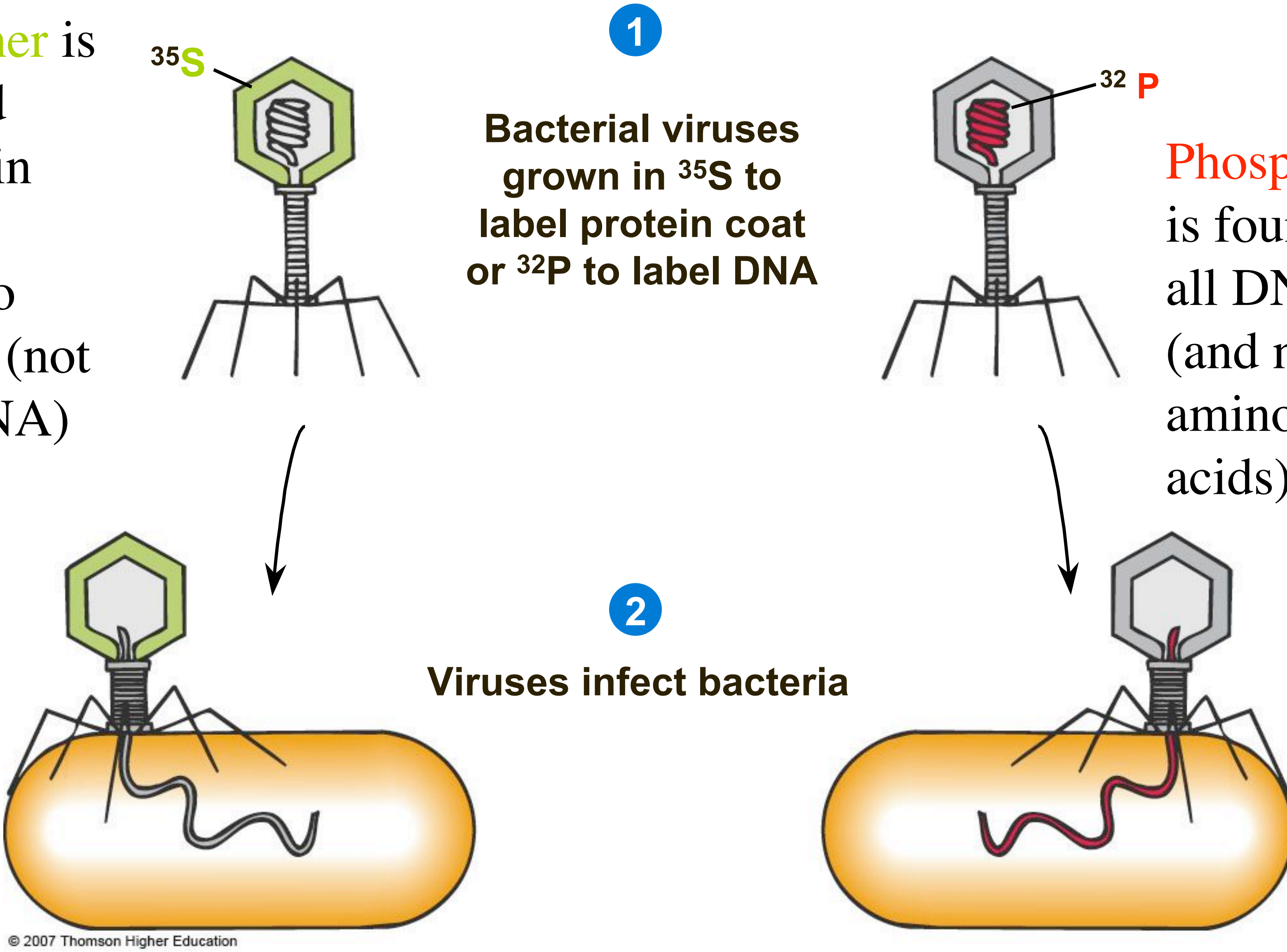
http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Keogh/plasmids.html



Hershey/Chase - Virus Labeling

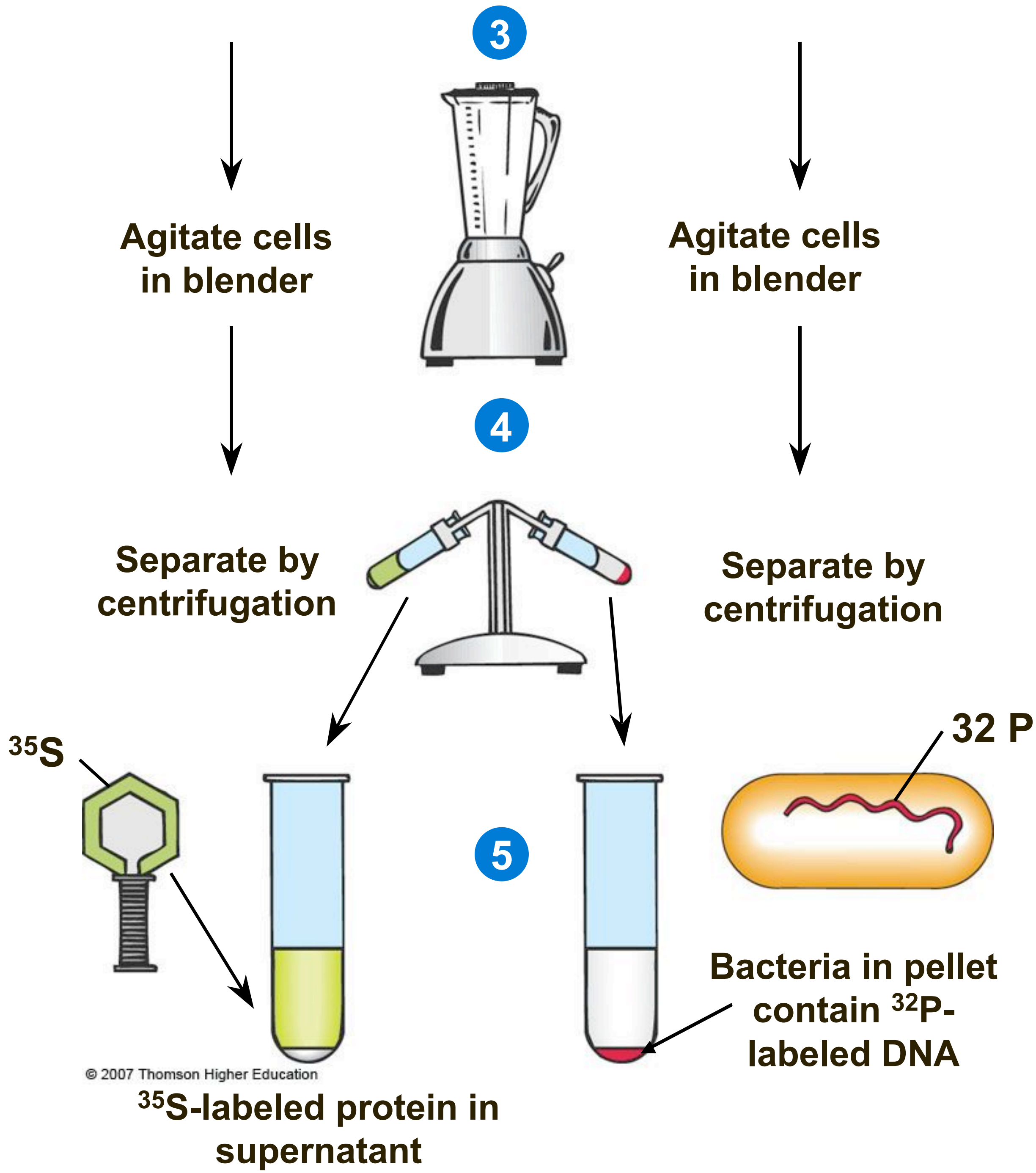
Fig. 12-2, p. 262

Sulpher is found only in some amino acids (not in DNA)



Phosphorus is found in all DNA (and not in amino acids)

Fig. 12-2, p. 262



3
Agitate cells in blender
Agitate cells in blender
4
Separate by centrifugation
Separate by centrifugation
35S
32 P
5
Bacteria in pellet contain 32P-labeled DNA
35S-labeled protein in supernatant

Fig. 12-2, p. 262

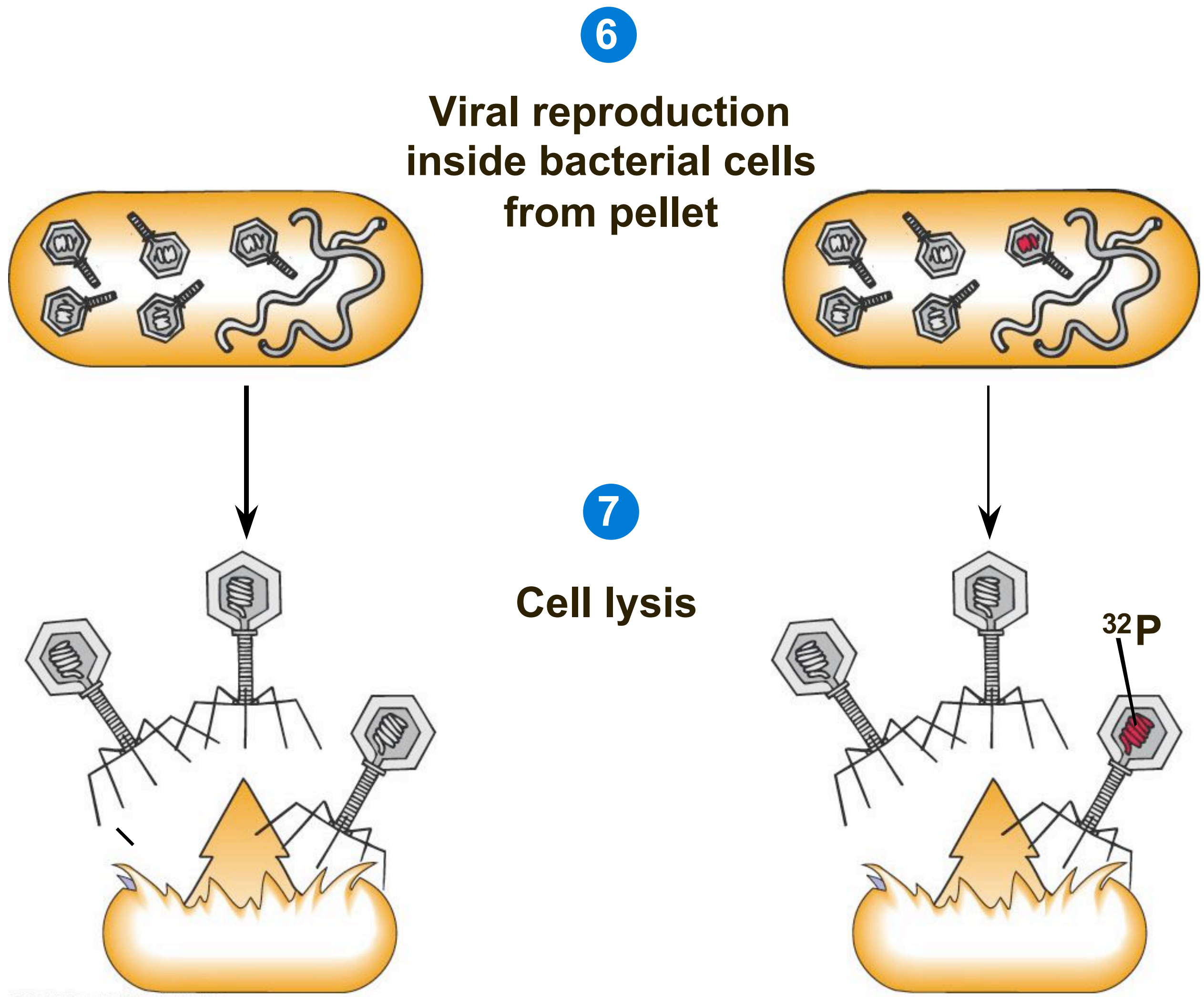

6
Viral reproduction inside bacterial cells from pellet
7
Cell lysis
^{32}P

Fig. 12-2, p. 262

Other clues to DNAs role and function

Chargaff (1949) - Quantitative relationships among DNA bases

The amount of each DNA base (A,T,CorG) varies from organism to organism, BUT

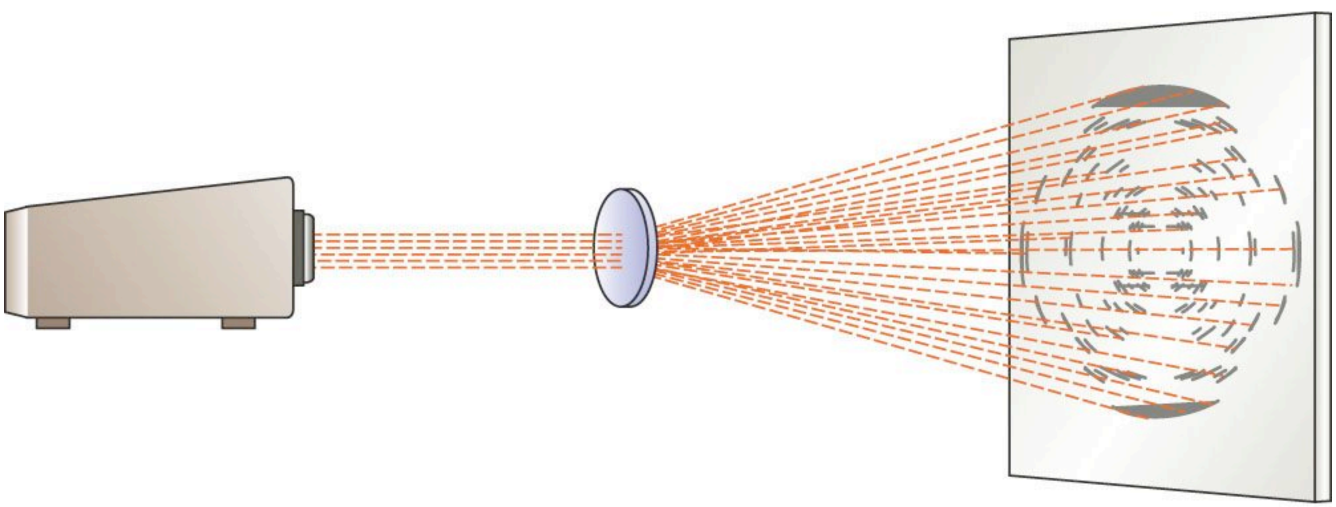
The amount of A = the amount of T;
The amount of G = the amount of C

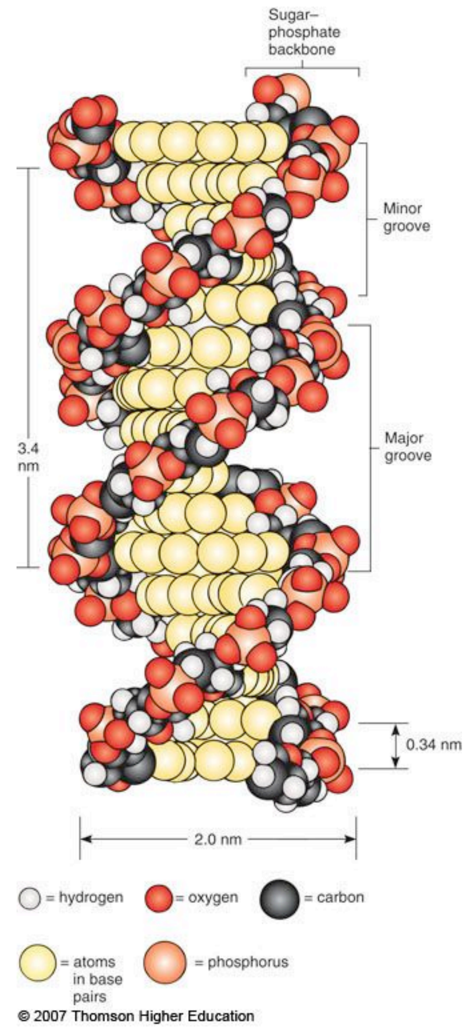
Early 1950s, Franklin, Watson, Crick, Wilkins

X-ray diffraction studies led to the

Double Helix

model





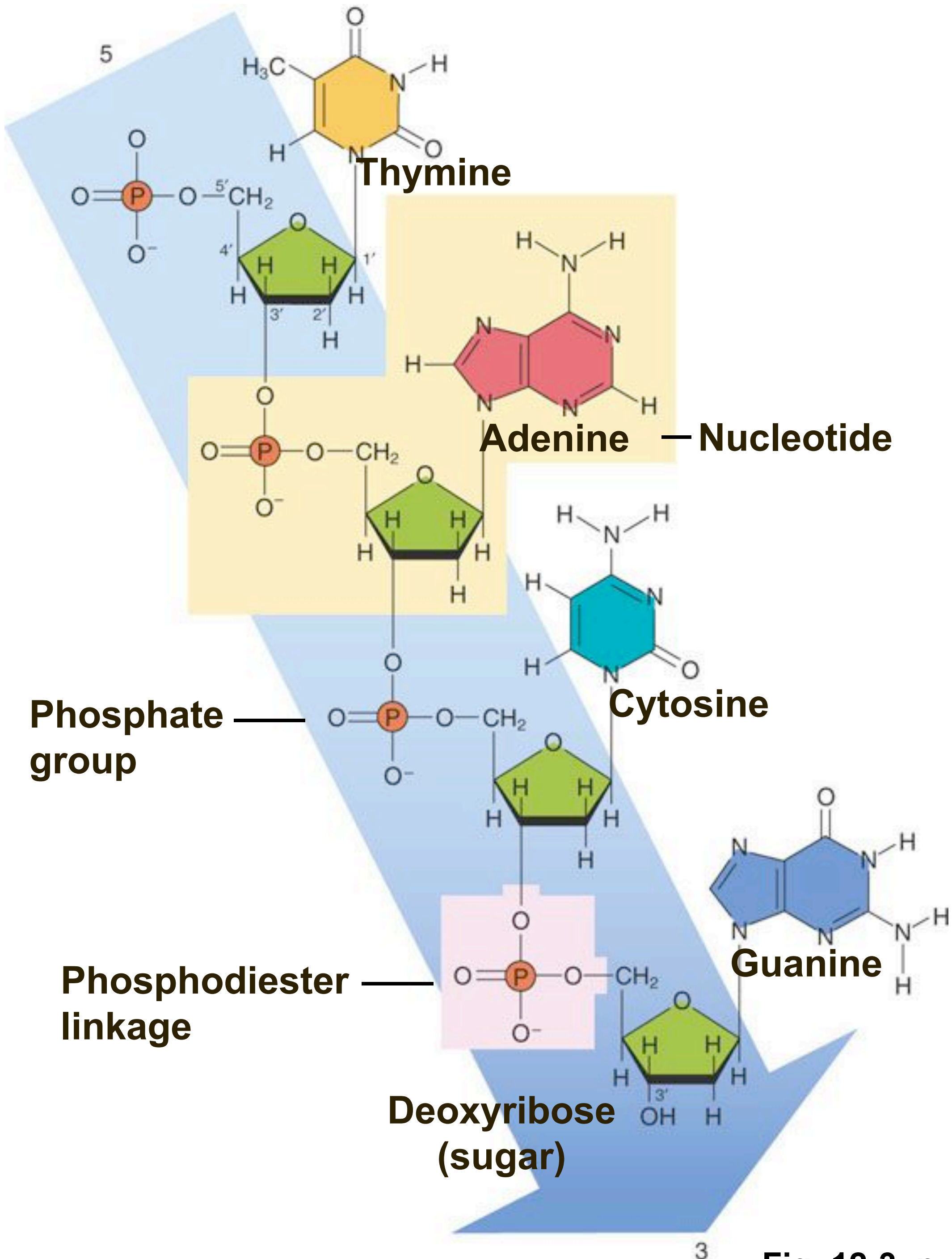
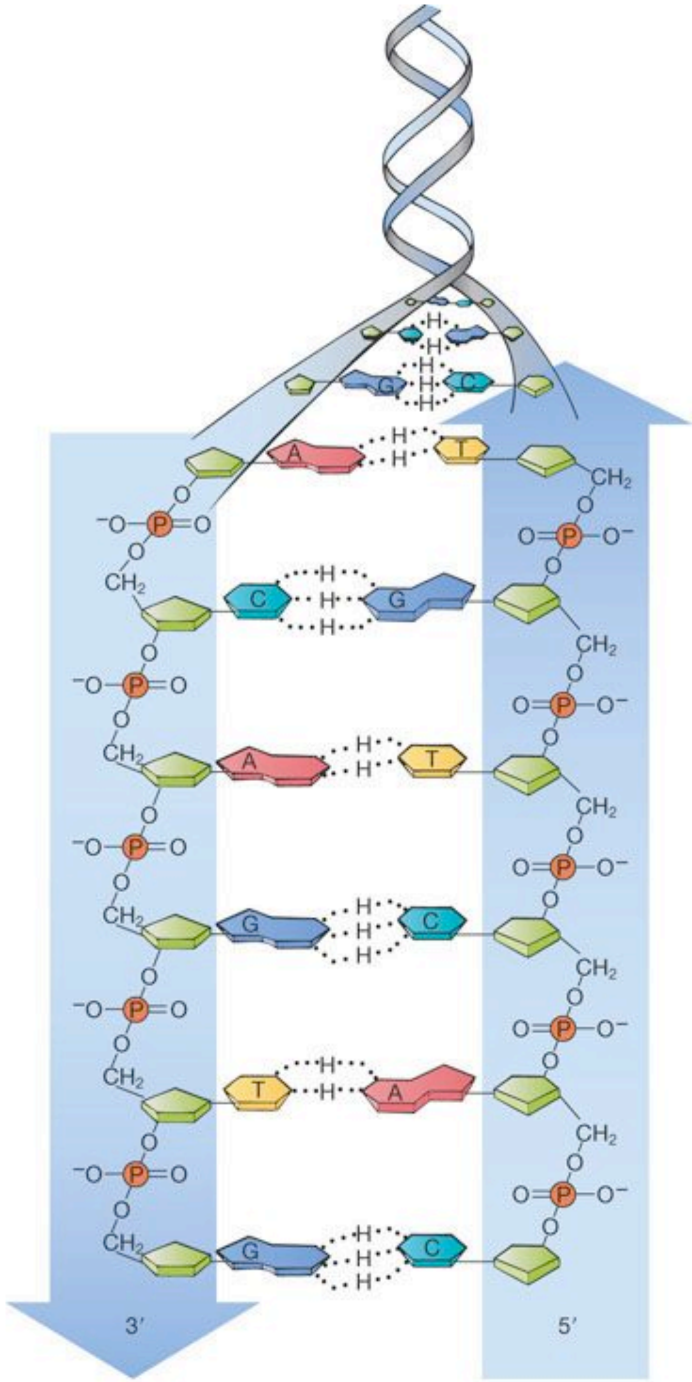


Fig. 12-3, p. 264

Each strand of the double helix is a polymer, with an orientation

Strands are **antiparallel**



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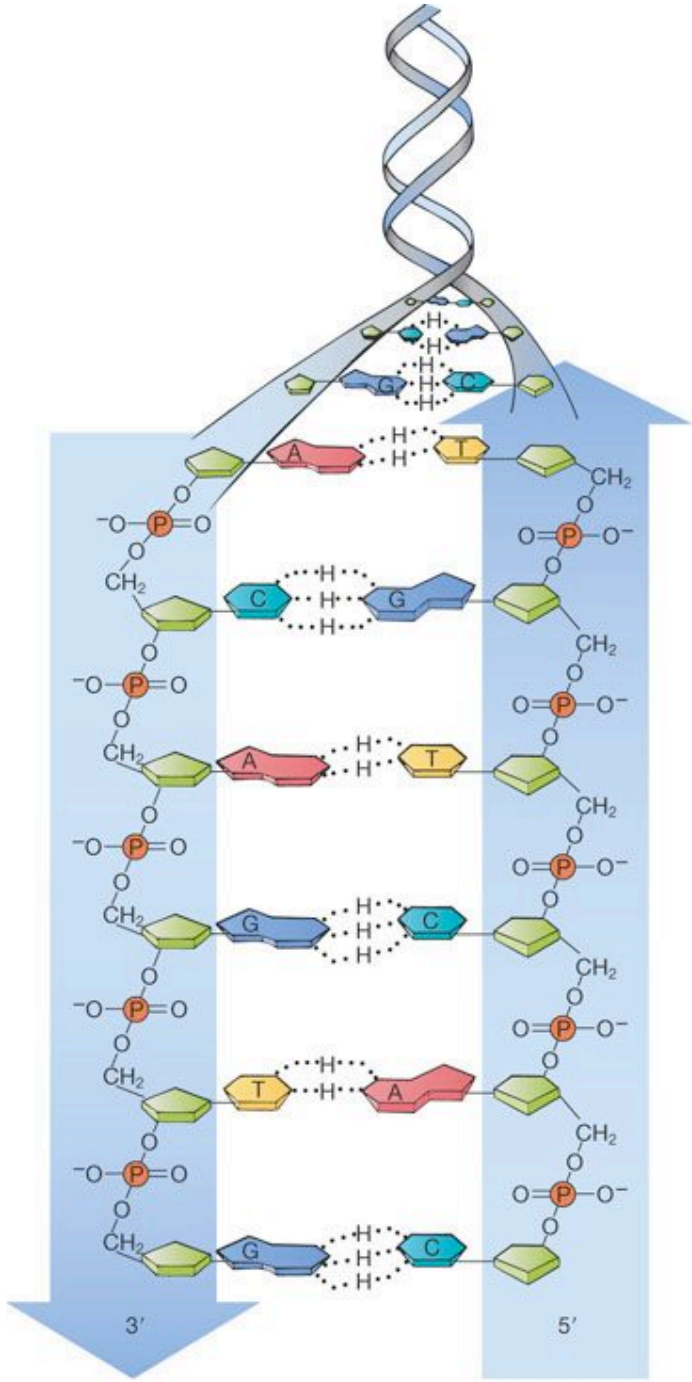


The bases are held to each other with 2 or 3 hydrogen bonds

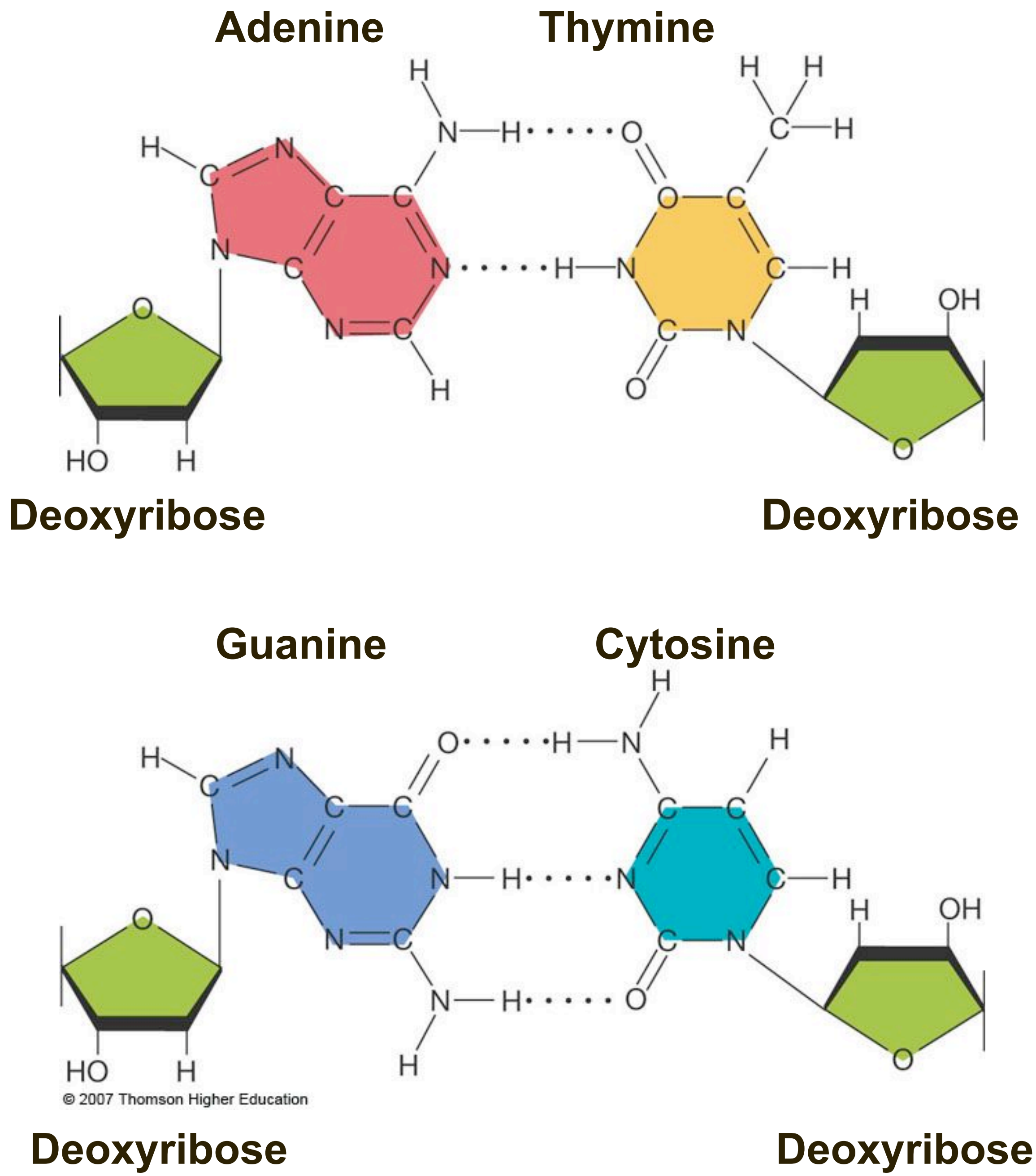
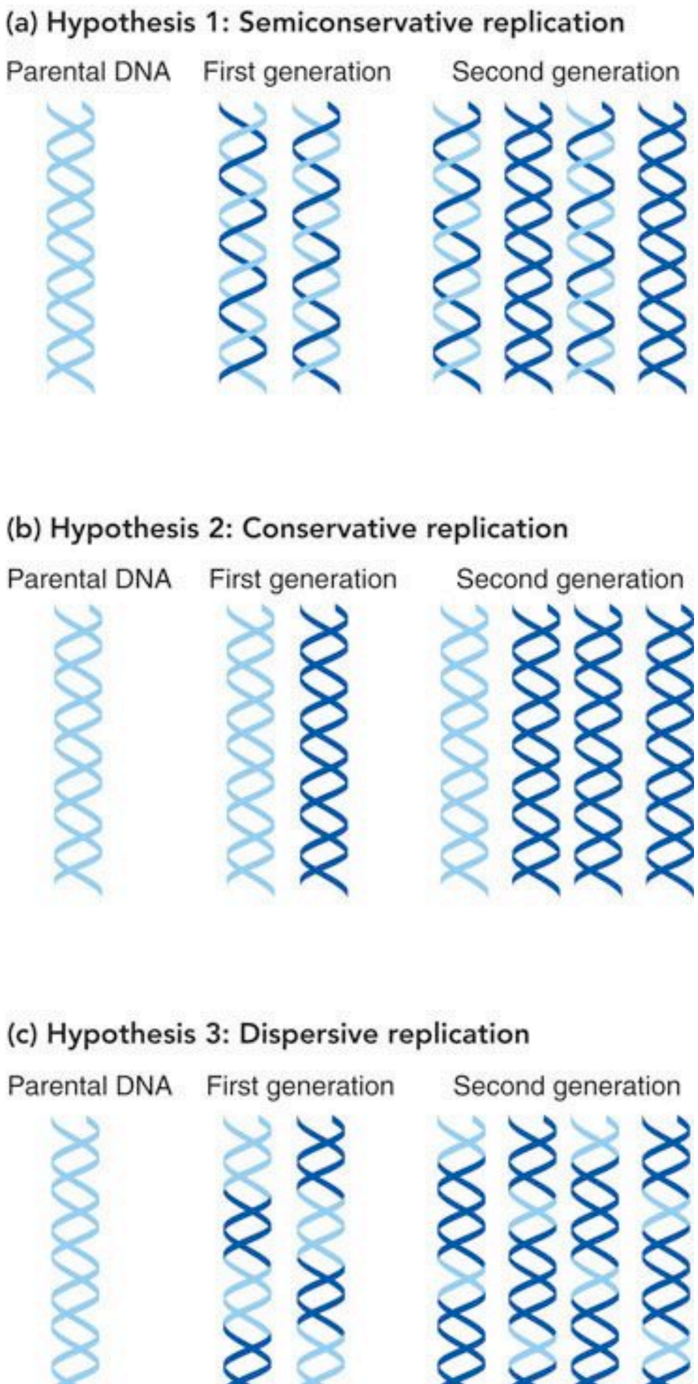


Fig. 12-6b, p. 267



DNA Replication is semi-conservative

Fig. 12-7, p. 268

(a) Hypothesis 1: Semiconservative replication

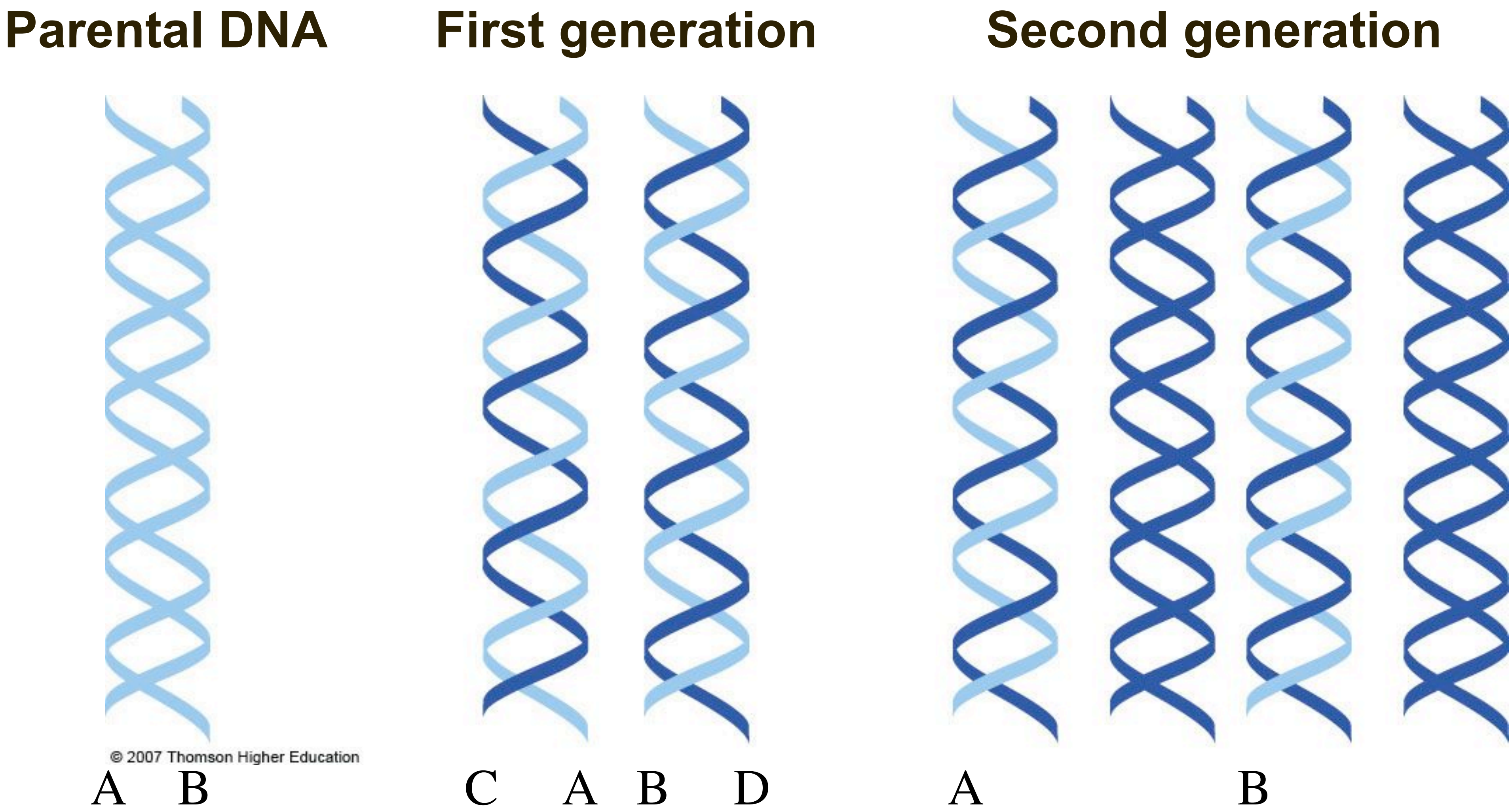


Fig. 12-7a, p. 268

TABLE 12-3

Proteins Involved in DNA Replication

Enzyme	Function
Helicase	Opens the double helix at replication forks by disrupting the hydrogen bonds that hold the two strands together.
Single-strand binding (SSB) protein	Binds to single strands of DNA and prevents the helix from re-forming before it can be used as a template for replication.
Topoisomerase	Breaks one or both DNA strands, preventing excessive coiling during replication, and then rejoins them in a more relaxed configuration.
DNA polymerase	Links nucleotide subunits to form a new DNA strand from a DNA template.
DNA primase	Synthesizes short RNA primers on the lagging strand. Begins replication of the leading strand.
DNA ligase	Links Okazaki fragments by joining the 3′ end of the new DNA fragment to the 5′ end of the adjoining DNA.
Telomerase	Lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes.

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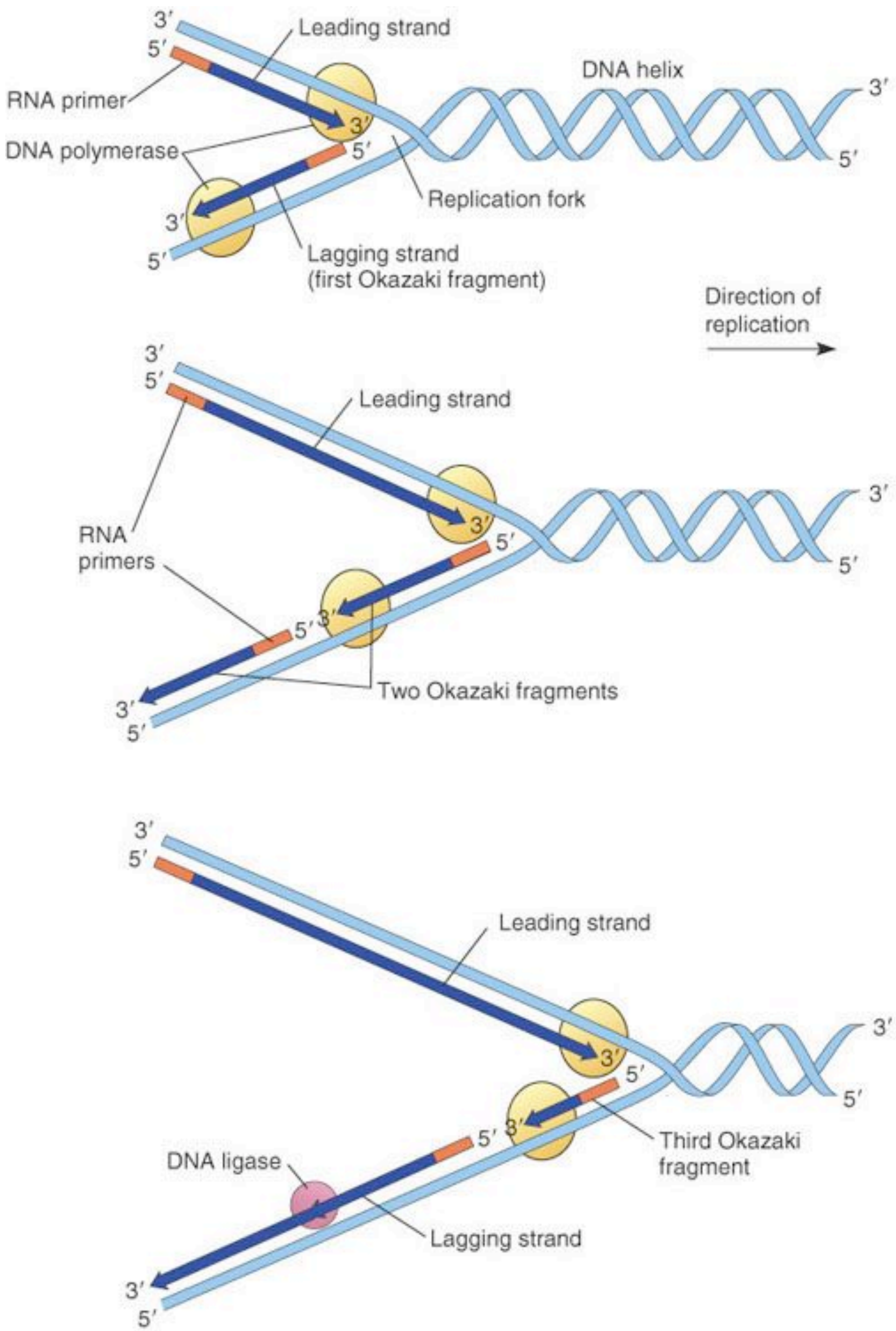
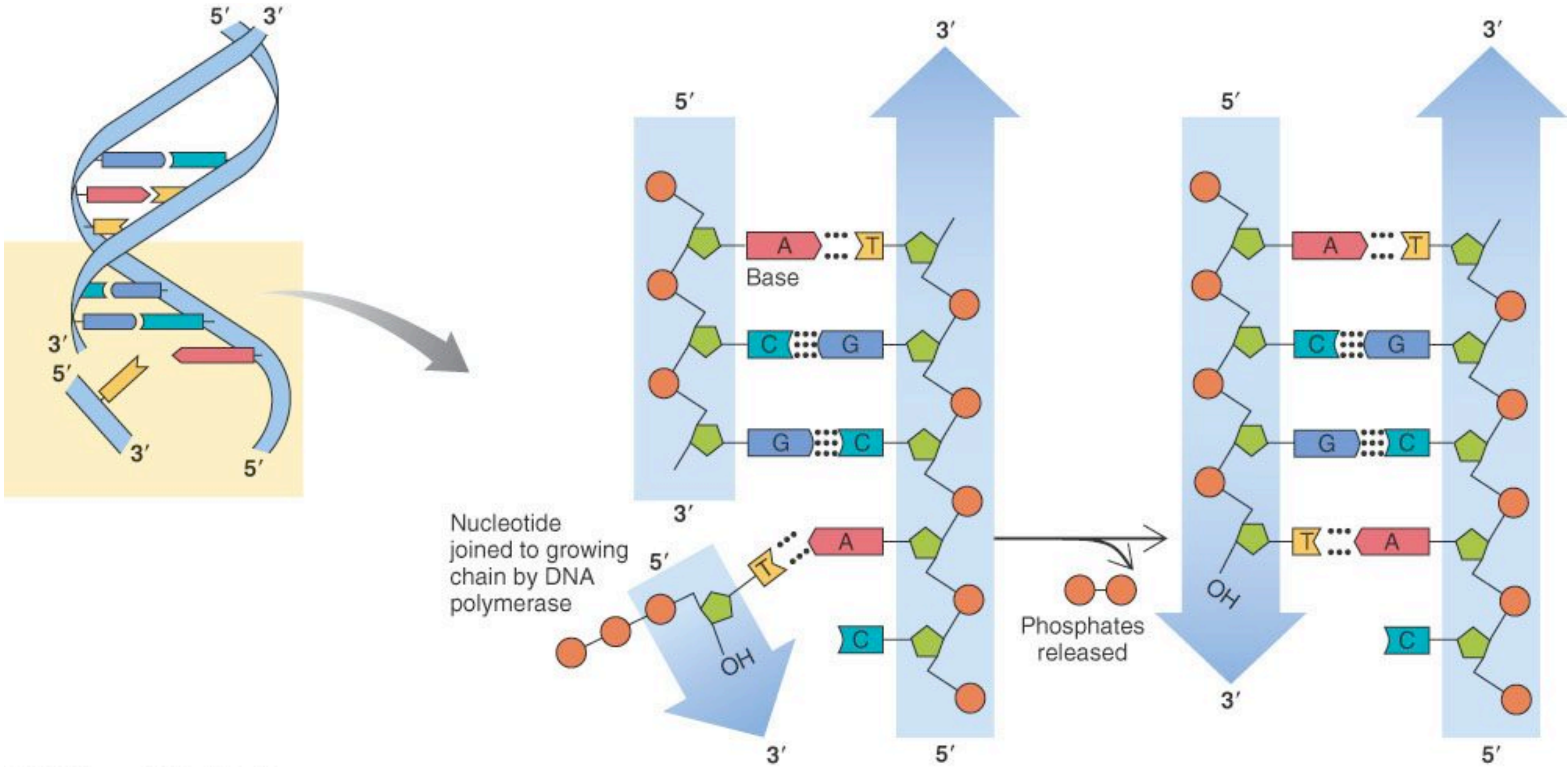


1. Leading strand is synthesized continuously in direction toward replication fork; lagging strand is synthesized in direction away from replication fork. Both strands require RNA primer for initiation of synthesis because DNA can be elongated only by addition to 3′ end of existing polynucleotide strand.

2. Lagging strand is synthesized as short Okazaki fragments. Okazaki fragment synthesis begins with the synthesis of RNA primer. Note that first Okazaki fragment synthesized is now at far left.

3. After each Okazaki fragment has been elongated by DNA polymerase, RNA primer is degraded, gaps are filled in with DNA, and adjoining fragments are linked by DNA ligase.

Fig. 12-12, p. 273


5′
3′
Base
Nucleotide joined to growing chain by DNA polymerase
OH
Phosphates released
A
T
C
G

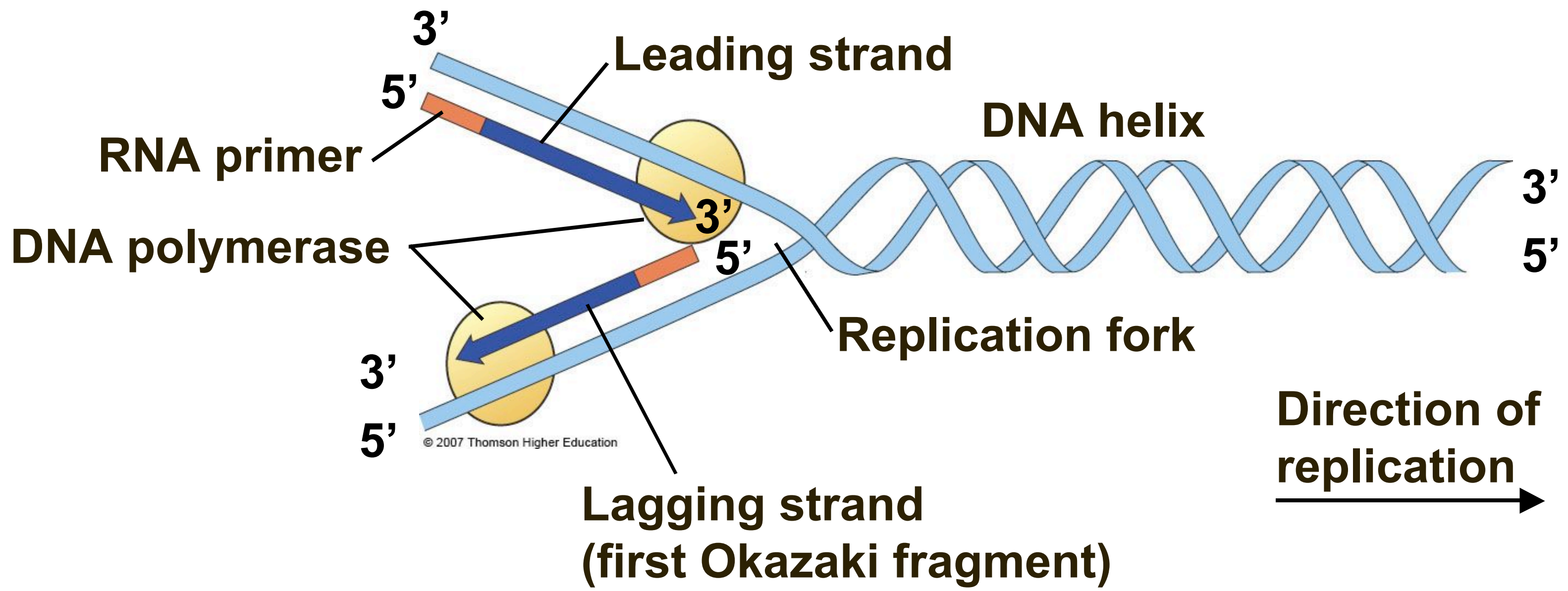


Fig. 12-12a, p. 273

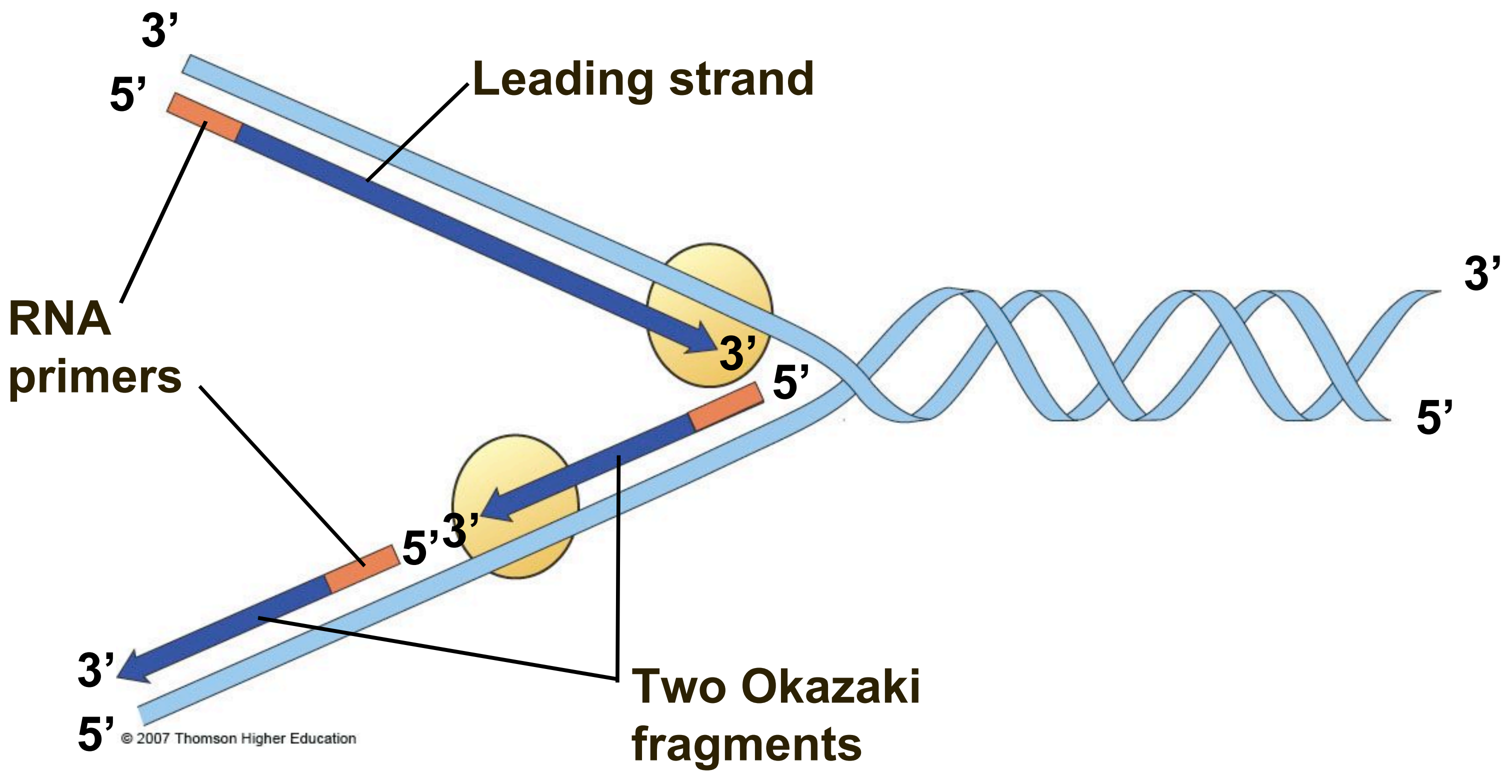


Fig. 12-12b, p. 273

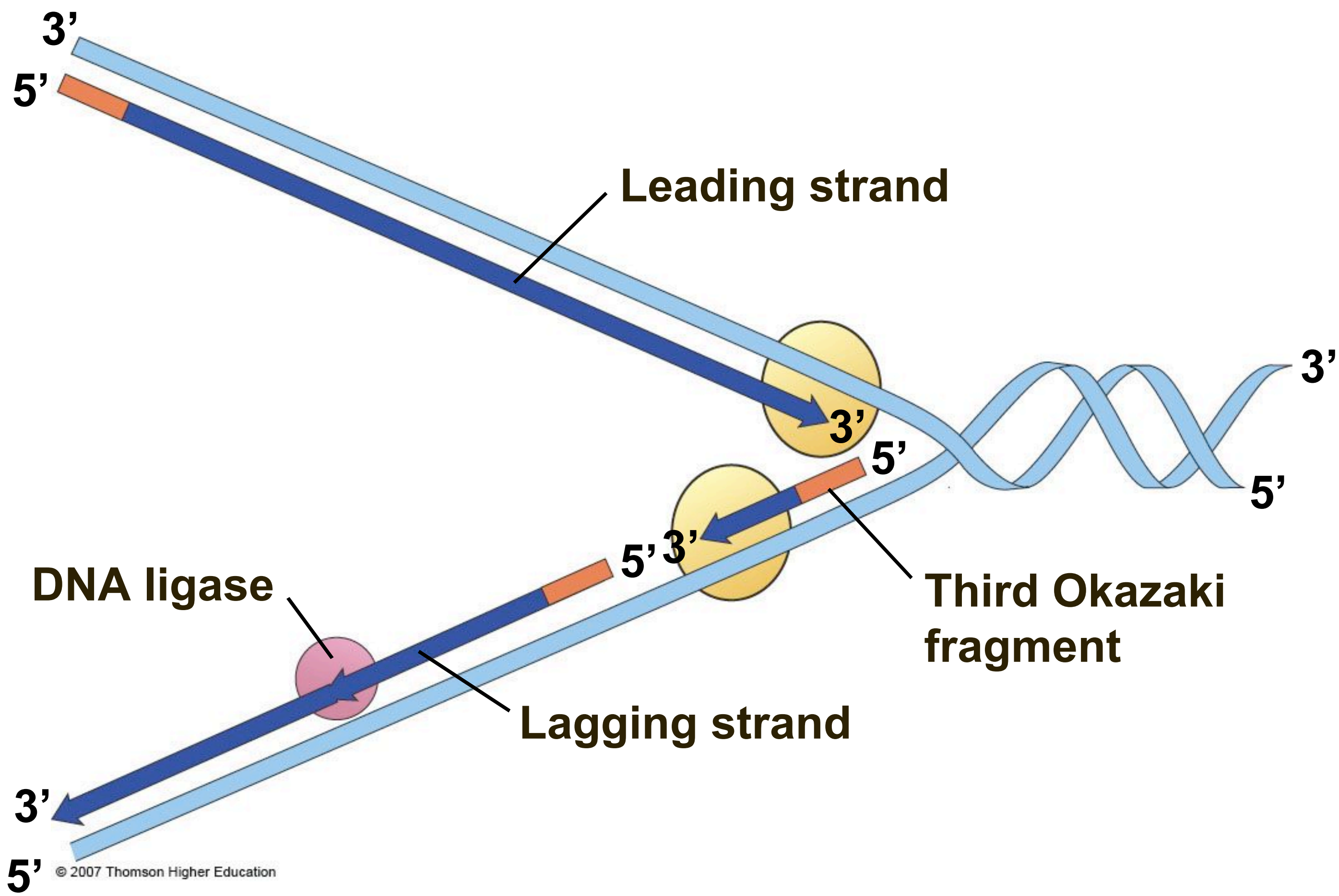


Fig. 12-12c, p. 273

Replication Rules

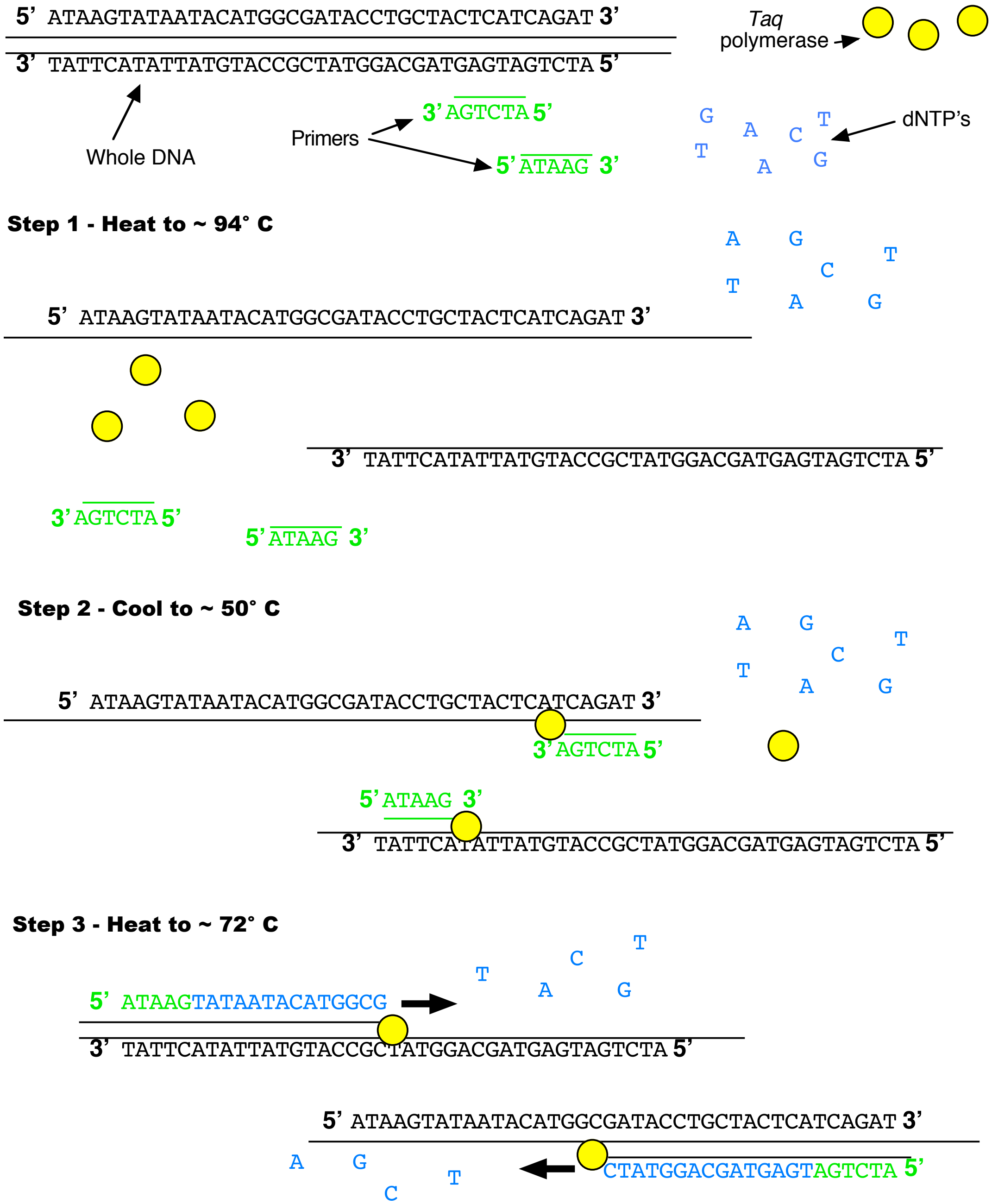
New bases can only be added on to the 3' end of the growing strand

A DNA or RNA primer is necessary

G always pairs with C, T with A

Strands are always antiparallel

Replication is Semi-conservative



PCR - uses a polymerase from a high temp. bacteria to make copies of DNA, *in vitro*

DNA Sequencing...

AATAAGACC**G**
TTATTCTGGCTAGTAGTTAGTTAGGCCCTGACCTTCAA

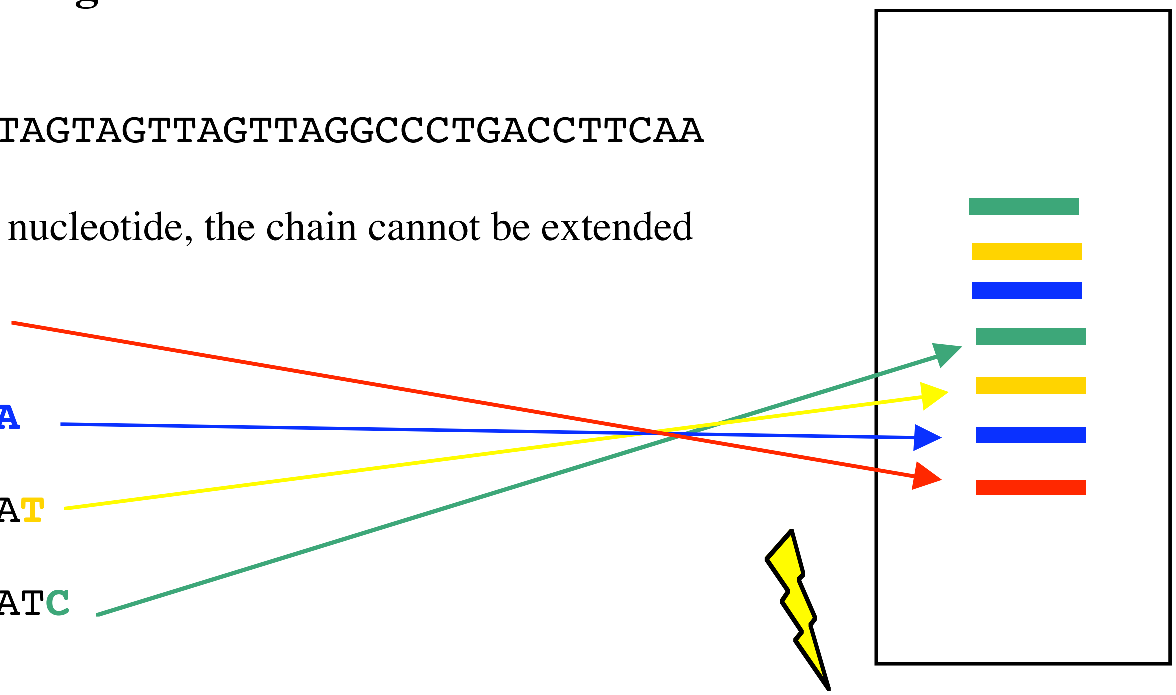
G = a **di**deoxy nucleotide, the chain cannot be extended

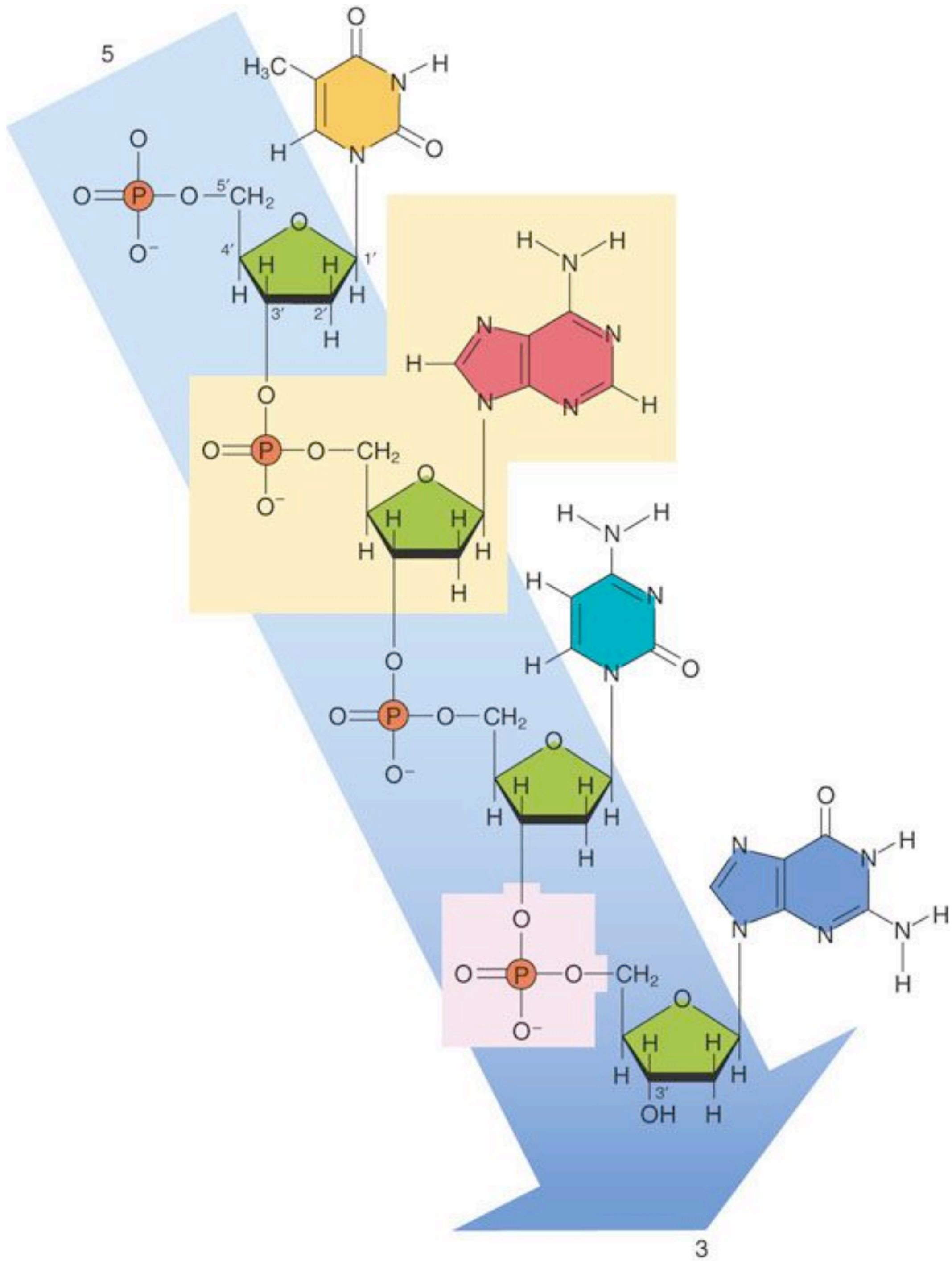
AATAAGACC**G**

AATAAGACCG**A**

AATAAGACCGA**T**

AATAAGACCGAT**C**




5
H3C
5′
CH2
4′
1′
3′
2′
OH
3

DNA Sequencing...

AATAAGACC**G**
TTATTCTGGCTAGTAGTTAGTTAGGCCCTGACCTTCAA

G = a **di**deoxy nucleotide, the chain cannot be extended

AATAAGACC**G**

AATAAGACCG**A**

AATAAGACCGA**T**

AATAAGACCGAT**C**