

Leading & Lagging strands



Theta model

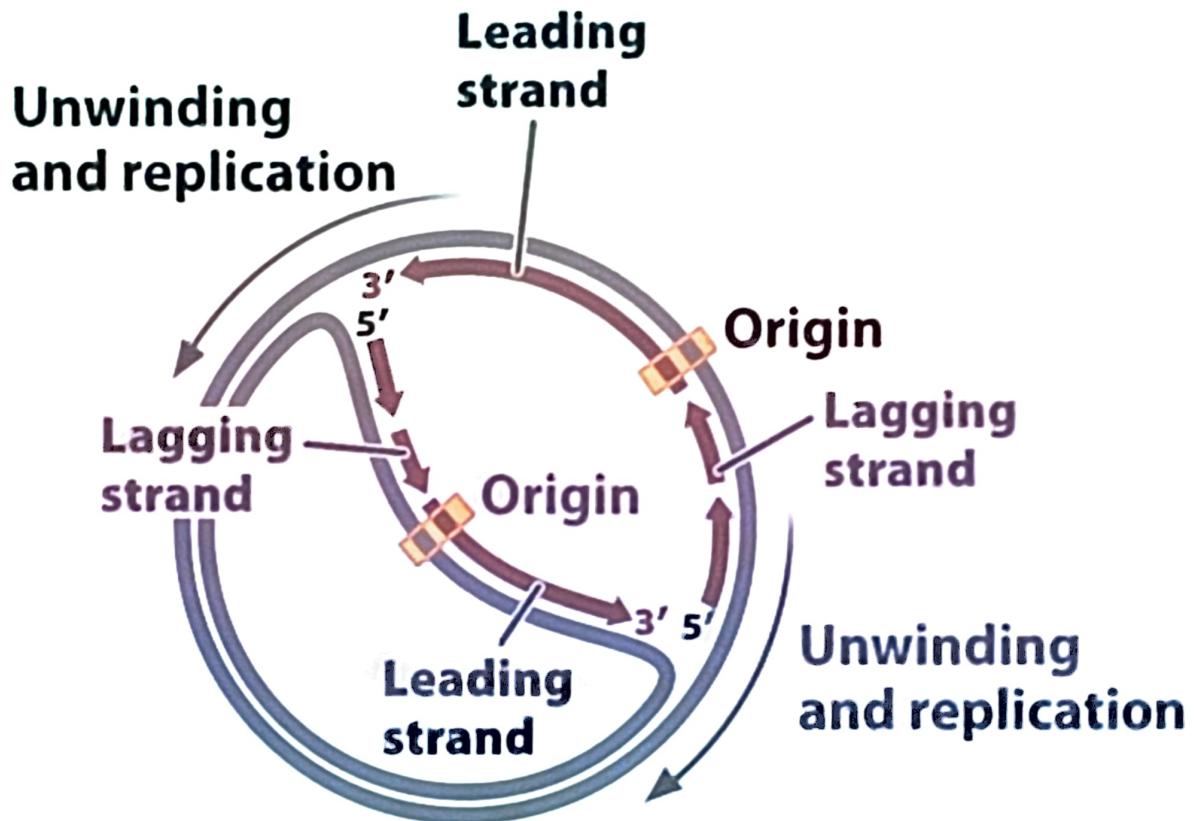
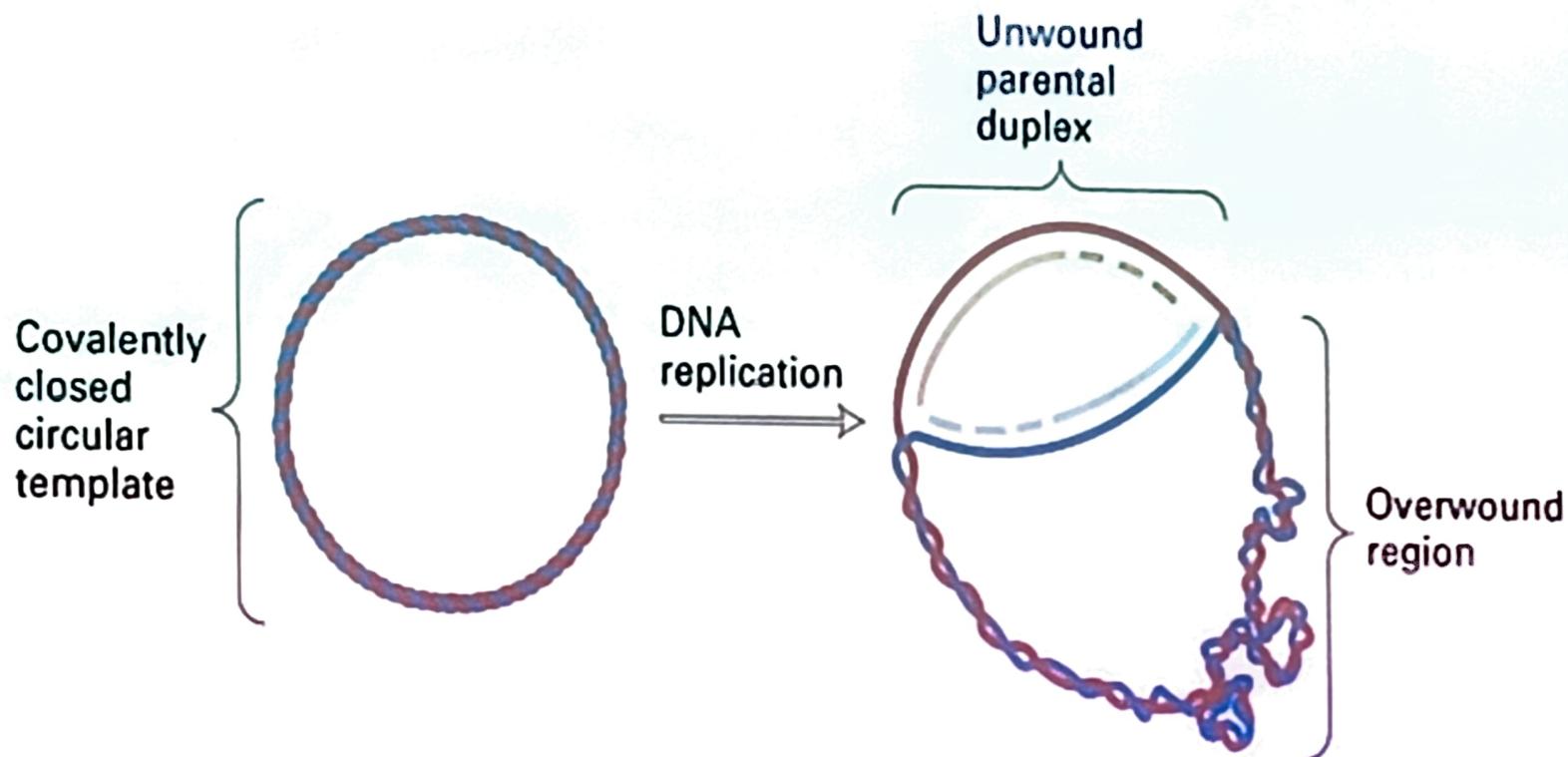


Figure 12-10a

Genetics: A Conceptual Approach, Third Edition
© 2009 W.H. Freeman and Company

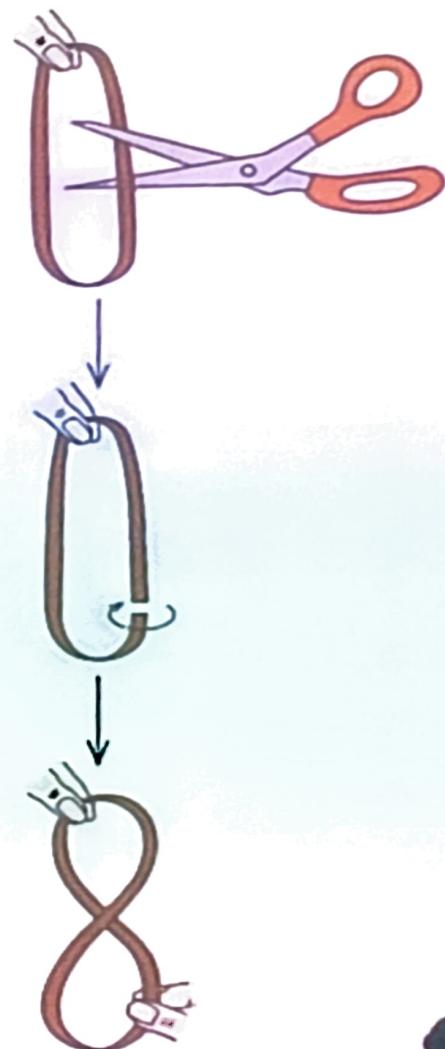
DNA Supercoiling



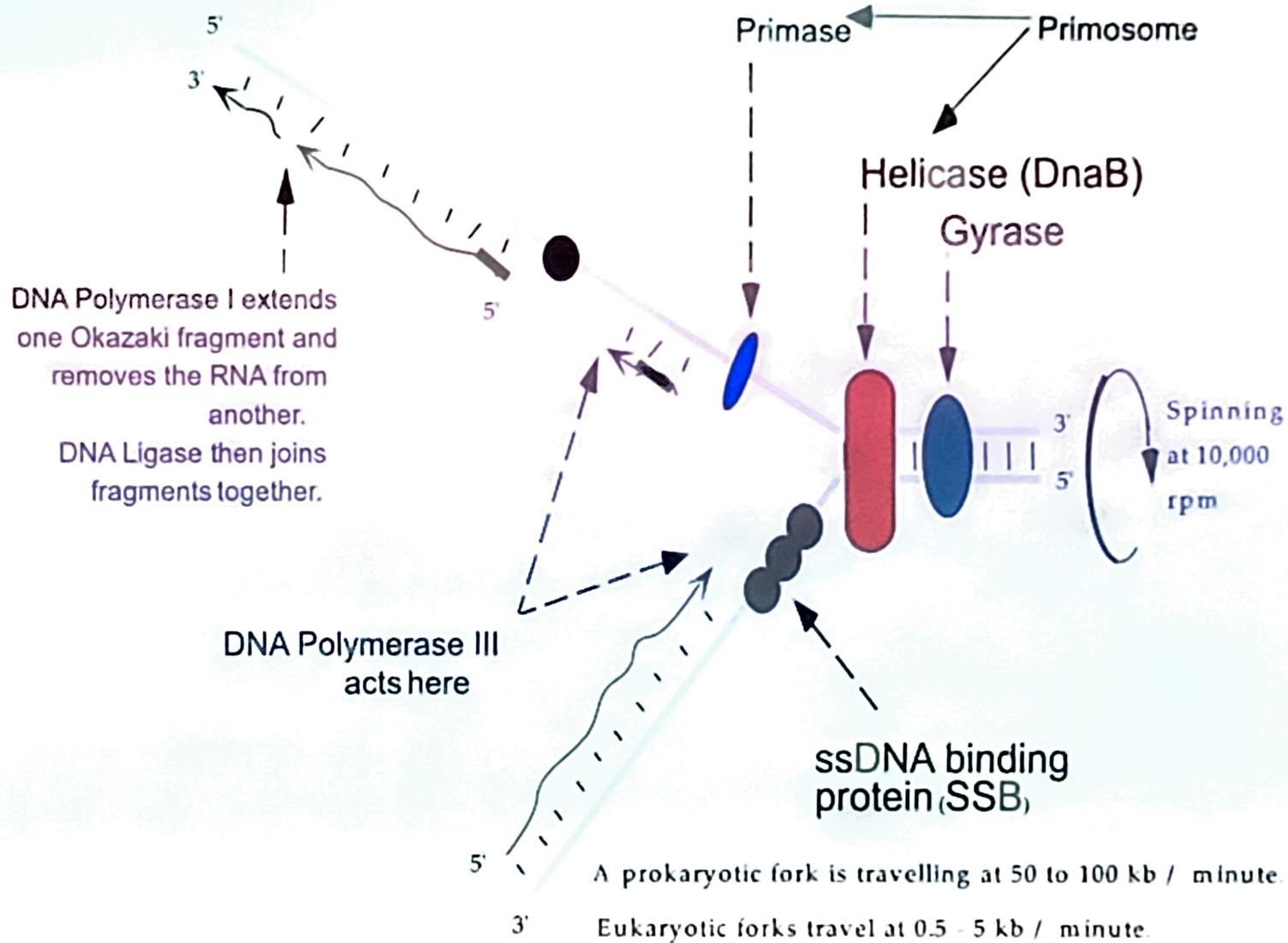
Rubber Band Model of Supercoiling DNA

DNA Gyrase relaxes positive supercoils by breaking and rejoining both DNA strands.

Figure
20.23



A Replisome



Bacterial DNA Replication

- **Initiation:** 245 bp in the *oriC* (single origin replicon); an initiation protein
 - Unwinding of DNA is performed by Helicase. Gyrase removes supercoiling ahead of the replication fork. Single stranded DNA is prevented from annealing by single stranded binding proteins.
 - **Primers:** an existing group of RNA nucleotides with a 3'-OH group to which a new nucleotide can be added; usually 10 ~ 12 nucleotides long

Primase: RNA polymerase

Linear eukaryotic replication

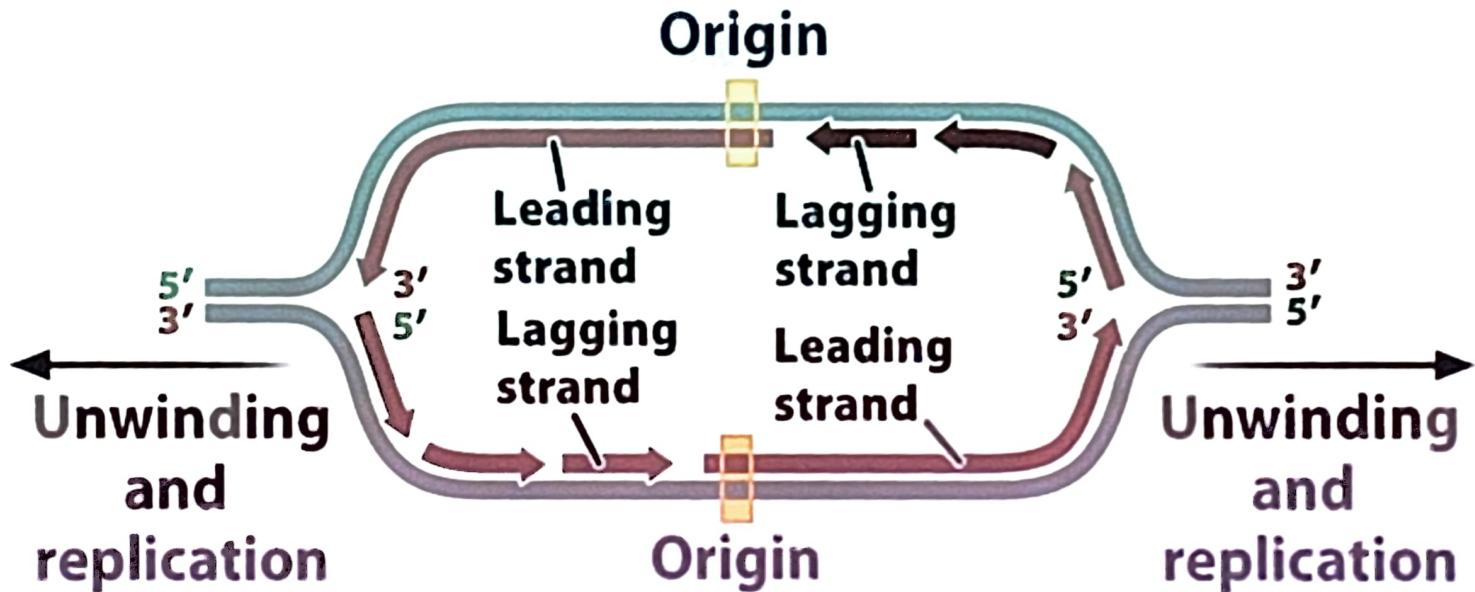


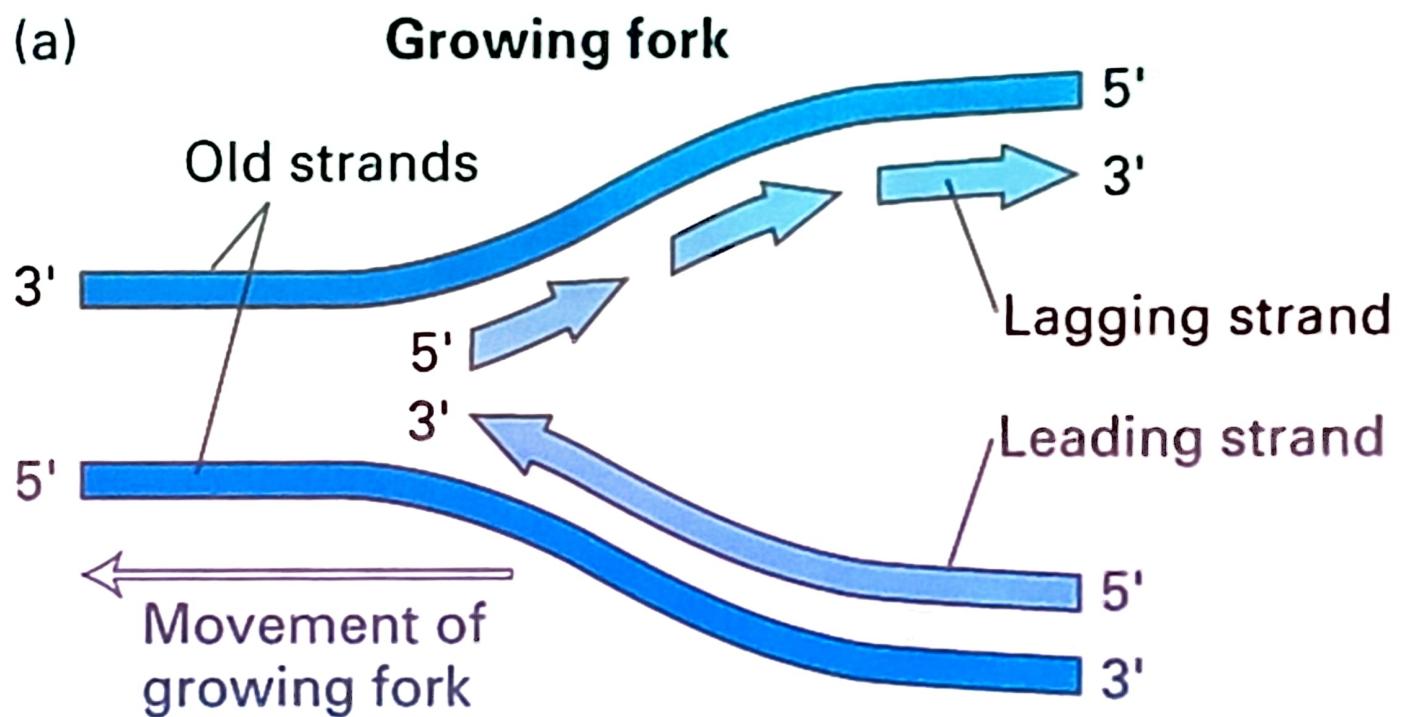
Figure 12-10c

Genetics: A Conceptual Approach, Third Edition

© 2009 W.H. Freeman and Company

If DNA polymerases only synthesize 5' to 3', how does the replication fork move directionally?

(a)



- Lagging strand synthesized as small (~100-1000 bp) fragments - “Okazaki fragments” .
- Okazaki fragments begin as very short 6-15 nt RNA primers synthesized by primase.

2. **Primase** - RNA polymerase that synthesizes the RNA primers (11-12 nt that start with pppAG) for both lagging and leading strand synthesis

Bacterial DNA Replication

- The fidelity of DNA replication
- Proofreading: DNA polymerase I: $3' \rightarrow 5'$ exonuclease activity removes the incorrectly paired nucleotide.
- Mismatch repair: correcting errors after replication is complete

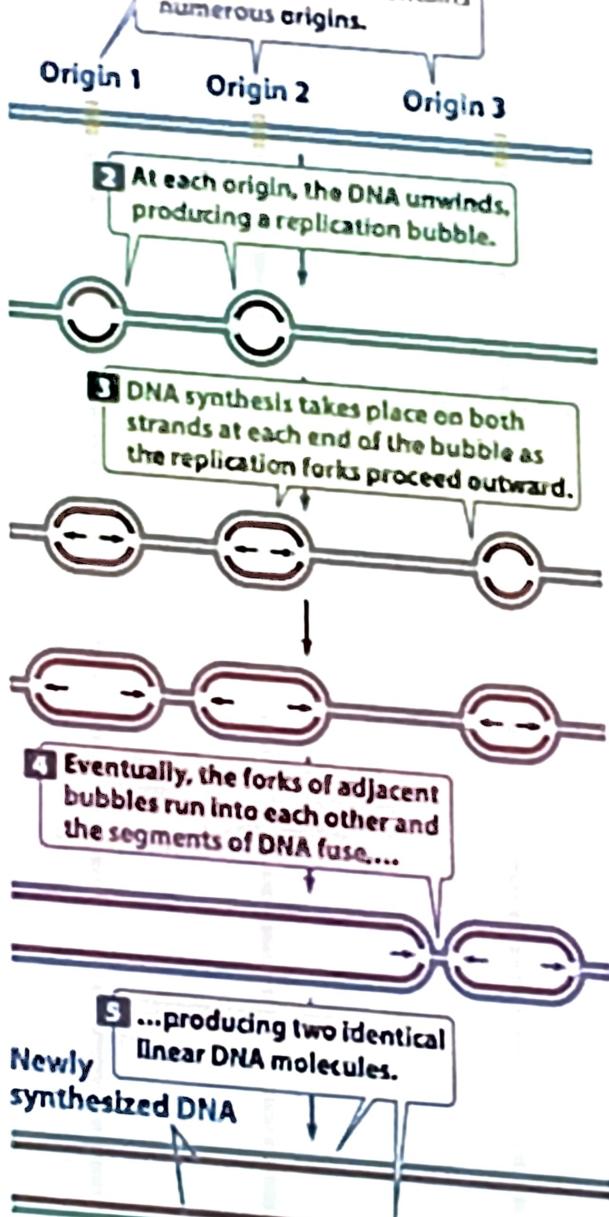
Proofreading Activity

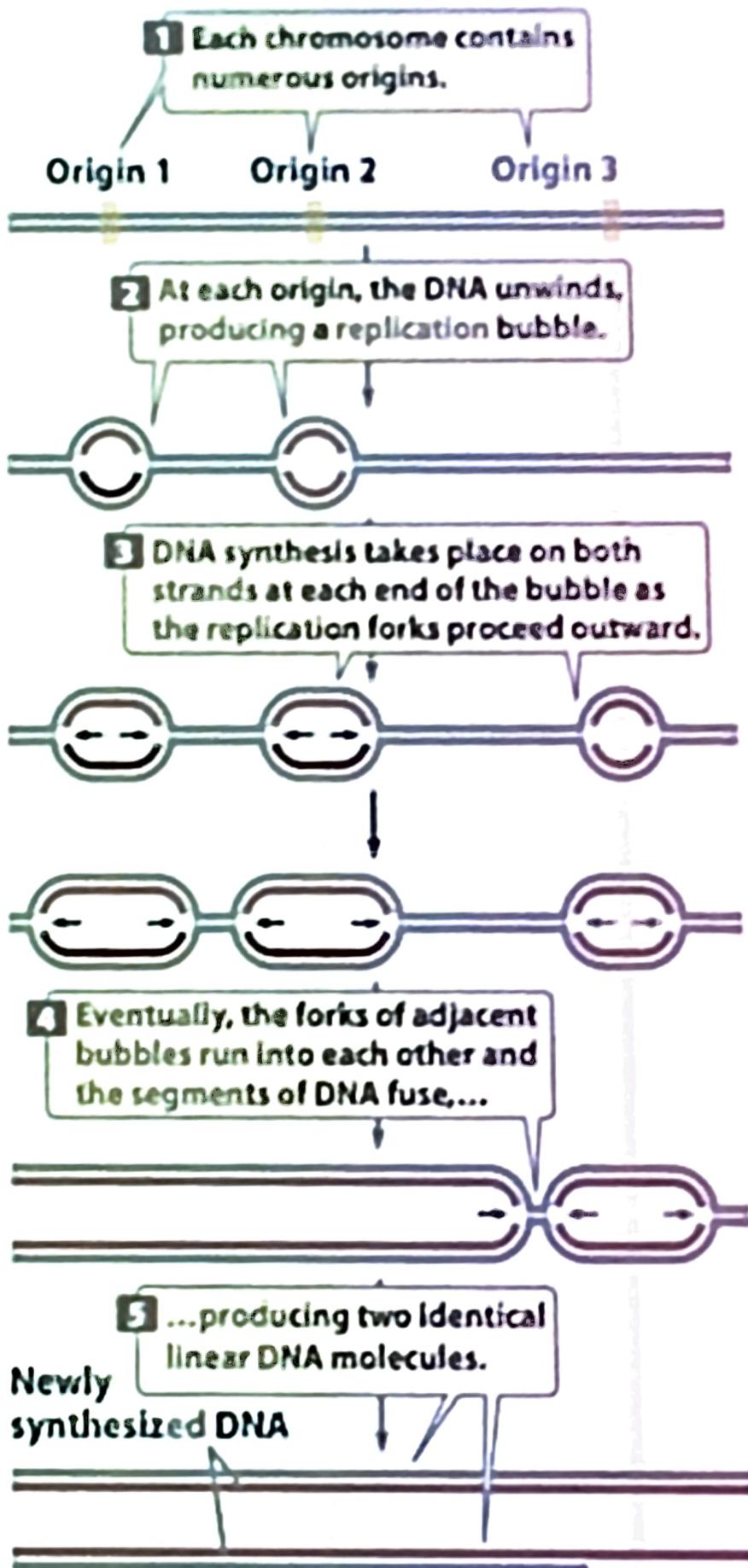


Insertion of the wrong nucleotide causes the DNA polymerase to stall, and then the 3'-to-5' exonuclease activity removes the mispaired A nt. The polymerase then continues adding nts to the primer.

The **3'-->5' exonuclease activity** intrinsic to several DNA polymerases plays a primary role in genetic stability; it acts as a first line of defense in correcting DNA polymerase errors. A mismatched basepair at the primer terminus is the preferred substrate for the exonuclease activity over a correct basepair.

Linear Eukaryotic Replication





Conclusion: The products of replication are two linear DNA

Figure 12-6
Genetics: A Conceptual Approach, Third Edition
© 2009 W.H. Freeman and Company

Linear Eukaryotic Replication

- Eukaryotic cells; thousands of origins; a typical replicon: 200,000 ~ 300,000 bp in length.
- Eukaryotic DNA polymerase
 - DNA polymerase α - acts like Primase to initiate
 - DNA polymerase δ - replicates lagging strand
 - DNA polymerase ϵ - replicates leading strand

Linear Eukaryotic Replication

- Replication is bi-directional and originates at multiple origins of replication (Ori C) in eukaryotes.
- DNA replication uses a semi-conservative method that results in a double-stranded DNA with one parental strand and a new daughter strand.
- It occurs only in the S phase and at many chromosomal origins.
- Takes place in the cell nucleus.

Linear Eukaryotic Replication

- Synthesis occurs only in the 5' to 3' direction.
- Individual strands of DNA are manufactured in different directions, producing a leading and a lagging strand.
- Lagging strands are created by the production of small DNA fragments called Okazaki fragments that are eventually joined together.
- Eukaryotic cells possess five types of polymerases involved in the replication process.

Enzymes for Eukaryotic DNA replication

- **DNA Polymerase**
- **DNA polymerase α** is a repair polymerase, with 3' to 5' exonucleases activities and 5' to 3' polymerase activities.
- **DNA Polymerase β** is a repair polymerase.
- **DNA Polymerase γ** shows polymerase activity 5' to 3' and exonucleases activity 3' to 5', it is involved in Mitochondrial DNA replication
- **DNA Polymerase δ** shows 3' to 5' exonuclease activity and 5' to 3' polymerase activity. This enzyme is involved in lagging strand synthesis.
- **DNA Polymerase ϵ** shows 3' to 5' and 5' to 3' exonucleases activities. This enzyme not only repairs but also synthesizes the leading strand efficiently in a 5' to 3' direction. It is the prime enzyme involved in DNA replication.

Enzymes for Eukaryotic DNA replication

- **Helicases:** Unwind the DNA helix at the start of replication.
- **SSB proteins:** Bind to the single strands of unwound DNA to prevent reformation of the DNA helix during replication.
- **DNA topoisomerase I:** Relaxes the DNA helix during replication through creation of a nick in one of the DNA strands.
- **DNA topoisomerase II:** Relieves the strain on the DNA helix during replication by forming supercoils in the helix through the creation of nicks in both strands of DNA.
- **DNA ligase:** Forms a 3'-5' phosphodiester bond between adjacent fragments of DNA.

Enzymes for Eukaryotic DNA replication

- **Telomerase**, a DNA polymerase that contains an integral RNA that acts as its own primer, is used to replicate DNA at the ends of chromosomes (telomeres).
- DNA Proofreading
- In eukaryotes only the polymerases that deal with the elongation (delta and epsilon) have proofreading ability ($3' \rightarrow 5'$ exonuclease activity).
- If an error is detected, the erroneous base is removed via $3'$ to $5'$ exonuclease activity replaced with the correct base.

Eukaryotic vs Prokaryotic DNA replication

S.N.	Eukaryotic DNA Replication	Prokaryotic DNA replication
1.	Occurs in eukaryotic cells.	Occurs in a prokaryotic cell.
2.	This process takes place in the cell's nucleus.	This process takes place in the cell's cytoplasm.
3.	There are multiple sites for the origin of replication per DNA molecule.	There is a single site for the origin of replication per DNA molecule.
4.	Initiation of DNA replication is carried out by multi-subunit proteins, origin recognition complex.	Initiation of DNA replication is carried out by protein DnaA and DnaB.
5.	Multiple replication forks are formed in a DNA molecule.	Only two replication forks are formed in a DNA molecule.

Eukaryotic vs Prokaryotic DNA replication

- | | | |
|-----|---|--|
| 6. | Okazaki fragments are short of around 100-200 nucleotides in length | Okazaki fragments are large, around 1000-2000 nucleotides in length. |
| 7. | It is a slow process with around 100 nucleotides added per second. | It is a fast process with around 2000 nucleotides added per second. |
| 8. | DNA is linear and double-stranded. | DNA is circular and double-stranded. |
| 9. | DNA polymerase involved in eukaryotic DNA replication is DNA polymerases ϵ , α , and δ . | DNA polymerase involved in prokaryotic DNA replication is DNA polymerase I, and III. |
| 10. | Eukaryotic cells have telomeres at the end of DNA thus they are replicated. | Prokaryotic cells have circular DNA thus they are not replicated. |
| 11. | DNA gyrase is needed. | DNA gyrase is not needed. |

What about the ends (or telomeres) of linear chromosomes?



DNA polymerase/ligase cannot fill gap at end of chromosome after RNA primer is removed. this gap is not filled, chromosomes would become shorter each round of replication!

Solution:

1. Eukaryotes have tandemly repeated sequences at the ends of their chromosomes.
2. Telomerase (composed of protein and RNA complementary to the telomere repeat) binds to the terminal telomere repeat and catalyzes the addition of new repeats.
3. Compensates by lengthening the chromosome.
4. Absence or mutation of telomerase activity results in chromosome shortening and limited cell division.

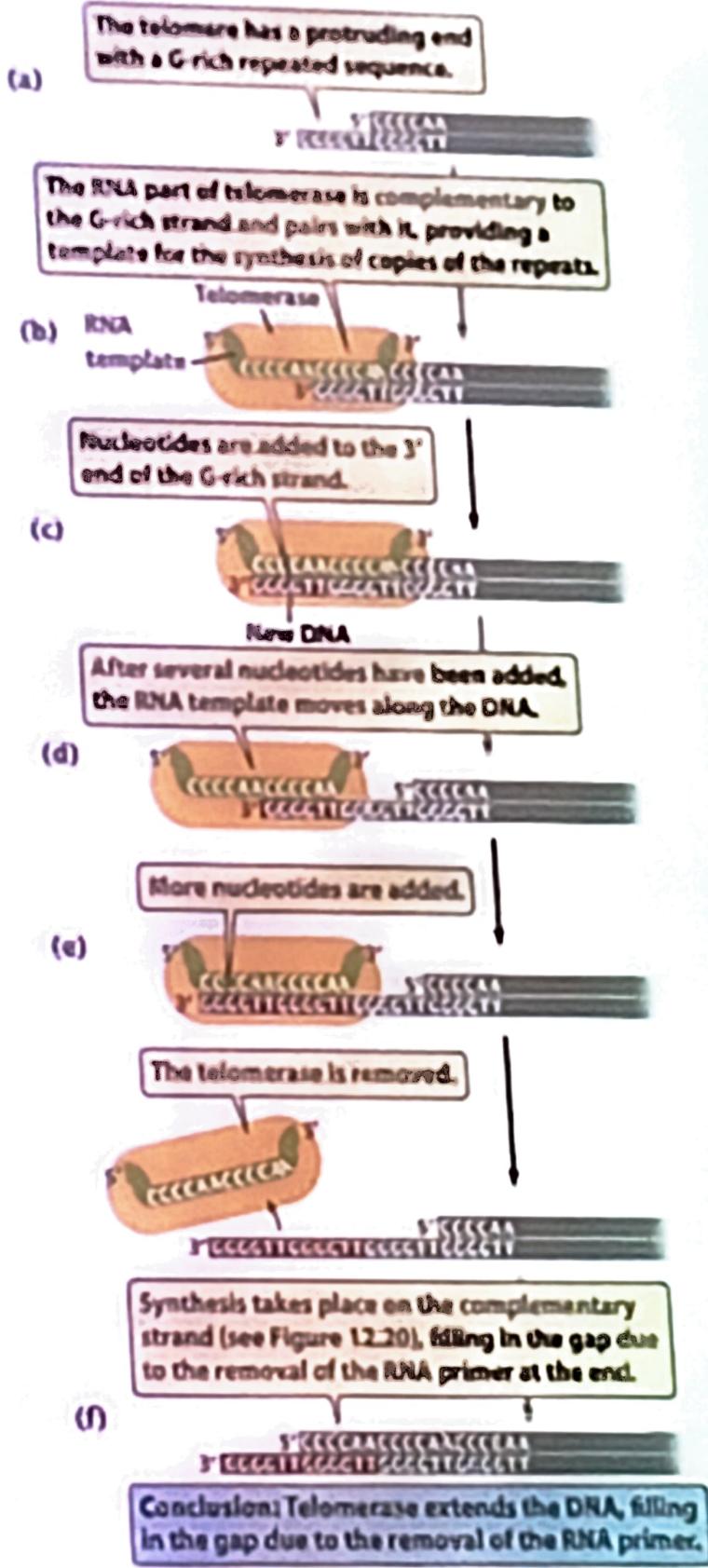
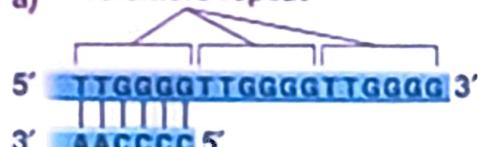


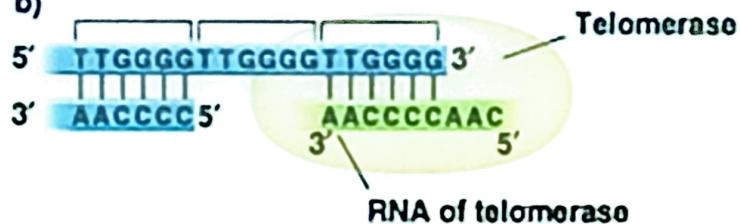
Figure 12-19
Genetics: A Conceptual Approach, Third Edition
© 2009 W.H. Freeman and Company

Fig. 3.16 Synthesis of telomeric DNA by telomerase

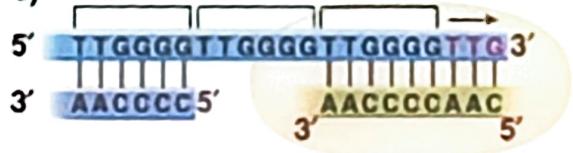
a) Telomere repeat



b)



c)



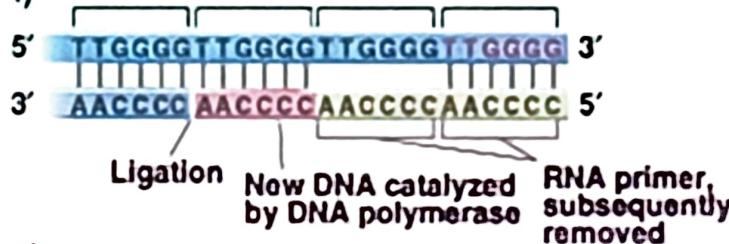
d)



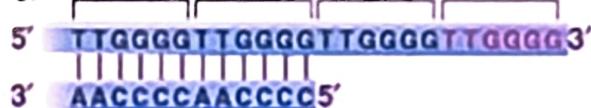
e)



f)



g)



Final Step - Assembly into Nucleosomes:

- As DNA unwinds, nucleosomes must disassemble.
- Histones and the associated chromatin proteins must be duplicated by new protein synthesis.
- Newly replicated DNA is assembled into nucleosomes almost immediately.
- Histone chaperone proteins control the assembly.

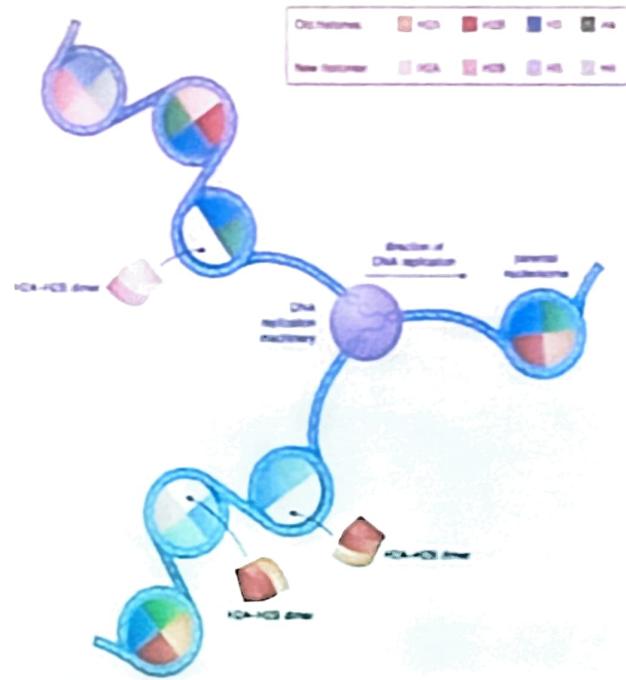


Fig. 3.17