# DNA REPLICATION

**Professor Sameeh Al-Sarayreh**
**Professor of Medical Biochemistry**
**Department of Biochemistry and Molecular Biology**
**Faculty of Medicine, Mutah University**

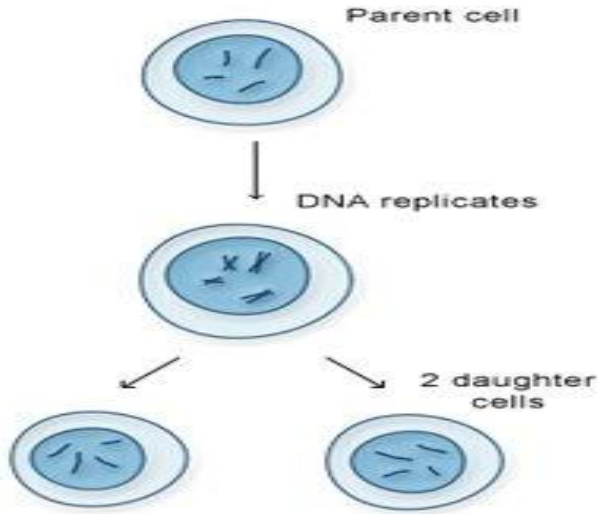
# DNA replication

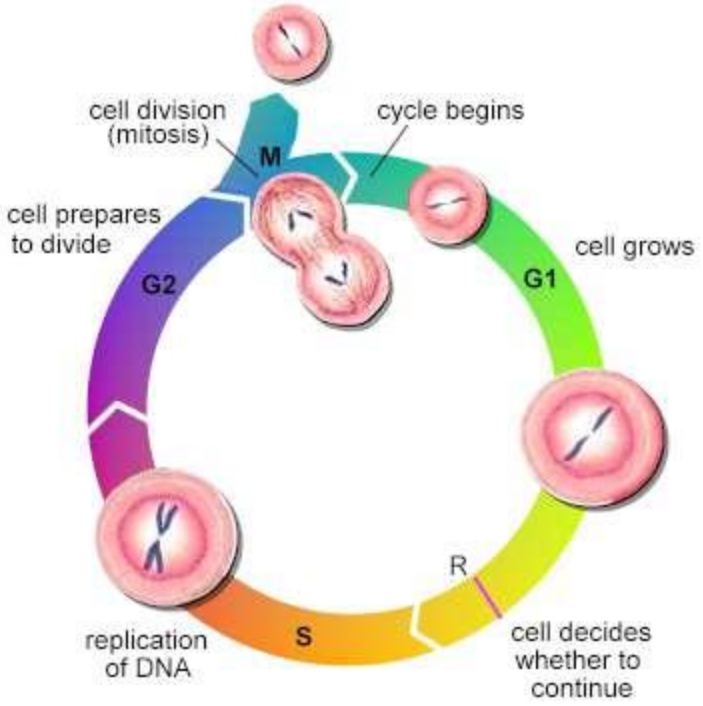
DNA replication is a biological process that occurs in all living organisms and copies their DNA; it is the basis for biological inheritance.

The double helix is unwound and each strand acts as a template for the next strand. Bases are matched to synthesize the new partner strands.

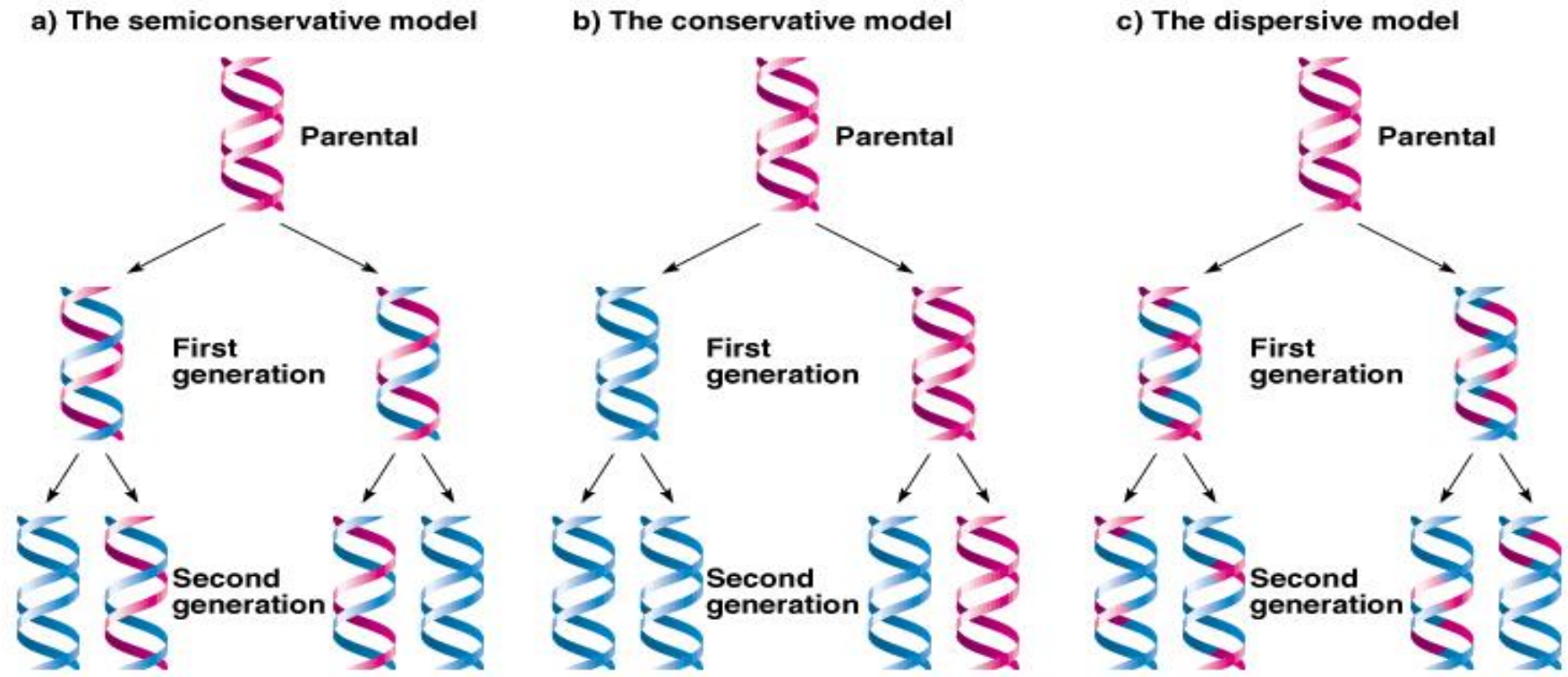
DNA replication occurring at S Phase (DNA synthesis phase)

The process starts when one double-stranded DNA molecule produces two identical copies of the molecule.



# The Three Possible DNA Replication Models




**Conservative- would leave the original strand intact and copy it.**

**Dispersive-would produce two DNA molecule with sections of both old and new along each strand.**

**Semiconservative –would produce DNA molecule with both one old strand and one new strand.**

# Semiconservative model:

**The idea was presented by Watson & Crick**

**The two strands of the parental molecule separate, and each acts as a template for a new complementary strand**

**New DNA consists of 1 PARENTAL (original) and 1 NEW strand of DNA**

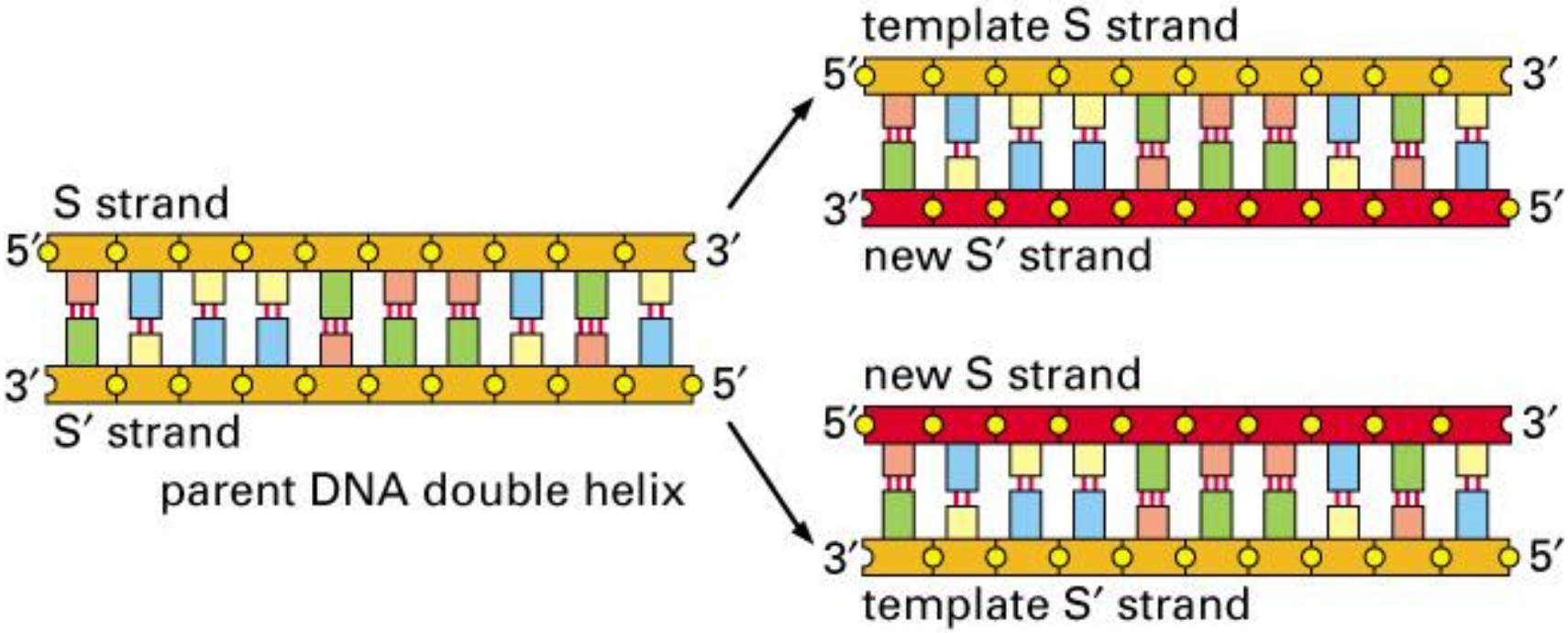



Figure 5–2. Molecular Biology of the Cell, 4th Edition.

# BACTERIAL REPLICATION

**DNA synthesis begins at a site termed the origin of replication**

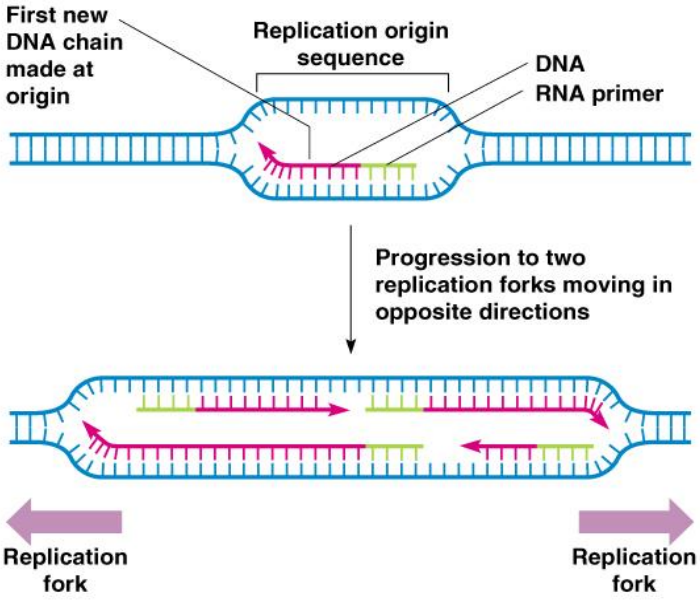
**Synthesis of DNA proceeds bidirectionally around the bacterial chromosome eventually meeting at the opposite side of the bacterial chromosome where replication ends**

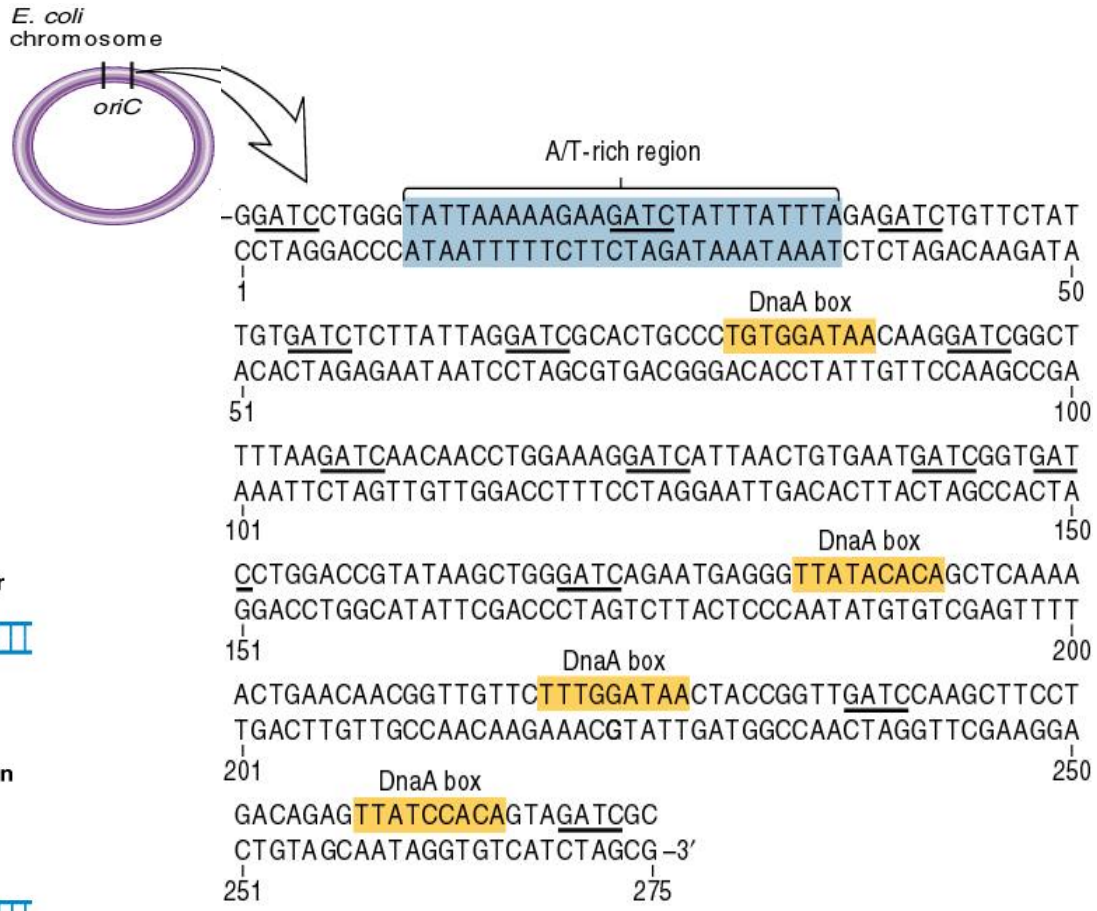
**The origin of replication in *E. coli* is termed *oriC***

**origin of Chromosomal replication**

**Important DNA sequences in *oriC* :**

- **AT-rich region**
- **DnaA boxes**



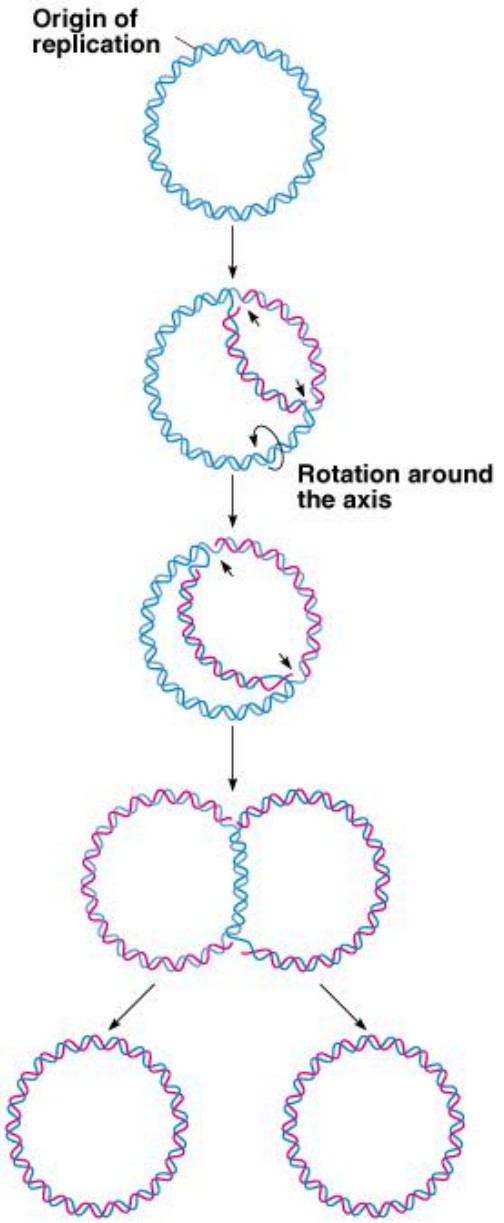
# Replication of circular DNA in *E. coli* :

- Replication begins with double-helix denaturing into single-strands thus exposing the bases.

- Exposes a replication bubble from which replication proceeds in both directions.

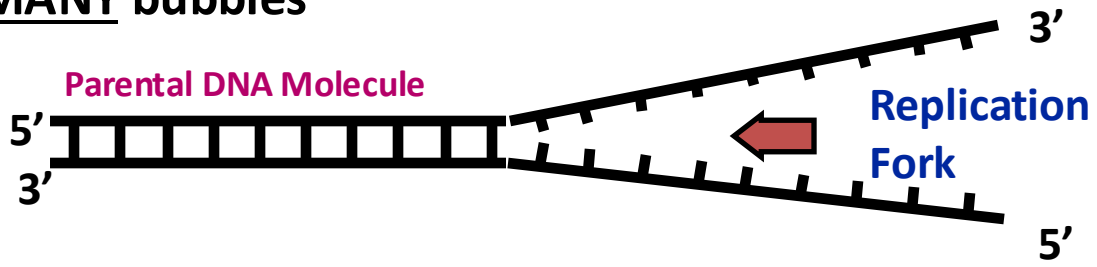
- Two replication forks result in a theta like (θ) structure.

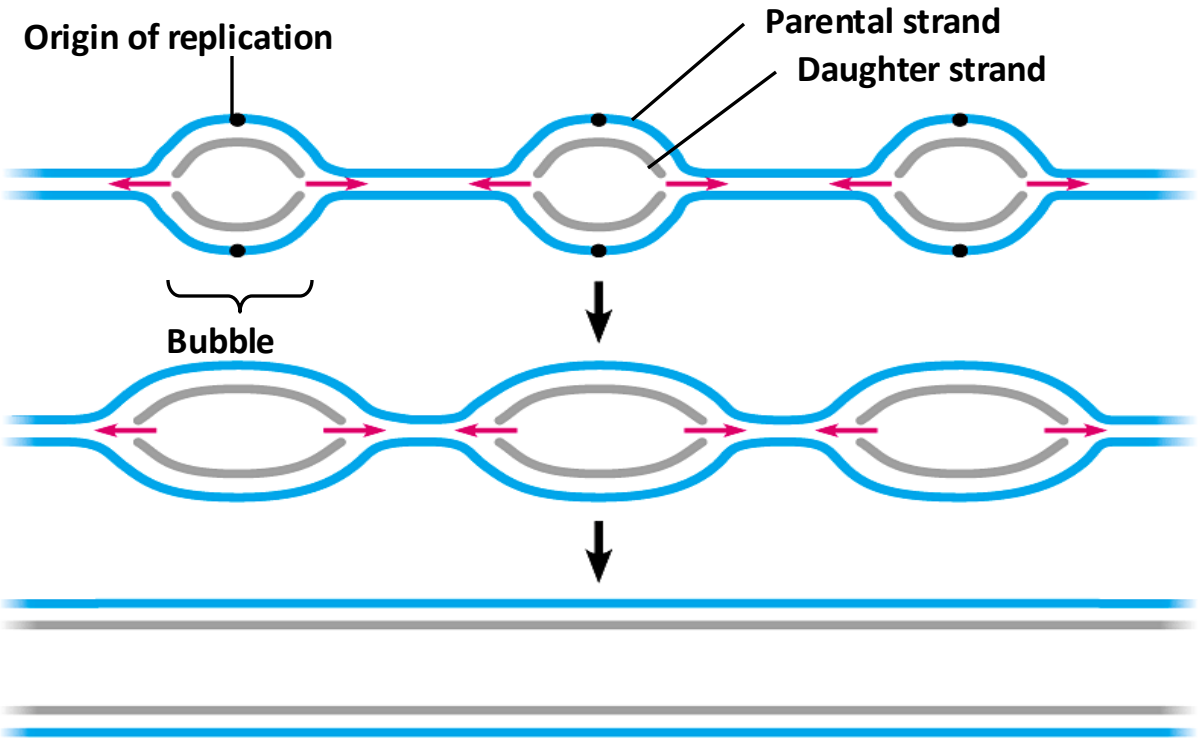
- Topoisomerases relieve tensions in the supercoils, allowing the DNA to continue to separate.



# How can entire chromosomes be replicated during S phase?

- **DNA replication begins at many specific sites**
  - **As the two DNA strands open at the origin, Replication Bubbles form**
  - **Prokaryotes (bacteria) have a single bubble**
  - **Eukaryotic chromosomes have <u>MANY</u> bubbles**



# Topoisomerases

**They relieve stress on the DNA molecule by allowing free rotation around a single strand.**

**Topoisomerases bind to either single-stranded or double-stranded DNA and cut the phosphate backbone of the DNA.**

**DNA topoisomerase I : cut one strand of double-stranded DNA, relax the strand, and reanneal the strands**

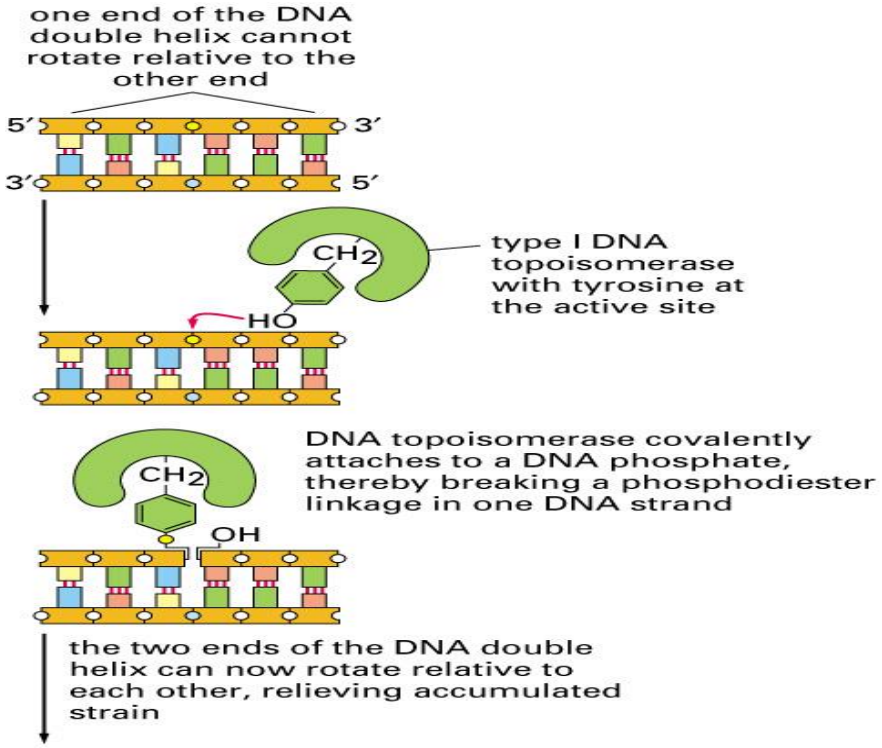



Figure 5–25 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

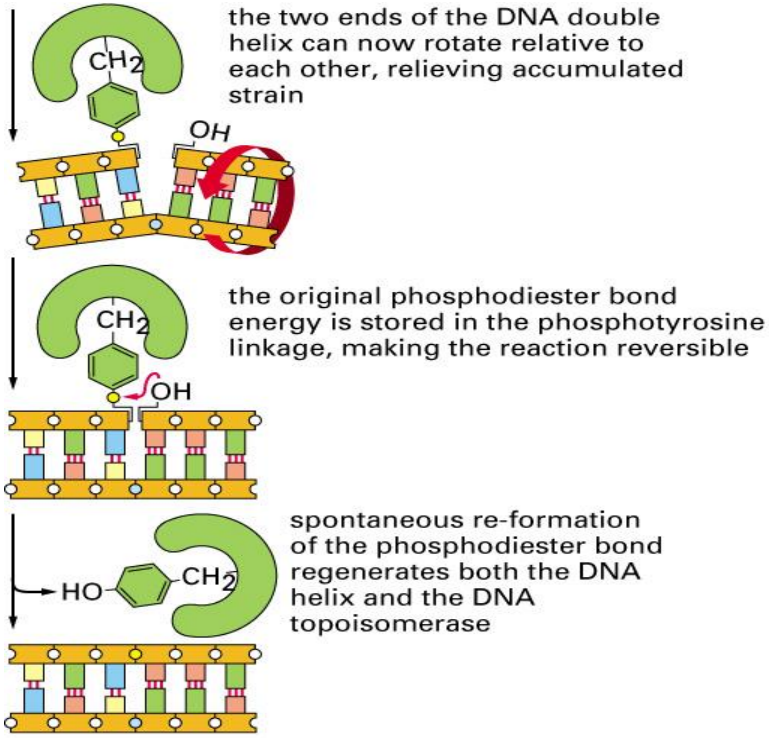



Figure 5–25 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

# DNA topoisomerase II

**cut both strands of the DNA helix simultaneously in order to manage DNA tangles and supercoils. They use the hydrolysis of ATP, unlike type I topoisomerase**

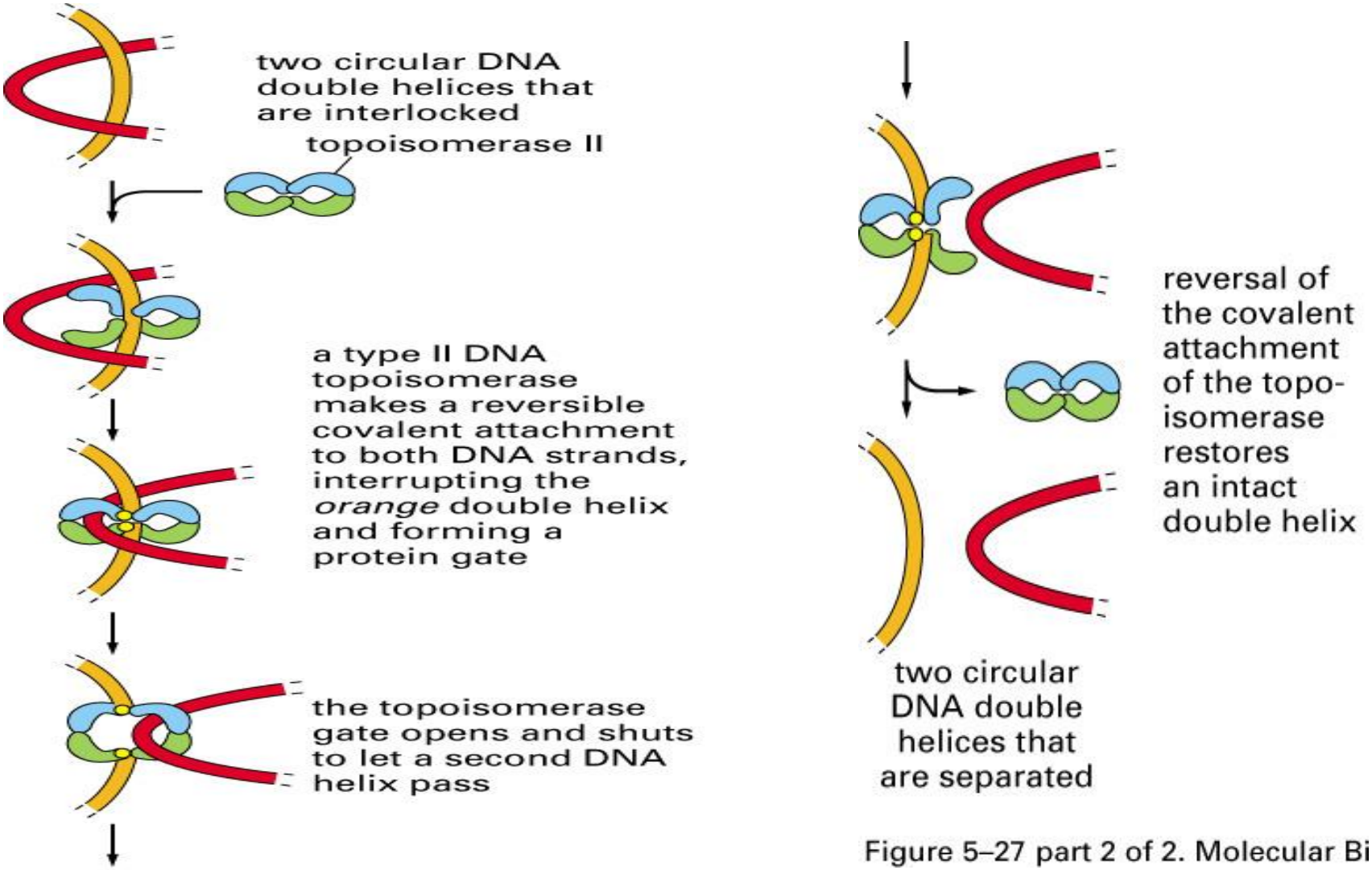



Figure 5–27 part 2 of 2. Molecular Bi

Figure 5–27 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

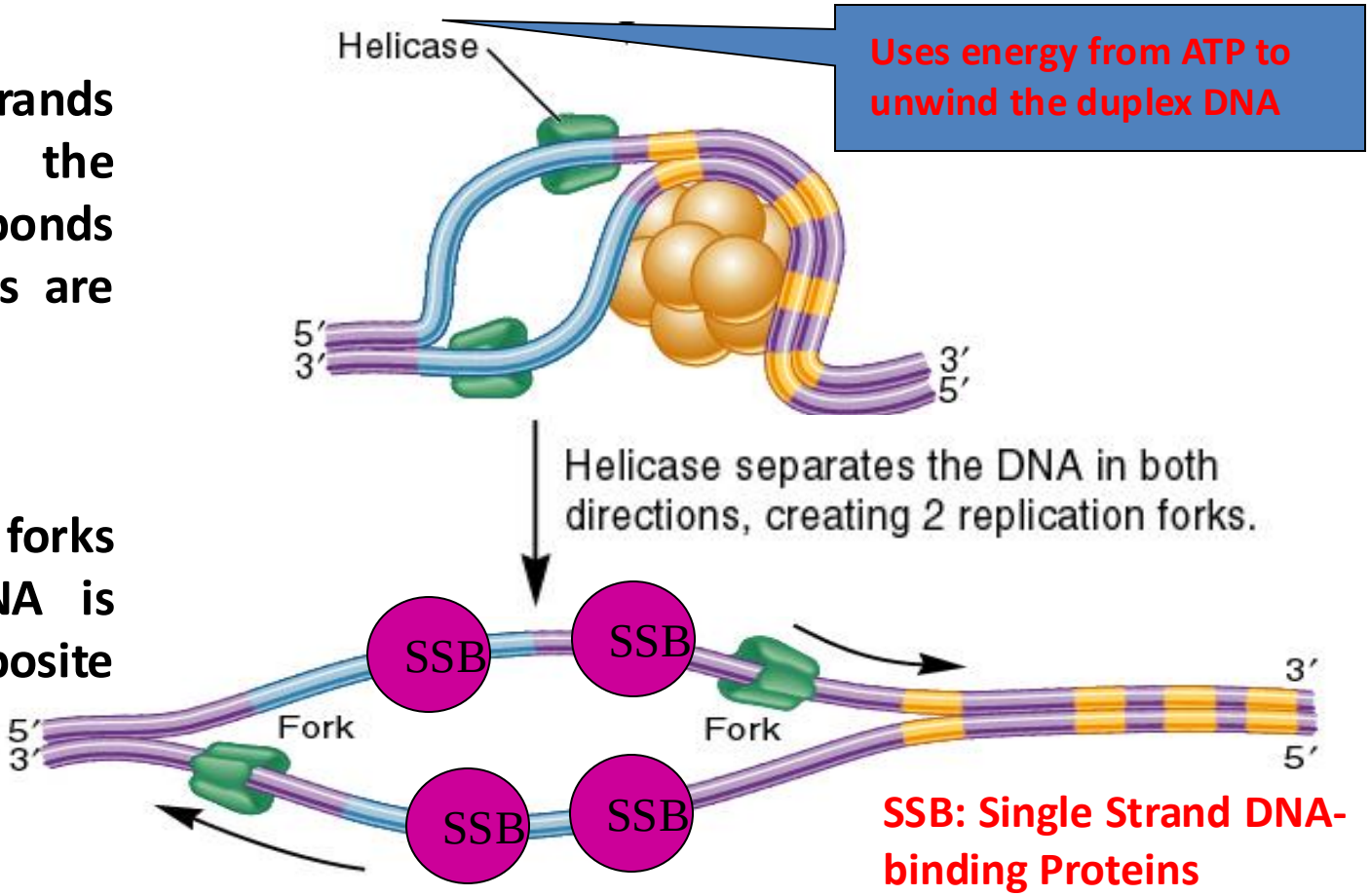
# DNA Helicase

**motor proteins that move directionally along a nucleic acid phosphodiester backbone, separating two annealed nucleic acid strands using energy derived from ATP hydrolysis**

**Helicase unwinds the double helix starting at a replication bubble.**

**The two strands separate as the hydrogen bonds between base pairs are broken.**

**Two replication forks form and the DNA is unwound in opposite directions.**



# SSB Proteins

**SSB: Single Stranded DNA-binding Proteins, bind to single-stranded regions of DNA to prevent premature annealing, and to protect the single-stranded DNA from being digested by nucleases.**

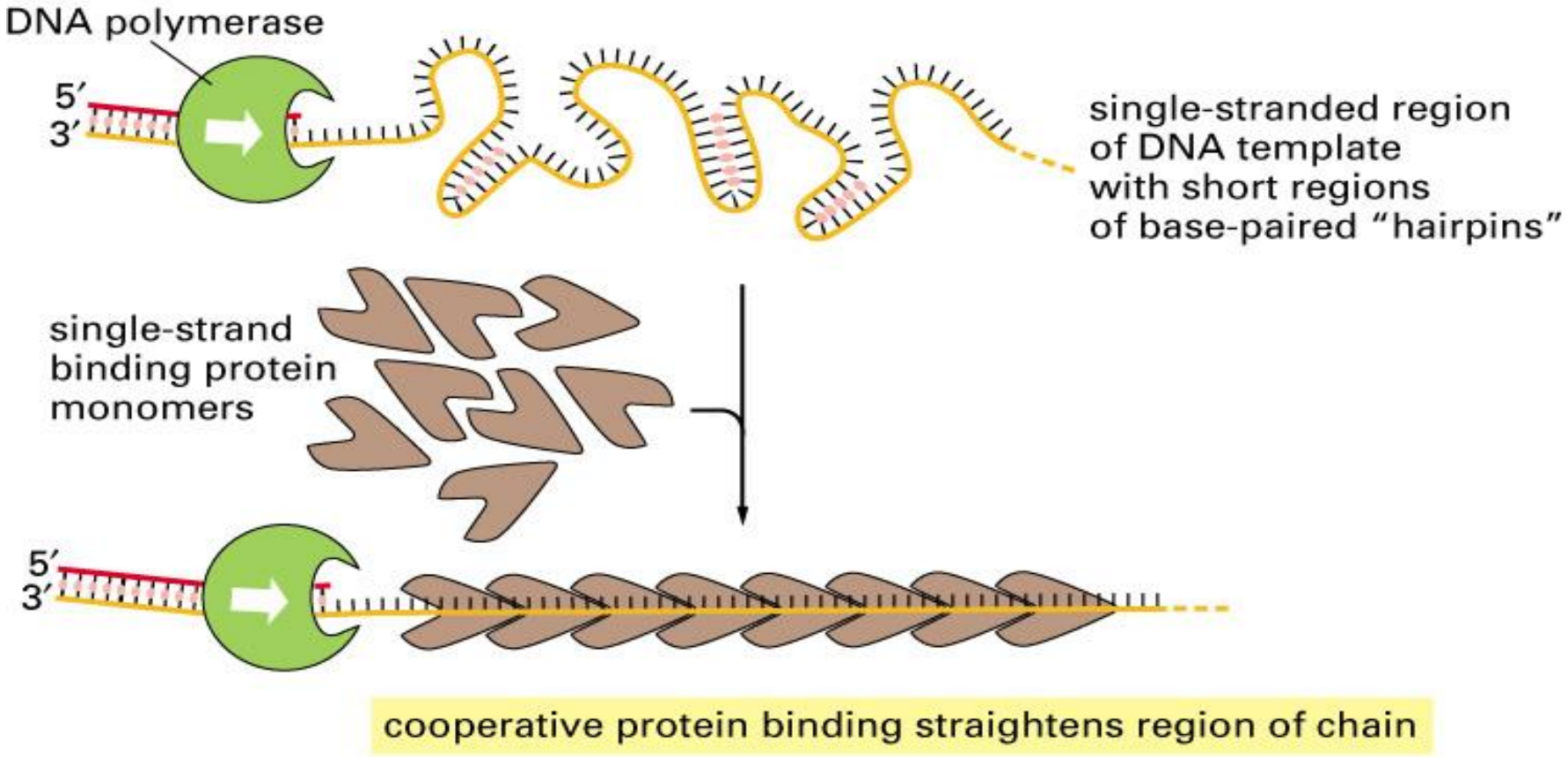



Figure 5–17. Molecular Biology of the Cell, 4th Edition.

# Starting DNA synthesis: RNA primers

**RNA primer built by DNA primase serves as starter sequence for DNA polymerase III**

**Before new DNA strands can form, there must be RNA primers present to start the addition of new nucleotides**

**DNA polymerase III can only build onto 3′ end of an existing DNA strand**

**In bacteria, DNA primase binds to the DNA helicase forming a complex called the primosome. DNA Primase is activated by DNA helicase where it then synthesizes a short RNA primer approximately 11 ± 1 nucleotides long, to which new nucleotides can be added by DNA polymerase.**

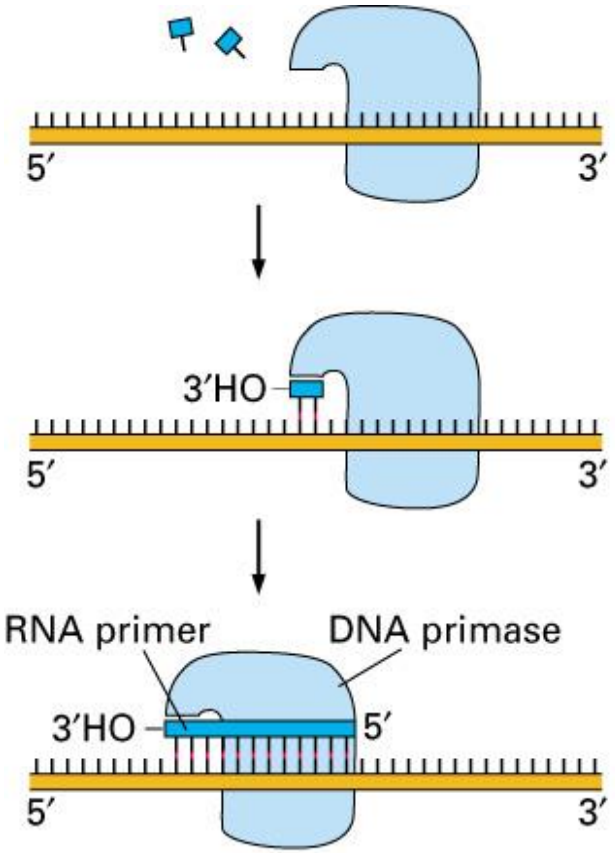



Figure 5–12. Molecular Biology of the Cell, 4th Edition.

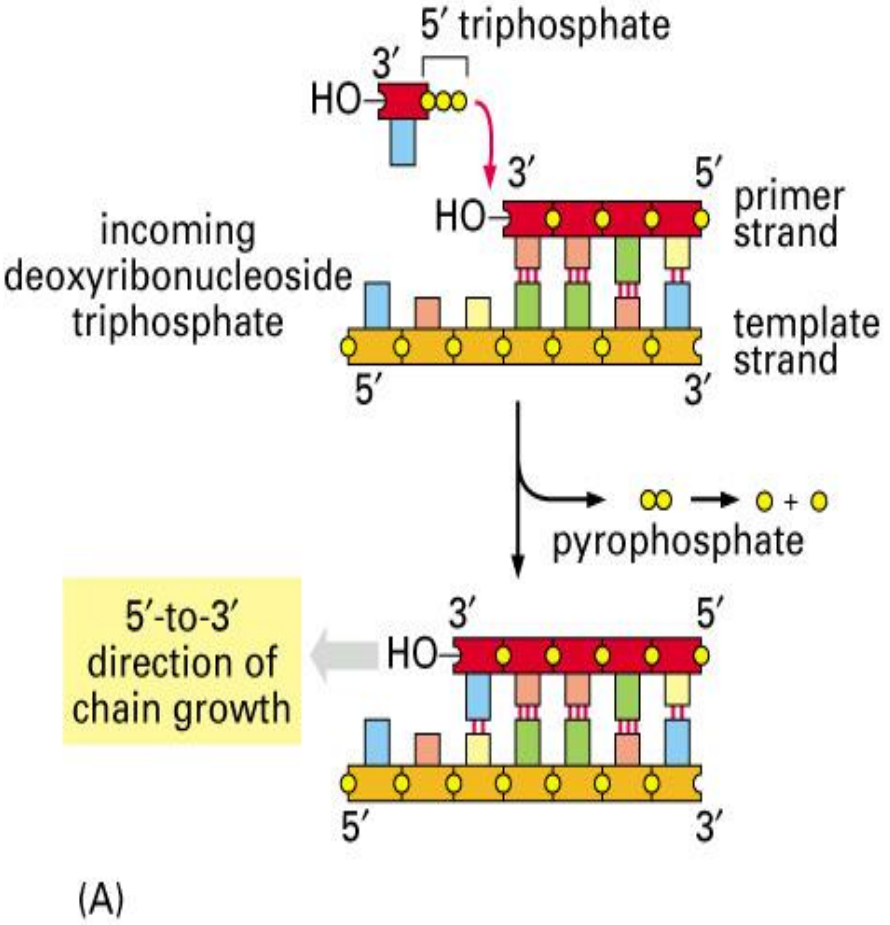
# DNA Synthesis by DNA polymerase

**DNA polymerase III catalyzes the formation of polynucleotide chains through the addition of successive nucleotides derived from deoxyribonucleoside triphosphates.**

**DNA polymerases III add nucleotides to the 3′ end of a polynucleotide chain.**

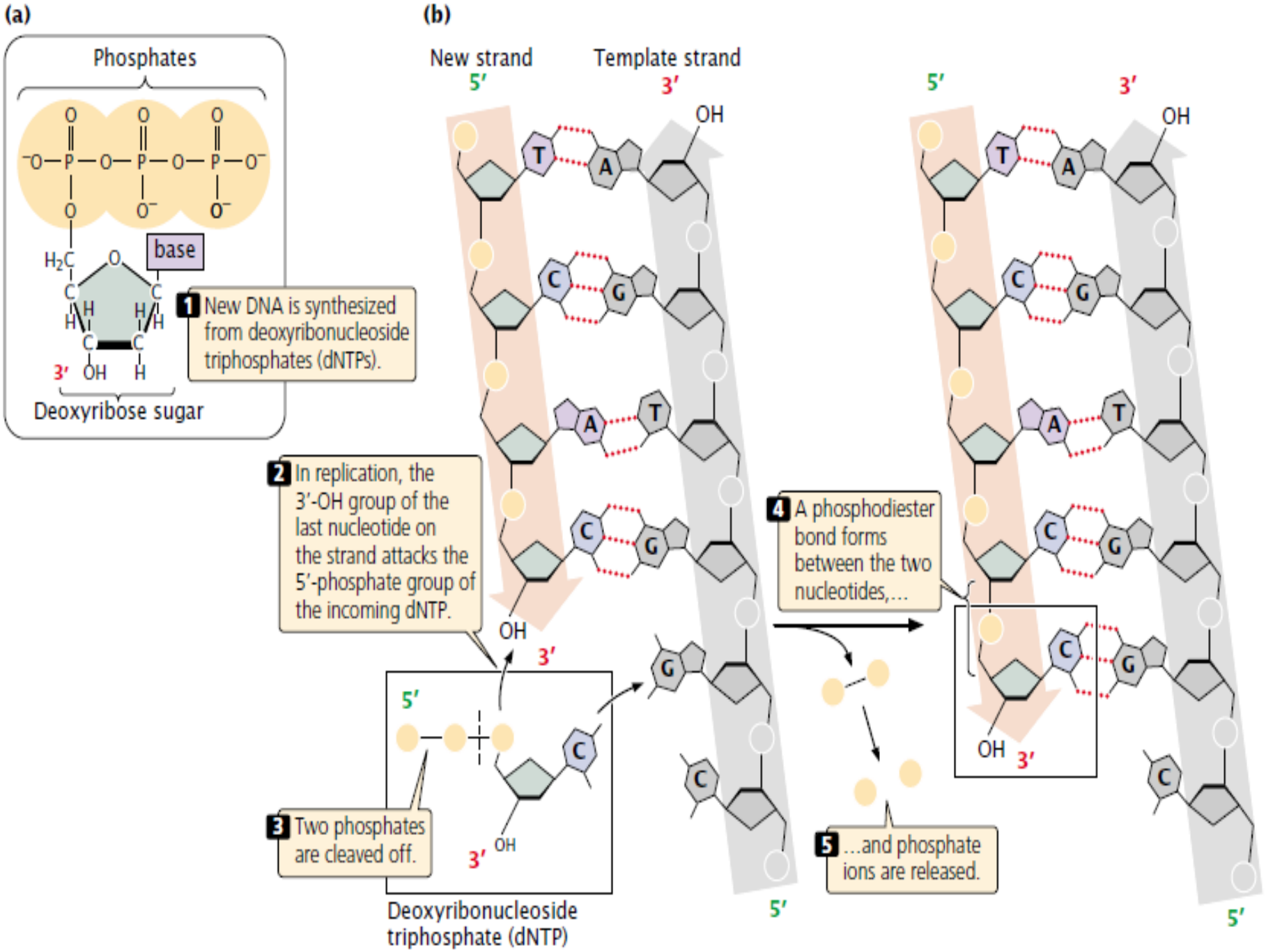
**The polymerase catalyzes the nucleophilic attack of the 3′-hydroxyl group terminus of the polynucleotide chain on the α-phosphate group of the nucleoside triphosphate to be added.**

**To initiate this reaction, DNA polymerases require a *primer* with a free 3′-hydroxyl group already base-paired to**




(A)

Figure 4–5 part 1 of 2. Molecular Biology of the Cell, 4th Edition.


(a)
Phosphates
base
Deoxyribose sugar
1 New DNA is synthesized from deoxyribonucleoside triphosphates (dNTPs).
(b)
New strand
Template strand
5′
3′
2 In replication, the 3′-OH group of the last nucleotide on the strand attacks the 5′-phosphate group of the incoming dNTP.
3 Two phosphates are cleaved off.
Deoxyribonucleoside triphosphate (dNTP)
4 A phosphodiester bond forms between the two nucleotides,...
5 ...and phosphate ions are released.

# Eukaryotic DNA polymerases

**A significant difference in the processes of bacterial and eukaryotic replication is in the number and functions of DNA polymerases. Eukaryotic cells contain a number of different DNA polymerases that function in replication, recombination, and DNA repair.**

**DNA polymerases in eukaryotic cells**

| DNA Polymerase | 5′→3′ Polymerase Activity | 3′→5′ Exonuclease Activity | Cellular Function |
|---|---|---|---|
| α (alpha) | Yes | No | Initiation of nuclear DNA synthesis and DNA repair |
| β (beta) | Yes | No | DNA repair and recombination of nuclear DNA |
| γ (gamma) | Yes | Yes | Replication of mitochondrial DNA |
| δ (delta) | Yes | Yes | Leading- and lagging-strand synthesis of nuclear DNA, DNA repair, and translesion DNA synthesis |

# Sliding clamp

**A DNA clamp, also known as a sliding clamp, is a protein fold that serves as a processivity-promoting factor in DNA replication.**

**As a critical component of the DNA polymerase III , the clamp protein binds DNA polymerase and prevents this enzyme from dissociating from the template DNA strand**

**The presence of the sliding clamp dramatically increases the number of nucleotides that can be added by DNA polymerase**

**The clamp-polymerase protein–protein interactions are stronger and more specific than the direct interactions between the polymerase and the template DNA strand**

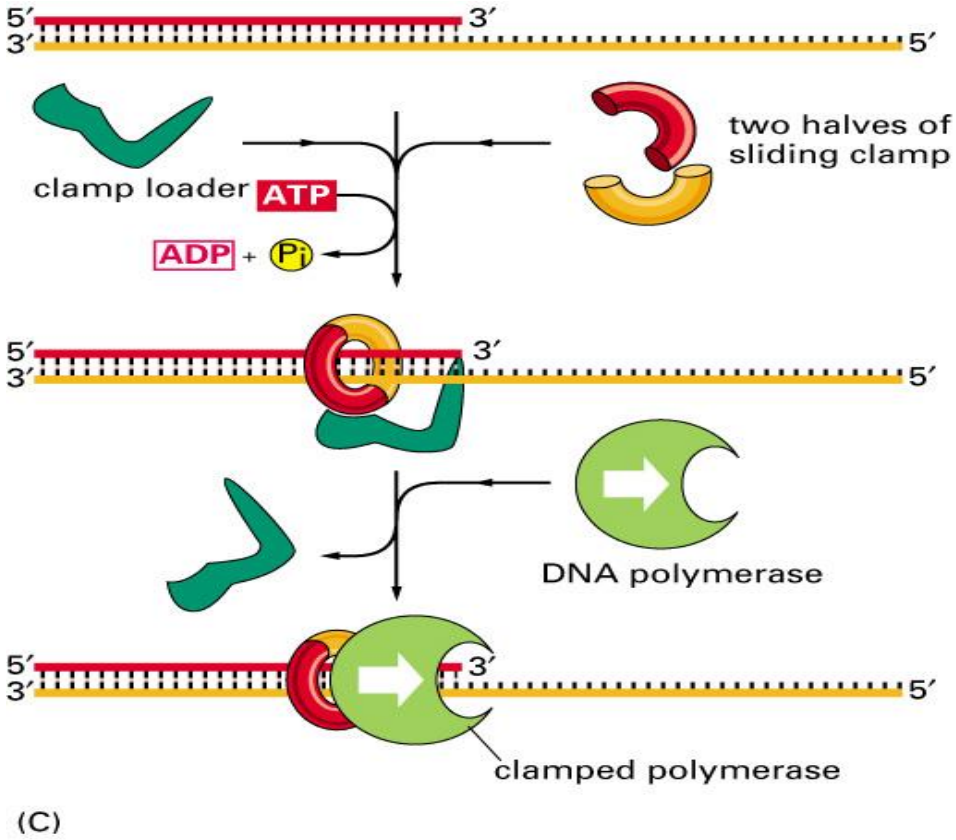
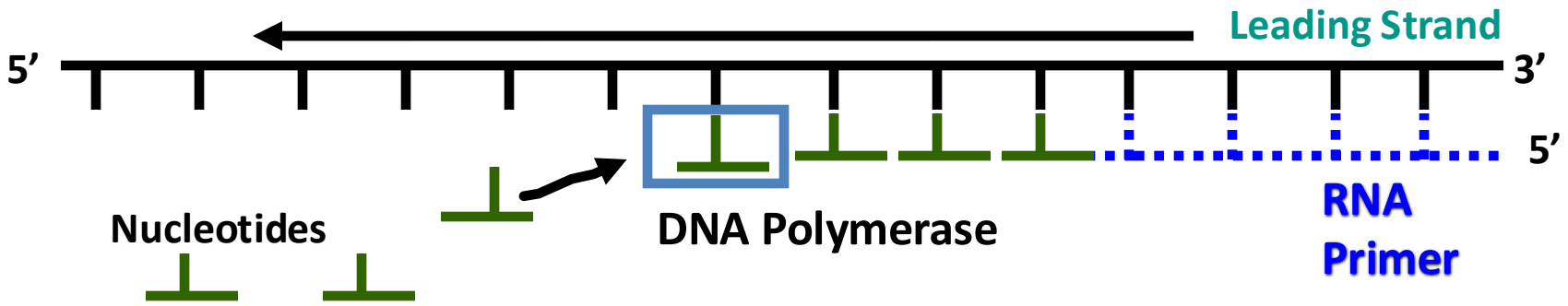
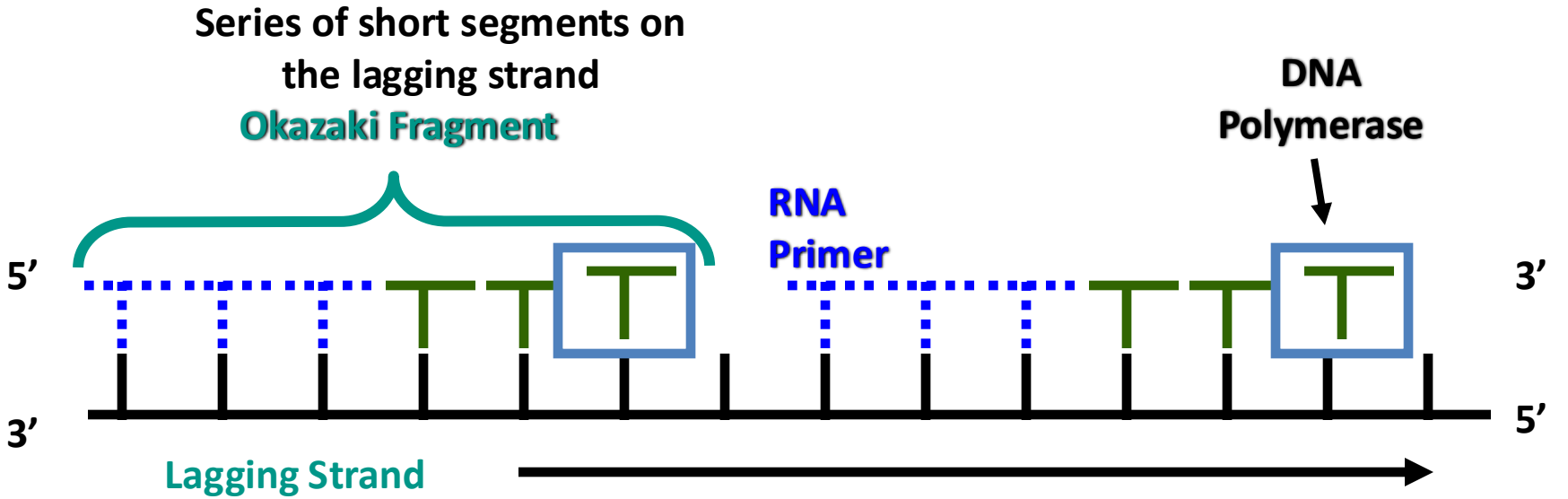



Figure 5–19 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

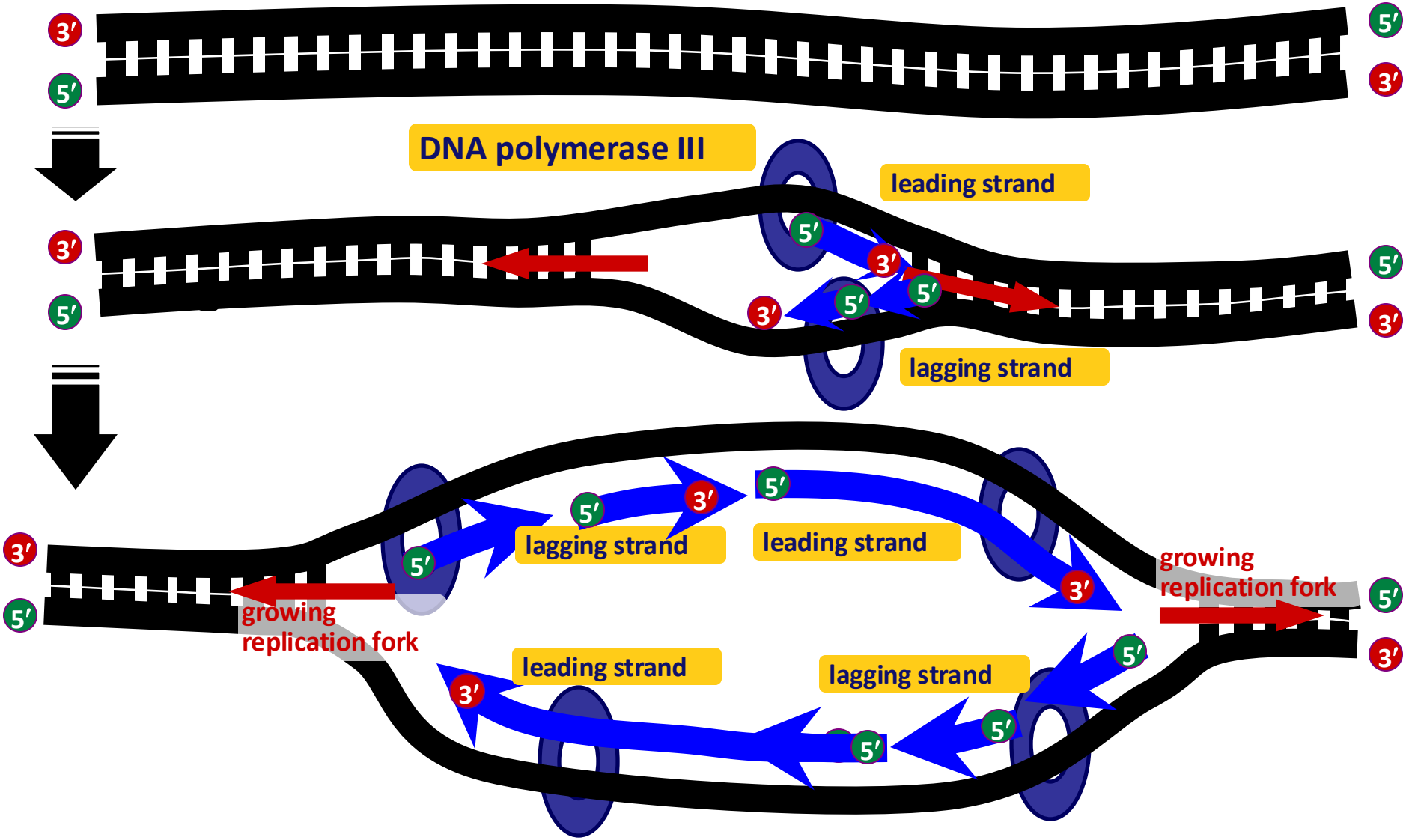
**Leading strand** is synthesized as a single polymer in the 5’ to 3’ direction




**Lagging strand** is also synthesized in the 5’ to 3’ direction but discontinuously against the overall direction of replication



# Replication fork / Replication bubble



# DNA Ligase

**Finally the gaps in the sugar phosphate backbone are sealed by DNA ligase.**

**DNA ligase is a specific type of enzyme, a ligase, that repairs single-stranded discontinuities in double stranded DNA molecules.**

**The mechanism of DNA ligase is to form two covalent phosphodiester bonds between 3' hydroxyl ends of one nucleotide, ("acceptor") with the 5' phosphate end of another ("donor").**

**ATP is required for the ligase reaction, which proceeds in three steps:**

**(1) adenylation (addition of AMP) of a residue in the active center of the enzyme, pyrophosphate is released;**

**(2) transfer of the AMP to the 5' phosphate of the so-called donor, formation of a pyrophosphate bond;**

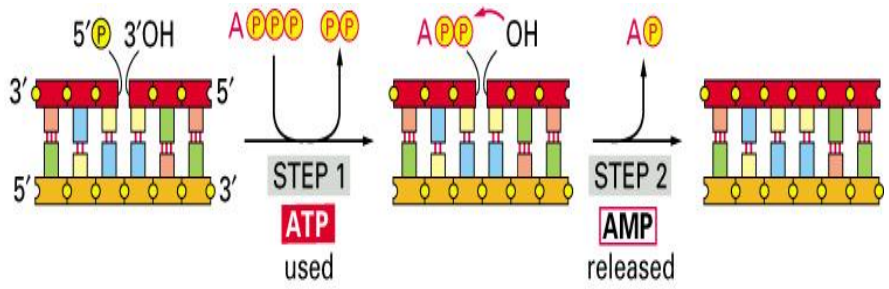
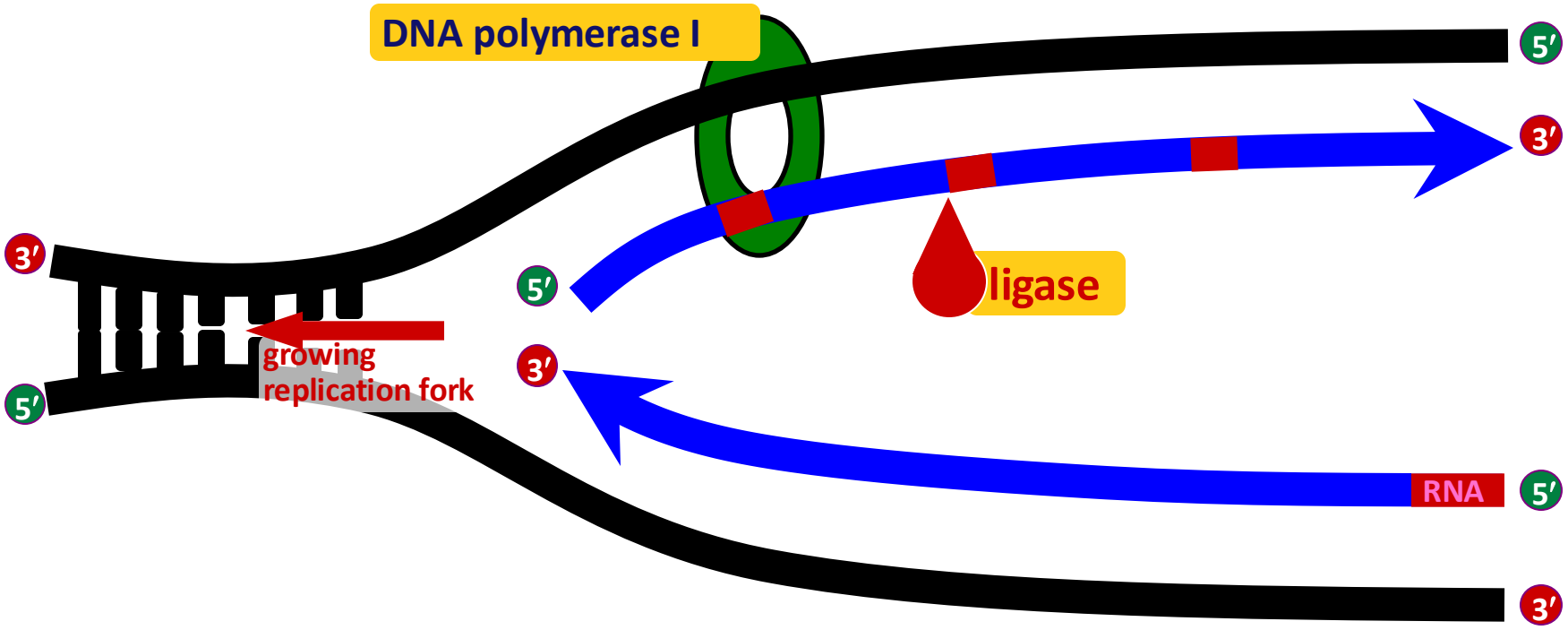



Figure 5–14. Molecular Biology of the Cell, 4th Edition.

**(3) formation of a phosphodiester bond between the 5' phosphate of the donor and the 3' hydroxyl of the acceptor**

# Replacing RNA primers with DNA

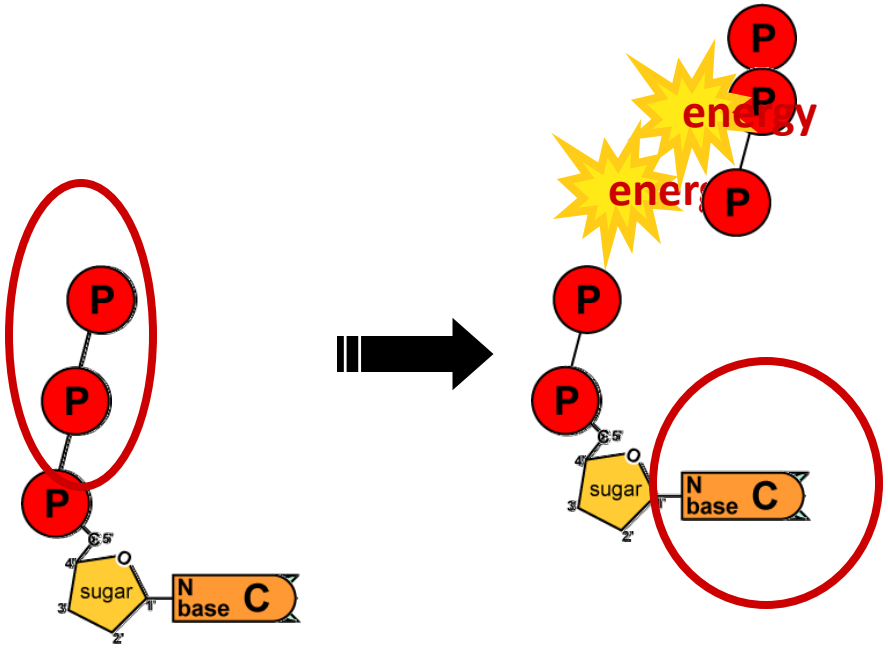
**In the replication process, DNA Polymerase I and Rnase H removes the RNA primer from the lagging strand and fills in the necessary nucleotides between the Okazaki fragments in 5' - 3' direction.**



# Energy of Replication

The nucleotides arrive as nucleosides

- DNA bases with P–P–P
- P-P-P = energy for bonding
- DNA bases arrive with their own energy source for bonding
- bonded by enzyme: DNA polymerase III




**CTP**

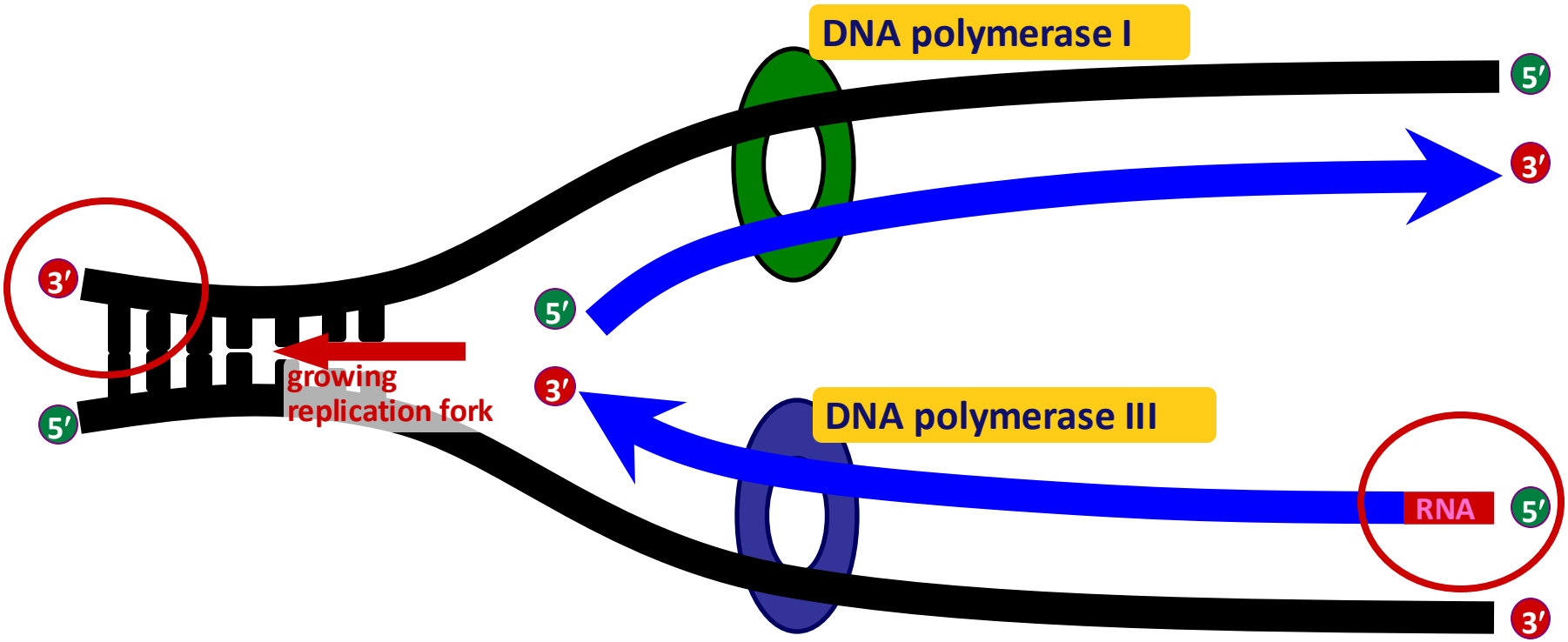
**modified nucleotide**

**CMP**

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

**Bacteria DNAs are circular, not a problem**
**There is a problem for eukaryote DNAs: ???**

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



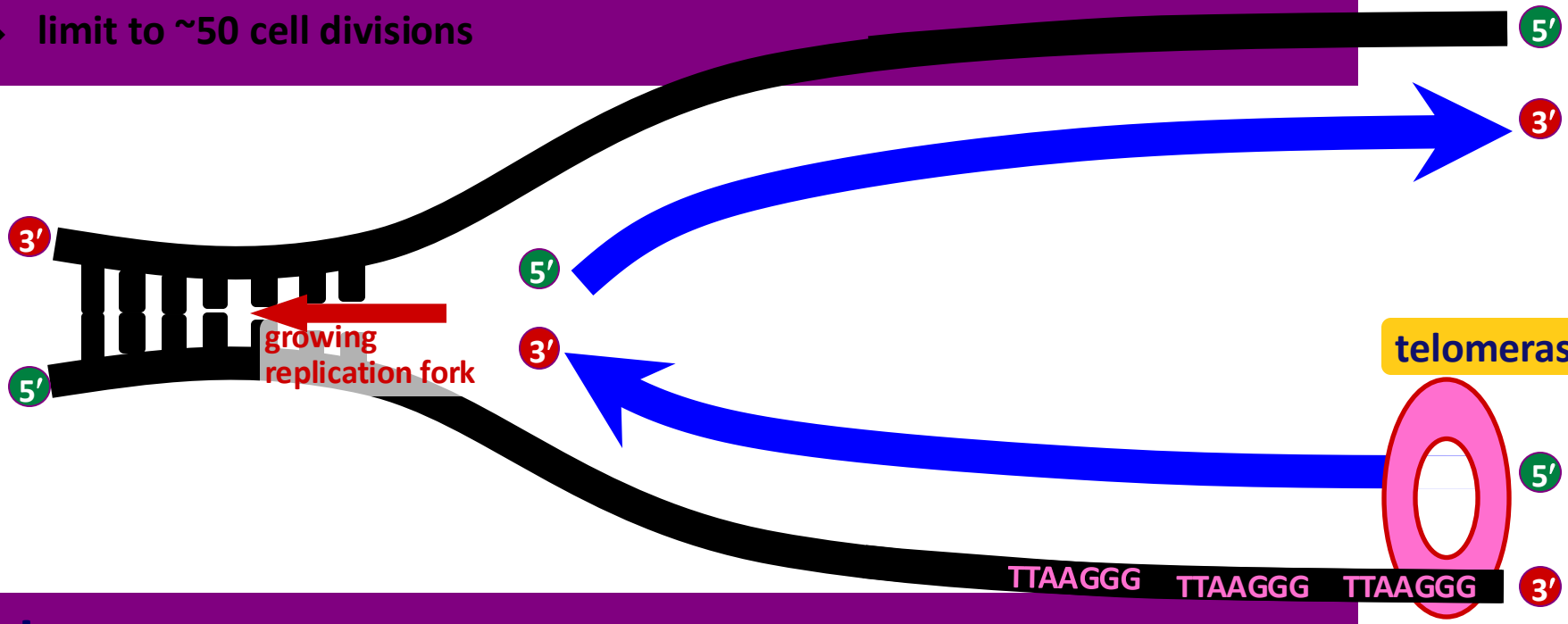

**Solution:**

**special telomere sequence: tandem repeats of TTAGGG (human)telomerase, a specific enzyme with integrated RNA template.**

# Telomeres

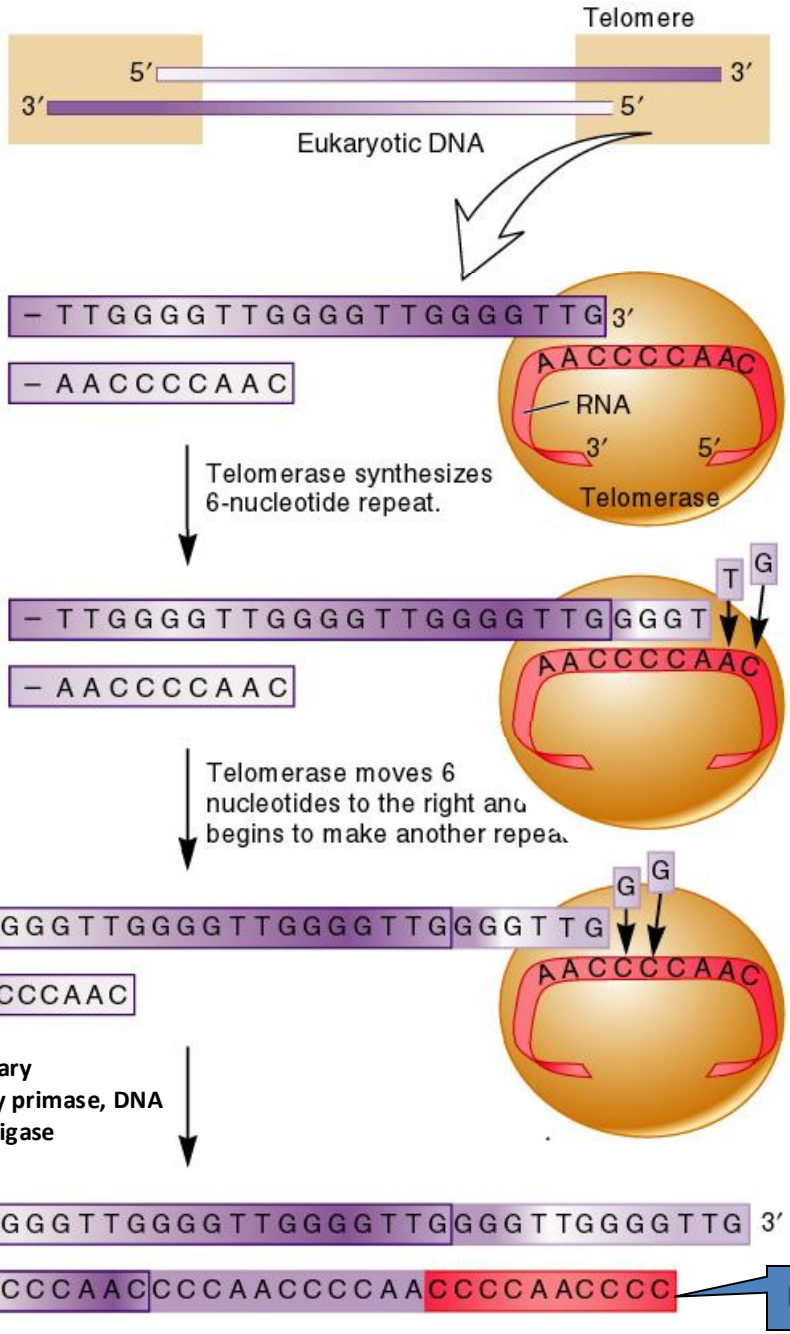
## Repeating, non-coding sequences at the end of chromosomes = protective cap

- limit to ~50 cell divisions




## Telomerase

- enzyme extends telomeres
- can add DNA bases at 5′ end
- different level of activity in different cells




The binding-polymerization-translocation cycle can occurs many times

This greatly lengthens one of the strands

Step 1 = Binding

Step 2 = Polymerization

Step 3 = Translocation

# Initiation of replication, major elements:

**topoisomerase relaxes the supercoiled DNA.**

**Initiator proteins and DNA helicase binds to the DNA at the replication fork and untwist the DNA using energy derived from ATP (adenosine triphosphate).**

**DNA primase next binds to helicase producing a complex called a primosome (primase is required for synthesis),**

**Primase synthesizes a short RNA primer of 10-12 nucleotides, to which DNA polymerase III adds nucleotides.**

**Polymerase III adds nucleotides 5' to 3' on both strands beginning at the RNA primer.**

**The RNA primer is removed and replaced with DNA by polymerase I, and the gap is sealed with DNA ligase.**

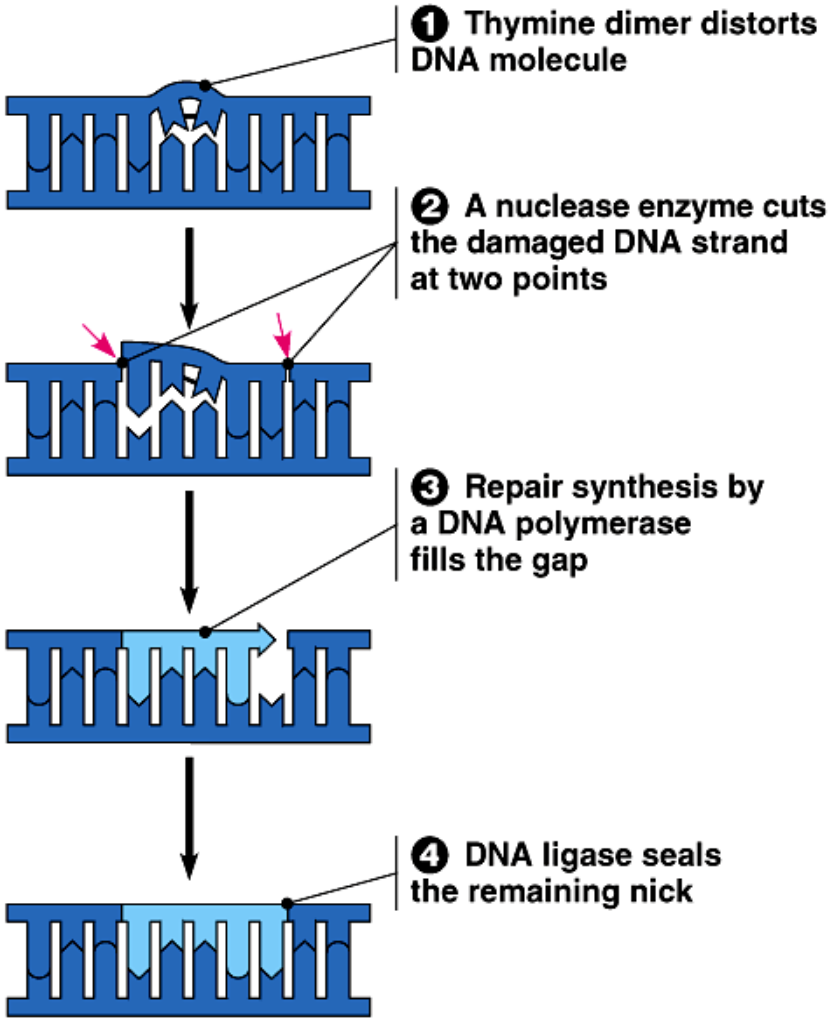
**Single-stranded DNA-binding (SSB) proteins stabilize the single-stranded template DNA during the process.**

# Proofreading DNA

**In bacteria, all three DNA polymerases (I, II, and III) have the ability to proofread, using 3'->5' exonuclease activity.**

**When an incorrect base pair is recognized, DNA polymerase reverses its direction by one base pair of DNA and excises the mismatched base.**

**Following base excision, the polymerase can re-insert the correct base and replication can continue.**



# Types of DNA Damage

**1- All four of the bases in DNA (A, T, C, G) can be covalently modified at various positions.**
**One of the most frequent is the loss of an amino group ("deamination") — resulting, for example, in a C being converted to a U.**

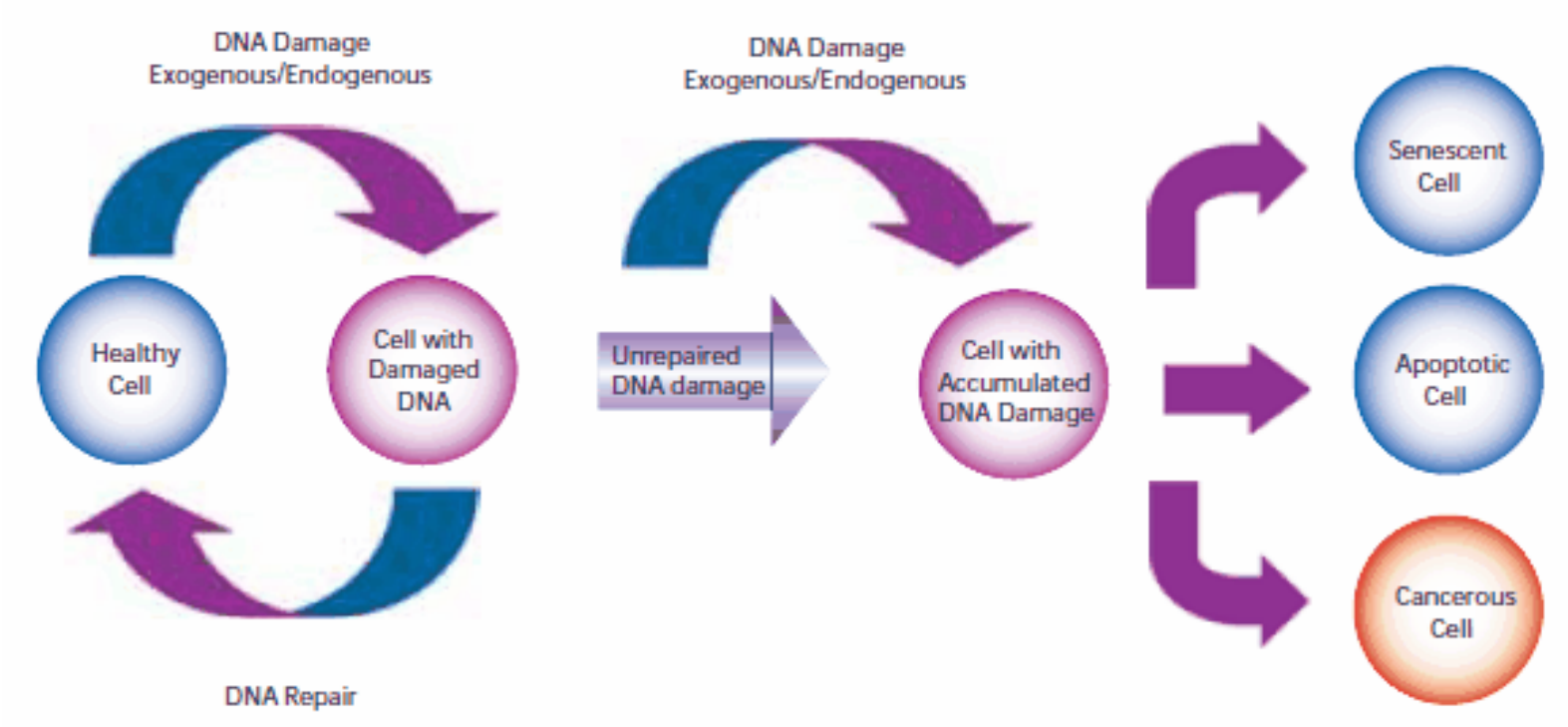
**2- Mismatches of the normal bases because of a failure of proofreading during DNA replication.**
**Common example: incorporation of the pyrimidine U (normally found only in RNA) instead of T.**

**3- Breaks in the backbone. Can be limited to one of the two strands (a single-stranded break) or on both strands (a double-stranded break ).**

**4- Crosslinks Covalent linkages can be formed between bases on the same DNA strand ("intrastrand") or on the opposite strand ("interstrand").**

**Once cells lose their ability to effectively repair damaged DNA, there are three possible responses :**

**1-The cell may become senescent, i.e., irreversibly dormant.**
**2-The cell may become apoptotic. Sufficient DNA damage may trigger an apoptotic signaling cascade, forcing the cell into programmed cell death.**
**3-The cell may become malignant, i.e., develop immortal characteristics and begin uncontrolled division.**



# Mechanisms of DNA Repair

## 1-Base Excision Repair

Base excision repair (BER) involves multiple enzymes to excise and replace a single damaged nucleotide base.

It is responsible primarily for removing small, non-helix-distorting base lesions from the genome.

## 2-Nucleotide Excision Repair (NER)

Nucleotide excision repair (NER) is a particularly important excision mechanism that removes mutations resulting from UV induced DNA damage. UV DNA damage results in bulky DNA adducts - these adducts are mostly thymine dimers and 6,4-photoproducts.

Recognition of the damage leads to removal of a short single-stranded DNA segment that contains the lesion.

## 3-Mismatch Repair (MMR)

is a system for recognizing and repairing erroneous insertion, deletion and mis-incorporation of bases that can arise during DNA replication and recombination, as well as repairing some forms of DNA damage

# 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.

## Addition of new histones

Chromatin assembly factors (CAFs) help to add and assemble new nucleosomes

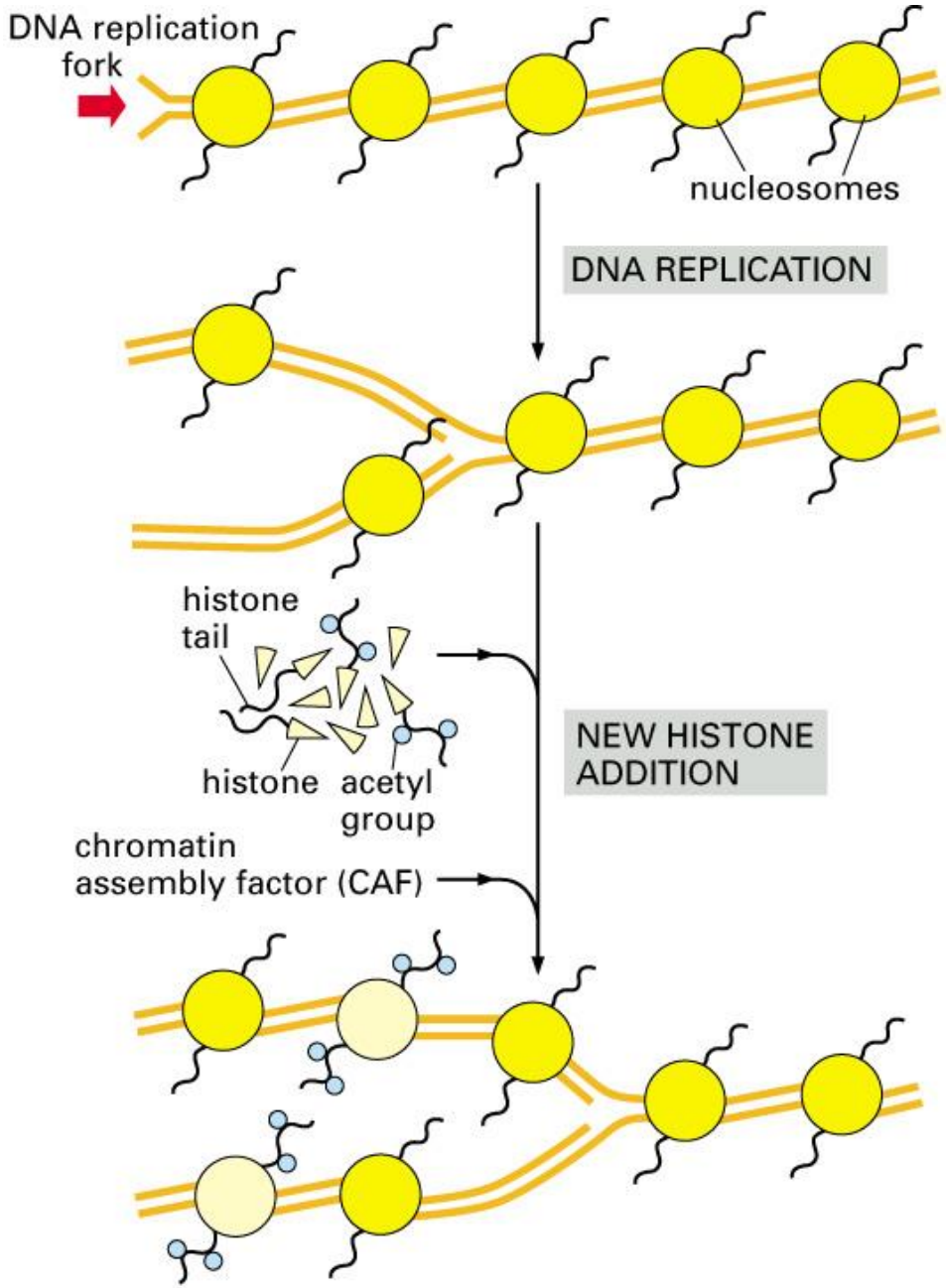



Figure 5–41 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

# A model for nucleosome replication