4. Recombination

- Recombination is essential for generating genetic diversity and for maintaining genome integrity (完整性).

重组的意义

- DNA recombination is the exchange of DNA strands to produce new nucleotide sequence arrangements.

DNA重组是指由于DNA链的交换形成新的核苷酸排列方式（新的DNA分子）的过程。

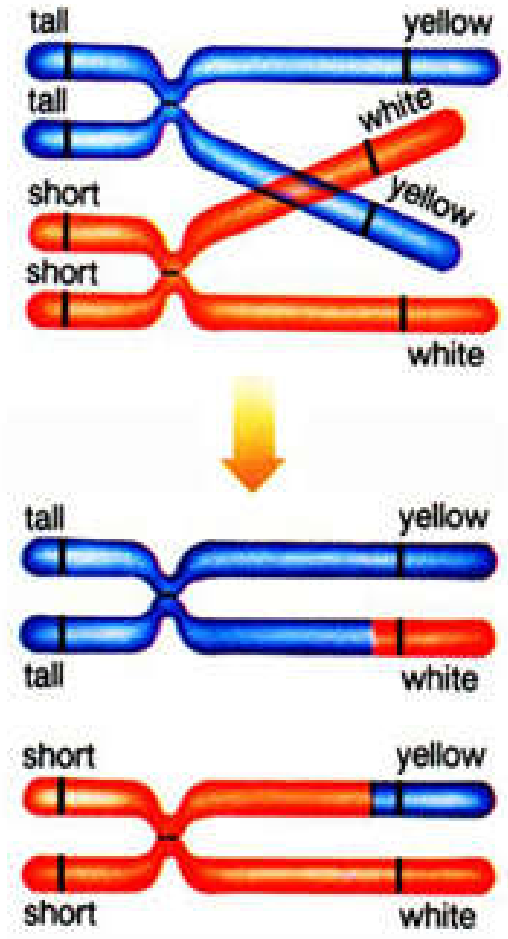
- Homologous recombination (同源重组)
- Site-specific recombination (位点特异性重组)
- Transposition (转座)

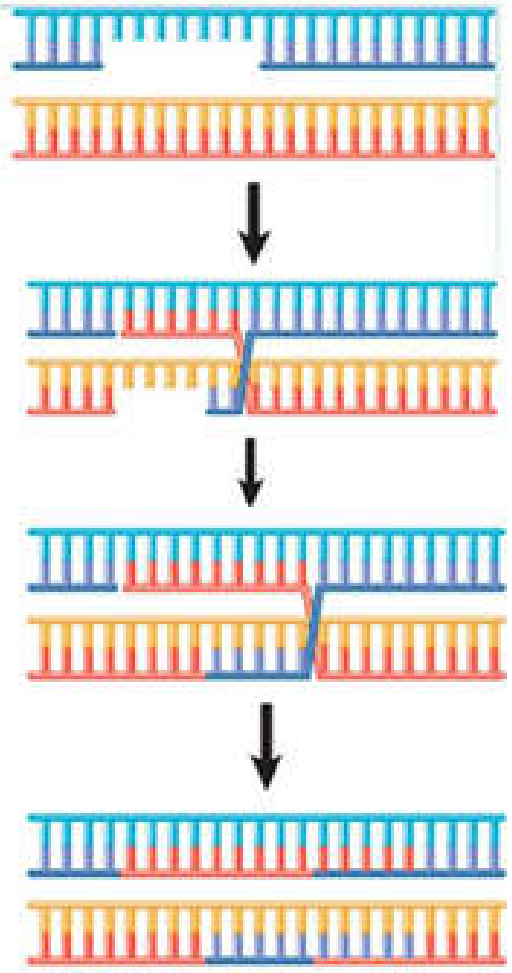
4.1 Homologous recombination (HR)

- **Homologous recombination** is the exchange of DNA strands of **similar or identical (homologous)** nucleotide sequence, also known as **general recombination**.

同源重组是相同或相似(同源)序列的DNA片段之间的交换，也称为**一般重组**。

- **HR is essential in meiosis（减数分裂）for generating diversity and for chromosome segregation, and in mitosis to repair DNA damage.**





4.1.1 Conditions for HR

(1) Homologous DNA sequences

(2) Synapsis (联会)

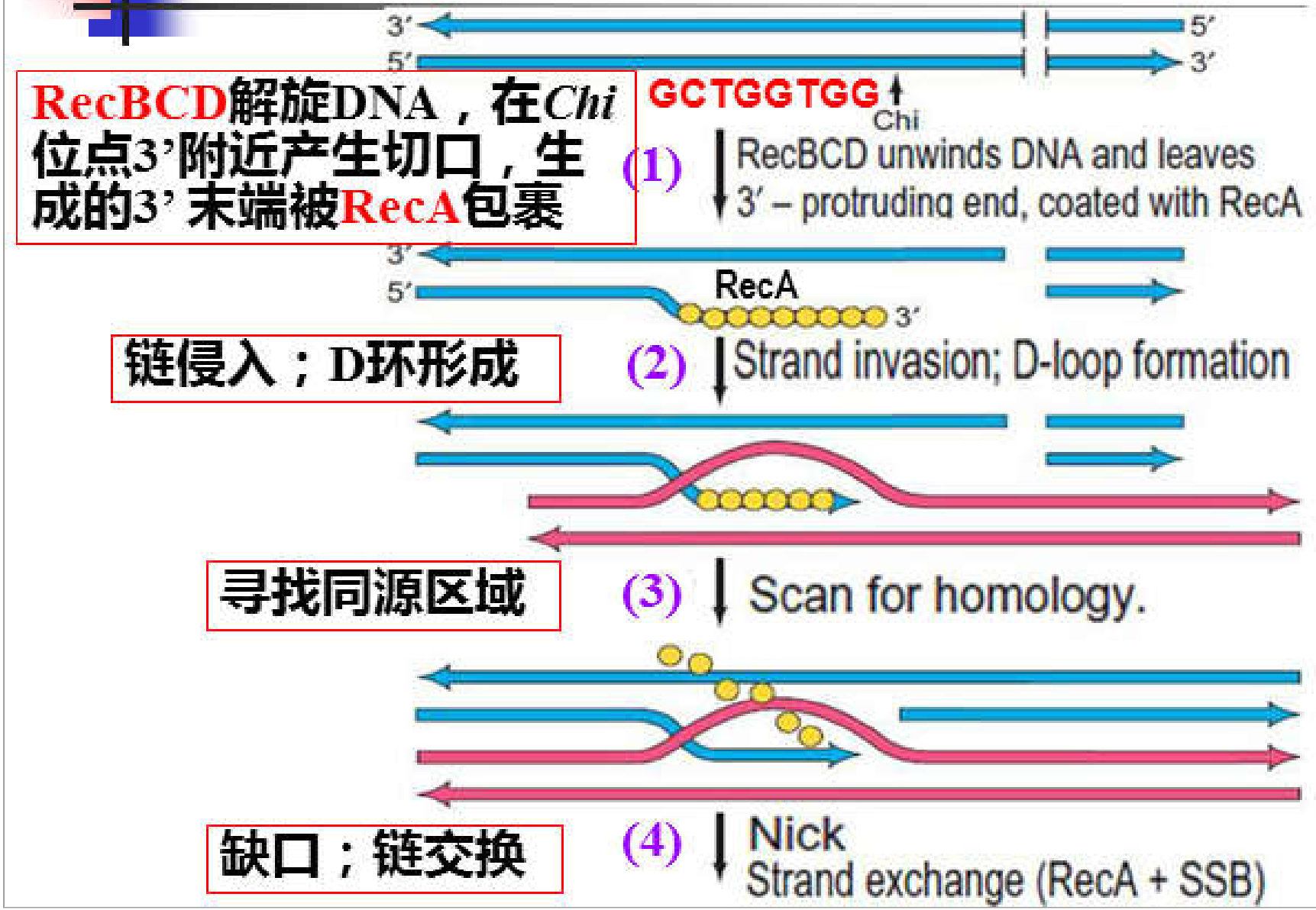
任意两个同源DNA片段在细胞内均可能重组，但是要做到这一点，两个同源双链DNA分子必须靠近，相对应的顺序方能交换，即发生联会。

(3) Nick or gap

重组时，两个DNA片段必须至少有一个发生断裂(形成nick)或有一段单链缺失(形成gap)。因此许多DNA损伤因素均可使重组频率增加。

(4) Recombination related proteins

4.1.2 Overview of RecBCD pathway



RecBCD解旋DNA，在*Chi*位点3’附近产生切口，生成的3’末端被RecA包裹

链侵入；D环形成

寻找同源区域

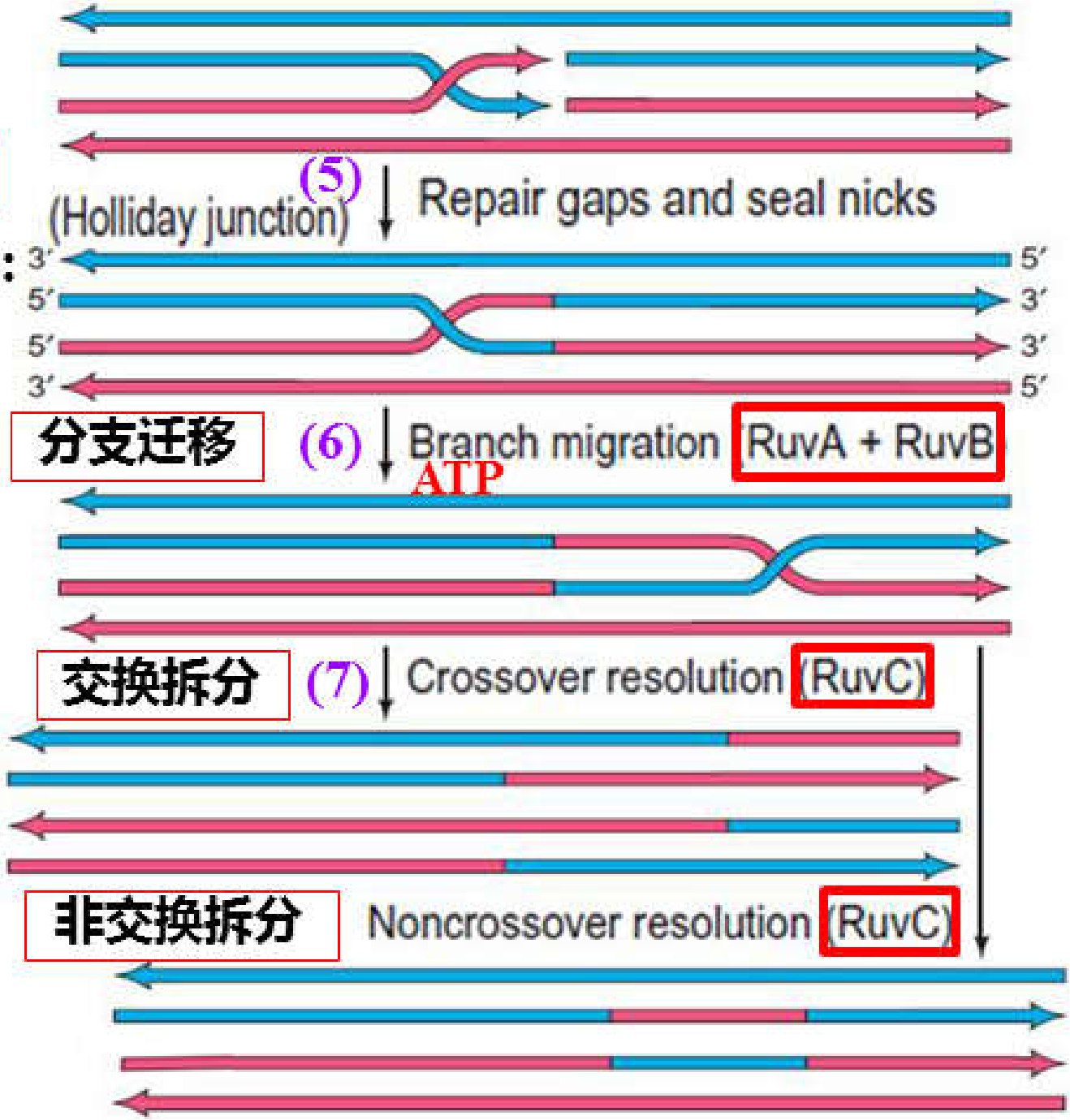
缺口；链交换

补缺

Holliday连接体：四股DNA所形成的交叉结构

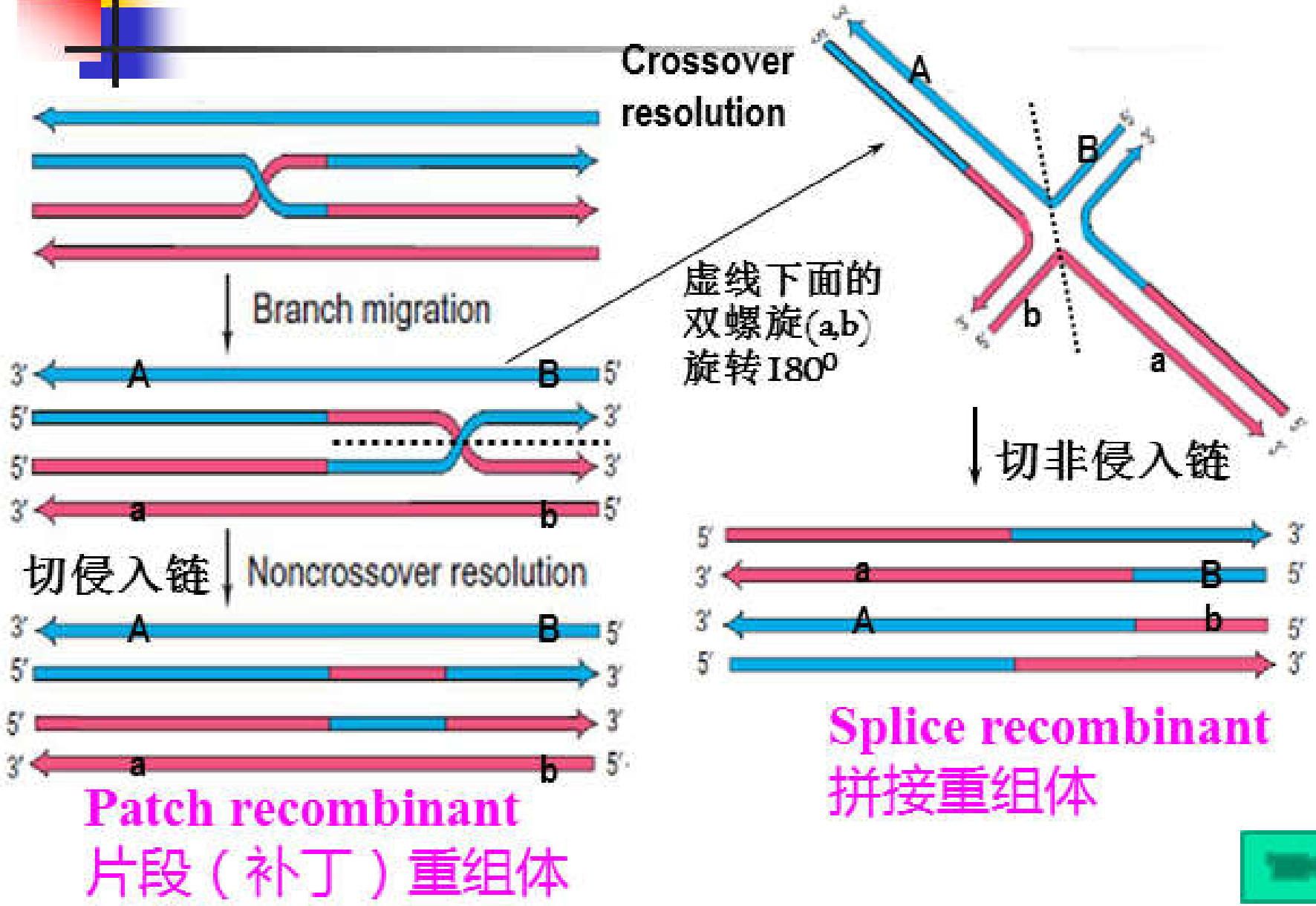
Proposed by **Robin Holliday** in 1964.

(A/T)TT↓(G/C)



Recombinants (重组体)

Holliday连接体的两种拆分方式



Patch recombinant
片段（补丁）重组体

Splice recombinant
拼接重组体

4.1.3 RecBCD

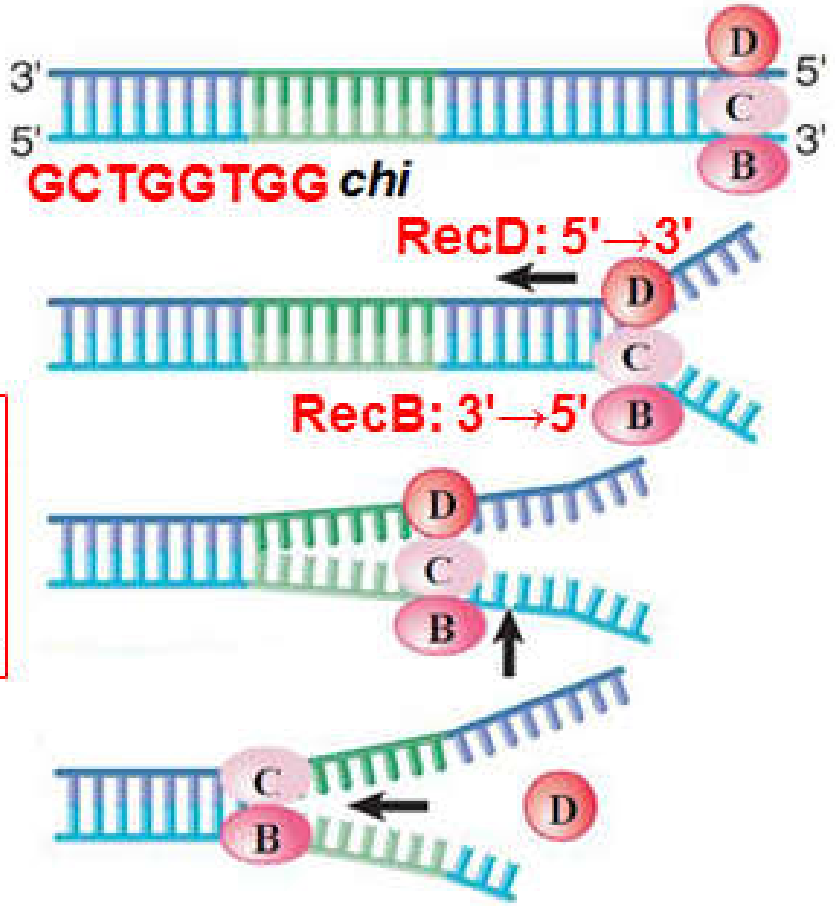
- The RecBCD complex has helicase and nuclease activities.

RecBCD binds a double-strand break.

RecBCD unwinds the duplex.

RecBCD cleaves single strand at a position 4-6 bases to the right of the *chi* site (重组频率较高的部位).

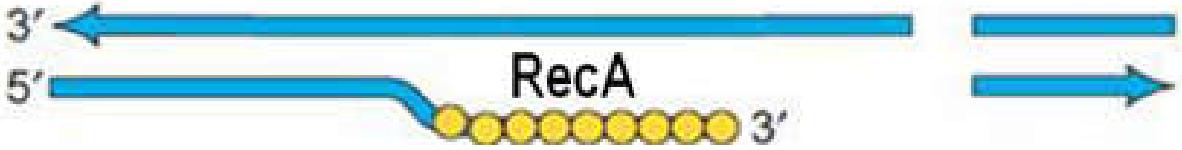
RecD dissociates and nuclease activity losses.



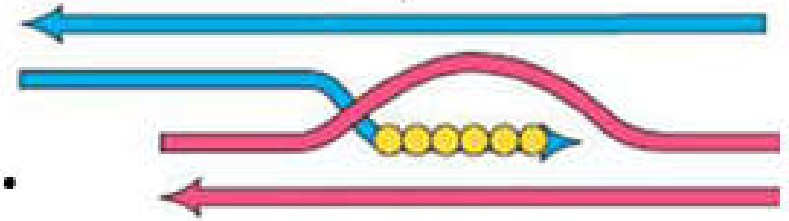
- RecBCD provides a single stranded region with a free 3' end.

4.1.4 RecA

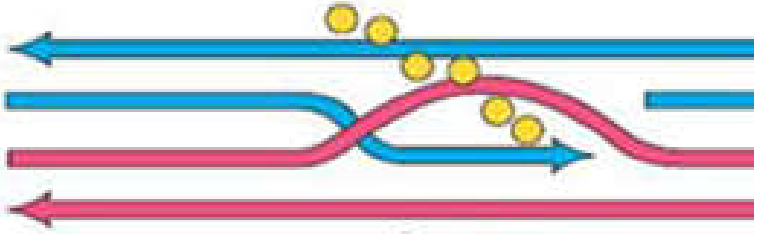
- In the presynapsis (联会前) step of recombination, RecA coats a single-stranded DNA that is participating in recombination.



- In synapsis, RecA promotes invasion (侵入) of another DNA duplex, forming a D-loop.

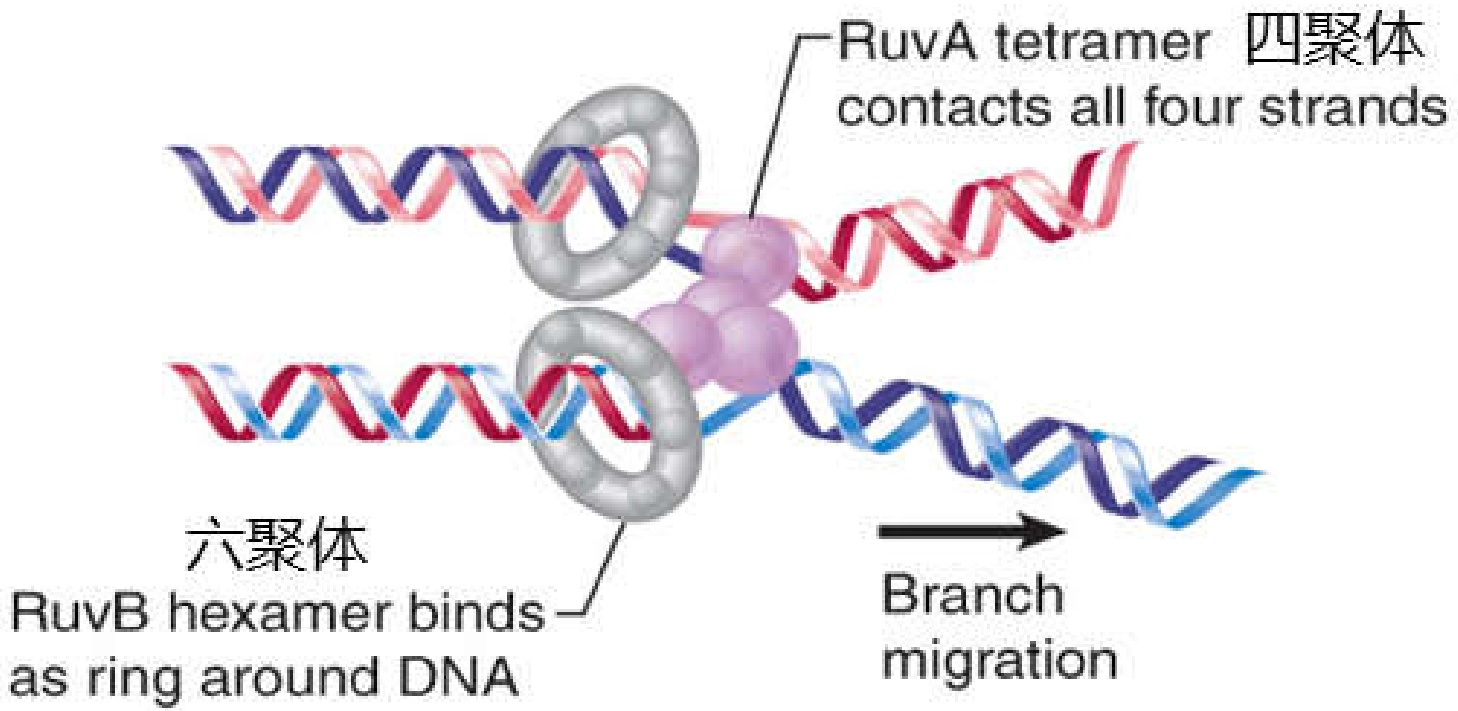


- RecA helps the invading strand scan for a region of homology in the recipient (受体) DNA duplex.



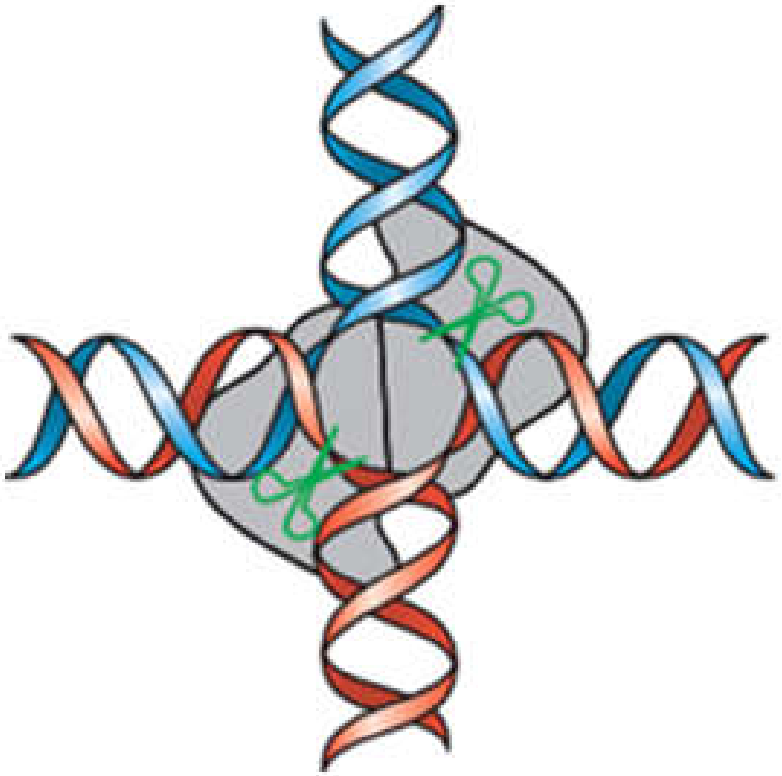
4.1.5 RuvA and RuvB

- **RuvA and RuvB form a DNA helicase that can drive branch migration.**
- **Need ATP. RuvB is a ATPase.**



4.1.6 RuvC

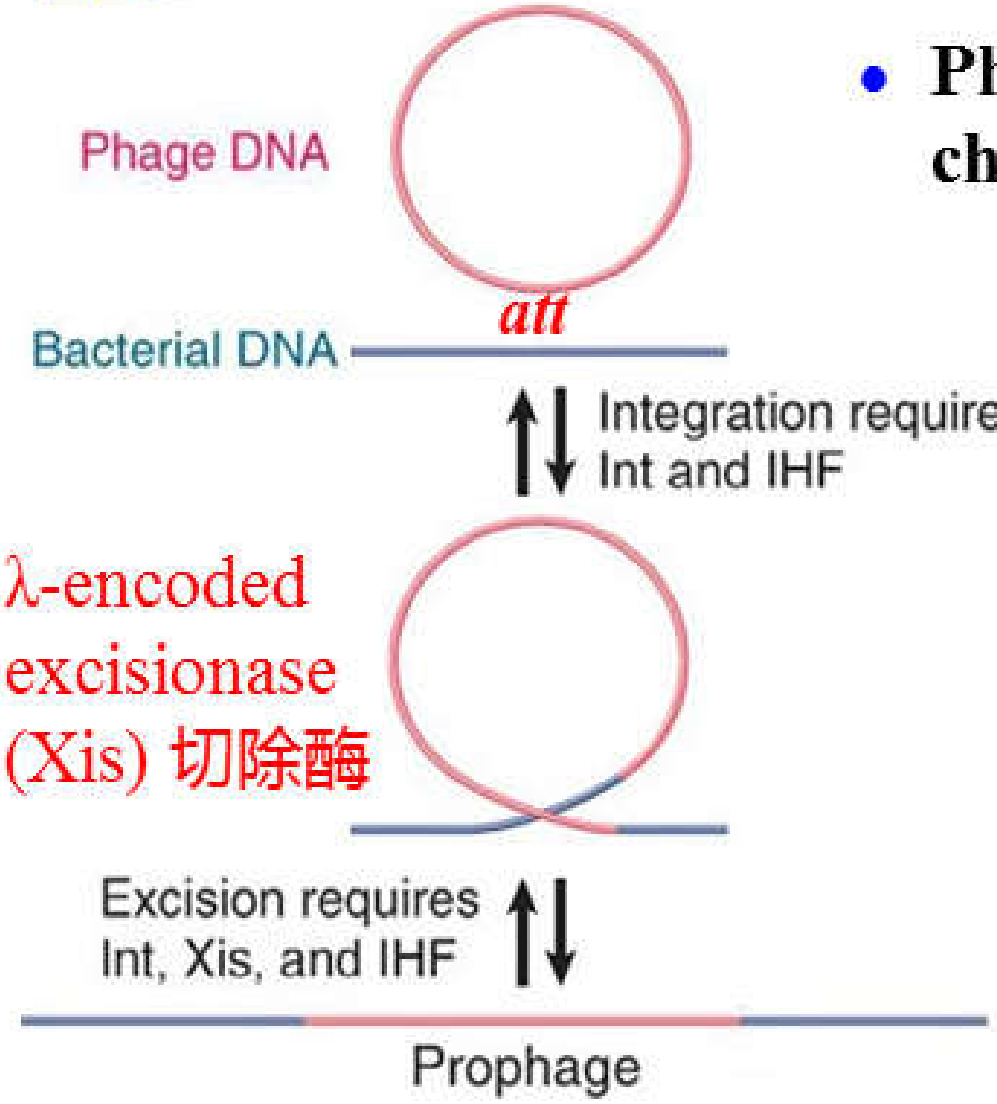
- **Resolution of Holliday junctions.**
- **The clipping occurs preferentially (优先地) at the consensus sequence (A/T)TT↓(G/C).**
- **Branch migration is essential for efficient resolution of Holliday junctions.**



4.2 Site-specific recombination

- **Site-specific recombination involves the exchange of nonhomologous but specific pieces of DNA and is mediated by integrase that recognize specific DNA sequences.**
  位点特异性重组是由能识别特定DNA序列的整合酶介导的非同源但序列特定的DNA交换。
- **It does not require RecA or ssDNA.**

4.2.1 Bacteriophage λ integration



- **Phage integrated in *E.coli* chromosome at *att* sites.**

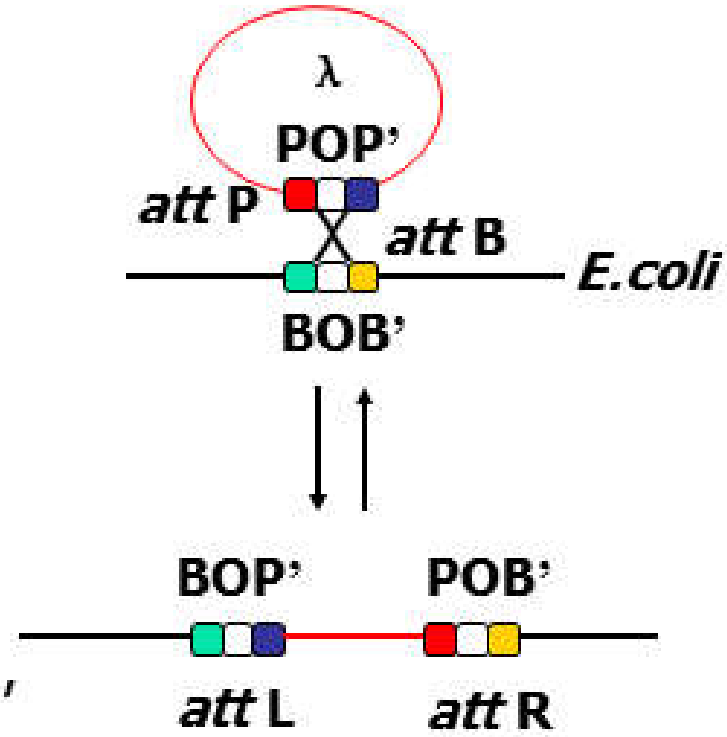
λ-encoded integrase (Int) 整合酶

Bacteria-encoded IHF (integration host factor) 整合宿主因子

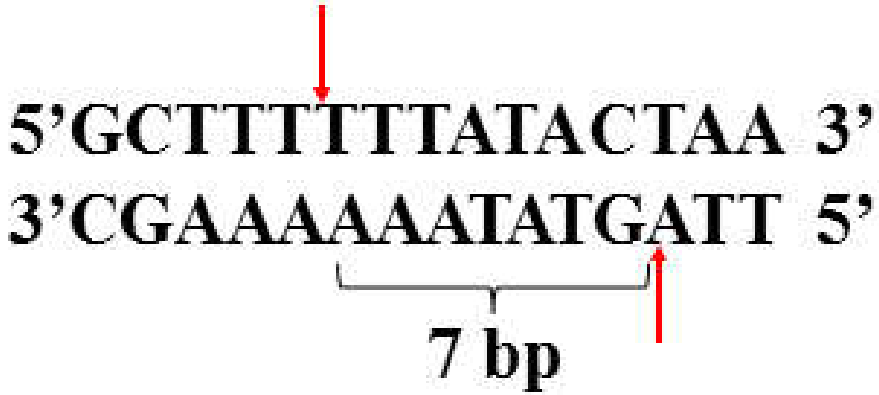
λ噬菌体DNA通过重组作用整合进*E. coli*染色体的特异位点，成为原噬菌体(prophage)。

4.2.2 The integration/attachment (*att*) site

- *att*B由称为BOB’的序列组成，而*att*P由POP’组成，O是核心序列，是*att*B和*att*P所共同的。
- 噬菌体DNA是环状的。重组时被整合到细菌染色体中，成为线性序列。原噬菌体的两侧是两个新的杂种*att*位点，左侧为*att*L，由BOP’组成，而右侧为*att*R，由POB’组成。

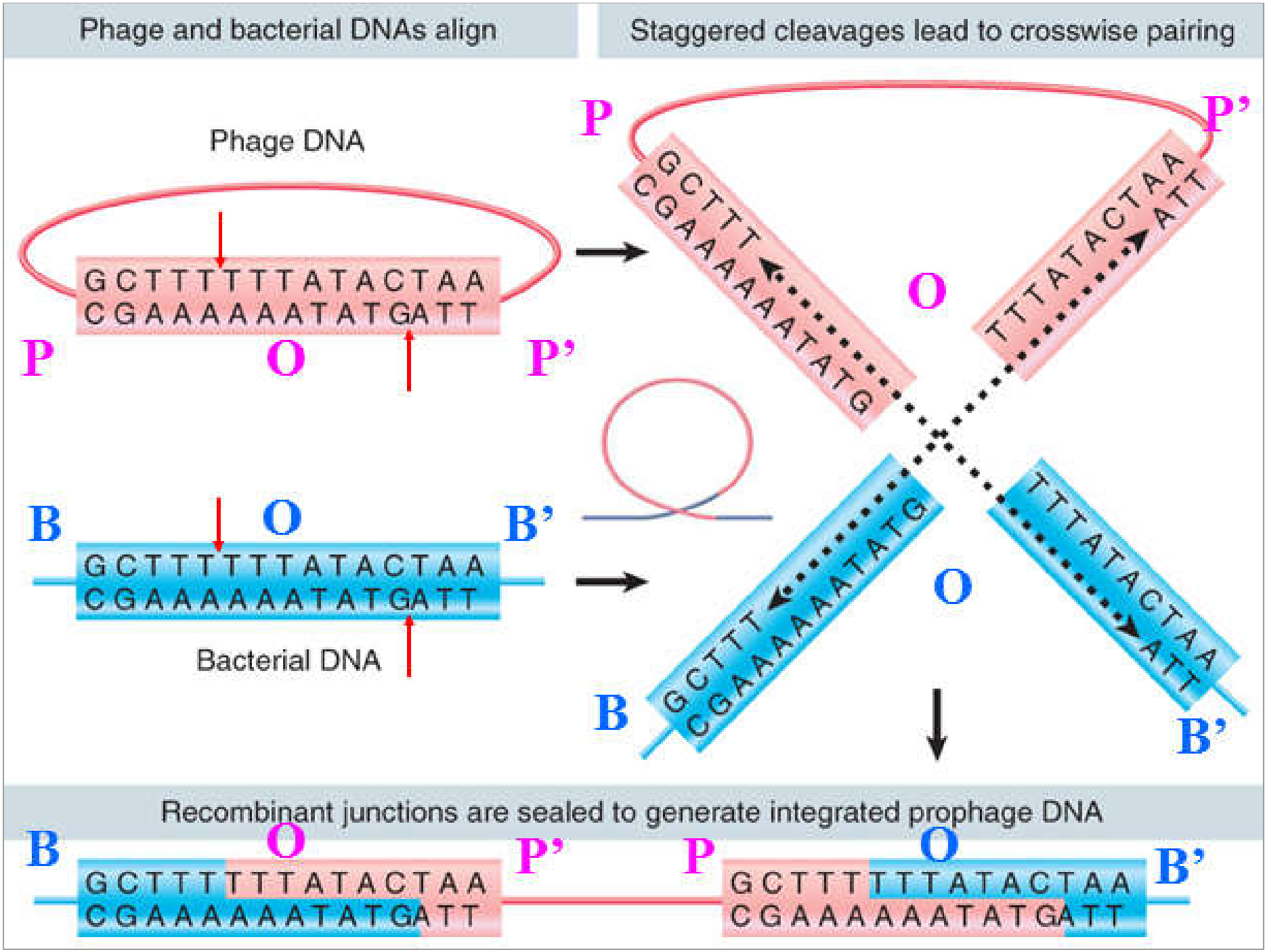


- **Core sequence** - the segment of DNA that is common to the attachment sites on both the λ phage and bacterial genomes.
- It is the **location of the recombination** event that **allows λ phage to integrate**.



15 bp

- **Integrase** makes **staggered cuts** (交错切) in the core sequence with 7 bp overhangs.



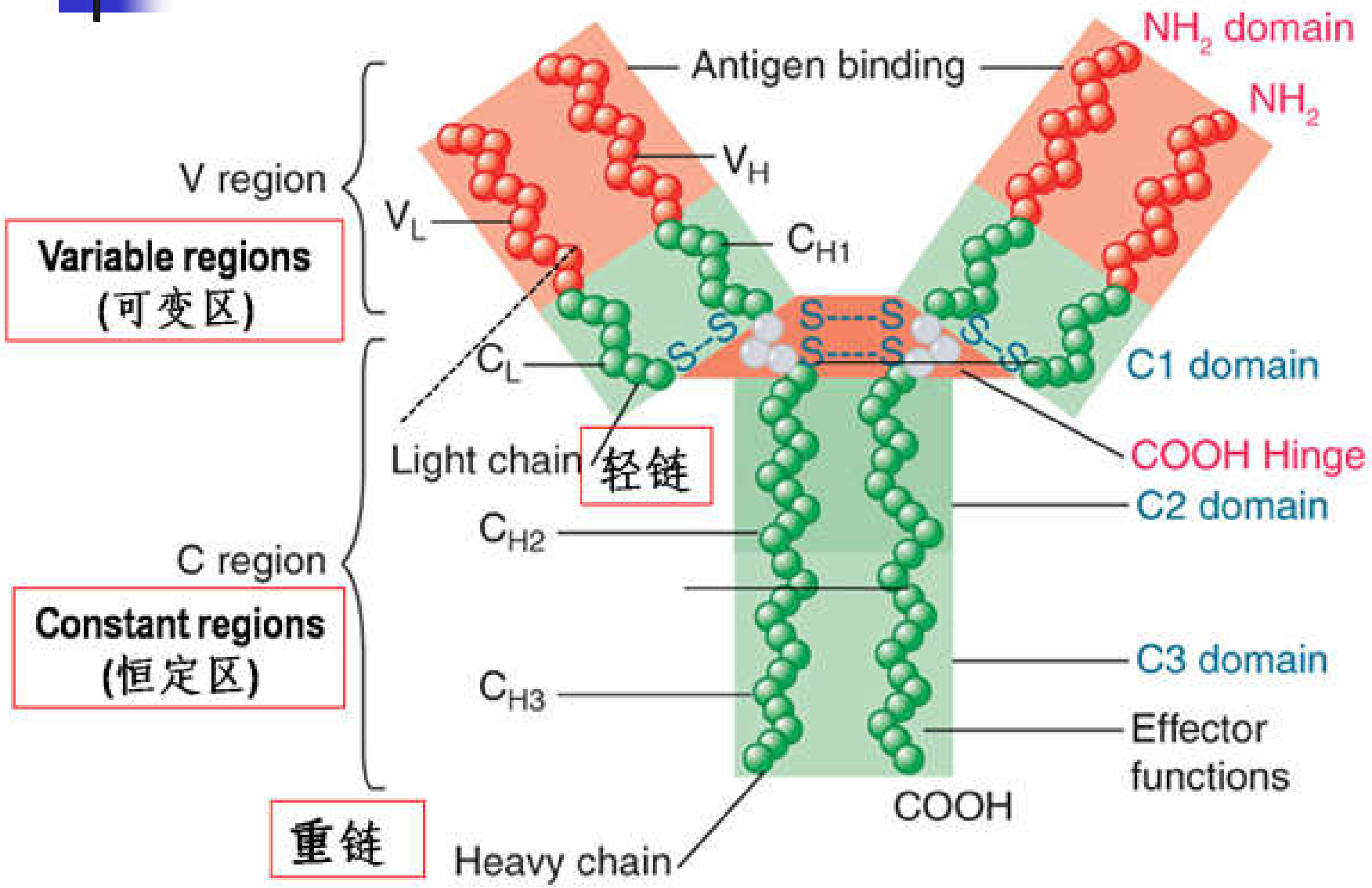
4.2.3 Features of λ phage integration

(1) 交换可逆，原先存在的DNA序列全部被保留下来，并无丢失。

(2) 噬菌体和细菌的DNA之间有一段很短的同源序列——核心序列（core sequence），重组交换必须通过这一段特定的核苷酸序列。

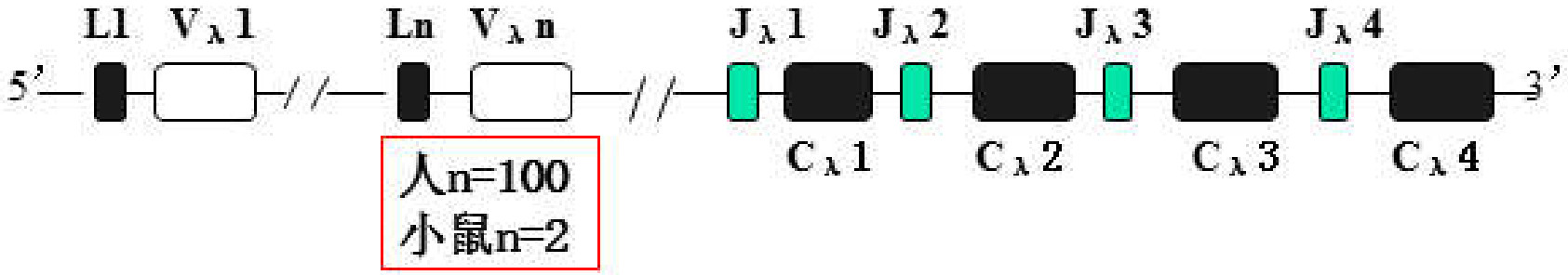
(3) λ噬菌体编码整合酶（Int），该酶具有拓扑异构酶性质，通过链的断裂和连接指导噬菌体DNA插入*E. coli*染色体中。

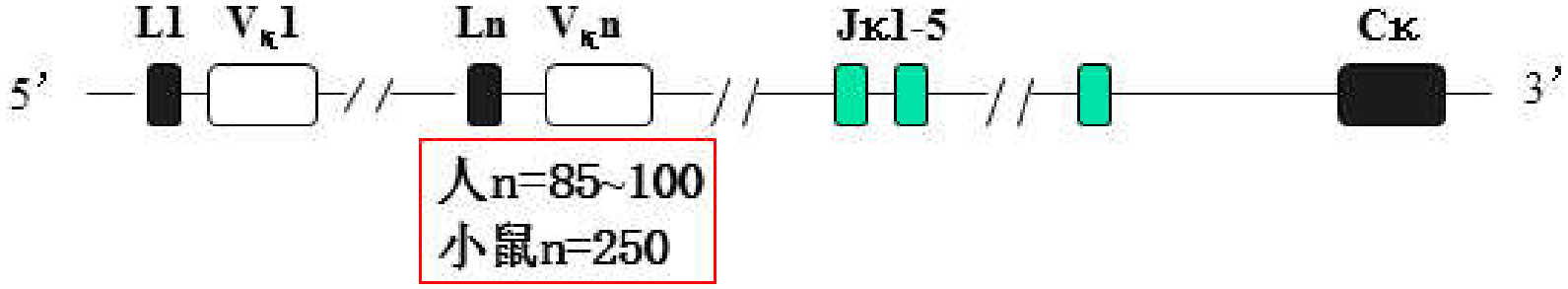
4.2.4 Rearrangement of immunoglobulin (Ig) genes



(1) Gene structure of Ig light chain

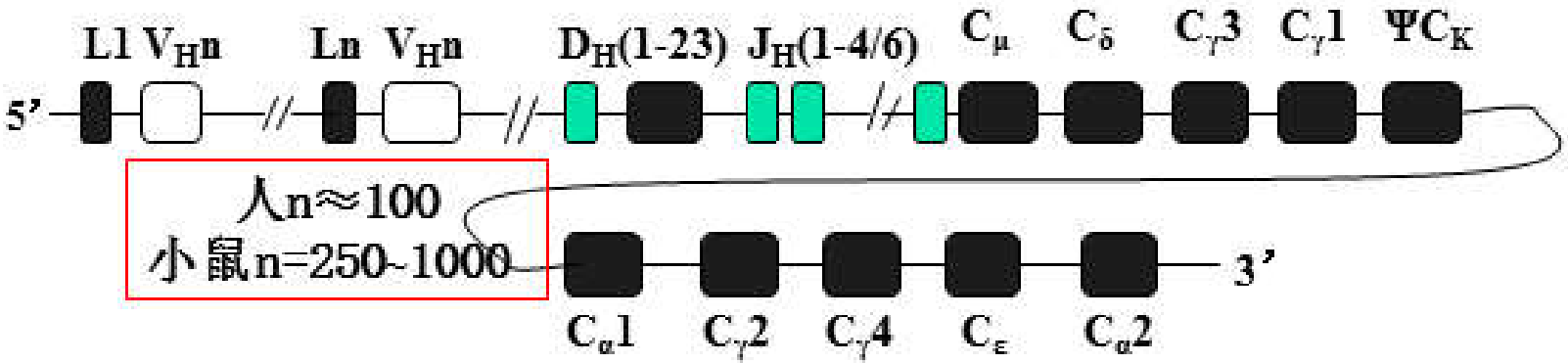
- 抗体轻链有λ和κ两个基因家族，分别位于人类22号和2号染色体上。λ和κ都由L (leader)、 V (variable)、J (joining)和C (constant)四种不同基因片段组成。





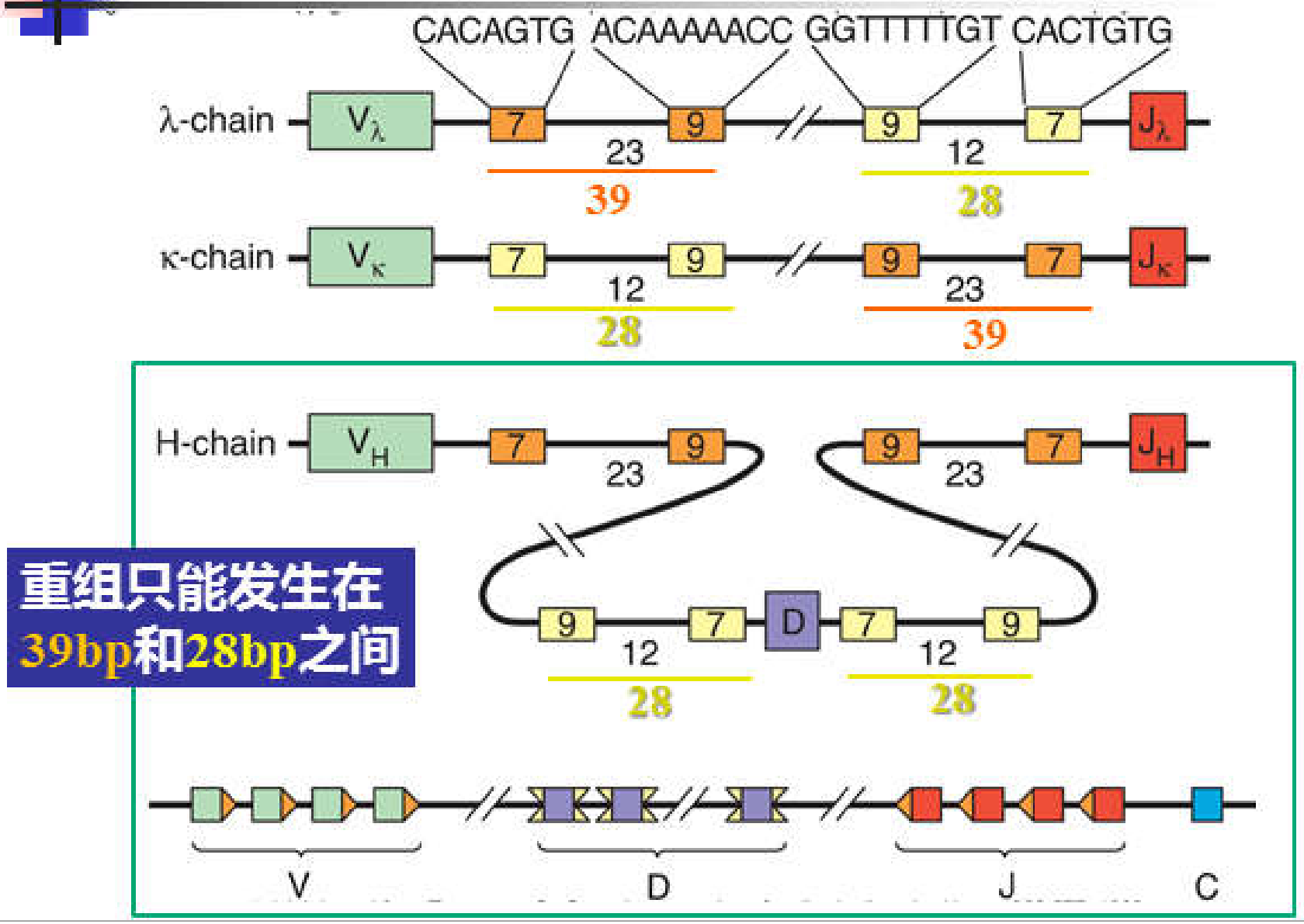
(2) Gene structure of Ig heavy chain

- 人类重链基因位于14号染色体。在重链基因中，DNA上除了有L、V、J、C片段外，在V_H和J_H之间还有约20个多样性片段（D片段）。



IgG (γ), IgM (μ), IgA (α), IgE (ε) and IgD (δ)

(3) Signals for V(D)J joining



重组只能发生在39bp和28bp之间

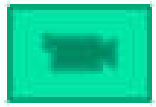
(4) Reasons for Ig diversity

- V区和(D)J区不同片段在DNA分子水平上的各种排列组合是形成Ig分子多态性的根本原因。

  L链: λ –V100 J4，有400种组合，κ-V100 J5，有500种组合。

  H链: V100 D20 J6，有$100\times20\times6 = 1.2\times10^4$

  H和L组合$(400+500)\times1.2\times10^4 = 1.08\times10^7$



- 体细胞超突变：免疫细胞增殖过程中可变区的突变频率比一般细胞高出10^4-10^5倍。突变发生的部位大都在V-D-J外显子内或其附近。

4.3 Transposition

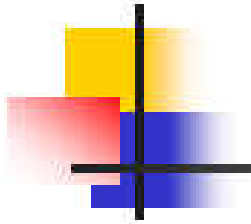
Barbara McClintock (1902-1992)

20世纪40年代发现

1983 Nobel Prize in Physiology or Medicine

- In transposition, a transposon (转座子), or transposable element (转座元件， 转位因子), moves from one DNA address to another.

- A transposon is a small DNA sequence that can move to virtually any position in a cell's genome.
  转座子是能够转移进细胞基因组内的几乎任何位置的短DNA序列。
- Requires no homology between sequences nor site-specific.
- Transposition has also been called illegitimate recombination (异常重组) .
- Inefficient



Insertion sequence
(IS元件, 插入序列)

Composite transposon
(复合型转座子, Tn)

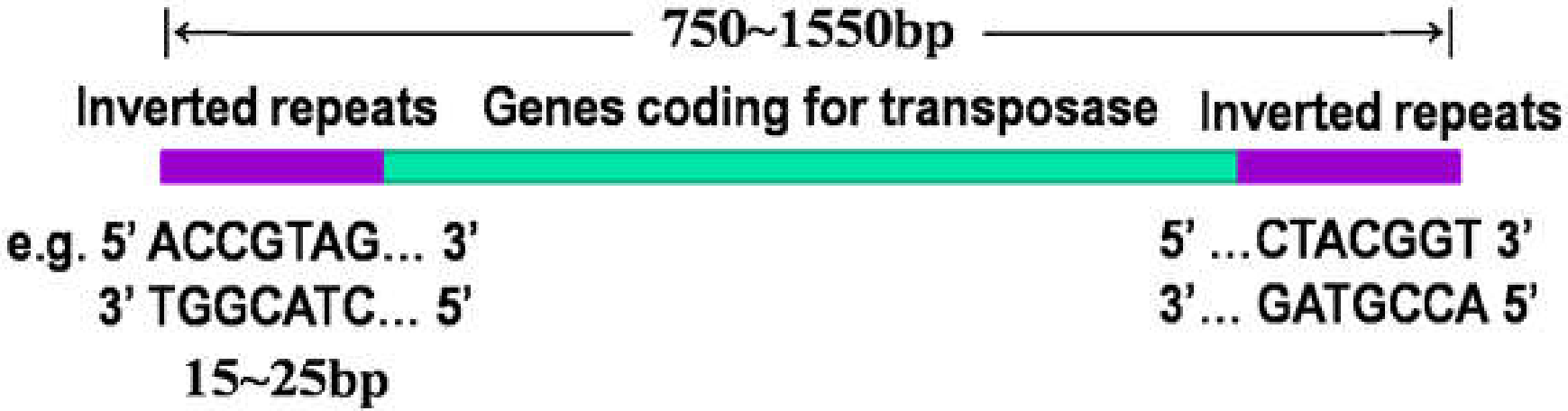
Mainly
Bacterial
transposons

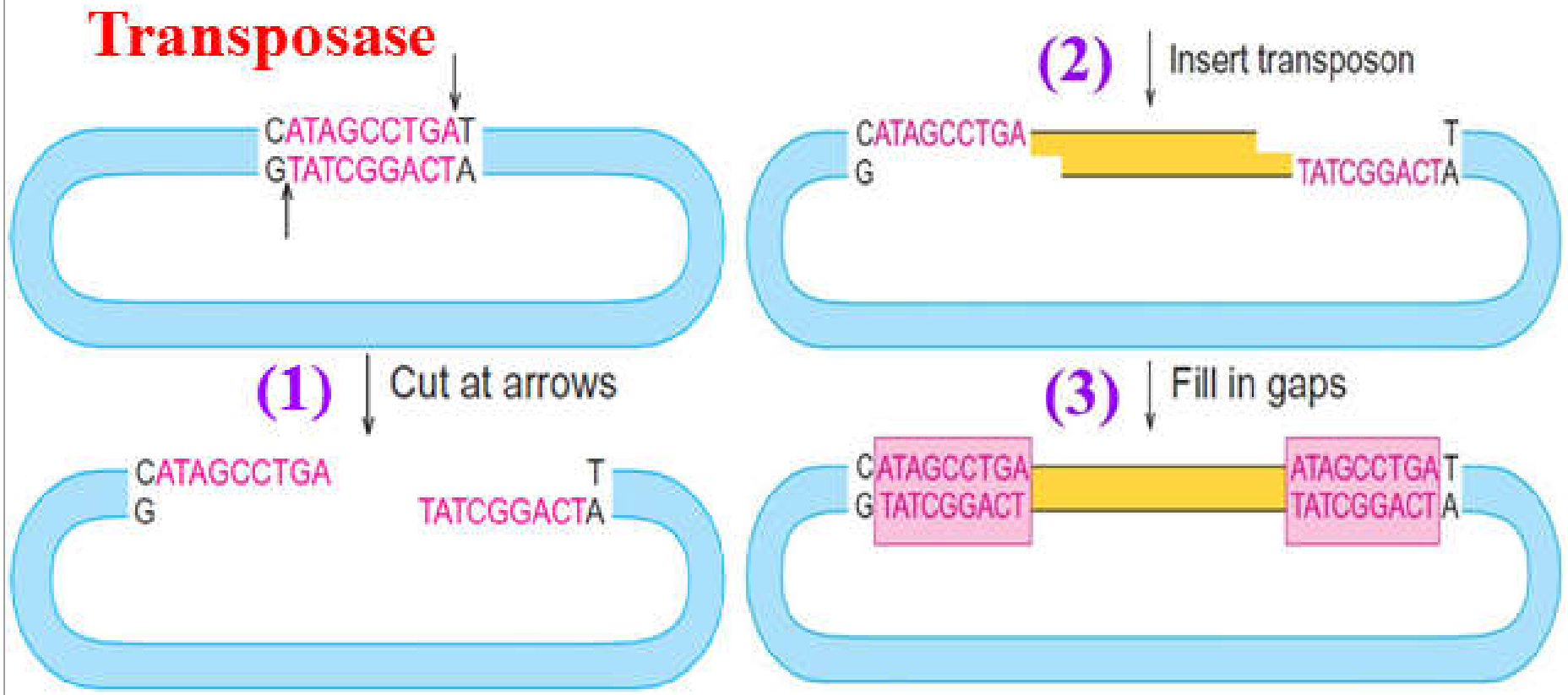
Retrotransposon
(反转录转座子)

Eukaryotic
transposons

4.3.1 Insertion sequences (IS elements)

- IS elements are the simplest transposons.
- Comprise transposase (转座酶) genes flanked by a short inverted terminal repeats (末端反向重复序列).



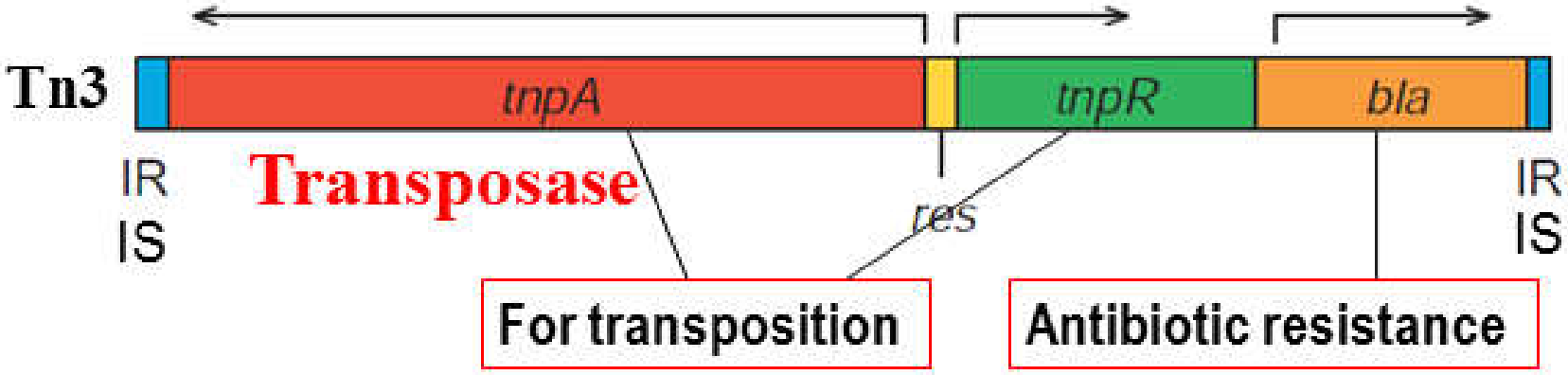


- **The target site at which an insertion sequence is inserted is duplicated during the insertion process to form direct repeats at the ends of the transposon.**
  转座子插入处生成了宿主DNA的正向重复。

4.3.2 Composite transposon (Tn)

(1) Structure of Tn 复合型转座子

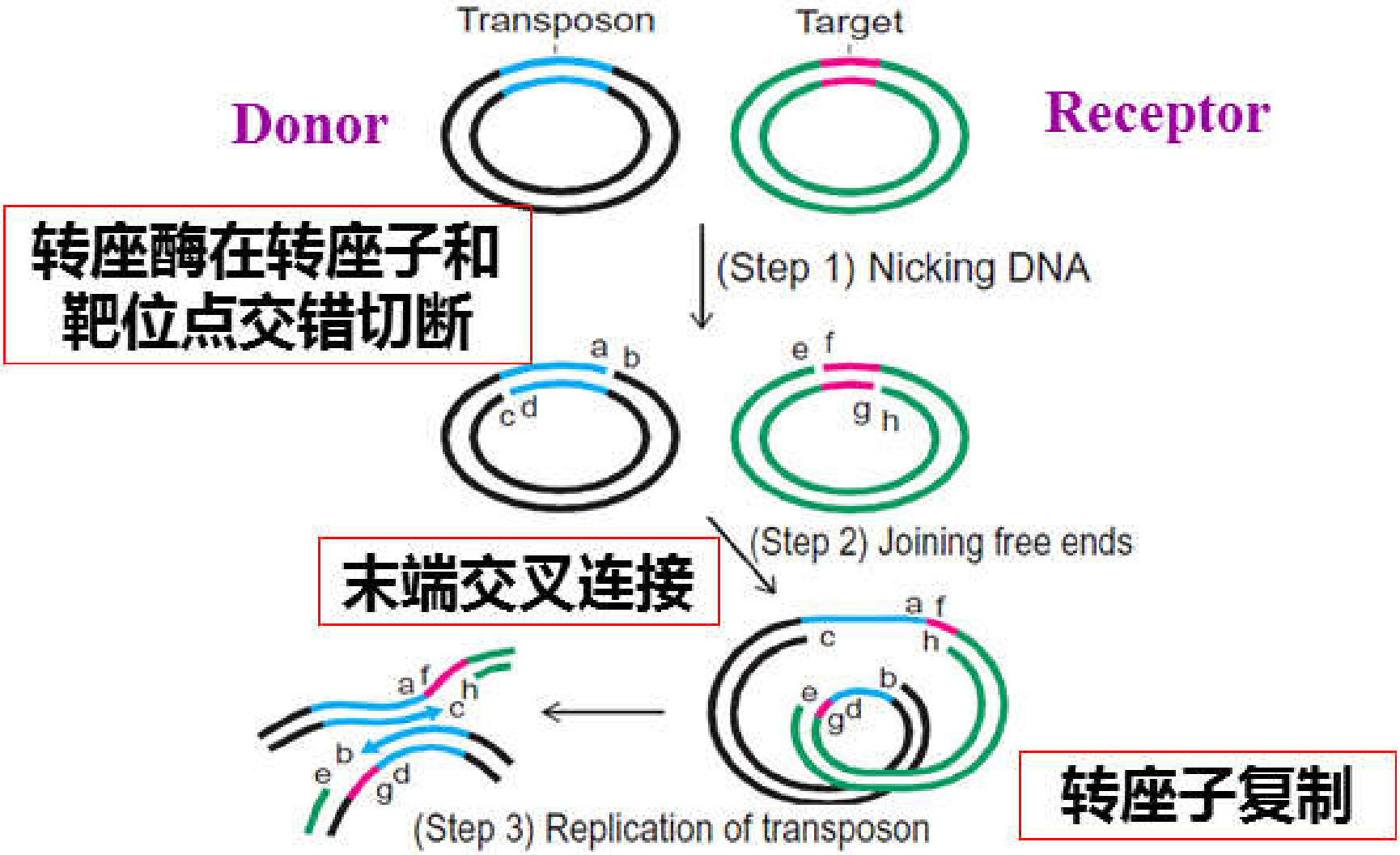
- Elements necessary for transposition
- Other genes (often conferring antibiotic resistance)

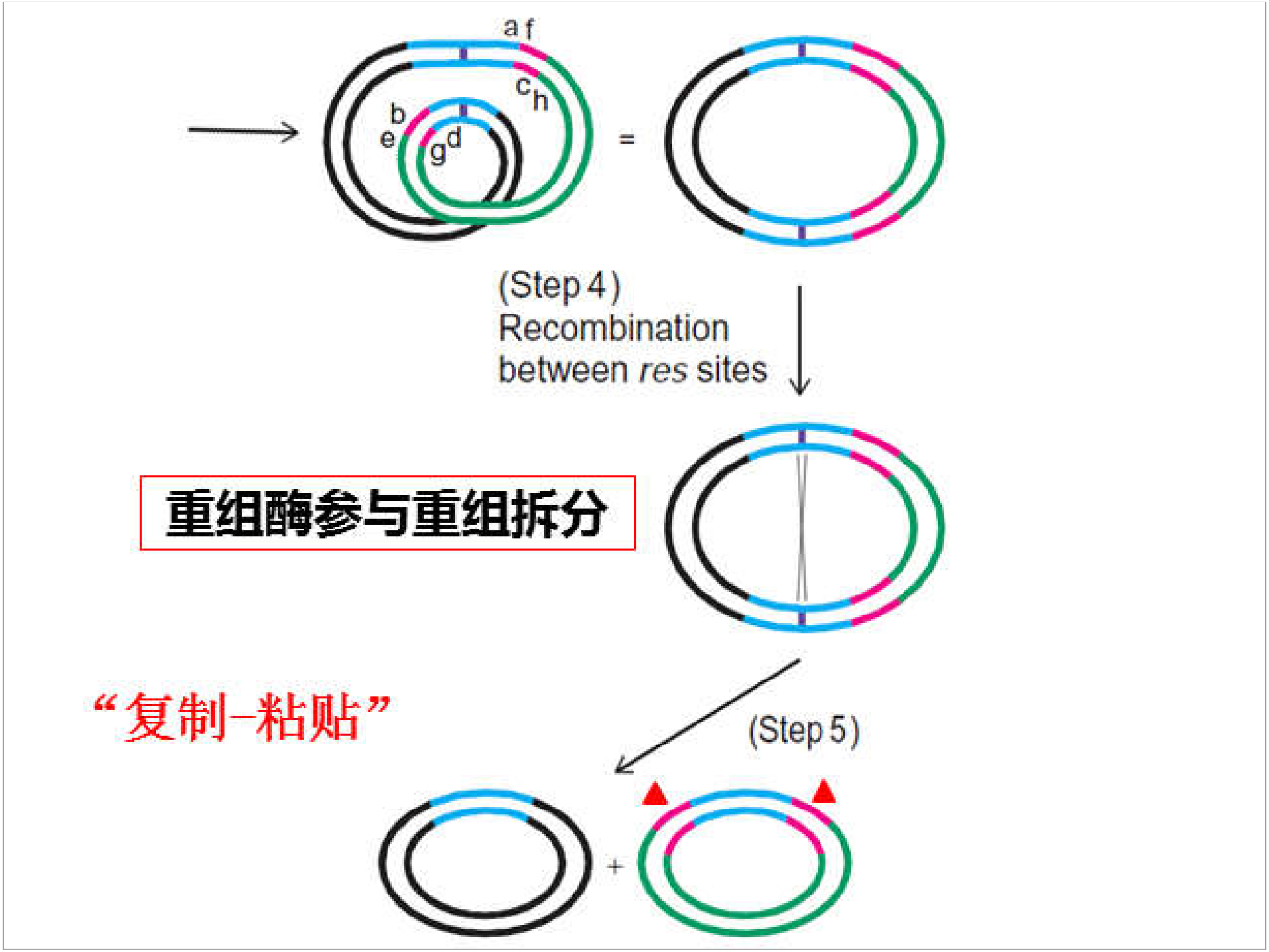


bla encodes β-lactamase (内酰胺酶), which protects bacteria against the antibiotic ampicillin (青霉素). This gene is also called Amp^r.

(2) Mechanisms of transposition

- **Replicative transposition (复制转座)**

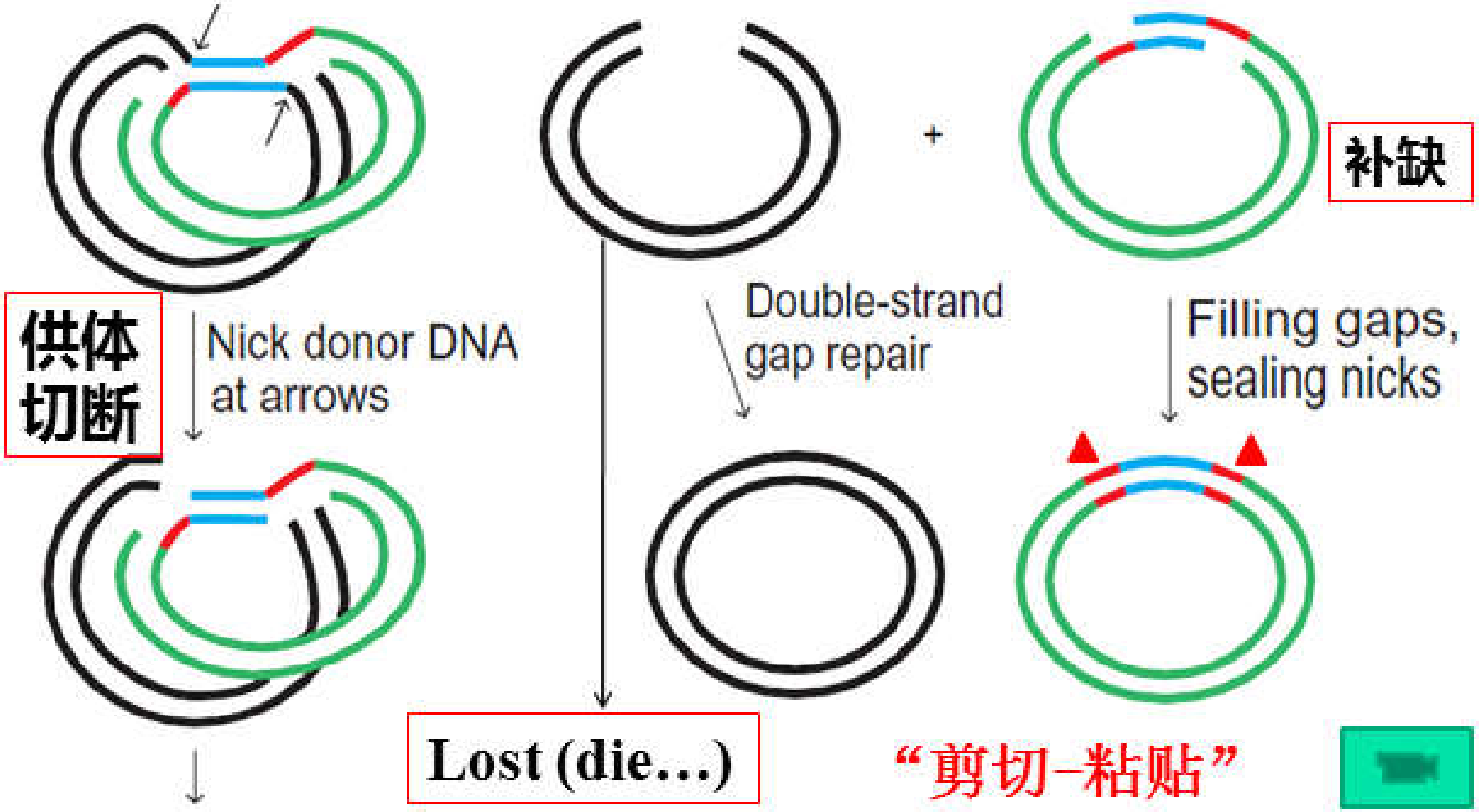



a f
c h
b
e
g d
=
(Step 4)
Recombination
between *res* sites
重组酶参与重组拆分
“复制-粘贴”
(Step 5)
+

- **Nonreplicative transposition (非复制转座)**

转座酶在转座子和靶位点交错切断,自由末端交叉连接

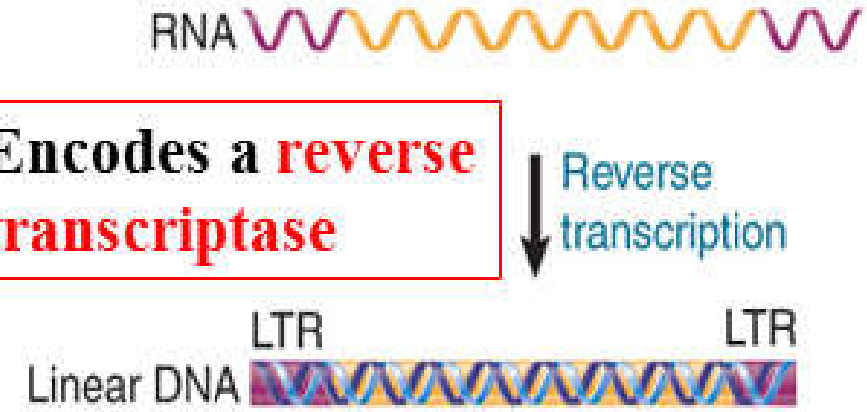
simple/conservative transposition



4.3.3 Retrotransposon

(1) LTR–containing retrotransposon

LTR (long terminal repeats, 长末端重复序列) is crucial for replication of most retrotransposons.



逆转录病毒(本身可看作转座子)

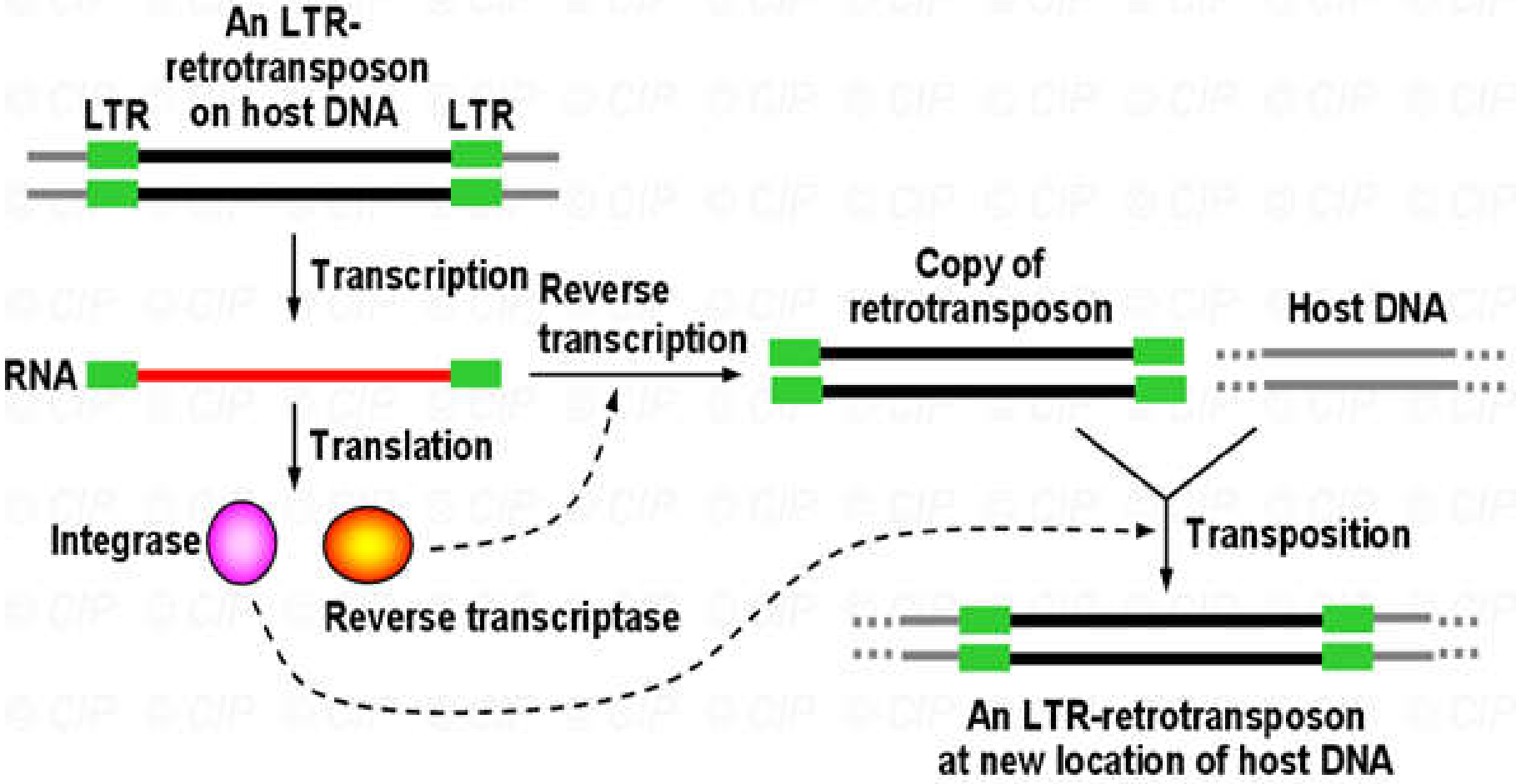
Eukaryotic transposons, many are retrotransposons.

e.g. Yest Ty element

***Drosophila copia* element**

DNA→RNA→DNA

DNA→RNA→DNA


An LTR-retrotransposon on host DNA
LTR
LTR
Transcription
RNA
Translation
Integrase
Reverse transcriptase
Reverse transcription
Copy of retrotransposon
Host DNA
Transposition
An LTR-retrotransposon at new location of host DNA

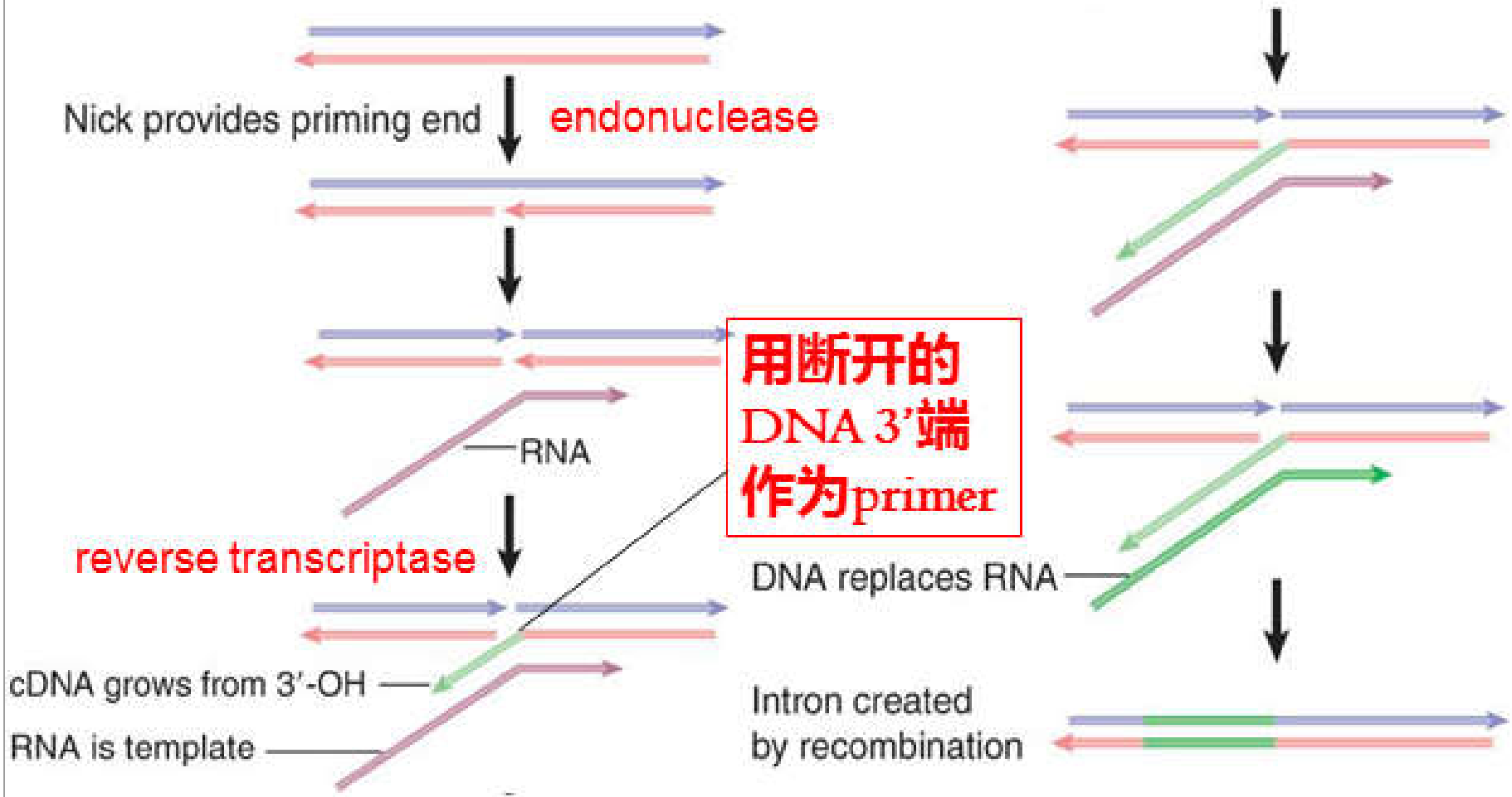
(2) LTR-free retrotransposons

Retrotransposons that lack LTRs are much more abundant than those with LTRs, at least in mammals.

① LINE

- The most abundant of LTR-free retrotransposons.
- They are autonomous (自主的) retrotransposons.

LINEs codes for an endonuclease (内切核酸酶) and reverse transcriptase.

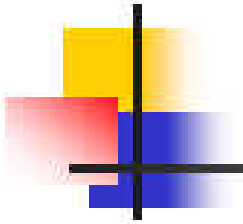


DNA → RNA → DNA

② SINE

- **nonautonomous retrotransposons.**
- **SINEs do not encode any proteins. Instead they take advantage of the retrotransposition machinery of other elements, such as LINEs.**
- **The transcripts of the *Alu* elements contain a domain that resembles (类似) the 7SL RNA component of signal recognition particle (信号识别颗粒).**

7SL RNA结构域通常是帮助特定核糖体结合到粗面内质网。7SL RNA结构域帮助*Alu*的RNA到翻译LINEs RNA的核糖体，使*Alu*的RNA能和所需要的蛋白结合，从而被反转录插入到新的位点。



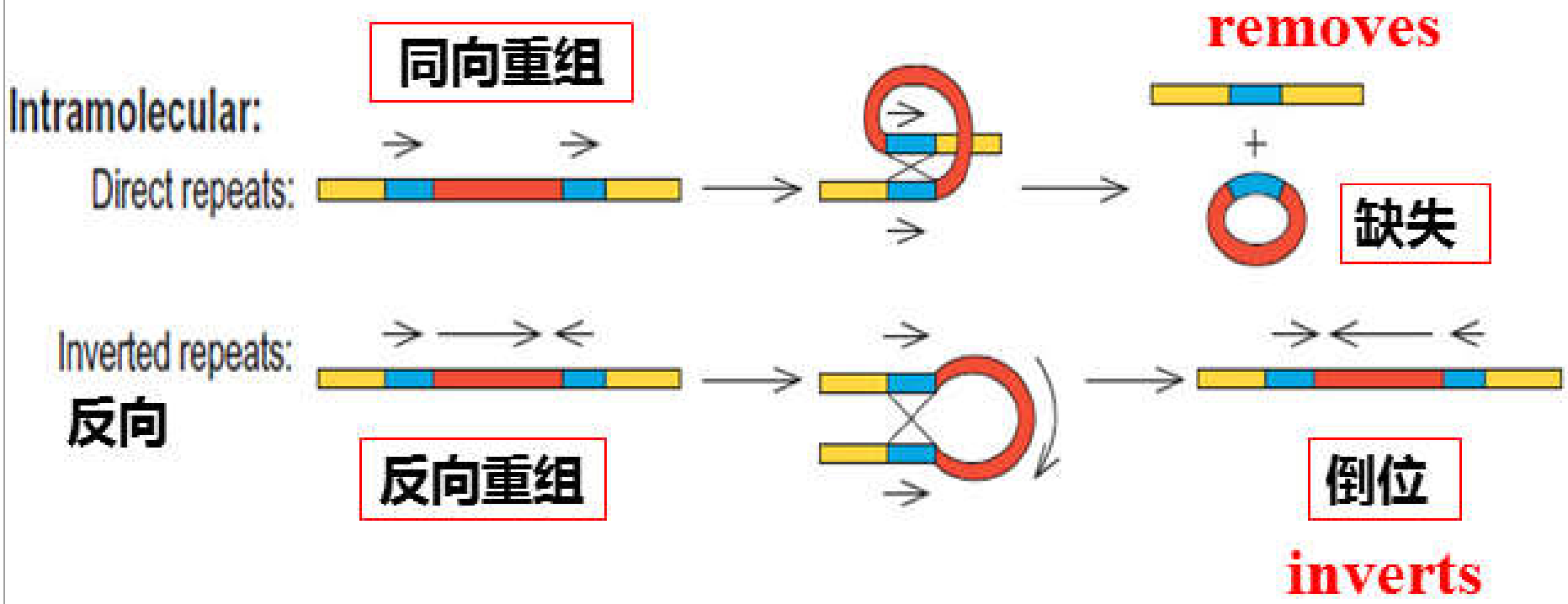
4.3.4 Genetic effects of transposition

(1) Insertion mutation

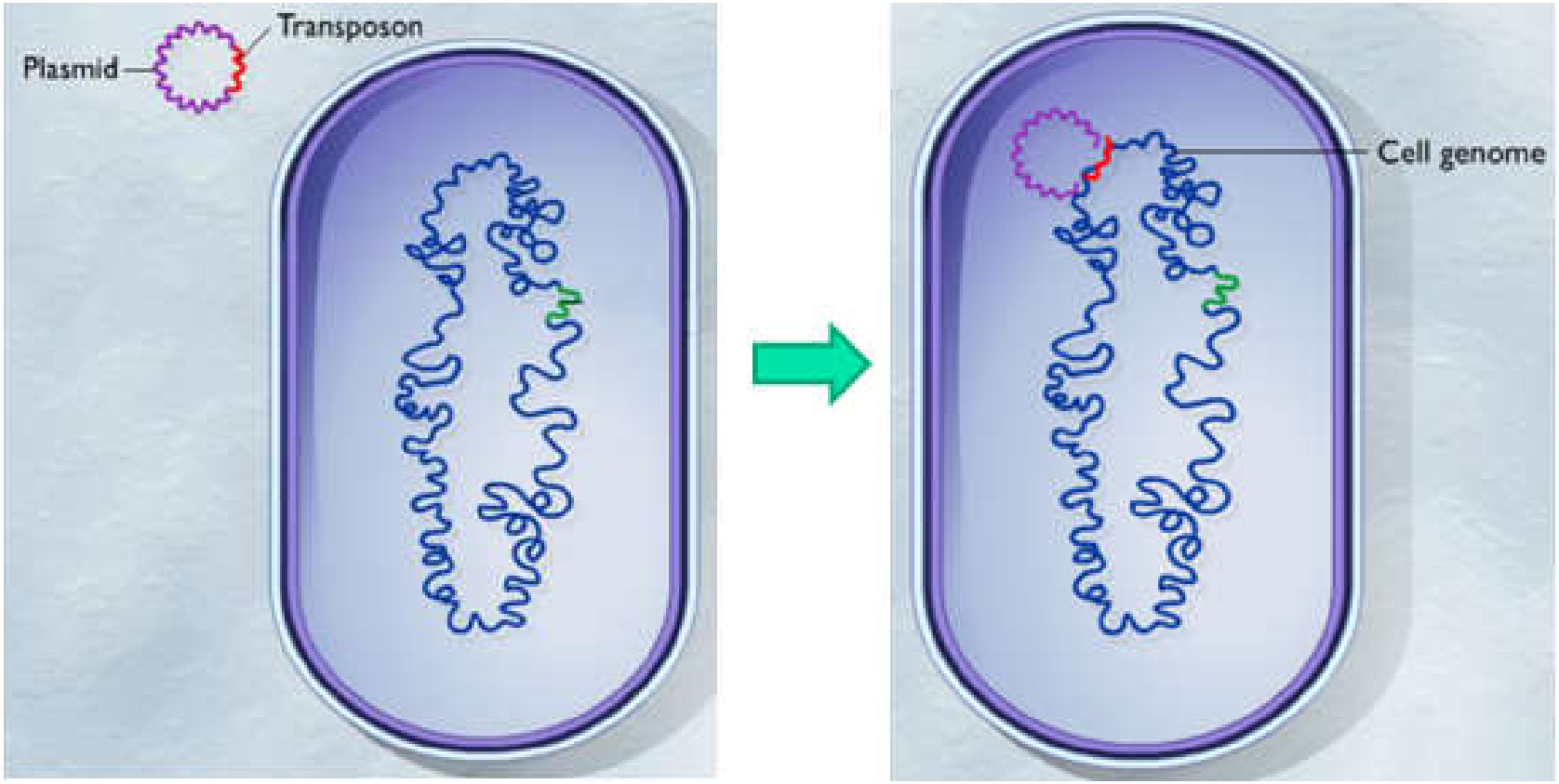
The gene into which the transposon inserts is usually inactivated.

(2) Deletion or inversion

Genes between two copies of a transposon can be deleted or inverted by recombination between them.


同向重组
removes
Intramolecular:
Direct repeats:
缺失
Inverted repeats:
反向
反向重组
倒位
inverts

(3) Introduction of new gene



Summary

1. Types and phenotypic effects of mutation
2. Replication fidelity
3. Factors and mechanisms of DNA damage
4. Relationship between DNA damage and mutation
5. DNA repair mechanisms
6. Definition of homologous recombination, site-specific recombination and transposition
7. Mechanisms of homologous recombination, site-specific recombination and transposition
8. Types and structural characteristics of transposons