DNA replication in eukaryotes

DNA Replication in Eukaryotes

- Shorter RNA primers and Okazaki fragments
- DNA replication only during S phase
- Multiple origins of replication
- Switch from initiation to elongation: polymerase α/ primase replaced by polymerase δ
- Nucleosomes
- RNA primers were removed by Ribonuclease
- Telomeres

Eukaryotic Replication Proteins

Pol α/primase	Synthesis of RNA-DNA primers (iDNA)
Pol δ (or Pol ε)	Replication leading strand and lagging strand and Make the gap between Okazaki fragments
RFC	Recognition the 3' end of the primer and Pol α/primase
PCNA	DNA binding and recruiting of Pol δ
RNAse H I/FEN1	Removal of the 5′- RNA primers of Okazaki fragments
DNA ligase Ⅰ	Covalently Closes Nicks in DNA
RPA	SSB like protein
Helicase (Mcm2-Mcm7)	Unwinds the Parental Double Helix
Topoisomerase	

Eukaryotic DNA Polymerases

at least 8 DNA polymerases

Eukaryotic	Number of subunits	Function
Pol α	4	Primer synthesis during DNA replication
Pol β	1	Base excision repair
Pol γ	3	Mitochondrial DNA replication and repair
Pol δ	2–3	DNA replication; nucleotide and base excision repair
Pol ε	4	DNA replication; nucleotide and base excision repair

- **Different DNA polymerases have different functions in the cell**
- **Rate of DNA synthesis in eukaryotic cells = 50 nt/s (~ 1/10 rate of bacterial DNA synthesis)**

Properties of mammalian DNA polymerases

Mammalian polymerases	α	β	γ	δ	ε
5'-3' polymerization	+	+	+	+	+
3'-5' exonuclease proofreading activity	-	-	+	+	+
Synthesis from					
RNA primer	-	-	-	+	-
DNA primer	+	+	+	+	+
Associated DNA primase	+	-	-	-	-
Sensitive to aphidicolin (inhibitor of cell DNA synthesis)	+	-	-	+	+
Cell location					
Nuclei	+	+	-	+	+
Mitochondria	-	-	+	-	-

When, during the cell cycle, can new replication origins be formed?

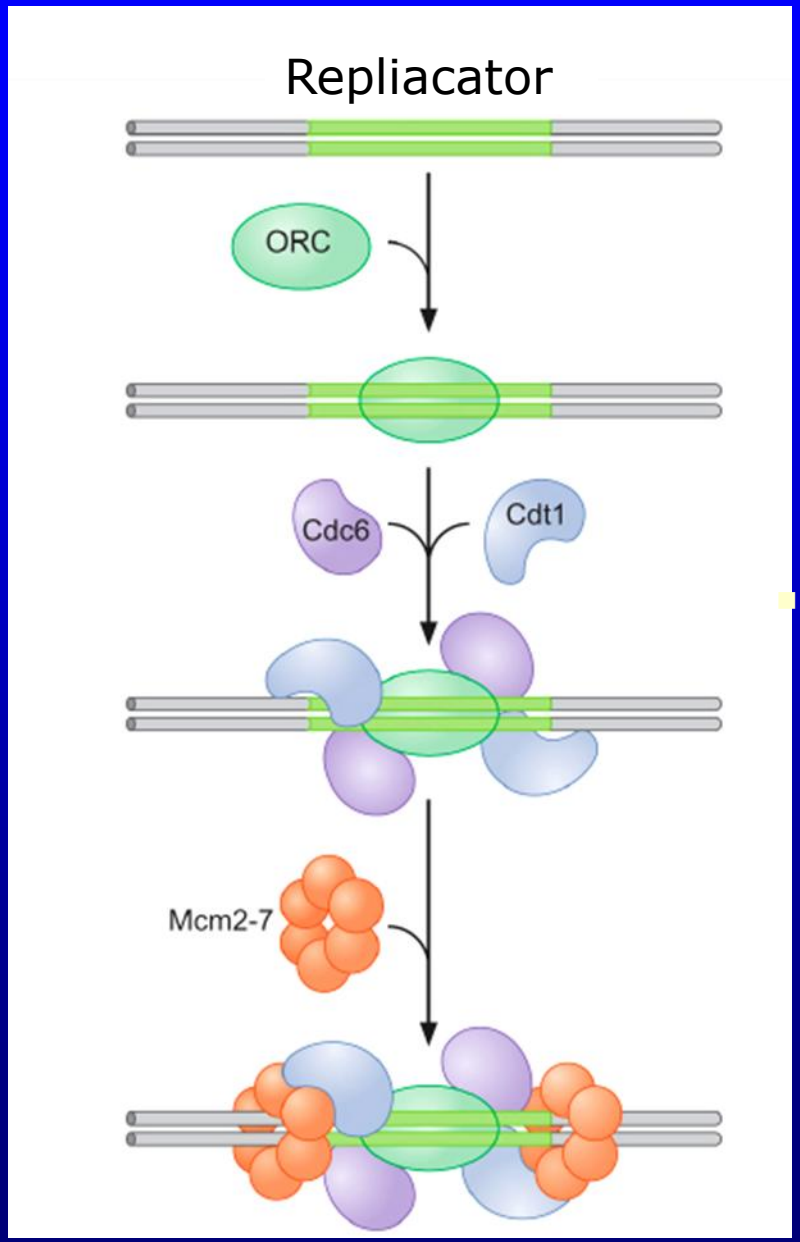
G1	S	G2
Pre-replication	replication	post replication
ARSs complex Into ORCs	DNA synthesis	DNA synthesis completed
Pre-RCs can form	No new pre-RCs	No new pre-RCs

ARS = autonomously replicating sequence = origin
ORC = origin recognition complex
Pre-RC = pre-replication complex

All the eukaryotic DNA replication occur in S phase，

and only once in one cell cycle。

Formation of pre-RC

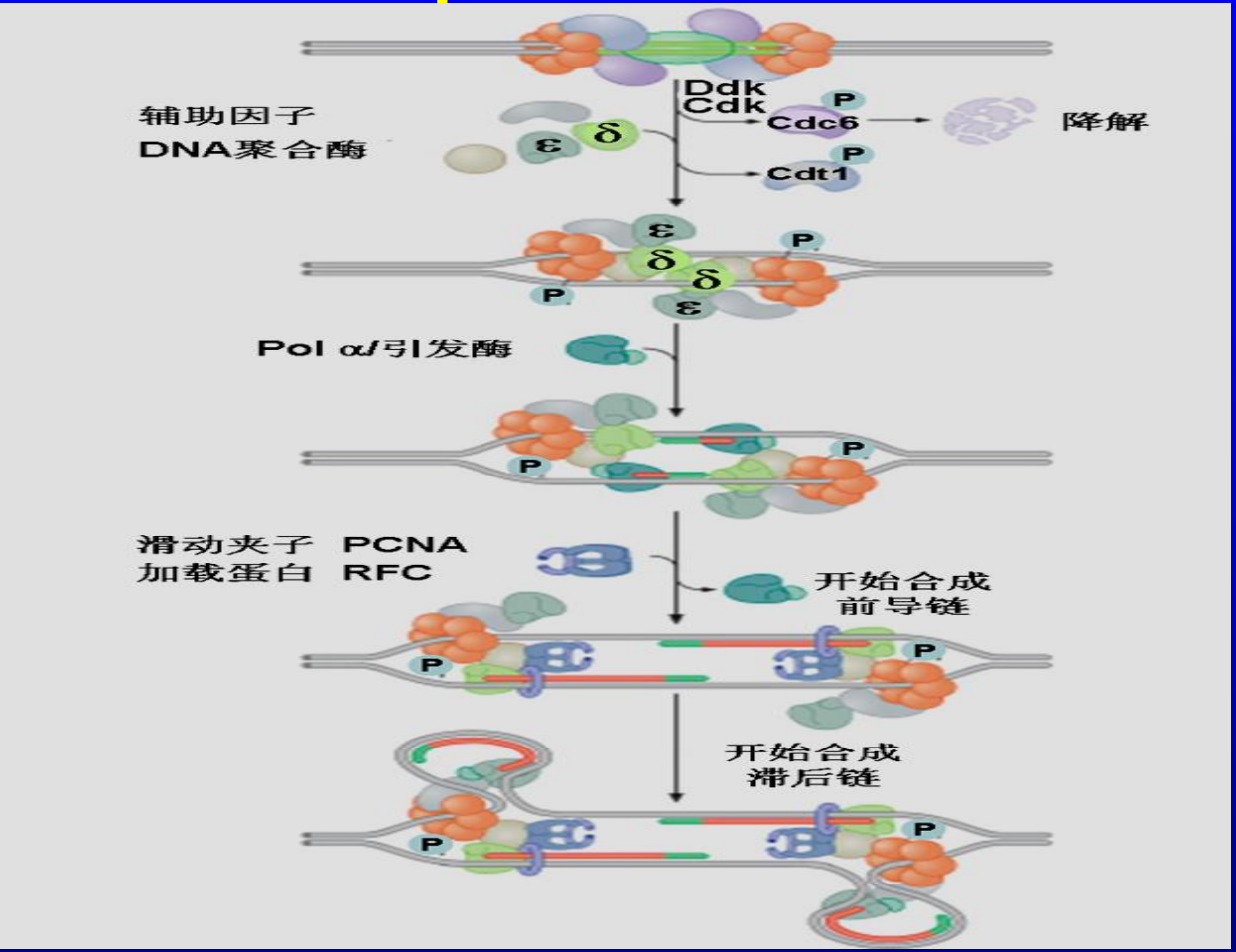


- **Origin recognition complex, (ORC) Binding to the replicator**
- **ORC recruits Helicase loading proteins : Cdc6 and Cdt1**
- **Helicase Mcm2-7 was recruited.。**

Pre-RC activation:

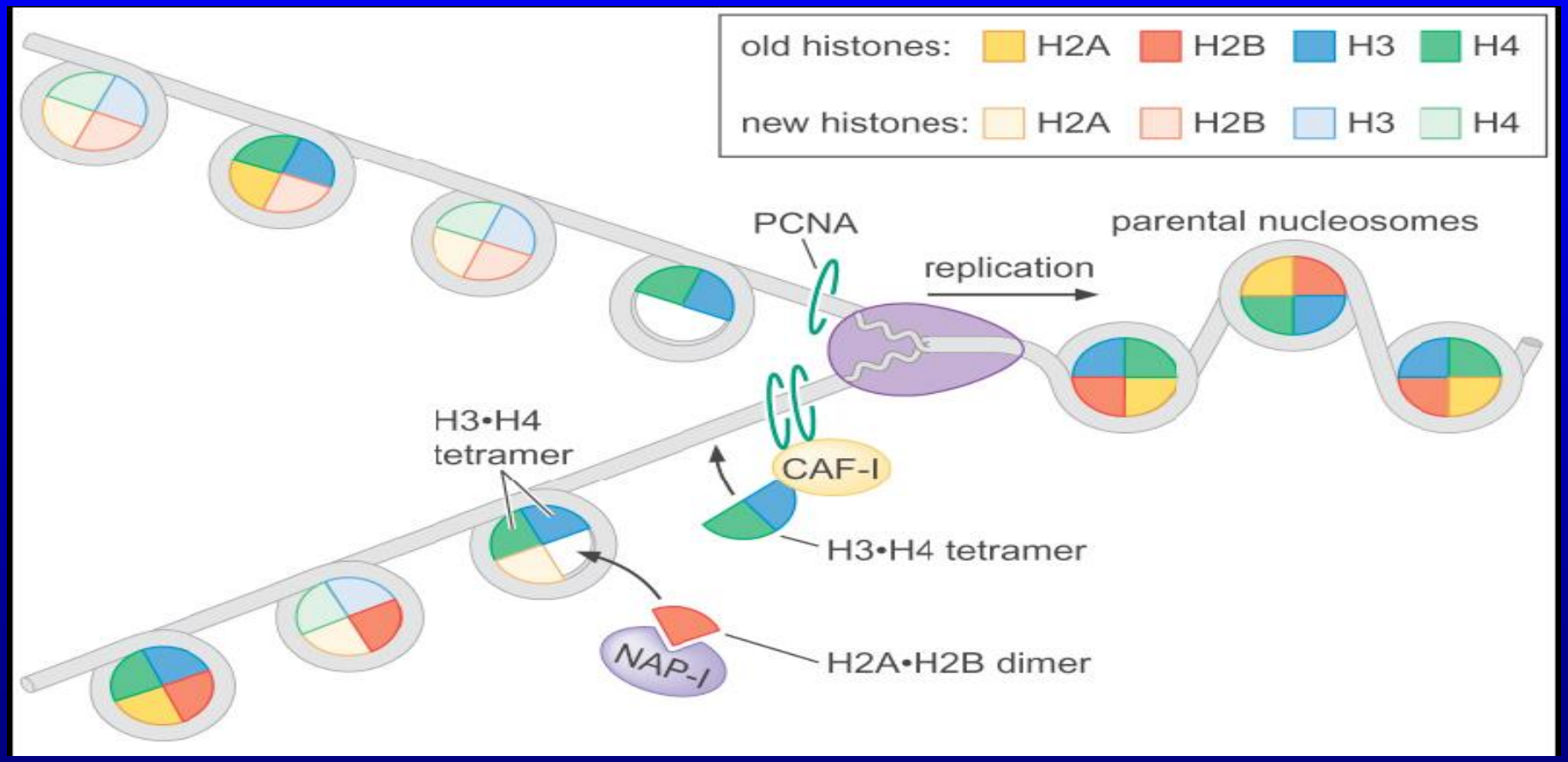
- Pre-Rcs are activated to initiate replication by two protein Kinase, CdK and DdK.
- High CdK activity is required for Pre-RC activation.
- CdK level is low in G1 phase but high in S,G2 and M phases.

Activation of pre-RC and formation of replication fork

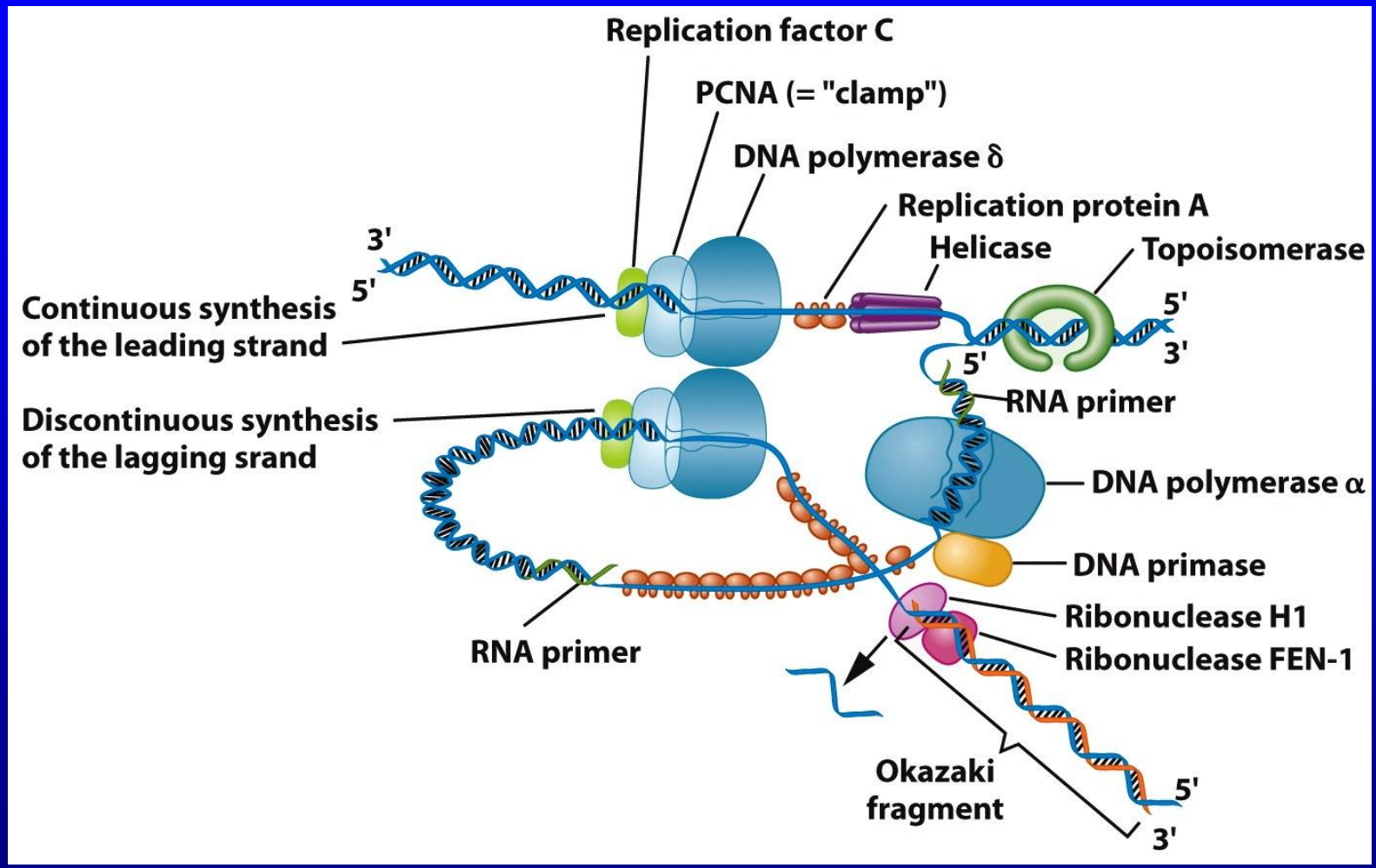


Cdk regulates the formation and activation of pre-RC by phosphorylation of DNA helicase.

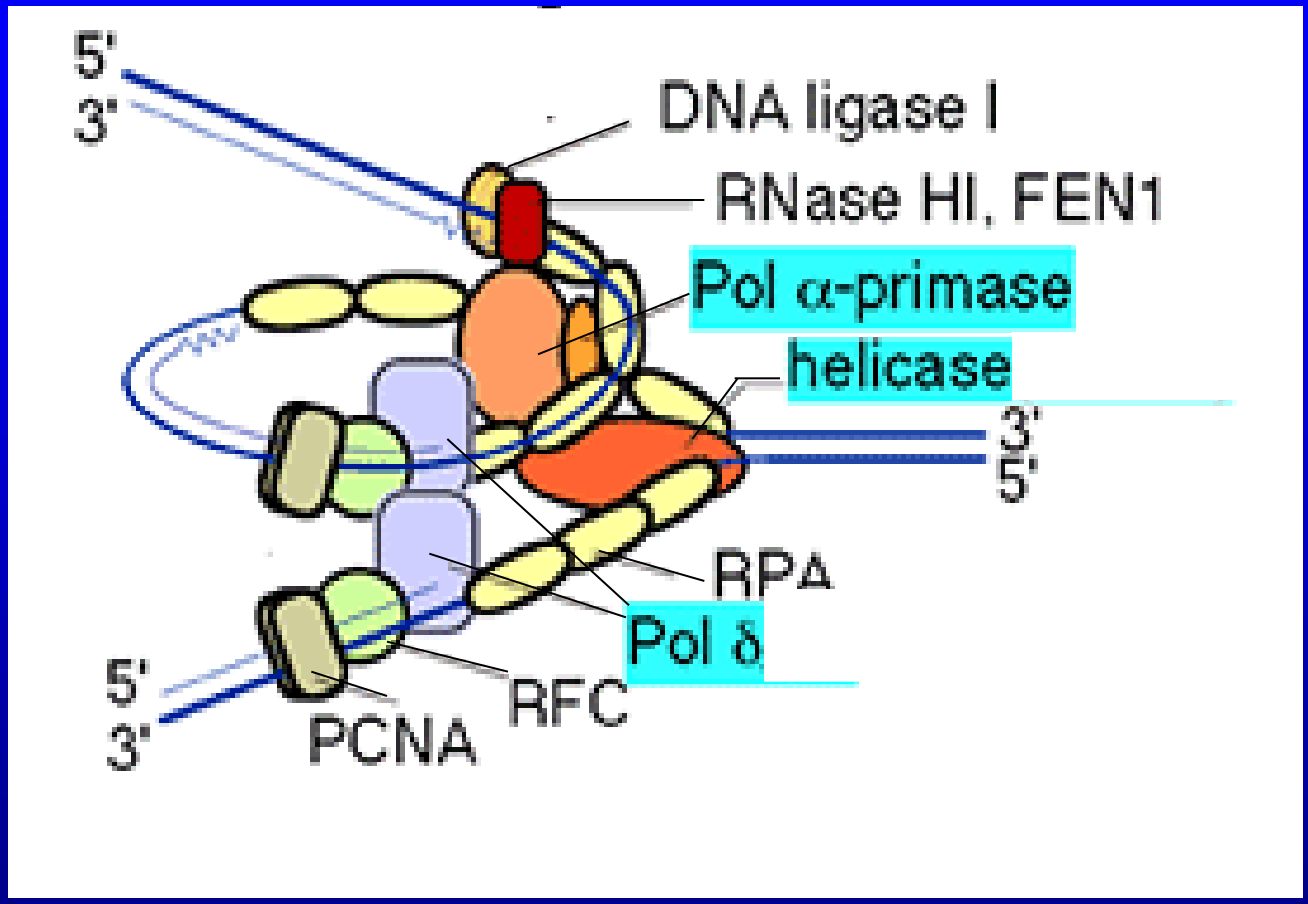
Sliding clamps attract histone chaperones



The Eukaryotic Replisome



Eukaryotic DNA replication fork



Switch from initiation to elongation: polymerase α/ primase replaced by polymerase δ

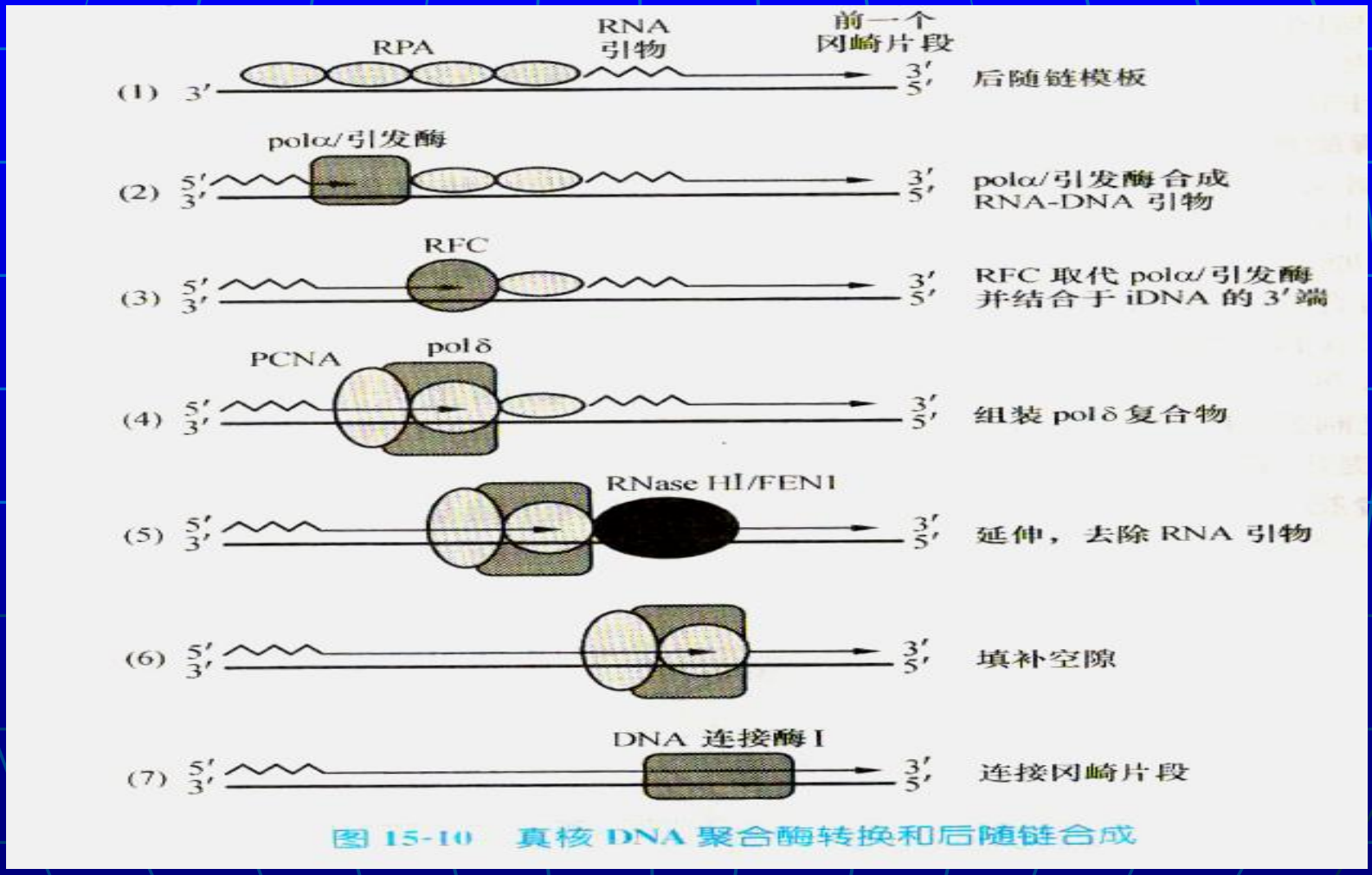
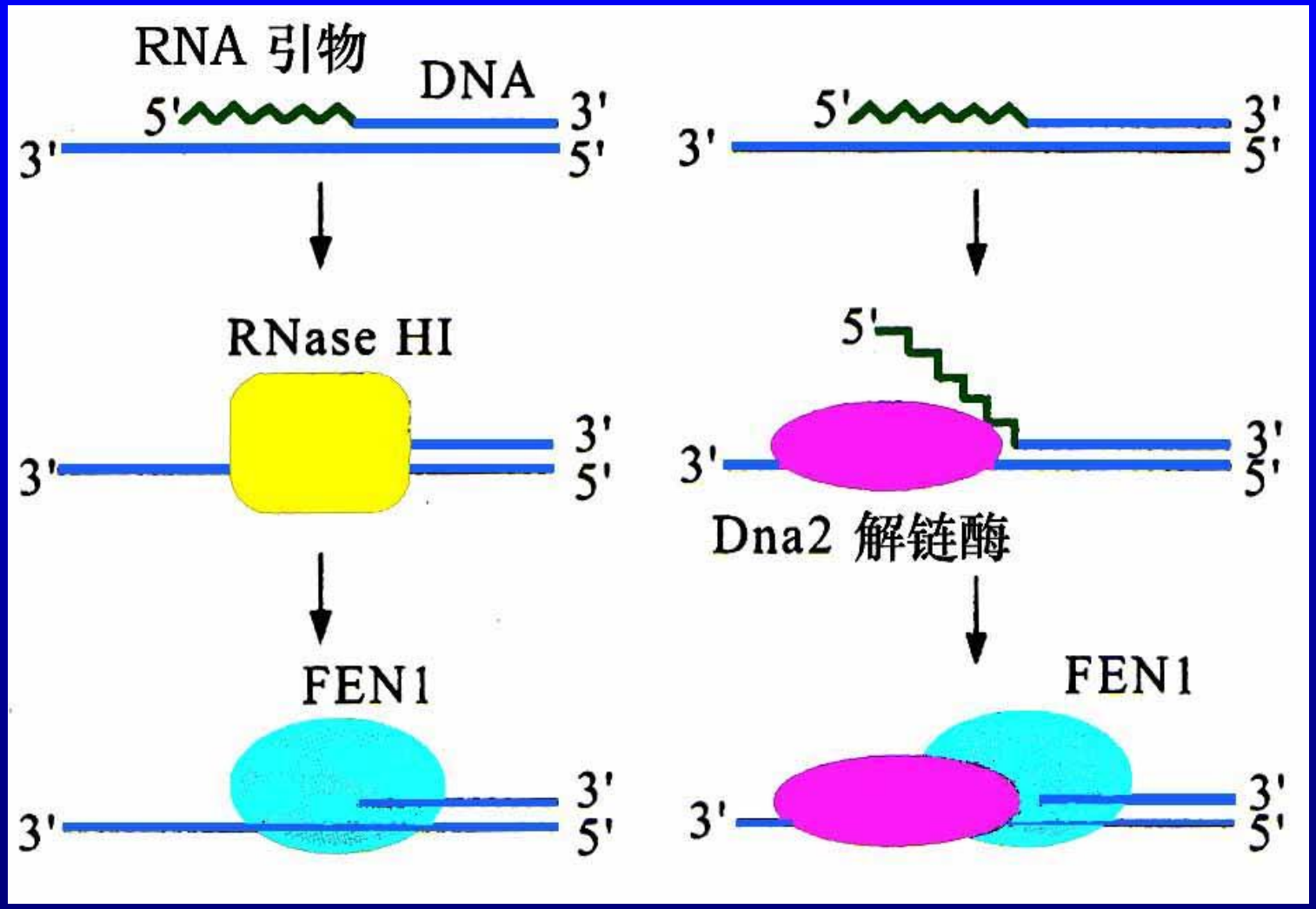
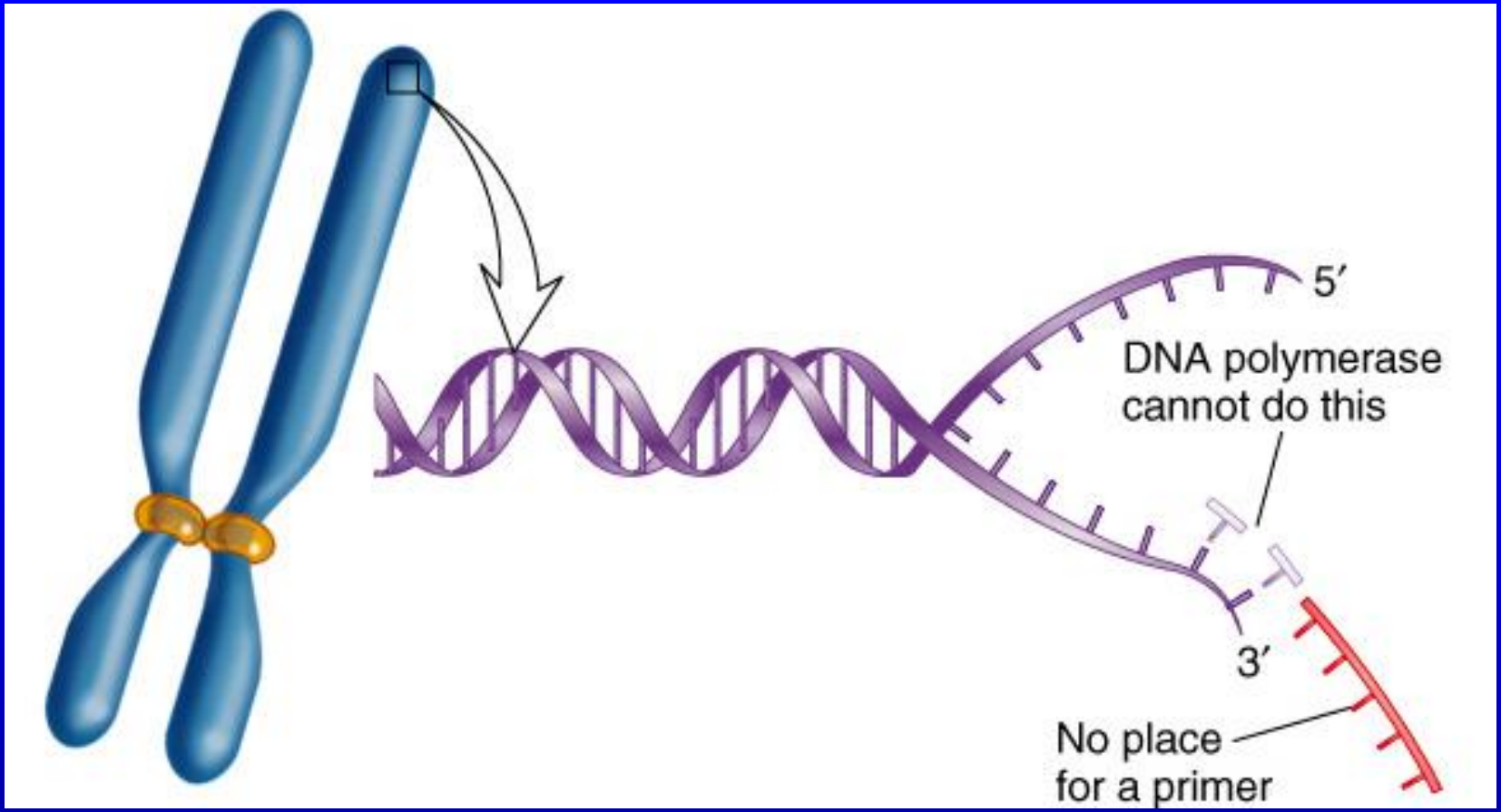


图 15-10 真核 DNA 聚合酶转换和后随链合成

Removal of RNA Primers

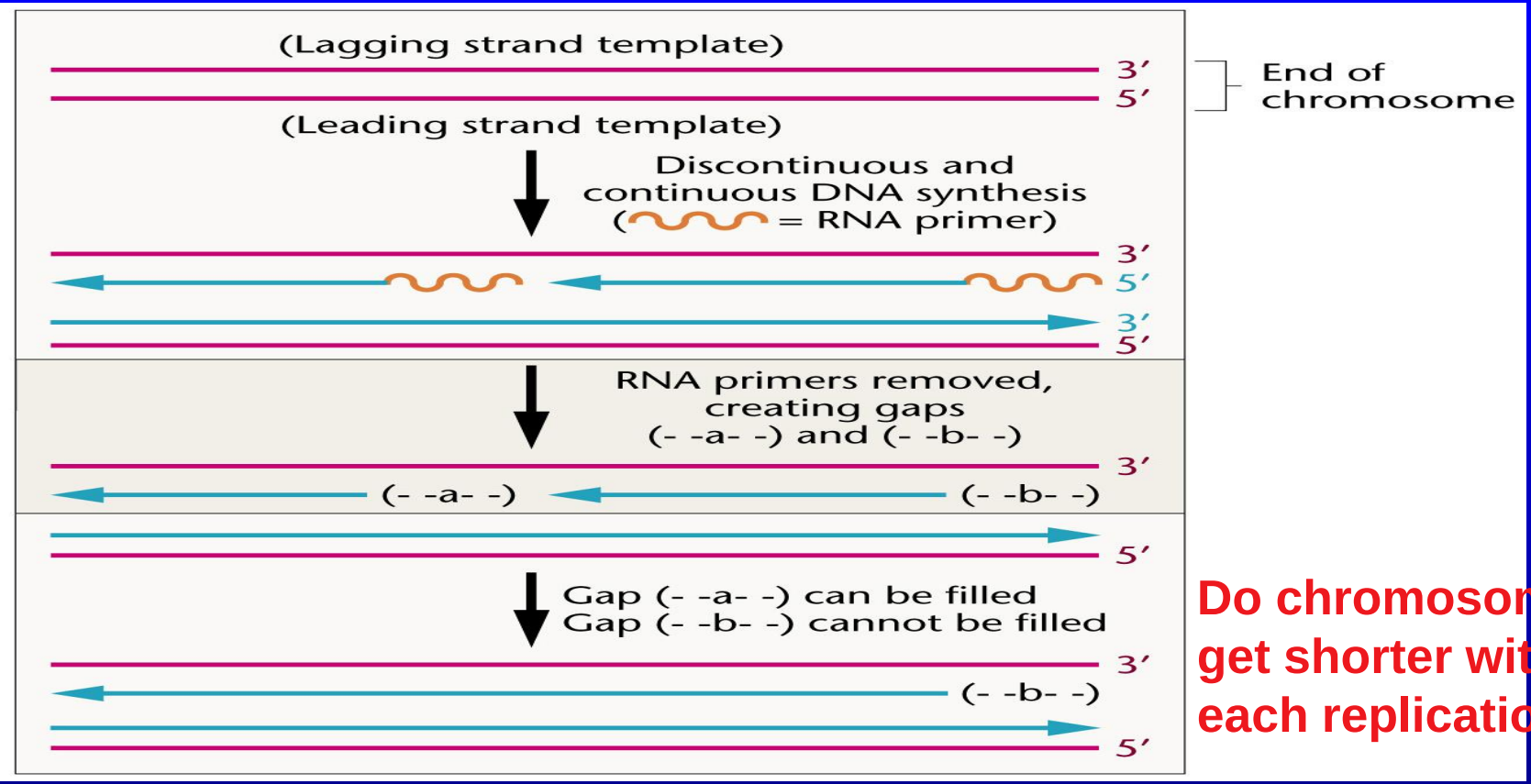
RNAse HI/FEN1 **Dna2/FEN1**





- DNA polymerases can only synthesize DNA only in the 5’ to 3’ direction and cannot initiate DNA synthesis
- These two features pose a problem at the 3’ end of linear chromosomes

The End Problem of Eukaryotic Replication

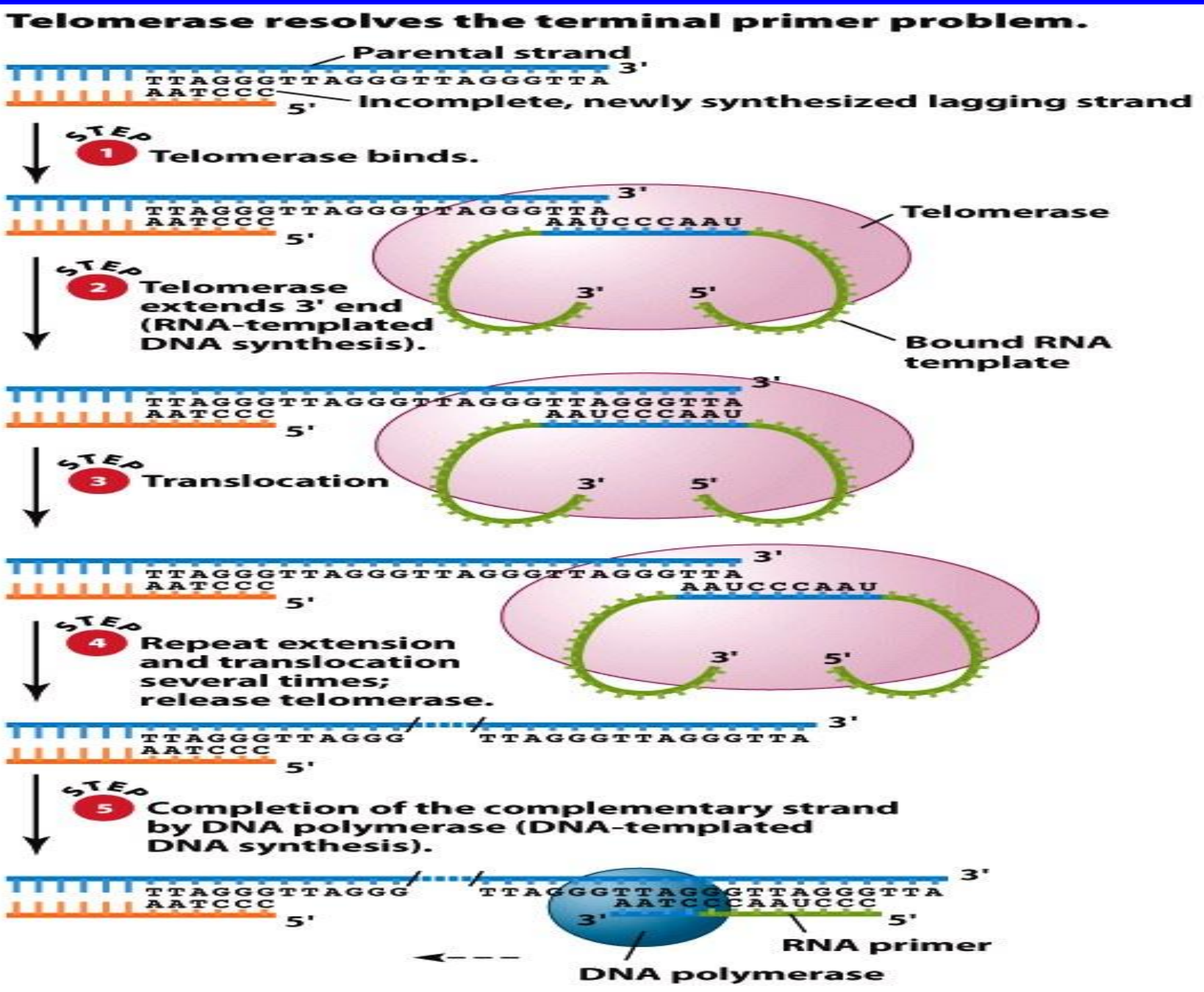


Do chromosomes get shorter with each replication???

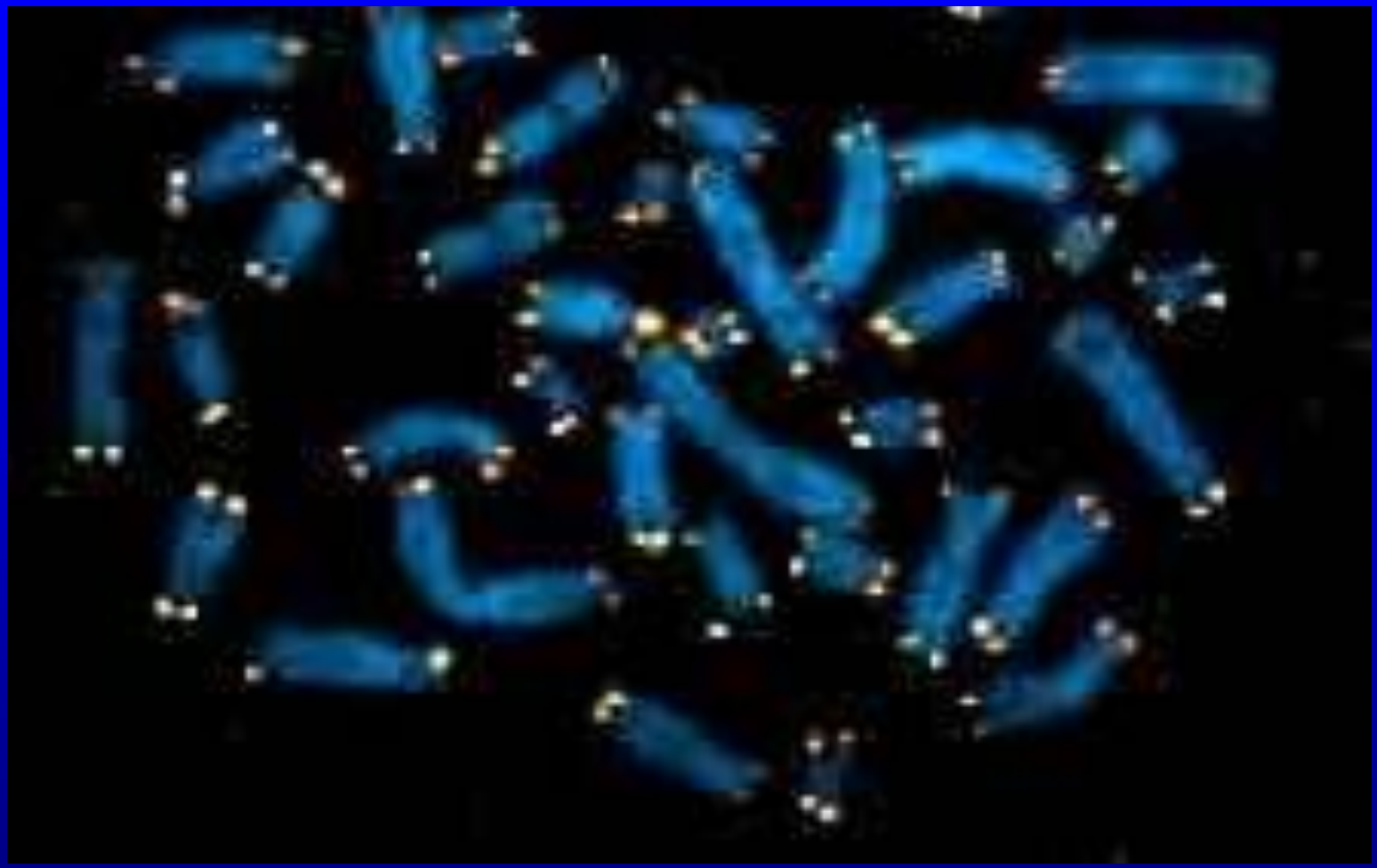
RNA primer near end of the chromosome on lagging strand can't be replaced with DNA since DNA polymerase must add to a primer sequence.

Solution to the Problem: Telomerase

- The cell solves this problem by adding DNA sequences to the ends of chromosome: **telomeres**
  - Small repeated TG rich $(TTAGGG)_n$ sequences
- Telomere specific proteins, eg. TRF1 & TRF2 bind to the repeat sequence and protect the ends
- Catalyzed by the enzyme **telomerase**
- Telomerase contains **protein** and **RNA**
  - The RNA functions as the template
  - complementary to the DNA sequence found in the telomeric repeat
    - This allows the telomerase to bind to the 3' overhang


Telomerase resolves the terminal primer problem.
Parental strand
3'
TTAGGGTTAGGGTTAGGGTTA
AATCCC
5'
Incomplete, newly synthesized lagging strand
STEP 1
Telomerase binds.
AAUCCCAAU
Telomerase
STEP 2
Telomerase extends 3' end (RNA-templated DNA synthesis).
Bound RNA template
STEP 3
Translocation
STEP 4
Repeat extension and translocation several times; release telomerase.
STEP 5
Completion of the complementary strand by DNA polymerase (DNA-templated DNA synthesis).
RNA primer
DNA polymerase

Chromosome Ends -Telomeres



Blue = chromosomes

White = Telomere protein

Telomerase is composed of both RNA and protein

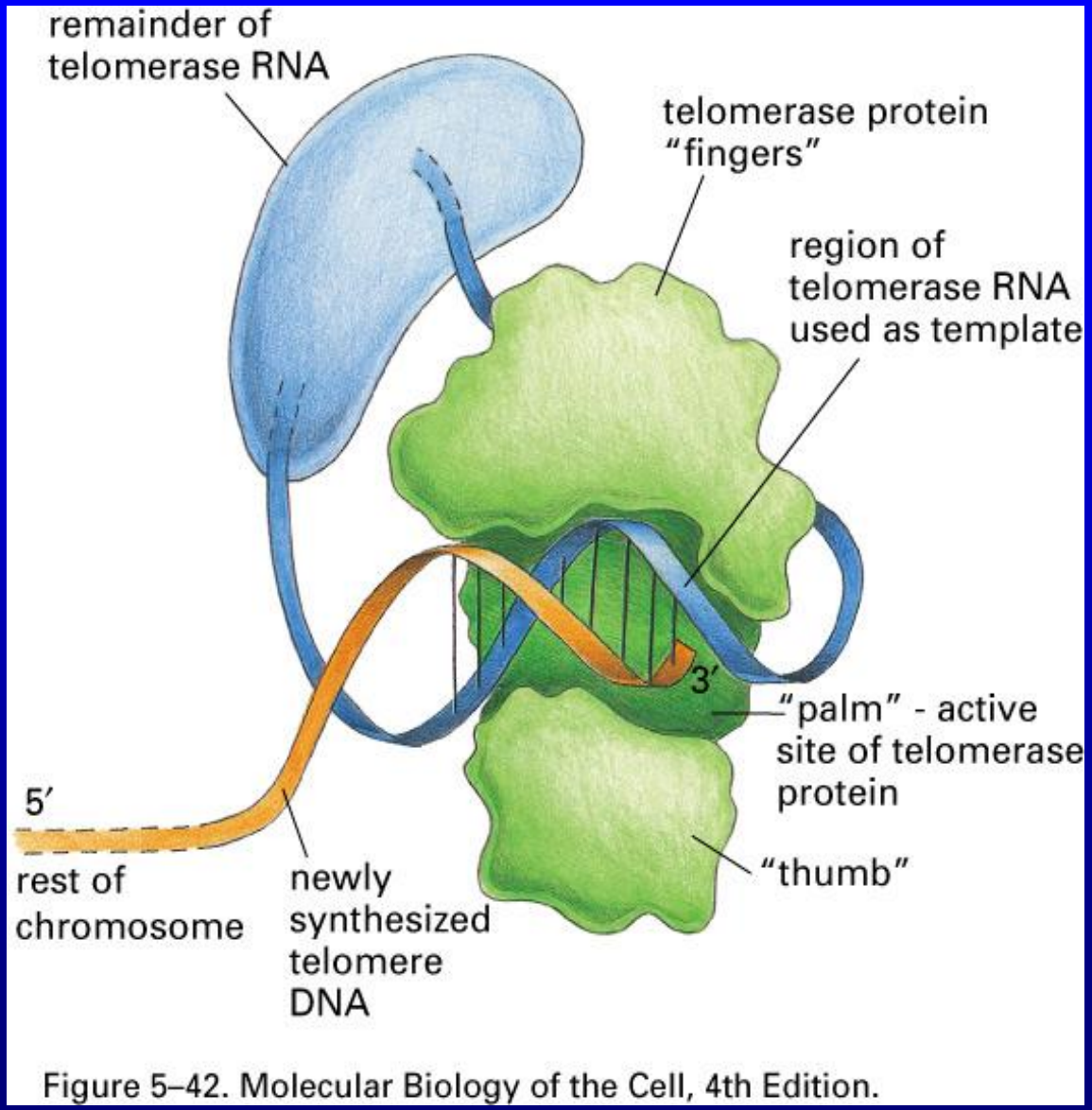


Figure 5–42. Molecular Biology of the Cell, 4th Edition.

Evidence that shortening of telomeres may be a signal to stop cellular division:

-Primary cells taken from the body and grown in vitro will divide a few times and then stop.

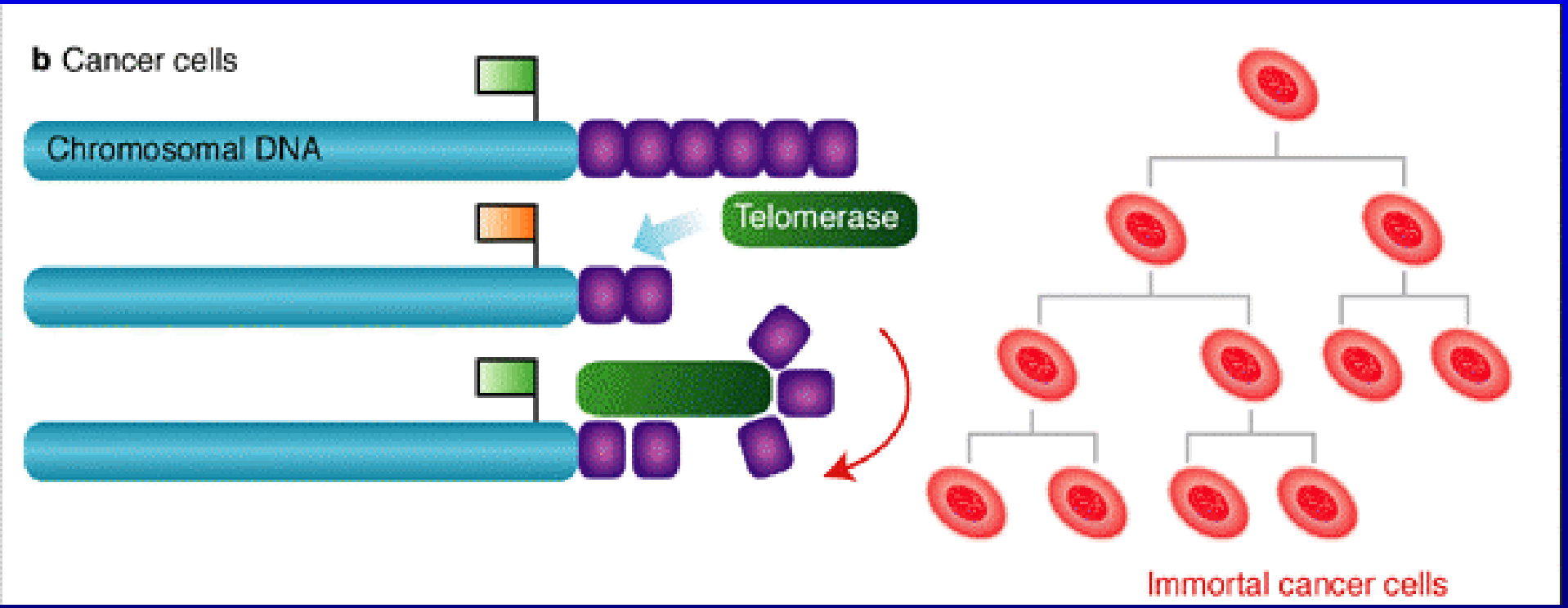
If the gene encoding the catalytic subunit of Telomerase is expressed in these primary cells, they grow for many more divisions.

-Proliferating cells in the body (stem cells / germ cells) express telomerase

-Cancer cells express telomerase

Telomerase and Cancer

The presence of telomerase in cancer cells allows them to maintain telomere length while they proliferate



Telomerase and Aging

Because there is very little telomerase in somatic tissues, older people have shorter telomeres

-particularly in actively regenerating tissues such as skin or intestinal ephithelia

This has generated a lot of interest in the possibility that telomere length could be tied to aging

Americans Win Nobel For Research On Aging

-National Public Radio

The Nobel Prize in Physiology or Medicine 2009

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

Elizabeth H. Blackburn
University of California San Francisco

Carol W. Greider
Johns Hopkins University School of Medicine

Jack W. Szostak
Harvard Medical School; Massachusetts General Hospital

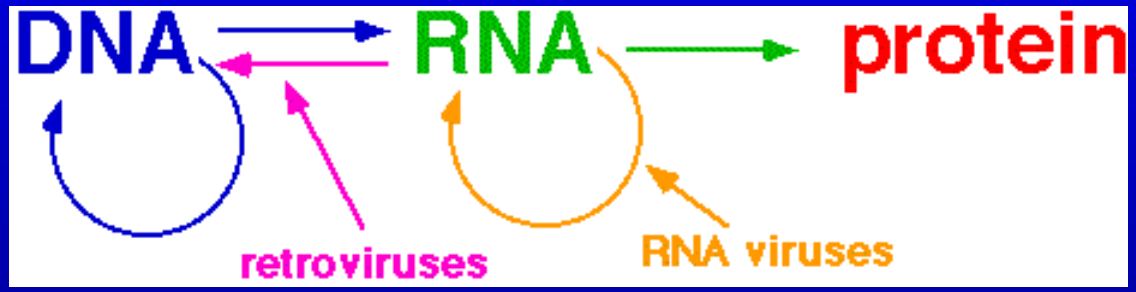
Summary of eukaryotic DNA replication

- The large DNA molecules in eukaryotic chromosome replicate bidirectionally from multiple origins.
- Two or three DNA polymerases (α, δ, and/or ε) are present at each replication fork in eukaryotes.
- Telomeres, the unique sequences at the ends of chromosomes, are added to chromosome by a unique enzyme called telomerase.

Section Three

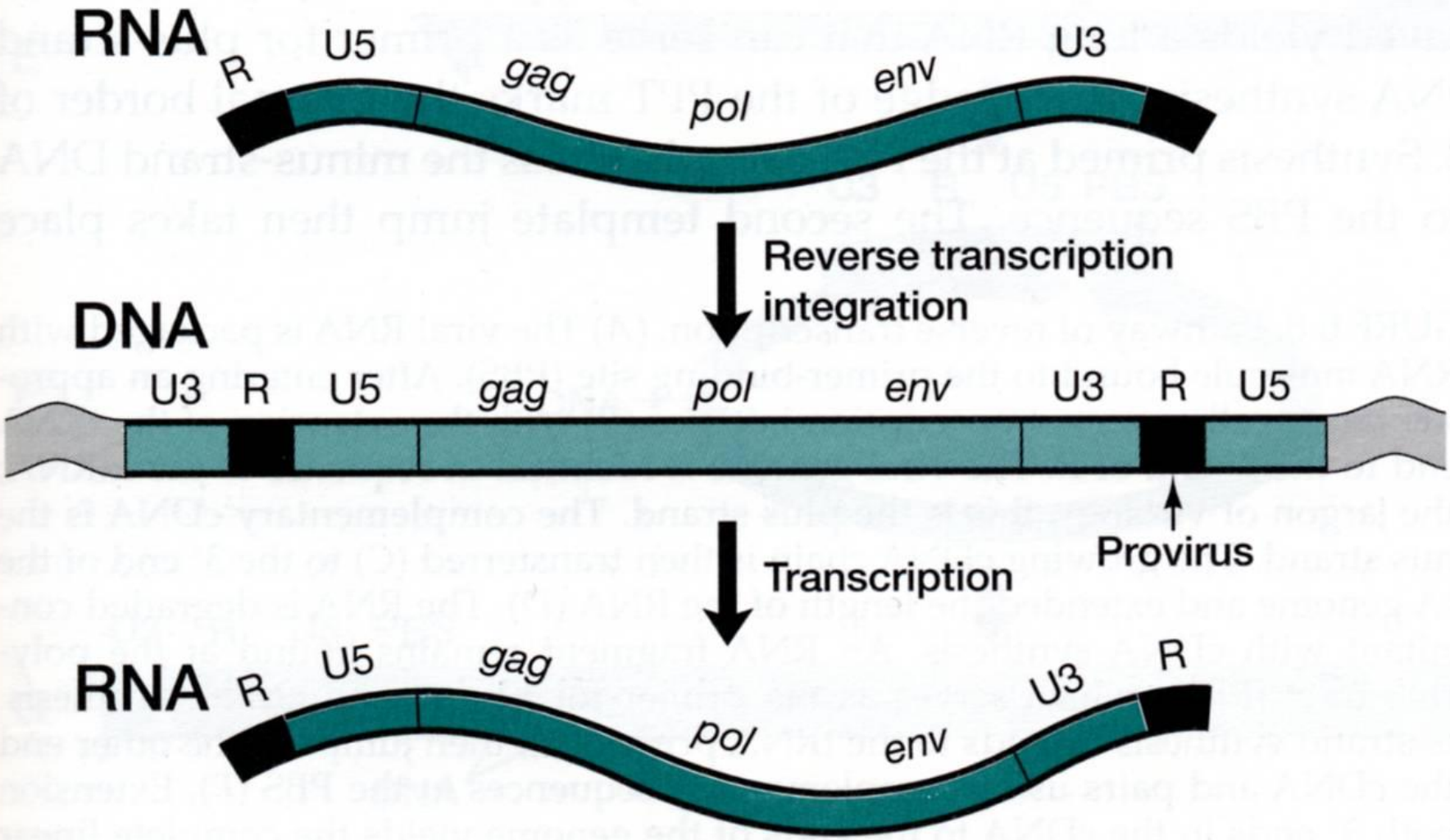
Reverse transcription

However, the "Central Dogma" has had to be revised a bit. It turns out that you CAN go back from RNA to DNA, and that RNA can also make copies of itself. *It is still not possible* to go from Proteins back to RNA or DNA, and no known mechanism has yet been demonstrated for proteins making copies of themselves.



- **In early 1970, Temin and Baltimore independently identified the unusual enzyme, reverse transcriptase and won the Nobel Prize in 1975 for their work**
- **Their discovery shattered the central dogma of molecular biology which stated the flow of genetic information was from DNA to RNA**

HIV integration:



Reverse Transcriptase

DNA Polymerase Activity

- Requires primer with 3’ OH termination
- Template either RNA or DNA
- Requires Mg^{++} (or Mn^{++})
- Lacks proof-reading function; high error rate (10^{-4} errors per base)

RNase H Activity

(Nuclease specific for RNA in RNA:DNA hybrids)

- Activity encoded in different domain than polymerase

Reverse Transcriptase From RNA to DNA

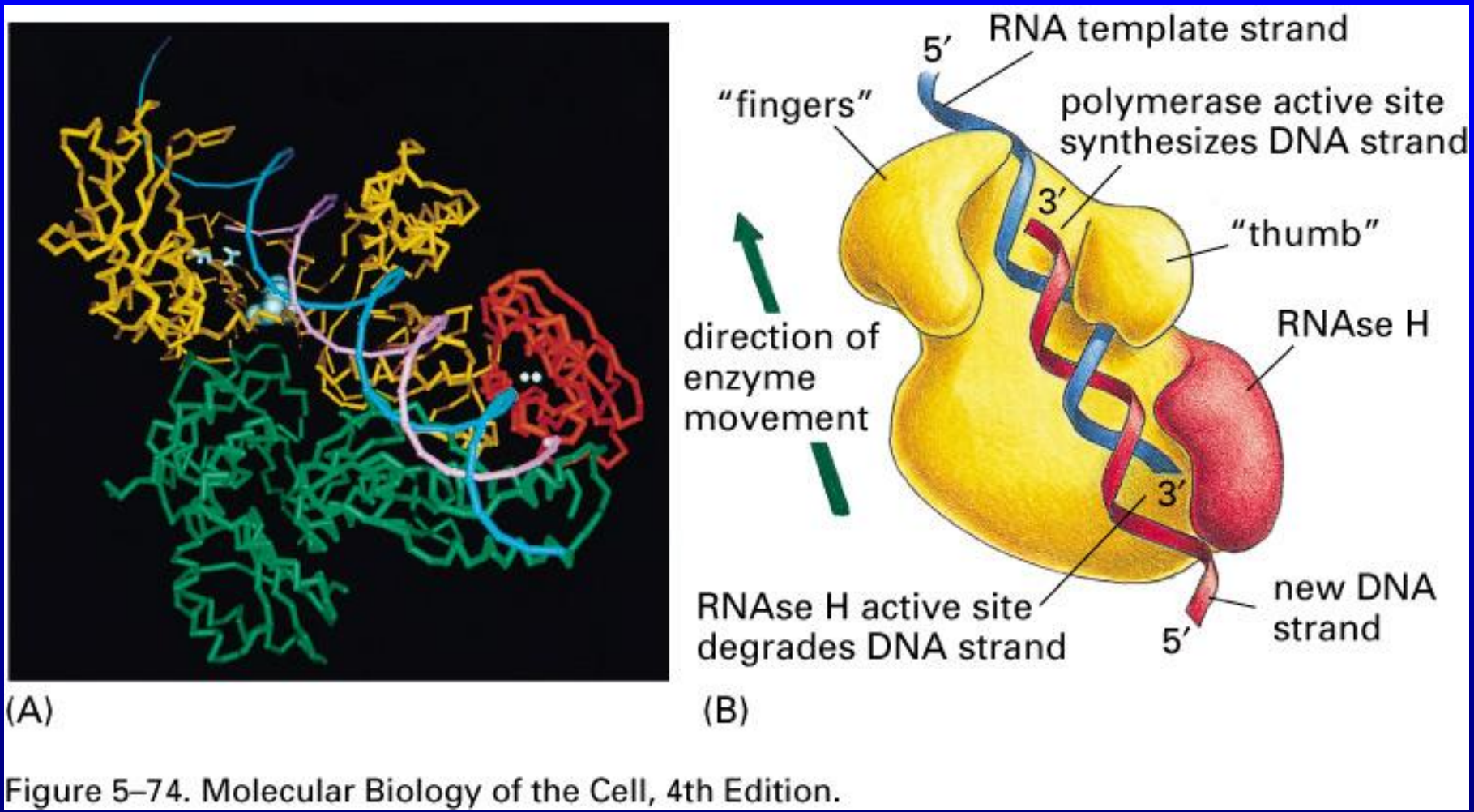
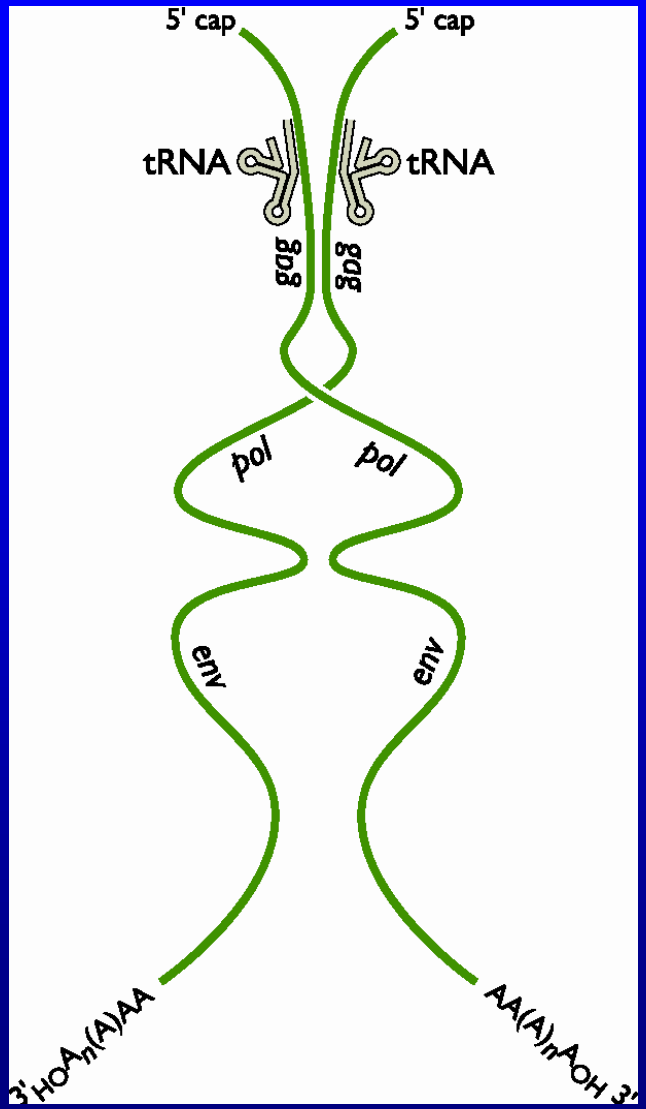


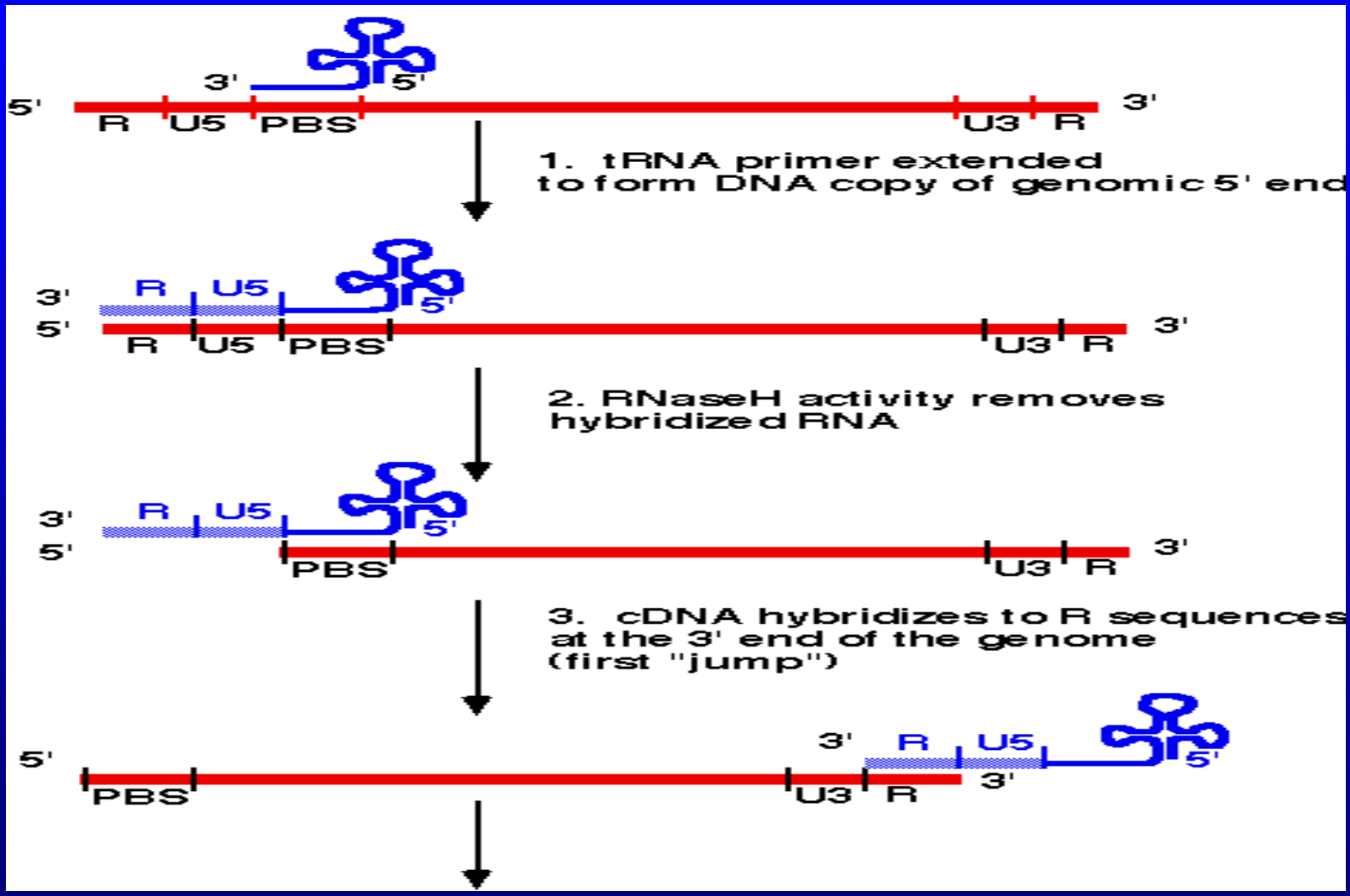
Figure 5–74. Molecular Biology of the Cell, 4th Edition.

Retroviral genome

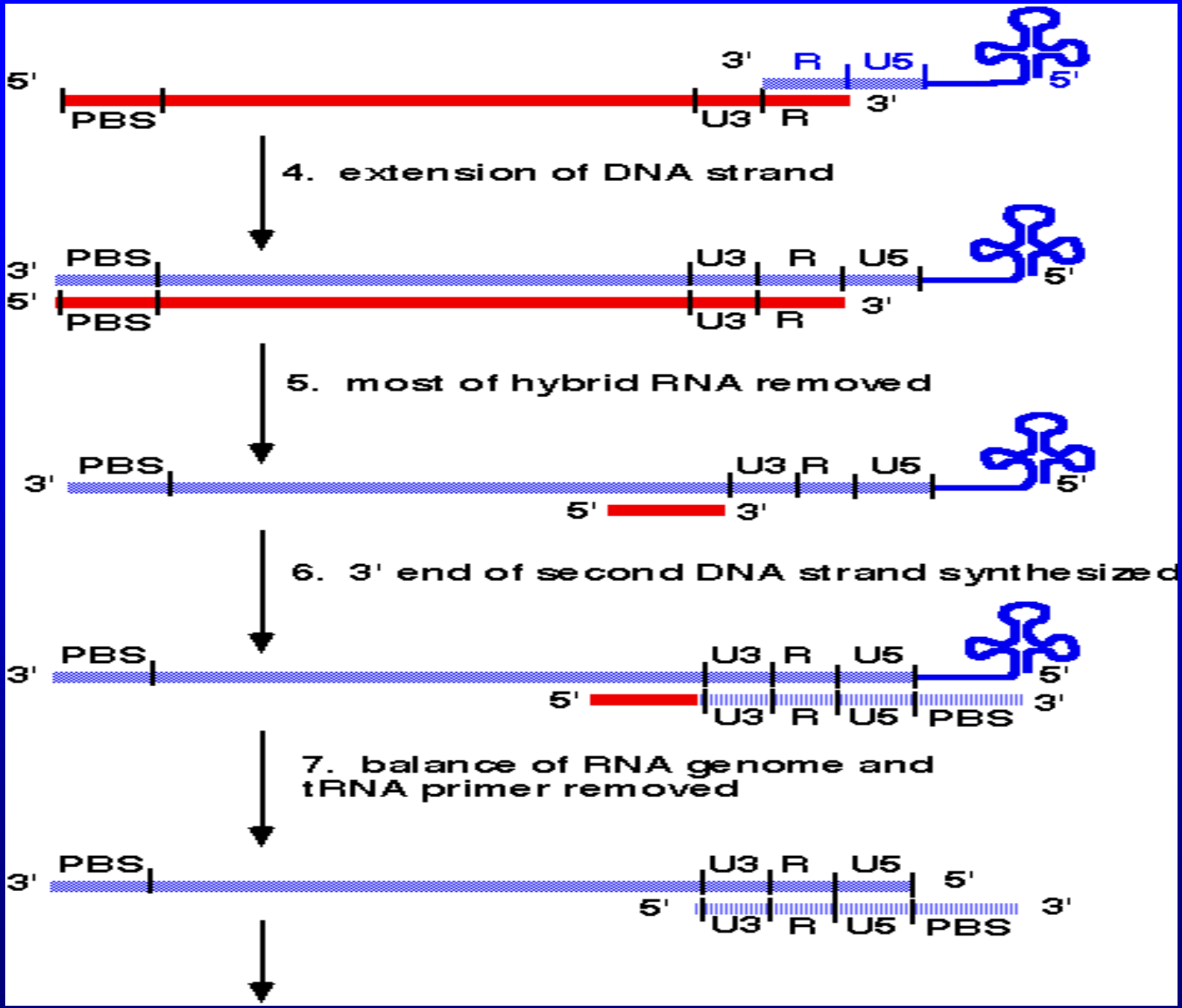


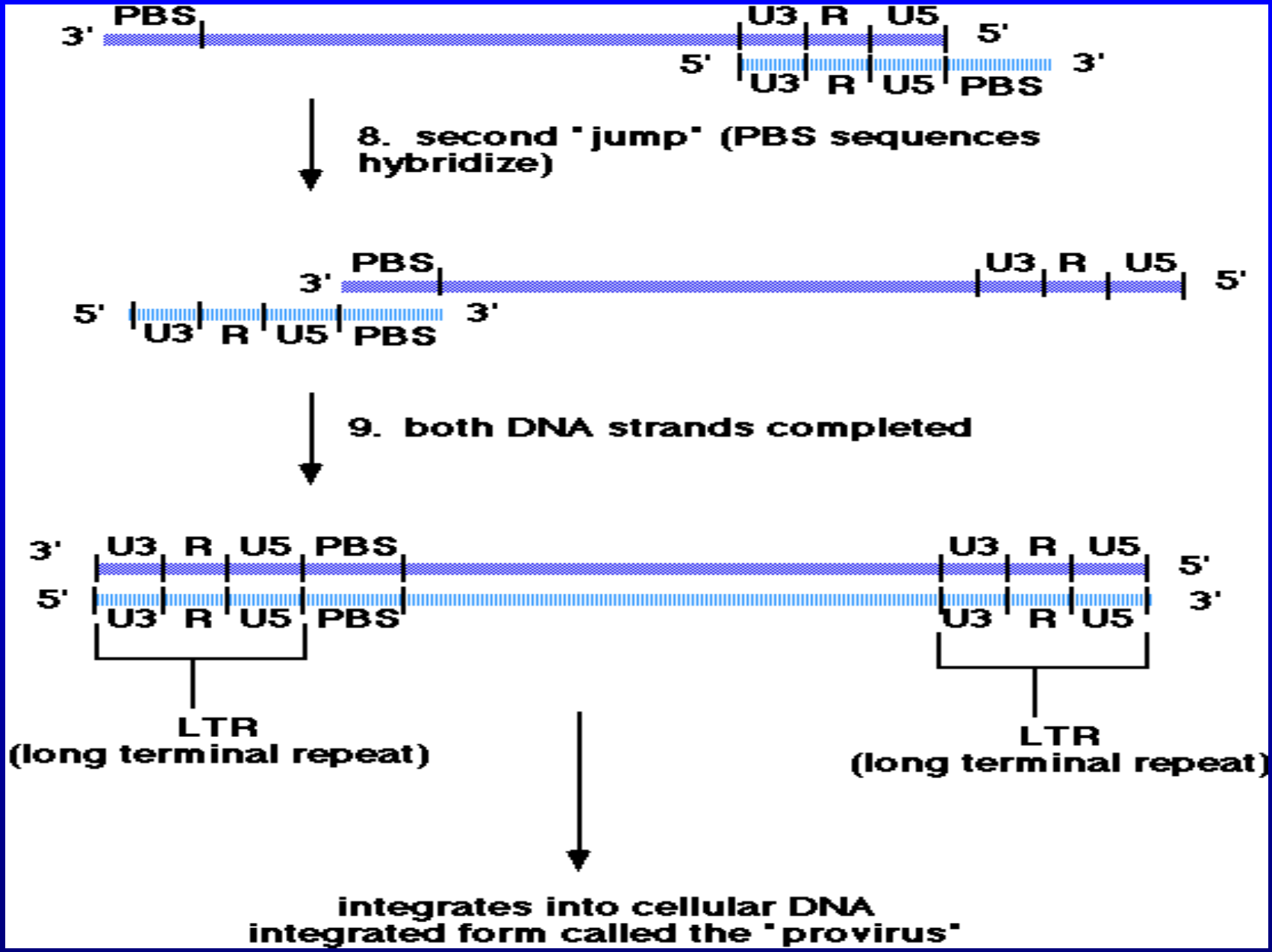
- Retroviruses contain two copies of the RNA genome held together by multiple regions of base pairing
- This RNA complex also includes two molecules of a specific cellular RNA ($tRNA_{lys}$) that serves as a primer for the initiation of reverse transcription
- The primer tRNA is partially unwound and hydrogen bonded near the 5' end of each RNA genome in a region called the primer binding site

Retrovirus replication carried out by reverse transcriptase

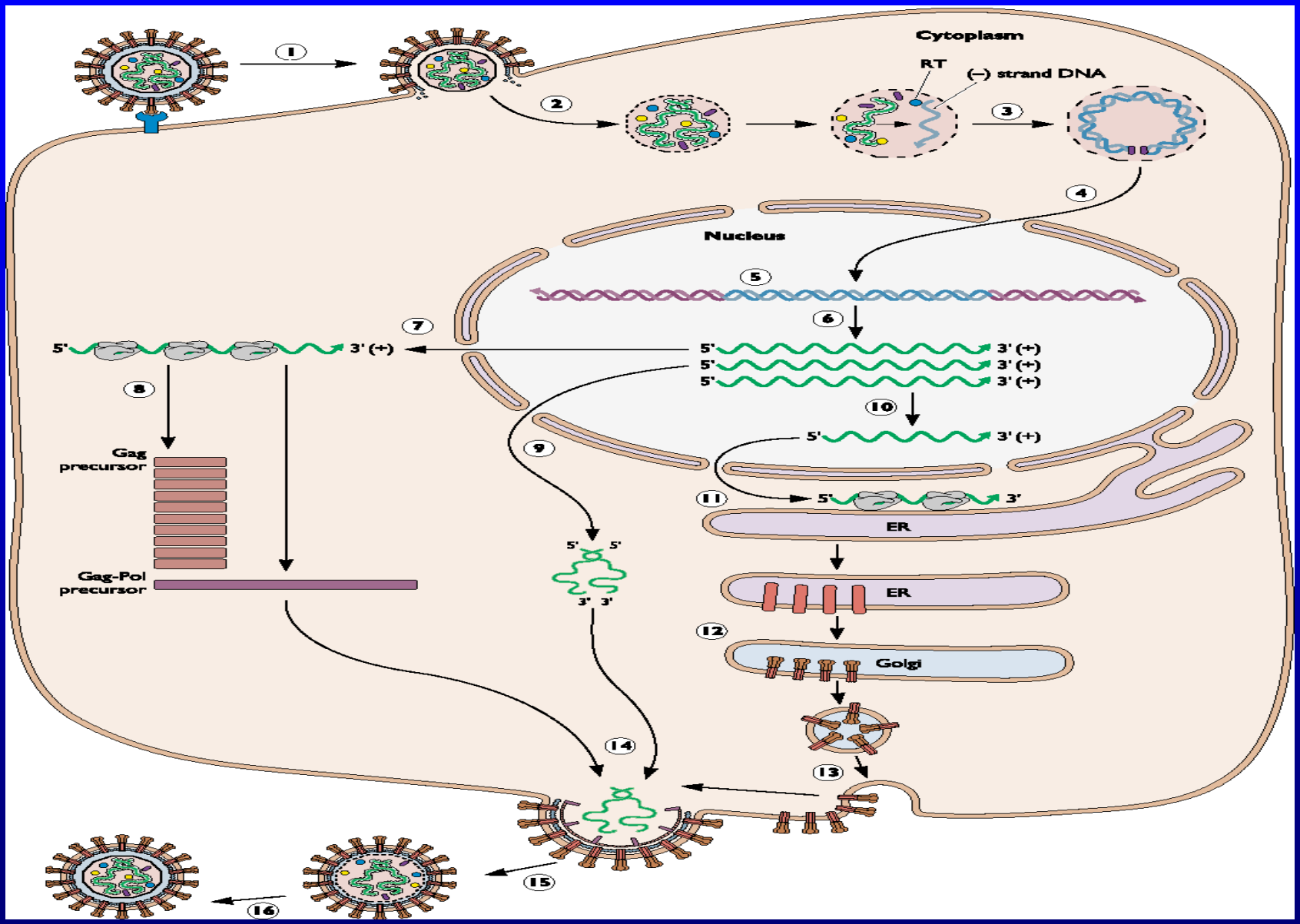


PBS:Primer binding site


5'
PBS
3'
R
U5
5'
3'
U3
R
4. extension of DNA strand
3'
PBS
U3
R
U5
5'
5'
PBS
3'
U3
R
5. most of hybrid RNA removed
3'
PBS
U3
R
U5
5'
5'
3'
6. 3' end of second DNA strand synthesized
3'
PBS
U3
R
U5
5'
5'
3'
U3
R
U5
PBS
7. balance of RNA genome and
tRNA primer removed
3'
PBS
U3
R
U5
5'
5'
3'
U3
R
U5
PBS


3'
PBS
U3
R
U5
5'
5'
U3
R
U5
PBS
3'
8. second "jump" (PBS sequences hybridize)
3'
PBS
U3
R
U5
5'
5'
U3
R
U5
PBS
3'
9. both DNA strands completed
3'
U3
R
U5
PBS
U3
R
U5
5'
5'
U3
R
U5
PBS
U3
R
U5
3'
LTR
(long terminal repeat)
LTR
(long terminal repeat)
integrates into cellular DNA
integrated form called the "provirus"

Retrovirus life cycle



Section Four

DNA damage and repair

DNA Damage

- Mistakes during DNA replication can lead to changes in the DNA sequence and DNA damage.
- DNA can also be damaged by chemical or physical agents called mutagens.
  - Ionizing radiation – breaks the backbone of the structure
  - Metabolites – alter base structure
  - UV Radiation – adjacent pyrimidines dimerise

- Mutations affect germ cells
  - modified trait passed on
- Mutations affect somatic cells
  - malignant transformation
  - aging

DNA security system

- **Mistakes during DNA replication：**
  - Proofreading by DNA polymerase
  - mismatch correction system
- **Damaged by chemical or physical agents**
  - Direct repair: Photoreactivation
  - Excision repair: BER and NER
  - Recombinant repair
  - SOS repair
- **Foreign DNA invasion**
  - Restriction modification system

DNA is the only cellular macromolecule that can be repaired, probably because the cost to the organism of mutated or damaged DNA far outweighs the energy spent to repair the defect.

Fidelity of DNA Replication

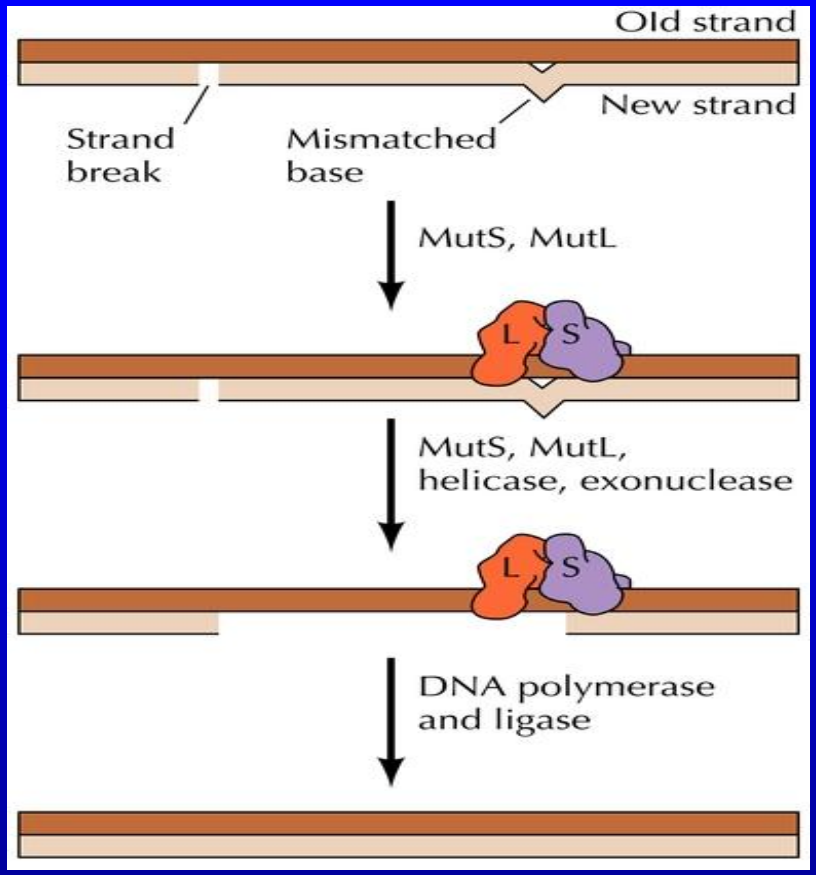
- **DNA replication is remarkably accurate**
  Errors occur only once every 10^9-10^{10} nucleotides incorporated

- **DNA polymerases are not so accurate**
  Errors occur once every 10^4-10^5 nucleotides incorporated

- **The proofreading activity of a polymerase will improve the overall error rate by 10^2-10^3**

- **This still leaves a difference of 10^2-10^3 between the error rates of DNA synthesis and replication**

Mismatch Repair



MutS recognizes mismatches by detecting distortion in DNA.The DNA distortion produces a conformational change in MutS

The complex attracts MutL

MutL activates MutH which nicks DNA

MutH is structurally related to restriction endonucleases

Exonuclease I (3'-> 5') or Exonuclease VII (5'-> 3') removes DNA between the the nick caused by MutH and the site of the mismatch.

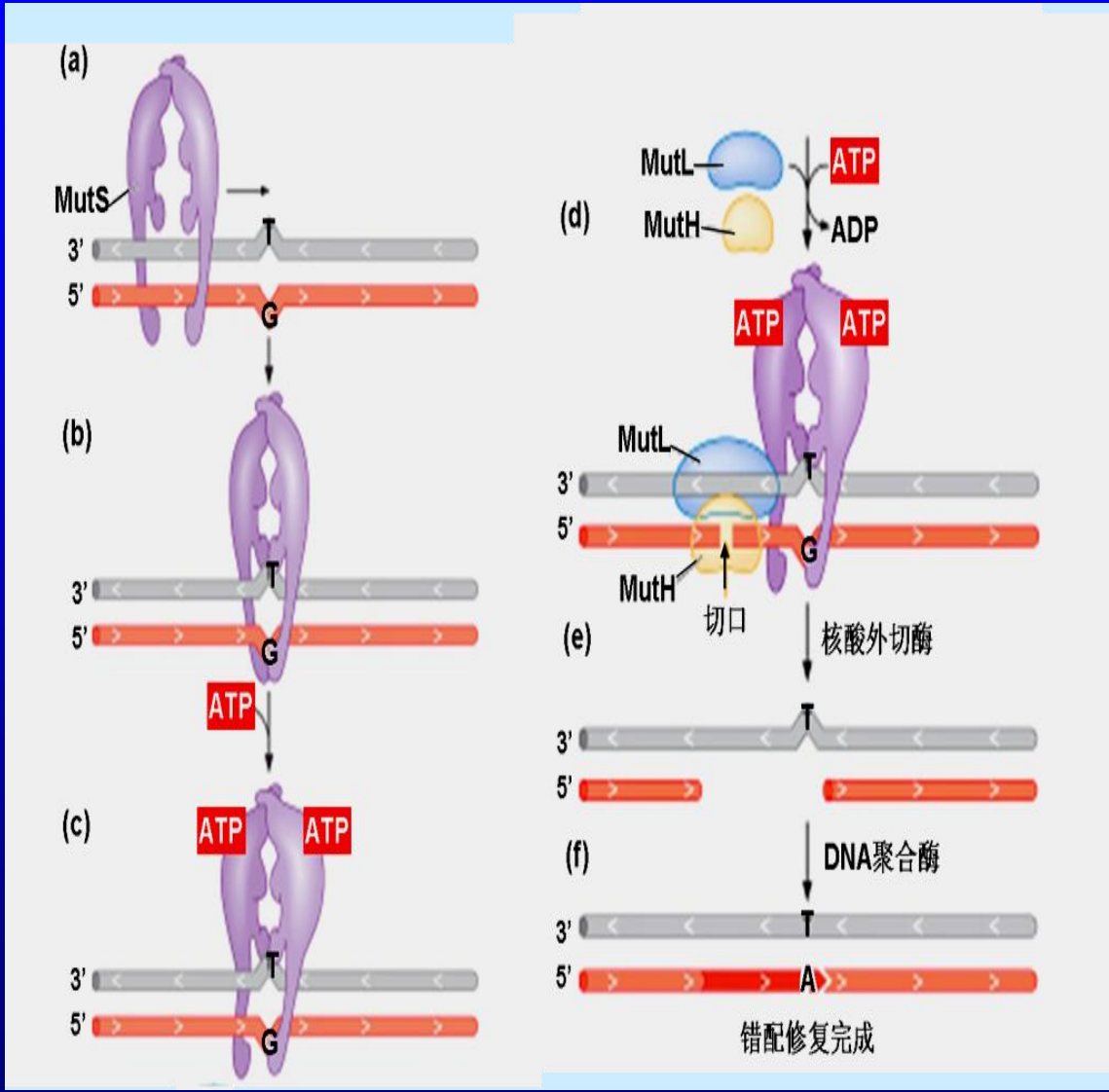
UvrD helicase unwinds DNA

The gap is repaired by DNA polymerase III and sealed by DNA ligase

Mismatch causes distortion of helix

- Recognized by a repair enzyme
- Repair system recognizes newly synthesized strand
- New strand recognized by presence of breaks

Mismatch Repair in *E. coli*



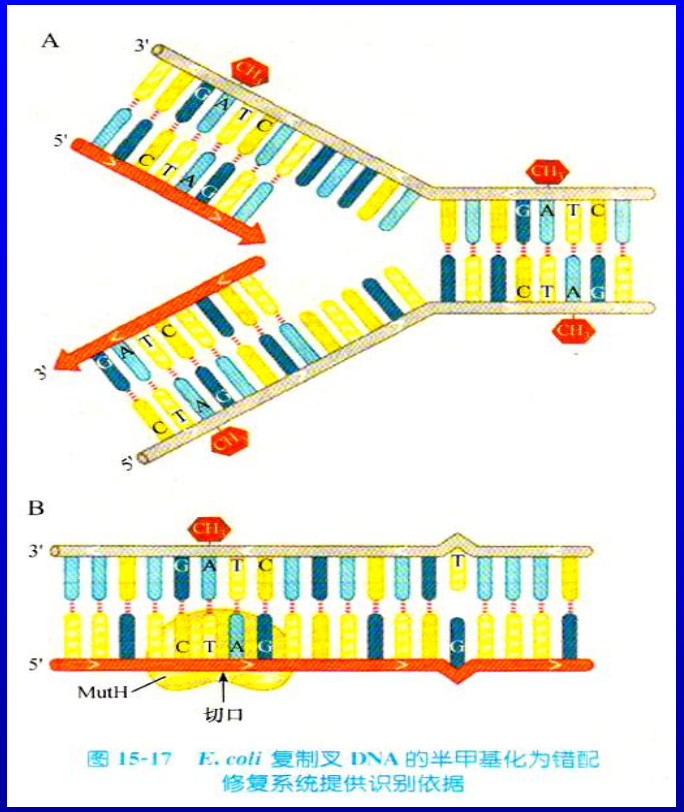


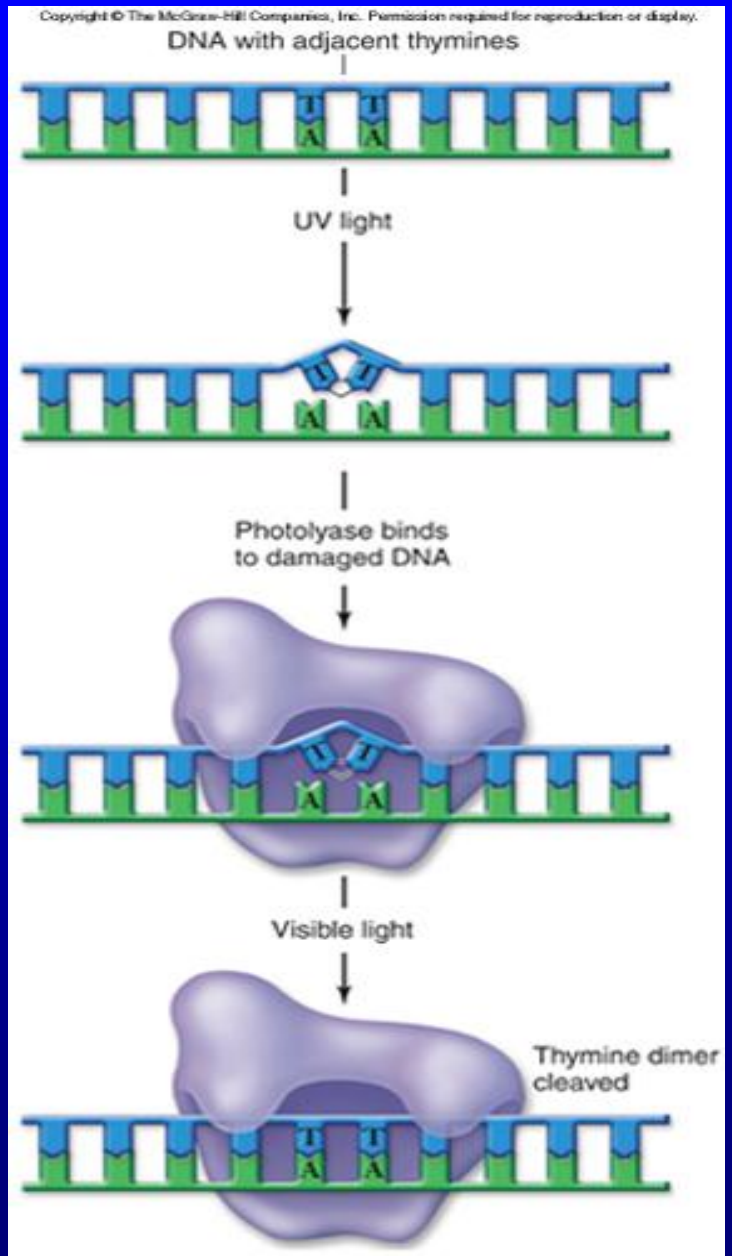
图 15-17 *E. coli* 复制叉 DNA 的半甲基化为错配修复系统提供识别依据

Mismatch is recognized by Dam methylase in E.coli. Because low density methylation of GATC is in new strand.

Direct Repair- Repair after photodimerization

photolyases

(光裂合酶)



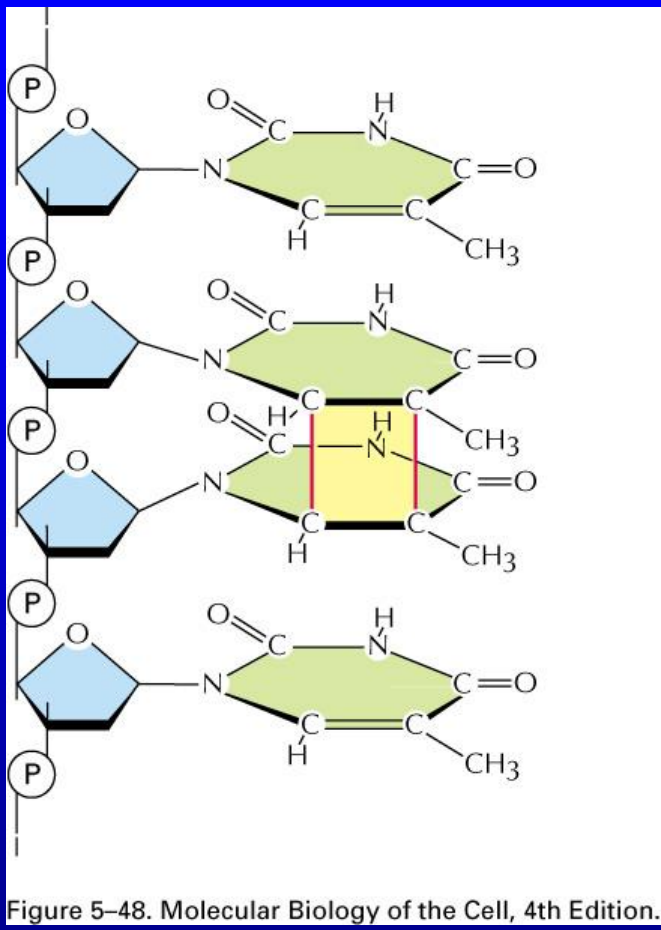


Figure 5–48. Molecular Biology of the Cell, 4th Edition.

Thymine dimer

Base Excision Repair (BER)

- Initiated by a DNA glycosylase (糖苷酶)
  - Recognizes alteration
  - Removes base by cleavage of glycosidic bond between the base and deoxyribose, leave a apurinic or apyrimidinic site (AP site)in DNA helix
- Several specific types of DNA glycosylase – for specific modifications
  - Uracil formed from hydrolytic removal of amino group of cytosine
  - Formation of 8-hydroxyguanine – by oxygen free radicals
  - Formation of 3-methyl adenine

Base Excision Repair (BER)

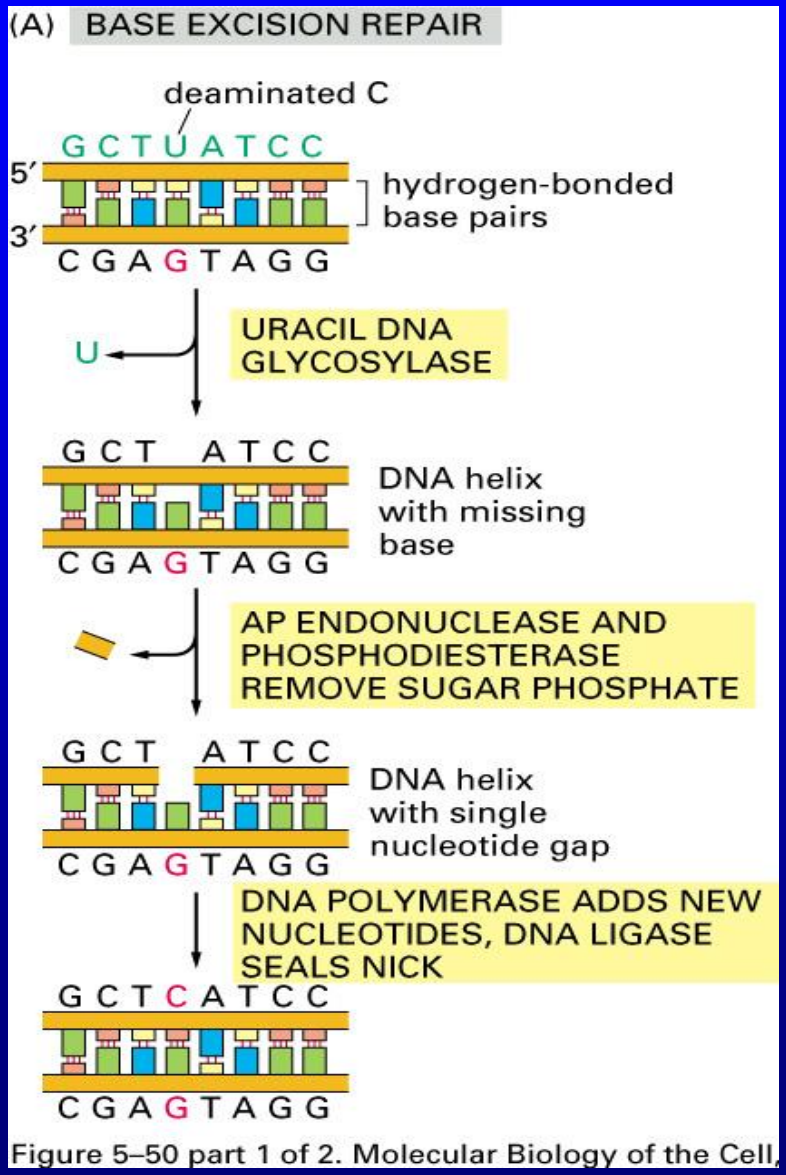


Figure 5–50 part 1 of 2. Molecular Biology of the Cell,

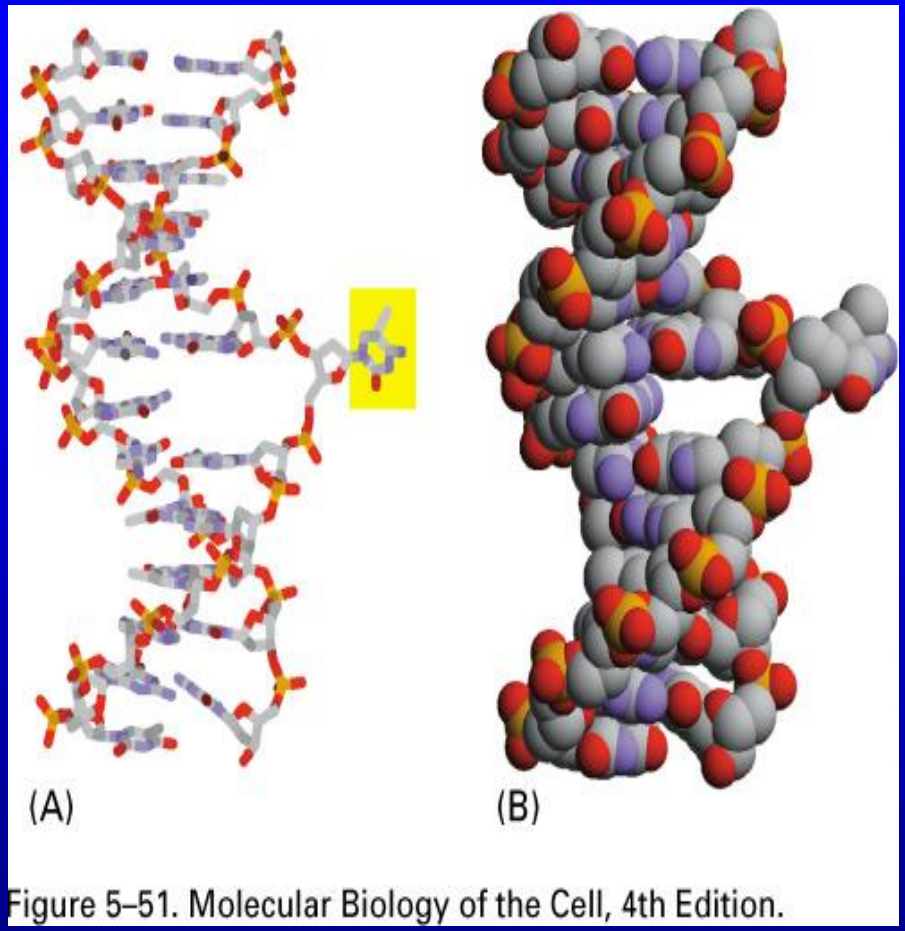
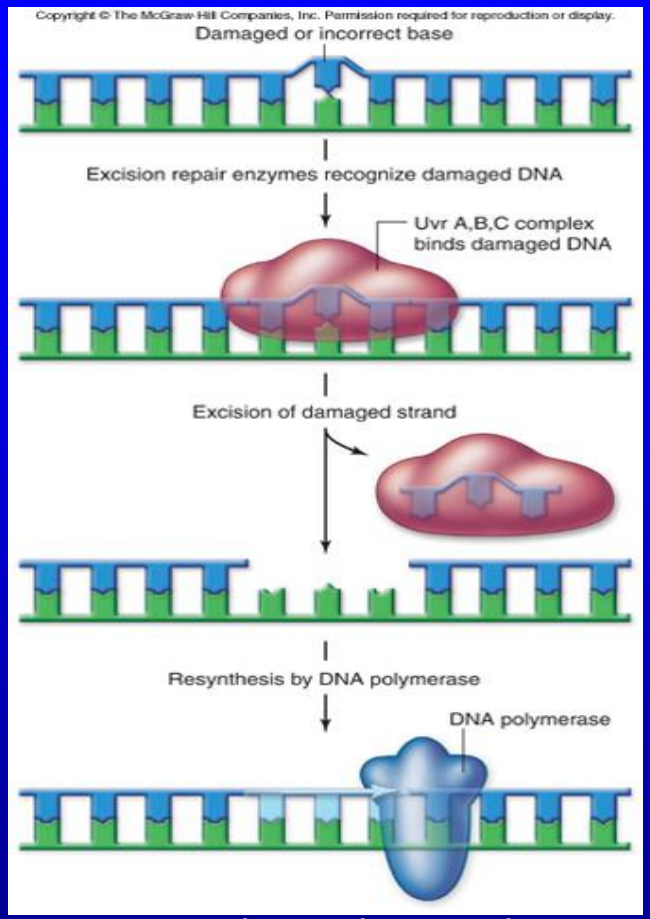


Figure 5–51. Molecular Biology of the Cell, 4th Edition.

Recognition of unusual nucleotide By base flipping recognized by DNA glycosylase family

Nucleotide Excision Repair (NER)



- A 'cut and patch' mechanism
- Removes bulky lesions
  - Pyrimidine dimers
  - Chemical groups attached
- Two pathways
  - Transcription coupled pathway
  - Global pathway

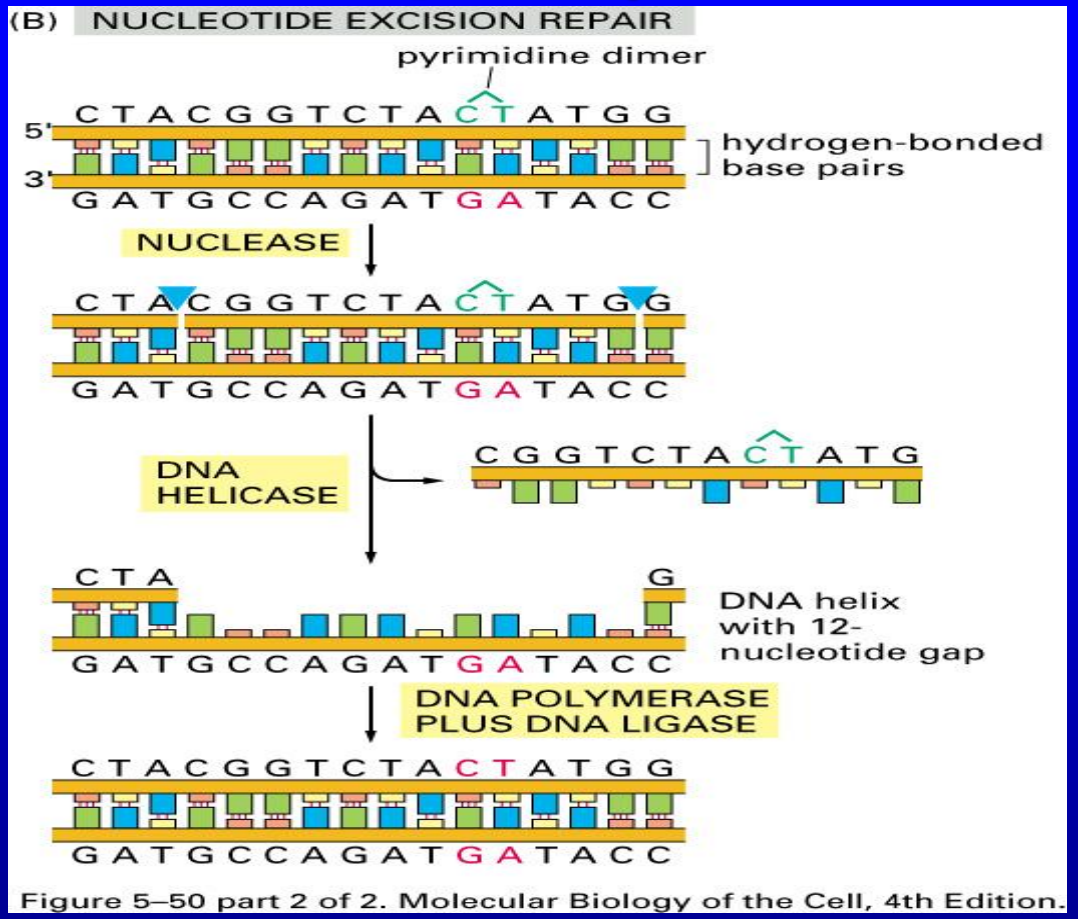


Figure 5–50 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

- NER in eukaryote is more complicated
- Xeroderma pigmentosum （XP）is related with several genes in NER

Transcription-coupled repair

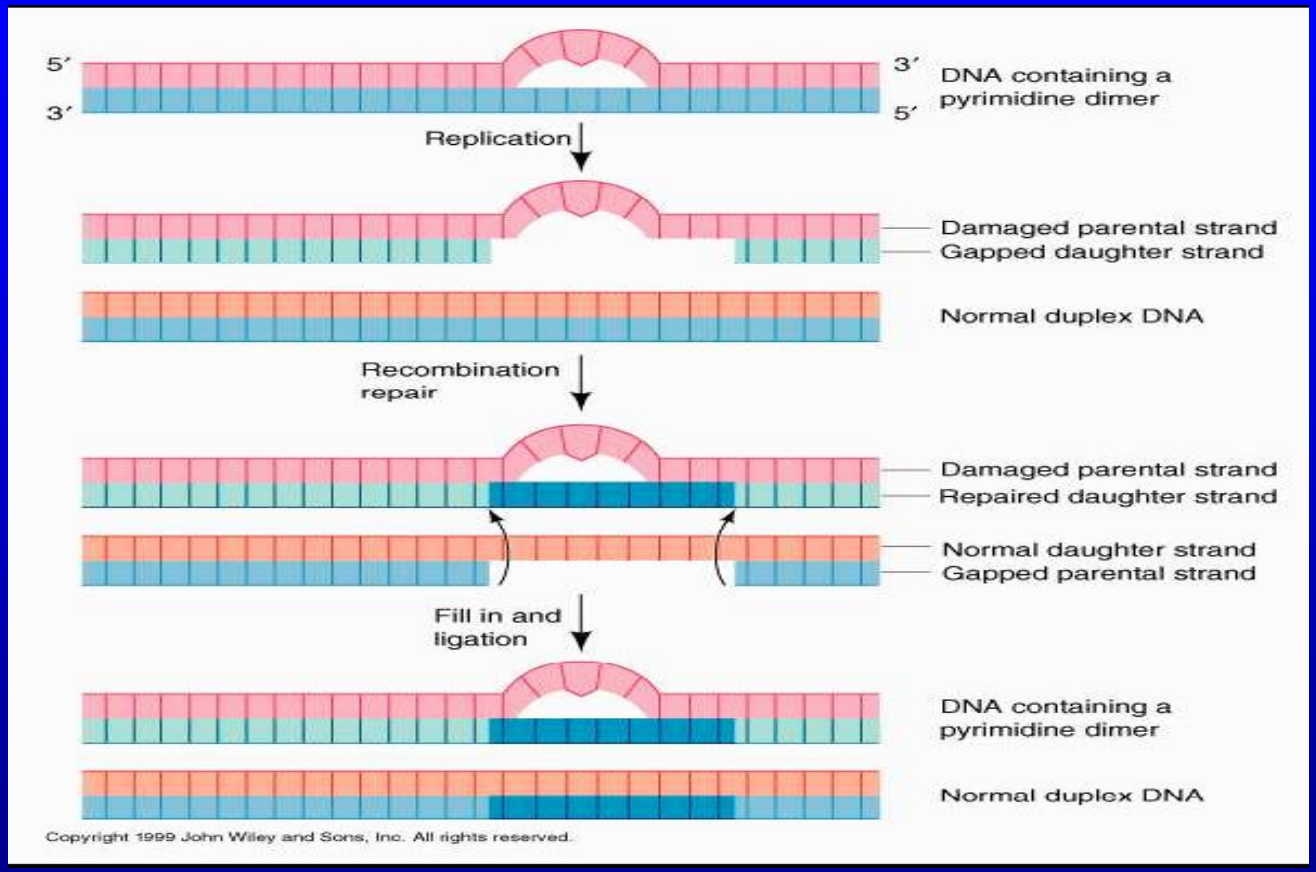
NER also takes part in transcription-coupled repair, saves RNA polymerase which paused by damage DNA temple.

NER proteins are recruited by paused RNA polymerase and repair the DNA damage 。

TFⅡH is essential for Transcription-coupled repair

1. Unwind DNA in NER
2. Unwind DNA temple in transcription

Recombination Repair Systems



Recombination involved in repair the mistake created in DNA replication

Emergency DNA Repair for Double helix break

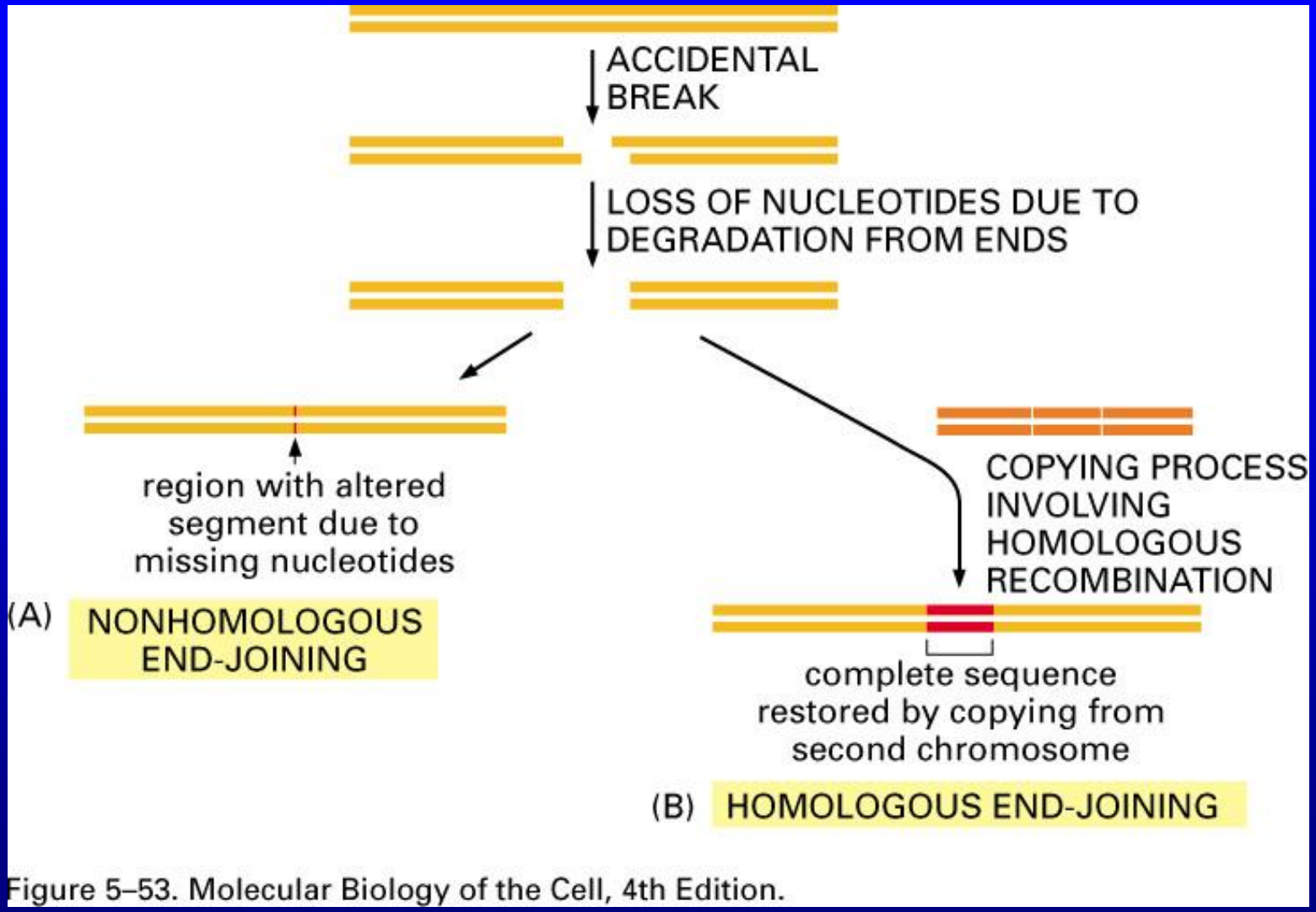
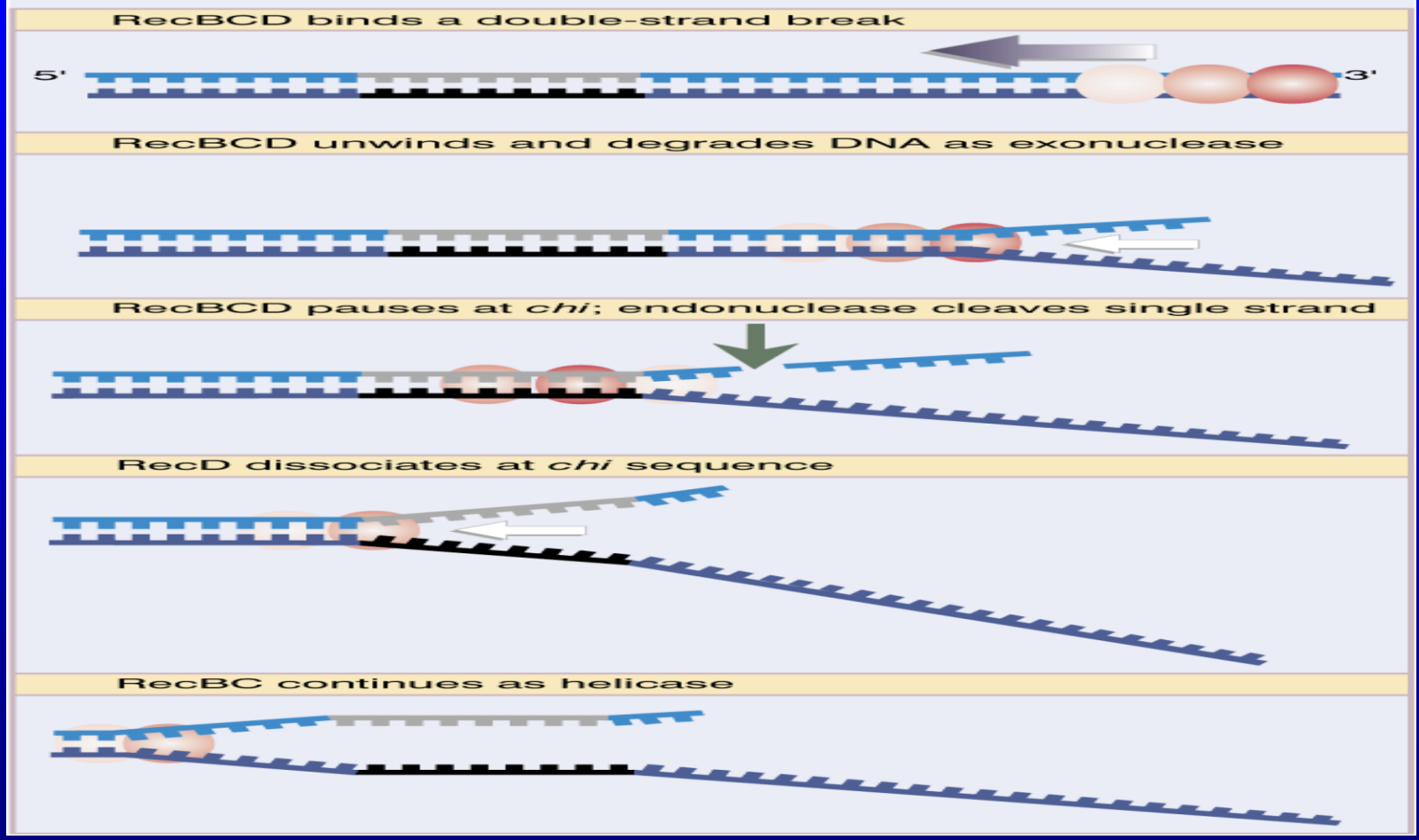


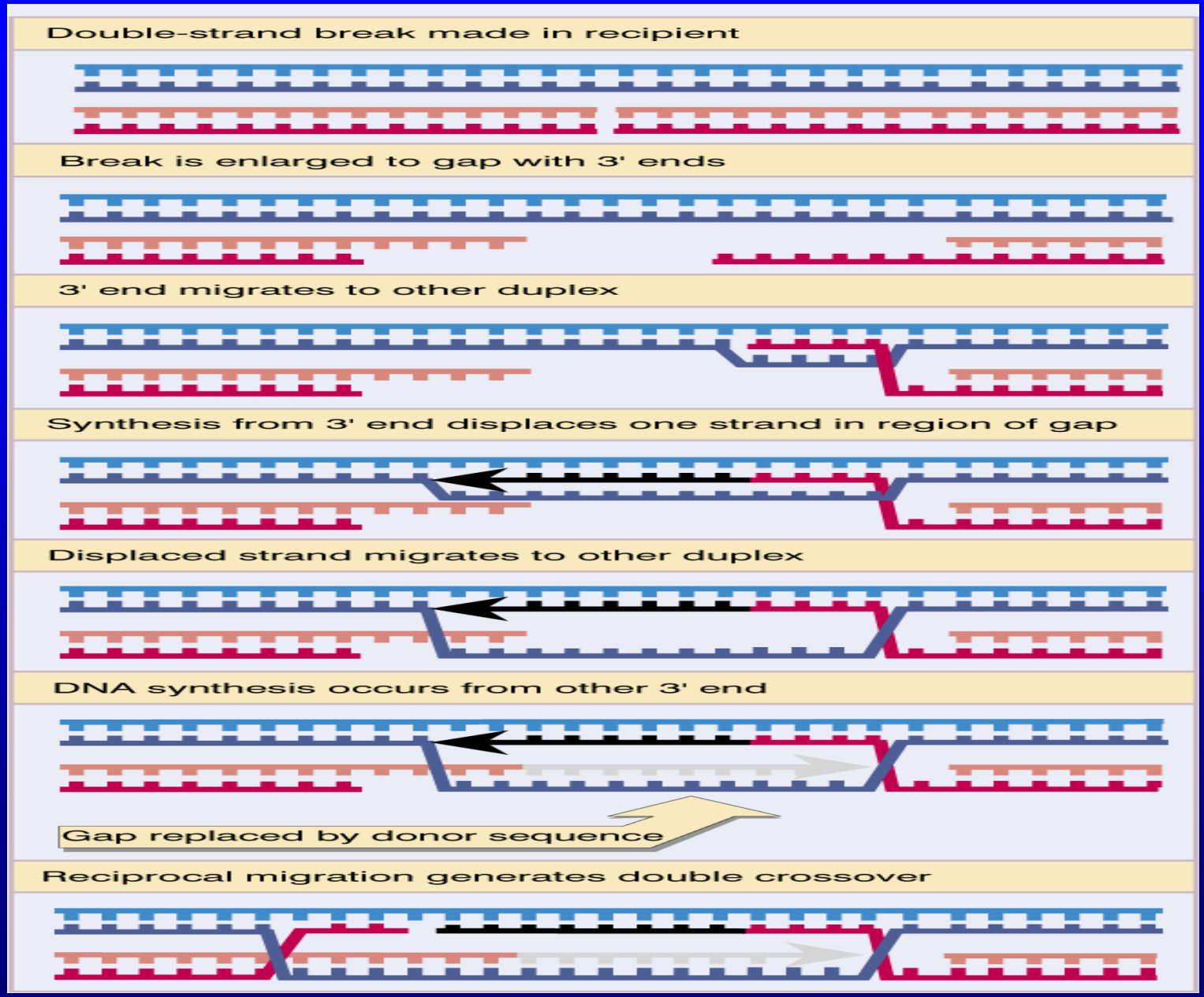
Figure 5–53. Molecular Biology of the Cell, 4th Edition.

Recombination repair *in E.coli*

Figure 14.9 RecBCD nuclease approaches a *chi* sequence from one side, degrading DNA as it proceeds; at the *chi* site, it makes an endonucleolytic cut, loses RecD, and retains only the helicase activity.



***Chi* sequence :5'GCTGGTGG3'**


Double-strand break made in recipient
Break is enlarged to gap with 3' ends
3' end migrates to other duplex
Synthesis from 3' end displaces one strand in region of gap
Displaced strand migrates to other duplex
DNA synthesis occurs from other 3' end
Gap replaced by donor sequence
Reciprocal migration generates double crossover

Translesion DNA Synthesis

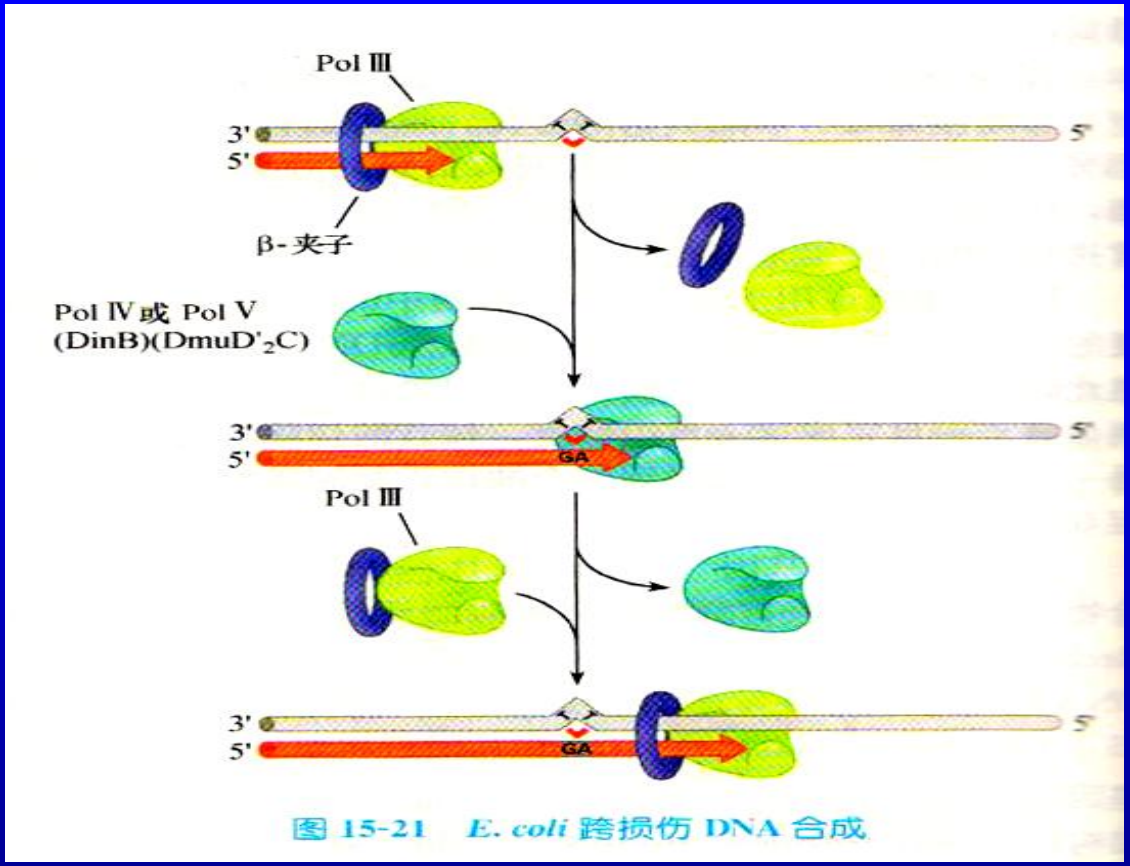


图 15-21 *E. coli* 跨损伤 DNA 合成

Translesion DNA synthesis is error-prone， and create mutation， but can overcome the block of replication.

DNA Repair Summary

- Spontaneous DNA damage: spontaneous alteration of bases, depurination and deamination, thymine dimer
- Pathways to remove DNA damage: base excision repair, nucleotide excision repair
- Damage detection: base flipping
- The repair of Double-strand break: nonhomolous end joining, homologous end joining
- DNA repair enzymes: heat shock proteins

Section Five

DNA recombination

DNA recombination

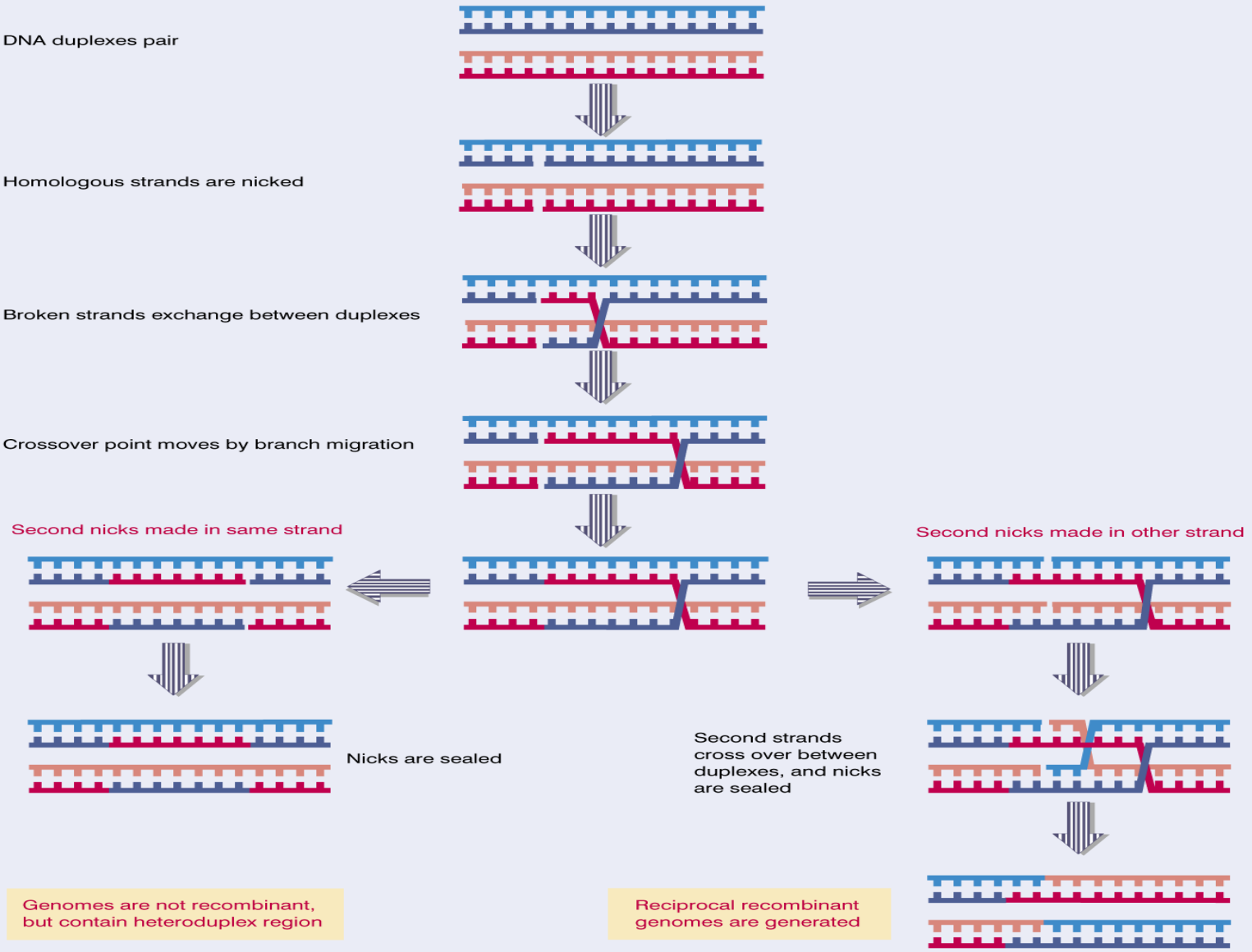
- homologous recombination
- site-specific recombination
- transposition

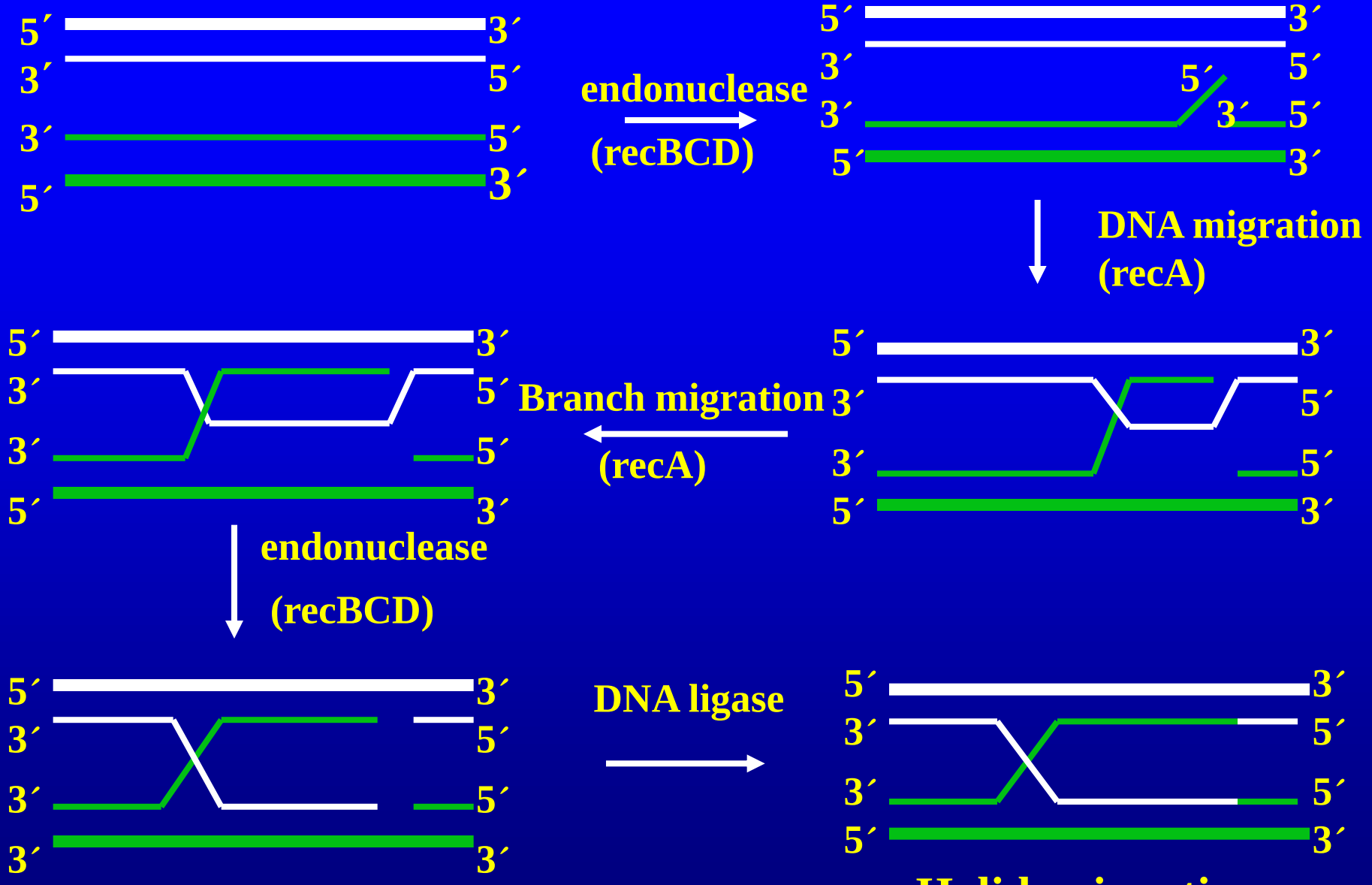
一、 homologous recombination

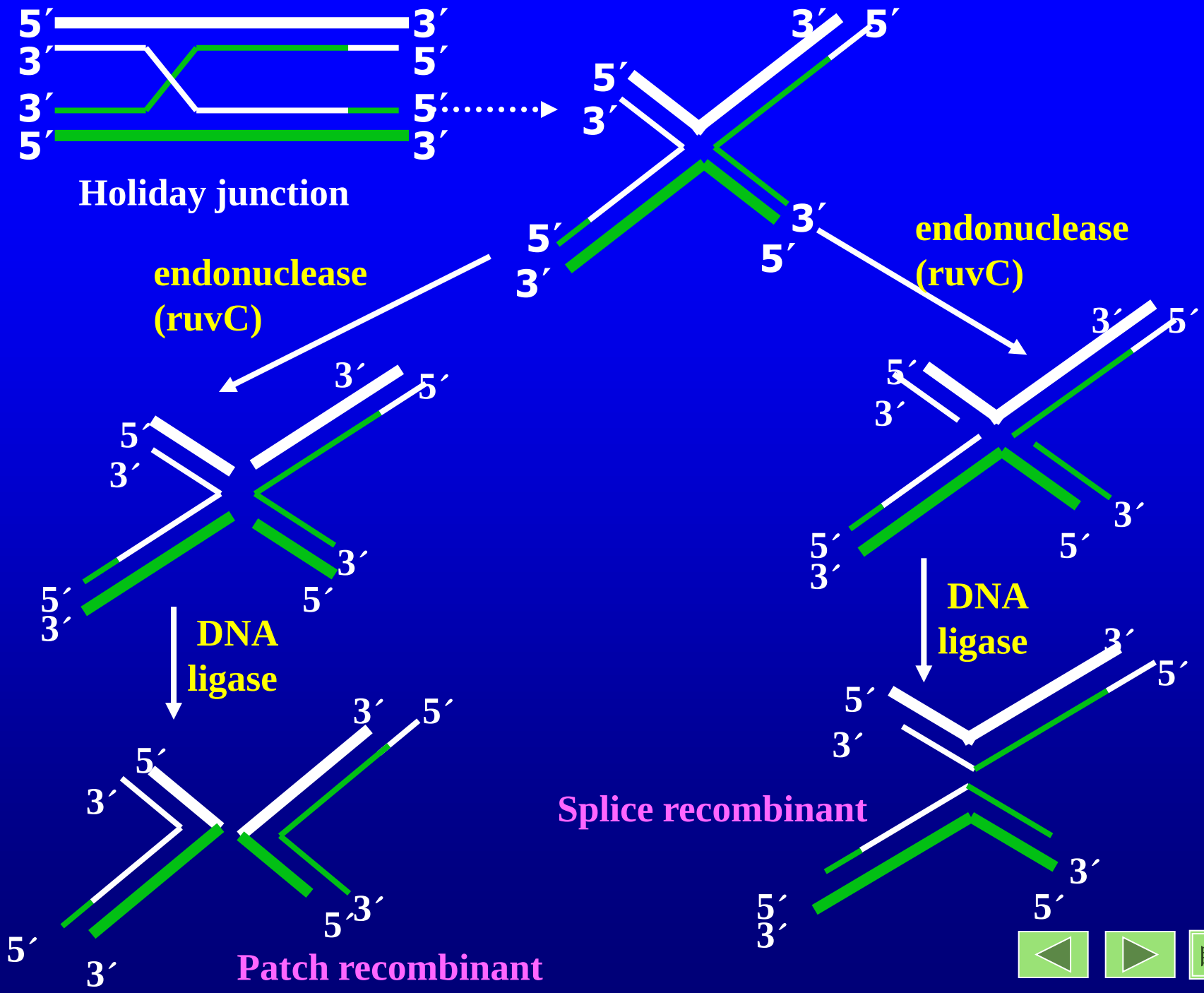
Recombination involving reaction between homologous sequences of DNA is called *homologous recombination*.

Homologous recombination is the most general recombination of DNA , in which two DNA duplex molecules are broken at corresponding points and then rejoined crosswise 。

Figure 14.2 Recombination between two paired duplex DNAs could involve reciprocal single-strand exchange, branch migration, and nicking.




5′
3′
3′
5′
endonuclease
(recBCD)
DNA migration
(recA)
Branch migration
(recA)
endonuclease
(recBCD)
DNA ligase
Holiday junction


Holiday junction
endonuclease
(ruvC)
endonuclease
(ruvC)
DNA
ligase
DNA
ligase
Patch recombinant
Splice recombinant
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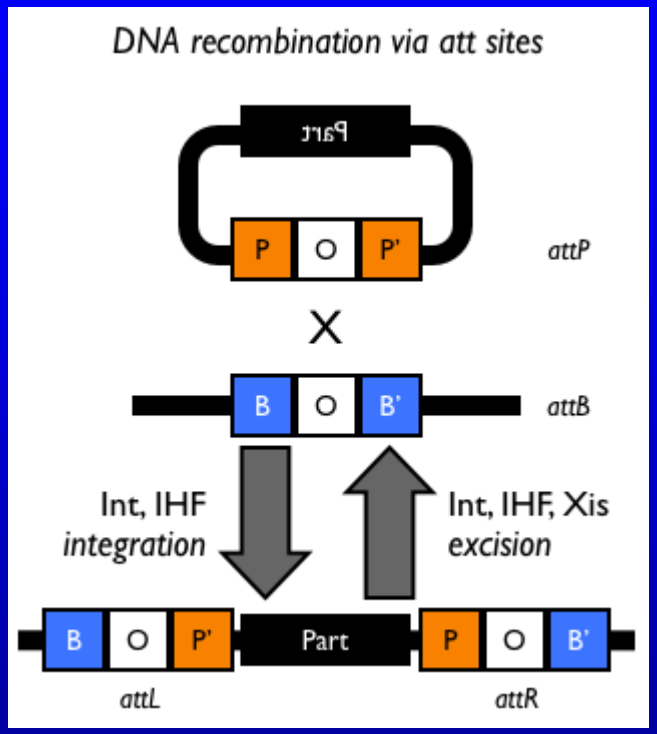
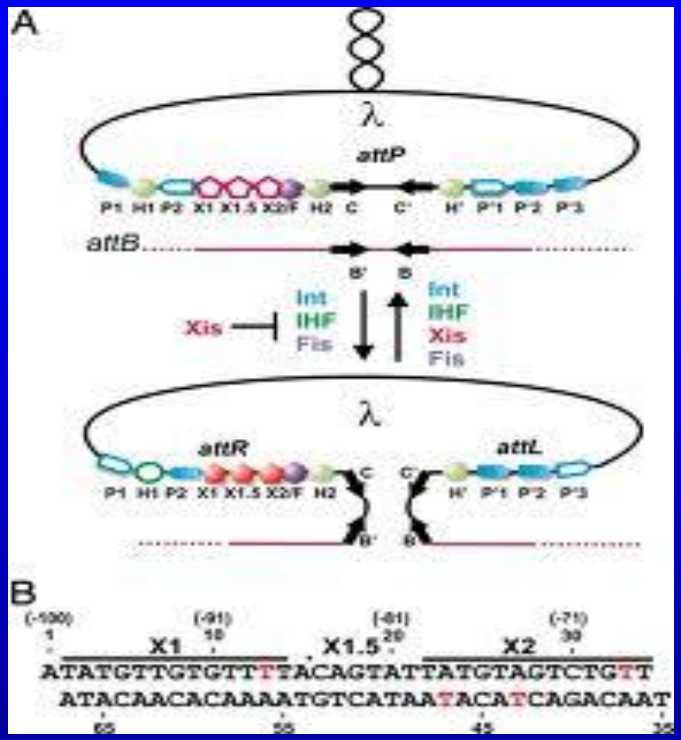
site-specific recombination

Site-specific recombination is responsible for the integration of two DNA sequences at specific sites by integrase (整合酶).

The recombination event involves specific sequences of the phage DNA and bacterial DNA, which include a short stretch of homology. The enzymes involved in this event act only on the particular pair of target sequences. Related reactions are responsible for inverting specific regions of the bacterial chromosome.

（一）The integration of λphage DNA

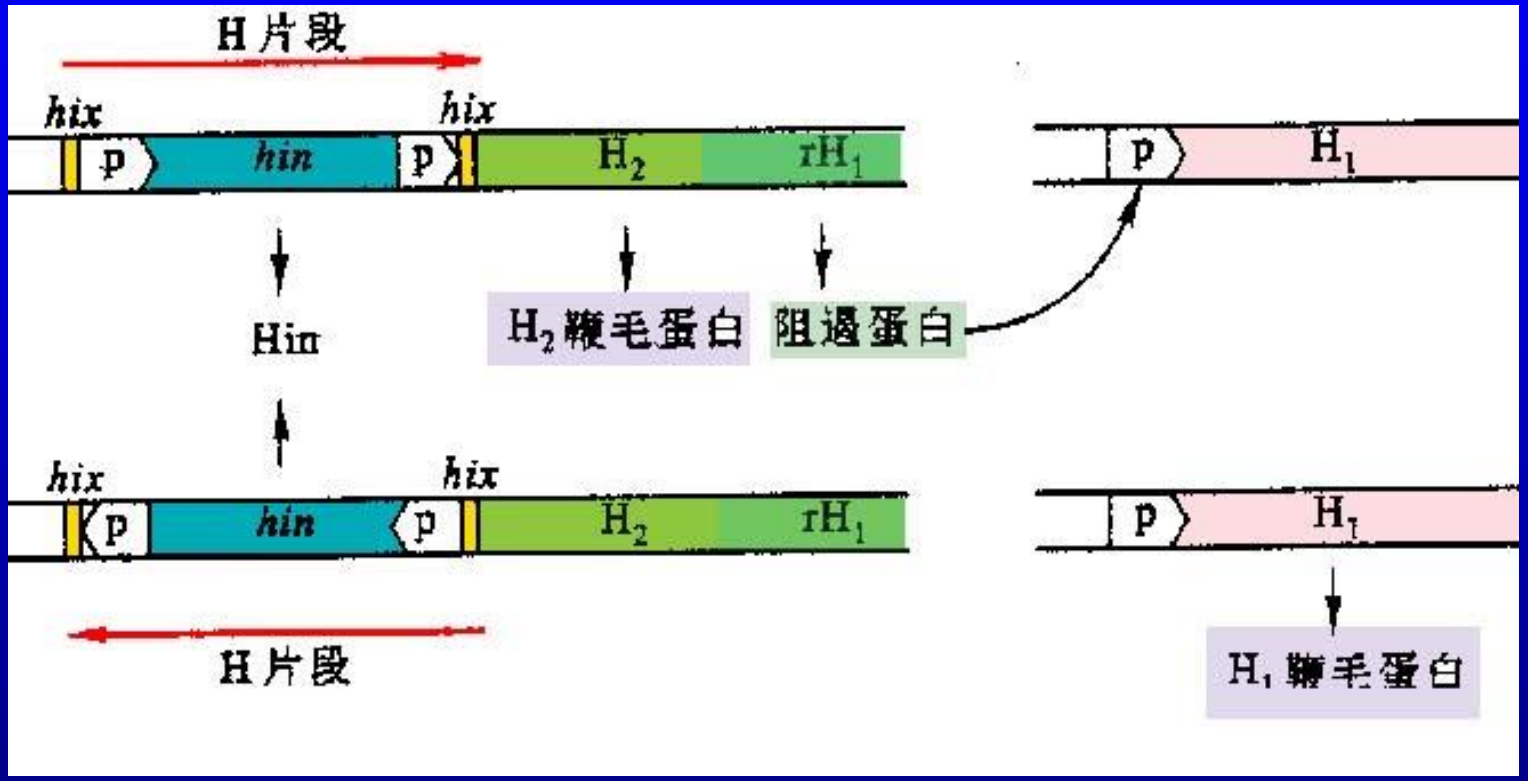




The integration of phage λ takes place at a special attachment site in the bacterial (*attB*) and phage genomes (*attλ*). integration itself is a sequential exchange via a Holliday junction and requires both the phage protein Int and the bacterial protein IHF (*integration host factor*).

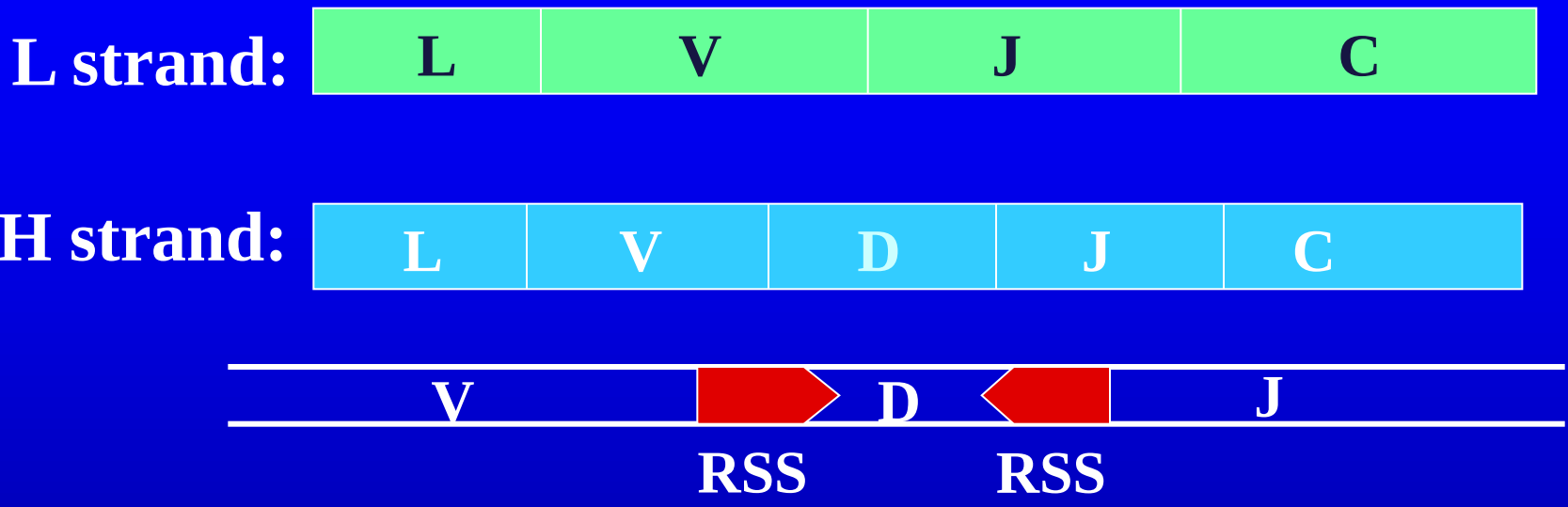
（二）Site-specific recombination in bacteria

H segment inversion make the difference of flagellum in *Samonella*

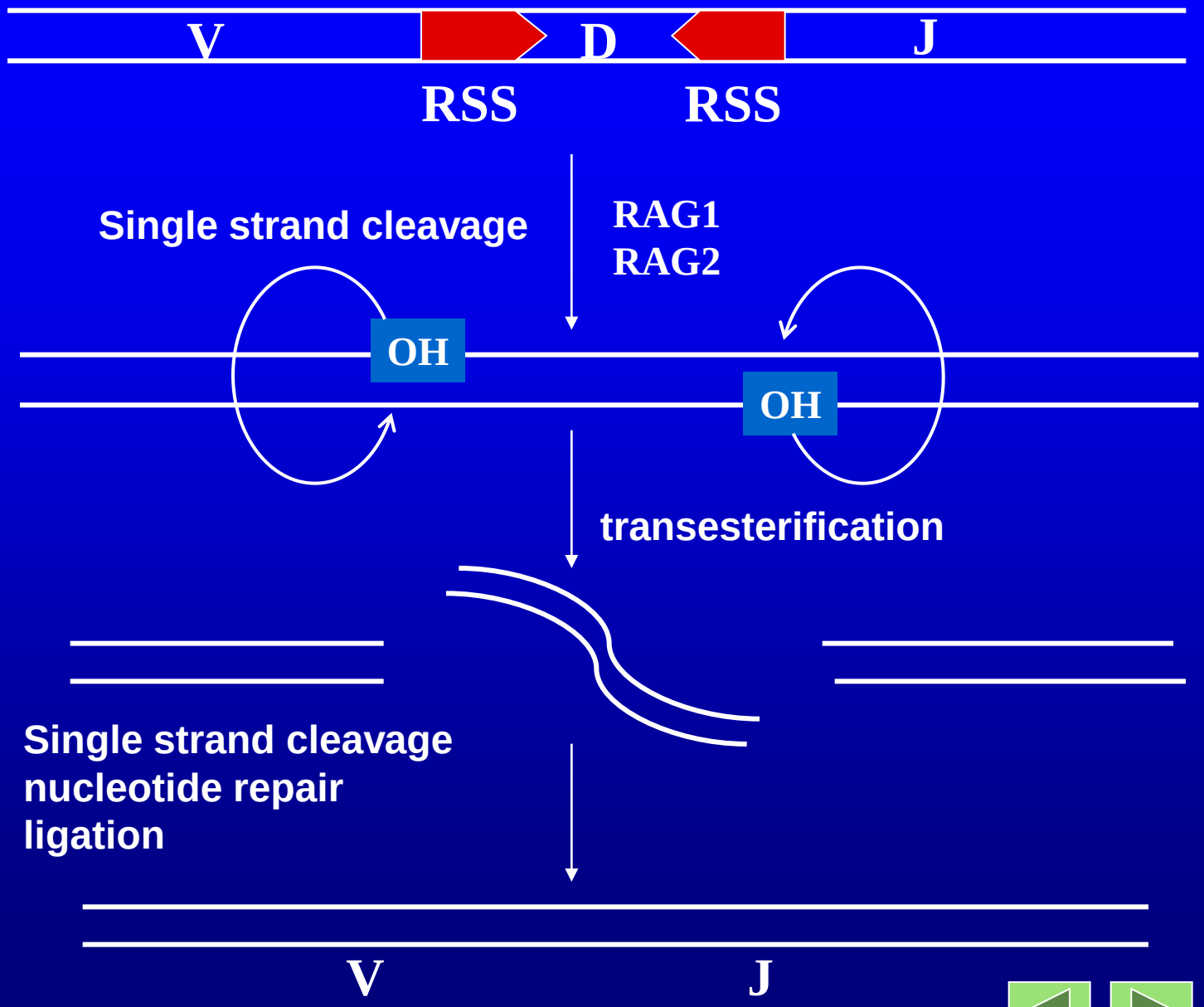


hix is inverted repeat sequence

（三）Immunoglobulin gene rearrange



Rearrange of V-D-J in IgH and V-J in IgL are take place at specific sites. There are recombination signal sequence (RSS) at the downstream of V, upstream of J and both end of D。Recombination activating gene are RAG1 and RAG2。


V
D
J
RSS
RSS
Single strand cleavage
RAG1
RAG2
OH
OH
transesterification
Single strand cleavage
nucleotide repair
ligation
V
J

三、Transposition

Transposition is a DNA movement which insert DNA segment at a new location in the genome .

- **Insertion sequences transposition**
- **Transposon**

（一）Insertion sequences transposition

Insertion sequences(IS) including

- **Two separate inverted repeats (IR)**
- **Direct repeats**
- **Transposase gene**

conservative transposition

replicative transposition

Figure 15.5 Replicative transposition creates a copy of the transposon, which inserts at a recipient site. The donor site remains unchanged, so both donor and recipient have a copy of the transposon.

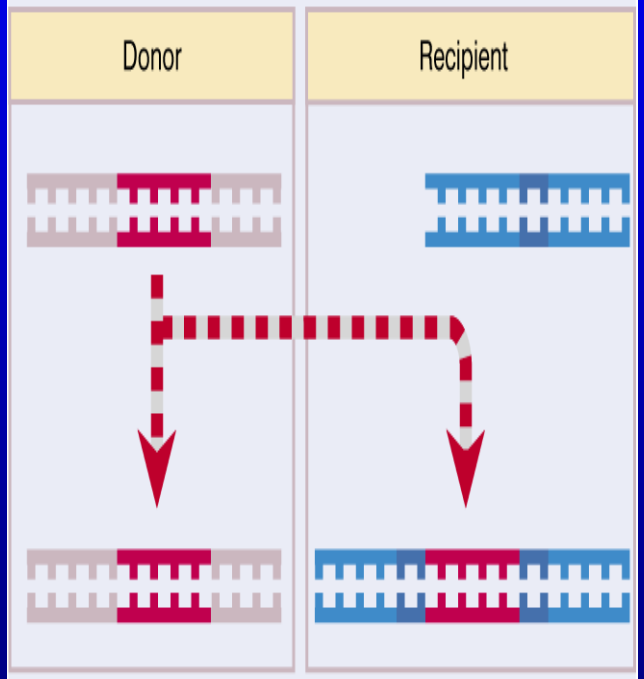


Figure 15.6 Nonreplicative transposition allows a transposon to move as a physical entity from a donor to a recipient site. This leaves a break at the donor site, which is lethal unless it can be repaired.

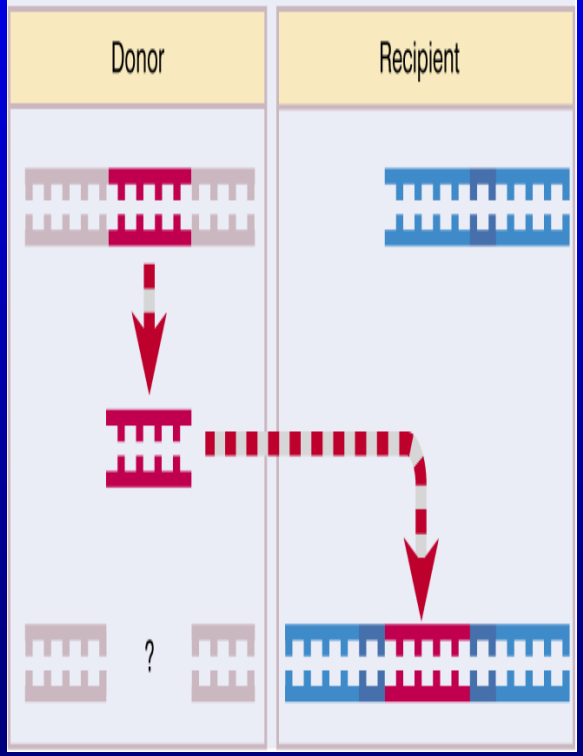
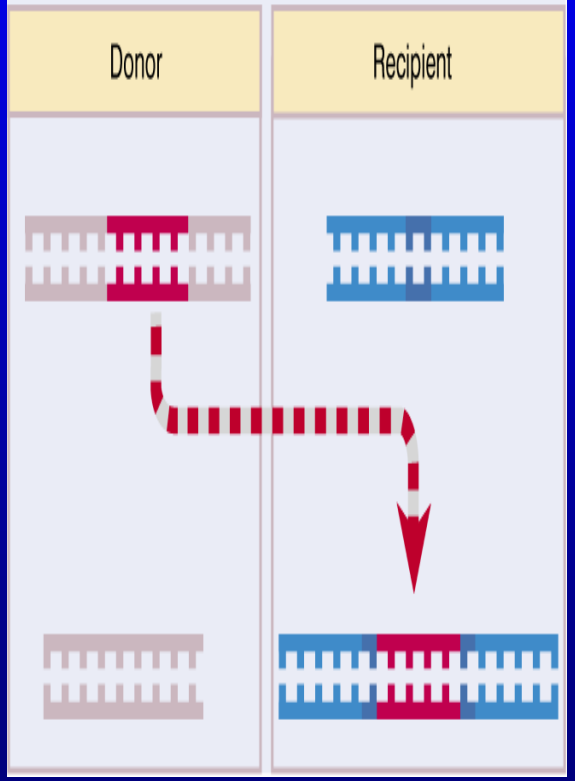
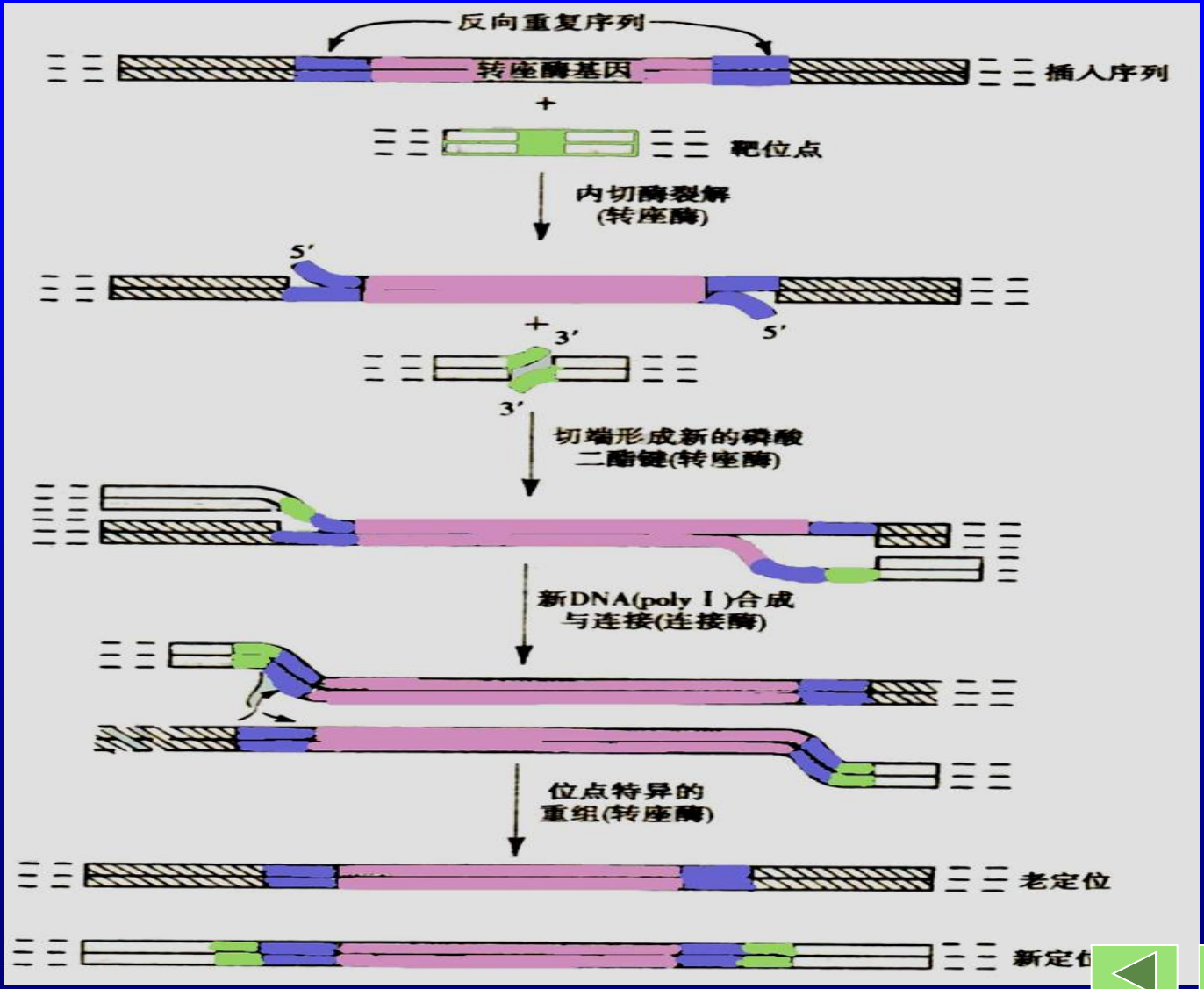


Figure 15.7 Conservative transposition involves direct movement with no loss of nucleotide bonds; compare with lambda integration and excision.



Duplicative transposition



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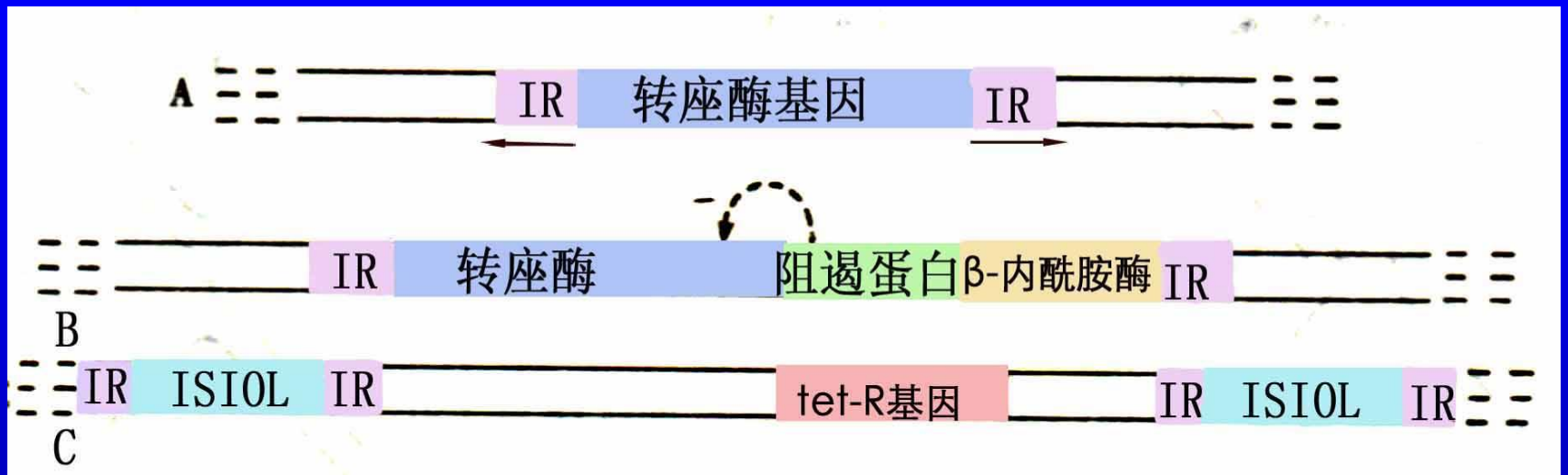
（二） Transposition by transposons

Transposon——is a DNA sequence able to insert itself at a new location in the genome (without any sequence relationship with the target locus).

including: Inverted repeats

Tansposase gene

other genes



细菌的可流动性元件

A. 插入序列：转座酶编码基因两侧连接反向末端重复序列(箭头所示)

B. 转座子 Tn3：含有转座酶、β-内酰胺酶及阻遏蛋白编码基因

C. 转座子 Tn10：含四环素抗性基因及两个相同的插入序列 IS10L

Transposition by transposons