DNA Replication

(CHAPTER 11- Brooker Text)

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BIO 184
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What are the structural features of DNA that enable its function?

- complementarity of DNA strands (AT/GC)
- The two DNA strands can come apart
- Each serves as a template strand for the synthesis of new strands
- Template strand also encodes for RNA

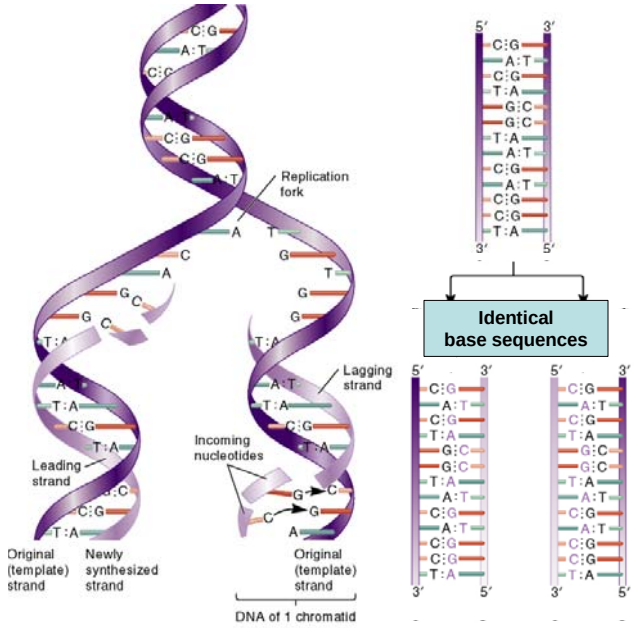


Figure 11.1 **(a)** The mechanism of DNA replication **(b)** The products of replication

Which Model of DNA Replication is Correct?

- In the late 1950s, three different mechanisms were proposed for the replication of DNA
  - Conservative model
    - Both parental strands stay together after DNA replication
  - Semiconservative model
    - The double-stranded DNA contains one parental and one daughter strand following replication
  - Dispersive model
    - Parental and daughter DNA are interspersed in both strands following replication

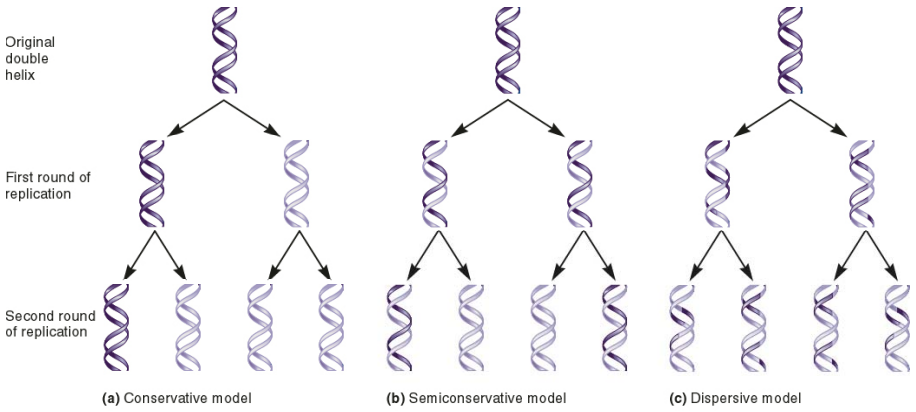


Figure 11.2

Meselson and Stahl Experiment (1958)

- Differentiated between the 3 different replication mechanisms by experimentally distinguishing daughter from parental strands
- Method
  - Grow *E. coli* in the presence of ^{15}N (a heavy isotope of Nitrogen) for many generations
    - The population of cells had heavy-labeled DNA
  - Switch *E. coli* to medium containing only ^{14}N (a light isotope of Nitrogen)
  - Collect sample of cells after various times
  - Analyze the density of the DNA by centrifugation using a CsCl gradient

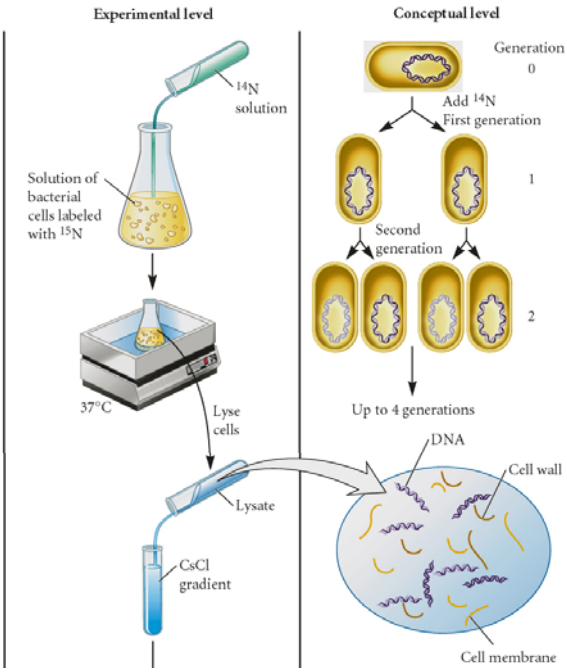


Figure 11.3

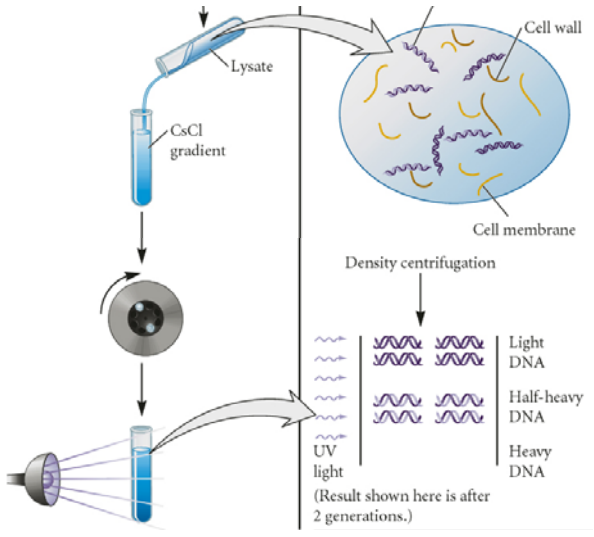
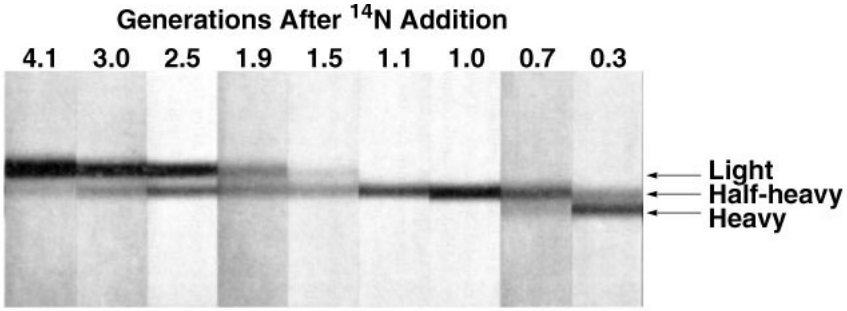


Figure 11.3

The Data



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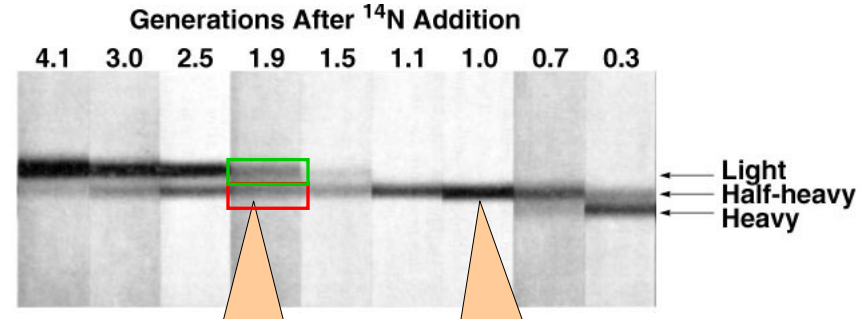
Interpreting the Data



After ~ two generations, DNA is of two types: "light" and "half-heavy"

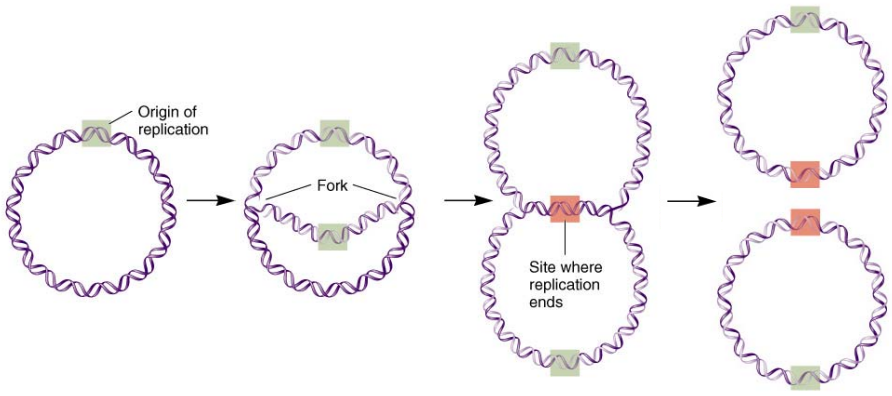
This is consistent with only the semi-conservative model

After one generation, DNA is "half-heavy"

This is consistent with both semi-conservative and dispersive models

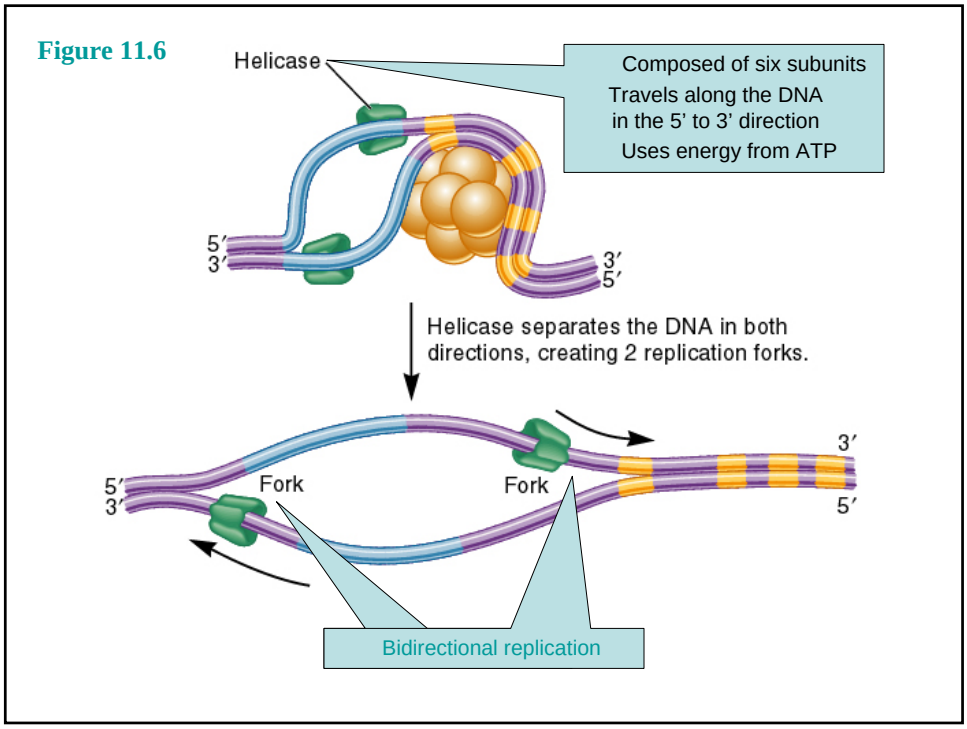
BACTERIAL DNA REPLICATION

- Overview
  - DNA synthesis begins at a site termed the origin of replication
    - Each bacterial chromosome has only one
  - Synthesis of DNA proceeds bidirectionally around the bacterial chromosome
  - The replication forks eventually meet at the opposite side of the bacterial chromosome
    - This ends replication



(a) Bacterial chromosome replication

Figure 11.4



- DNA helicase separates the two DNA strands by breaking the hydrogen bonds between them
- This generates positive supercoiling ahead of each replication fork
  - DNA gyrase travels ahead of the helicase and alleviates these supercoils
- Single-strand binding proteins bind to the separated DNA strands to keep them apart
- Then short (10 to 12 nucleotides) RNA primers are synthesized by DNA primase
  - These short RNA strands start, or prime, DNA synthesis
    - They are later removed and replaced with DNA

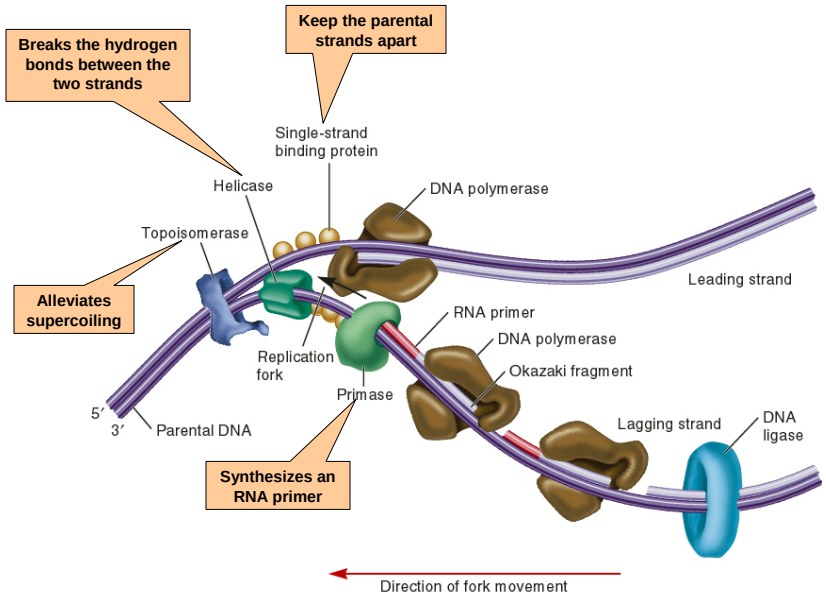


Figure 11.7

DNA Polymerases

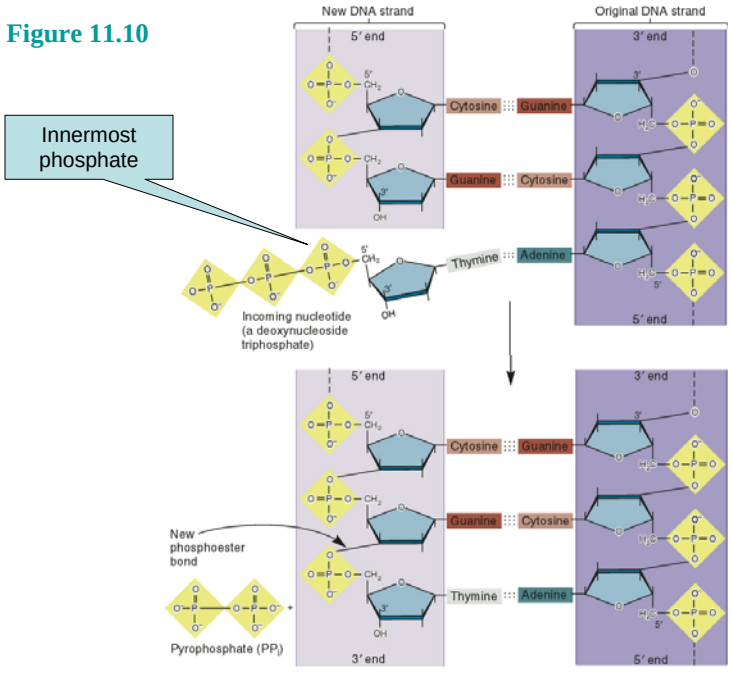
- DNA polymerases are the enzymes that catalyze the attachment of nucleotides to make new DNA

- DNA pol I
  - Composed of a single polypeptide
  - Removes the RNA primers and replaces them with DNA

- DNA pol III
  - Composed of 10 different subunits
  - The complex of all 10 is referred to as the DNA pol III holoenzyme
  - It is the workhorse of replication

The Reaction of DNA Polymerase

- DNA polymerases catalyzes a phosphodiester bond between the
  - Innermost phosphate group of the incoming deoxynucleoside triphosphate
    - AND
  - 3'-OH of the sugar of the previous deoxynucleotide
- In the process, the last two phosphates of the incoming nucleotide are released
  - In the form of pyrophosphate (PP_i)



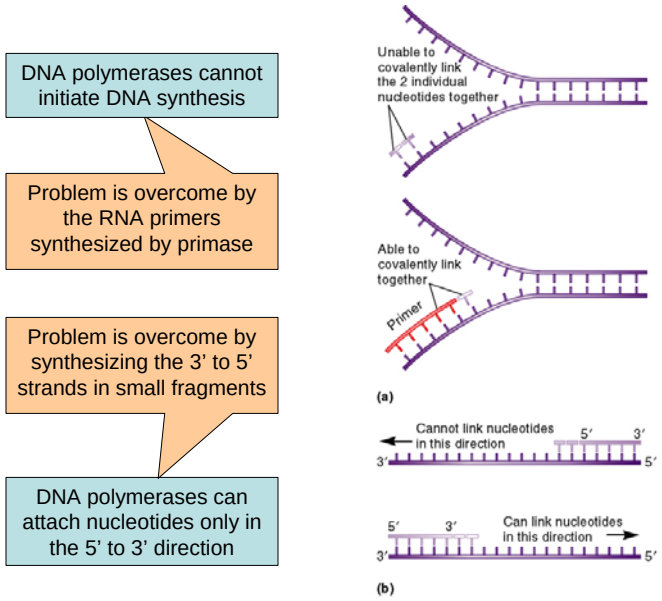


Figure 11.9

- The two new daughter strands are synthesized in different ways
  - Leading strand
    - One RNA primer is made at the origin
    - DNA pol III attaches nucleotides in a 5' to 3' direction as it slides toward the opening of the replication fork
  - Lagging strand
    - Synthesis is also in the 5' to 3' direction
      - However it occurs away from the replication fork
    - Many RNA primers are required
    - DNA pol III uses the RNA primers to synthesize small DNA fragments (1000 to 2000 nucleotides each)
      - These are termed Okazaki fragments after their discoverers

- DNA pol I removes the RNA primers and fills the resulting gap with DNA
  - It uses its 5’ to 3’ exonuclease activity to digest the RNA and its 5’ to 3’ polymerase activity to replace it with DNA
- After the gap is filled a covalent bond is still missing
- DNA ligase catalyzes a phosphodiester bond
  - Thereby connecting the DNA fragments

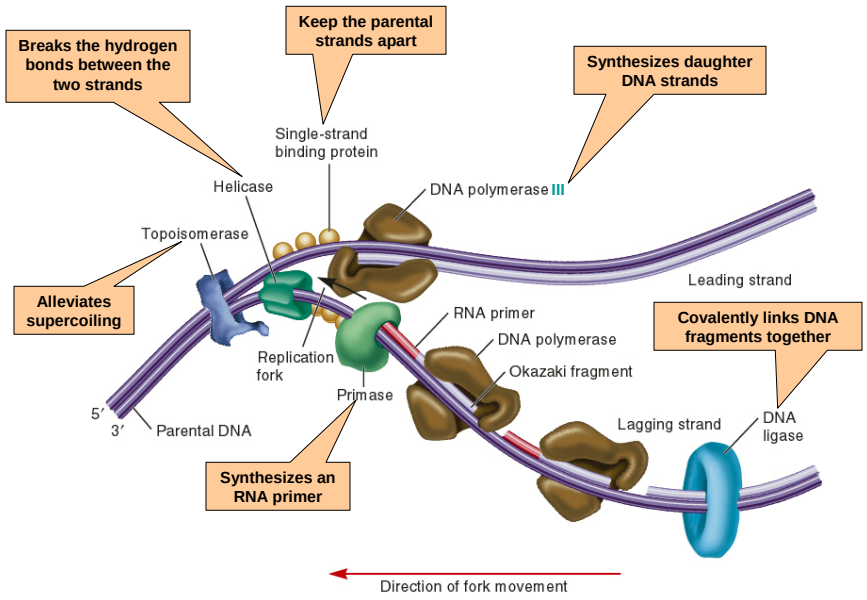


Figure 11.7

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Termination of Replication

- Opposite to *oriC* is a pair of termination sequences called *ter* sequences
- A termination protein binds to these sequences
  - It can then stop the movement of the replication forks
- DNA replication ends when oppositely advancing forks meet (usually at *T1* or *T2*)
- DNA replication often results in two intertwined molecules
  - Intertwined circular molecules are termed catenanes
  - These are separated by the action of topoisomerases

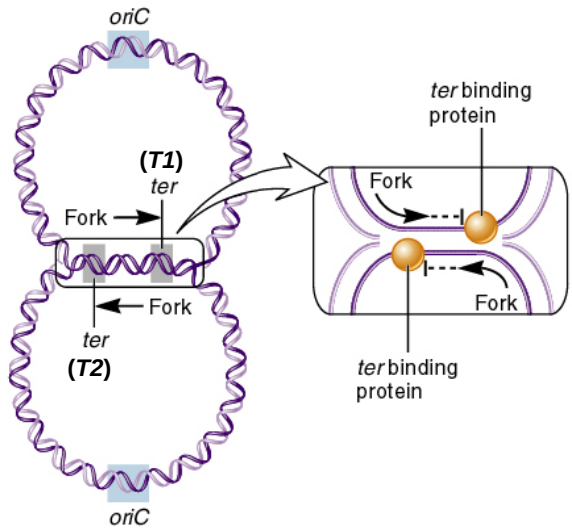
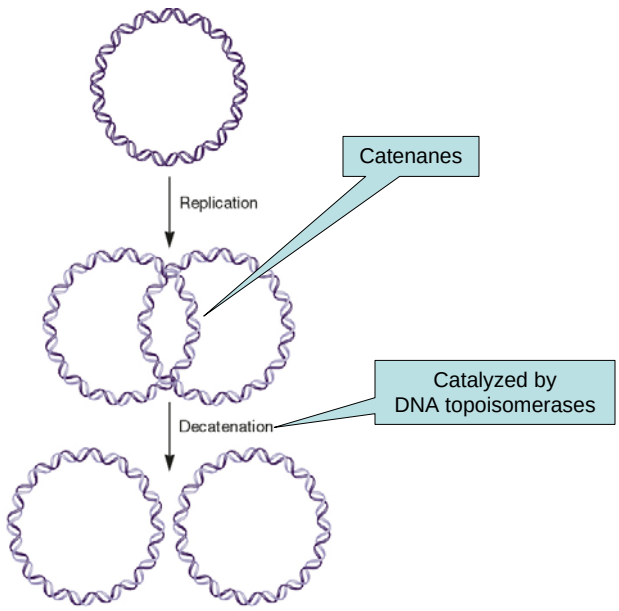


Figure 11.12



Proofreading Mechanisms

- DNA replication exhibits a high degree of fidelity
  - Mistakes during the process are extremely rare
    - DNA pol III makes only one mistake per 10^8 bases made
- There are several reasons why fidelity is high
  - 1. Instability of mismatched pairs
  - 2. Configuration of the DNA polymerase active site
  - 3. Proofreading function of DNA polymerase

Proofreading Mechanisms

- 1. Instability of mismatched pairs
  - Complementary base pairs have much higher stability than mismatched pairs
  - This feature only accounts for part of the fidelity
    - It has an error rate of 1 per 1,000 nucleotides
- 2. Configuration of the DNA polymerase active site
  - DNA polymerase is unlikely to catalyze bond formation between mismatched pairs
  - This induced-fit phenomenon decreases the error rate to a range of 1 in 100,000 to 1 million

Proofreading Mechanisms

- 3. Proofreading function of DNA polymerase
  - DNA polymerases can identify a mismatched nucleotide and remove it from the daughter strand
  - The enzyme uses its 3' to 5' exonuclease activity to remove the incorrect nucleotide
  - It then changes direction and resumes DNA synthesis in the 5' to 3' direction

Bacterial DNA Replication is Coordinated with Cell Division

- Bacterial cells can divide into two daughter cells at an amazing rate
  - *E. coli* → 20 to 30 minutes
  - Therefore it is critical that DNA replication take place only when a cell is about to divide
- Bacterial cells regulate the DNA replication process by controlling the initiation of replication at *oriC*

Eukaryotic DNA Replication

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EUKARYOTIC DNA REPLICATION

- Eukaryotic DNA replication is not as well understood as bacterial replication
  - The two processes do have extensive similarities,
    - The bacterial enzymes discussed have also been found in eukaryotes
  - Nevertheless, DNA replication in eukaryotes is more complex
    - Large linear chromosomes
    - Tight packaging within nucleosomes
    - More complicated cell cycle regulation

Multiple Origins of Replication

- Eukaryotes have long linear chromosomes
  - They therefore require multiple origins of replication
    - To ensure that the DNA can be replicated in a reasonable time
- DNA replication proceeds bidirectionally from many origins of replication

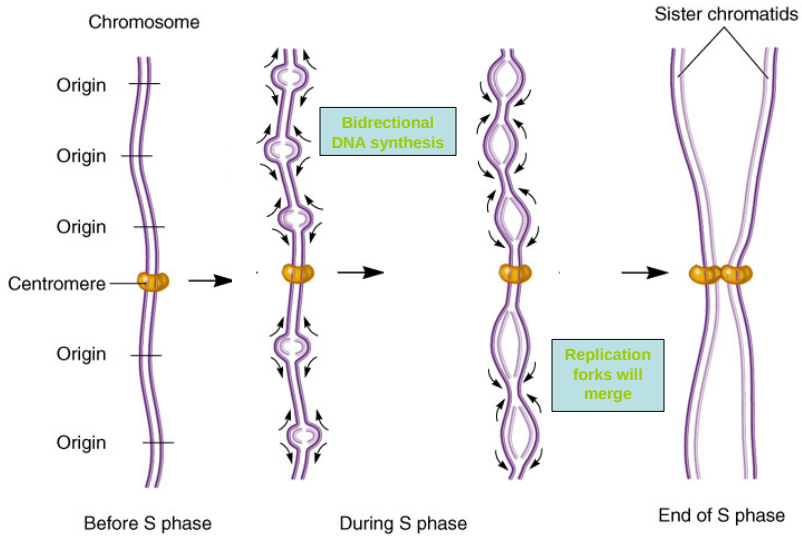


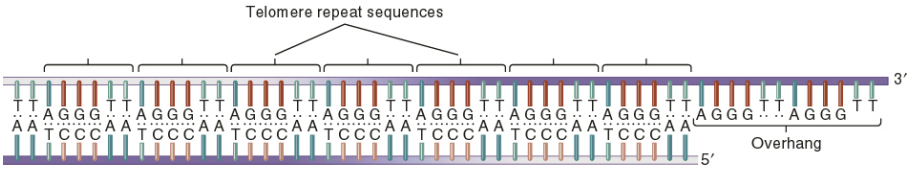
Figure 11.20 **(a)** DNA replication from multiple origins of replication

Part (b) shows a micrograph of a replicating DNA chromosome

Telomeres and DNA Replication

- Linear eukaryotic chromosomes have telomeres at both ends
- The term telomere refers to the complex of telomeric DNA sequences and bound proteins
- Telomeric sequences consist of
  - Moderately repetitive tandem arrays
  - 3' overhang that is 12-16 nucleotides long

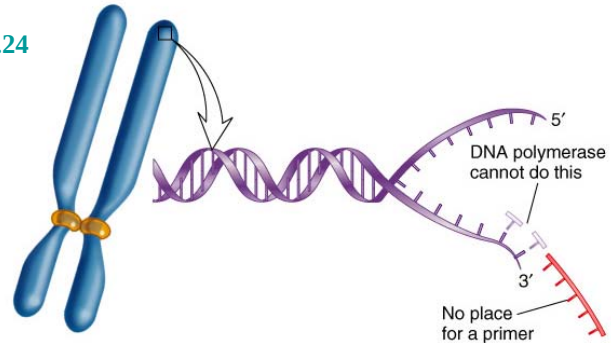
Figure 11.23



- Telomeric sequences typically consist of
  - Several guanine nucleotides
  - Often many thymine nucleotides
  - Differ between species

- DNA polymerases possess two unusual features
  - 1. They synthesize DNA only in the 5’ to 3’ direction
  - 2. They cannot initiate DNA synthesis
- These two features pose a problem at the 3’ end of linear chromosomes

Figure 11.24



- The linear chromosome becomes progressively shorter with each round of DNA replication if not solved
- Solution= adding DNA sequences to the ends of telomeres
- Requires a specialized mechanism catalyzed by the enzyme telomerase (e.g. stem cells, cancer)
- Telomerase contains protein and RNA
  - The RNA is complementary to the DNA sequence found in the telomeric repeat (binds to the 3' overhang)

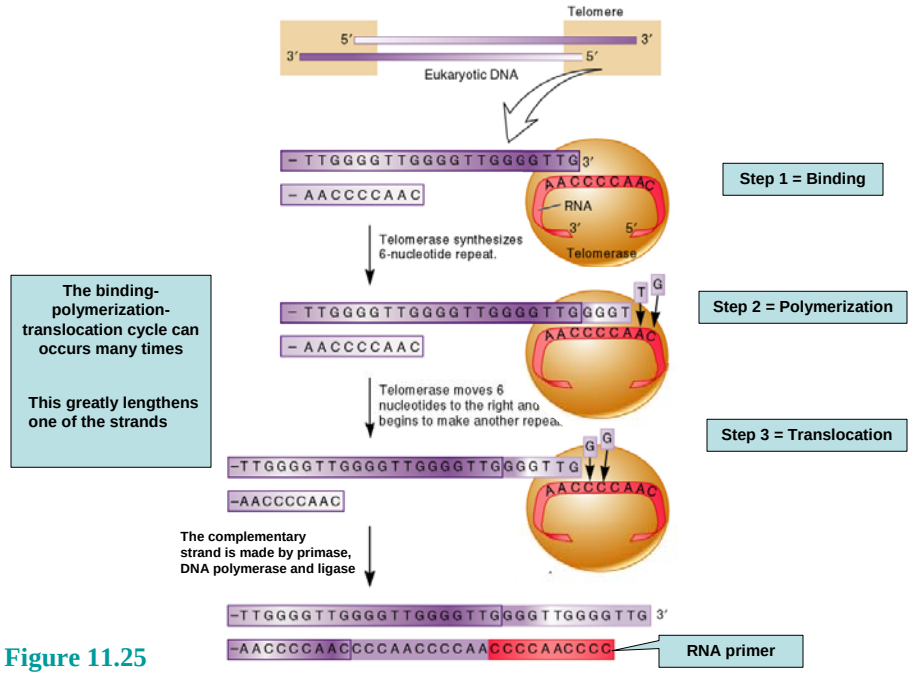


Figure 11.25