Mutation:

A permanent change in the nucleotide sequence.

1- Substitution mutation:

Replacement of one/more base pair with another.

2- Insertion or deletion mutation:

addition or deletion of one/more base pairs.

3- Silent mutation:

mutation affects non essential DNA, or has neglieable effect.

Mutations and Cancer

Strong correlation between carcinogens and Mutagens.

90% of carcinogens are mutagens.

Ames Test for Carcinogens:

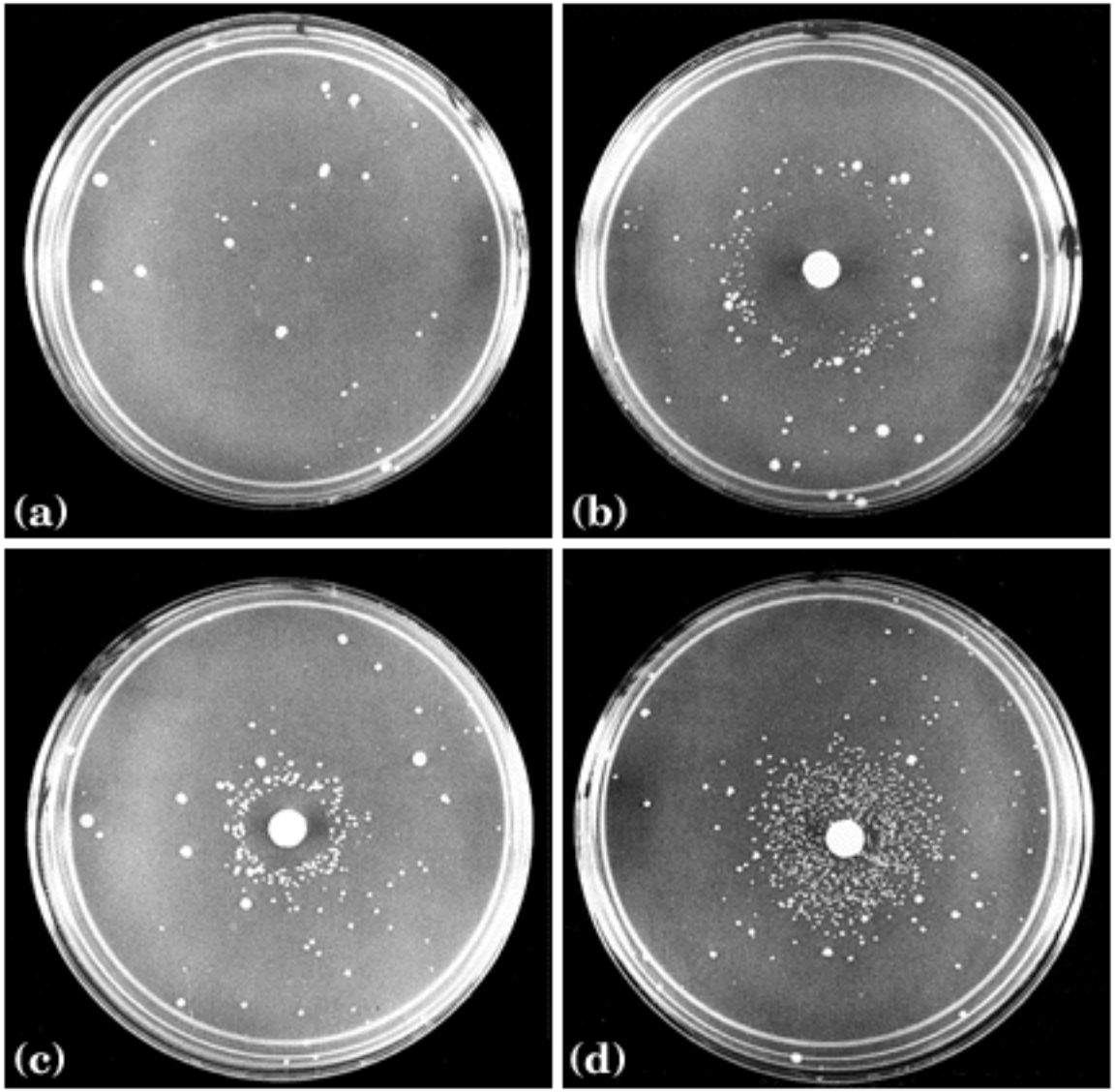
Measure the potential of a chemical to induce mutations in bacteria.

Rapid and inexpensive Screen important in industry:

Salmonella carry mutations in genes involved in histidine synthesis (they require histidine for growth).

Plate incubated for 48h.

Mutagenicity of a substance is proportional to the # of colonies observed.



Number and diversity of DNA repair pathways reflects

1) various sources of DNA damage

2) importance of repair pathways.

table 25–5

Types of DNA Repair Systems in *E. coli*

Enzymes/proteins	Type of damage
Mismatch repair	
Dam methylase	Mismatches
MutH, MutL, MutS proteins	
DNA helicase II	
SSB	
DNA polymerase III	
Exonuclease I	
Exonuclease VII	
RecJ nuclease	
Exonuclease X	
DNA ligase	
Base-excision repair	
DNA glycosylases	Abnormal bases (uracil, hypoxanthine, xanthine); alkylated bases; pyrimidine dimers in some other organisms *
AP endonucleases	
DNA polymerase I	
DNA ligase	
Nucleotide-excision repair	
ABC excinuclease	DNA lesions that cause large structural changes (e.g., pyrimidine dimers) *
DNA polymerase I	
DNA ligase	
Direct repair	
DNA photolyases	Pyrimidine dimers*
O^6-Methylguanine-DNA methyltransferase	O^6-Methylguanine

1) Mismatch Repair:

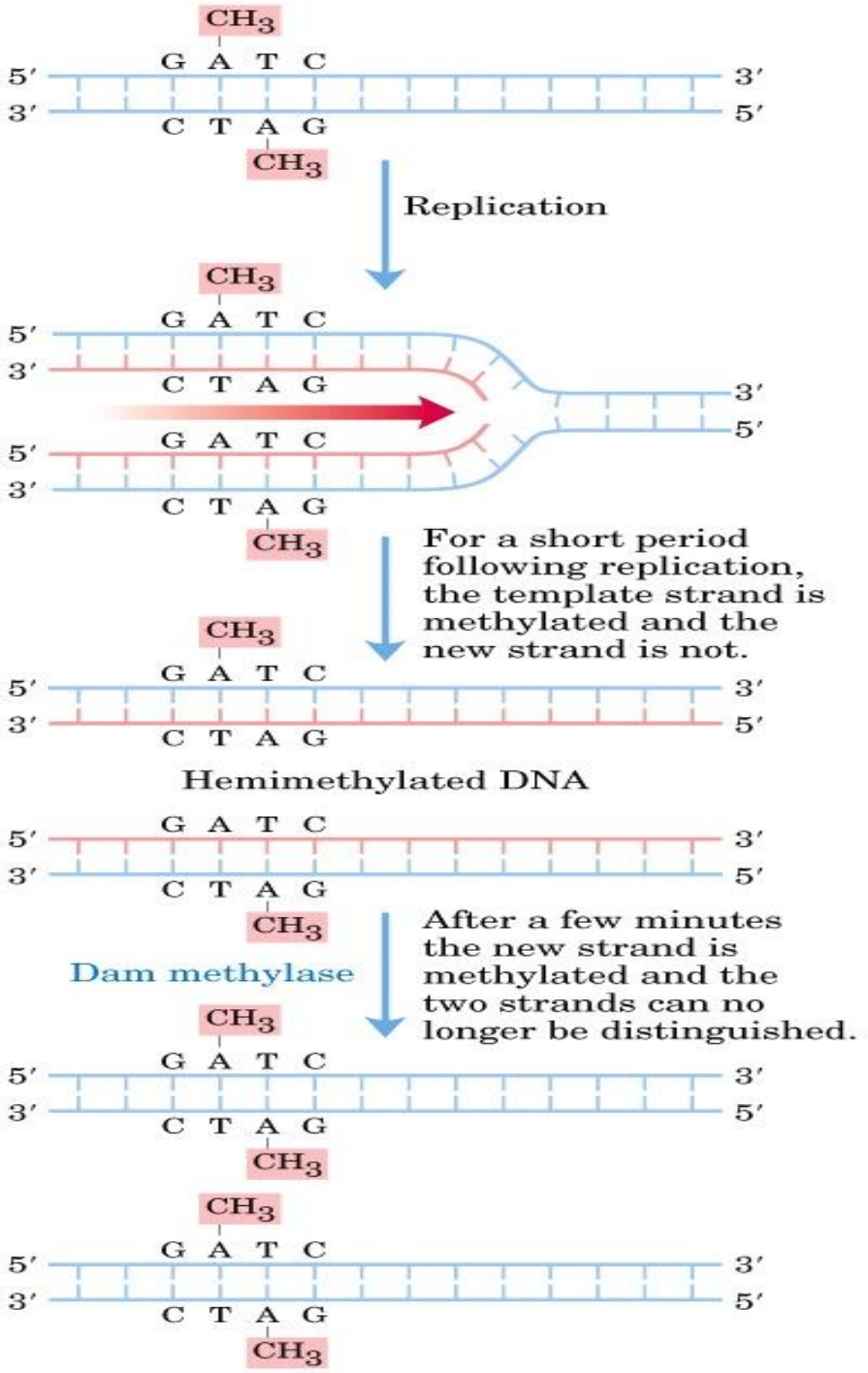
Improves fidelity by a factor of 10^2-10^3

Discrimination between template and newly synthesized strand by tagging the template with methyl groups.

Repair occurs in this period→→→

lag period = 10min.

Dam methylase 5'-GATC-3' in newly synthesized. So both strands are methylated and indistinguishable.



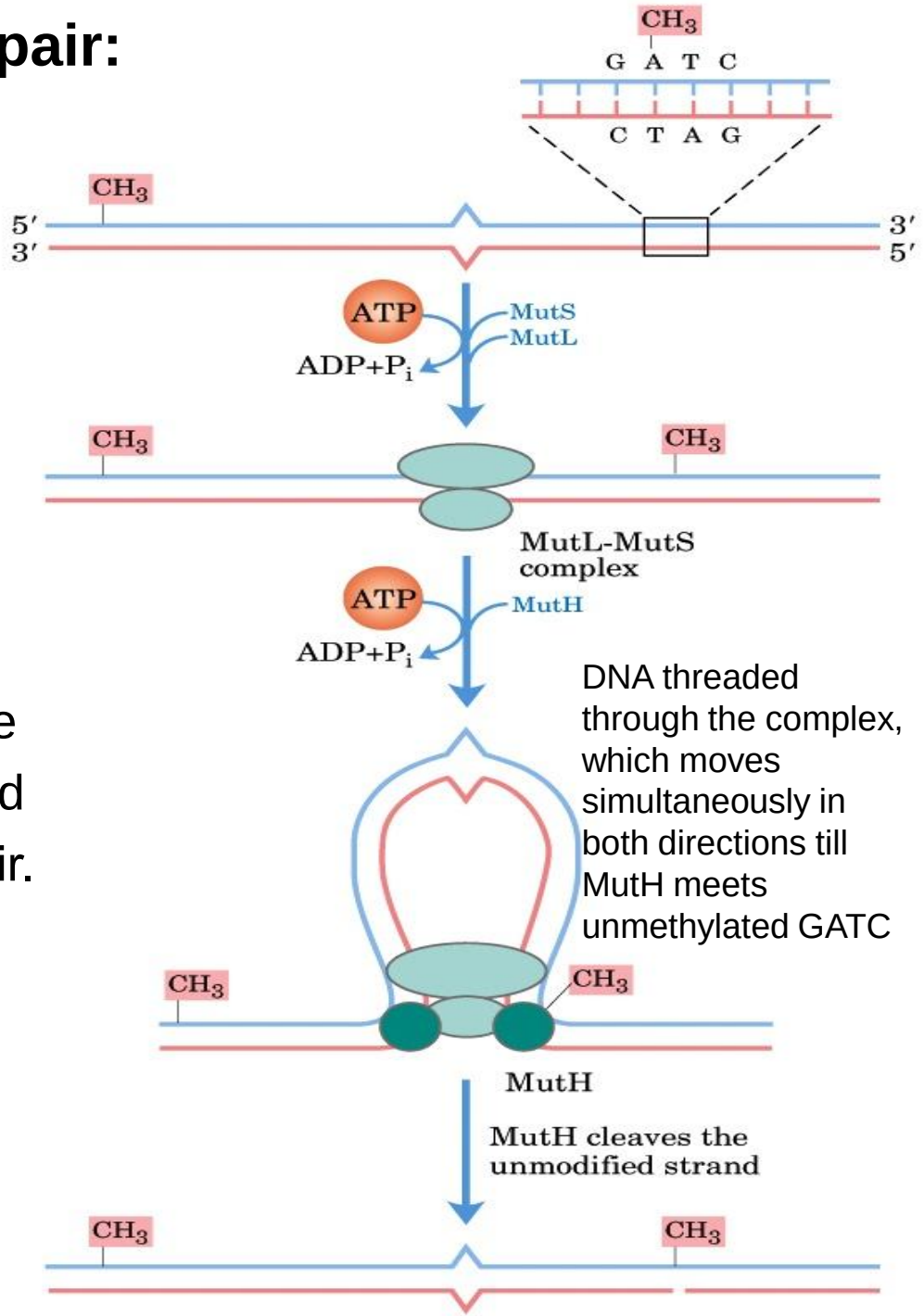
Methyl- directed mismatch repair:

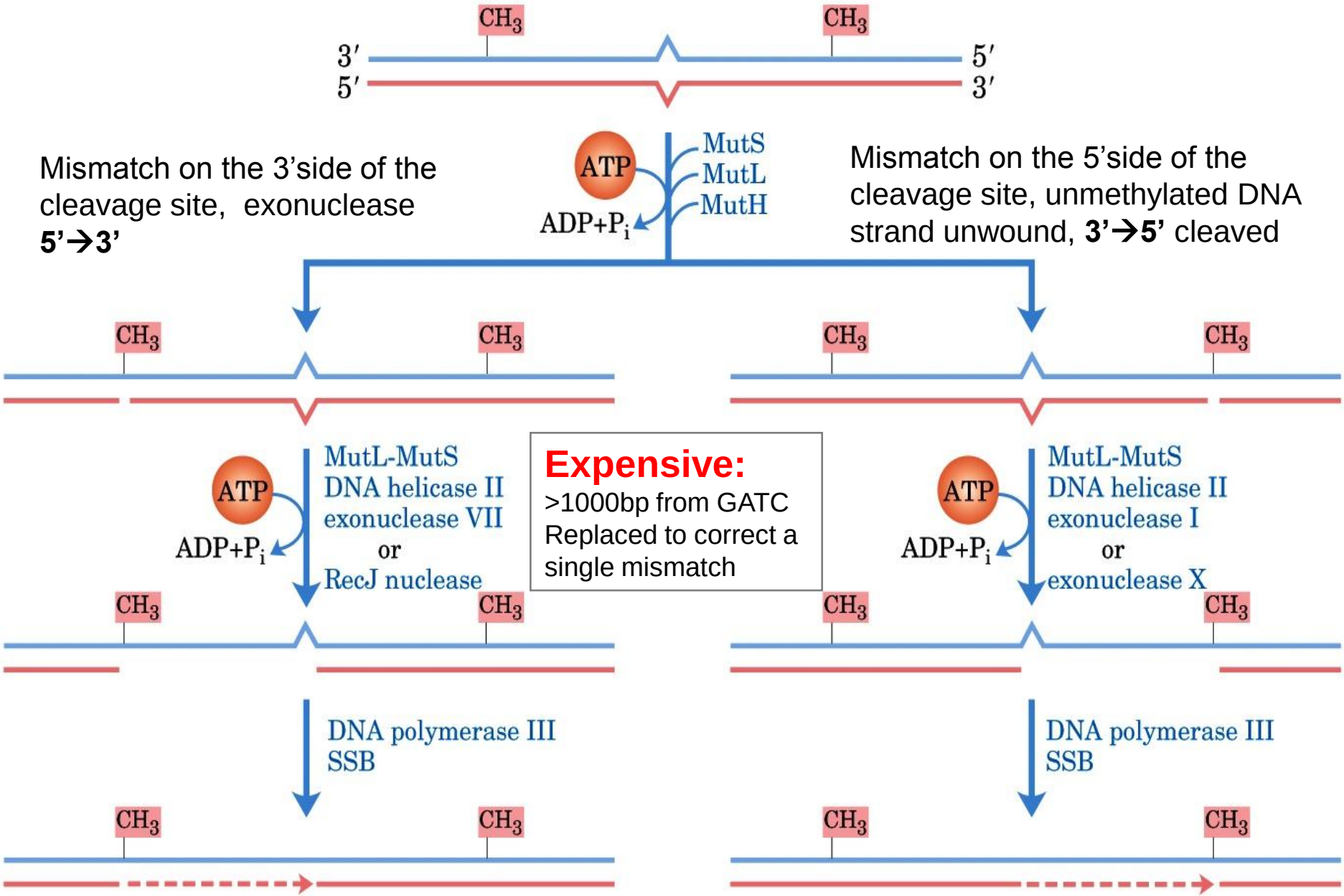
Repairs up to 1000bp from hemimethylated GATC sequence.

MutS +MutL protein repair complex binds all mismatched base pair.

MutH binds MutL.

MutH has a site specific endonuclease activity inactive till meets unmethylated 5’-GATC-3’ (marks the strand for repair.




CH3
CH3
3′
5′
5′
3′
ATP
ADP+Pi
MutS
MutL
MutH
Mismatch on the 3'side of the cleavage site, exonuclease 5'→3'
Mismatch on the 5'side of the cleavage site, unmethylated DNA strand unwound, 3'→5' cleaved
MutL-MutS
DNA helicase II
exonuclease VII
or
RecJ nuclease
MutL-MutS
DNA helicase II
exonuclease I
or
exonuclease X
Expensive:
>1000bp from GATC
Replaced to correct a single mismatch
DNA polymerase III
SSB

Eukaryotes:

- Similar to MutS and MutL.
- MutS homologs for eukaryotes from yeast to humans.

  MSH2 (Muts homolog 2) MSH3, MSH6.

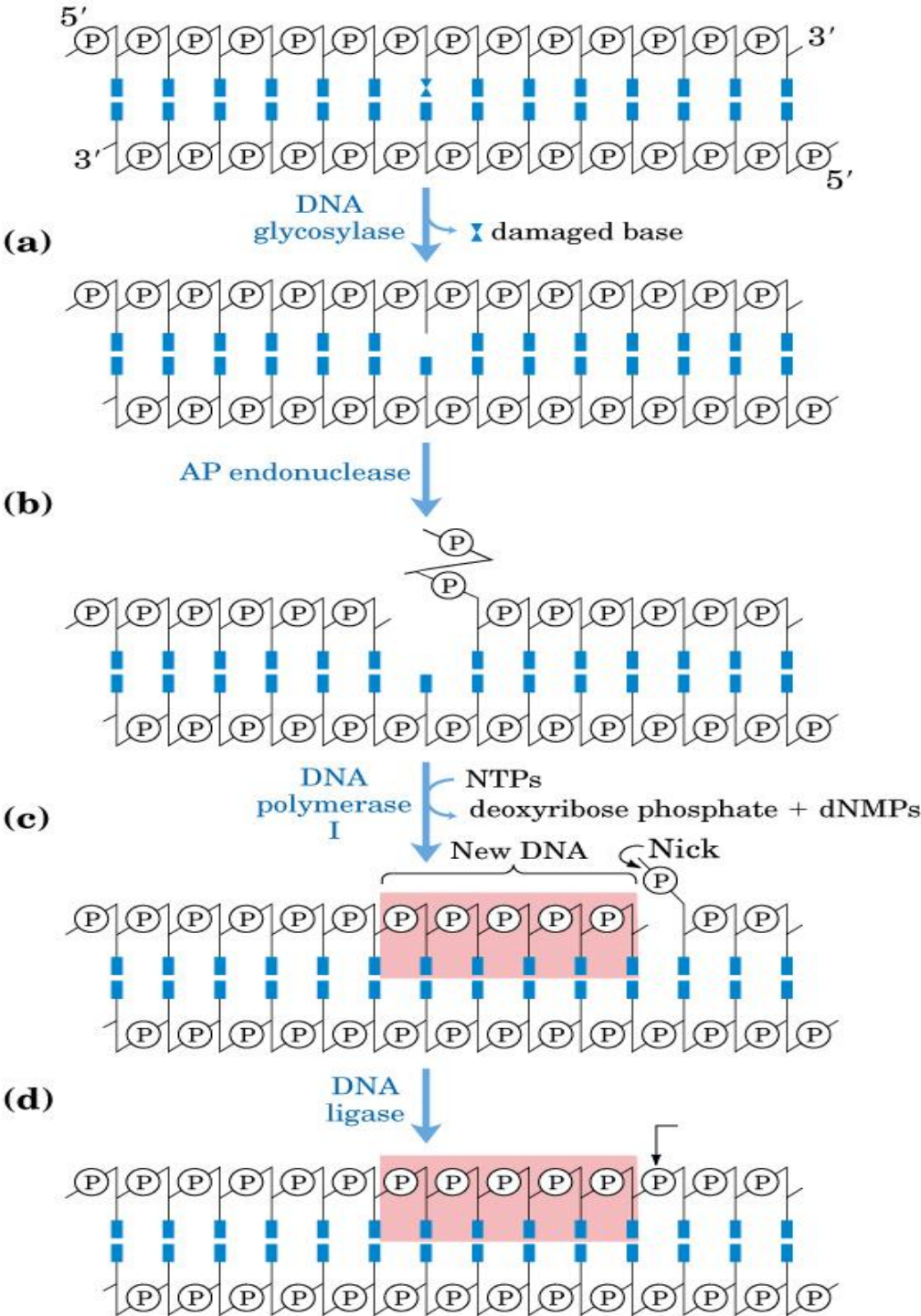
- Mutated in CANCER.

2) Base- Excision Repair:

DNA glycosylases recognize DNA lesions (products of A and C deamination.

cleaving N-glycosyl bond bw base and deoxyribose → apurinic/apyrimidinic base = Abasic site= AP site.

AP endonuclease (cut DNA strand containing AP site)



- Uracil DNA glycosylase (UNG)→ removes U formed from deamination of C.

- Highly selective distinguish T from U (act only on DNA not RNA)

- Bacteria has one type, humans 4.

3) Nucleotide Excision Repair: (critical for all organisms survival.

DNA lesions cause large distortion in DNA helical structure.

Multisubunit enzyme hydrolyses 2 phosphodiester bonds one on either side of the distorsion.

Excinuclease= catalyze 2 specific endonucleolytic cleavages. (not standard endonuclease)

E.coli + prokaryotes:

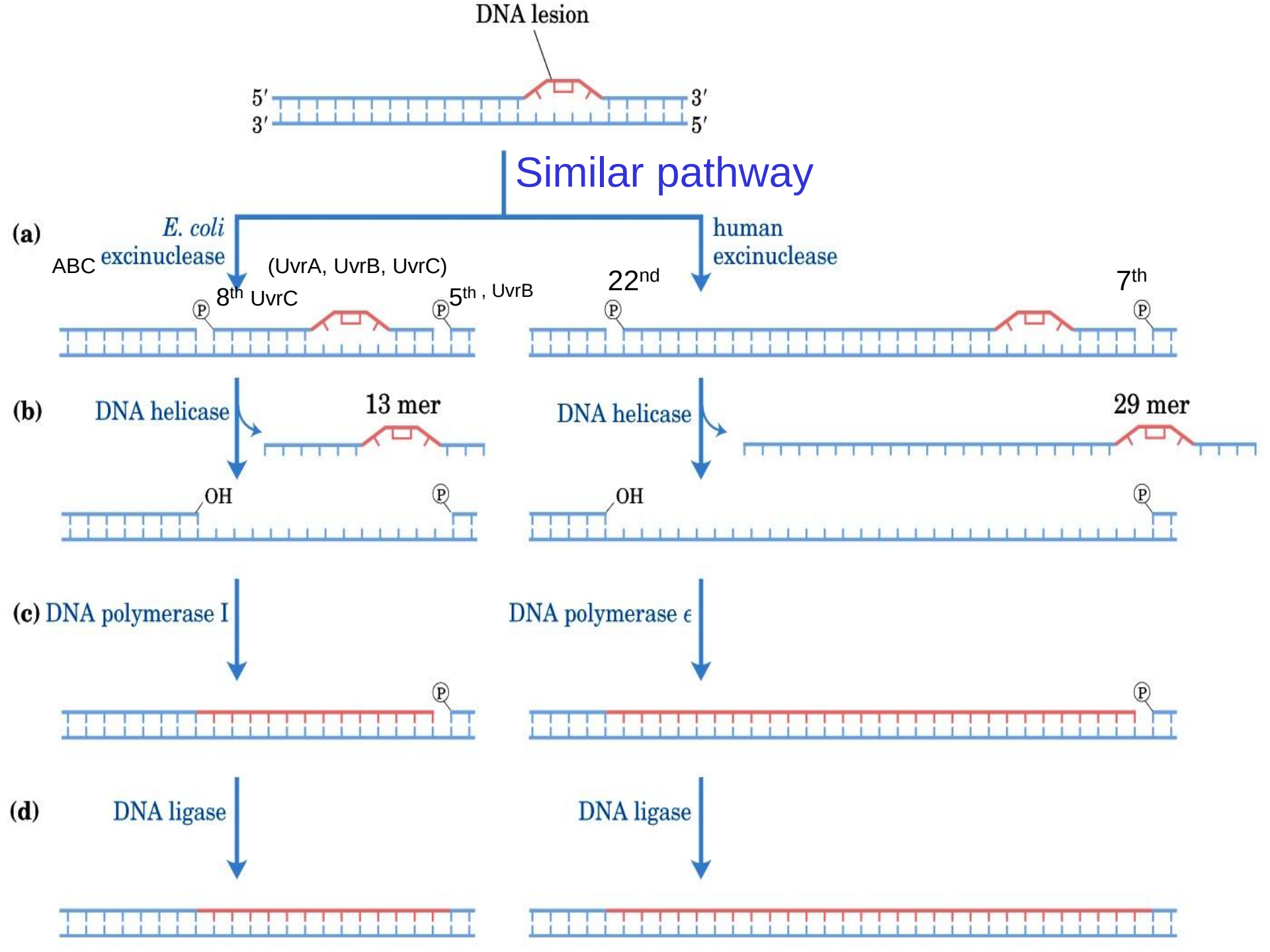
Hydrolyses the 5^{th} phosphodiester bond on 3' side+ 8^{th} phosphodiester bond on 5' side = A fragment 13 nucleotide, removed by helicase

gap filled by DNA pol I , nick sealed by ligase.

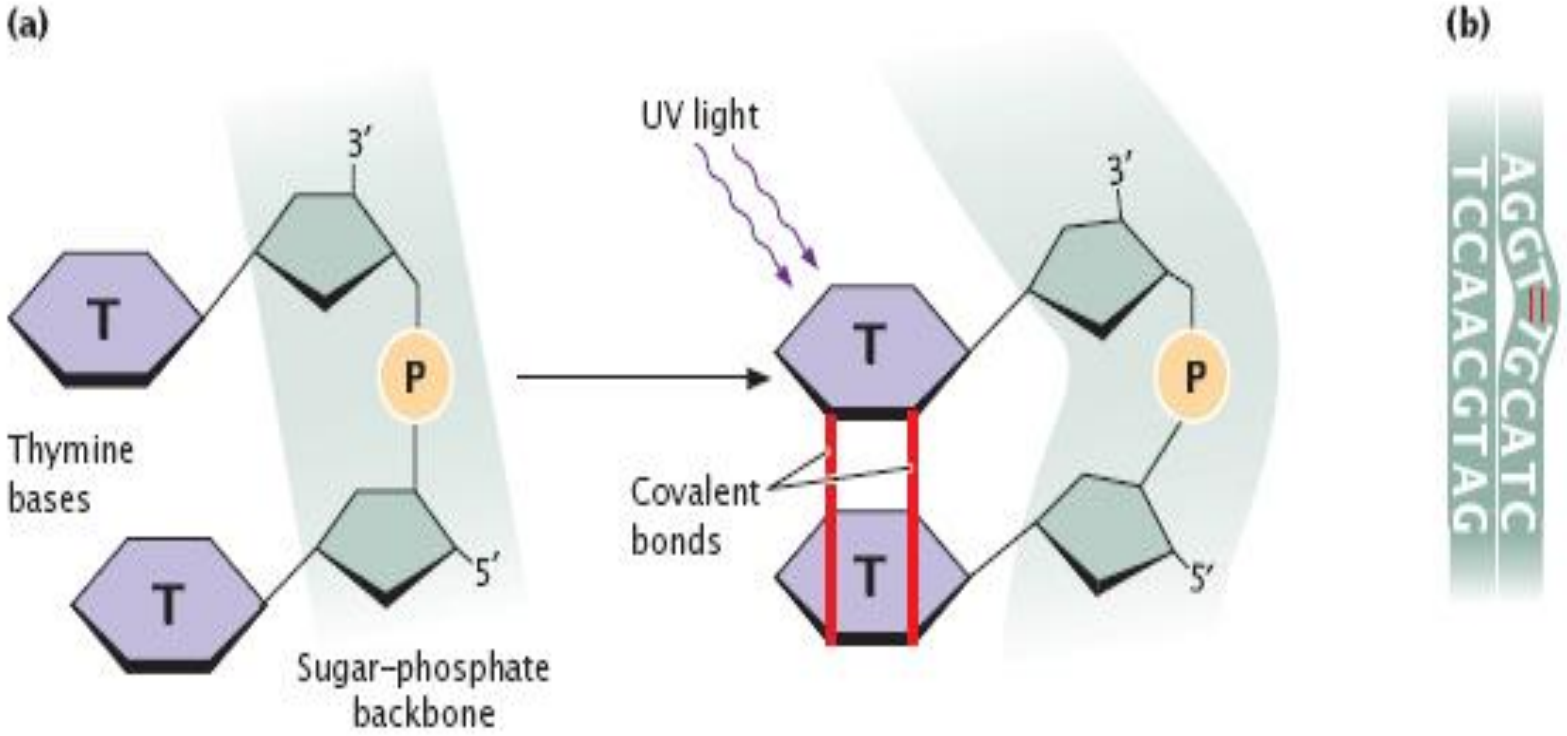
Humans + eukaryotes

6^{th} bond on 3' side + 22^{nd} bond on 5' side = 27-29 nucleotide fragment.

gap filled by DNA pol ε , nick sealed by ligase


DNA lesion
5′
3′
3′
5′
Similar pathway
(a)
E. coli
ABC excinuclease
(UvrA, UvrB, UvrC)
8th UvrC
5th , UvrB
human
excinuclease
22nd
7th
(b)
DNA helicase
13 mer
DNA helicase
29 mer
OH
OH
(c) DNA polymerase I
DNA polymerase ϵ
(d)
DNA ligase
DNA ligase

PYRIMIDINE DIMER , T=T or C=C



UvrA and UvrB scans the DNA and bind the lesion site.

UvrA dimer dissociates → UvrB-DNA tight bonding

UvrC binds to UvrB →UvrB makes incision at 5th phosphodiester bond. UvrC at 8th bond on 5' site .

This pathway the primary route for many lesion types :

1- pyrimidine dimers (T, C).

2- benzo pyrene-guanine (formed in DNA by **cigarette smoke**).

Eukaryotes similar to E. coli mechanism but 16 polypeptide.

Xeroderma pigmentosum (XP):

rare inherited disease (pigmented lesions on skin + skin canc) due to mutations in NER system.

4) **Direct Repair:**

Repair with no excision / removal of a base or a nucleotide.

a) photoreactivation of pyrimidine dimers by DNA photolyase.

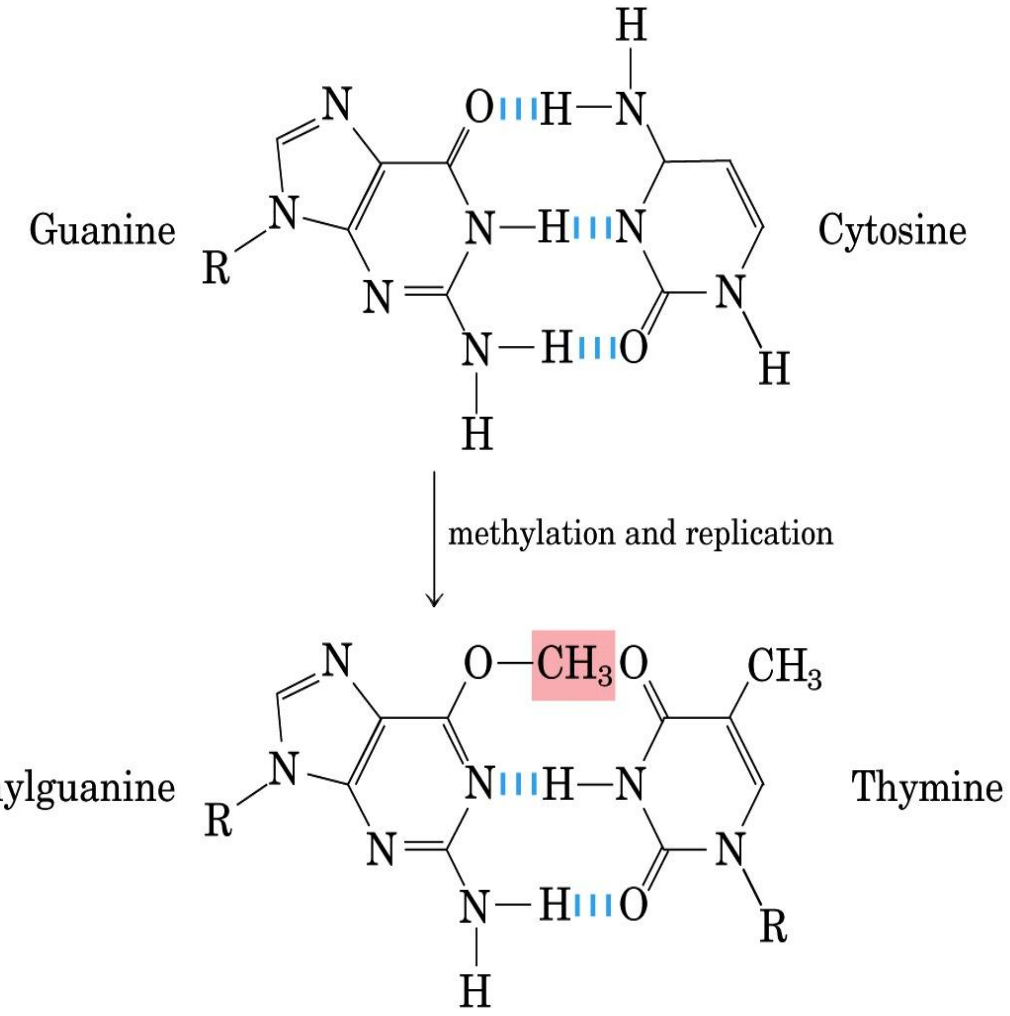
Pyrimidine dimers result from UV-light induced rxn

Photolyase contain light absorbing agent = **chromophore**.

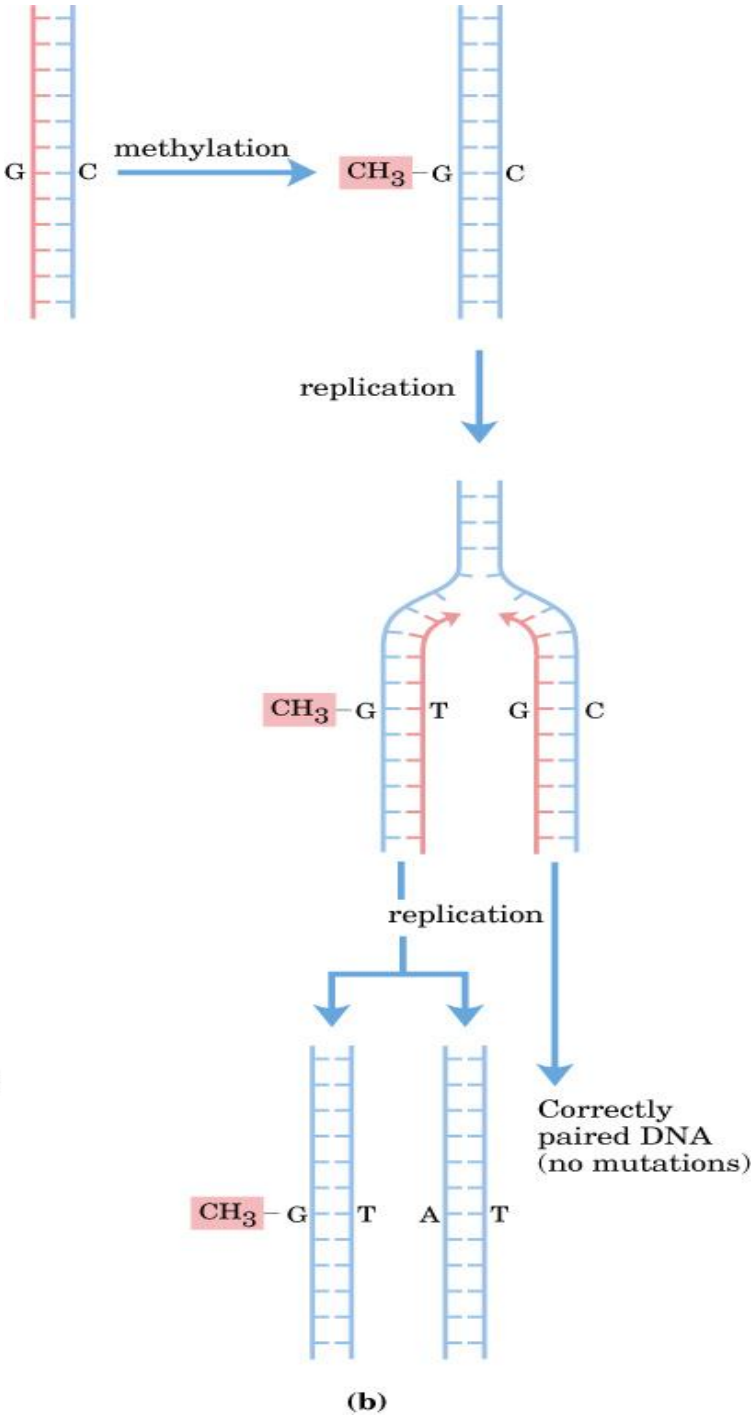
DNA photolyase not present in placental mammals e.g. humans.

DNA damage cause mutations:

The methylation product of alkylating agents pairs with T rather than C during replication.



(a)



(b)

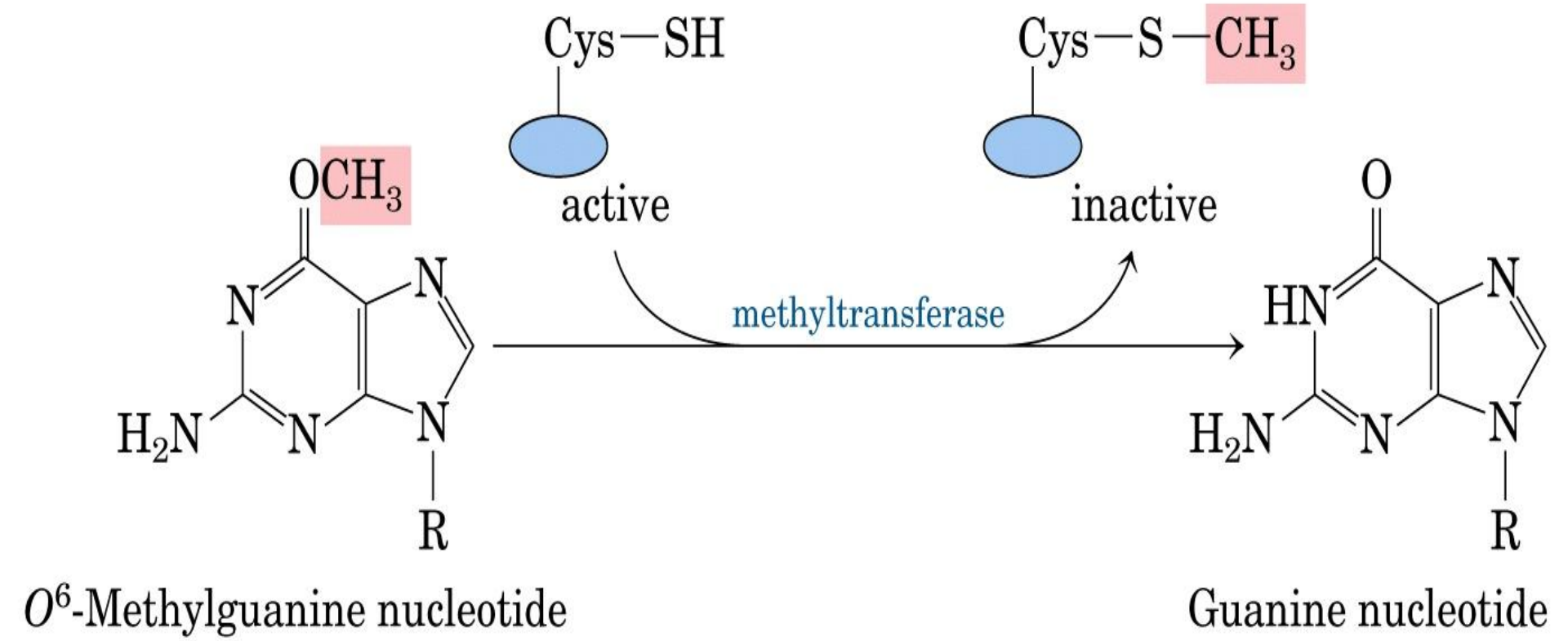
b-Direct repair : O_6- methylguanine- DNA methyltransferase.

Not strictly an enzyme.

A single methyl transfer event permanently methylates the protein.

An entire protein consumed to correct a single damaged base →

Priority given to maintain the integrity of cellular DNA.



c- Direct Repair: AlkB

The amino group of A /C methylated in ssDNA→ improper base pairing.

Oxidative demethylation by ALkB (α-KG- Fe2+-dependent dioxygenase)

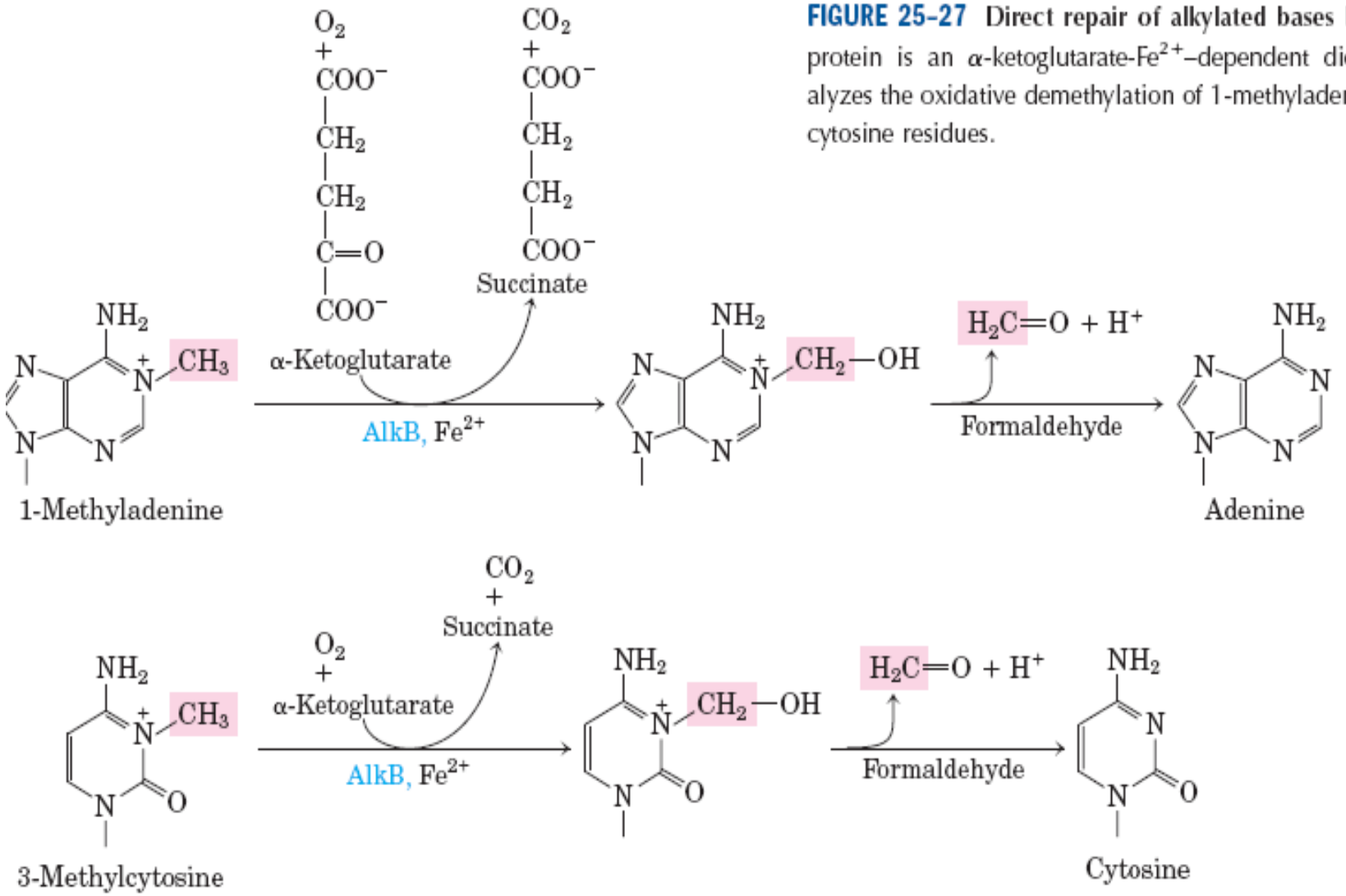
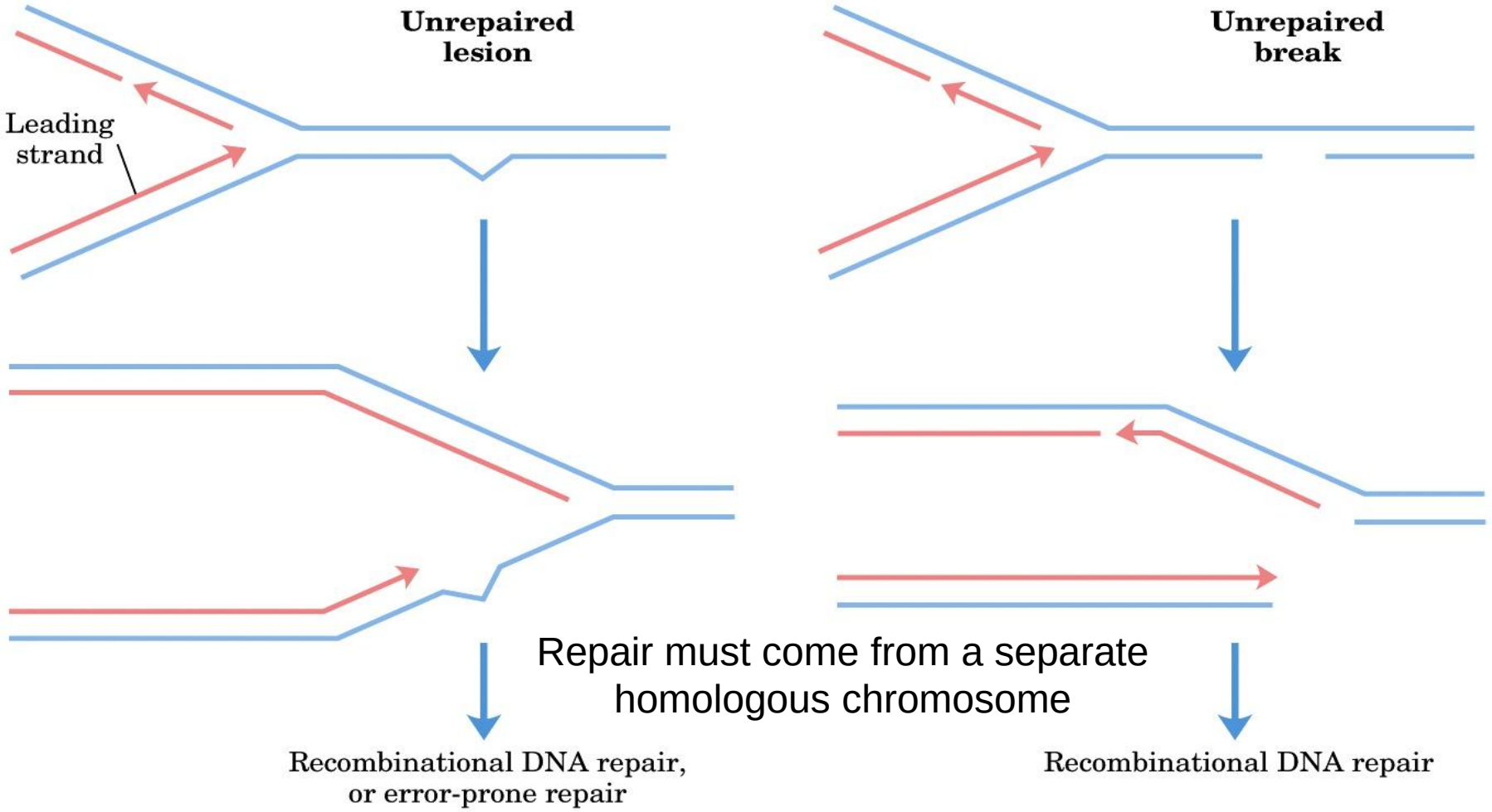


FIGURE 25–27 **Direct repair of alkylated bases by AlkB.** The AlkB protein is an α-ketoglutarate-Fe^{2+}–dependent dioxygenase. It catalyzes the oxidative demethylation of 1-methyladenine and 3-methylcytosine residues.

The above repair systems work only if template is present + undamaged providing the correct genetic material to restore the wrong/damaged one.

Due to unrepaired lesion / unrepaired break → template absent / damaged (radiation, oxidative rxns).



1-**Recombinational DNA repair**.

2-**Error prone translesion DNA synthesis** (TLS).

Error prone = mutations often occur. Part of SOS response, SOS proteins:

UvrA, UvrB, UmuD = unmutable (lack of it eliminates SOS)

UmuD→ cleaved to a shorter segment →UmuD'

UmuD' complex with **UmuC→ DNA pol V** replicate past / trans lesions that normally would block replication.

- Proper base pairing is nearly impossible→ **inaccurate repair + high mutation rate.**
- **Lack proofreading exonuclease activity.**
- **Fidelity reduced** by a factor of 10^2 →**lowering fidelity to 1 error/ 1000 nucleotide.**
- SOS activated UmuD' +UmuC only <u>when all replication forks blocked</u> { result of extensive DNA damage}.
- Other polymerases in eukaryotes **eta, iota.**

table 25–6

Genes Induced as Part of the SOS Response in *E. coli*

Gene name	Protein encoded and/or role in DNA repair
Genes of known function	
*pol*B (*din*A)	Encodes polymerization subunit of DNA polymerase II, required for replication restart in recombinational DNA repair
*uvr*A, *uvr*B	Encode ABC excinuclease subunits UvrA and UvrB
*umu*C, *umu*D	Encode DNA polymerase V
*sul*A	Encodes protein that inhibits cell division, possibly to allow time for DNA repair
*rec*A	Encodes RecA protein required for error-prone repair and recombinational repair
*din*B	Encodes DNA polymerase IV
Genes involved in DNA metabolism, but role in DNA repair unknown	
ssb	Encodes single-stranded DNA-binding protein (SSB)
*uvr*D	Encodes DNA helicase II (DNA-unwinding protein)
*him*A	Encodes subunit of integration host factor, involved in site-specific recombination, replication, transposition, regulation of gene expression
*rec*N	Required for recombinational repair
Genes of unknown function	
*din*D	
*din*F	

Note: Some of these genes and their functions are further discussed in Chapter 28.

DNA Recombination

DNA recombination refers to the process that a DNA segment moves from one DNA molecule to another.

1) Homologous / general recombination/ DNA cross over:

It occurs between two homologous DNA molecules.

2) Site-specific recombination:

It occurs at a specific particular DNA sequence present in both non-homologous DNA molecules.

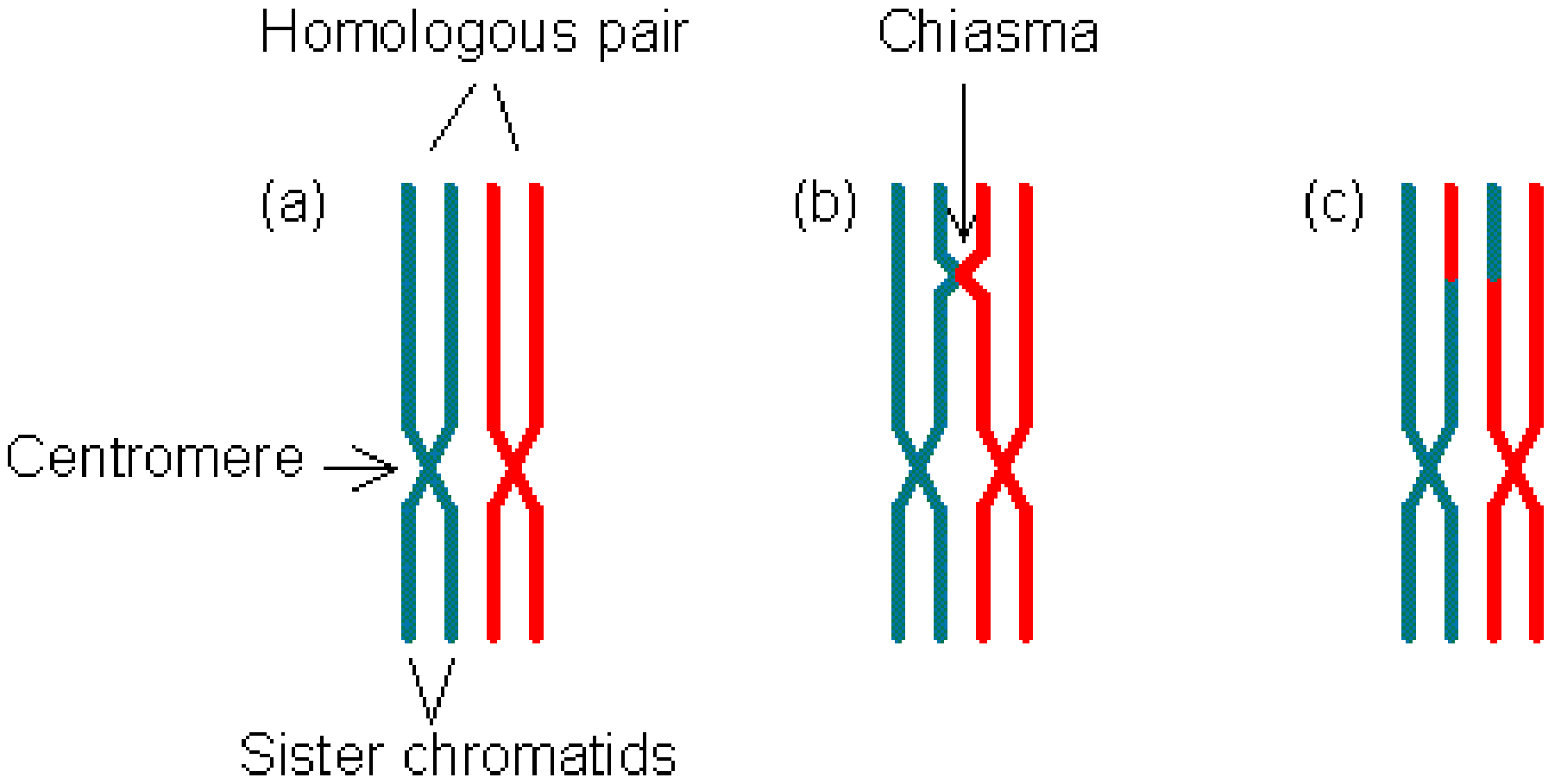
3) Transpositional recombination:

A mobile element / short segment is inserted into a target DNA.

1) **Homologous recombination/ DNA crossover**.

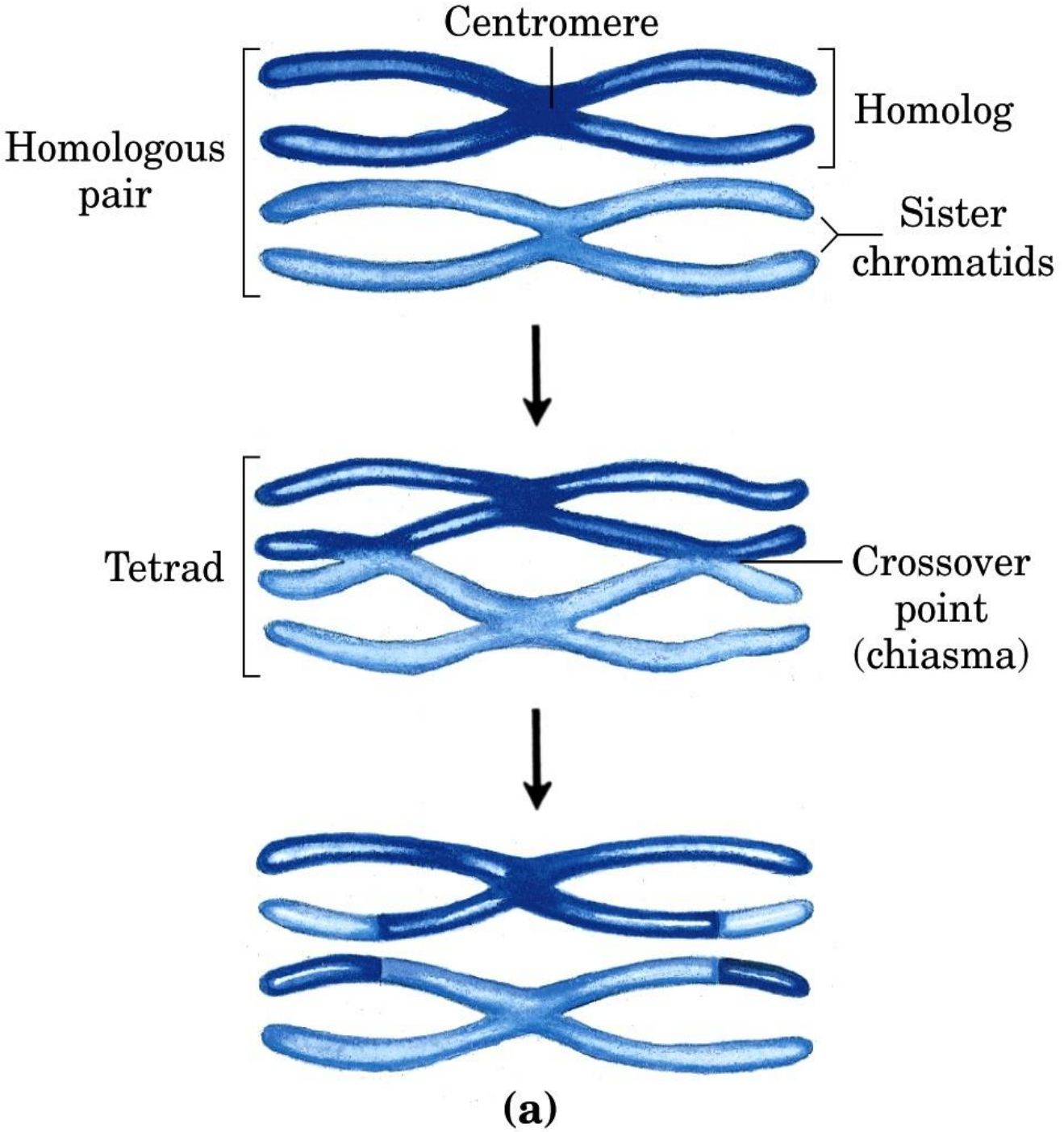
Due to significant sequence homology,

Two homologous pairs of sister chromatids align side by side. **(b)** The two homologs are connected at a certain point called **chiasma**. **(c)** The two homologs exchange the DNA segment from the chiasma to the end of chromosomes.

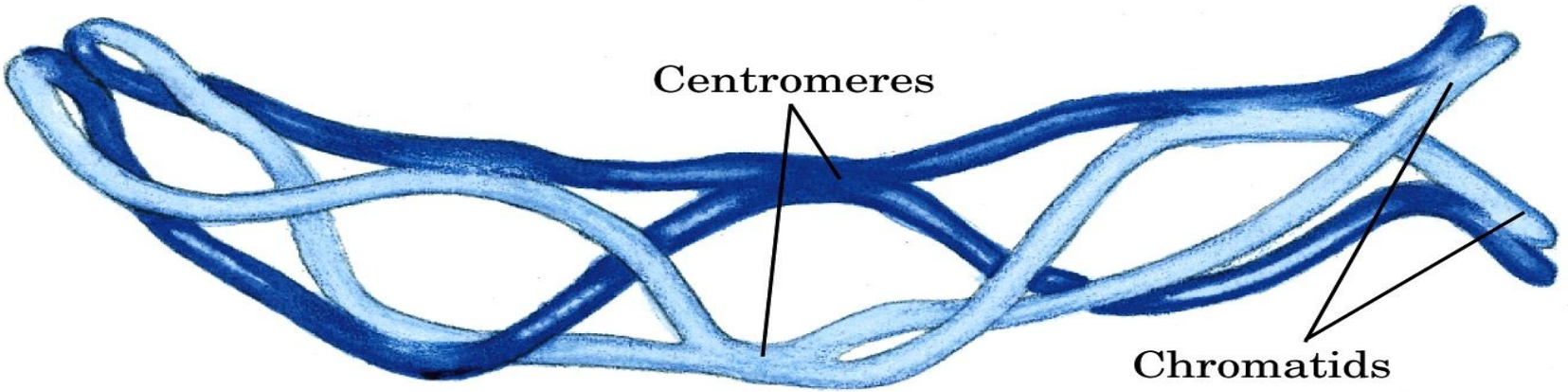


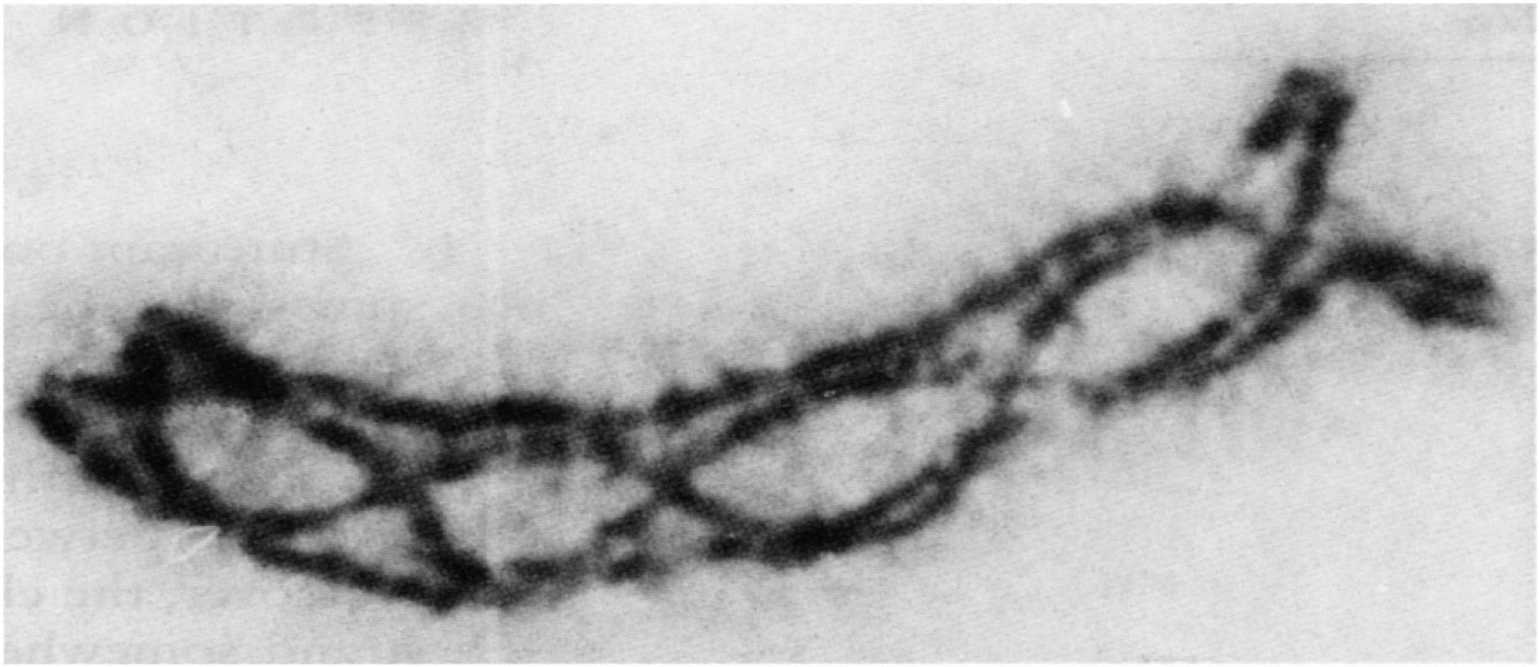
Crossing Over:

Produces exchange of genetic material.



Many points of joining chiasmata





Most DNA damage repaired BER/NER but replication fork encounters breaks.

ds Break Repair System:

Two homologous chromosomes

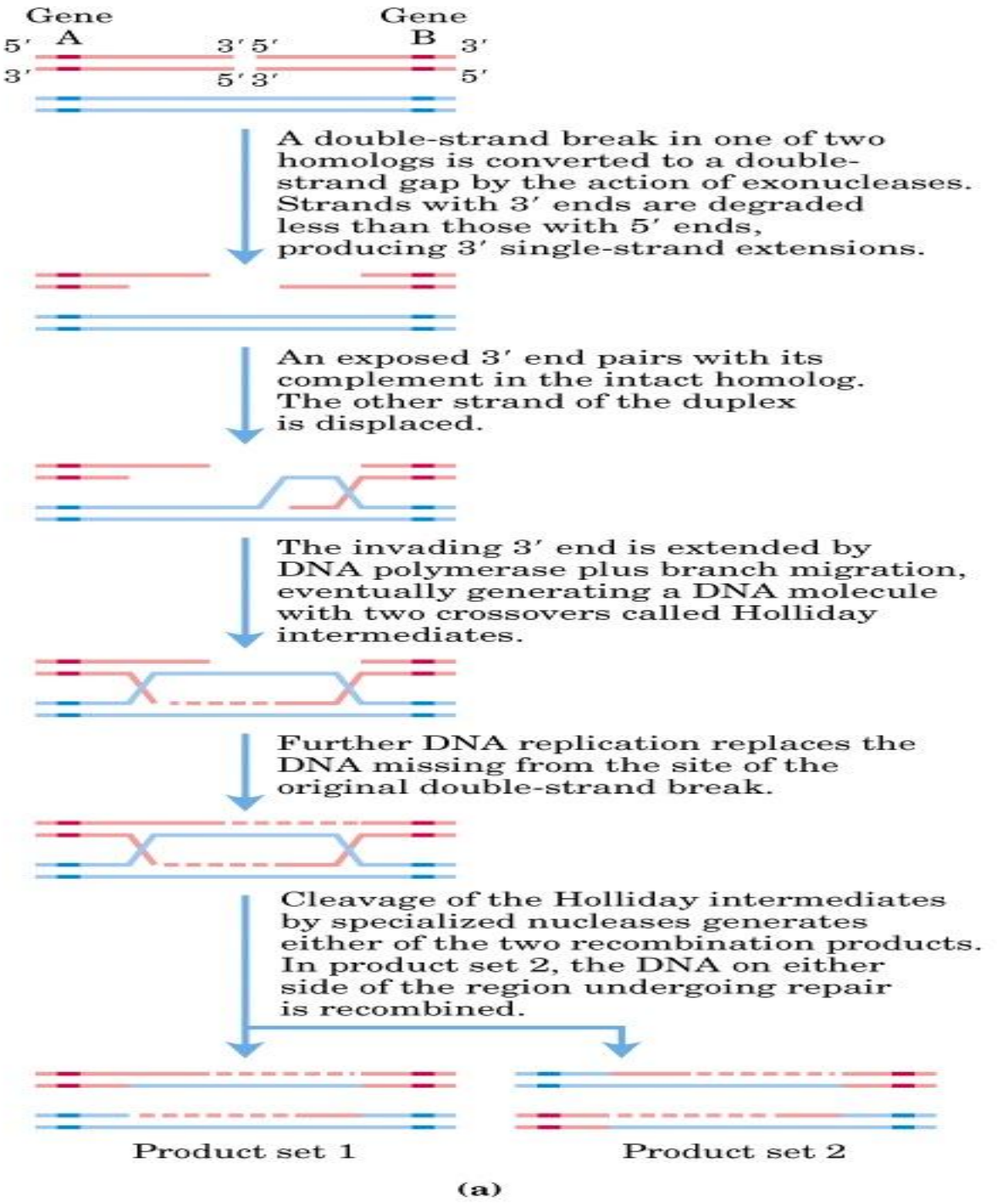
1- break→gap by exonuclease (more at 5' end)→3'ss extensions.

2- extension invades, pairs with its complementary strand in intact homolog.

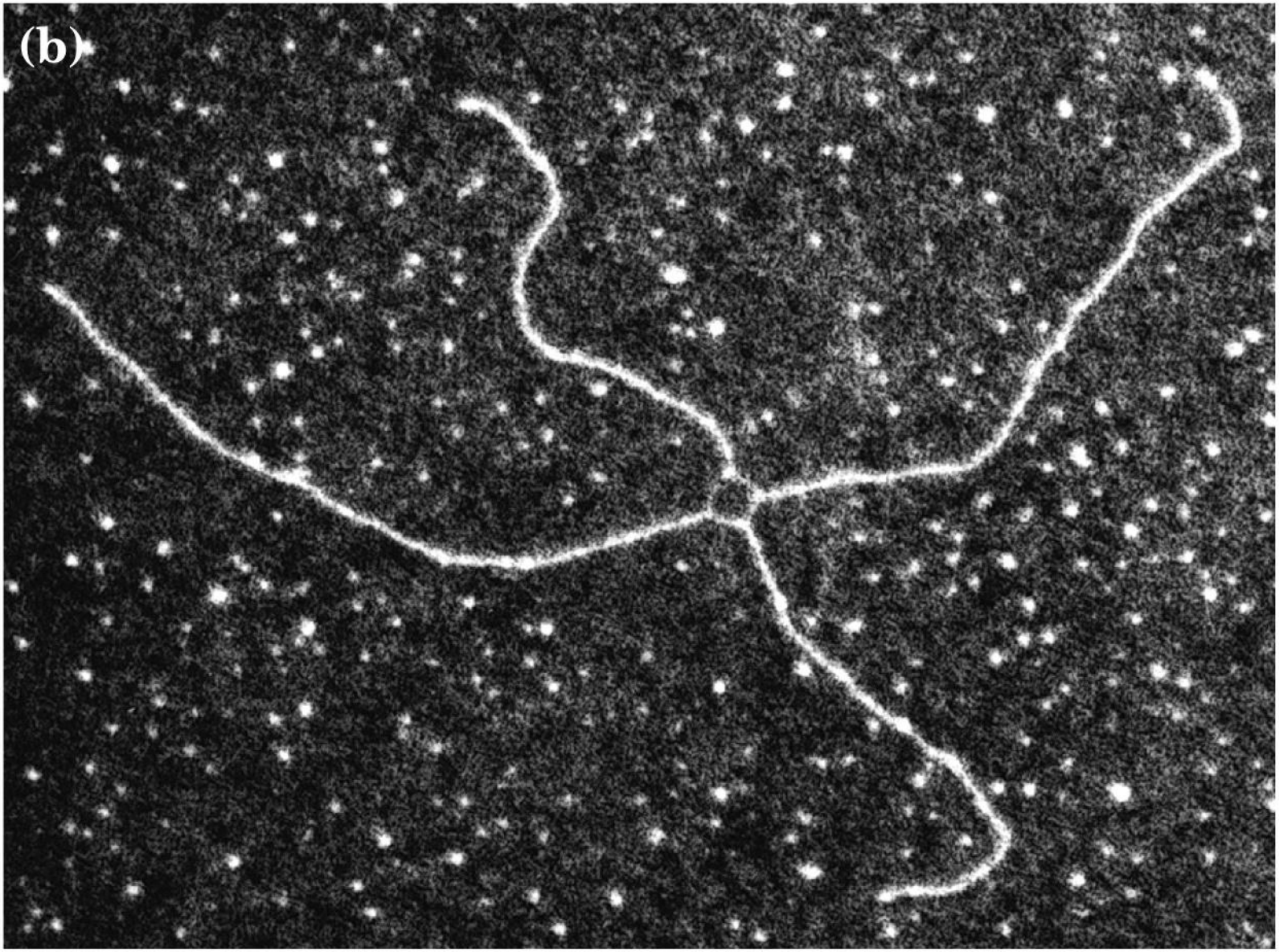
3- extended by DNA pol + branch migration.

4- two Holliday junctions.

5- specialized nucleases cleave Holiday junctions.



Holliday intermediate/ crossover junction.



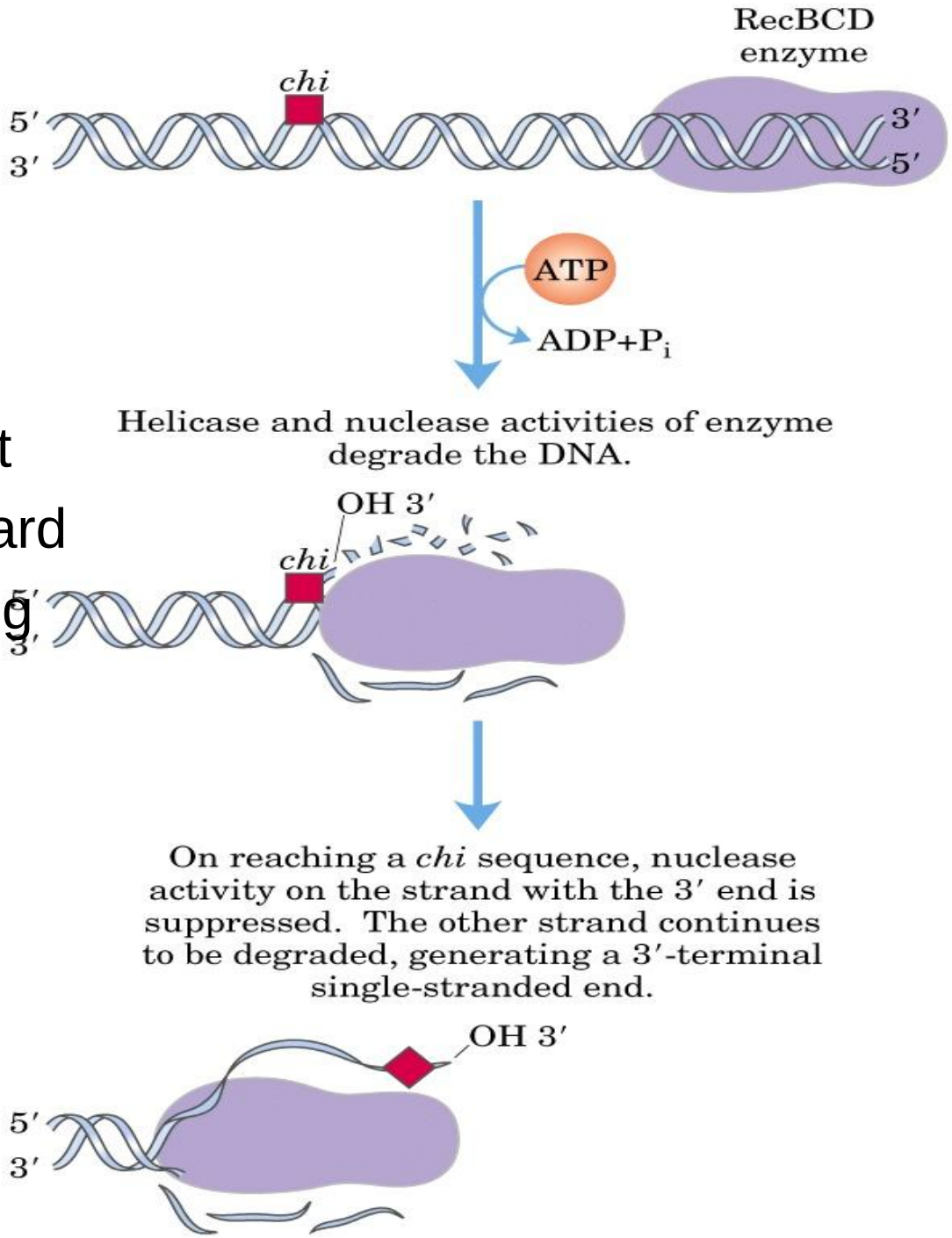
Formation of 3'ssDNA extensions:

By helicase and nuclease activity:

RecBCD binds linear DNA at free broken end moving inward along double helix, unwinding + degrading.

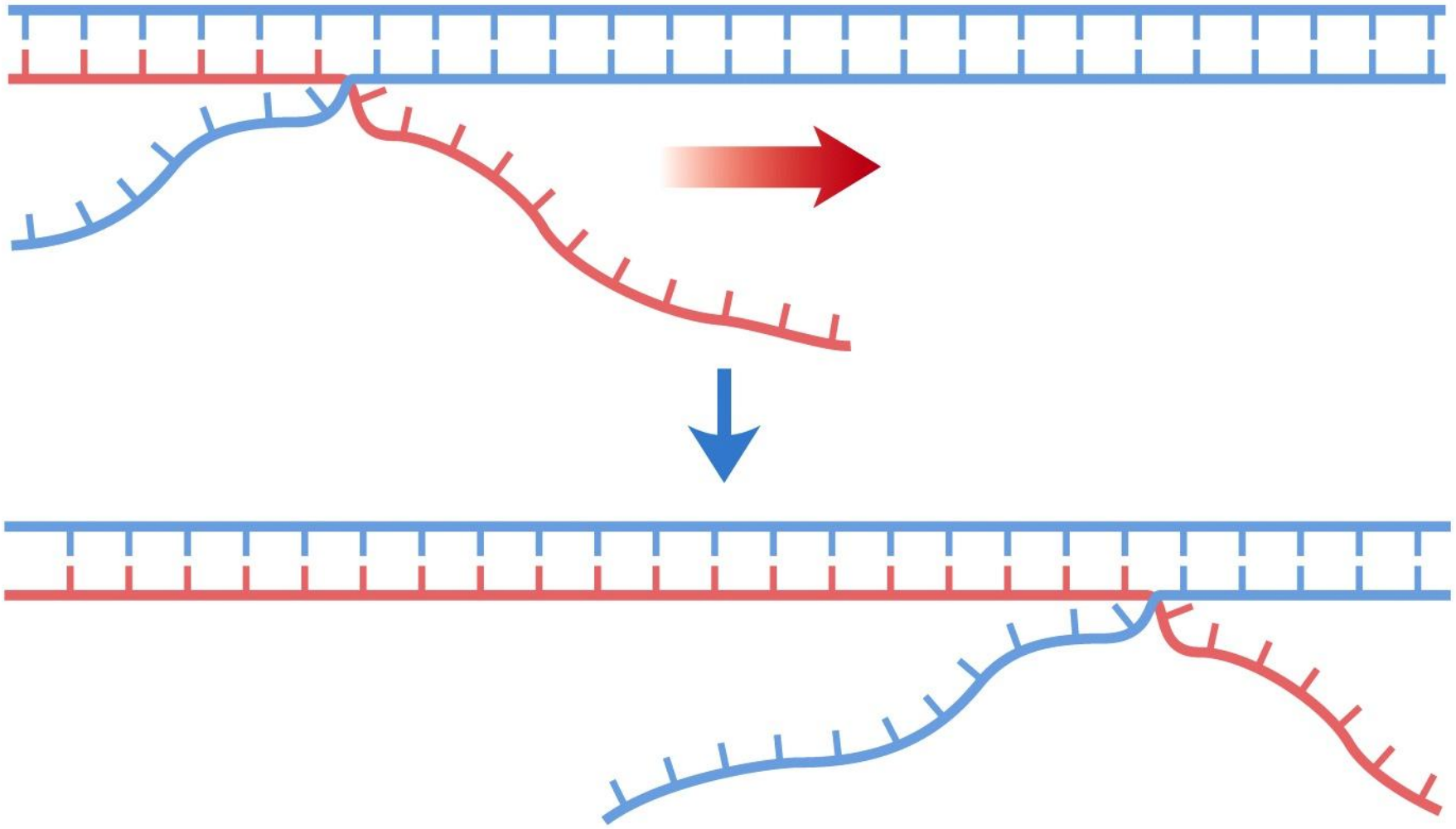
Chi = 5'-GCTGGTGG-3'

Around 1009 chi in E.coli enhances the frequency of recombination 5-10 folds.



Branch migration:

When base pairing with one of the complementary strands is broken and replaced with base pairing to the other strand.

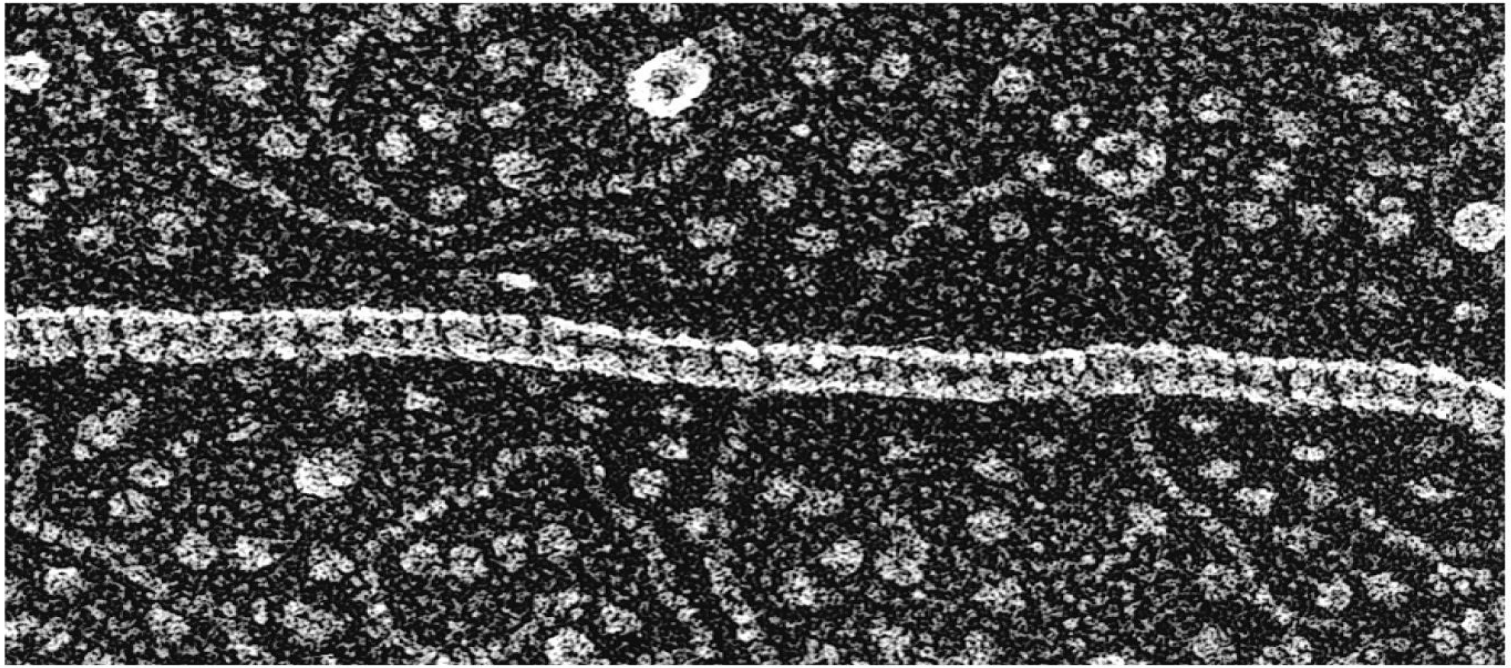


RecA filament protein = 6 subunit/turn , right handed helix

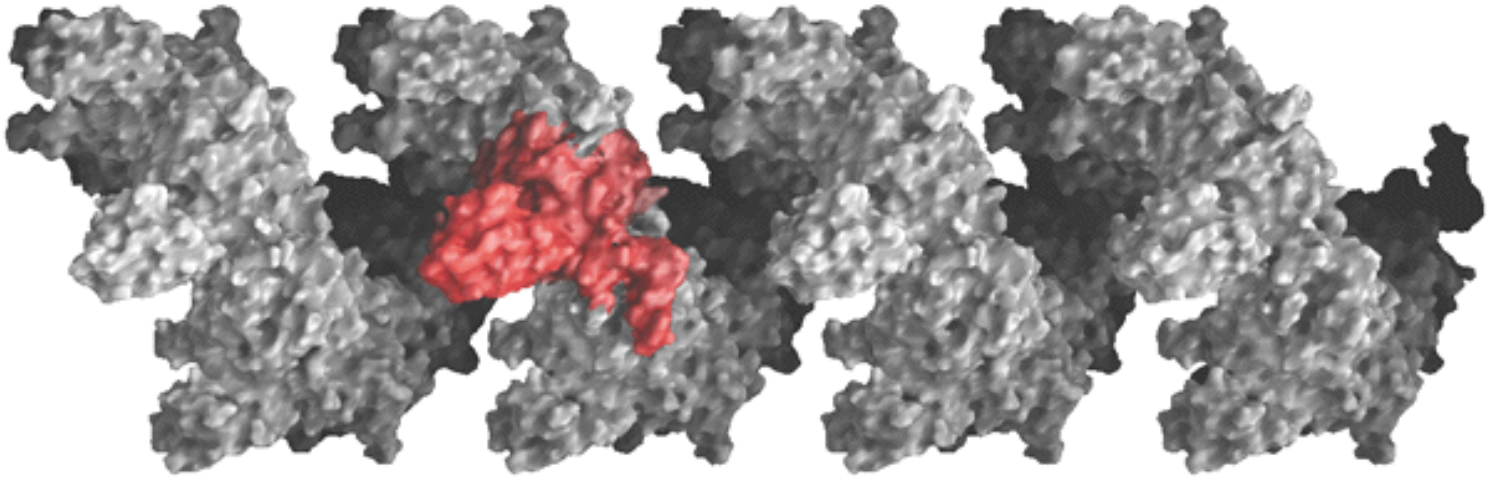
The active form of Rec A is ordered of thousands of RecA monomers.

Rec F, O, R regulate assembly and deassembly of the RecA

Since DNA ordered helical structure. Strand exchange needs ordered rotation of the 2 aligned DNA.
= spooling action



(a)



(b)

Model for strand exchange:

a) RecA protein forms filaments on ssDNA

b) A homologous duplex incorporates into this complex.

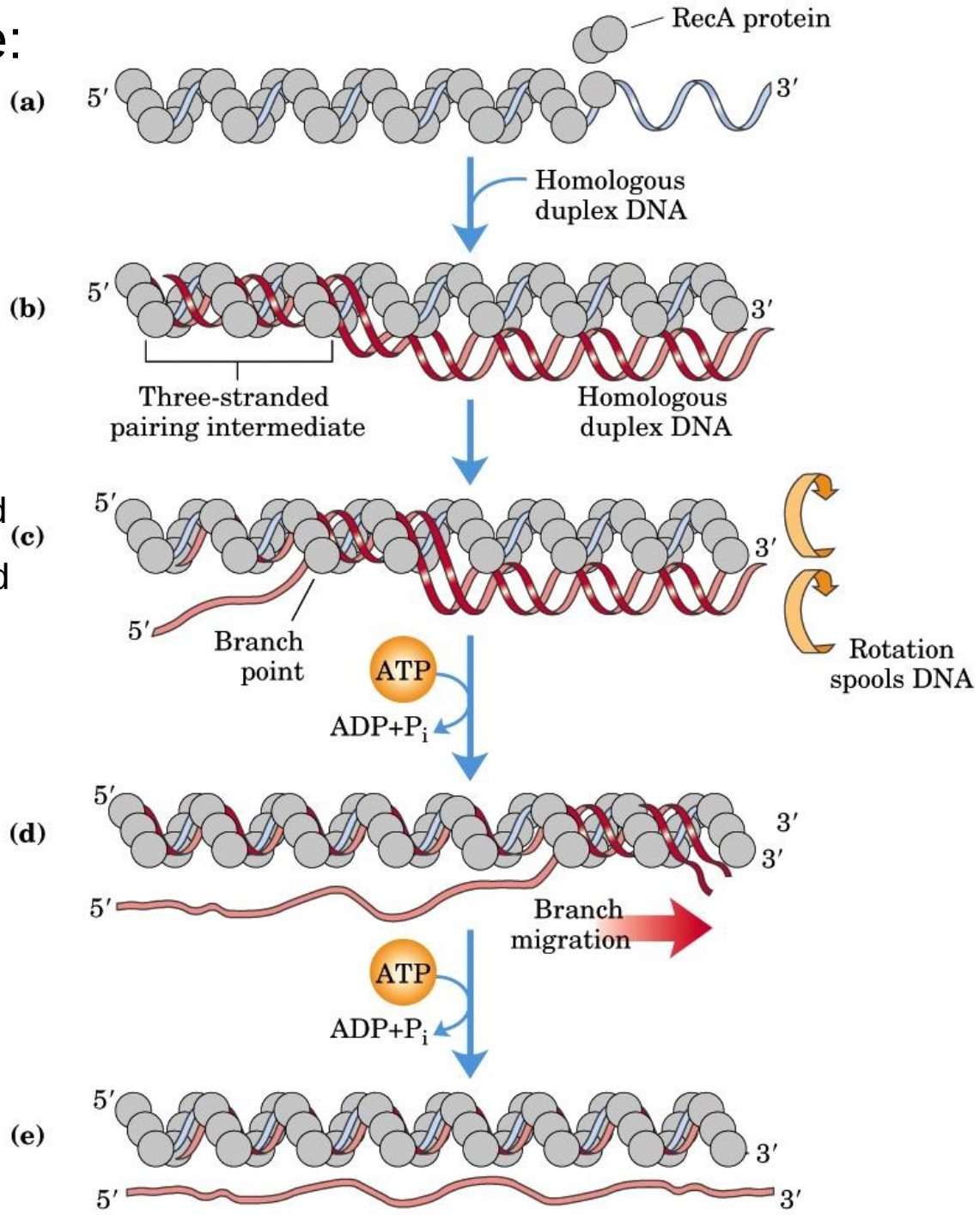
c) Spooling shifts 3 stranded chain left →right

d) One of the duplex strands transferred to the duplex strand, the other displaced

-A new duplex forms in the filament.

e) Rotation continues, displaced strand separates entirely.

-Hydrolysis of ATP by RecA rotates DNA strands from left → right.



ds Break Repair System:

Two homologous chromosomes

1- break→gap by exonuclease (more at 5' end)→3'ss extensions.

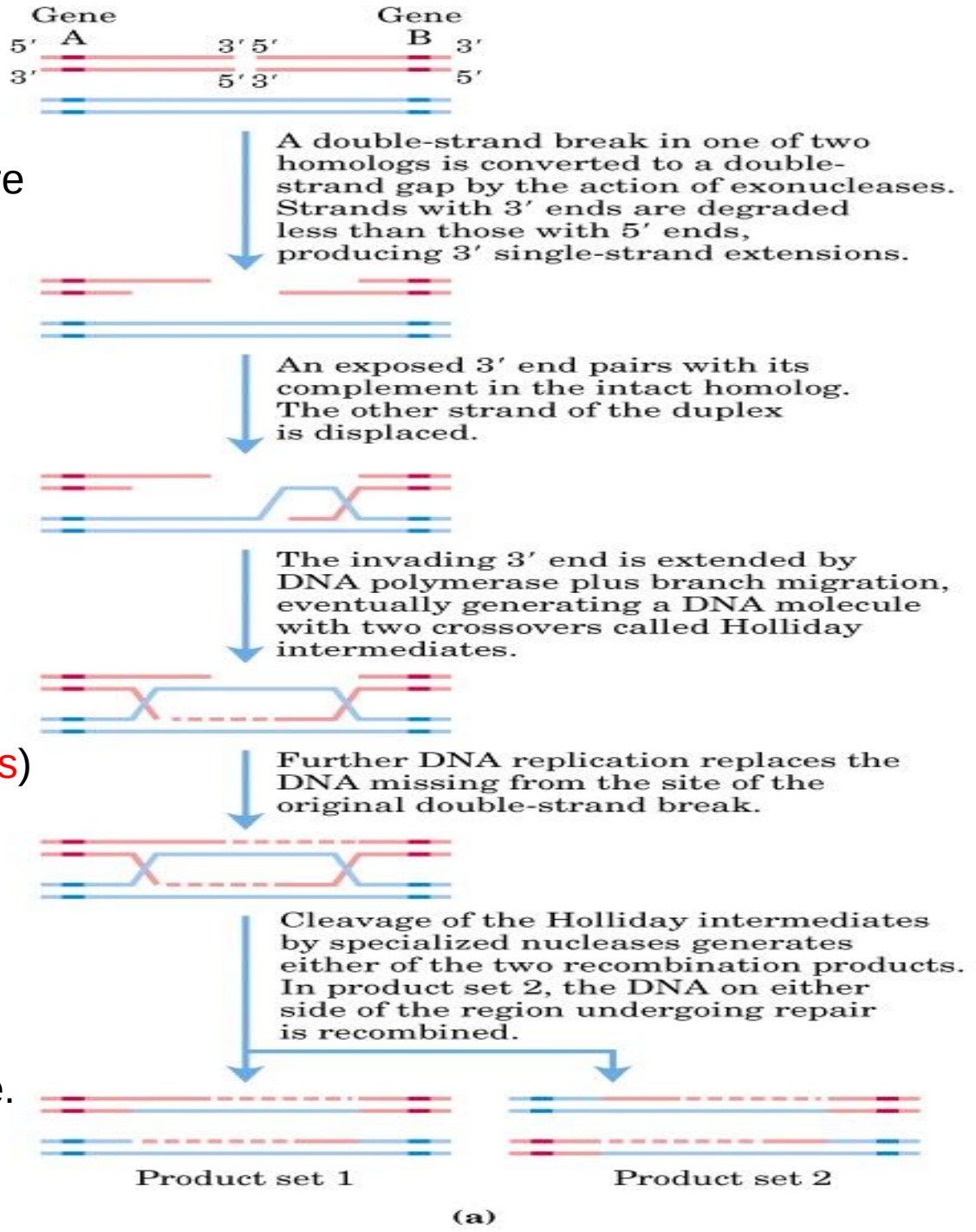
2- extension invades, pairs with its complementary strand in intact homolog

3- extended by DNA pol + branch migration.

4- two Holliday junctions.

5- specialized nucleases (resolvases) cleave Holiday junctions.

Once Holliday formed a set of proteins and enzymes required topoisomerase, DNA pol, ligase.



- Homologous repair may vary in many details from one species to another, but most the steps are common.

- Both cleavage products are seen in vivo in eukaryotes and prokaryotes.
- Contribute to genomic diversity→
- Two homologous chromosomes are not identical→ differ slightly in different alleles.

- One contains the allele of hemoglobin A the other hemoglobin S (sickle cell anemia).

- The difference one bp among millions.

- Most DNA damage repaired by BER/NER but replication fork - in its journey from origin to terminus - encounters DNA ds breaks / lesions.

- DNA pol III can't continue → **Recombinational DNA repair**
  Origin-independent restart of replication:
  Complex of 7 proteins **DNA pol II**.

- Repair of stalled/ blocked replication fork :Transition from replication→ recombinational repair → replication.

Site specific recombination:

Recombinase/integrase + recombination site (DNA segment 20-200bp)

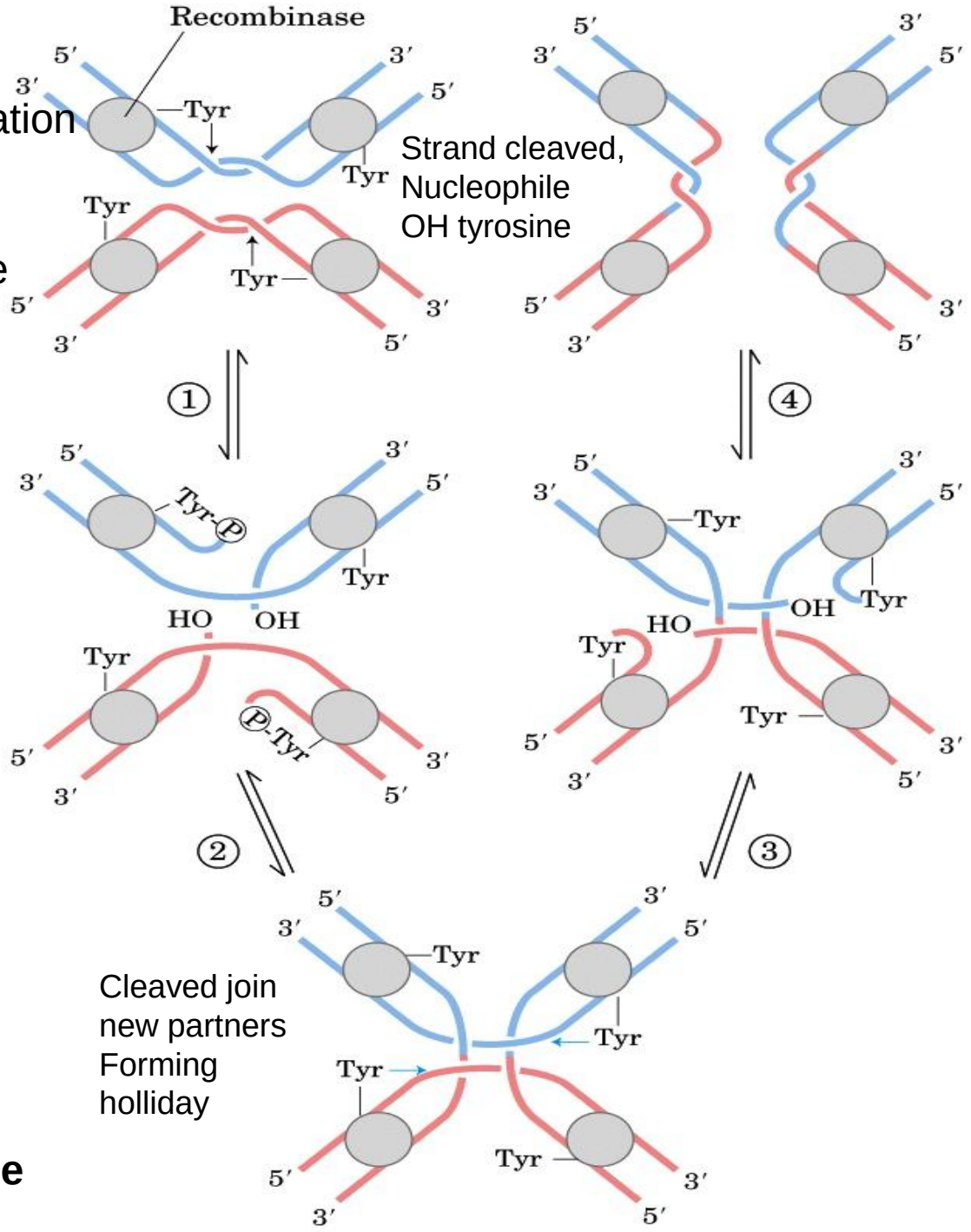
Site specific recombinase / integrase tetramer of identical units.

One recombinase recognize+bind each of the 2 recombination sites through phosphotyrosine.

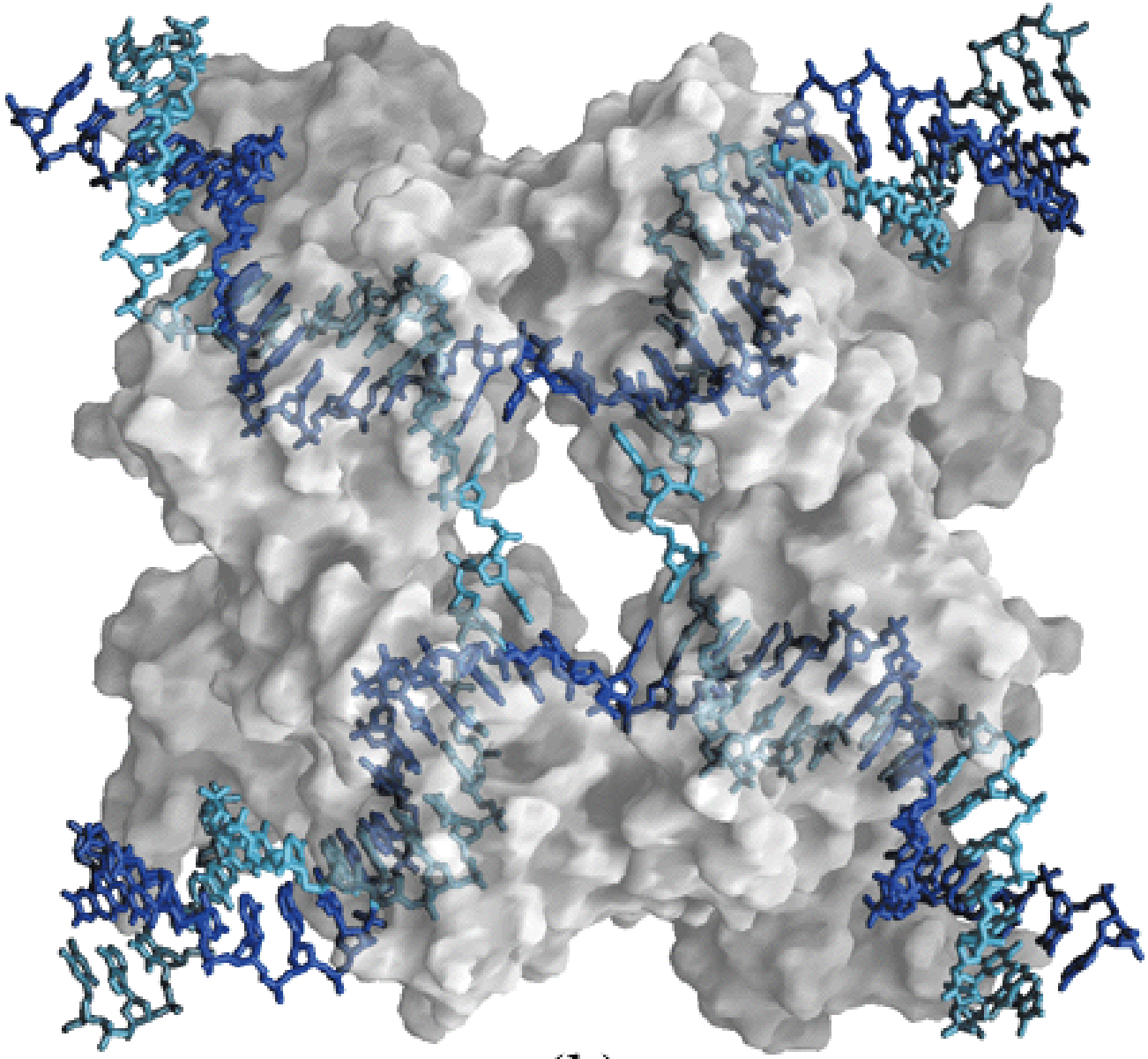
Transient protein-DNA covalent linkage preserve phosphodiester bond by phosphoserine/ phosphotyrosine bond.

Recombinase=

site specific endonuclease + ligase



Recombinase bound to Holliday intermediate:



(b)

3) Transpositional recombination:

a process in which a **mobile element** is inserted into a target DNA.

It may occur by one of two mechanisms:

(1) directly as DNA, (**transposons).**

(2) through RNA. (**retrotransposons)**.

Transposable Genetic Elements:

Simple transposons:

contains only the sequence required for transposition. The genes for transposase.

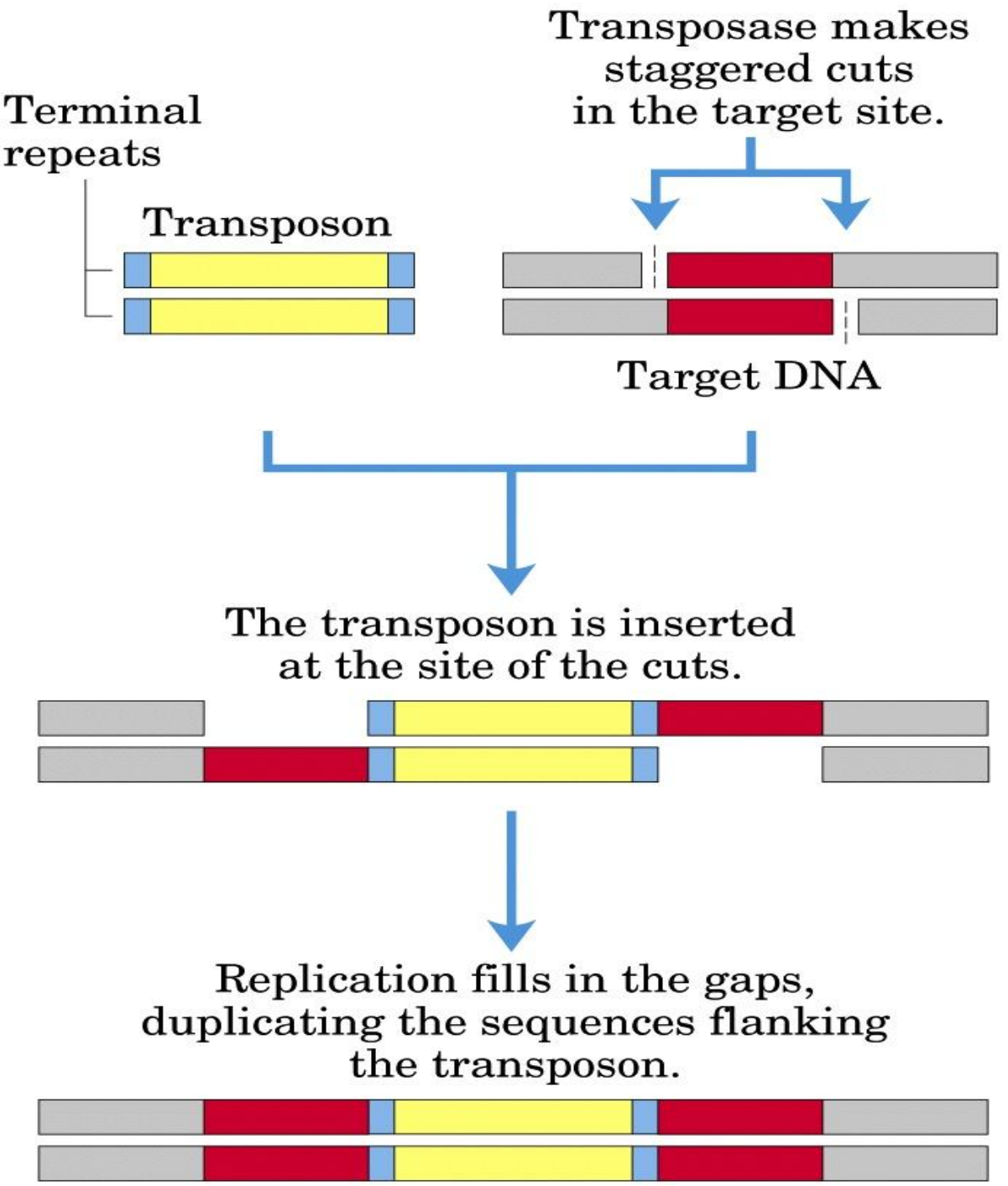
Complex transposons:

contains one/ more genes in addition to those needed for transposition.

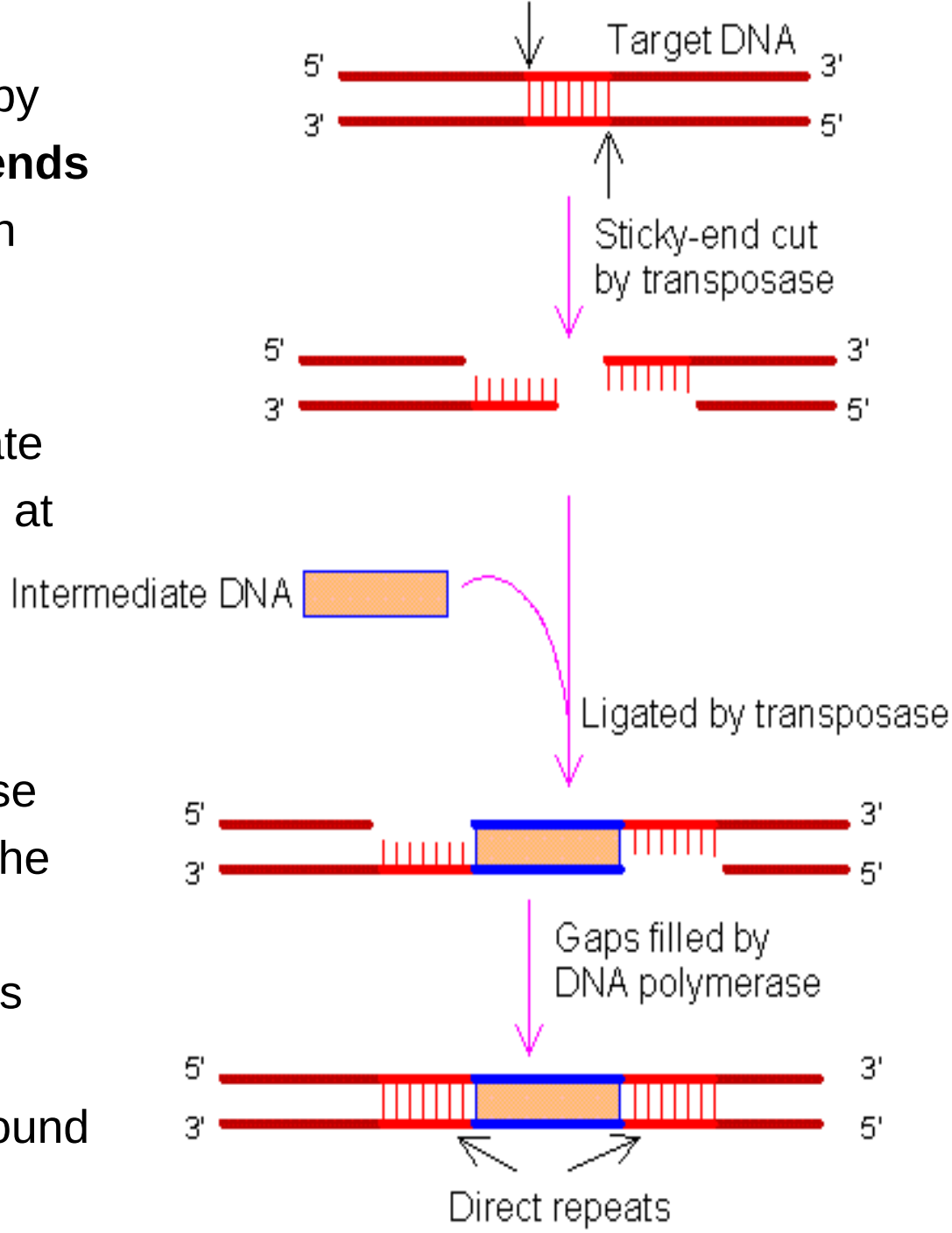
e.g. confer resistance to antibiotics.

Transposons vary in structure, but most have short repeated sequences at each end = binding sites for transposase.

When transposition occurs a short sequence 5-10bp duplicates to form an additional short repeated sequence flanking each end of the inserted transposons.



- In bacteria, the target DNA cut by transposase, produces **sticky ends** (single strands, easy to pair with complementary sequences).
- Transposase has ligase activity which may ligate the intermediate DNA of transposons. The gaps at sticky ends are filled by DNA polymerase, generating **direct repeats**.
- Other organisms expected to use the same mechanism to insert the DNA intermediates of either transposons or retrotransposons into target DNA. direct repeats generated by this mechanism found in **ALL** mobile elements.



- **Mechanisms of transpositional recombination**

a) For transposons. The transposon in donor DNA is cut by a special enzyme and then inserted into a target DNA. enzyme = transposase has both nuclease and ligase activities.

b) For retrotransposons. The retrotransposon in donor DNA is first transcribed into RNA and then reverse-transcribed into DNA, which is inserted into a target DNA by the same recombination mechanism as the DNA intermediates of transposons.