DNA replication:

- Copying genetic information for transmission to the next generation
- Occurs in S phase of cell cycle
- Process of DNA duplicating itself
- Begins with the unwinding of the double helix to expose the bases in each strand of DNA
- Each unpaired nucleotide will attract a complementary nucleotide from the medium
  - will form base pairing via hydrogen bonding.
- Enzymes link the aligned nucleotides by phosphodiester bonds to form a continuous strand.

DNA replication:

- First question asked was whether duplication was semiconservative or conservative

  - Meselson and Stahl expt

  - Semiconservative -
    - one strand from parent in each new strand

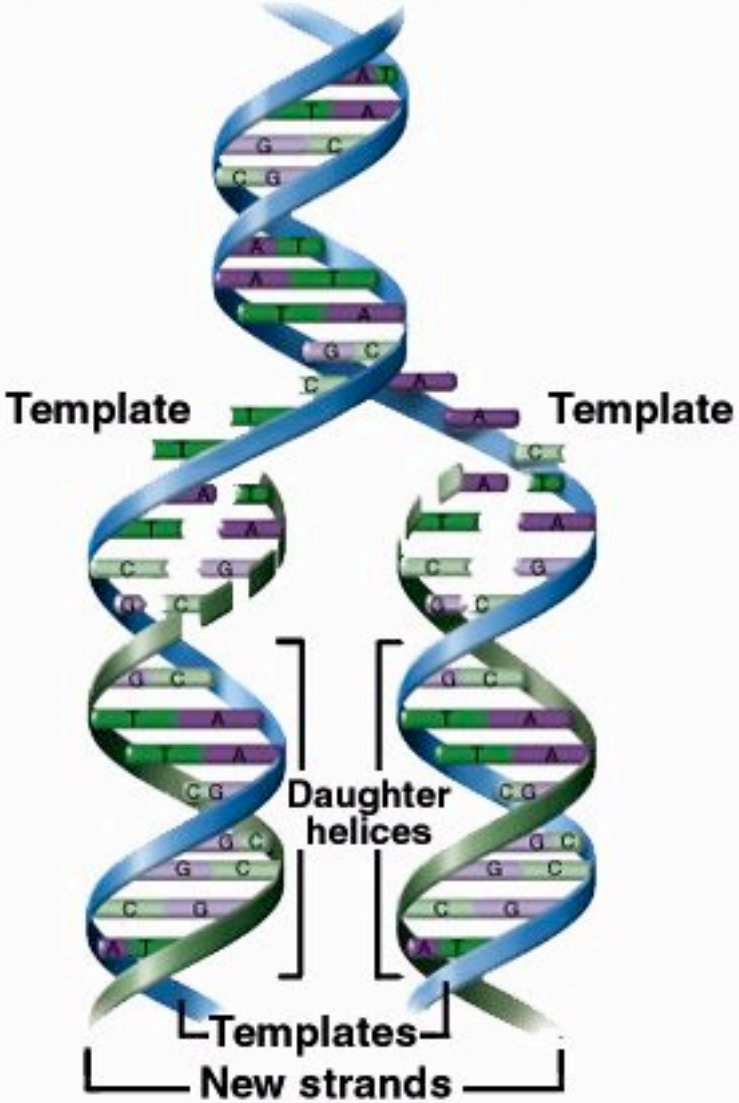
  - Conservative-
    - both strands from parent and other is all new strands

DNA replication:

- Complementary base pairing produces semiconservative replication
  - Double helix unwinds
  - Each strand acts as template
  - Complementary base pairing ensures that T signals addition of A on new strand, and G signals addition of C
  - Two daughter helices produced after replication

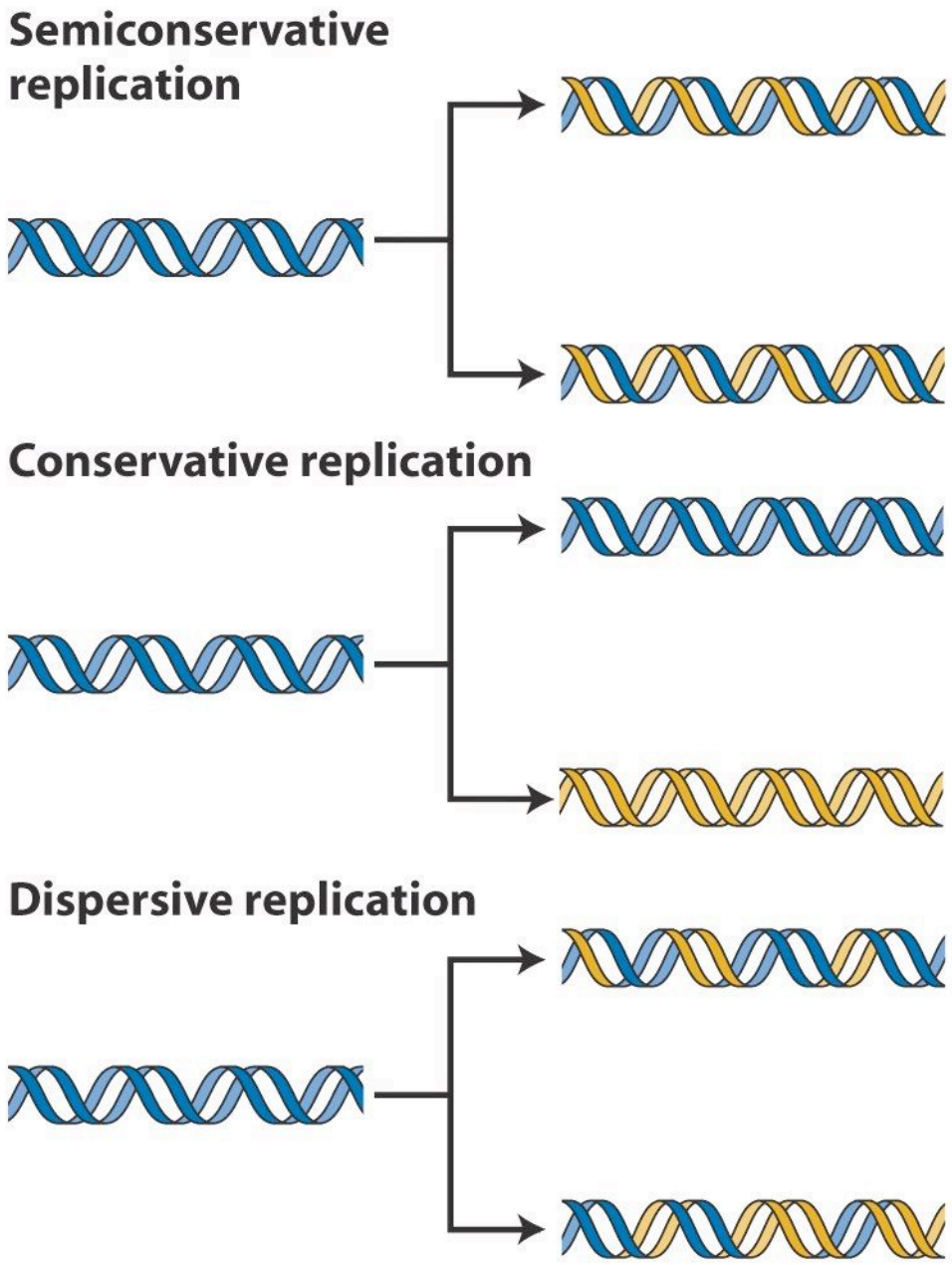
DNA replication: an overview

1. Original double helix
2. Strands separate
3. Complementary bases align opposite templates
4. Enzymes link sugar-phosphate elements of aligned nucleotides into a continuous new strand



Experimental proof of semiconservative replication – three possible models

- Semiconservative replication –
  - Watson and Crick model
- Conservative replication:
  - The parental double helix remains intact;
  - both strands of the daughter double helix are newly synthesized
- Dispersive replication:
  - At completion, both strands of both double helices contain both original and newly synthesized material.


Semiconservative replication
Conservative replication
Dispersive replication

Meselson-Stahl experiments confirm semiconservative replication

- Experiment allowed differentiation of parental and newly formed DNA.
- Bacteria were grown in media containing either normal isotope of nitrogen (^{14}N) or the heavy isotope (^{15}N).
- DNA banded after equilibrium density gradient centrifugation at a position which matched the density of the DNA:
  - heavy DNA was at a higher density than normal DNA.

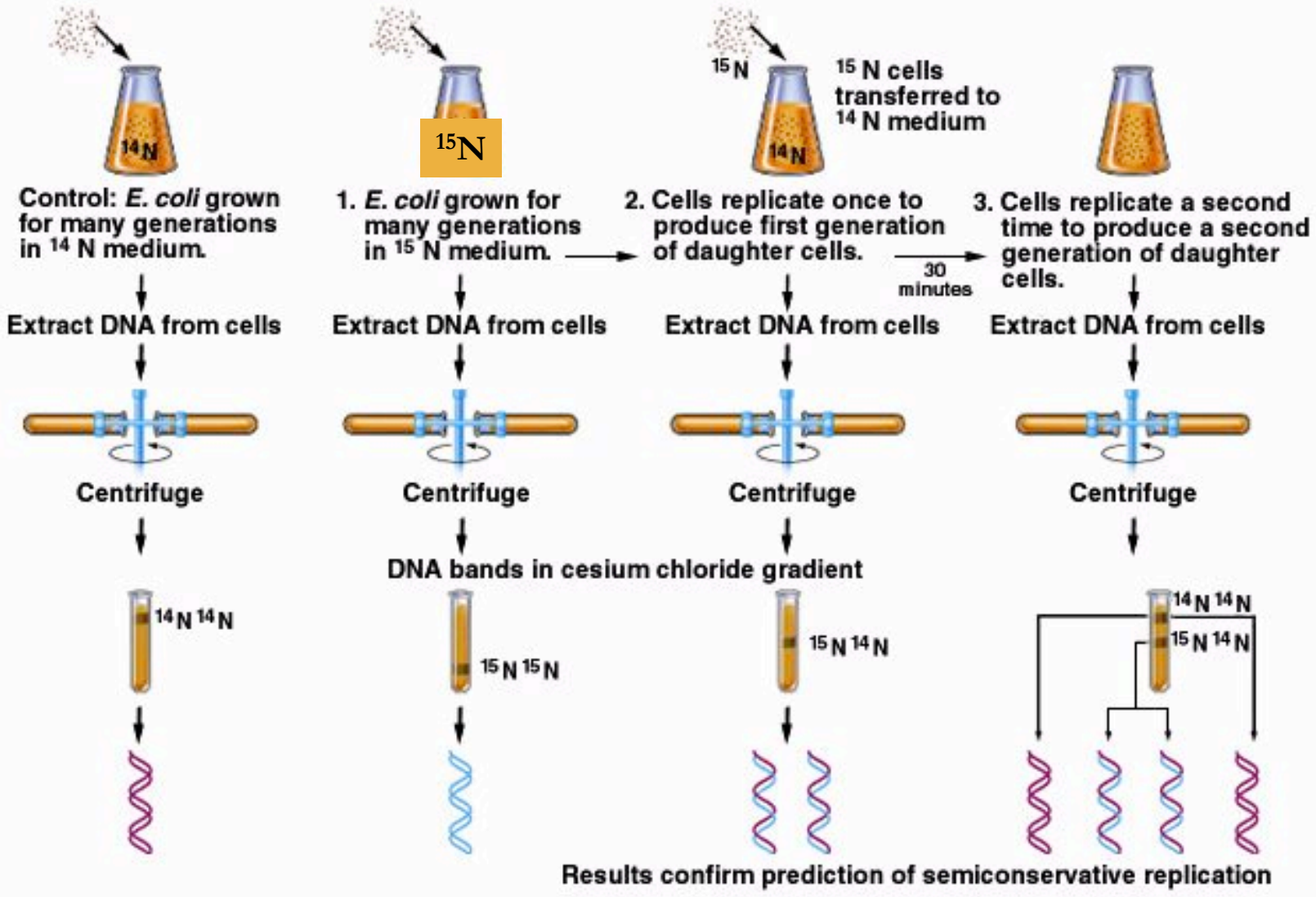
Fig. 6.16

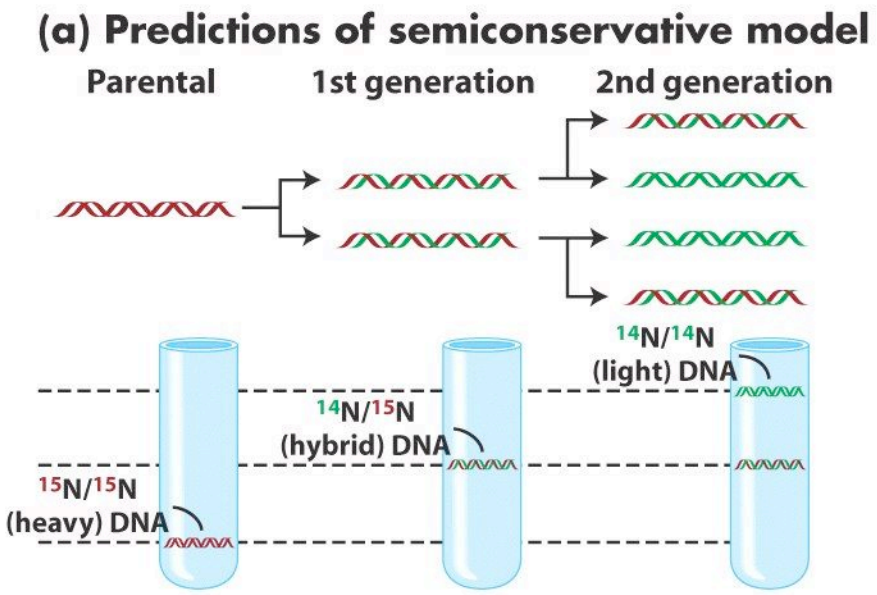
Meselson-Stahl experiments confirm semiconservative replication

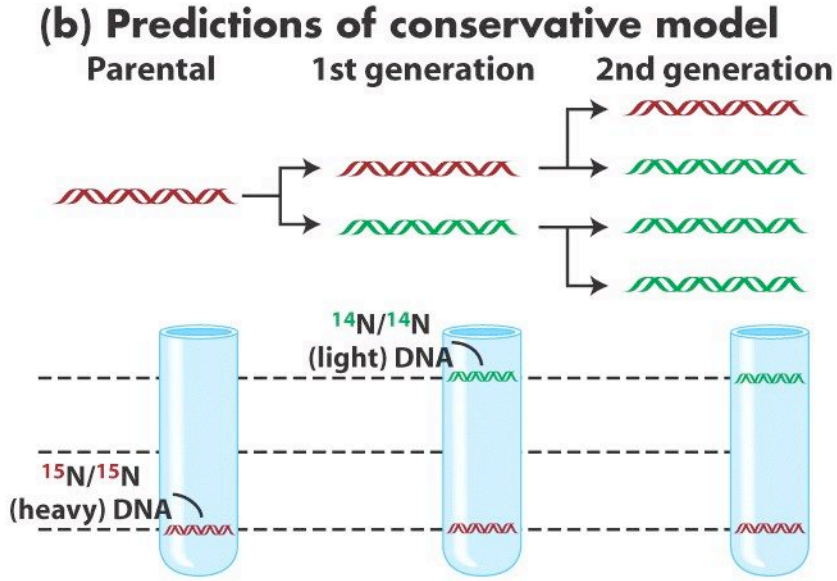
- When bacteria grown in ^{15}N were transferred to normal ^{14}N containing medium,
  - the newly synthesized DNA strand had the ^{14}N while the parental strand had ^{15}N.

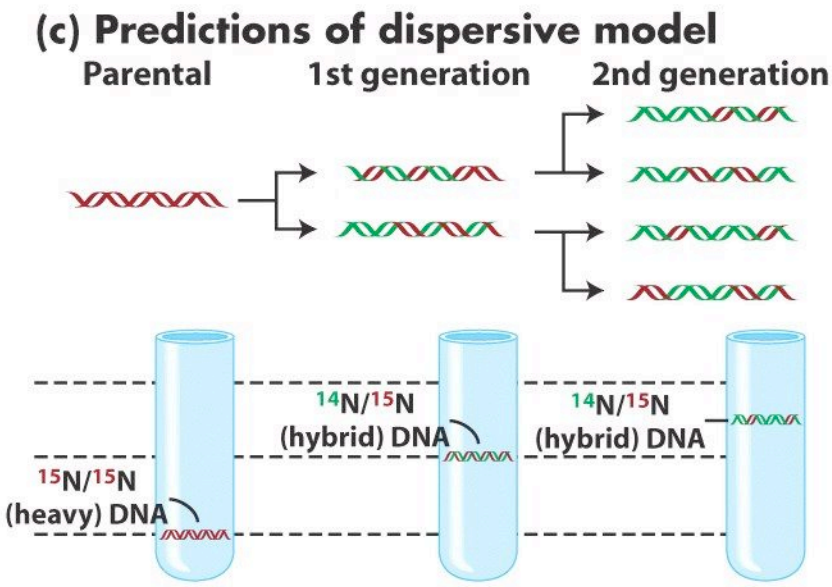
- They checked the composition of the resulting DNA molecules by density gradient centrifugation,
  - found an intermediate band,
  - indicating a hybrid molecule
  - containing both ^{14}N and ^{15}N DNA.

The Meselson-Stahl experiment




(a) Predictions of semiconservative model
Parental
1st generation
2nd generation
14N/14N
(light) DNA
14N/15N
(hybrid) DNA
15N/15N
(heavy) DNA


(b) Predictions of conservative model
Parental
1st generation
2nd generation
14N/14N
(light) DNA
15N/15N
(heavy) DNA


(c) Predictions of dispersive model
Parental
1st generation
2nd generation
14N/15N
(hybrid) DNA
14N/15N
(hybrid) DNA
15N/15N
(heavy) DNA

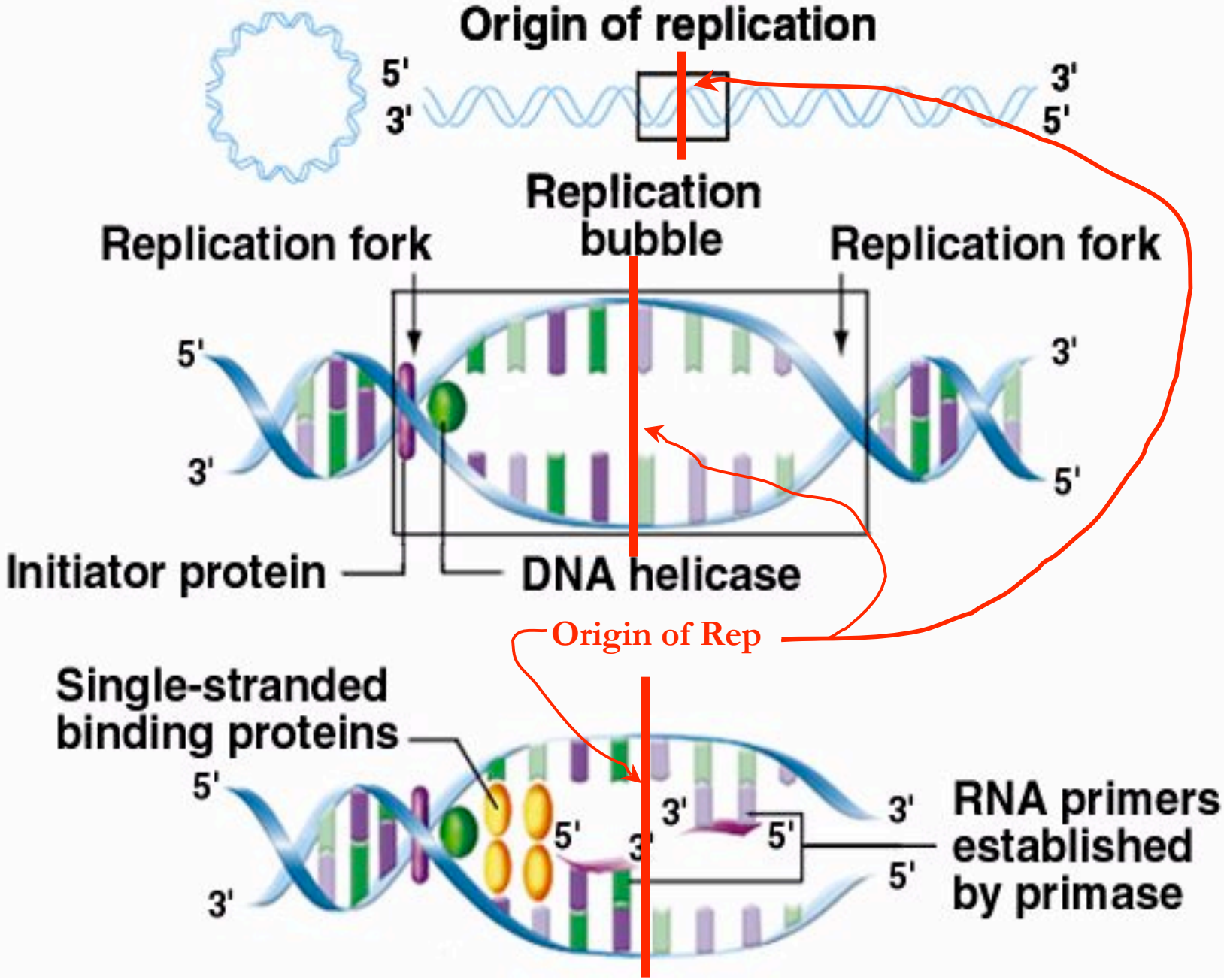
The mechanism of DNA replication

- Tightly controlled process,
  - occurs at specific times during the cell cycle.
- Requires:
  - a set of proteins and enzymes,
  - and requires energy in the form of ATP.
- Two basic steps:
  - Initiation
  - Elongation.
- Two basic components:
  - template
  - primer.

The mechanism of DNA replication (prokaryotic)

- DNA polymerase
  - the enzyme that extends the primer;
  - Pol III –
    - produces new stands of complementary DNA
  - Pol I –
    - fills in gaps between newly synthesized Okazaki segments
- additional enzymes/proteins
  - i) DNA helicase –
    - unwinds double helix
  - ii) Single-stranded binding proteins –
    - keep helix open
  - iii) Primase –
    - creates RNA primers to initiate synthesis
  - iv) Ligase –
    - welds together Okazaki fragments

Mechanism of DNA replication, 1



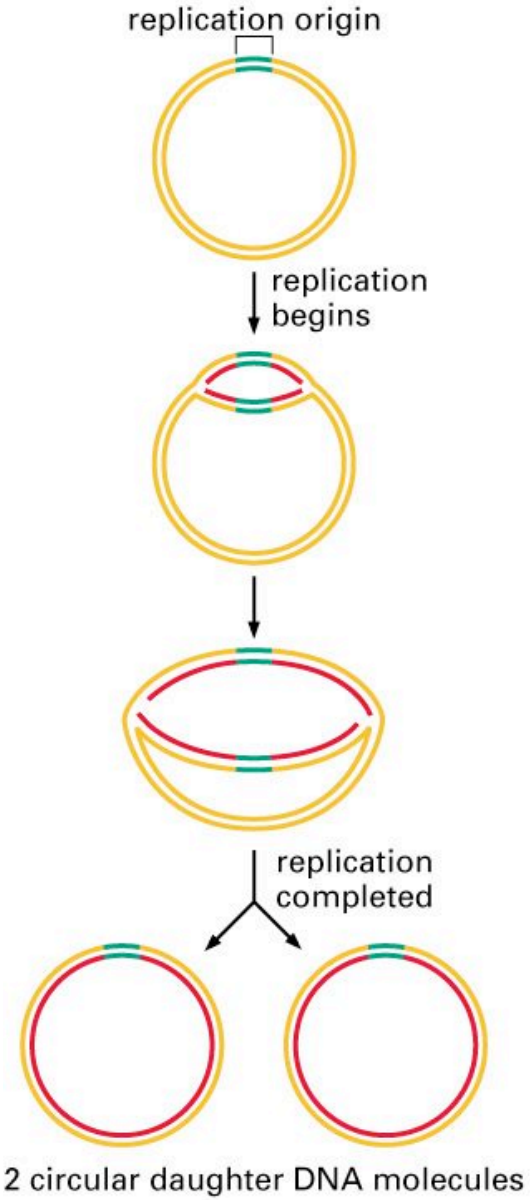
Origins of Replication

- Replication proceeds in both directions (bidirectionally) from a single origin of replication on the prokaryotic circular chromosome

- Replication proceeds in both directions (bidirectionally) from hundreds or thousands of origins of replication on each of the linear eukaryotic chromosomes.

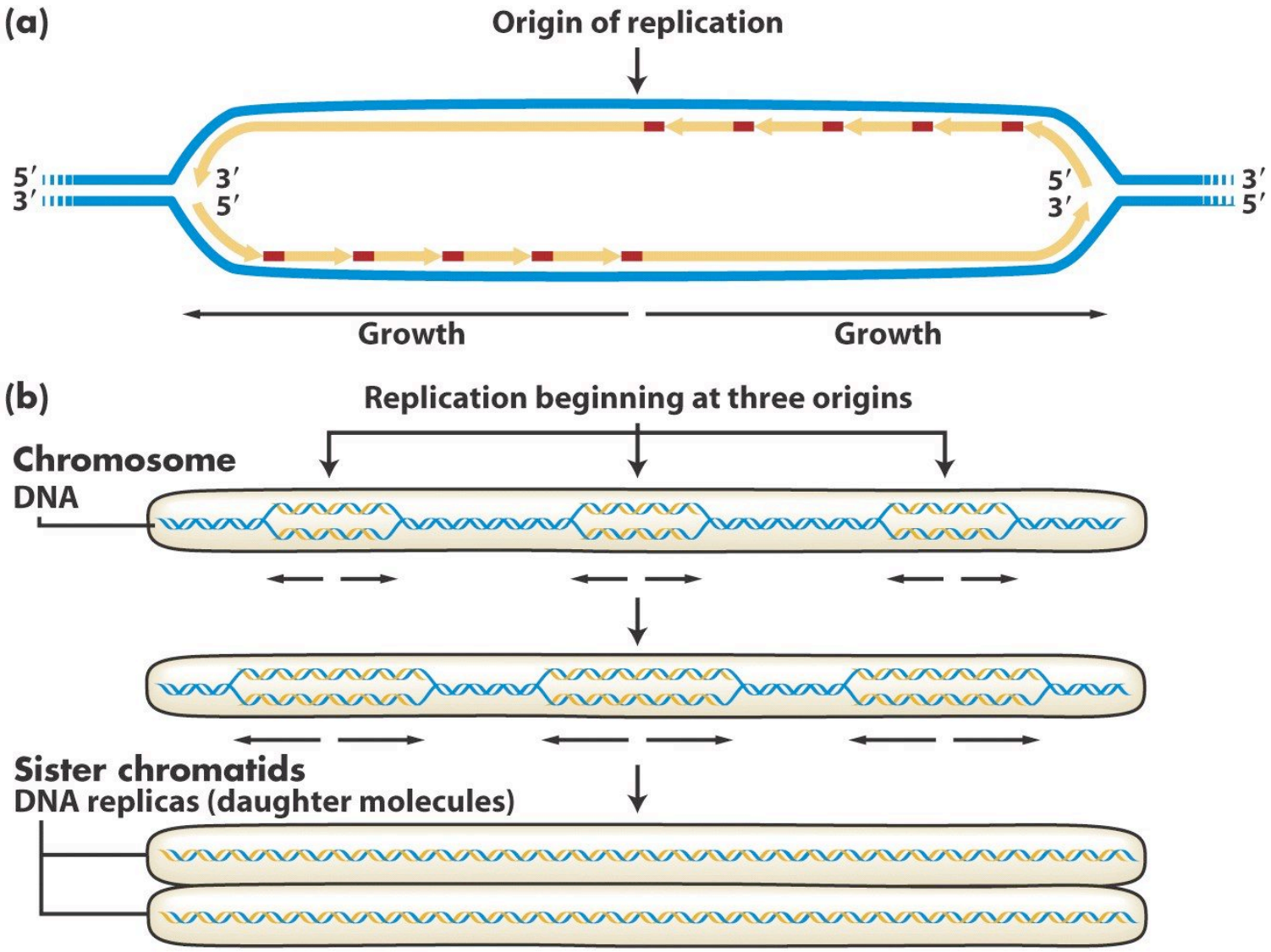
Origins of Replication

- Bacteria have 1 origin of replication per one chromosome
- They only have one chromosome = 1 origin!



Molecular Biology of the Cell, 4th Edition.

Eukaryotic Origins of Replication



Replication Initiation

- DNA **origin** of replication
- Initiator proteins bind
- Recruits DNA **helicase**
- Opening of DNA strands

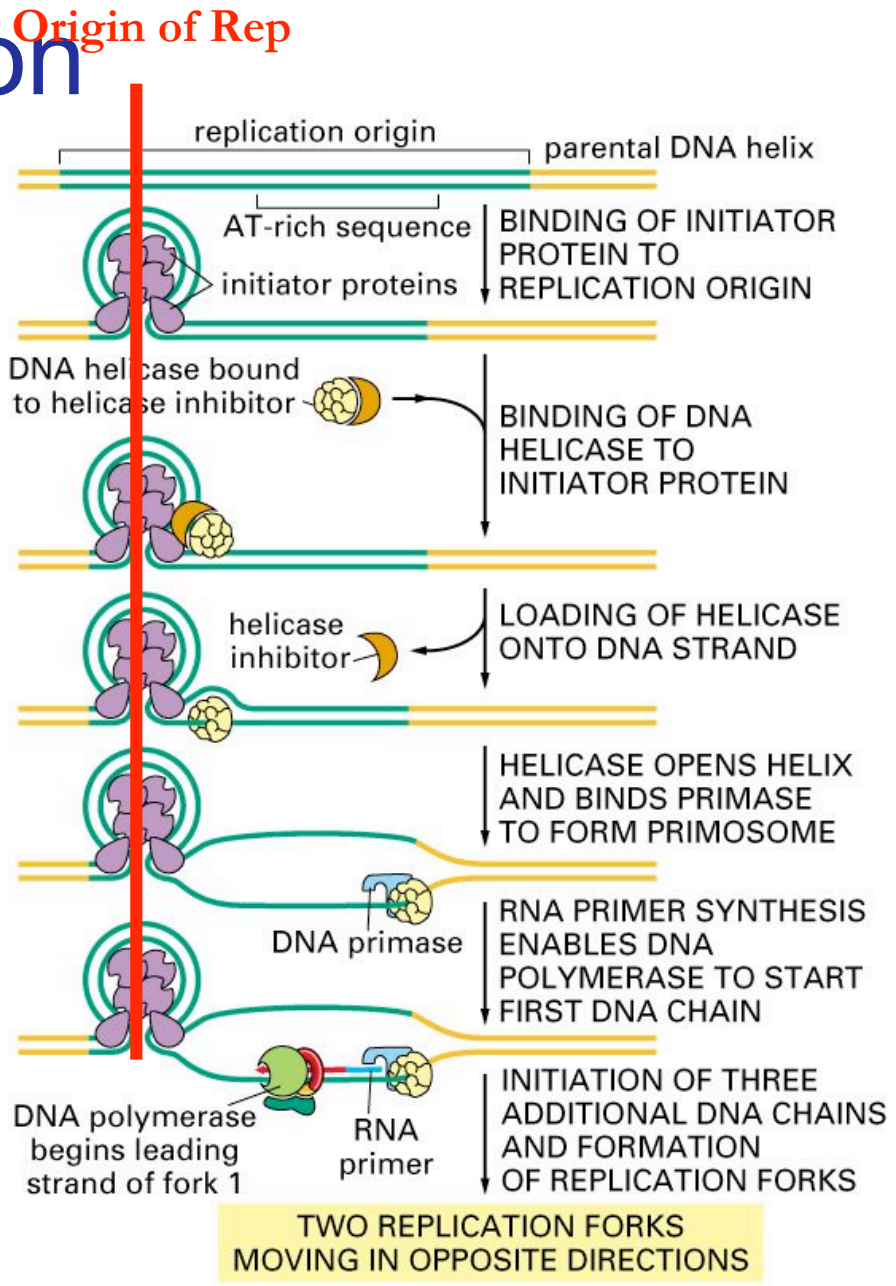
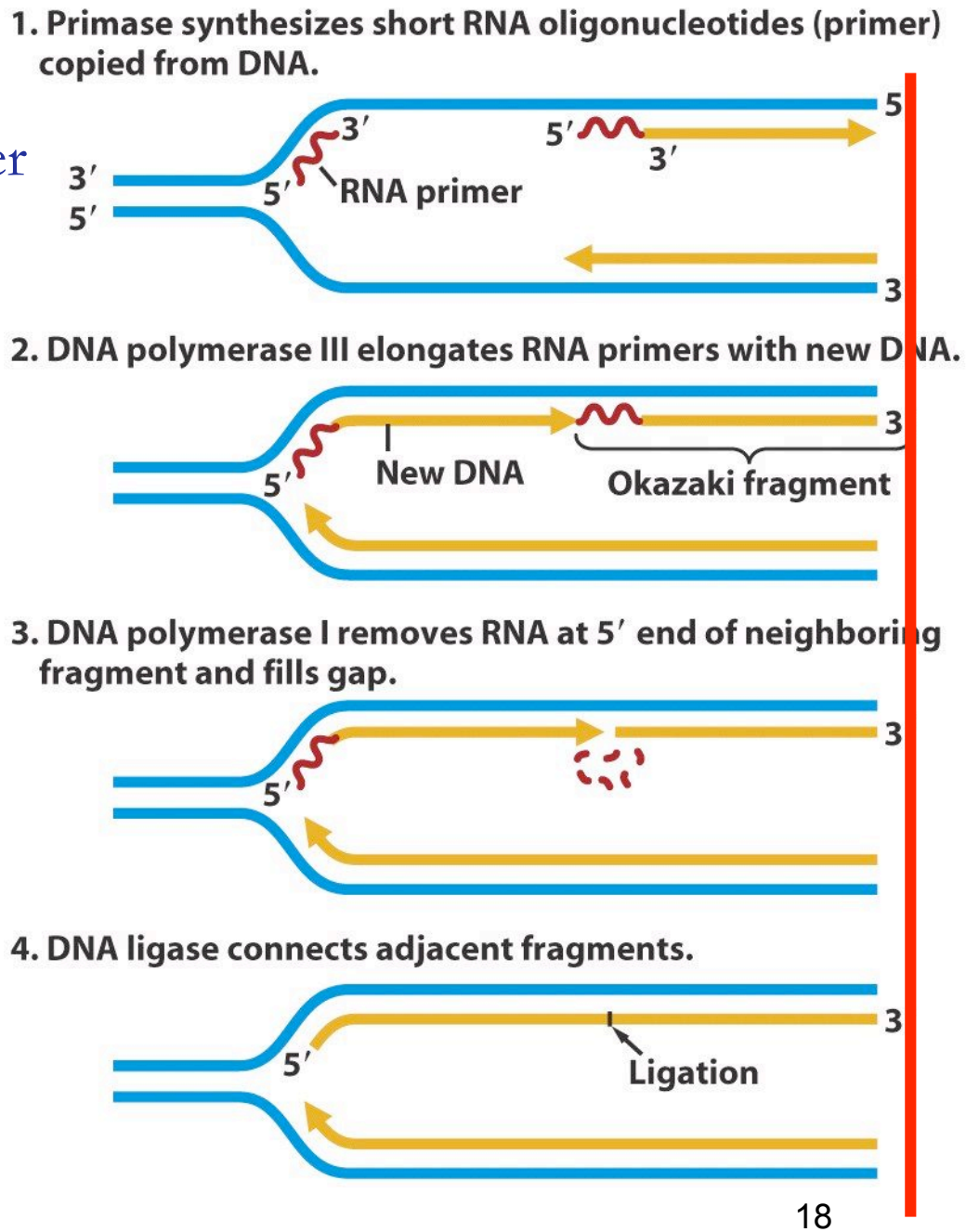


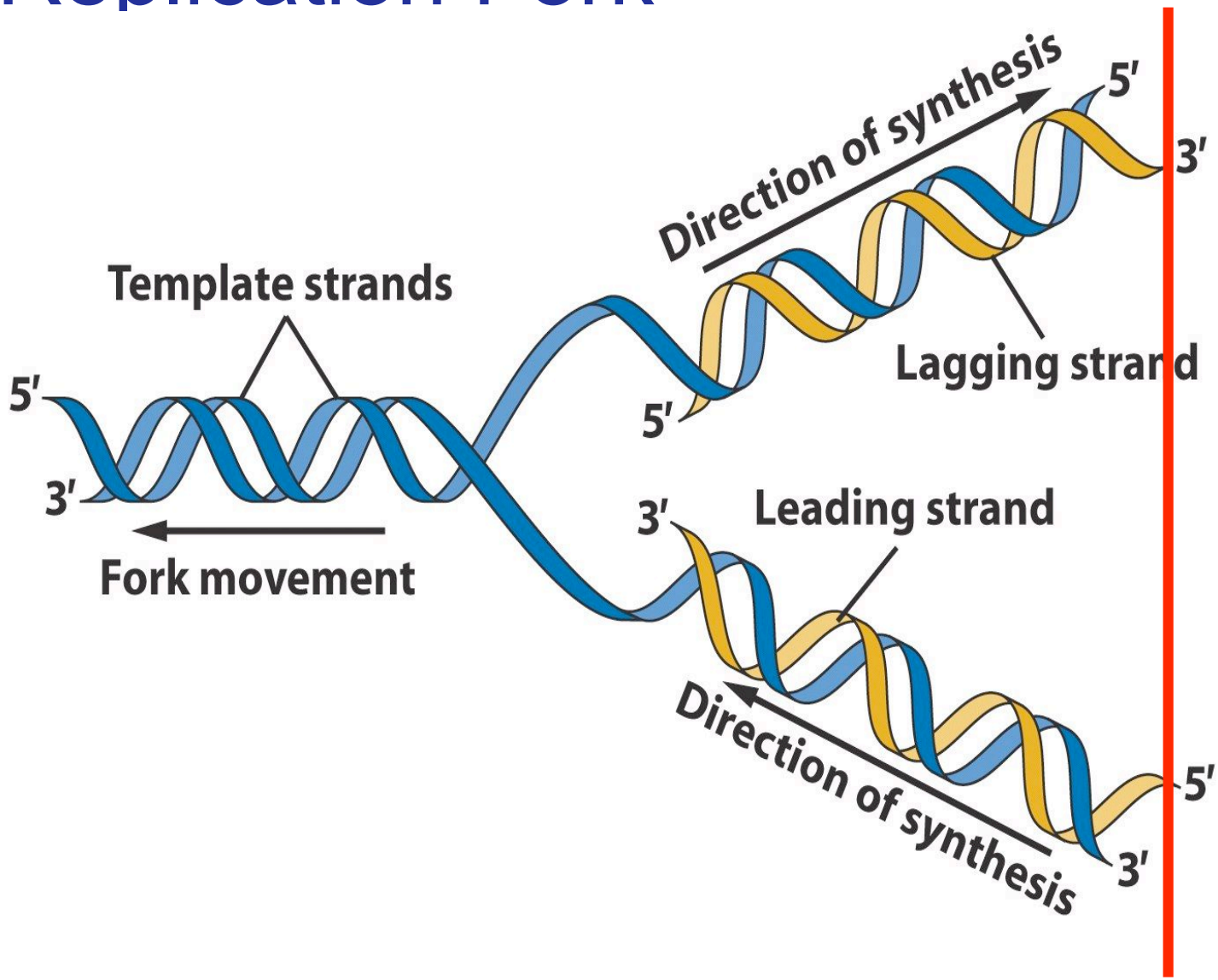
Figure 5–31. Molecular Biology of the Cell, 4th Edition.

- Replication Initiation:
  - Primase and the RNA Primer
- Replication Elongation:
  - DNA polIII
    - Must have 3' to add to
- Replication is Finished:
  - DNA polI removes primer
  - Fills gap using 3'ends
  - DNA ligase connects frags
    - Uses 5' ends!



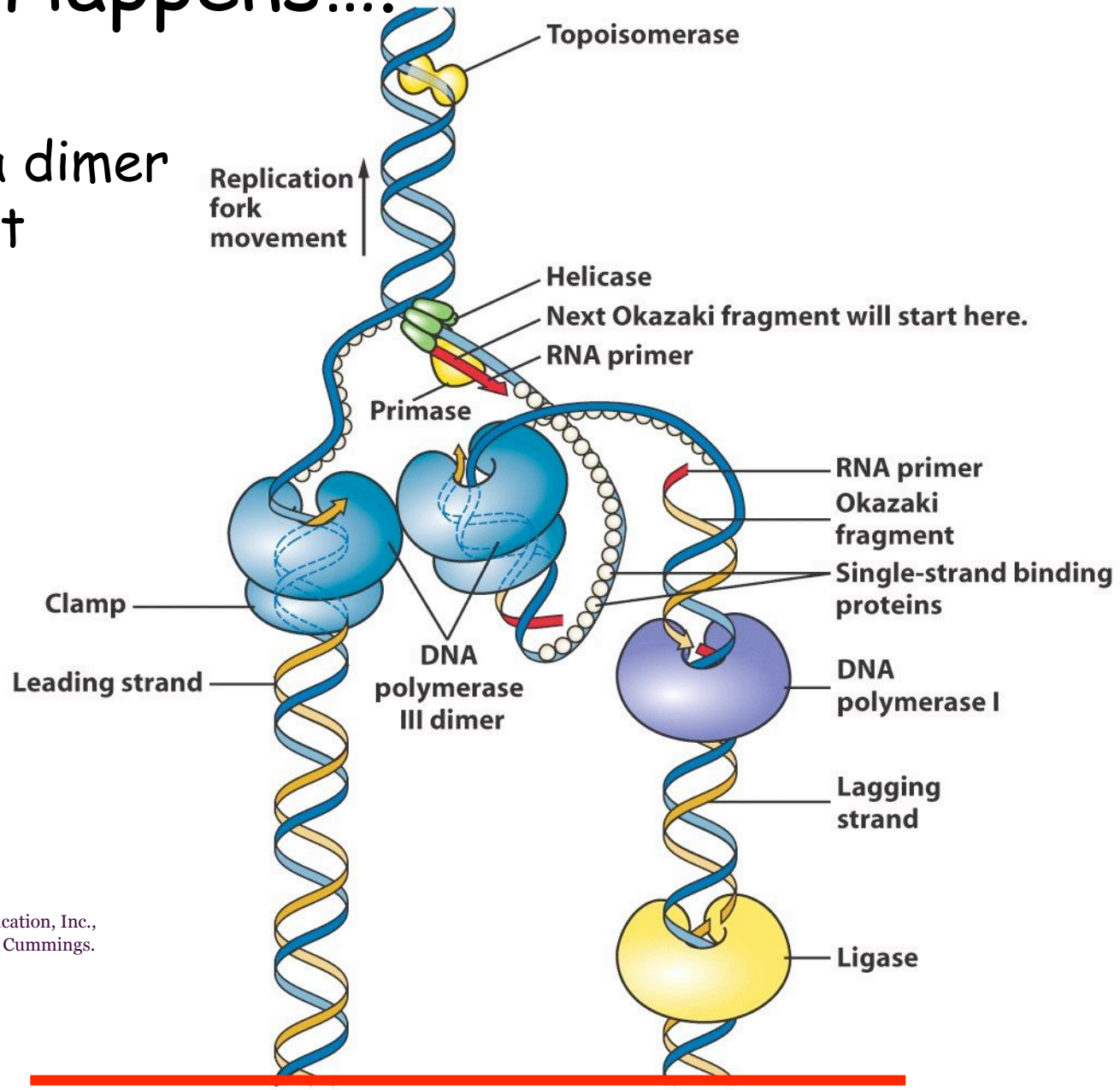
Origin of Rep

Replication Fork



What Really Happens….

DNA pol works as a dimer
Lagging strand must loop around to accommodate dimerization



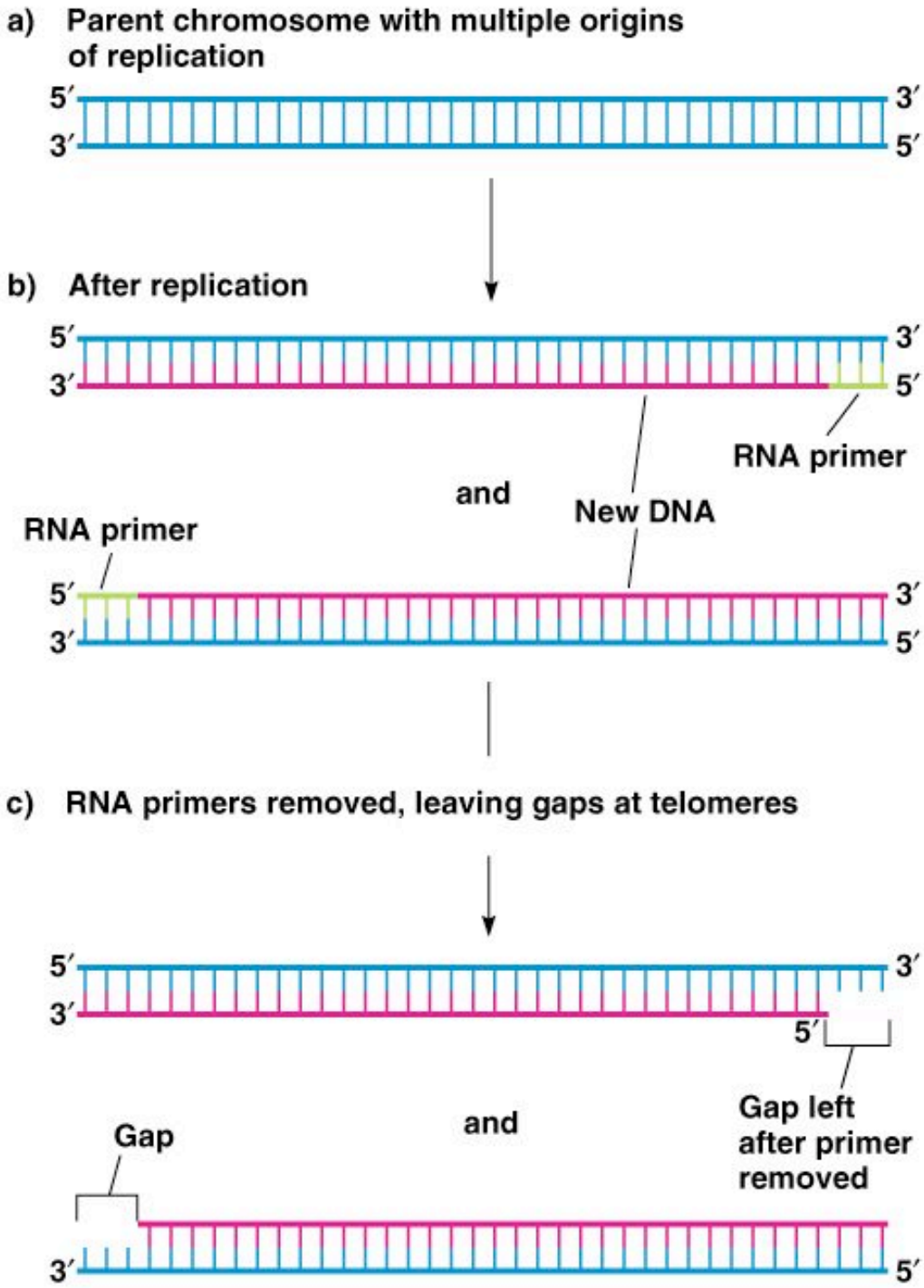
Origin of Rep

Replication Termination

- The ends of chromosomes (telomeres) cannot be replicated on the lagging strand because there is no primer available.
- **Telomerases**
  - enzymes that contain RNA primers which extend the ends of chromosomes (not normally expressed in significant levels)
    - Telomeres form a sort of single stranded cap around the chromosome ends to protect them from being degraded
  - chromosome ends are progressively shortened with each round of replication.
  - "old" cells with shortened telomeres undergo apoptosis -
    - Protective for normal cells
    - Kill the old and possibly mutated
  - Telomerase is over expressed in cancer cells
  - Hypothesis is that cancer cells do not undergo apoptosis because their telomeres do not shorten over time.
    - No death signal

Fig. 11.14

The problem of replicating completely a linear chromosome in eukaryotes

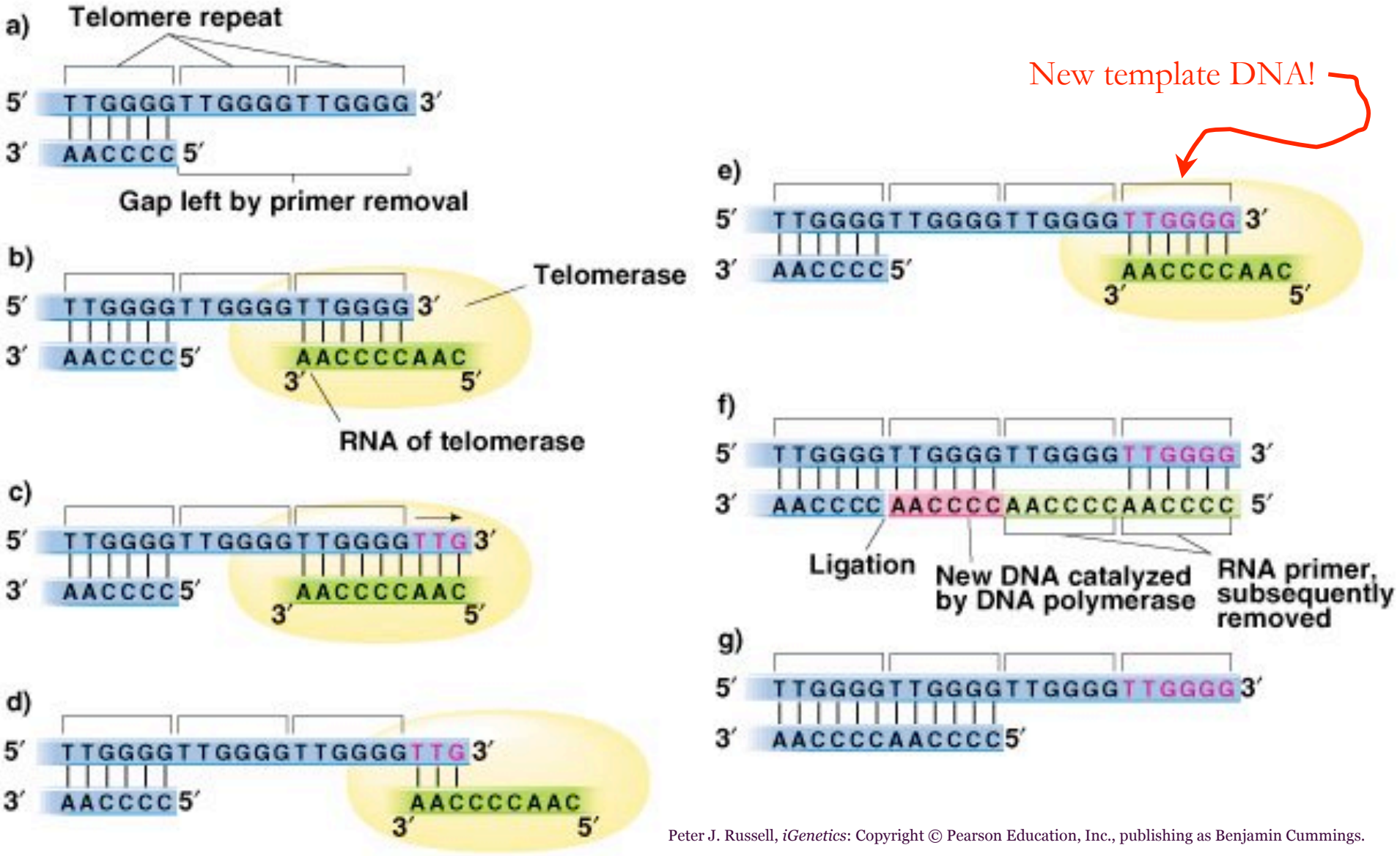


Replicating the Ends of Chromosomes

- telomerase adds an RNA primer complementary to telomere sequences
  - chromosomal replication proceeds by adding to the 3' end of the primer
- Fills the gap left behind by replication
- Telomerase enzyme can also add DNA basepairs to the TEMPLATE DNA
  - complementary to the RNA primer basepairs

  - Using an RNA template to make DNA, telomerase functions as a reverse transcriptase called TERT (telomerase reverse transcriptase).
    - This goes against the Central Dogma….
    - Evolutionarily thought to be derived from a Retrovirus

Fig. 3.19
Synthesis of telomeric DNA by telomerase



Replication at the chromosomal level

- Replication is bidirectional.
- For circular DNA (and linear chromosomes)
  - the unwinding at the replication forks causes supercoiling.
- **DNA topoisomerases**
  - enzymes that help relax the DNA by nicking the strands
  - releasing the twists
  - then rejoining the DNA ends.
  - Example is DNA gyrase

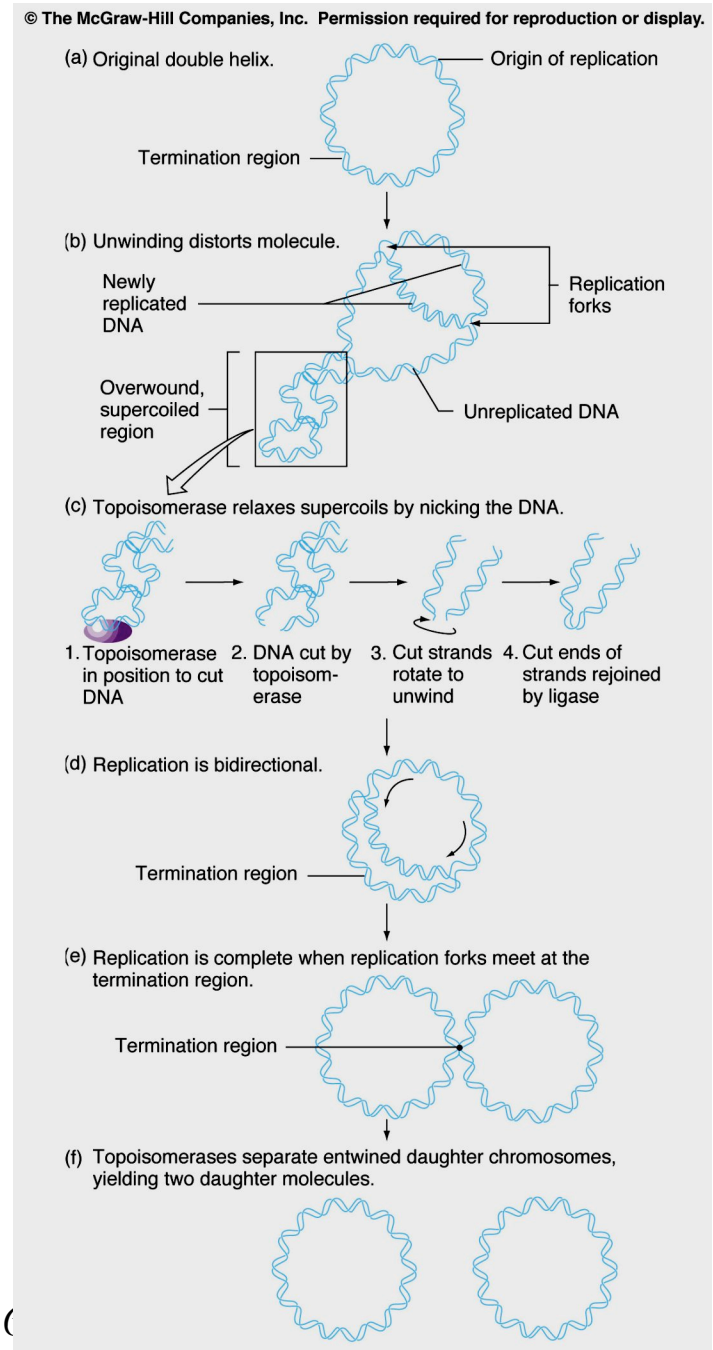
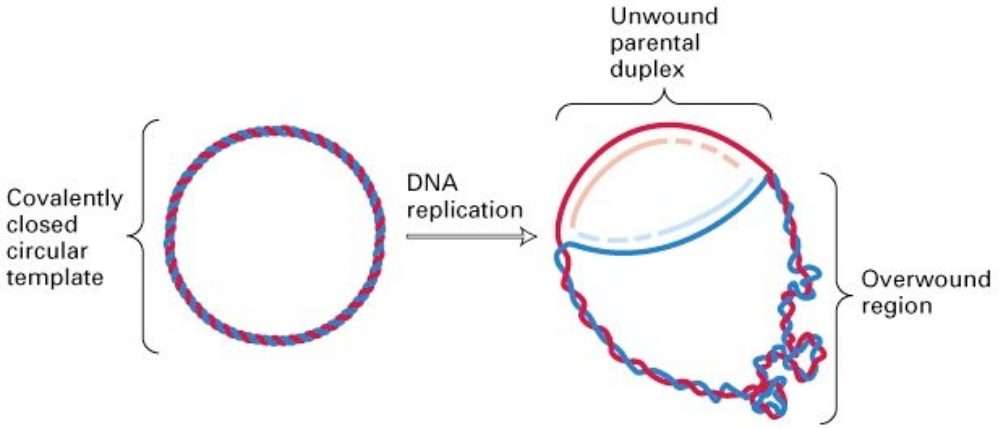


Fig. (

The bidirectional replication of a circular chromosome (Prokaryotic)



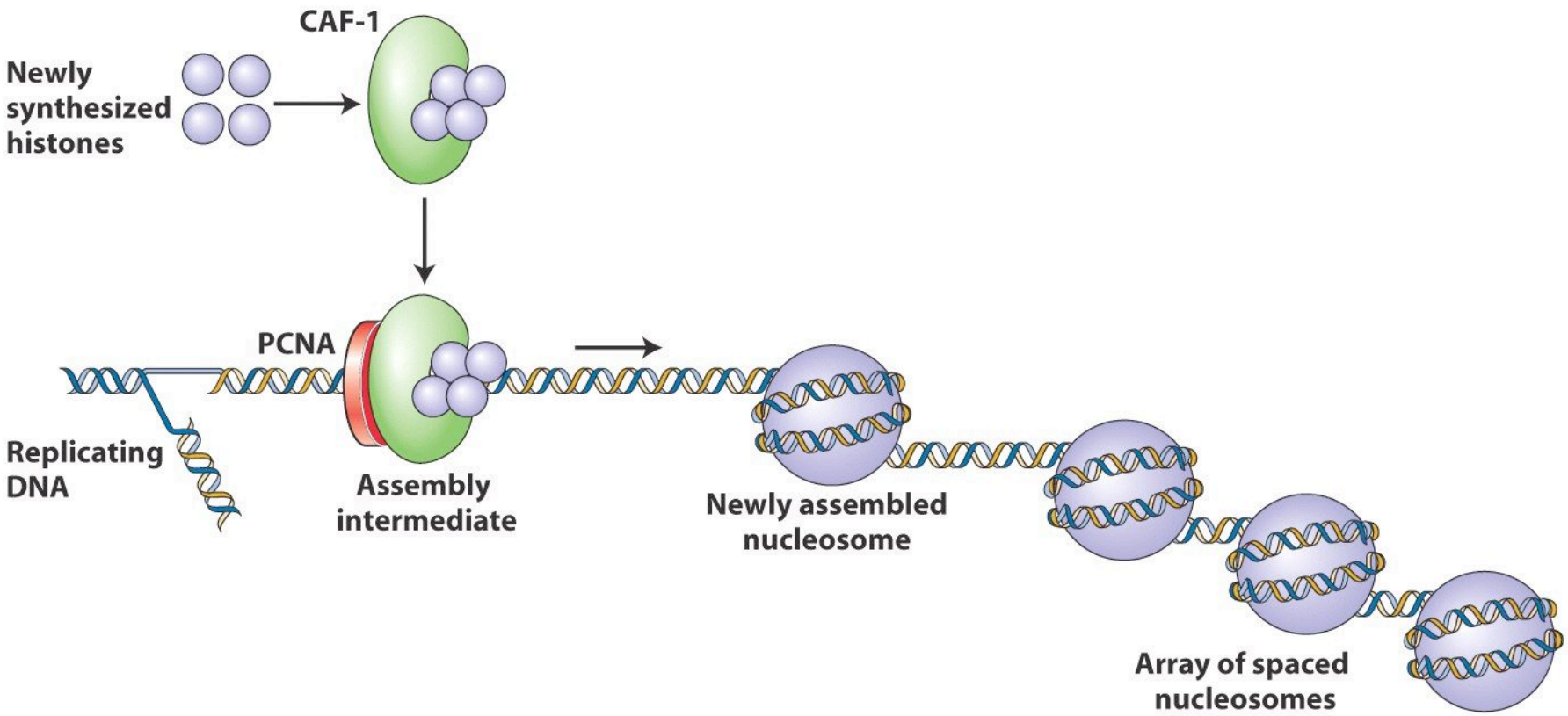
Assembling Newly Replicated DNA into Nucleosomes

- When eukaryotic DNA is replicated, it complexes with histones.
  - This requires synthesis of histone proteins and assembly of new nucleosomes.

- Transcription of histone genes is initiated near the end of G1 phase, and translation of histone proteins occurs throughout S phase.

- Assembly of newly replicated DNA into nucleosomes is shown in Figure 11.16.

The Assembly of Nucleosomes after Replication



Homework Problems

Chapter 11

4, 11