DNA Repair and Recombination

Tong-Jin Zhao

School of Life Sciences, Xiamen University

Part I. DNA Repair

What We Have Learned Previously

TABLE 5–1 The Three Steps That Give Rise to High-Fidelity DNA Synthesis

Replication step	Errors per nucleotide added
5′ → 3′ polymerization	1 in 10^5
3′ → 5′ exonucleolytic proofreading	1 in 10^2
Strand-directed mismatch repair	1 in 10^3
Combined	1 in 10^{10}

The third step, strand-directed mismatch repair, is described later in this chapter. For the polymerization step, "errors per nucleotide added" describes the probability that an incorrect nucleotide will be added to the growing chain. For the other two steps, "errors per nucleotide added" describes the probability that an error will not be corrected. Each step therefore reduces the chance of a final error by the factor shown.

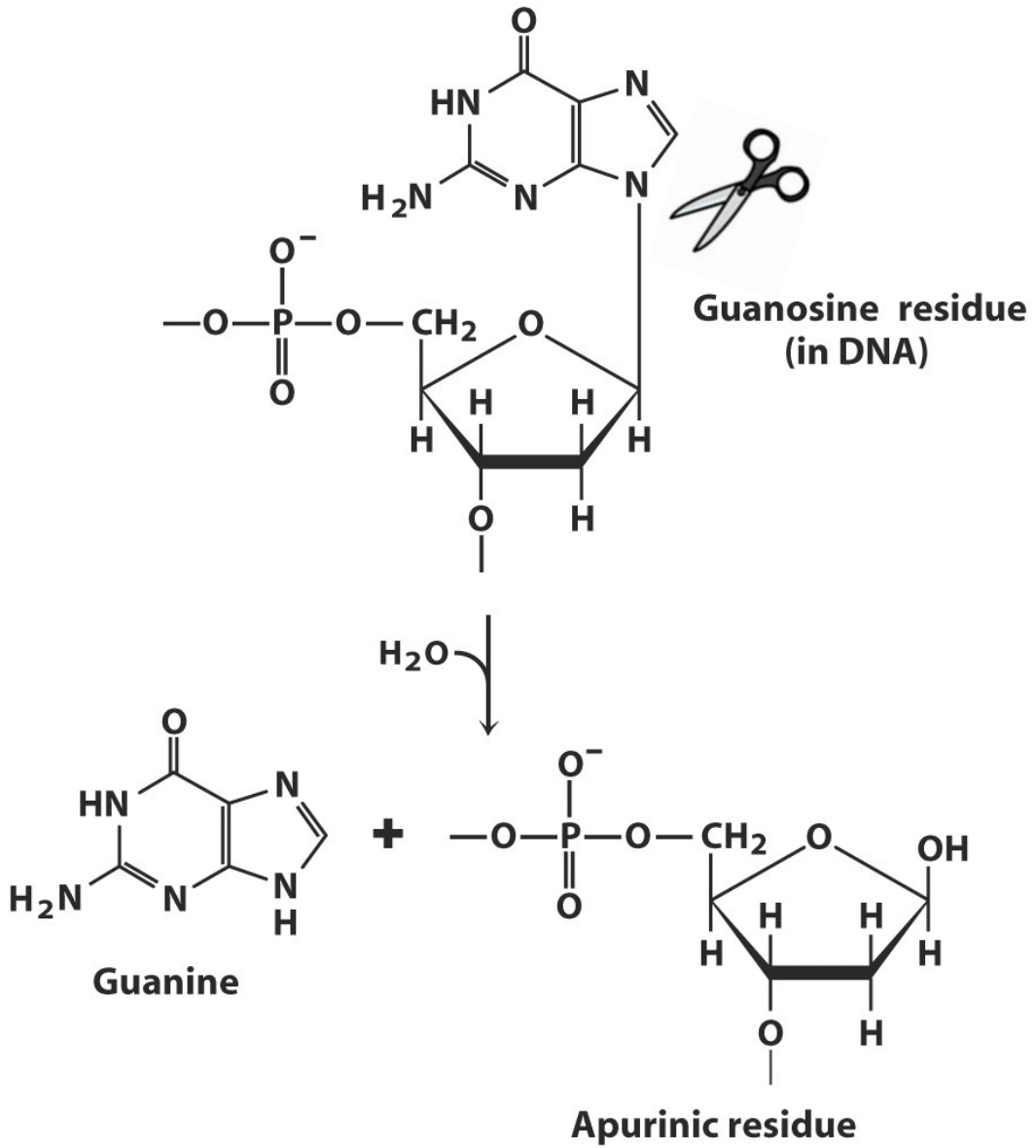
Part I

Spontaneous DNA Damage

DNA lesion	Number repaired in 24 h
Hydrolysis	
Depurination	18,000
Depyrimidination	600
Cytosine deamination	100
5-Methylcytosine deamination	10
Oxidation	
8-oxo G	1500
Ring-saturated pyrimidines (thymine glycol, cytosine hydrates)	2000
Lipid peroxidation products (M1G, etheno-A, etheno-C)	1000
Nonenzymatic methylation by *S*-adenosylmethionine	
7-Methylguanine	6000
3-Methyladenine	1200
Nonenzymatic methylation by nitrosated polyamines and peptides	
O^6-Methylguanine	20–100

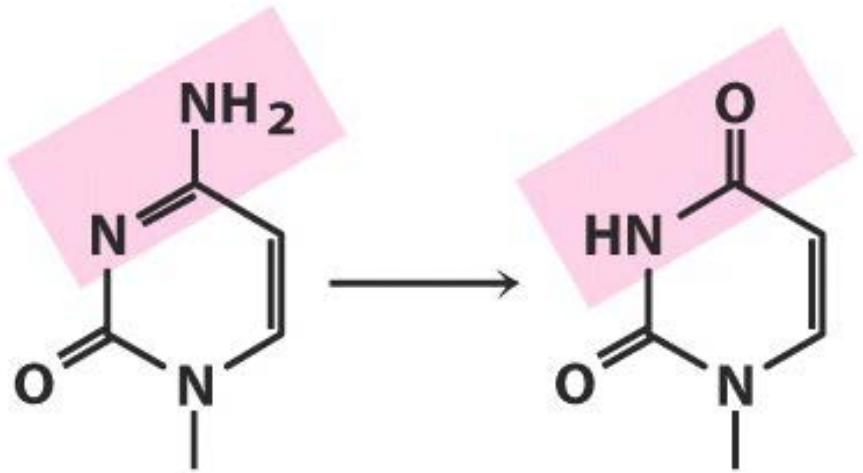
Part I

Depurination



Part I

Deamination

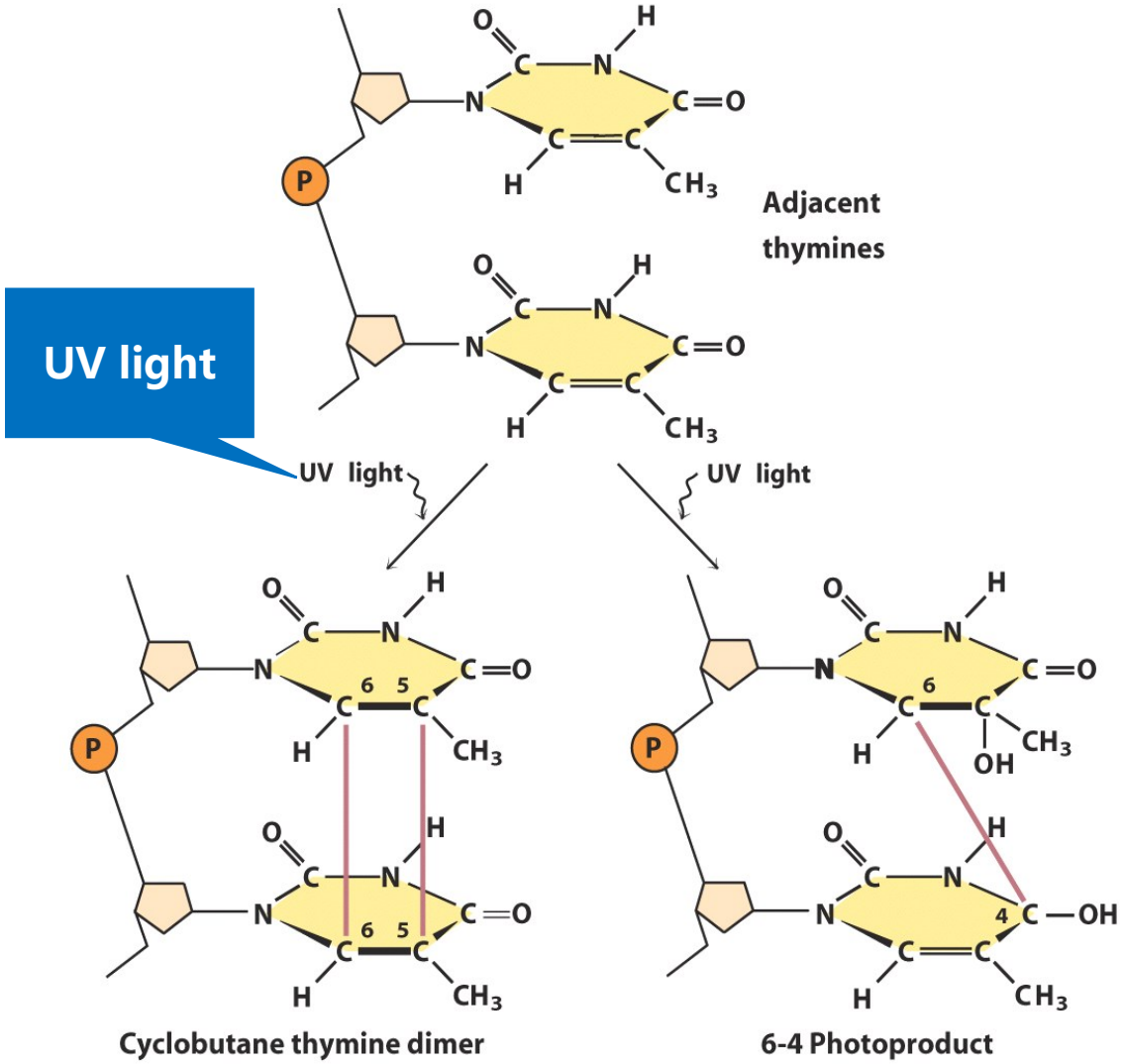


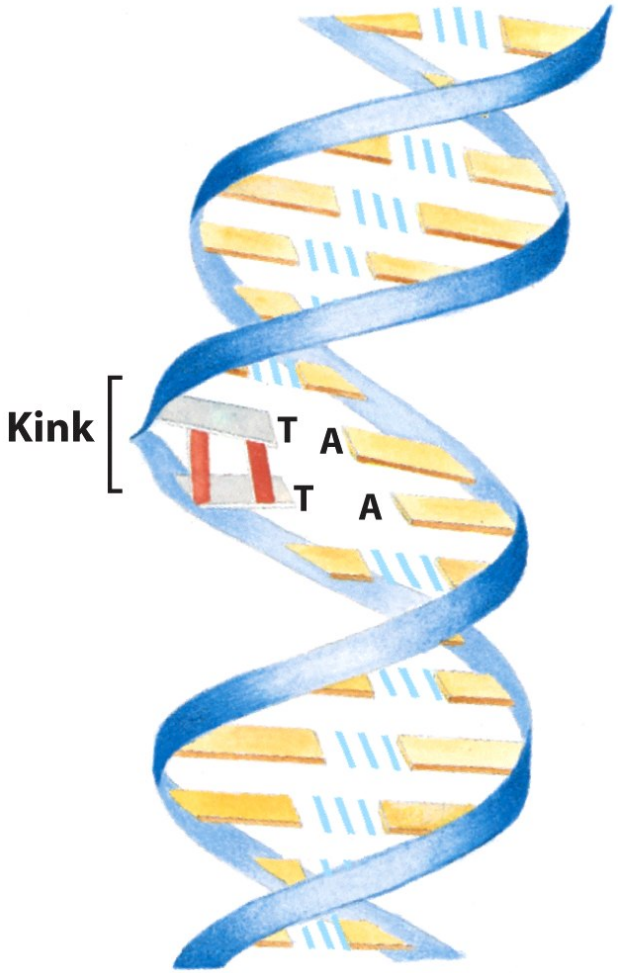
Cytosine Uracil

G≡C ⟶ A=U

Part I

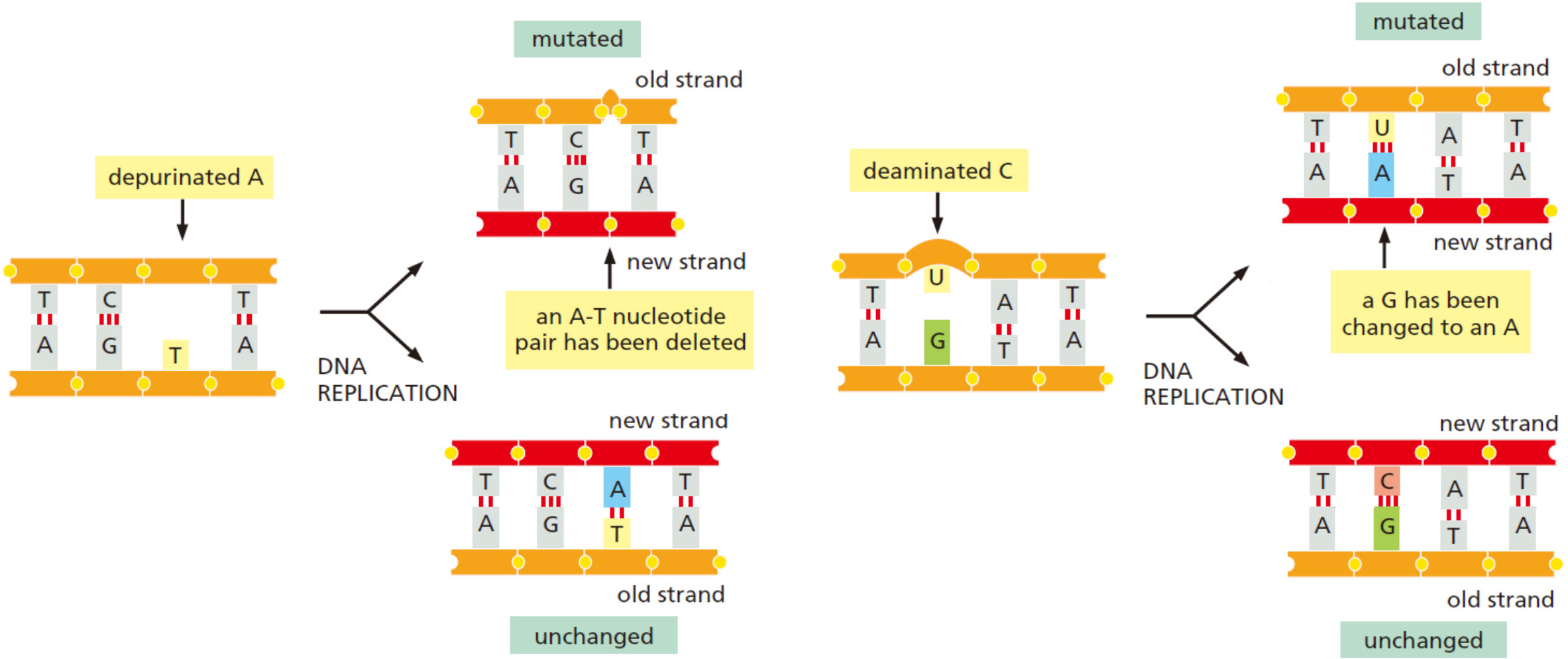
Formation of Pyrimidine Dimers





Part I

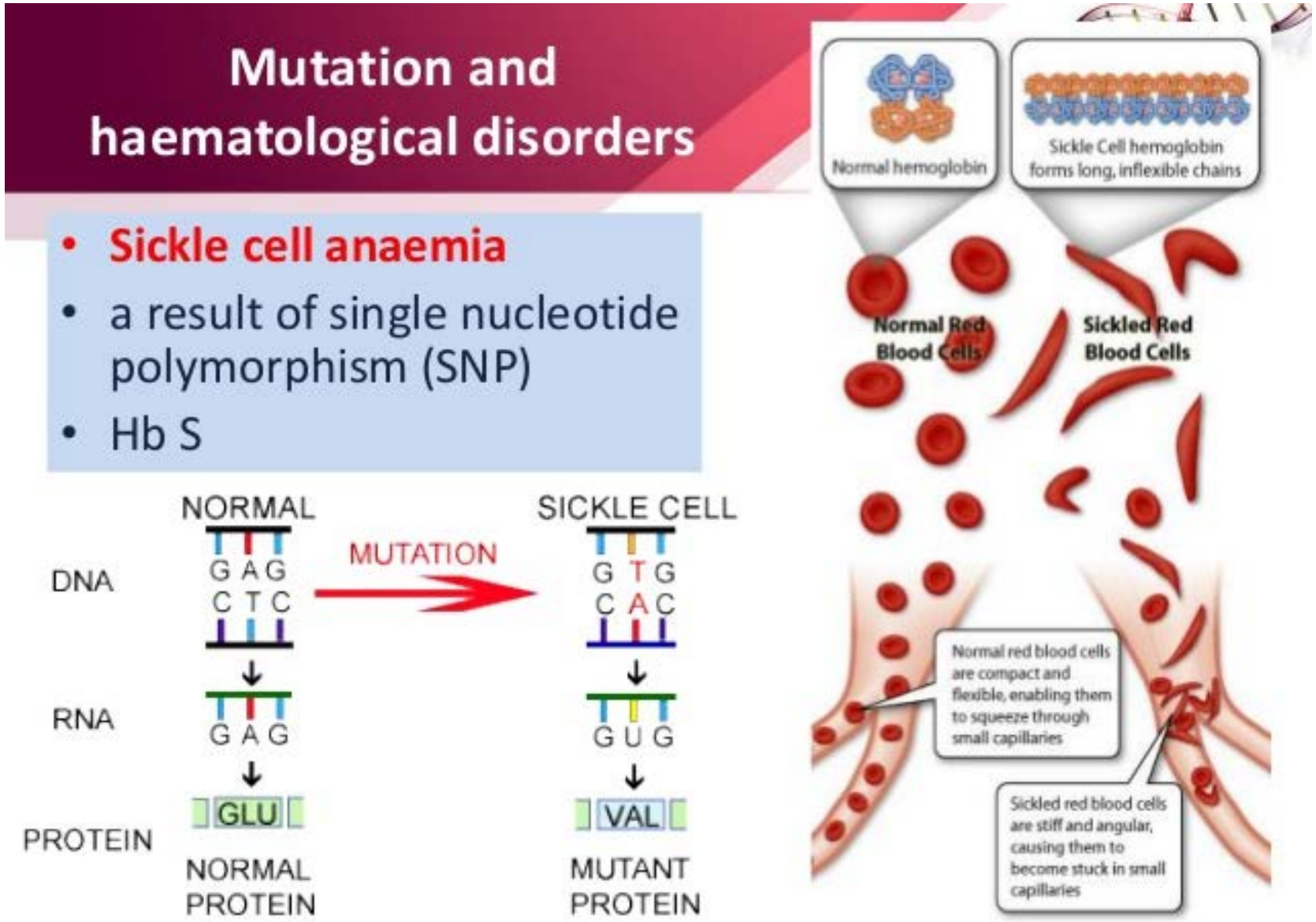
How Chemical Modifications of Nucleotides Produce Mutations



Part I

What Are the Consequences?

—— Various genetic diseases and cancer



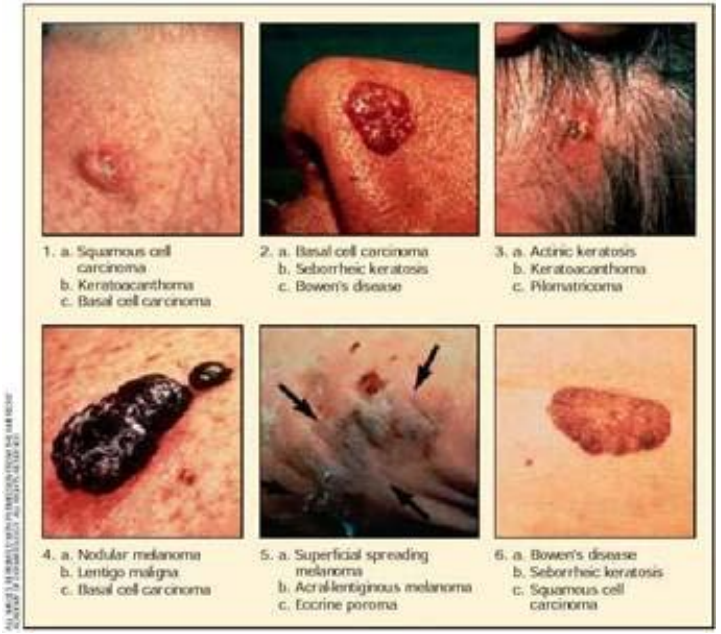
Example 1

Part I

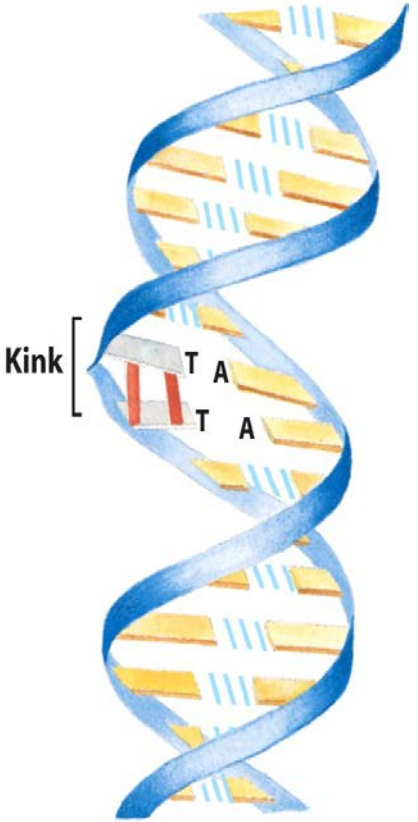
What Are the Consequences?

—— Various genetic diseases and cancer

Getting tanned



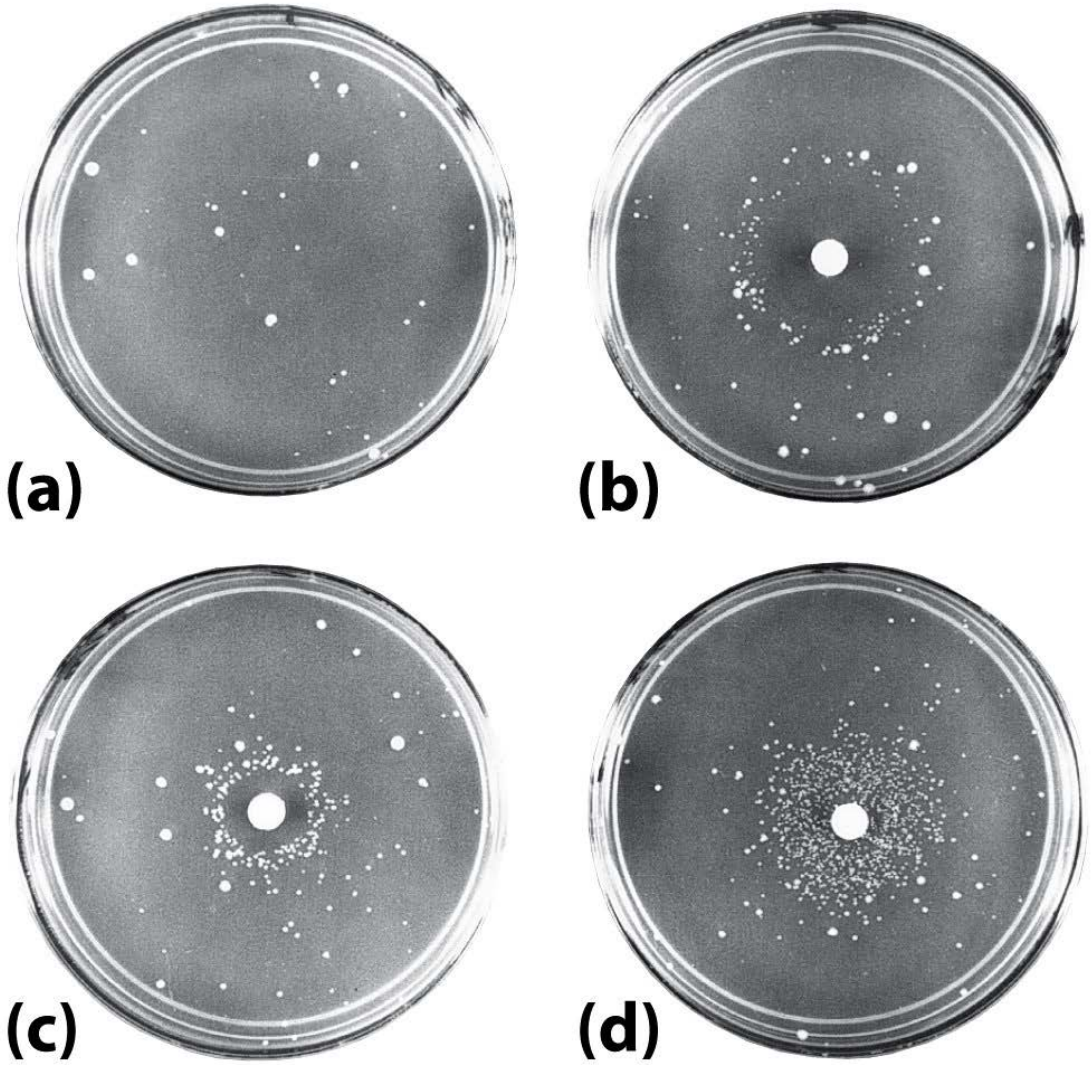
Skin cancer



Example 2

Part I

Ames Test for Carcinogens, Based on Their Mutagenicity



Part I

How Does a Living Organism Respond to Mutations?

—— DNA Repair

Part I

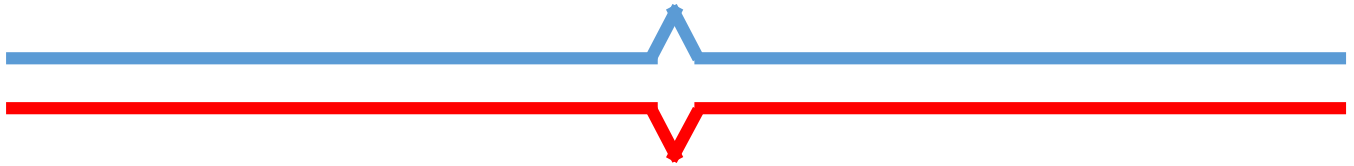
Types of DNA Repair System in *E. Coli*

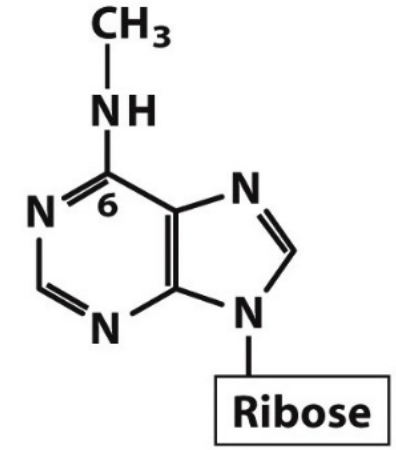
- Mismatch repair
- Base-excision repair
- Nucleotide-excision repair
- Direct repair

Part I

Which Strand to Repair?

— An Issue of Strand Discrimination



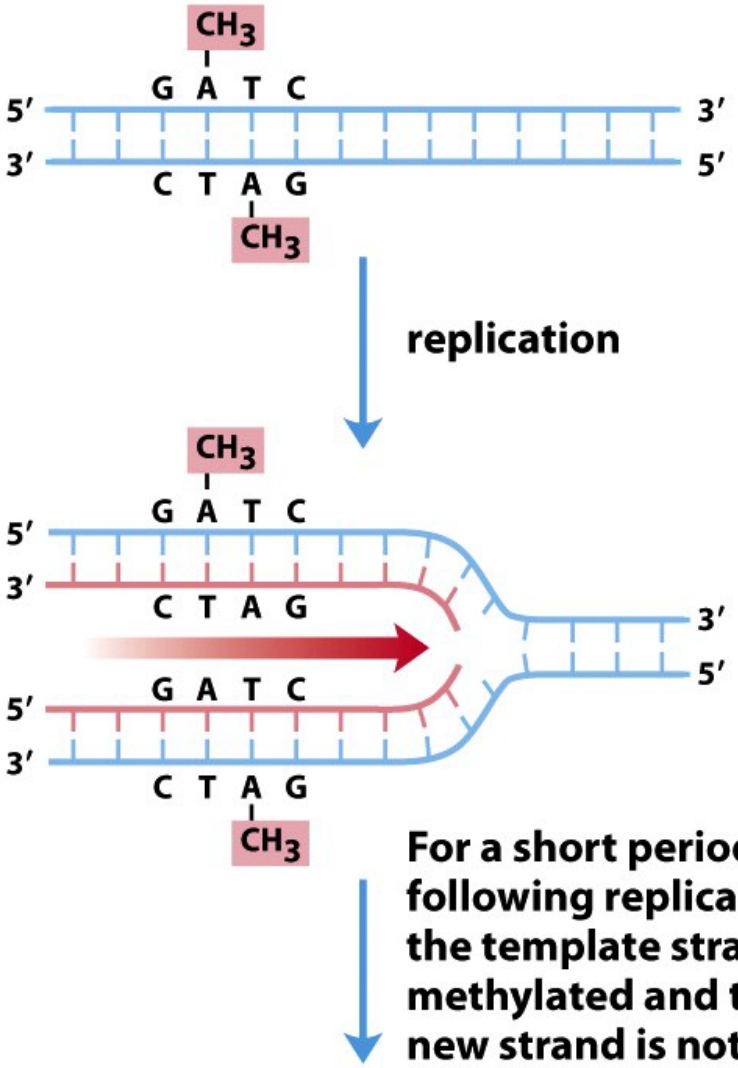


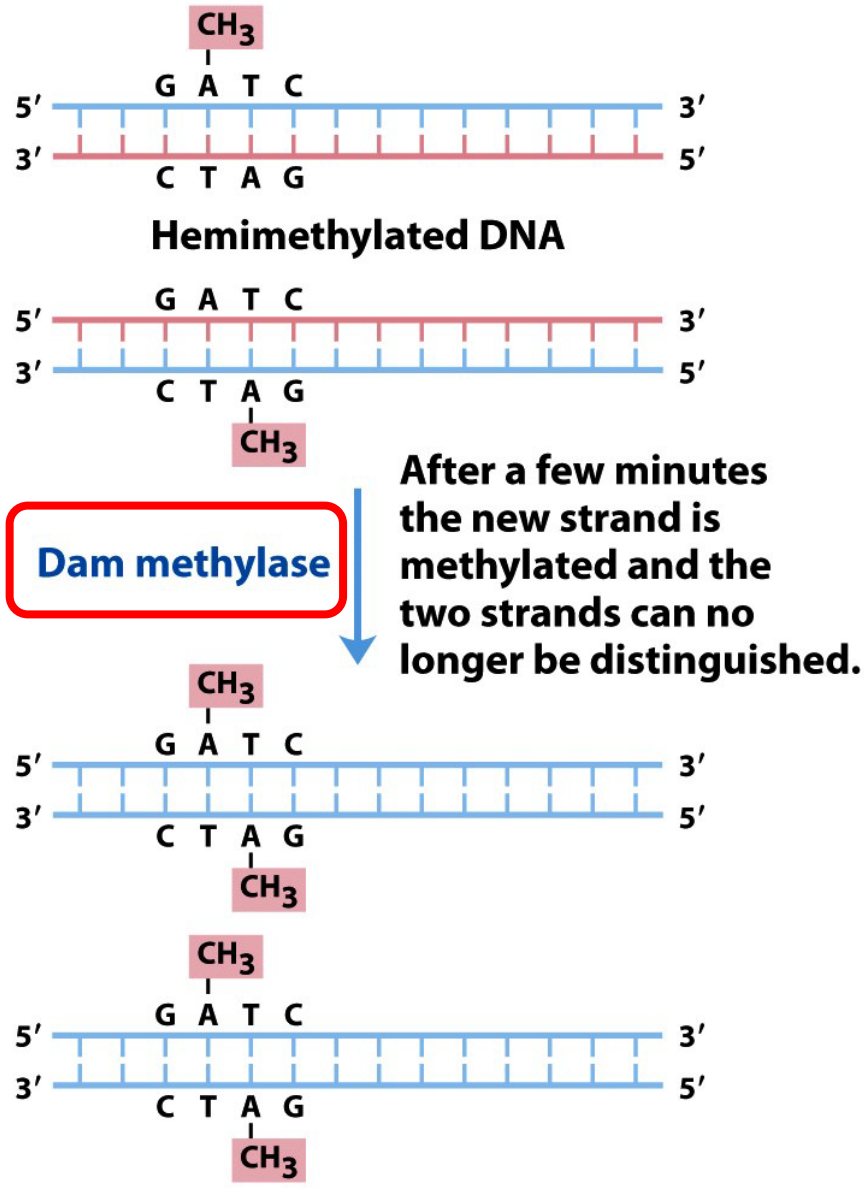
N^6-Methyladenosine

In *E. Coli*, Dam methylase methylates DNA at the N^6 position of all adenines within (5')GATC sequence.

Part I

Methylation and Mismatch Repair

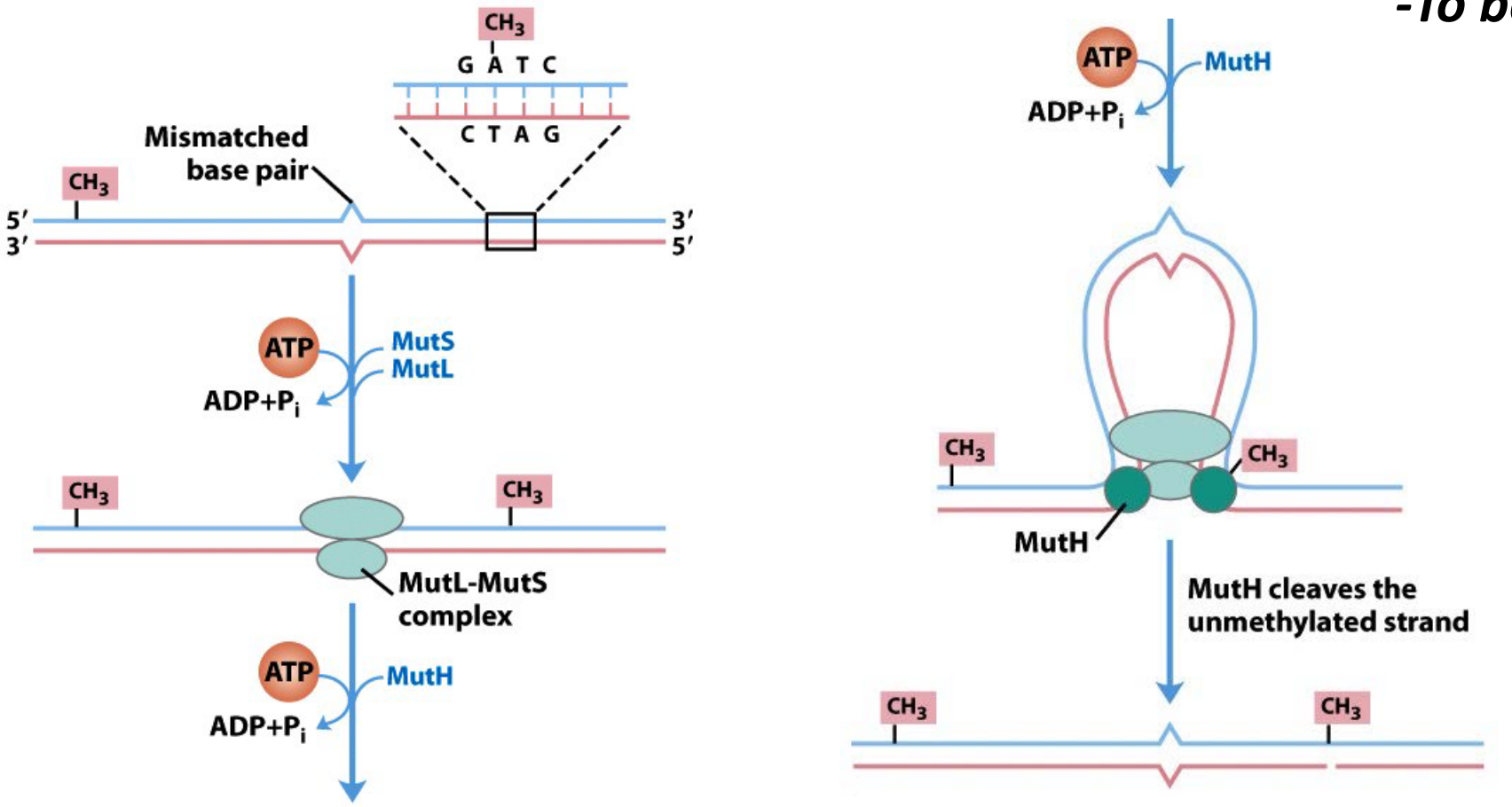




Part I

Methyl-Directed Mismatch Repair

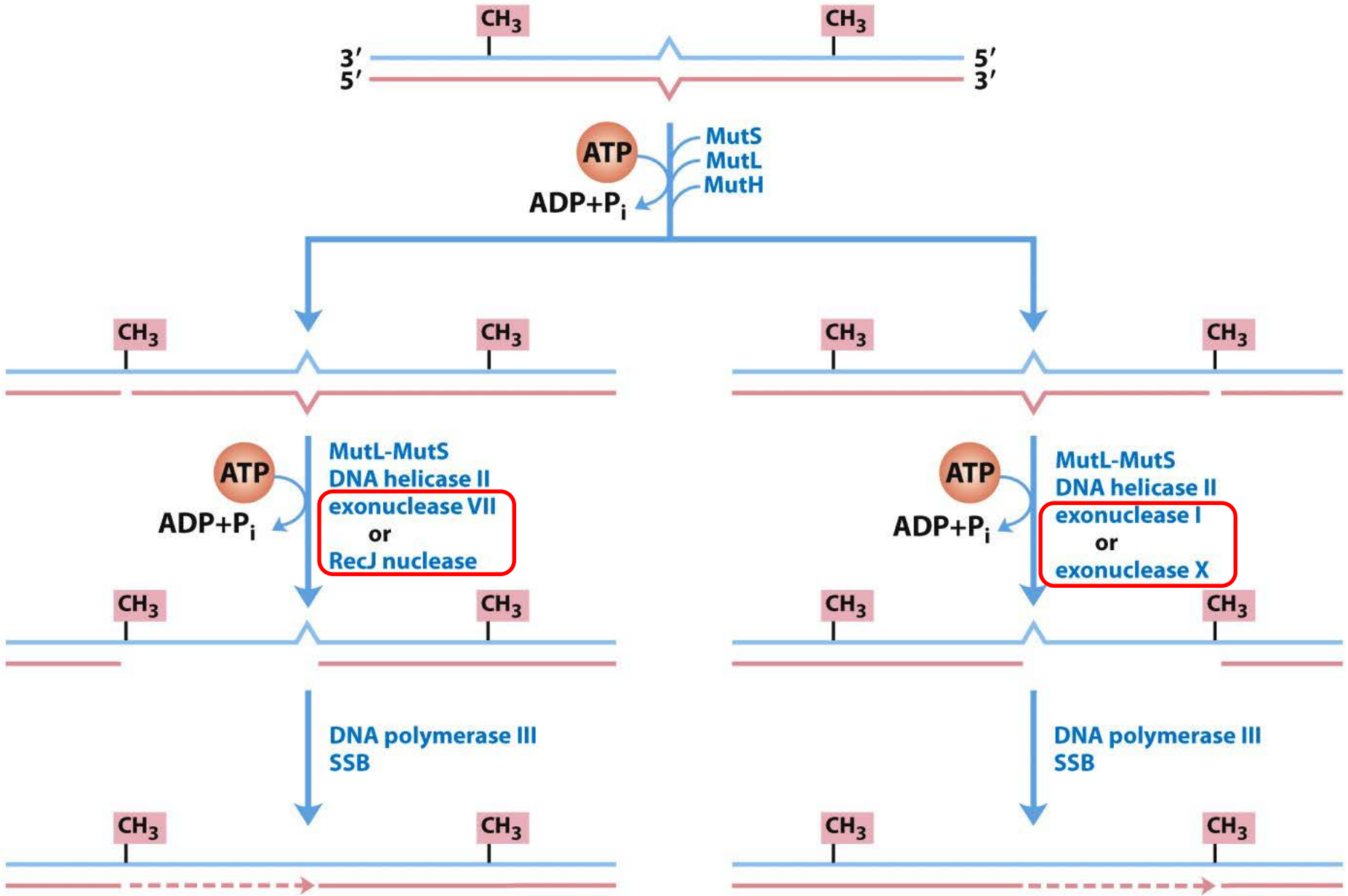
-To be continued



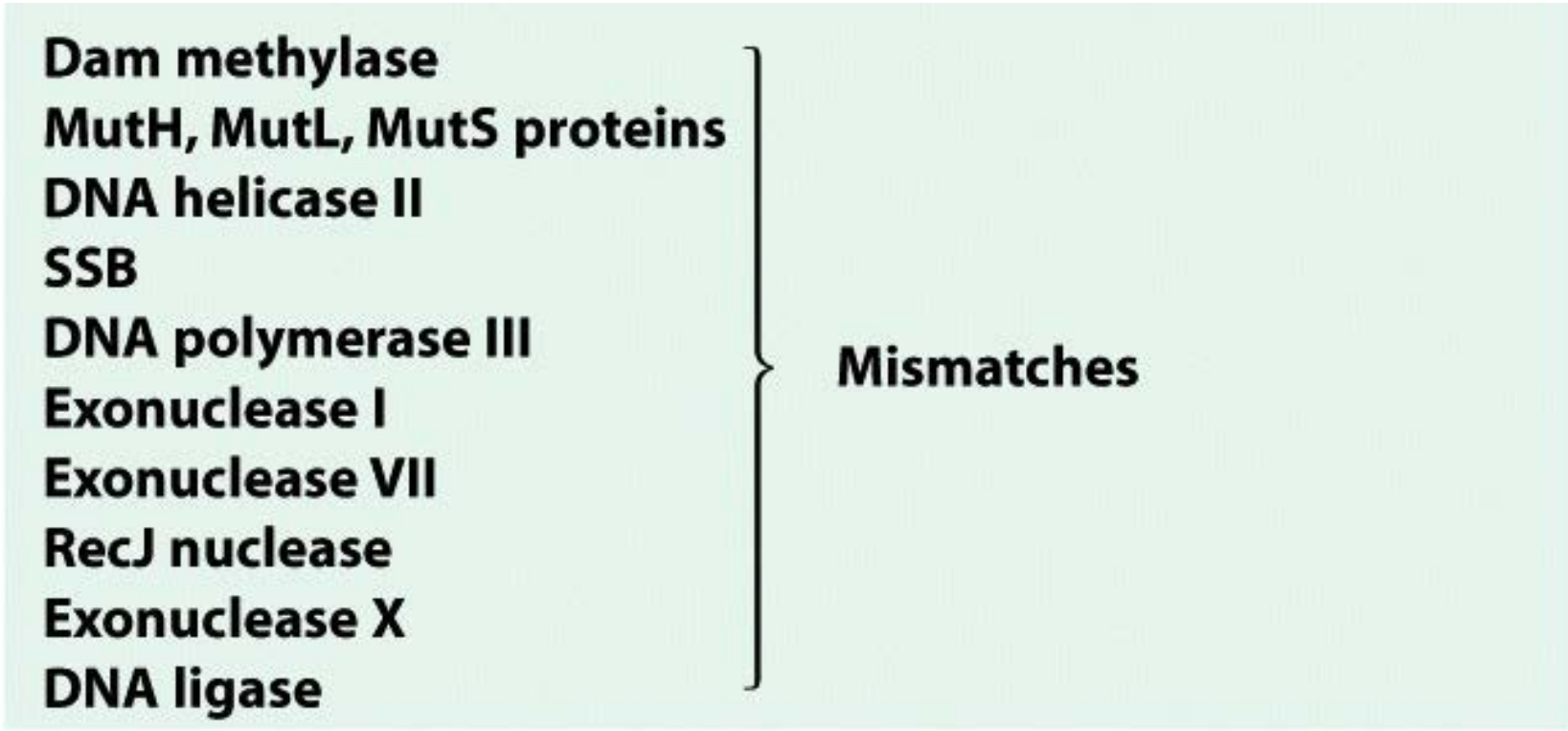
MutH has a site-specific endonuclease activity. It is inactive until the complex encounters a hemimethylated GATC sequence. MutH cuts at the 5'-side of the G in the GATC sequence in the unmethylated strand, which marks the strand for repair.

Part I

Methyl-Directed Mismatch Repair *-continued*

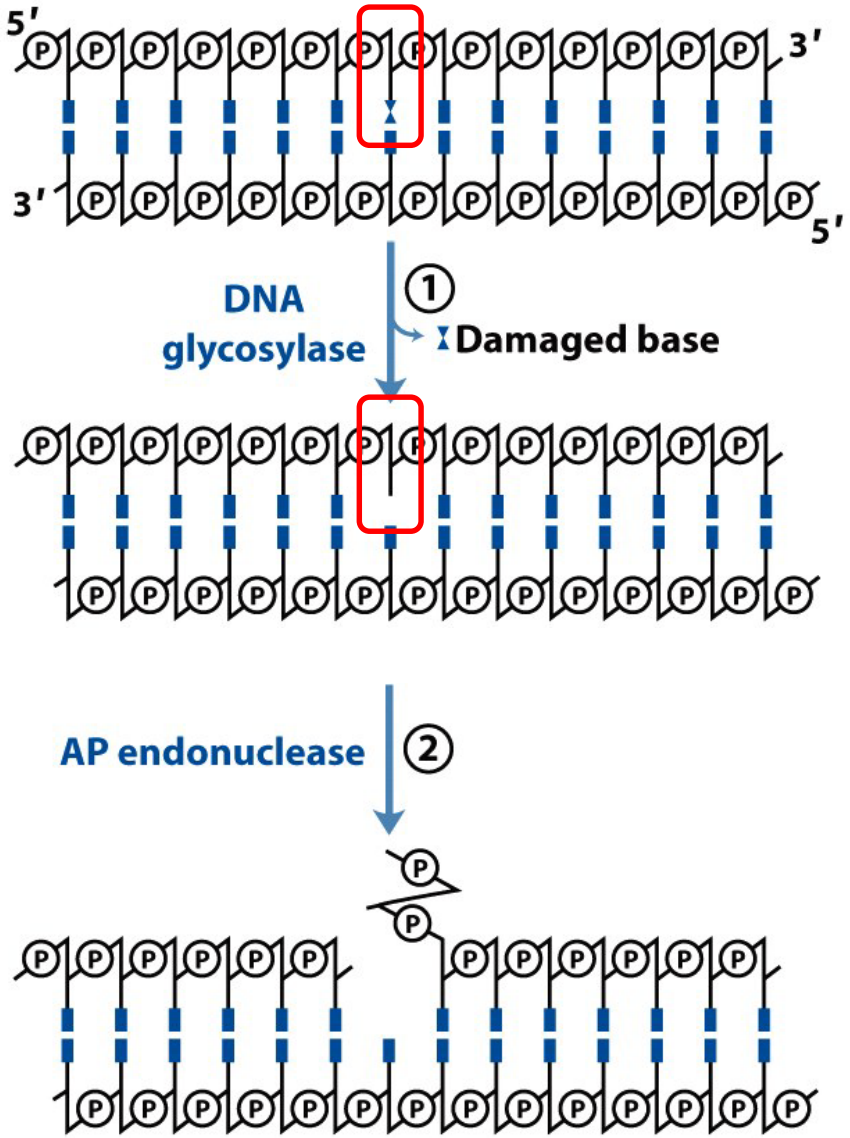


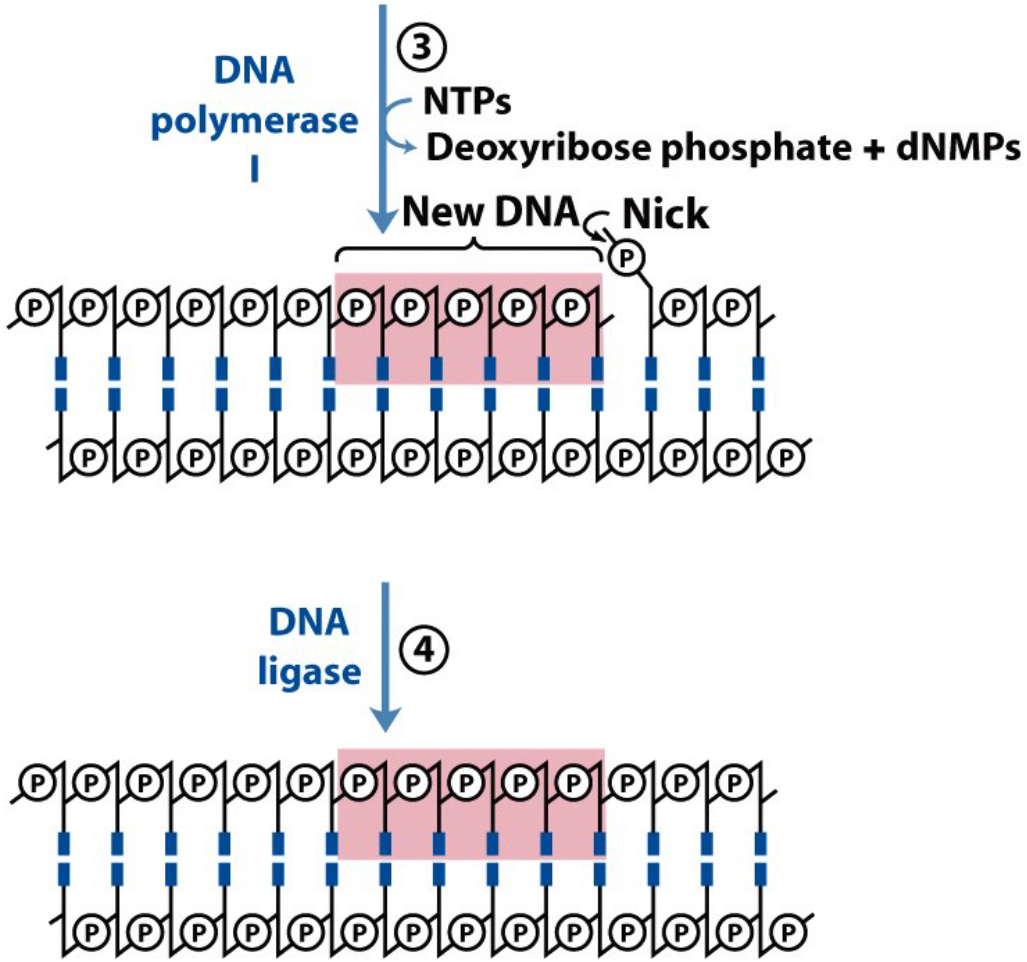
Proteins Required for Methyl-Directed Mismatch Repair



Part I

Base-Excision Repair

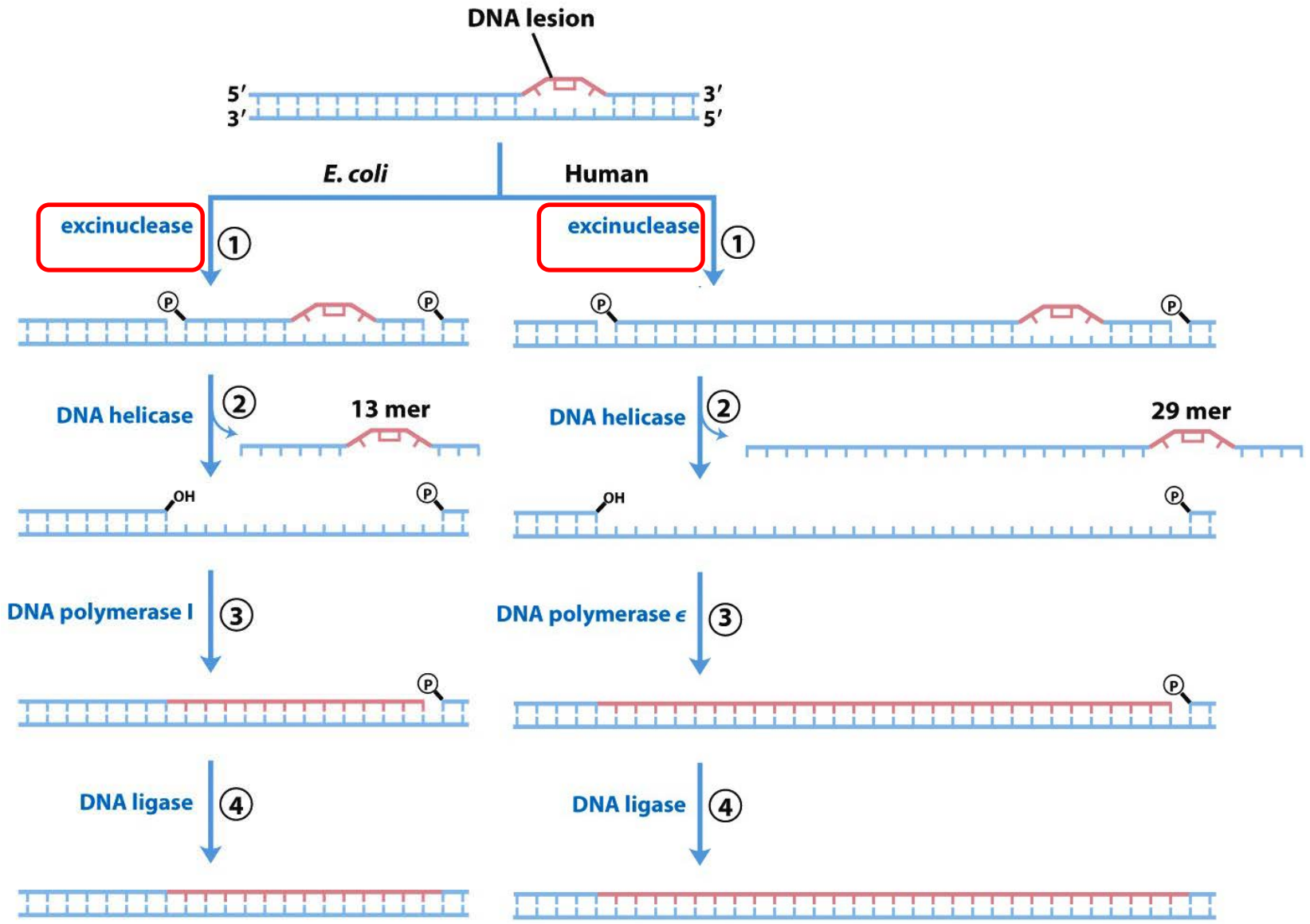




AP site: Apurinic or apyrimidinic site, or abasic site

Part I

Nucleotide-Excision Repair



Excinuclease

Multisubunit complex (UvrA, UvrB and UvrC)

Catalyze two specific endonucleolytic cleavages

Part I

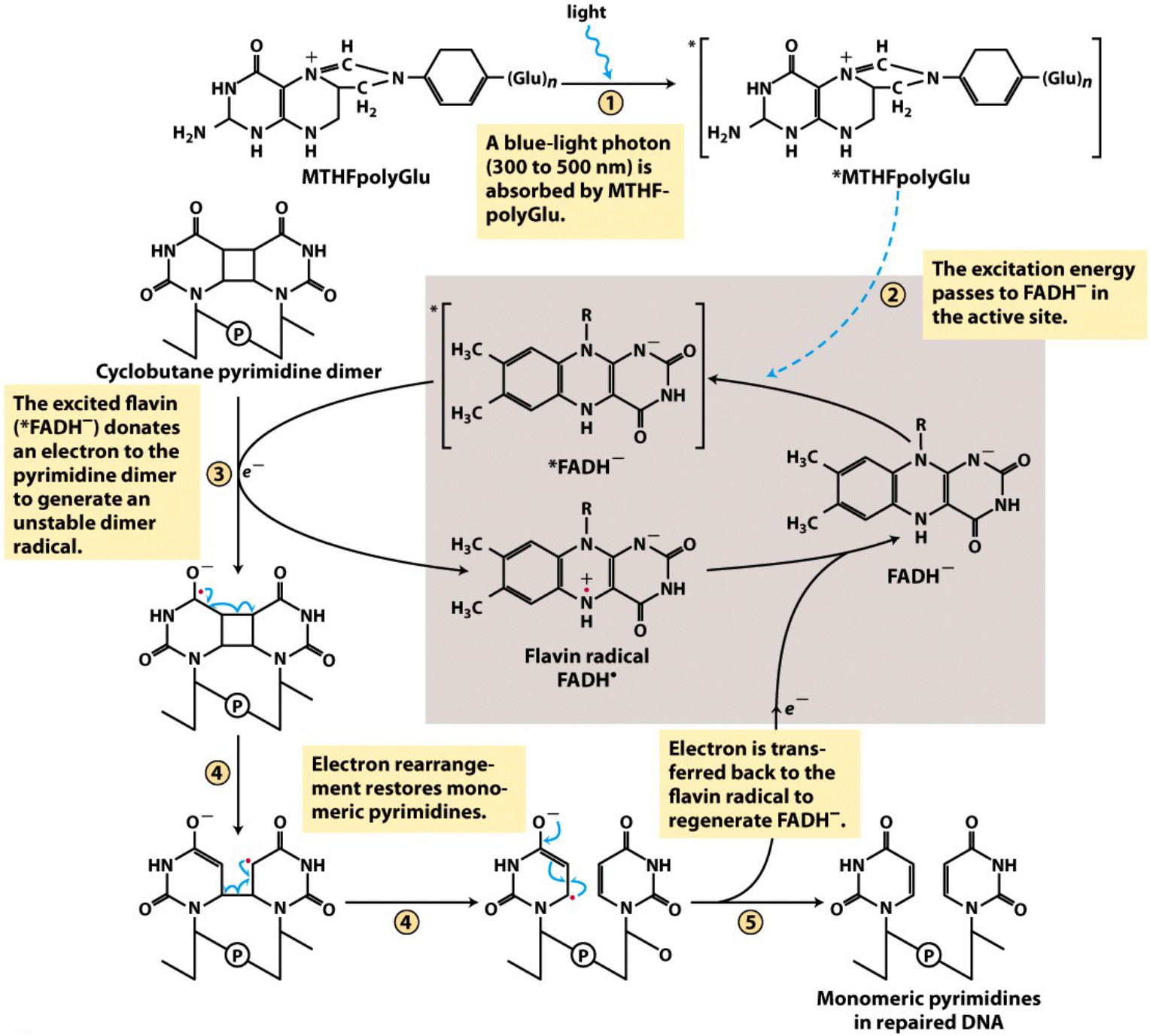
Direct Repair

DNA photolyase

Two chromophores:

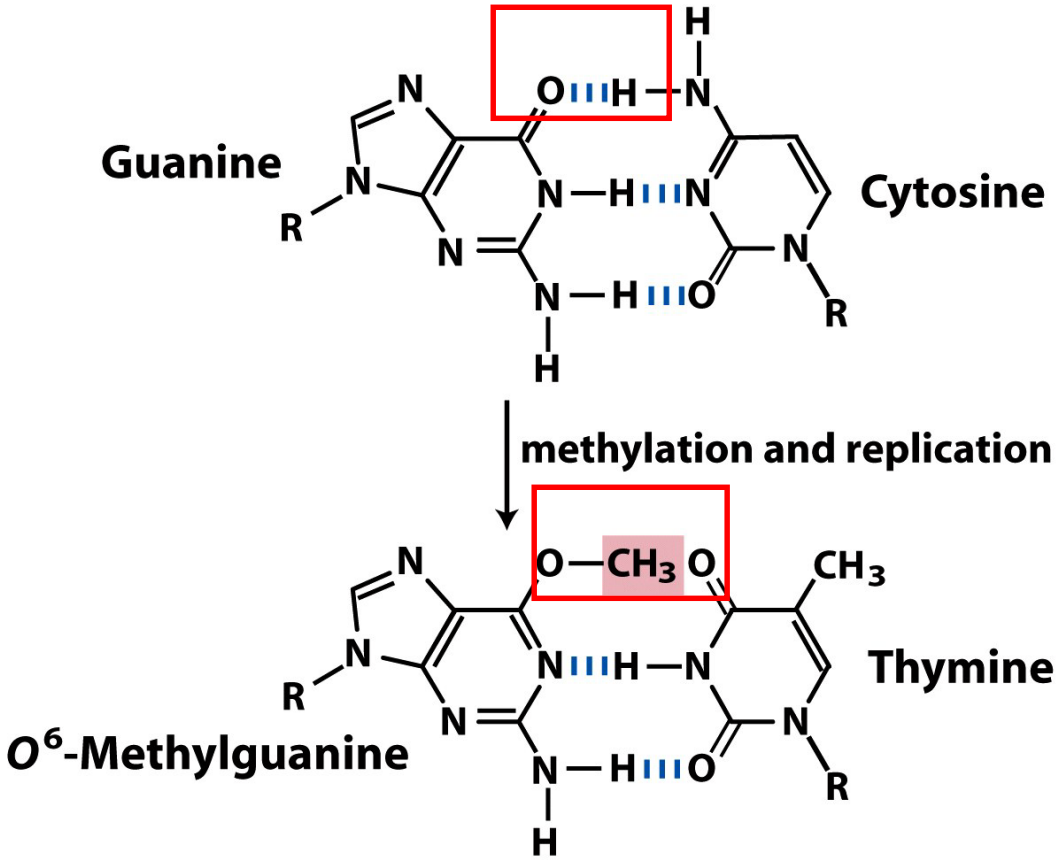
MTHFpolyglu (folate)

FADH$^-$

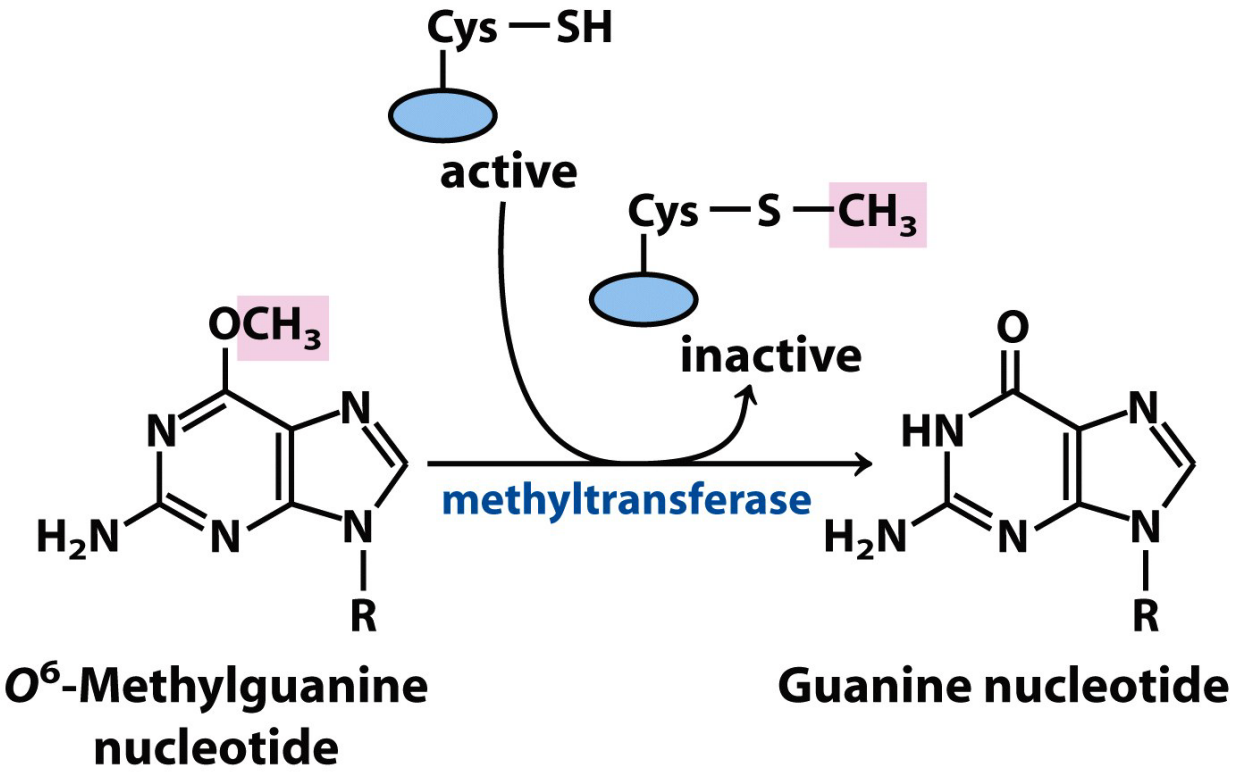


Part I

Direct Repair of O^6-methylguanidine



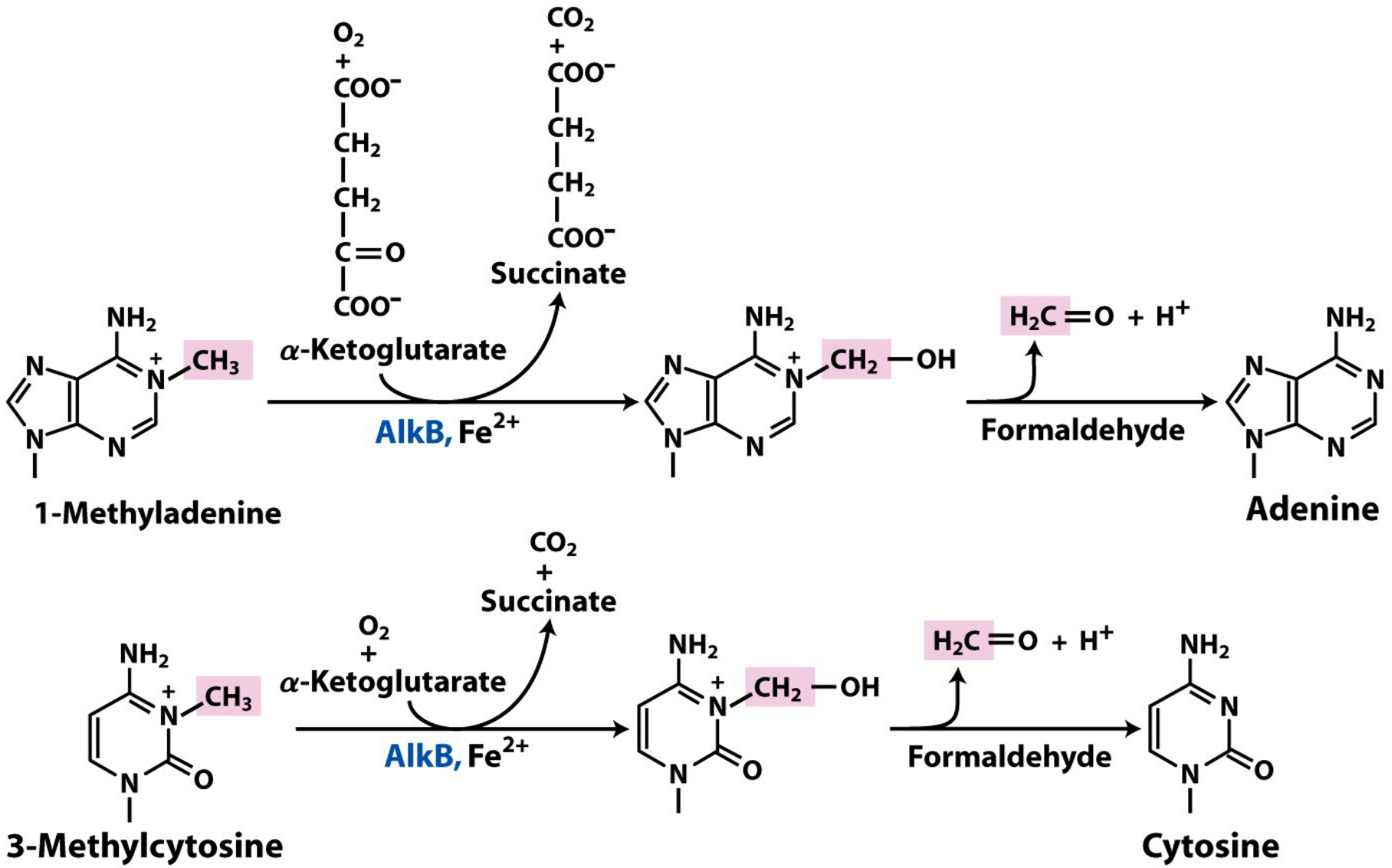
Mutation



Repair

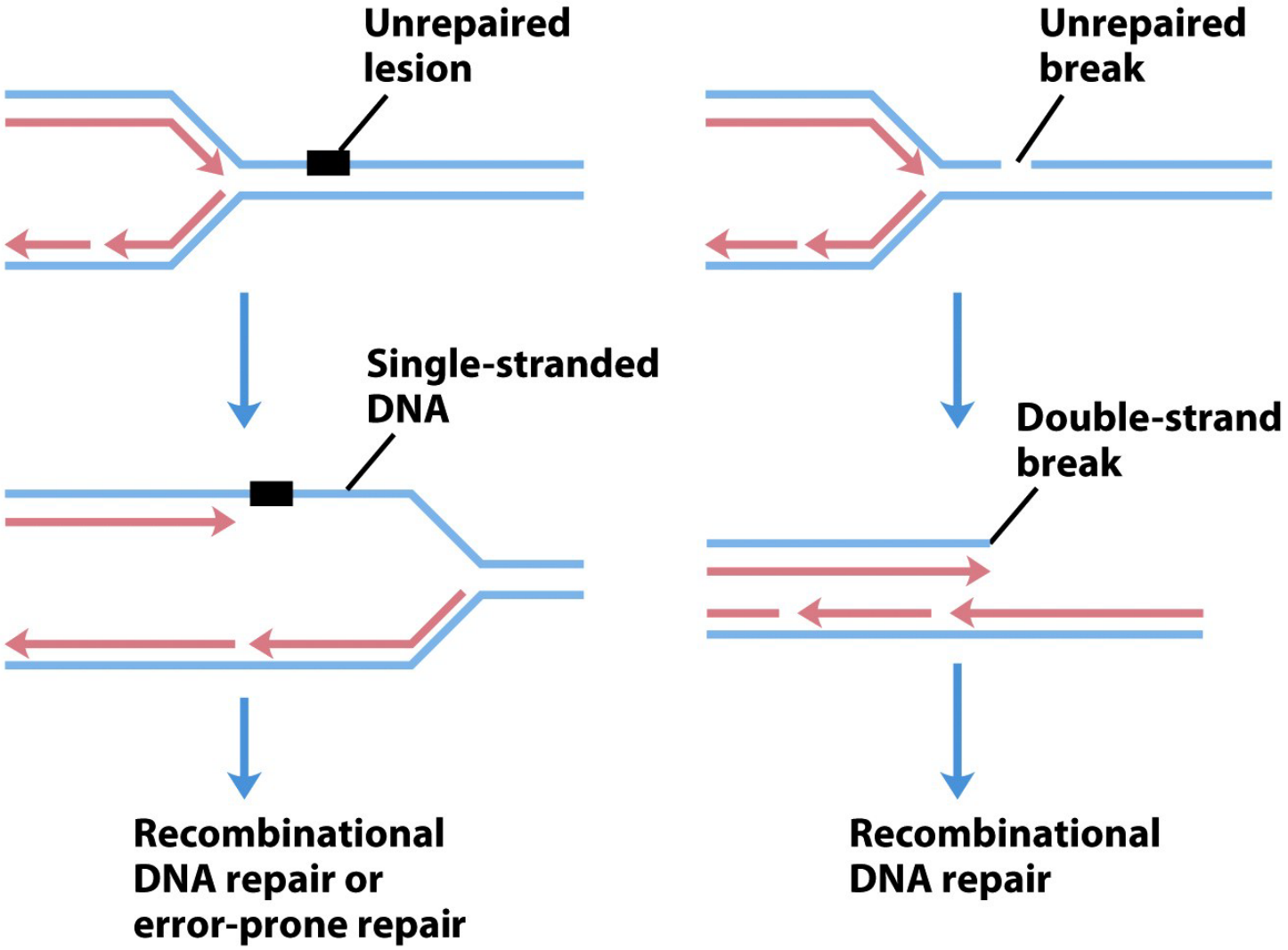
Part I

Direct Repair of Alkylated Bases by AlkB



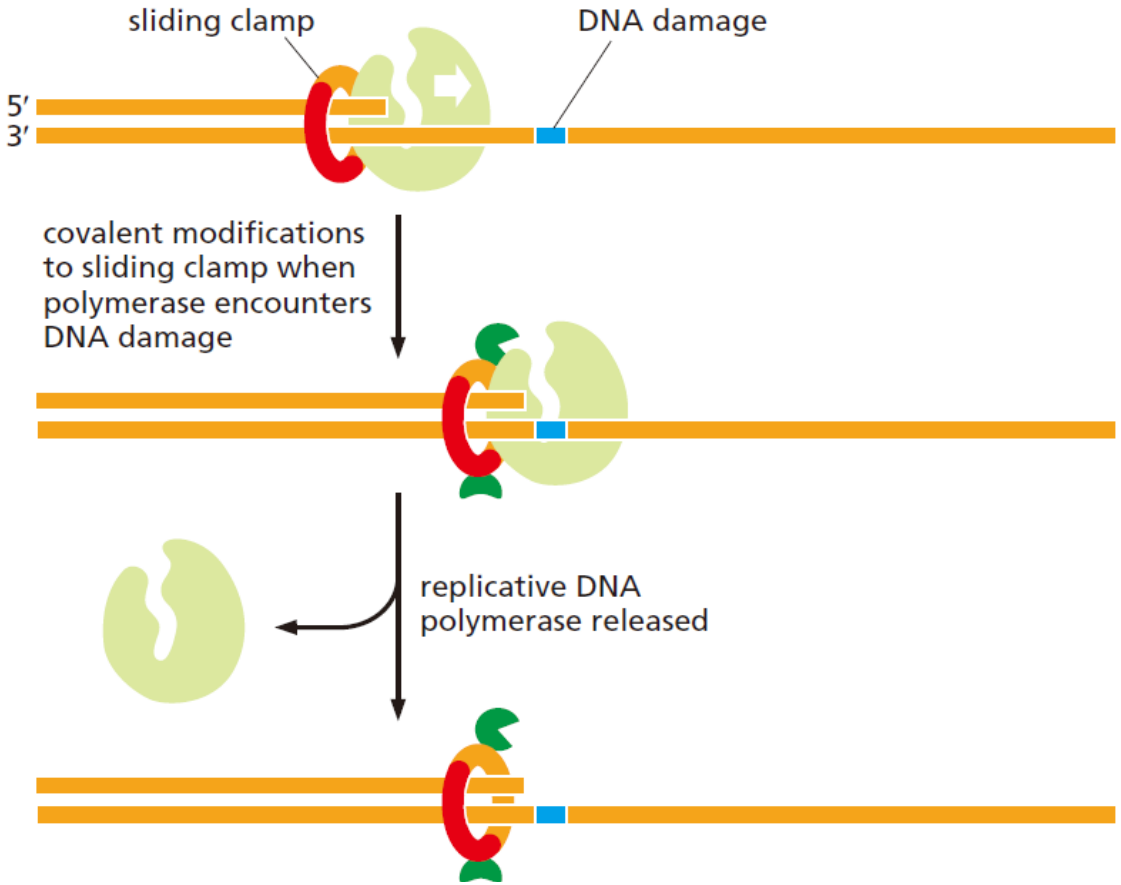
Part I

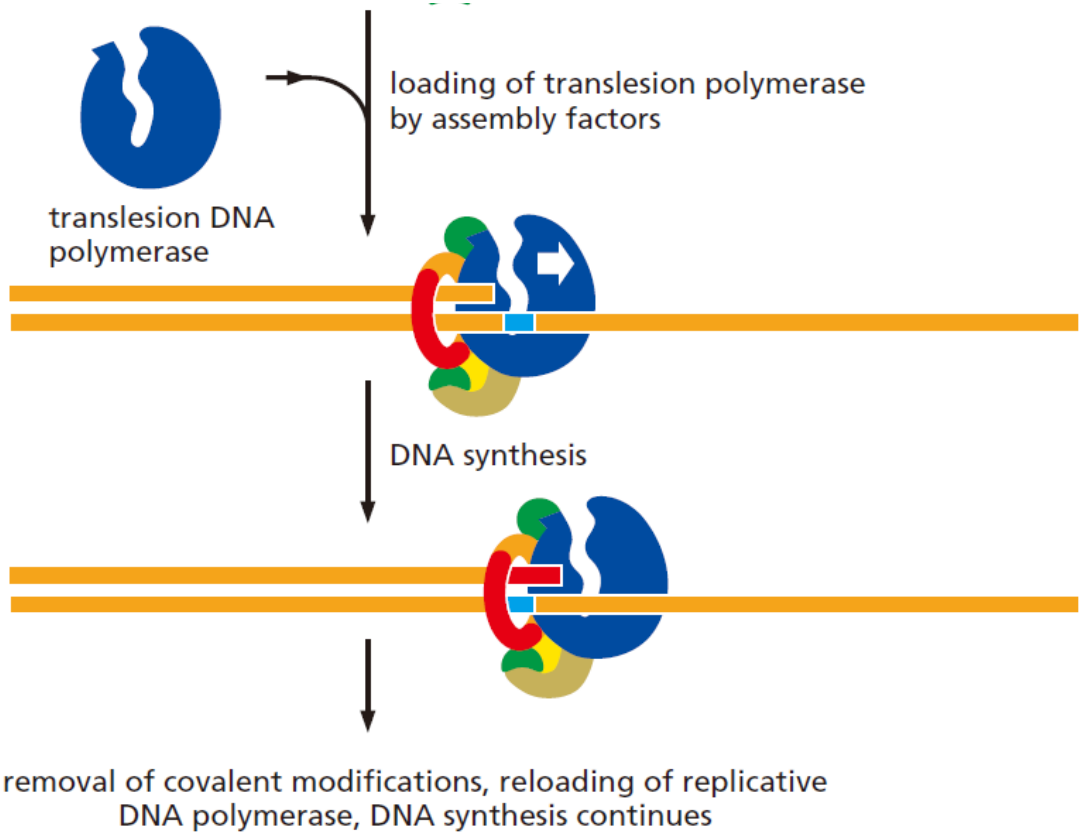
Interaction of Replication Forks with DNA Damage Can Lead to Error-Prone Translesion DNA Synthesis



Part I

Translesion DNA Polymerases Can Use Damaged Templates

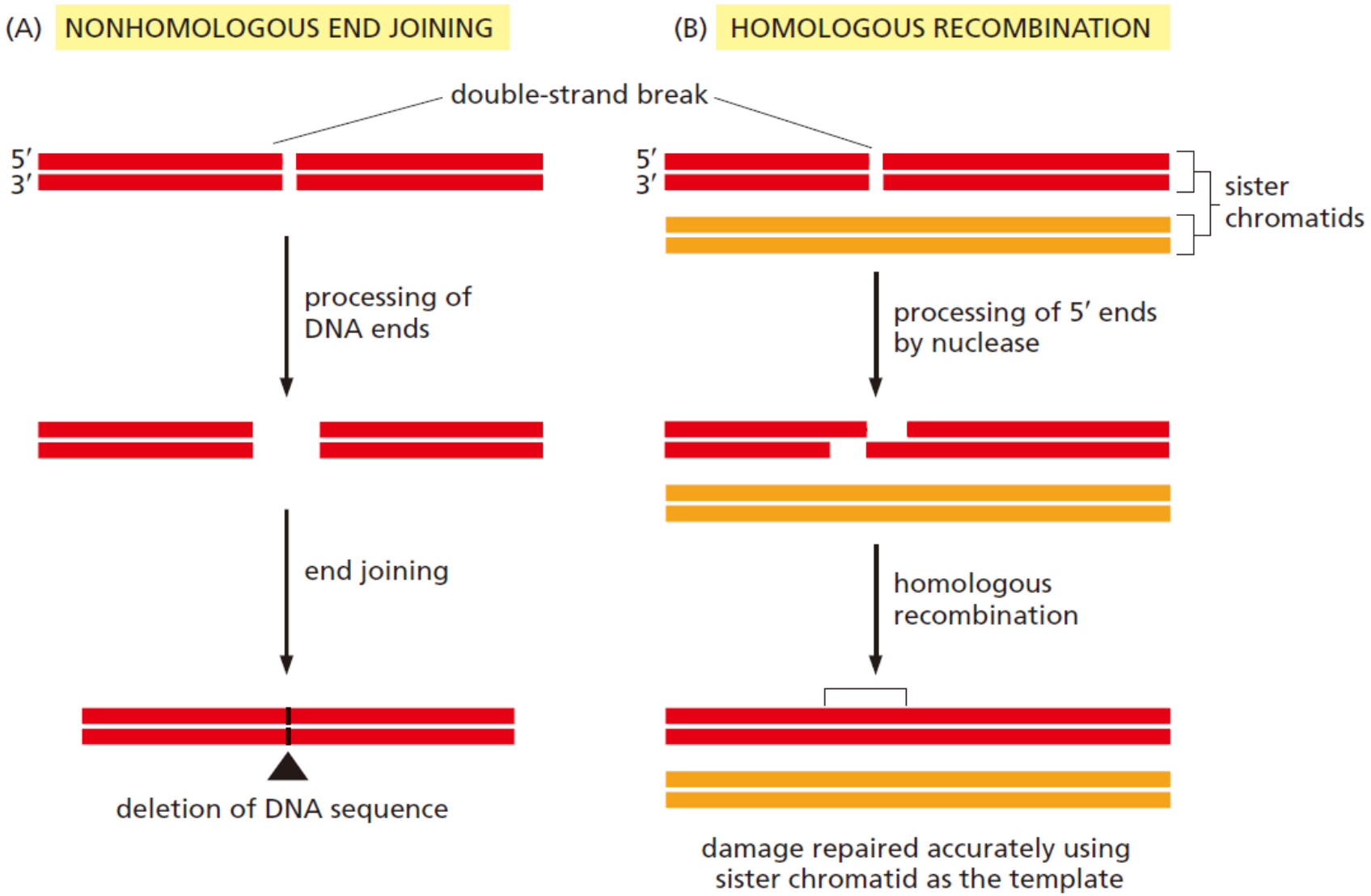




Translesion DNA Synthesis (TLS) is part of SOS Response.

Part I

Two Ways to Repair Double-Strand Breaks



Summary

Mismatch repair in E. Coli is directed by transient nonmethylation of the (5’)GATC sequence on the newly synthesized strand.

Base-excision repair systems recognize and repair damage caused by environmental reagents and spontaneous reaction of nucleotides.

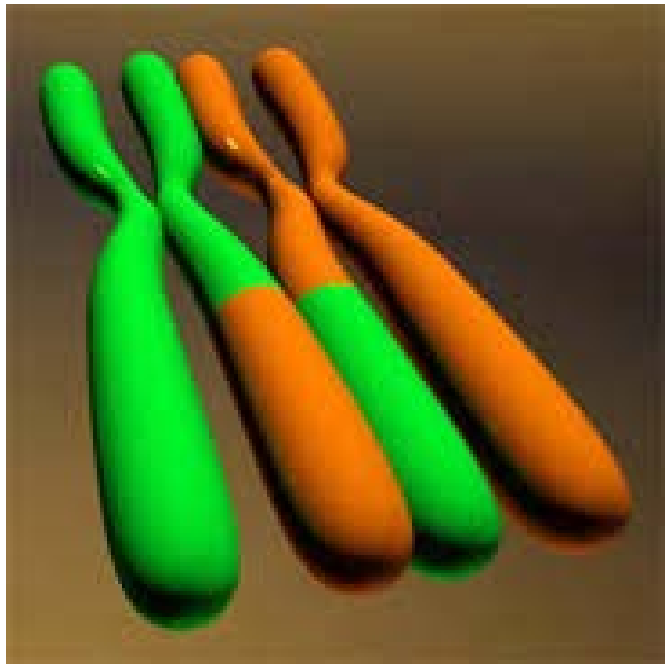
Nucleotide-excision repair systems recognize and remove a variety of bulky lesion and pyrimidine dimers.

Direct repair system works through direct reversal of the reaction causing the damage.

In bacteria, error-prone translesion DNA synthesis occurs in response to very heavy DNA damage.

Two ways to repair double-strand breaks: nonhomologous end joining and homologous recombination.

Part II. DNA Recombination



Part I

Genetic Recombination

Homologous Genetic Recombination

Site-Specific Recombination

DNA Transposition

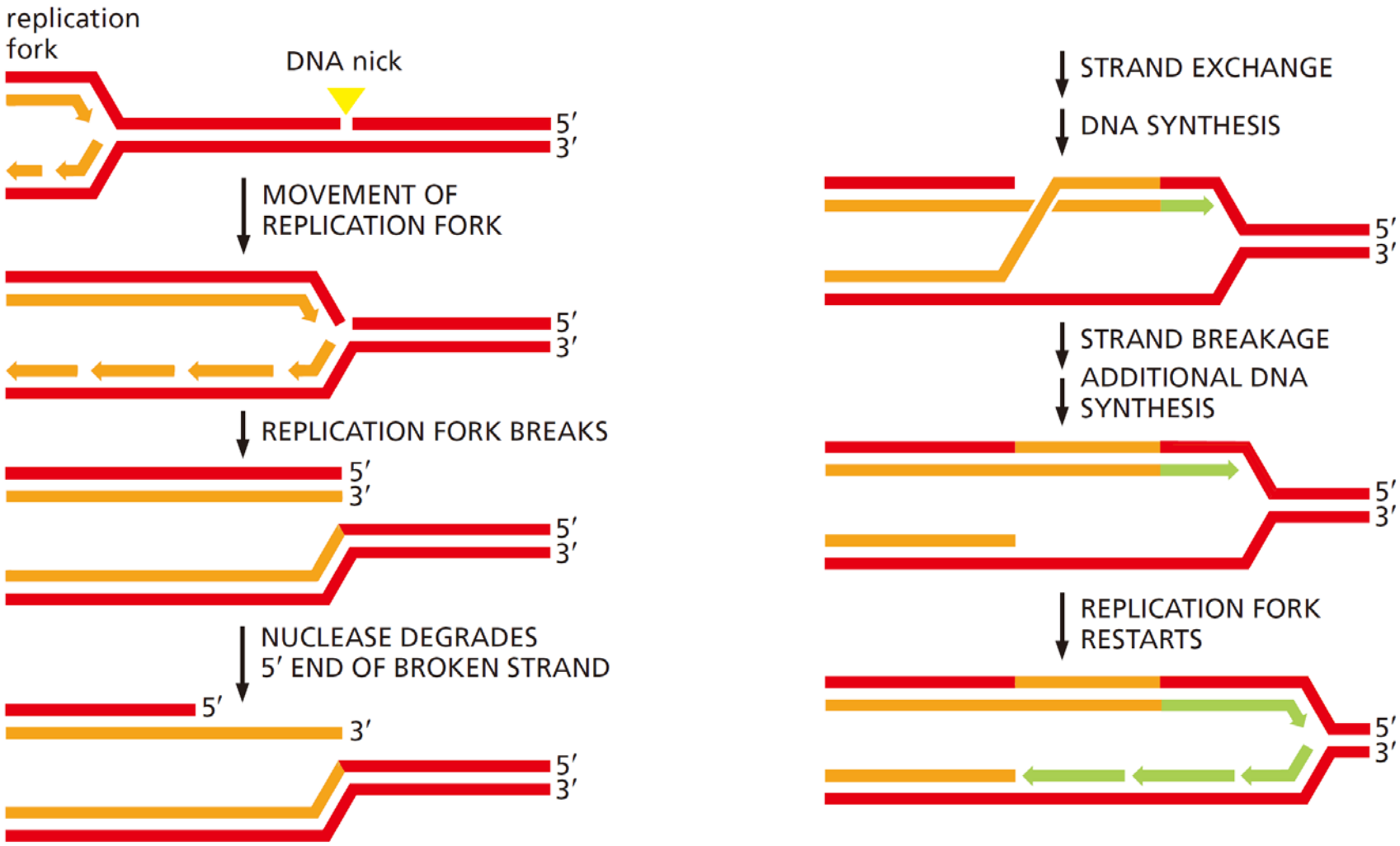
Homologous Recombination Has Several Functions

In bacteria, it is mainly recombinational repair, which is directed at the reconstruction of replication forks stalled at the site of DNA damage.

In eukaryotes, homologous recombination has several roles in replication and cell division, including the repair of stalled replication forks.
Recombination occurs with highest frequency during meiosis.

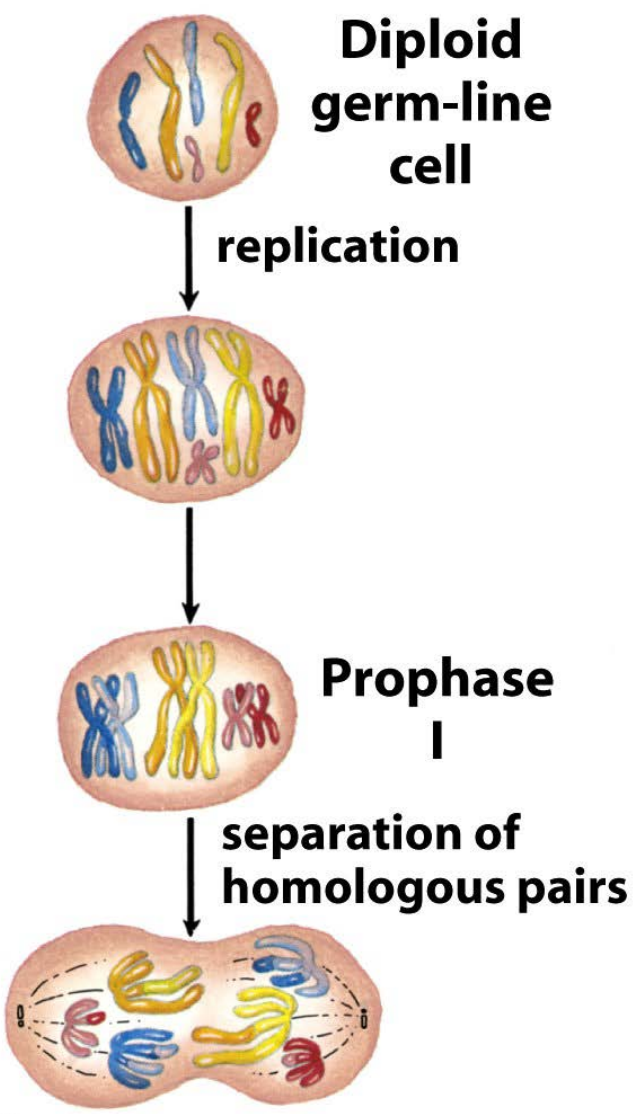
Part II

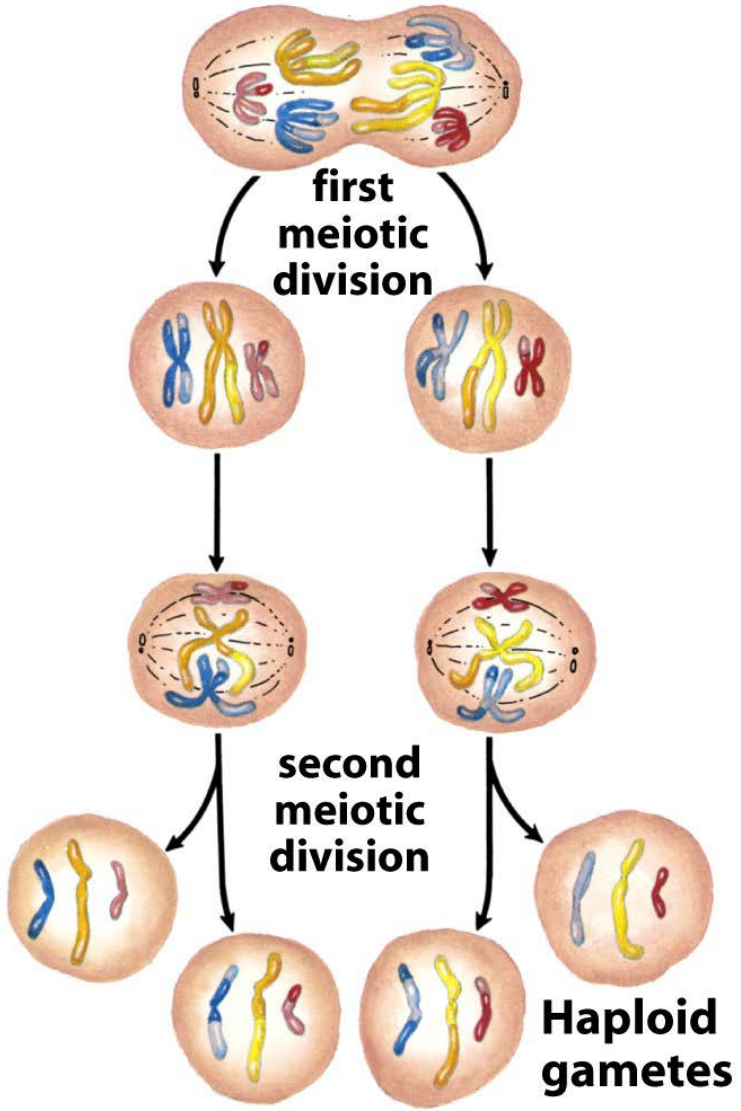
Repair of a Broken Replication Fork by Homologous Recombination



Part II

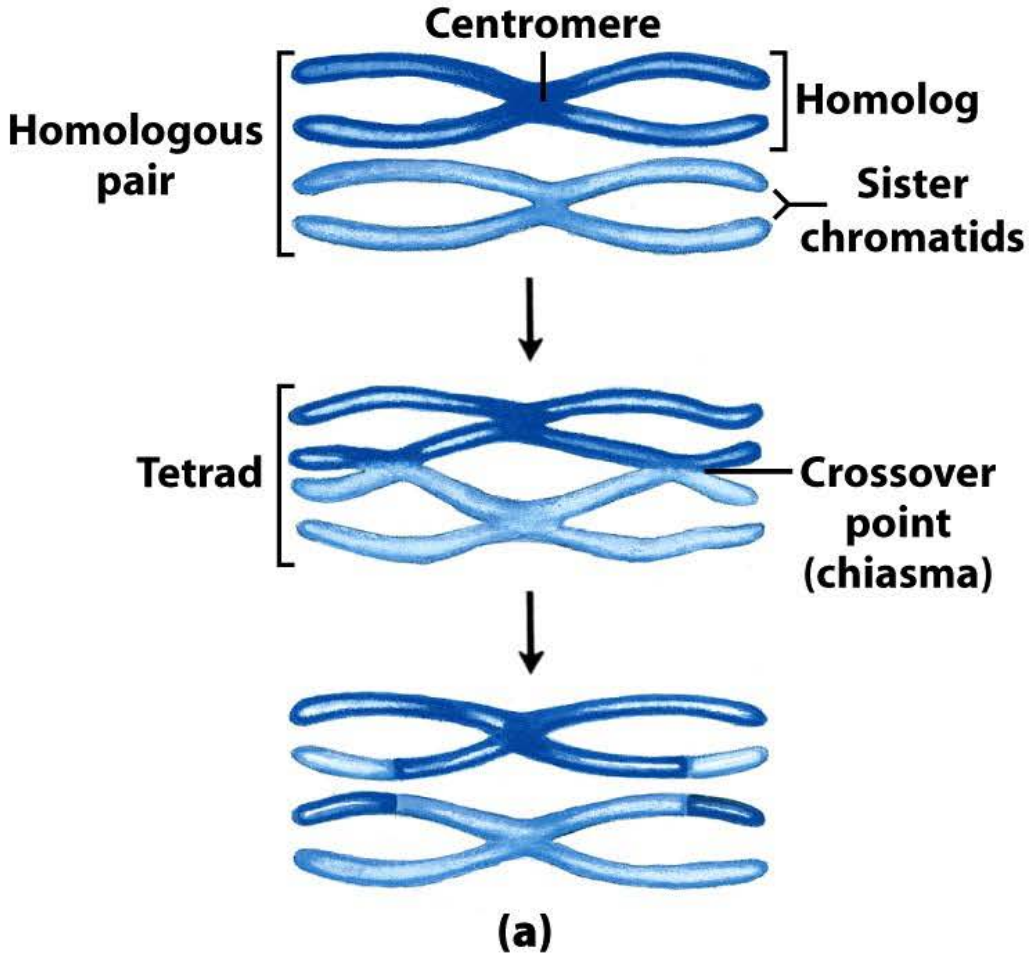
Meiosis in Animal Germ Cells

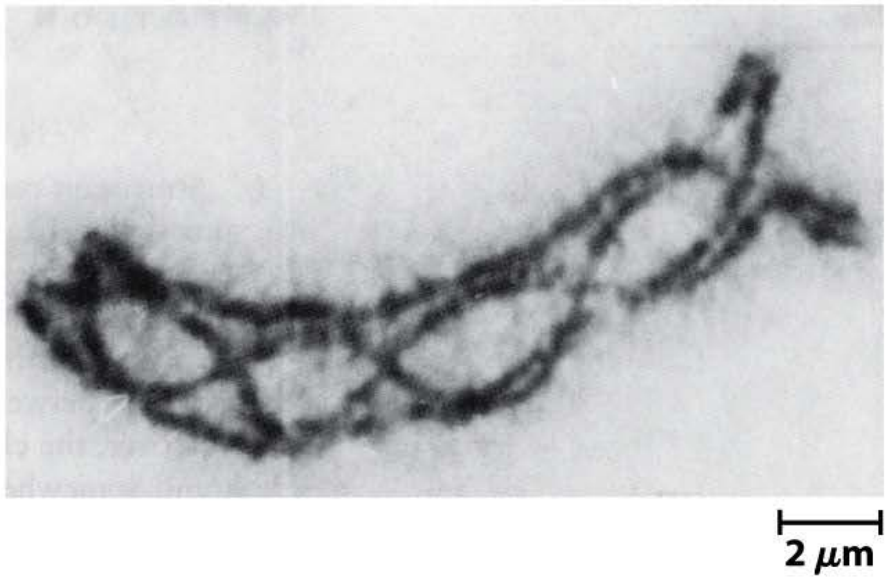


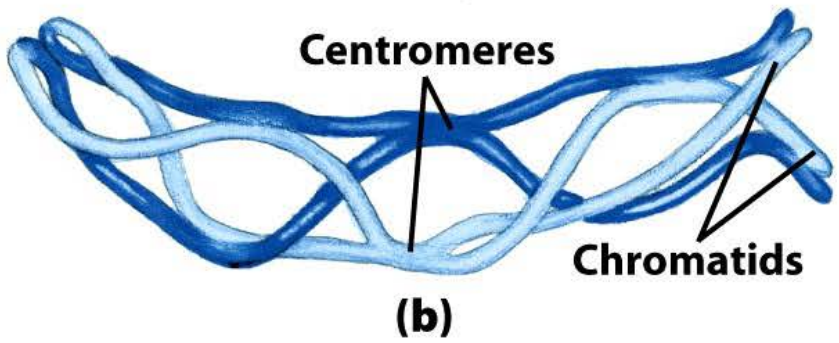


Part II

Crossing Over







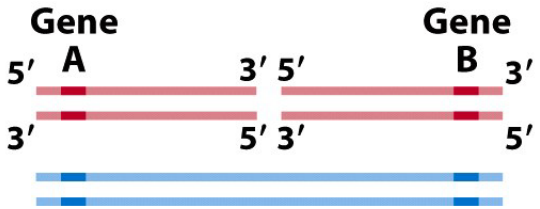
Hot Spots

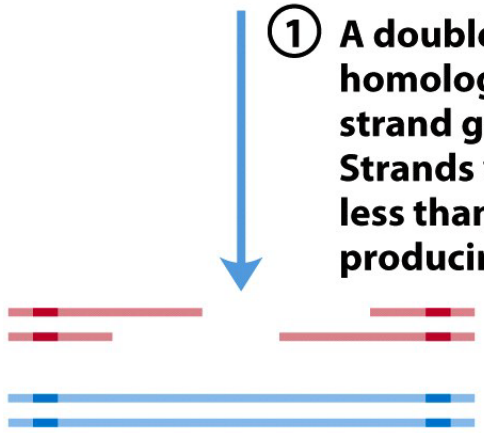
Homologous Recombination Has at Least Three Identifiable Functions

- It contributes to the repair of several types of DNA damage
- It provides, in eukaryotic cells, a transient physical link between chromatids that promotes the orderly segregation of chromosomes at the first meiotic cell division
- It enhances genetic diversity in population

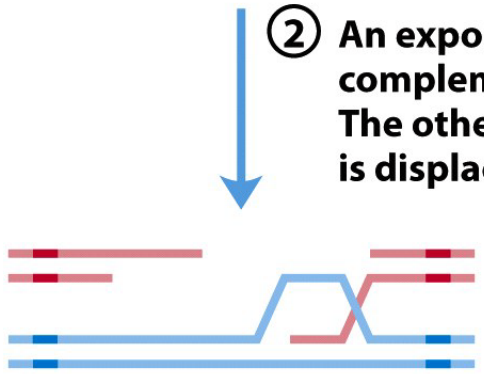
Part II

Recombination During Meiosis





① A double-strand break in one of two homologs is converted to a double-strand gap by the action of exonucleases. Strands with 3′ ends are degraded less than those with 5′ ends, producing 3′ single-strand extensions.

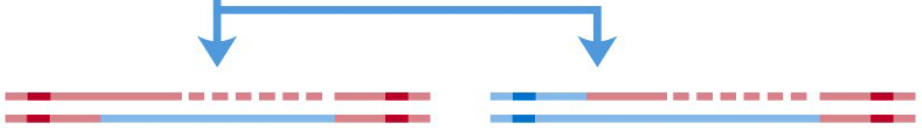


② An exposed 3′ end pairs with its complement in the intact homolog. The other strand of the duplex is displaced.

③ The invading 3′ end is extended by DNA polymerase plus branch migration, eventually generating a DNA molecule with two crossovers called Holliday intermediates.

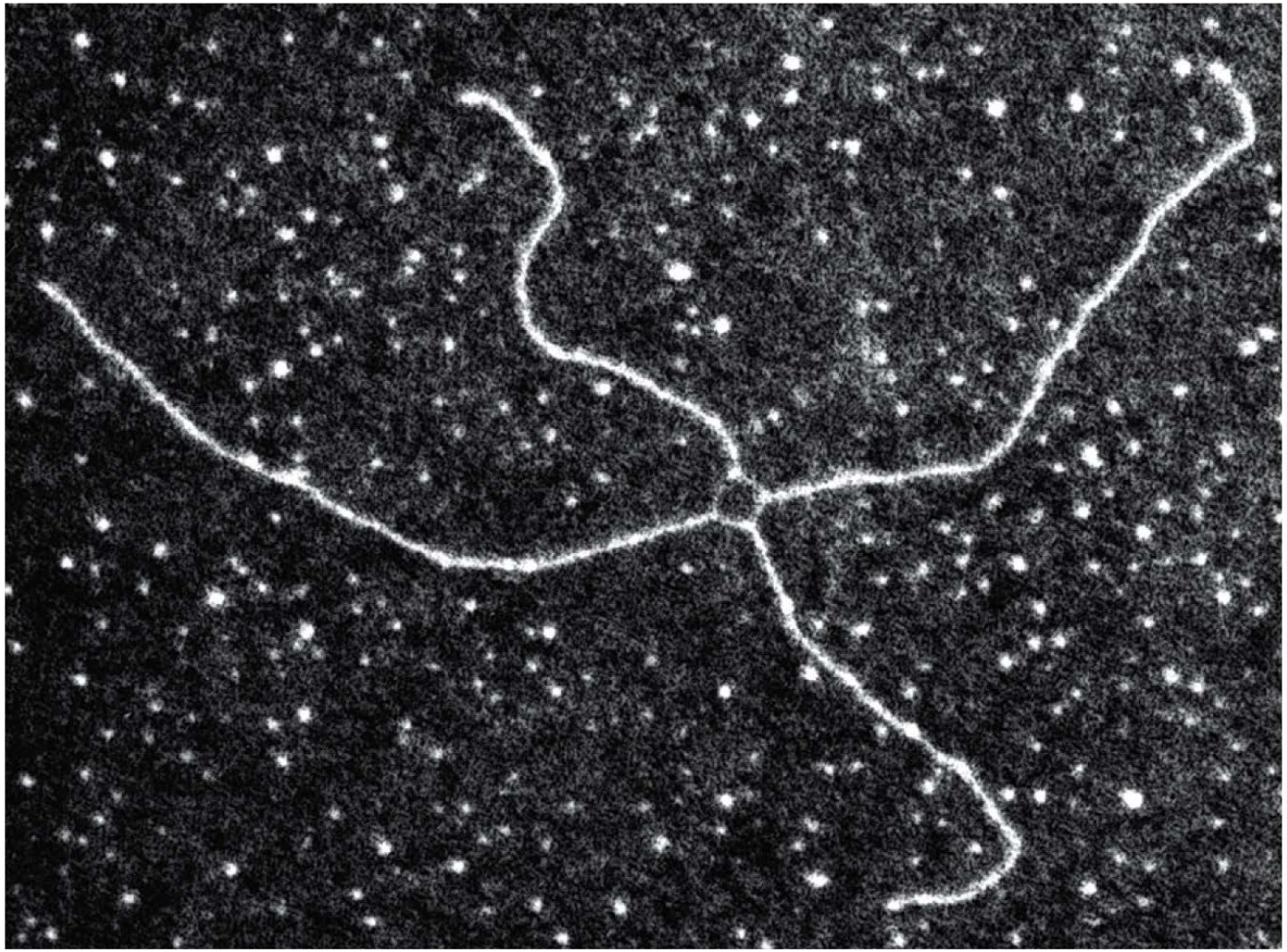
④ Further DNA replication replaces the DNA missing from the site of the original double-strand break.

⑤ Cleavage of the Holliday intermediates by specialized nucleases generates either of the two recombination products. In product set 2, the DNA on either side of the region undergoing repair is recombined.



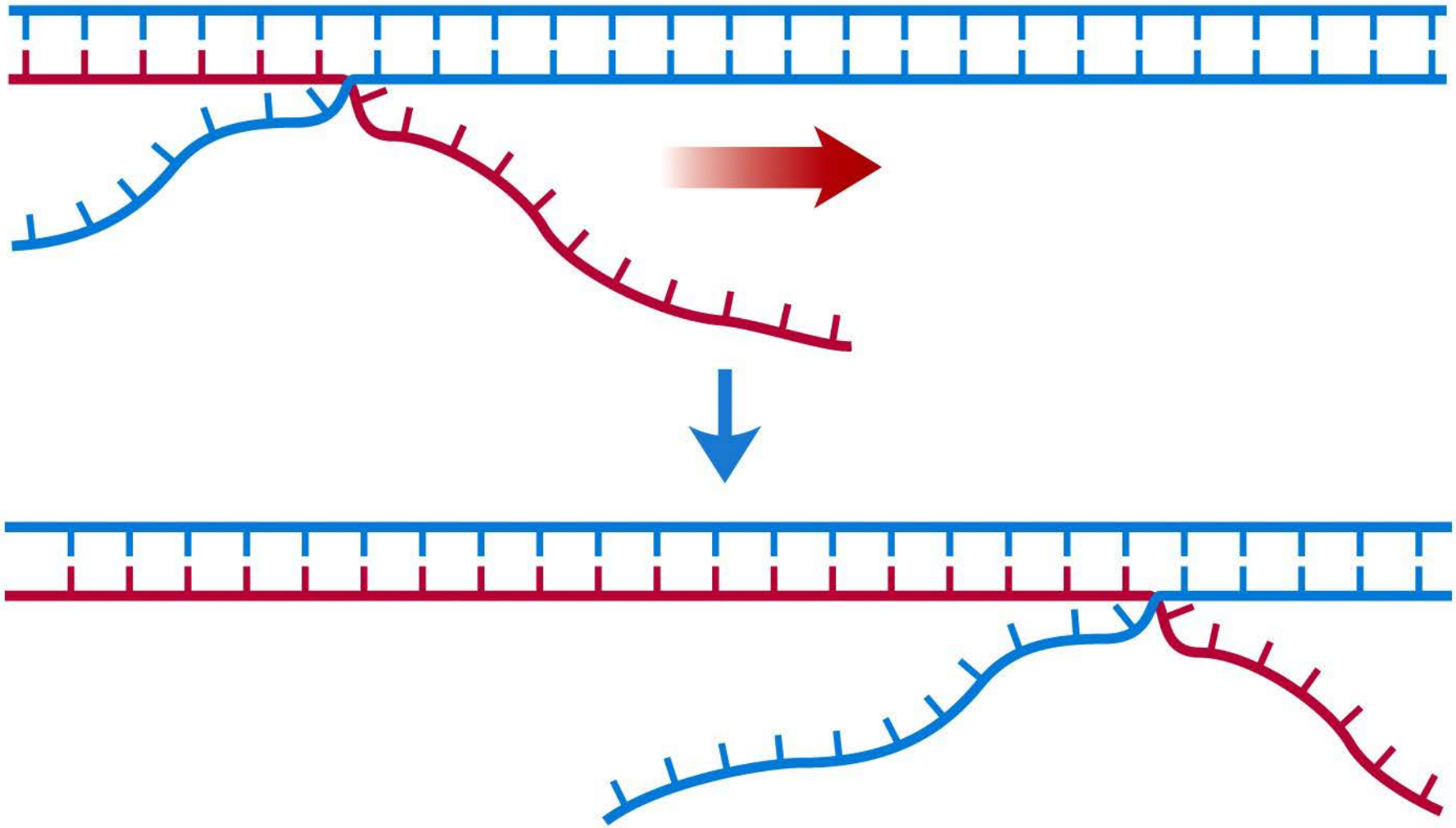
Part II

Holliday Intermediates Are a Feature of Homologous Recombination Pathways in All Organisms



Part II

Branch Migration



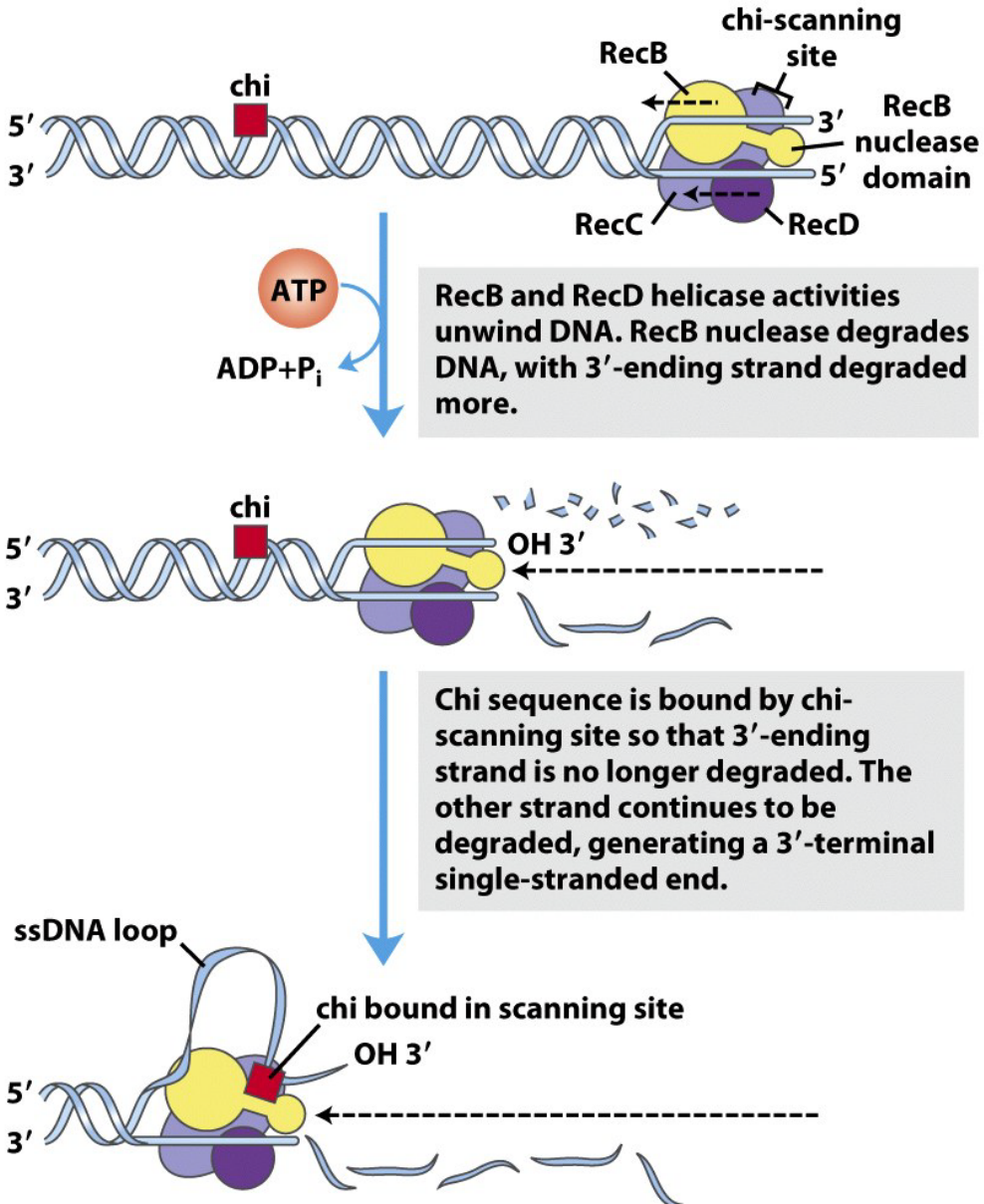
Part II

Recombination Requires a Host of Enzymes and Other Proteins

- In E. Coli, RecB, RecC and RecD form a heterotrimeric RecBCD, which has both helicase and nuclease activities.

Part II

Helicase and Nuclease Activities of the RecBCD Enzyme



RecB and RecD are helicase motors:
RecB moves 3’-to-5’ on one strand
RecD moves 5’-to-3’ on the other strand

Chi: (5’)GCTGGTGG
Chi binds tightly to a site on RecC subunit

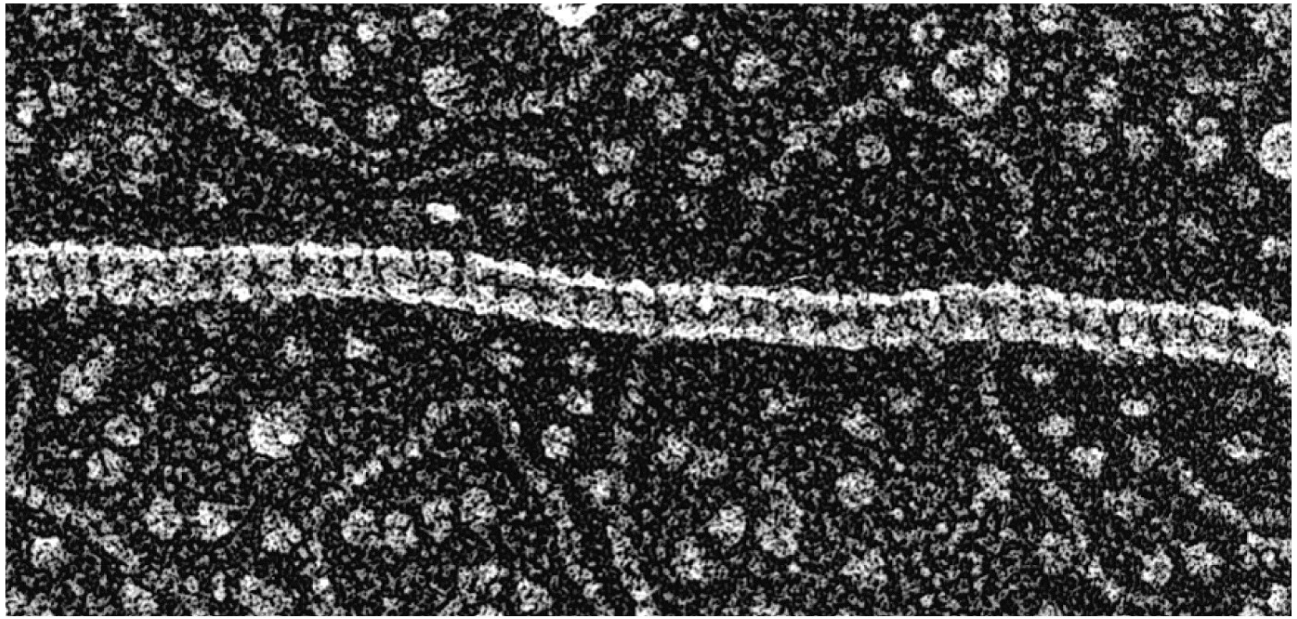
Recombination Requires a Host of Enzymes and Other Proteins

- In E. Coli, RecD, RecC and RecD form a heterotrimeric RecBCD, which has both helicase and nuclease activities.

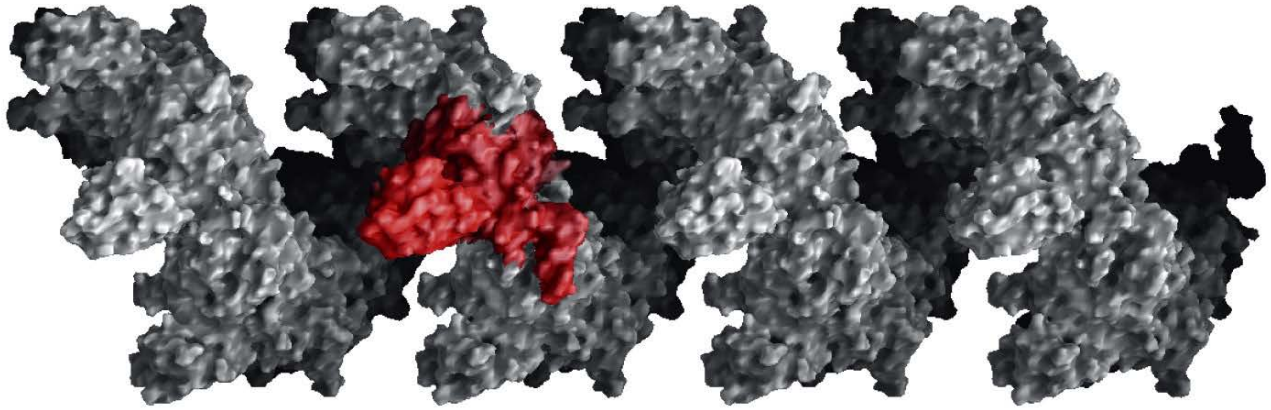
- The RecA promotes all central steps in homologous recombination process: the pairing of two DNAs, formation of Holliday intermediates, and branch migration.

Part II

The Active Form of RecA Protein Is an Ordered Helical Filament



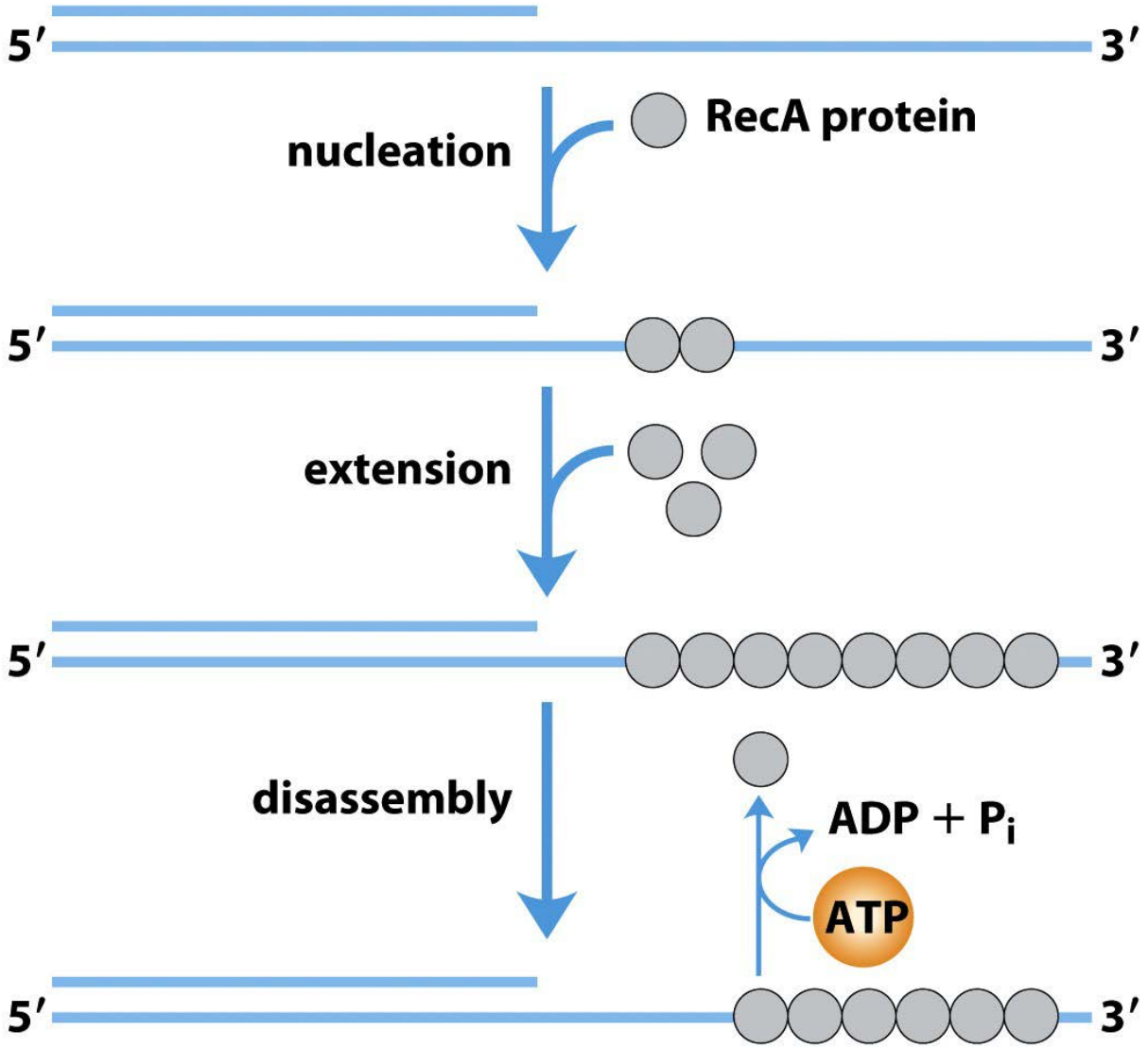
Up to several thousand subunits



24-subunit filament

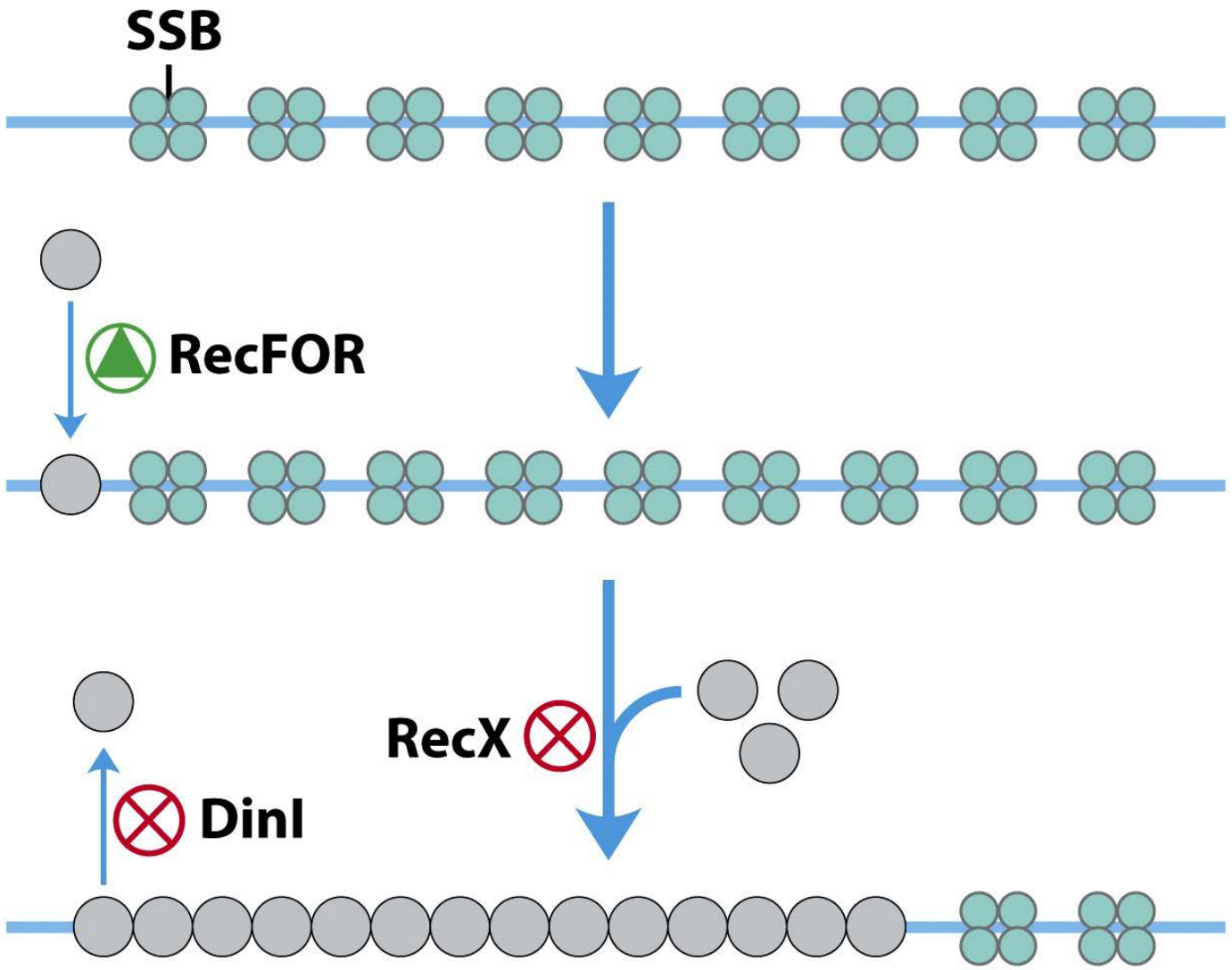
Part II

RecA Filaments Are Extended or Disassembled in the 5’-to-3’ Direction



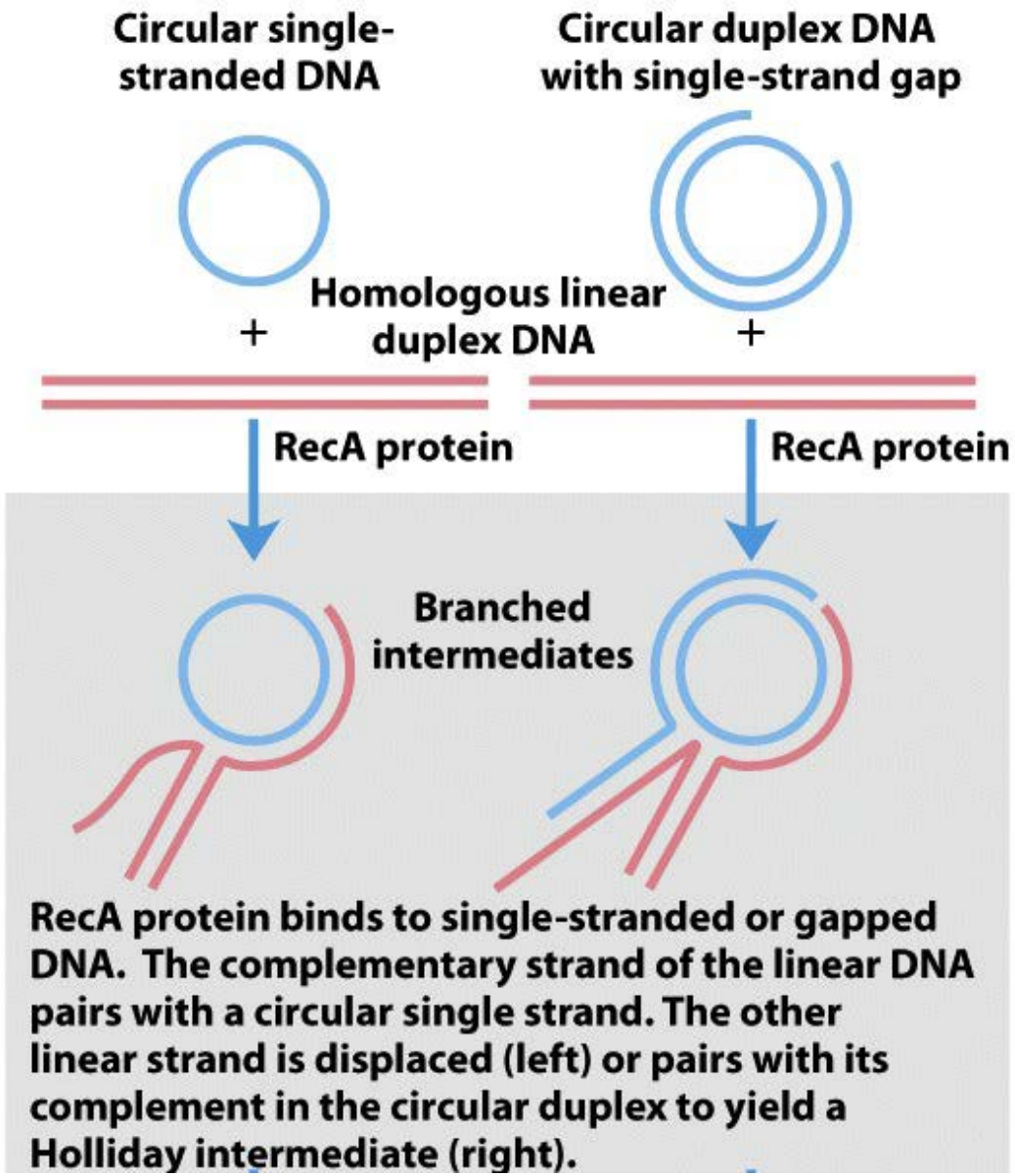
Part II

Filament Assembly Is Assisted by RecF, RecO and RecR, and Inhibited by RecX

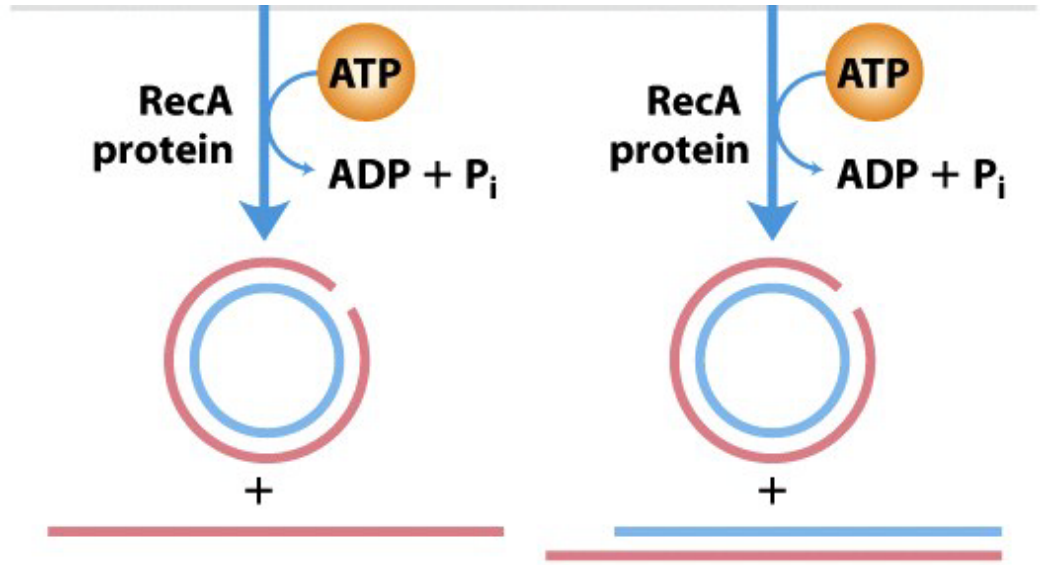


Part II

RecA-Promoted DNA Strand Exchange in vitro



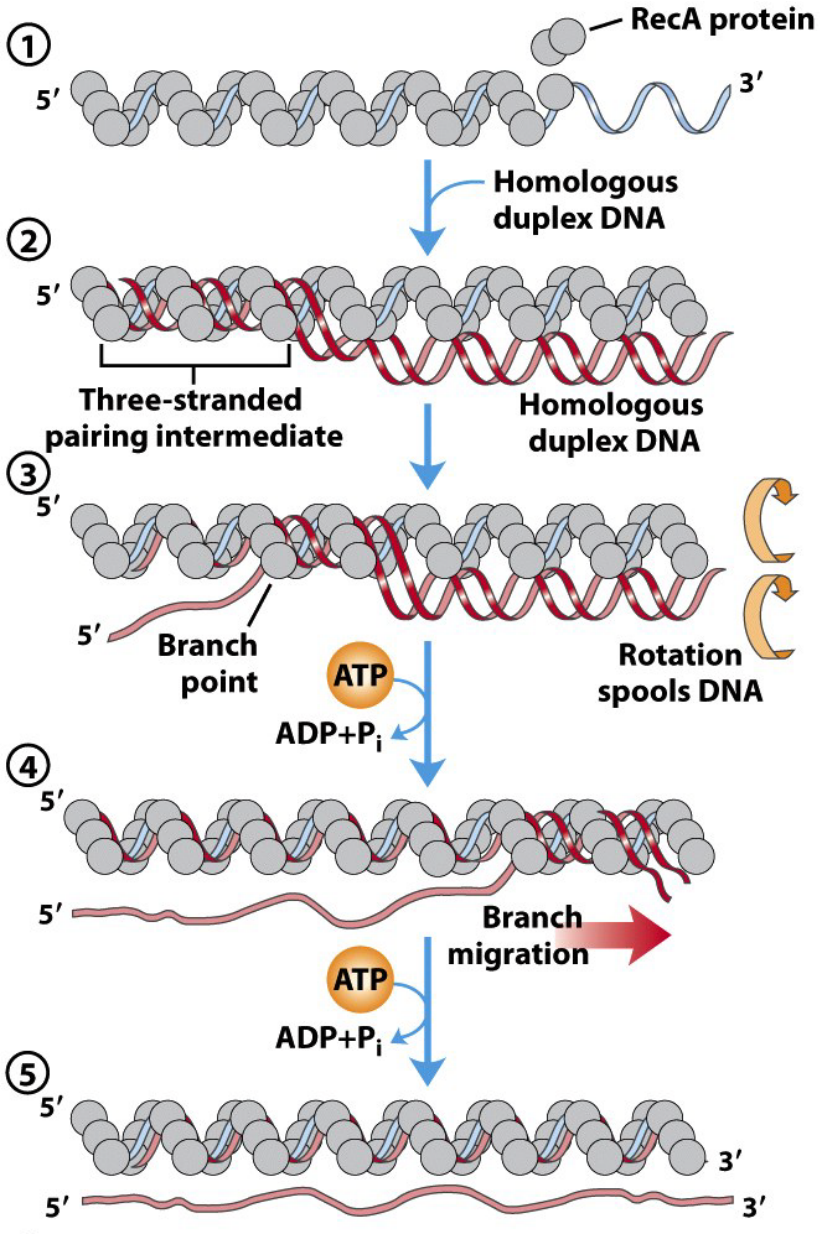
RecA protein binds to single-stranded or gapped DNA. The complementary strand of the linear DNA pairs with a circular single strand. The other linear strand is displaced (left) or pairs with its complement in the circular duplex to yield a Holliday intermediate (right).



Continued branch migration yields a circular duplex with a nick and either a displaced linear strand (left) or a partially single-stranded linear duplex (right).

Part II

Model for RecA-Mediated DNA Strand Exchange



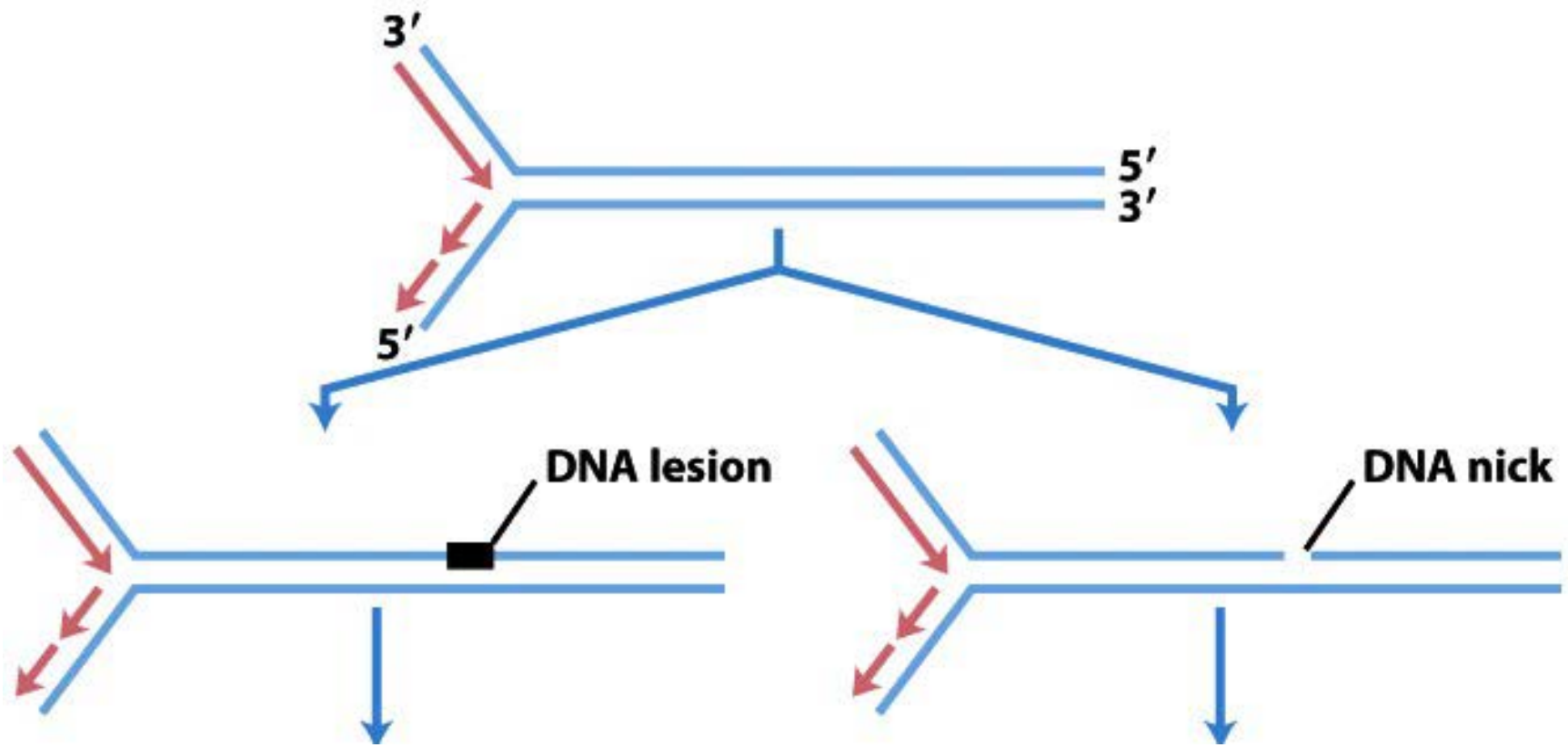
Part II

Recombination Requires a Host of Enzymes and Other Proteins

- In E. Coli, RecD, RecC and RecD form a heterotrimeric RecBCD, which has both helicase and nuclease activities.
- The RecA promotes all central steps in homologous recombination process: the pairing of two DNAs, formation of Holliday intermediates, and branch migration.
- The RuvA and RuvB (*r*epair of *UV* damage) form a complex that binds to Holliday complex, displaces RecA proteins, and promotes branch migration at higher rates than does RecA.
- Nucleases, often called resolvases, specifically cleave Holliday intermediates. RuvC is one of the at least two such nucleases in E. Coli.

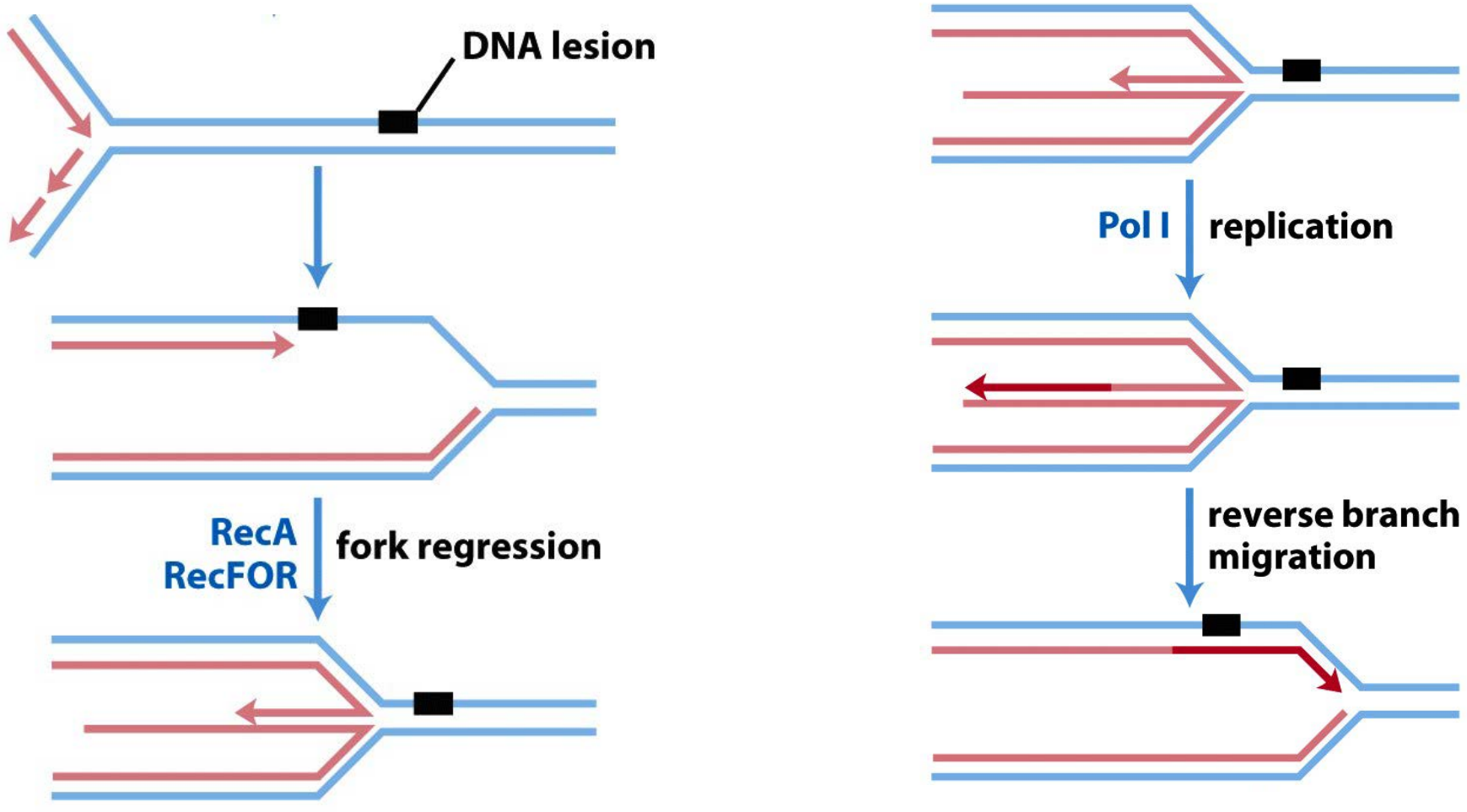
Part II

All Aspects of DNA Metabolism Come Together to Repair Stalled Replication Forks



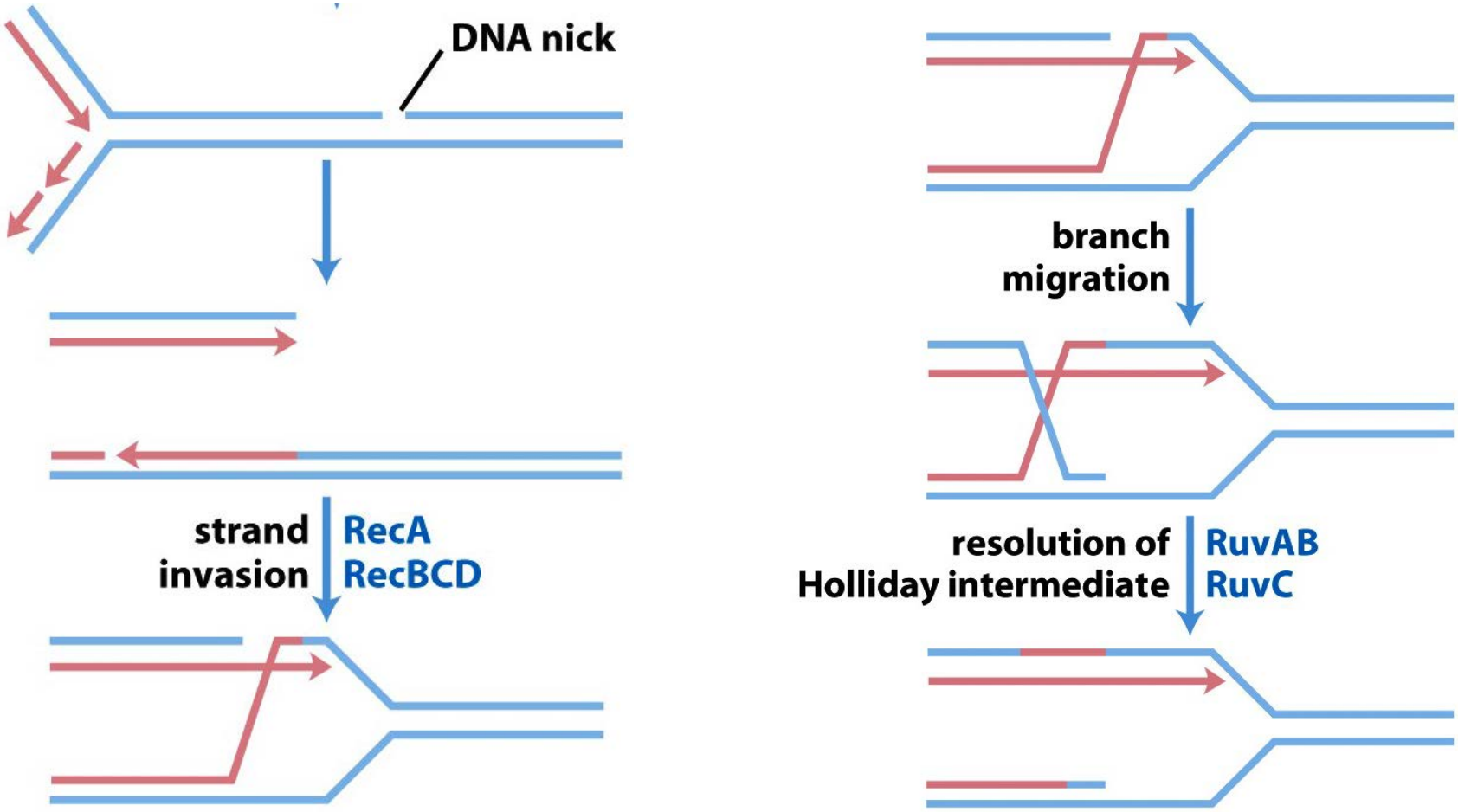
Part II

I. Recombinational DNA Repair of DNA Lesion in Stalled Replication Fork



Part II

II. Recombinational DNA Repair of DNA Nick in Stalled Replication Fork



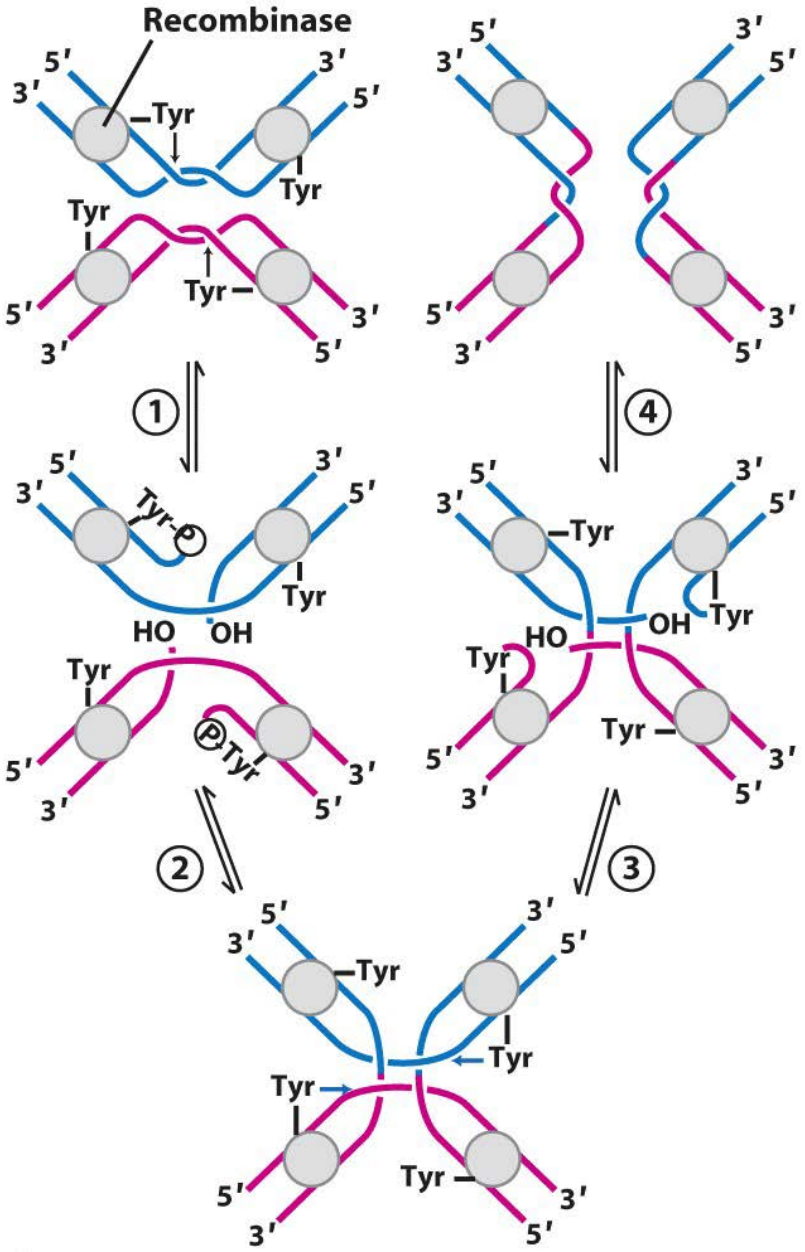
Origin-Independent Replication Restart

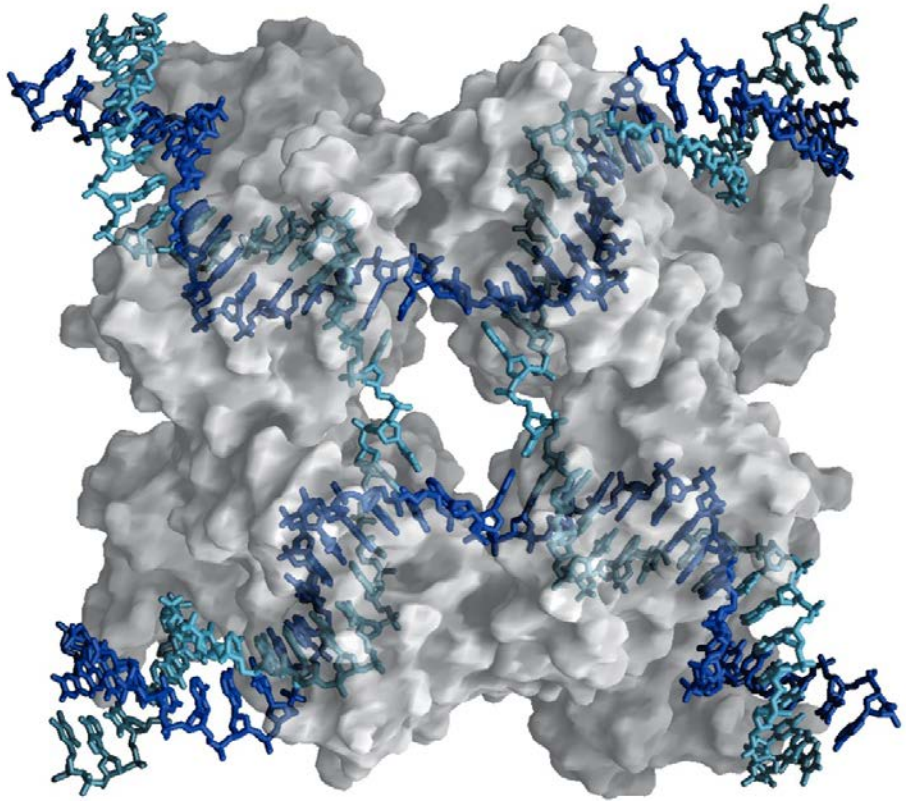
Site-Specific Recombination

- Each site-specific recombination system consists of
  - An enzyme called recombinase
  - A short (20 to 200 bp), unique DNA sequence where the recombinase acts
  - One or more of the auxiliary proteins that regulate the timing or outcoming
- Two general classes of site-specific recombination systems
  - Rely on Tyr in the active site
  - Rely on Ser in the active site

Part II

A Site-Specific Recombination Reaction





Active site: Tyr

Part II

Site-Specific Recombination

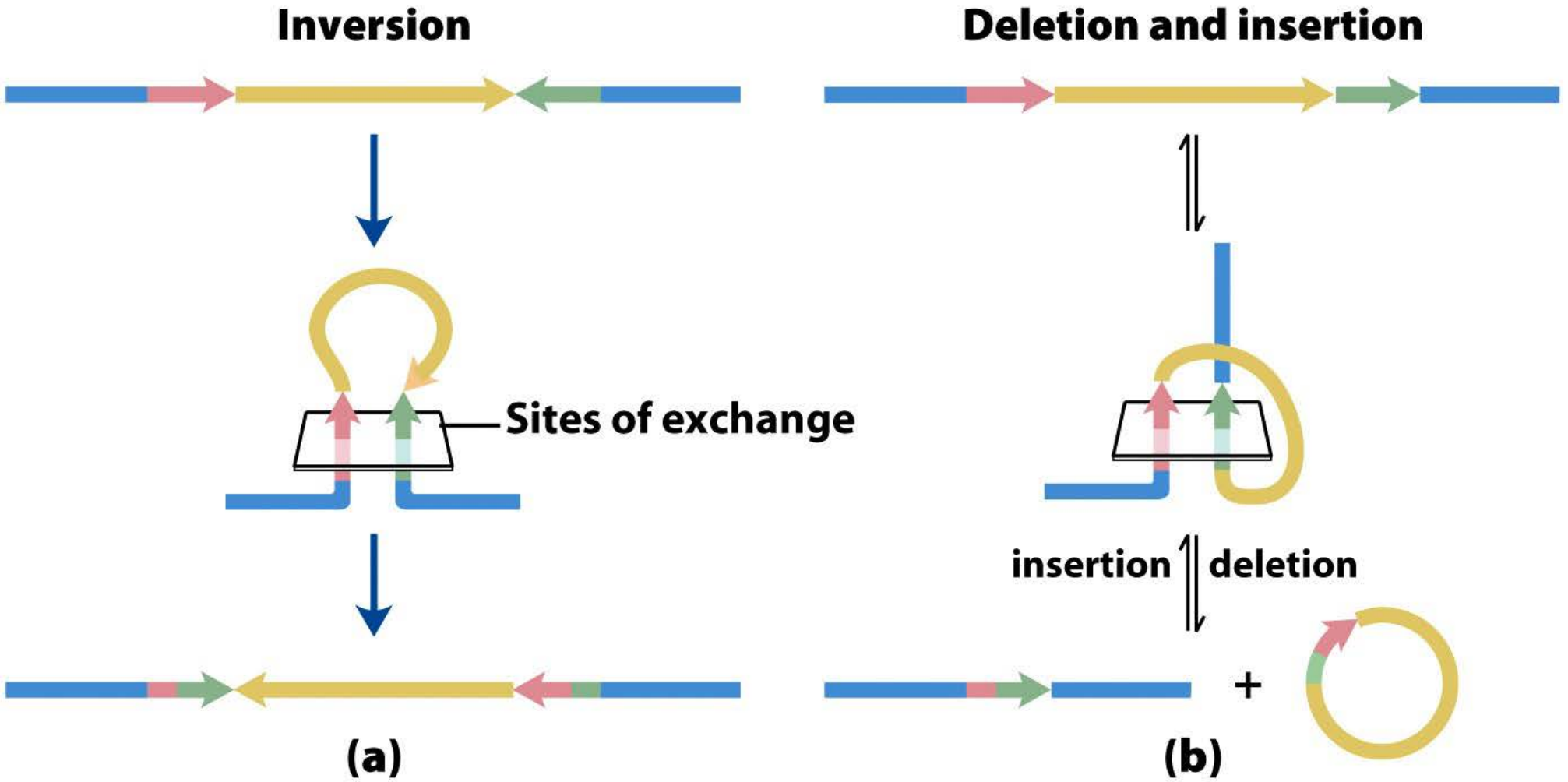
In systems that employ an active-site Ser residue, both strands are cut concurrently and rejoined to the new partners without forming Holliday intermediate.

The exchange is always reciprocal and precise, regenerating the recombination sites when the reaction is complete.

The two recombination sites align in the same orientation during the recombination reaction.

Part II

Effects of Site-Specific Recombination



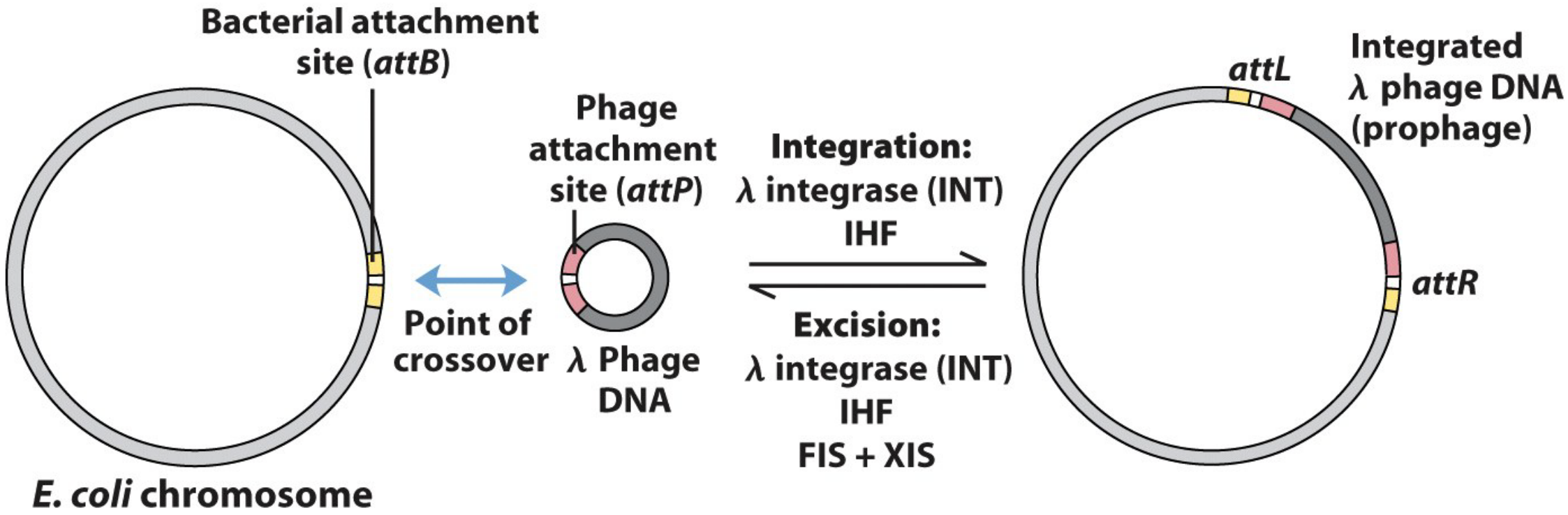
Part II

Outcomes Depends on the Location and Orientation of the Recombination Sites

- Two sites on the same DNA: inversion or deletion
- Two sites on different DNAs: Intermolecular
  - If one or both are circular : Insertion

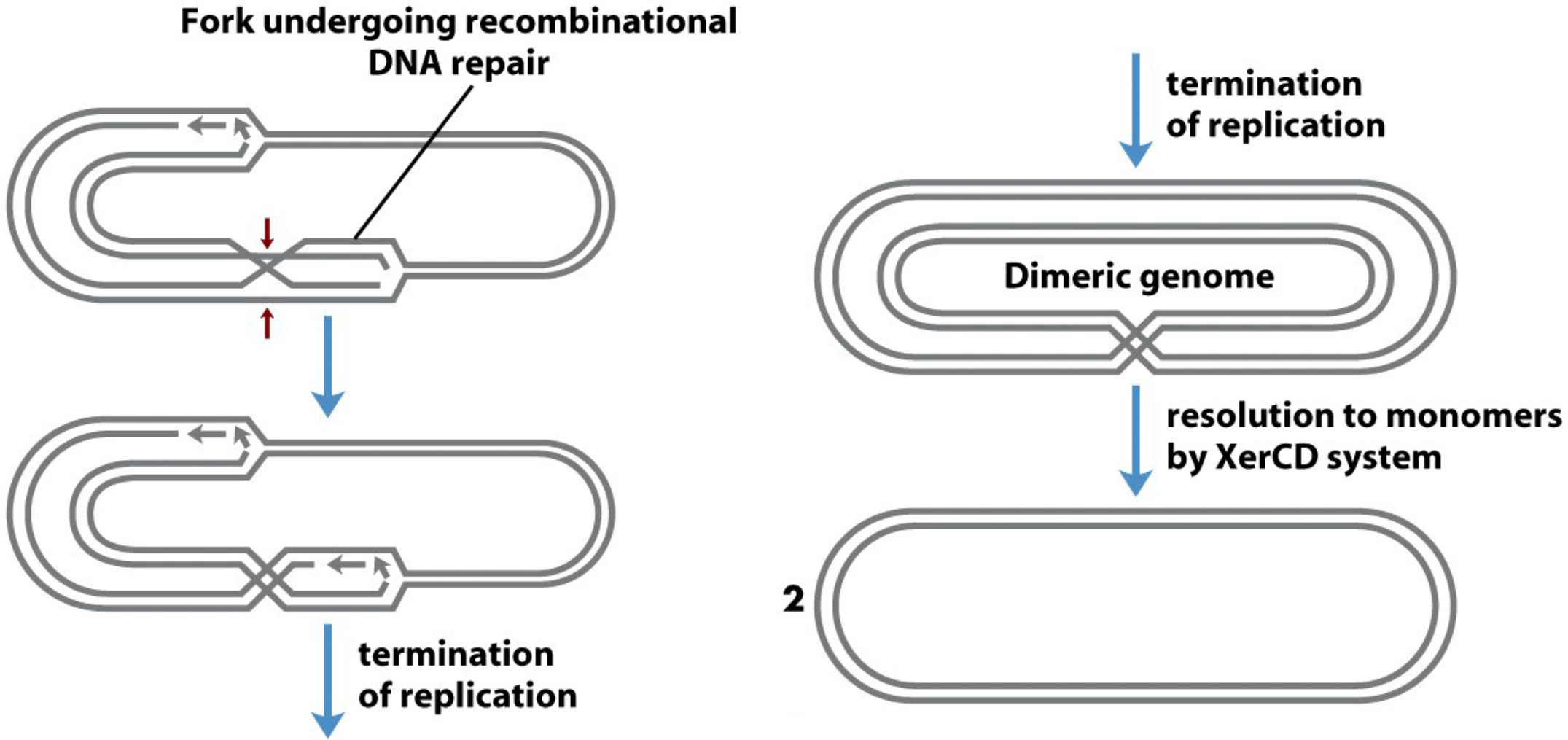
Part II

Integration and Excision of Bacteriophage λ DNA at the Chromosomal Target Site



Part II

Complete Chromosome Replication Can Require Site-Specific Recombination



Part II

Transposition

Barbara McClintock
1902–1992

Jumping genes in maize

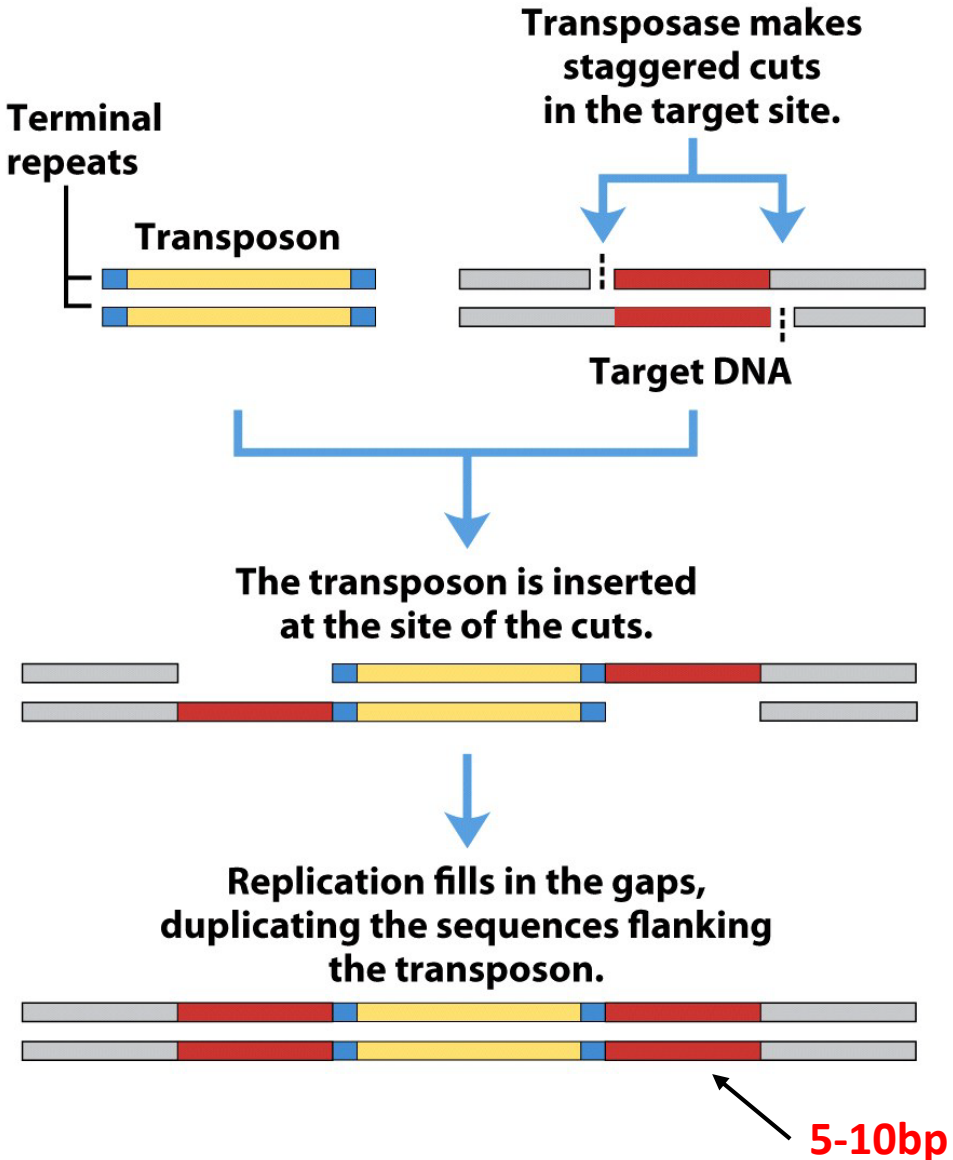
Part II

Transposable Genetic Elements Move from One Location to Another

- Transposition is a recombination that allows the movement of transposable elements, or transposons.
- DNA sequence homology is usually not required for this movement, called transpostition; the new location is determined more or less randomly.
- Bacteria have two classes of transposons.
  - Insertion sequences (simple transposons) contain only the sequences required for transposition and transposases.
  - Complex transposons contain one or more genes in addition to those needed for transposition.

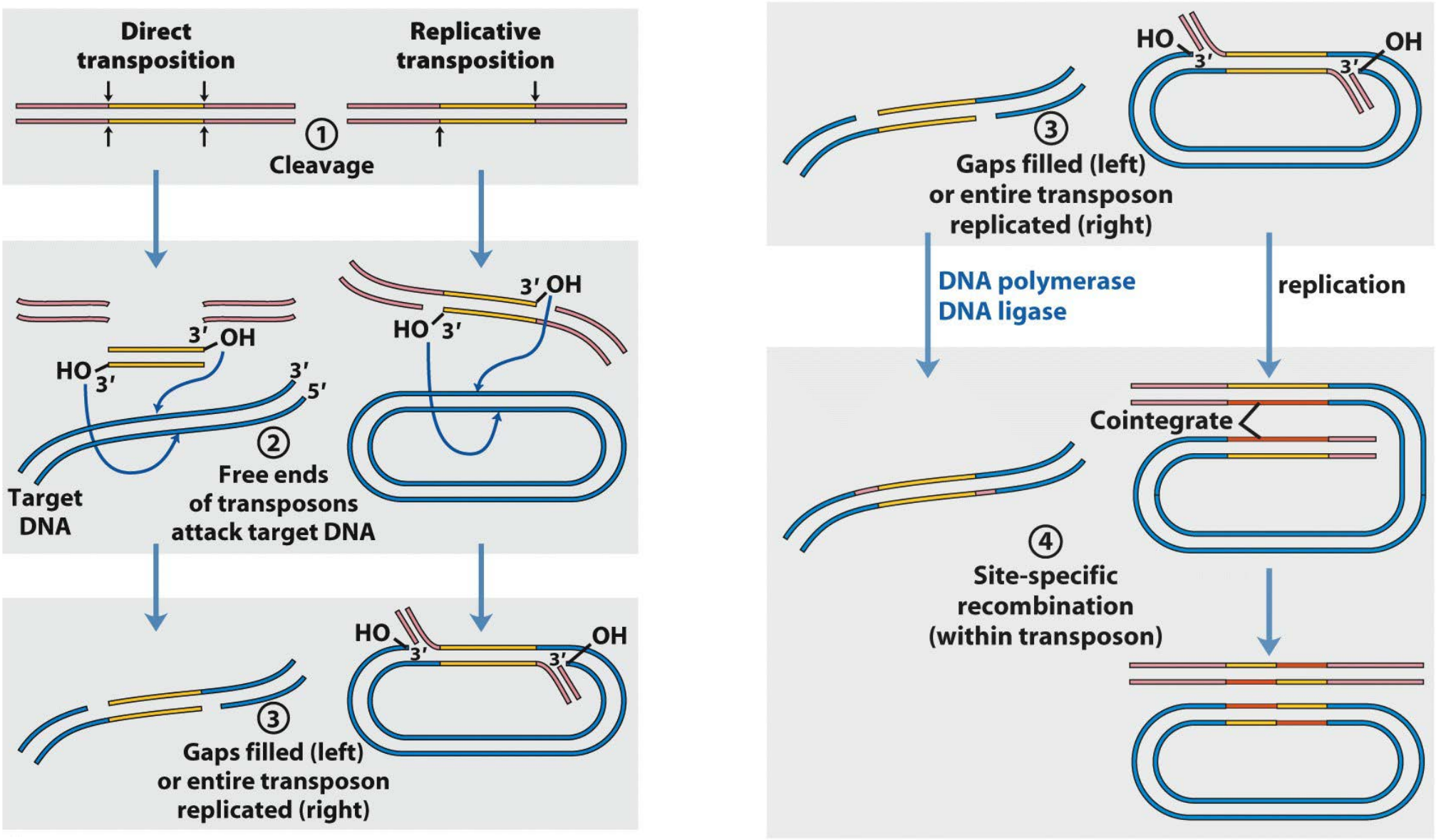
Part II

Duplication of the DNA Sequence at a Target Site When a Transposon Is Inserted



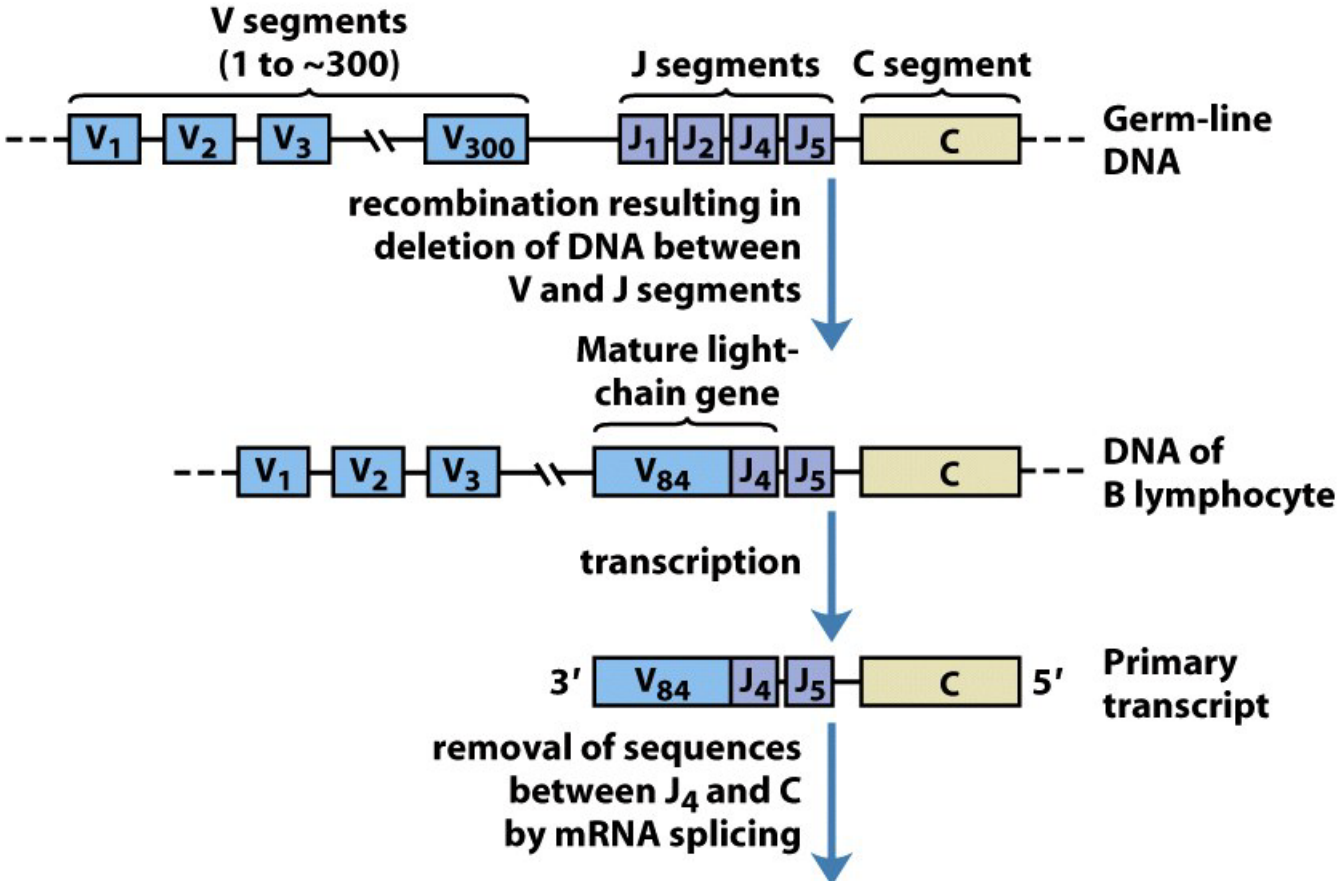
Part II

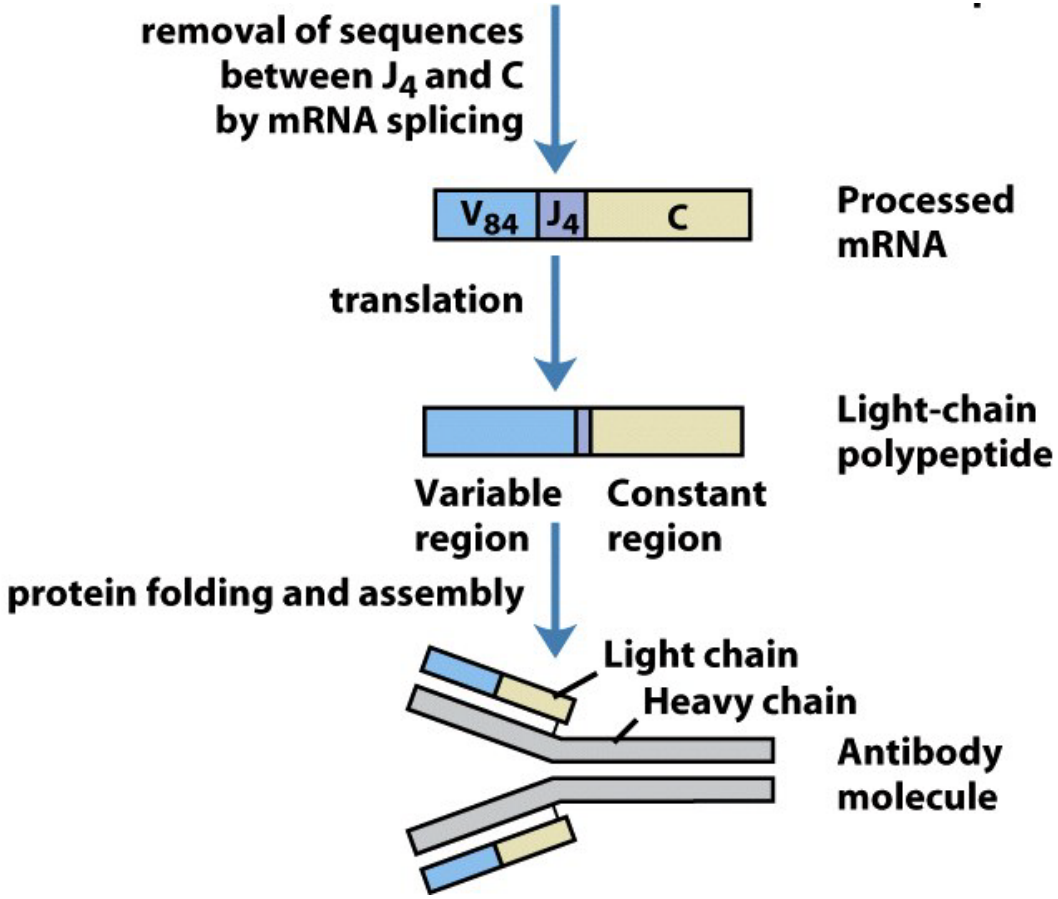
Two General Pathways for Transposition: Direct and Replicative



Part II

Immunoglobulin Genes Assemble by Recombination



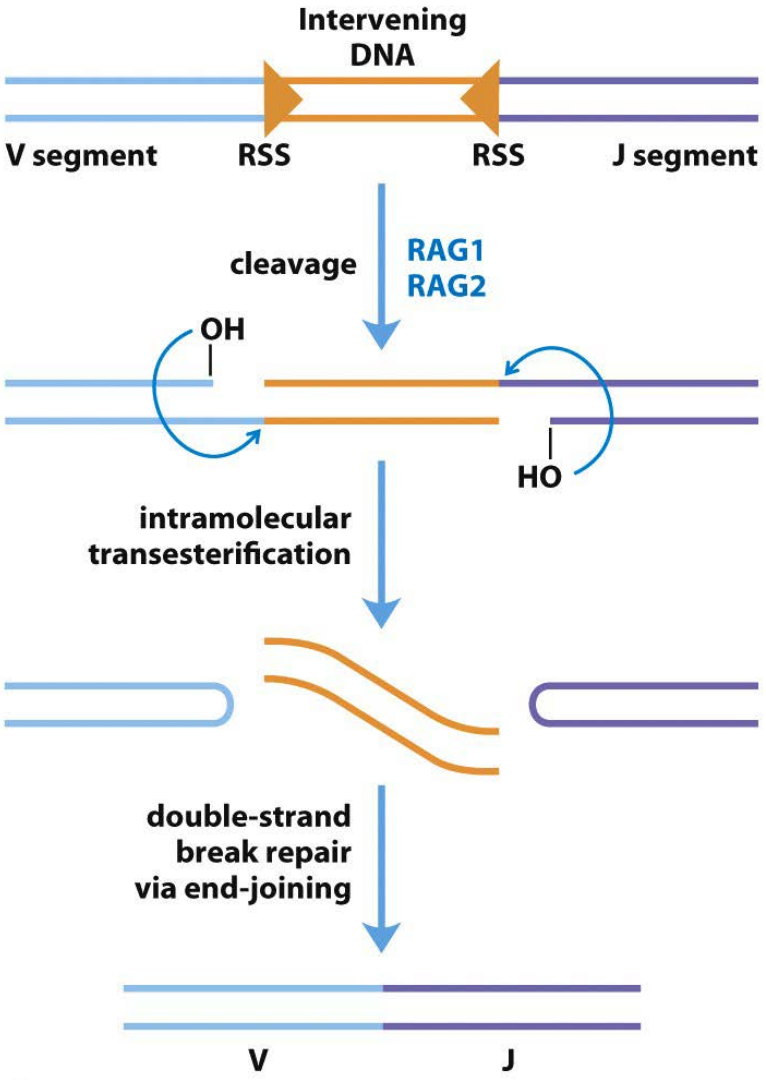


V (variable); J (joining); C (constant).

1.5×10^7 possible IGs in human.

Part II

Mechanism of Immunoglobulin Gene Rearrangement



RSS: Recombinational signal sequence
Just beyond each V segment
and just before each J segment

RAG: Recombination Activating Gene

Summary

- DNA sequences are rearranged in recombinational reactions
- Homologous genetic recombination can take place between any DNA molecules that share sequence homology.
  - In meiosis, it helps to ensure accurate chromosomal segregation and create genetic diversity.
  - In both bacteria and eukaryotes, it serves in the repair of stalled replication forks.
  - A Holliday intermediate forms during homologous recombination.
- Site-specific recombination occurs at specific target sequences and this process can also involve Holliday intermediate.
- In virtually any cells, transposons use recombination to move within or between chromosomes.