12.1 DNA Mutation and Classification

12.2 Detection and Isolation of Mutants

12.3 Bacterial DNA Repair

12.4 Transposable Elements

12.5 DNA Transformation in Bacteria

12.6 Bacteria Conjugation

12.7 Transduction in Bacteria

厦门大学
生|命|科|学|学|院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

MICROBIOLOGY

Chapter 12

12.1 DNA Mutation and Classification
DNA突变和分类

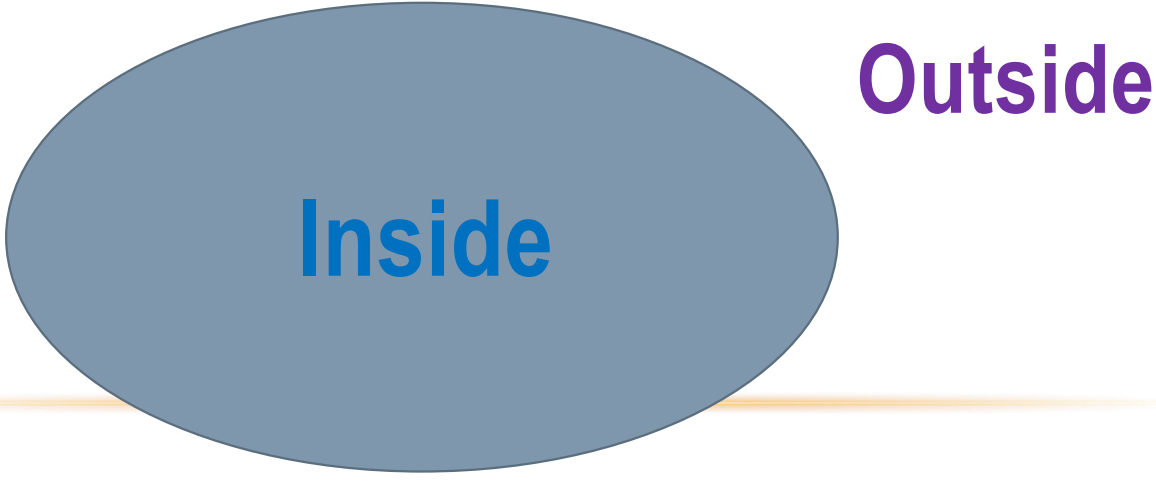
Genetic Information in nucleotide sequence of DNA

DNA Mutations

- Stable and heritable changes in sequence of bases in DNA
  - Changes in sequence of bases in DNA
  - Stable and heritable

Mutation Source

- Spontaneous mutation 自发产生
- Induced 诱变产生

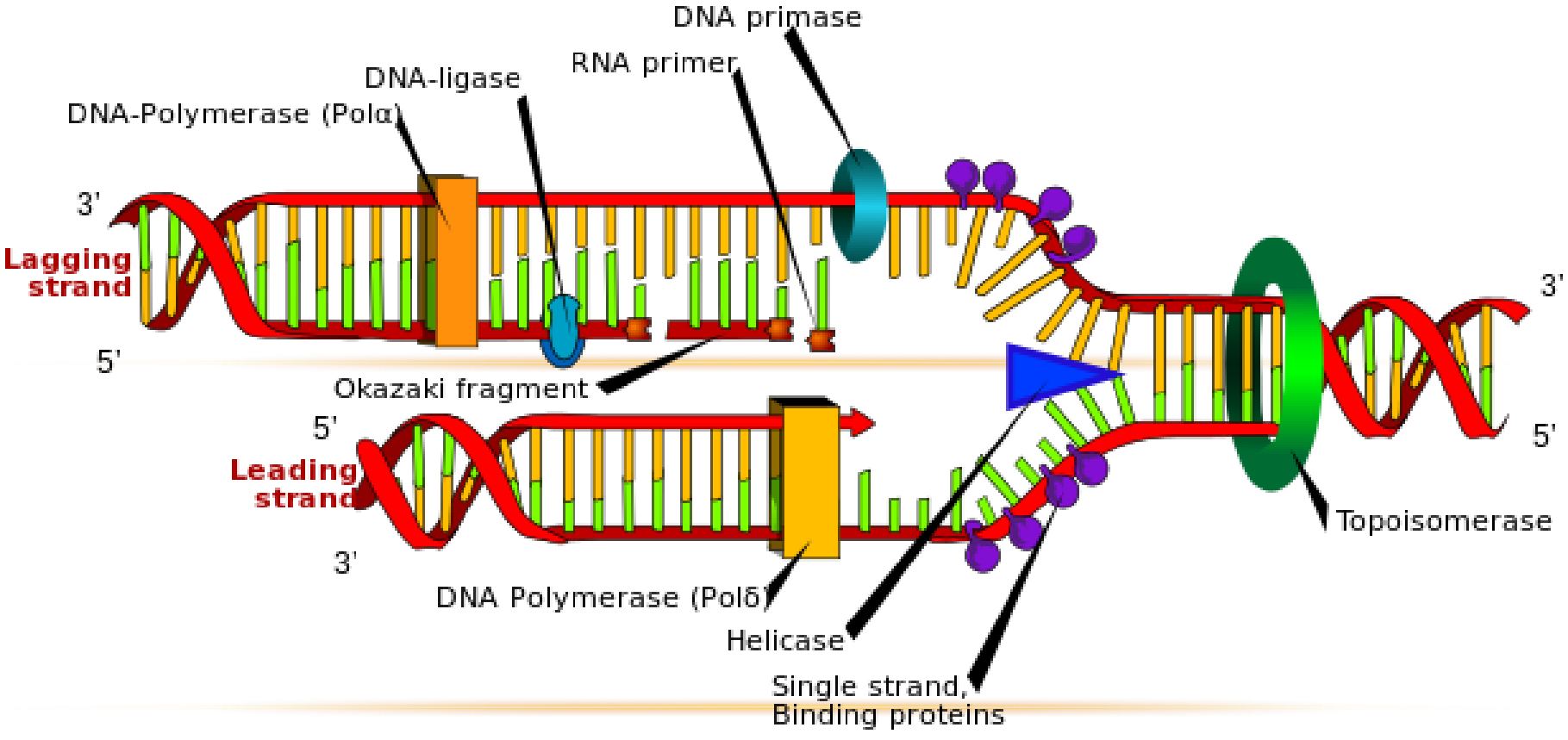


Spontaneous mutations 自发突变

- May result from errors in DNA replication
  - Transition and transversion mutations
  - insertion or deletion of nucleotides

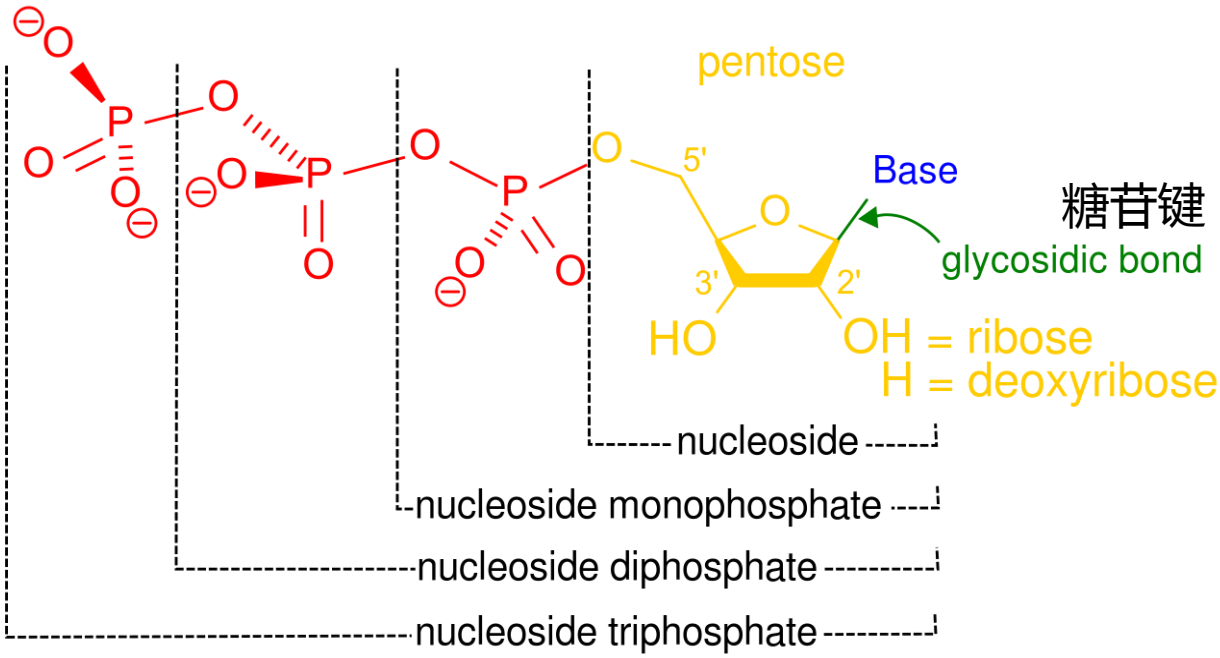
- May also result from the action of mobile genetic elements such as transposons

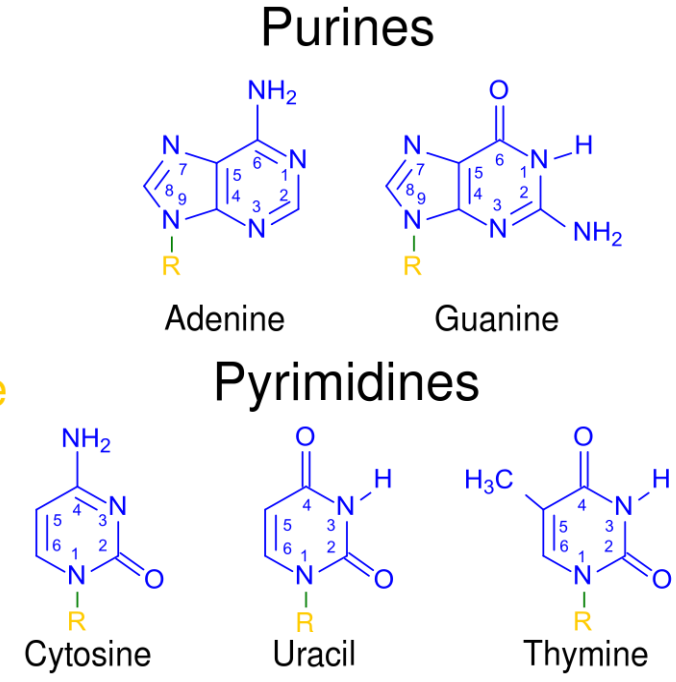
DNA replication



Base, Nucleoside, Nucleotide

碱基
核苷
核苷酸



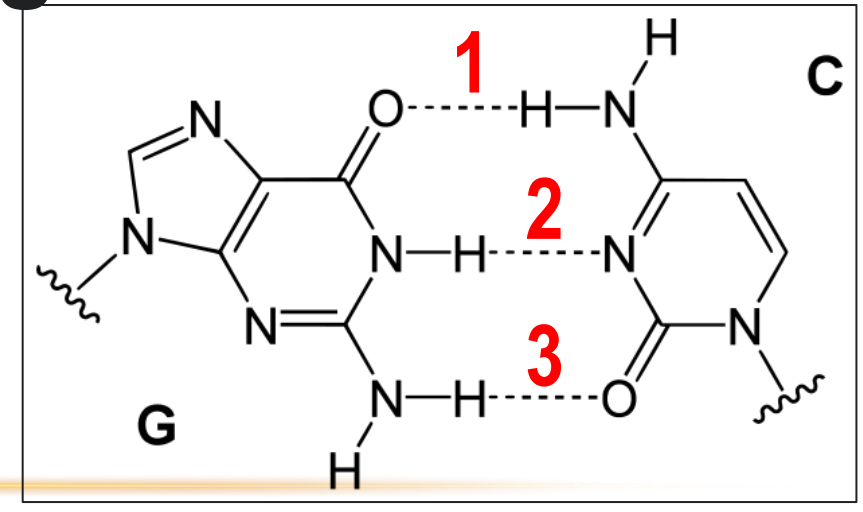


腺嘌呤 A---- 胸腺嘧啶 T

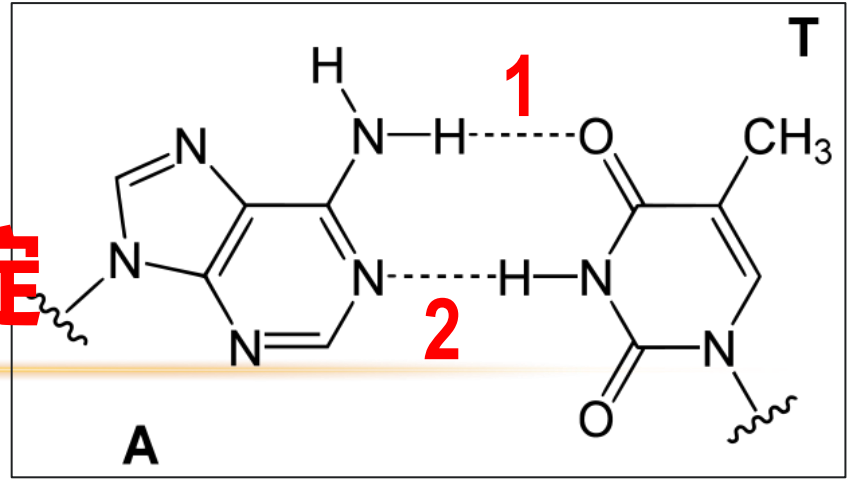
胞嘧啶 C---- 鸟嘌呤 G

Base Pairing of DNA

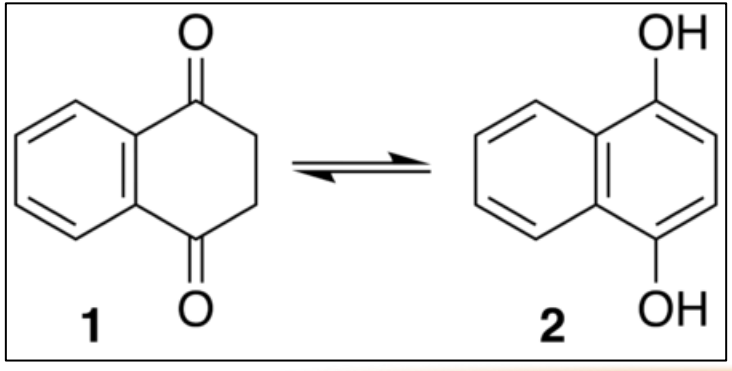
胞嘧啶 C ≡≡≡ G鸟嘌呤



腺嘌呤 A ≡≡≡T胸腺嘧啶



Keto–Enol Tautomerism 互变异构现象



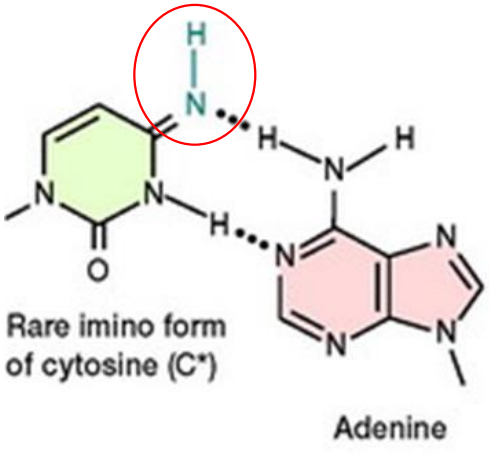
Keto form: 酮式

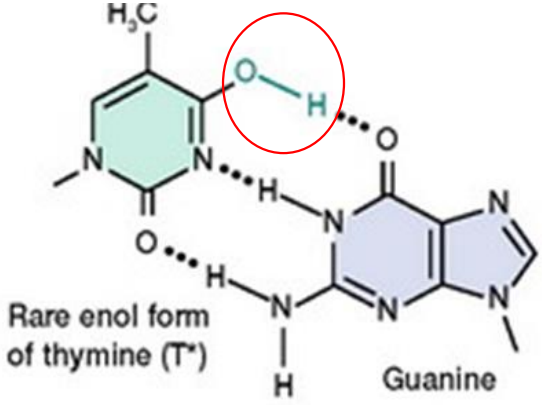
Enol form: 烯醇式形式

Imino form: 亚氨基形式

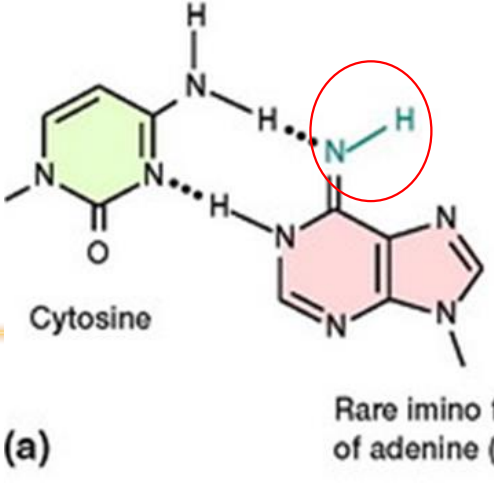
腺嘌呤 A---- 胸腺嘧啶 T

胞嘧啶 C---- 鸟嘌呤 G

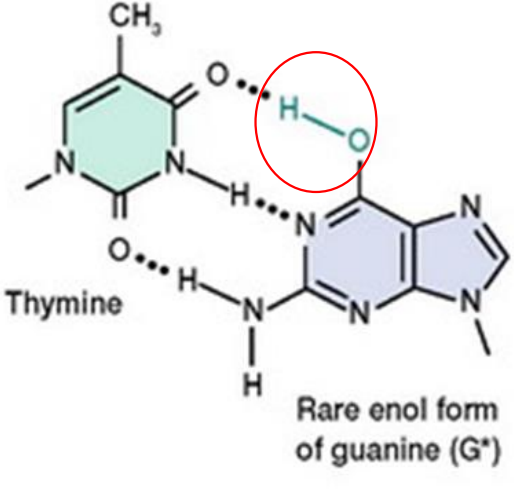


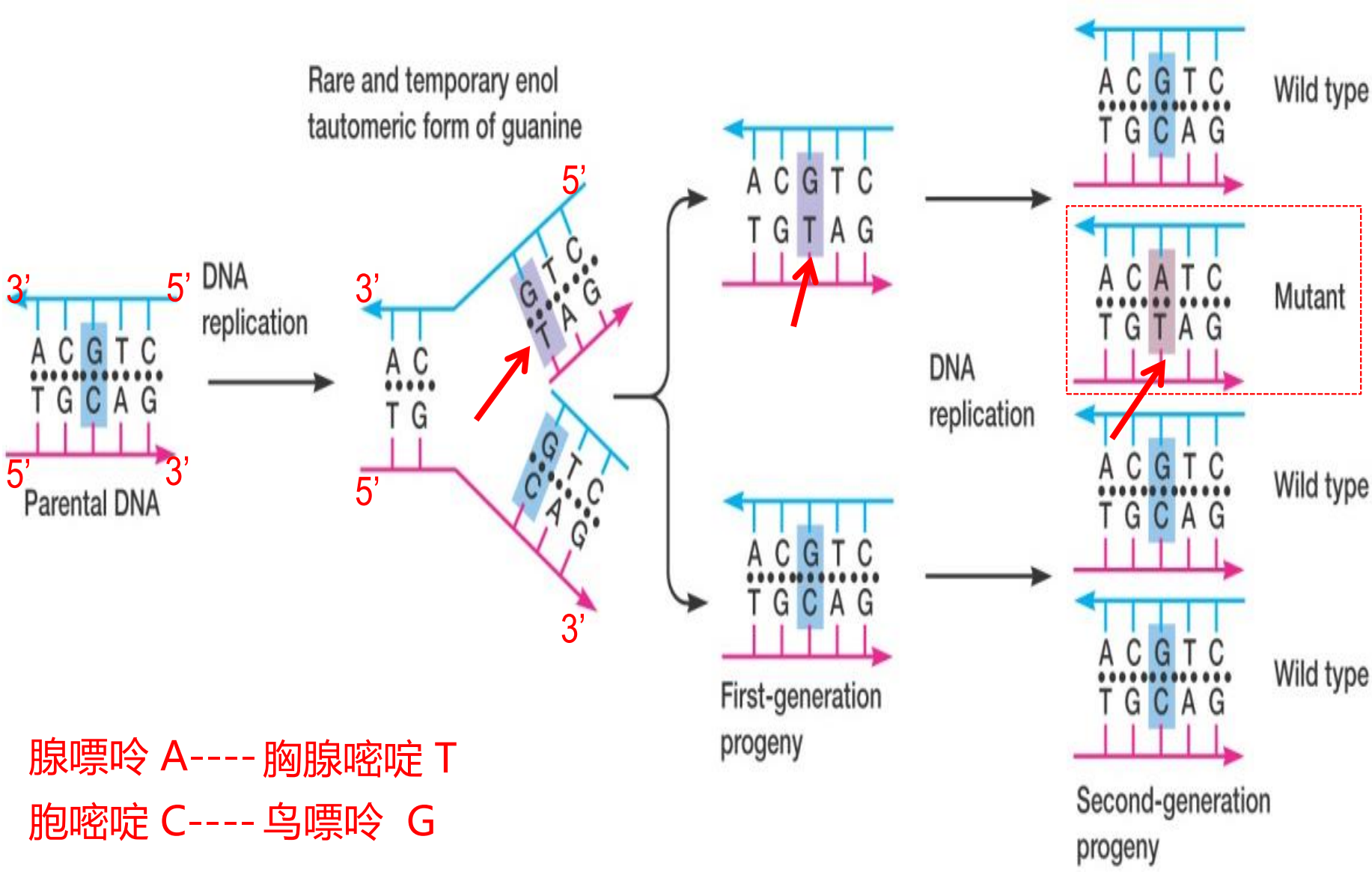


C----A



T----G

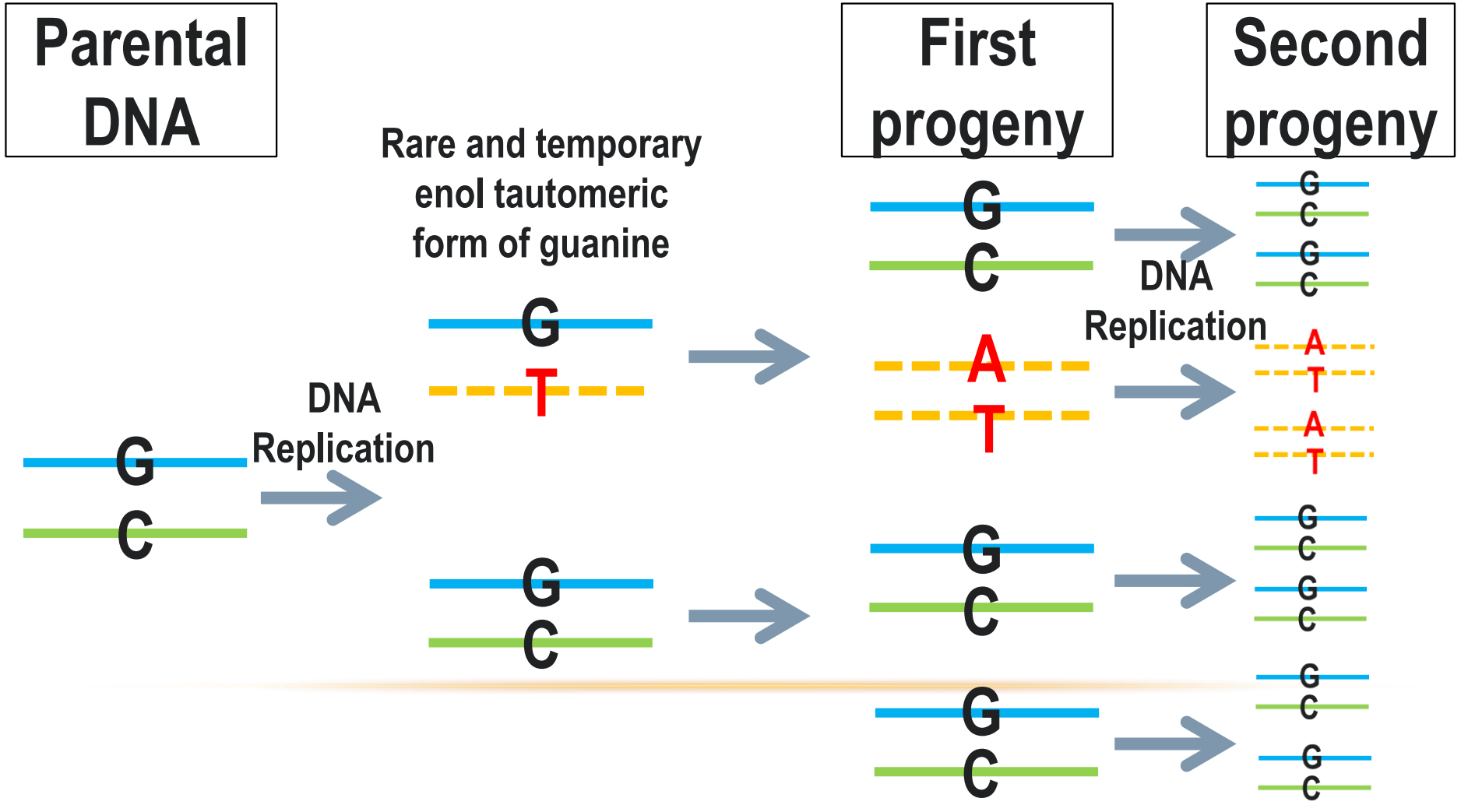




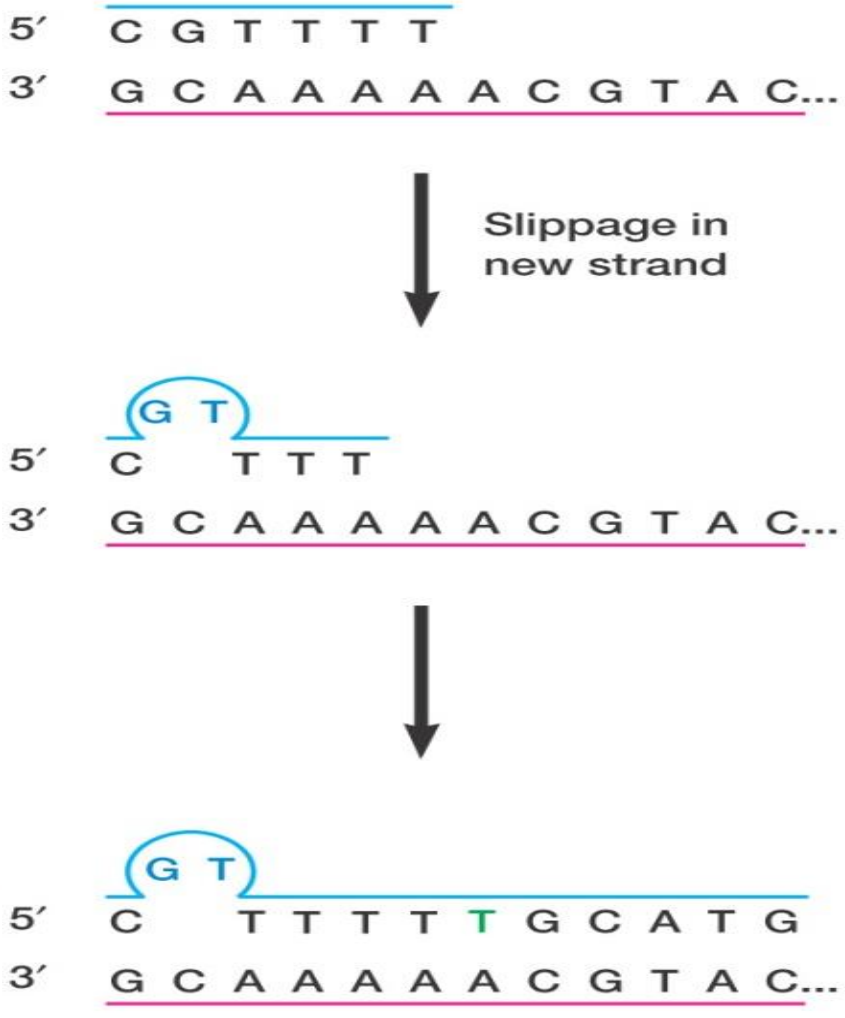
腺嘌呤 A---- 胸腺嘧啶 T

胞嘧啶 C---- 鸟嘌呤 G

Tautomerization and Transition Mutation

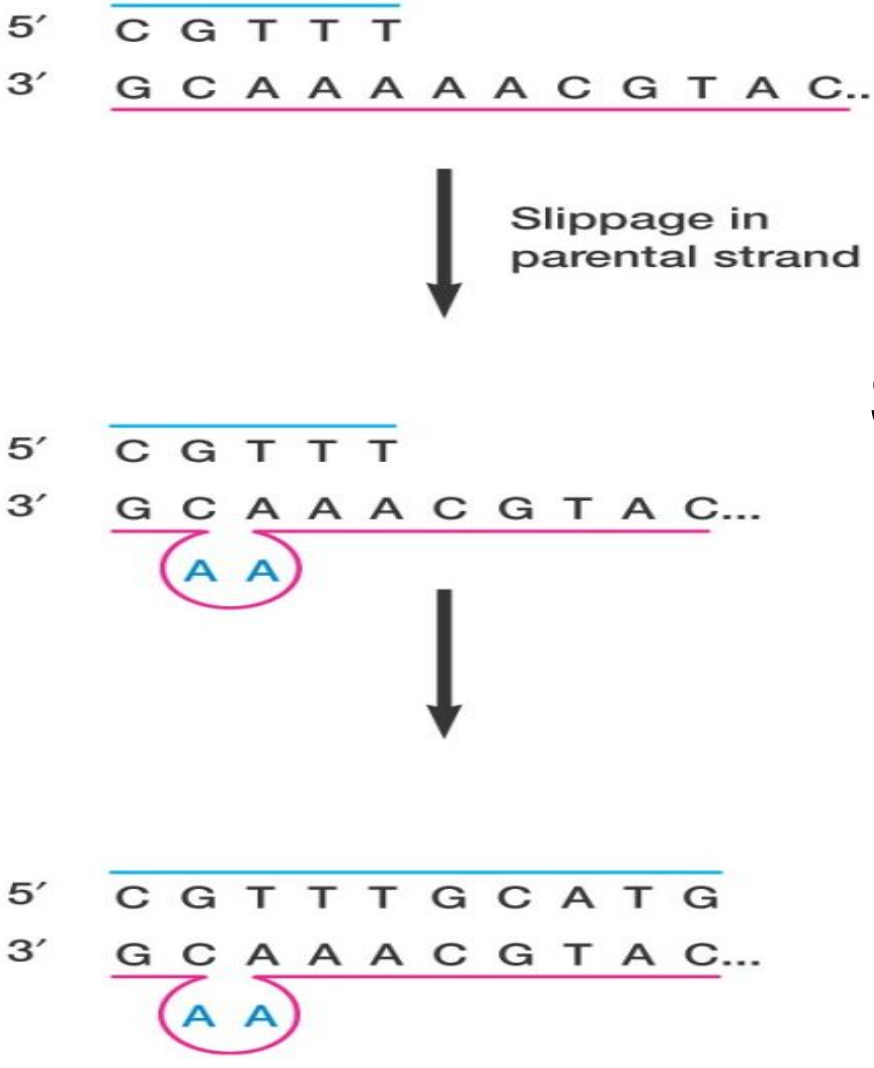


Insertion/Deletion by DNA Polymerase Slipping



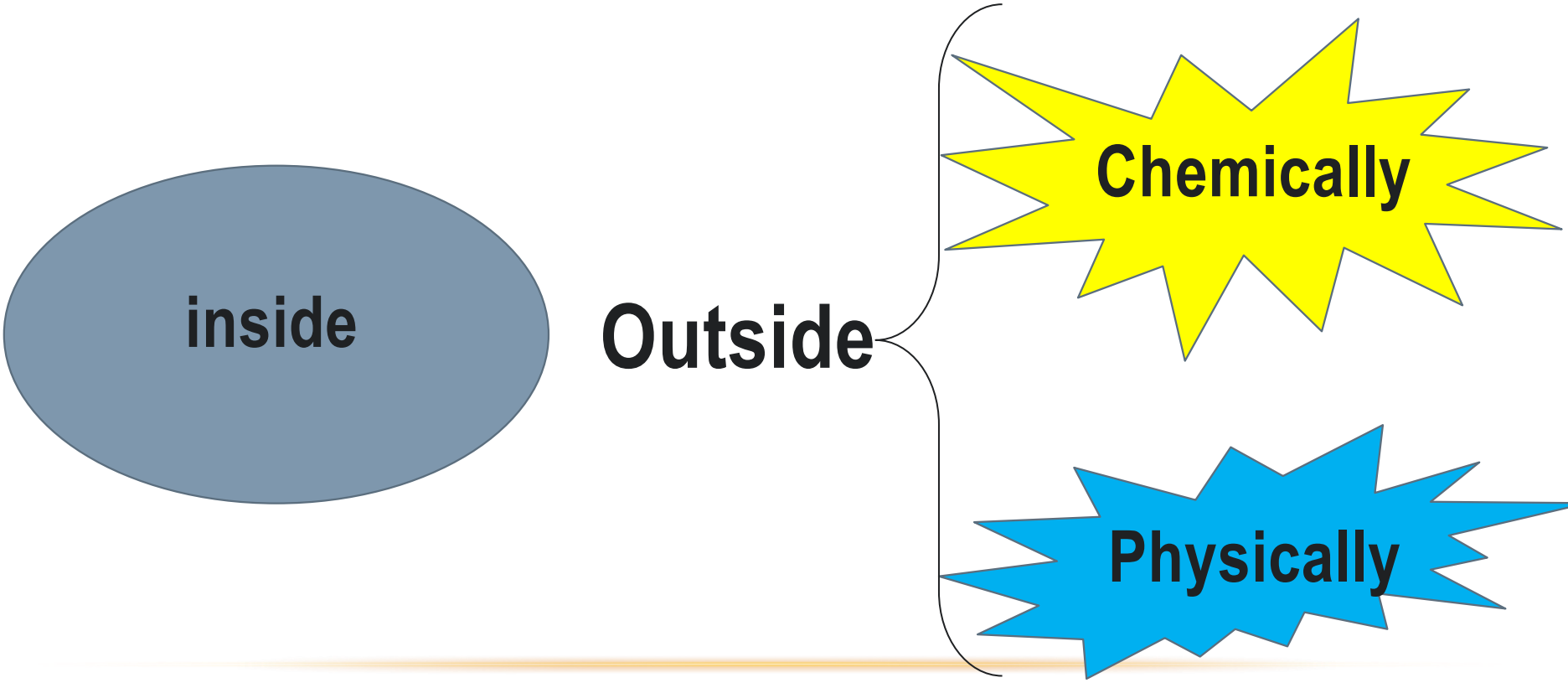
Slippage in new strand leading to an insertion

Insertion/Deletion by DNA Polymerase Slipping



Slippage in parental strand leading to an deletion

Induced Mutations 诱变

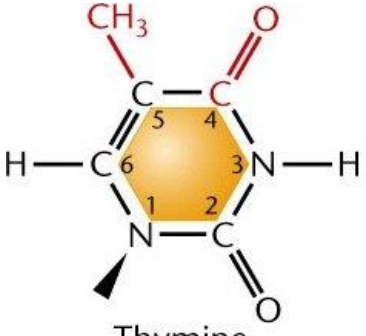


Chemically Induced Mutations

Caused by agents that directly damage DNA

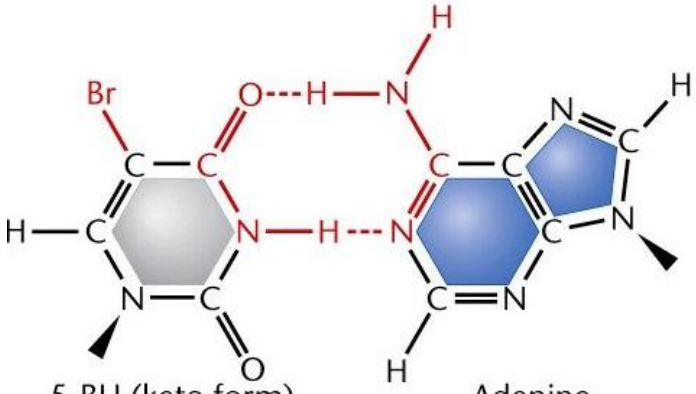
- Base analogs 碱基类似物
  - structurally similar to normal nitrogenous bases
  - mistakes occur when they are incorporated into growing polynucleotide chain
- DNA modifying agents
  - Mutagen change a base's structure and alter its base paring characteristics.
- Intercalating agents 插入剂
  - Distort DNA to induce single nucleotide pair insertions and deletions

Mutations induced by 5'-Bromouracil 溴尿嘧啶



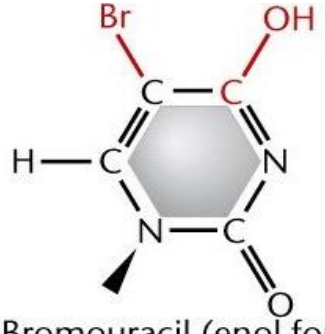
Thymine

5-Bromouracil (keto form)



5-BU (keto form) Adenine

酮式



5-Bromouracil (enol form)

5-BU (enol form) Guanine

烯醇式

腺嘌呤 A---- 胸腺嘧啶 T

胞嘧啶 C---- 鸟嘌呤 G

T------ A

Br------ A

Br'------ G

C ------ G

Mutations induced by methy-nitrosoguanidine 亚硝基胍

C-----G

↓

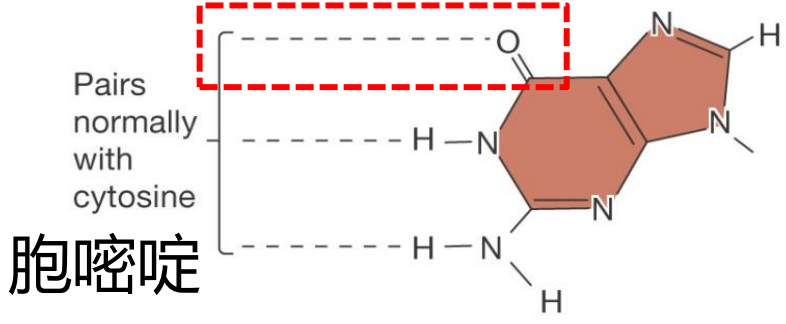
C-----G’

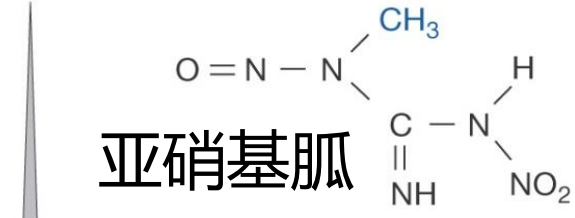
↓

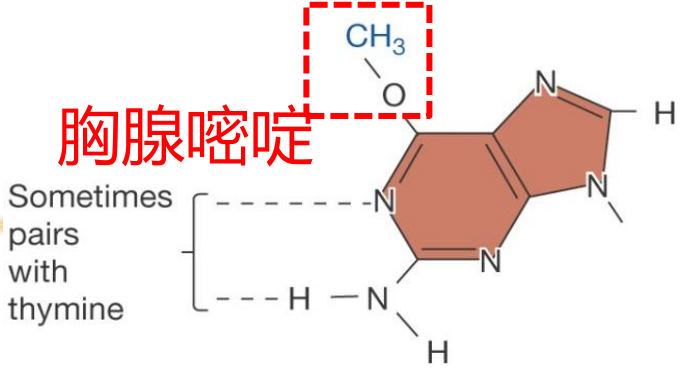
T-----G’

↓

T-----A







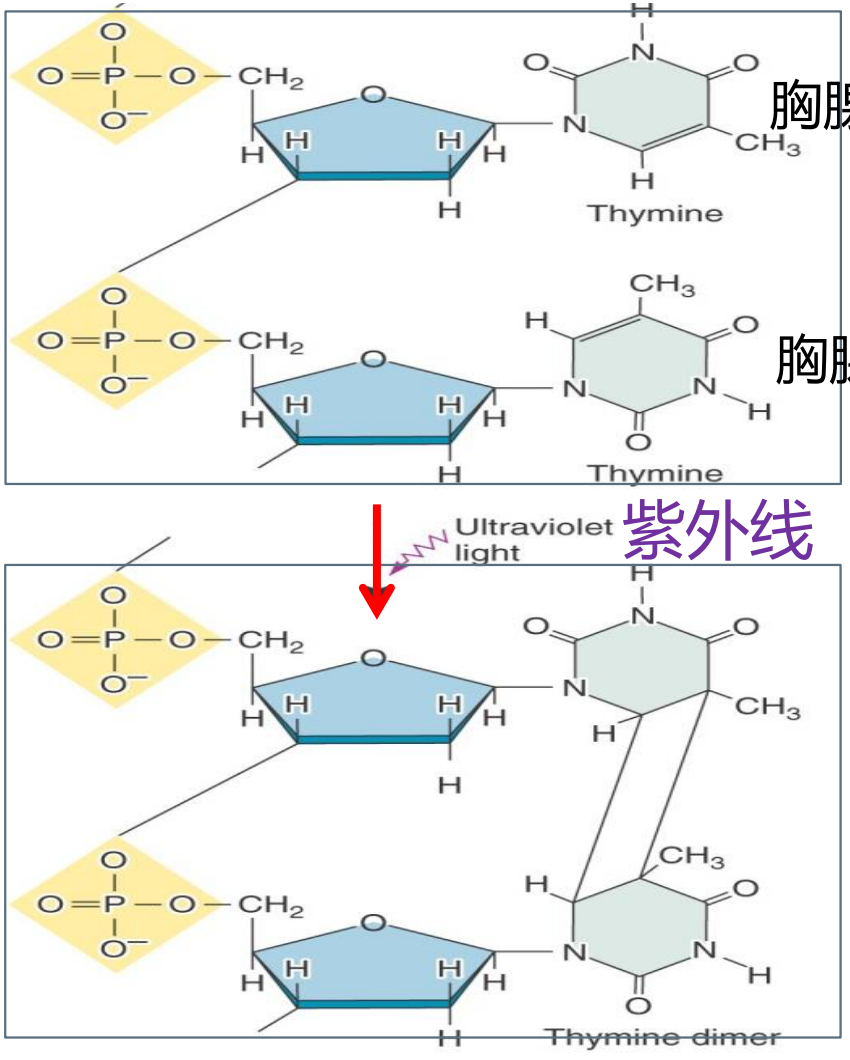
腺嘌呤 A---胸腺嘧啶 T

胞嘧啶 C---鸟嘌呤 G

Physically Induced mutations

- Ultraviolet radiation
- Ionizing radiation, such as X-rays and gamma rays

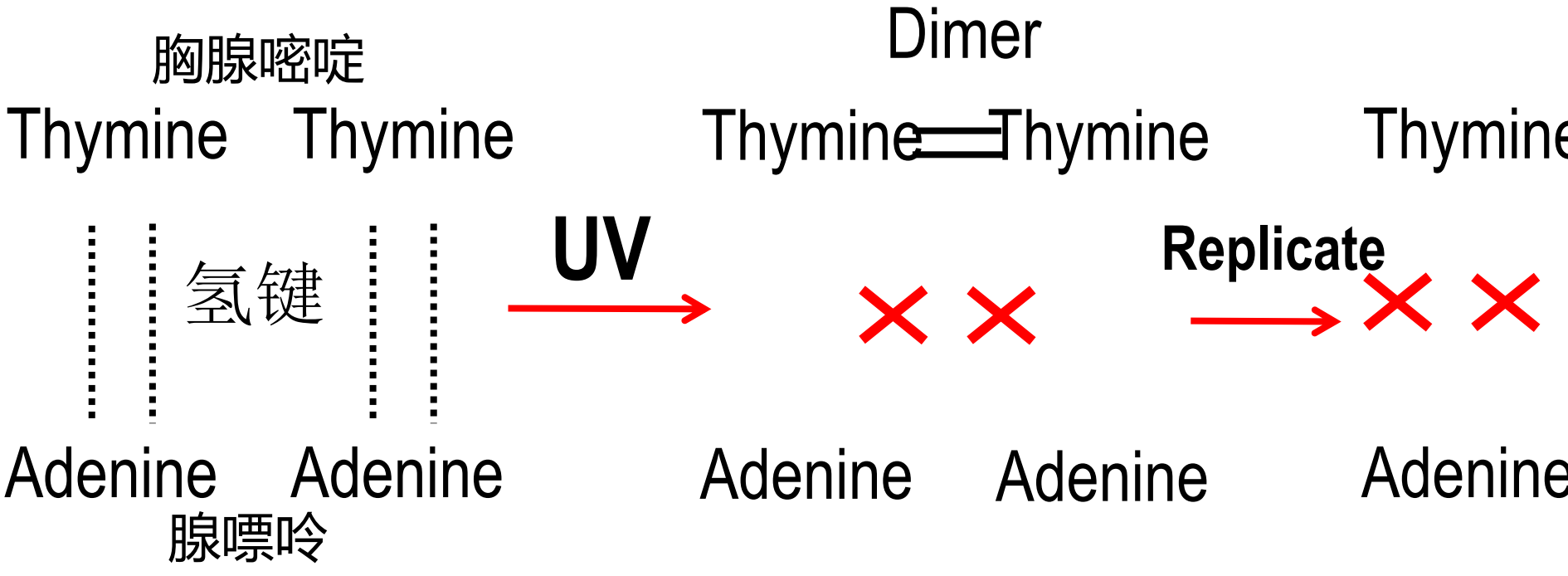
Thymine Dimer induced by UV Radiation



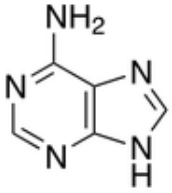
Thymine·····Adenine
胸腺嘧啶·····腺嘌呤

二聚体

Thymine Dimer induced by UV Radiation

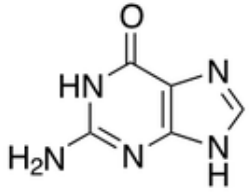


腺嘌呤



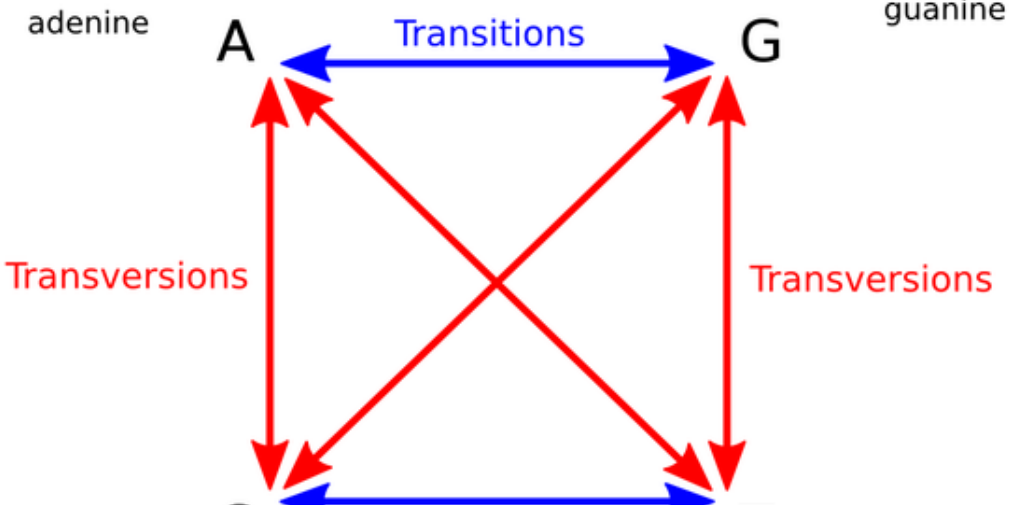
adenine

purines

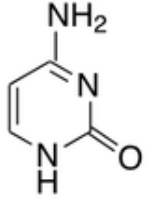


guanine

鸟嘌呤

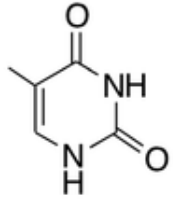


胞嘧啶



cytosine

pyrimidines

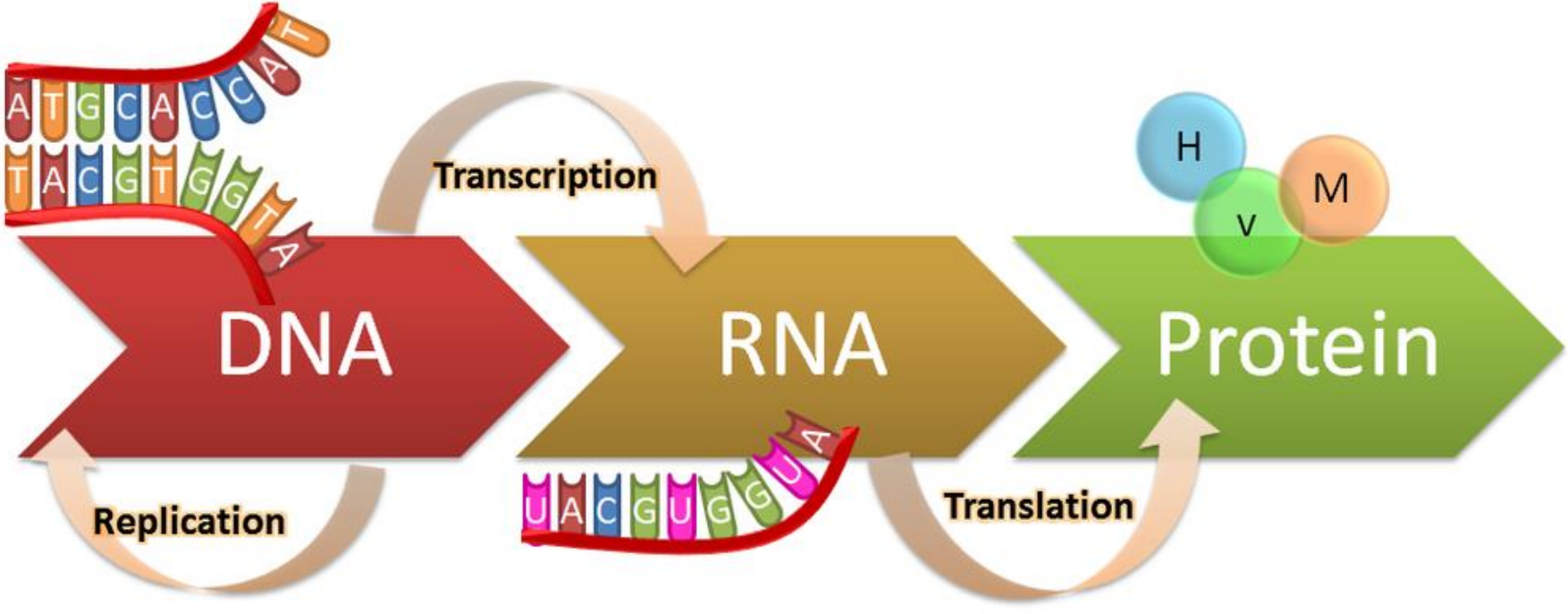


thymine

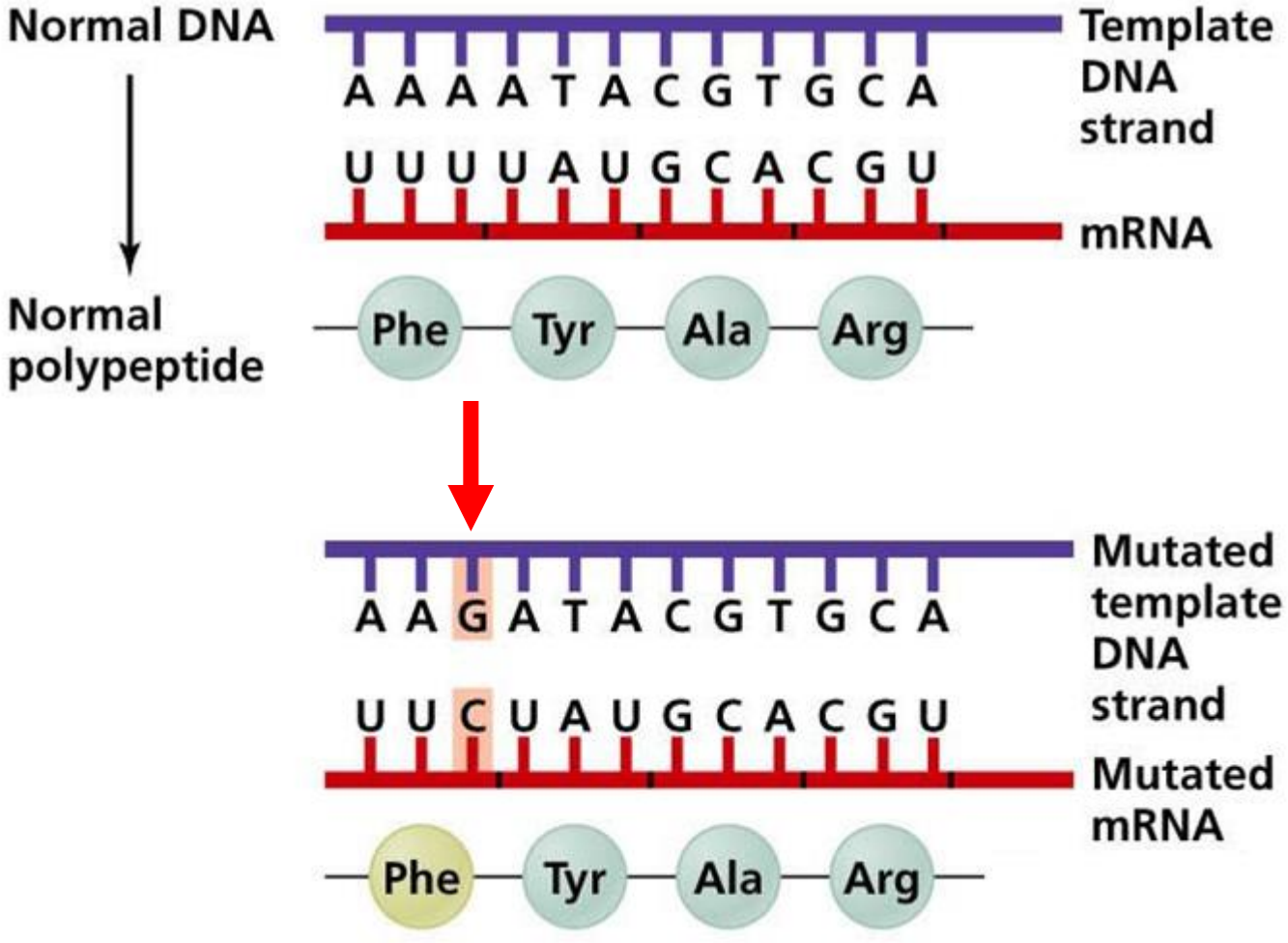
胸腺嘧啶

http://en.wikipedia.org/wiki/File:Transitions-transversions-v3.png

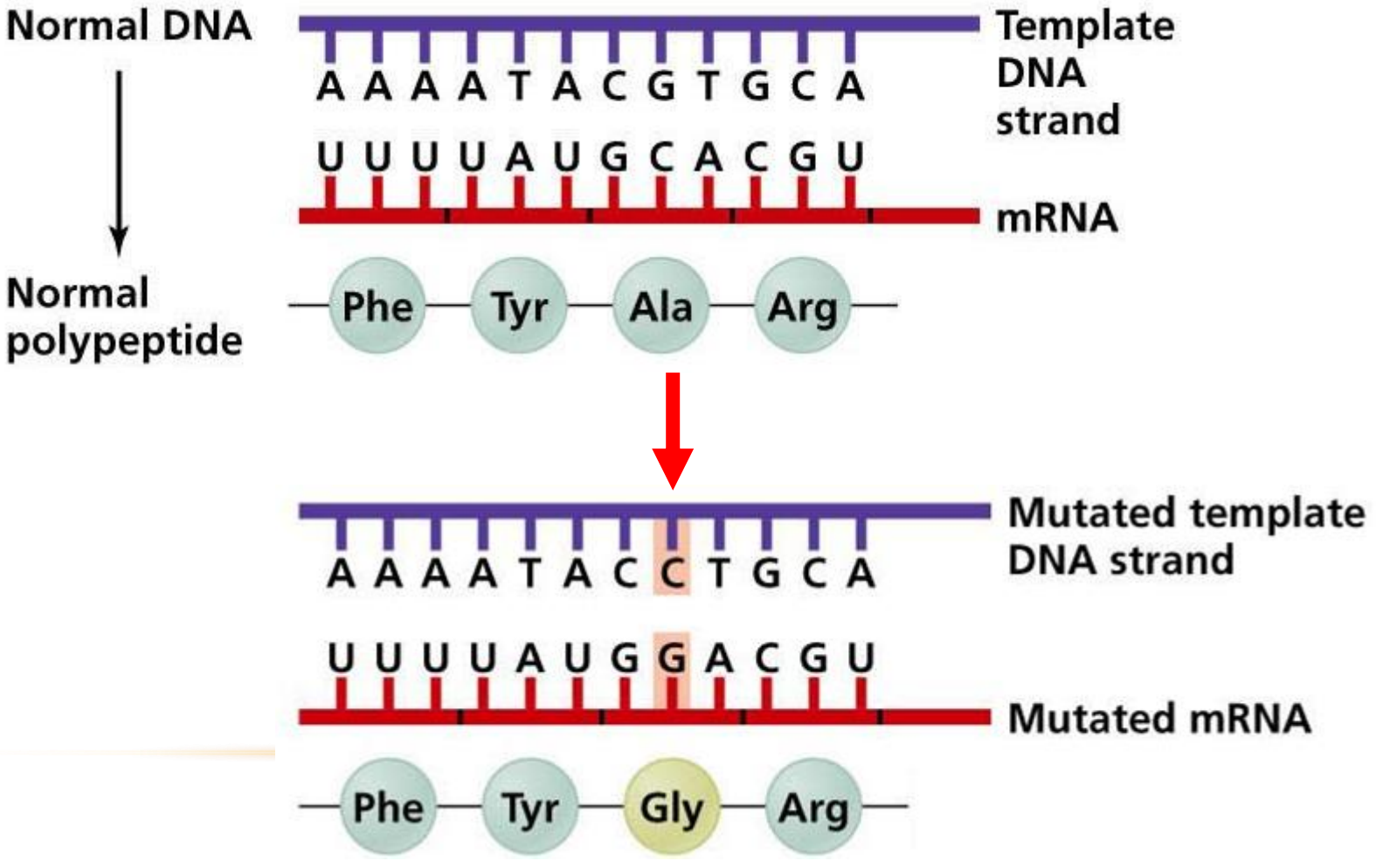
DNA mutation in Protein Coding Gene



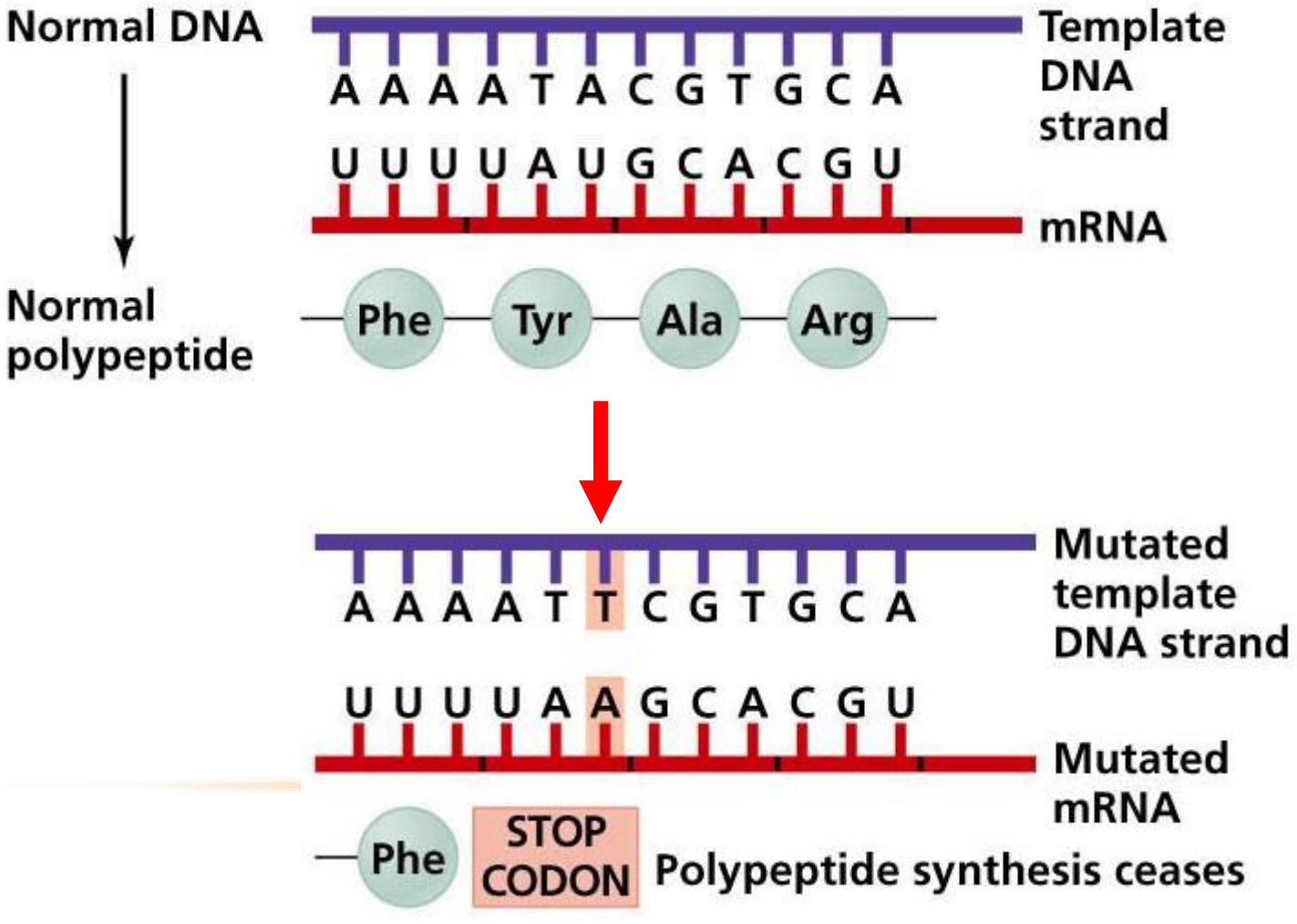
Silent Mutation: No change in amino acid sequence



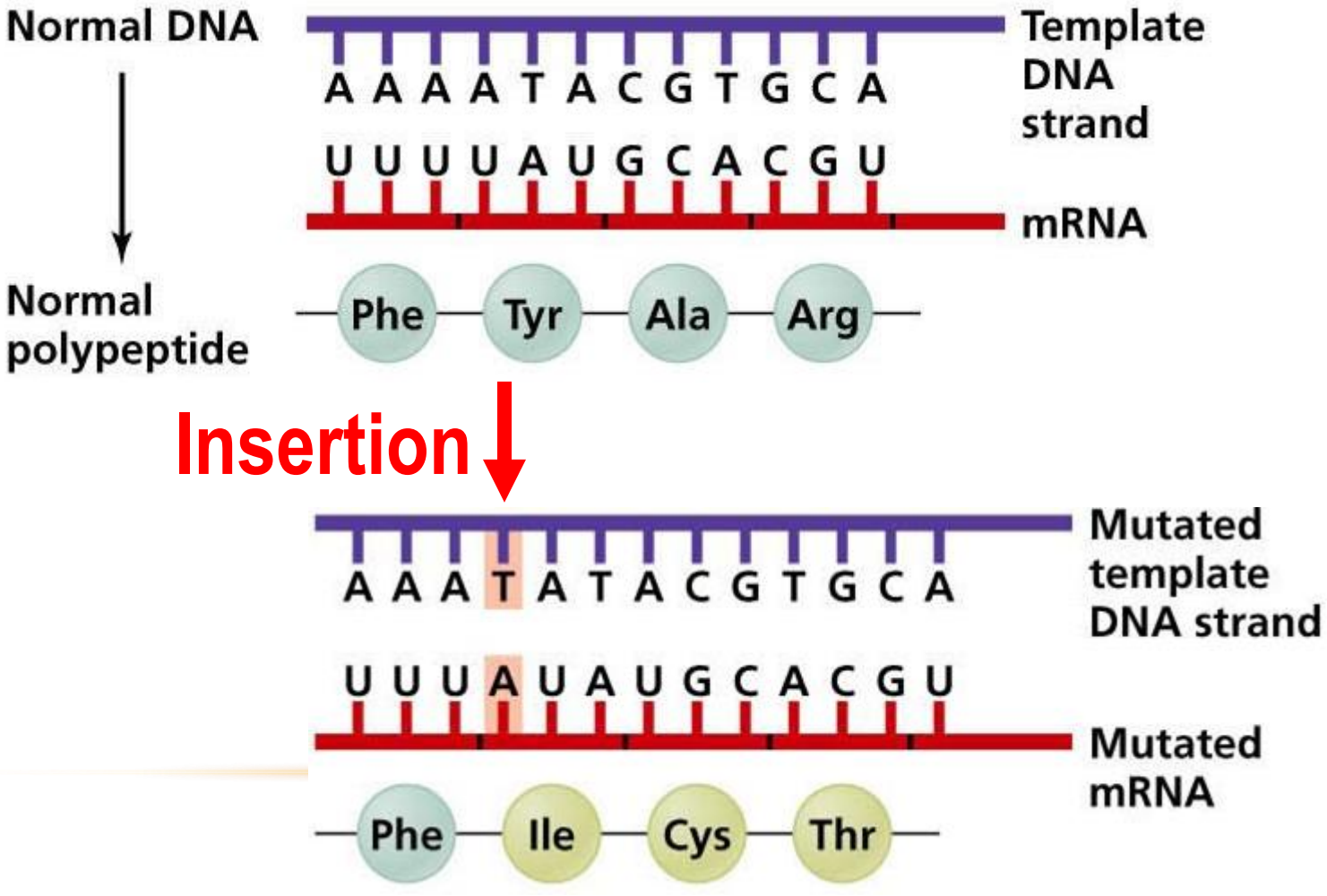
Missense Mutation: change in one amino acid(AA)



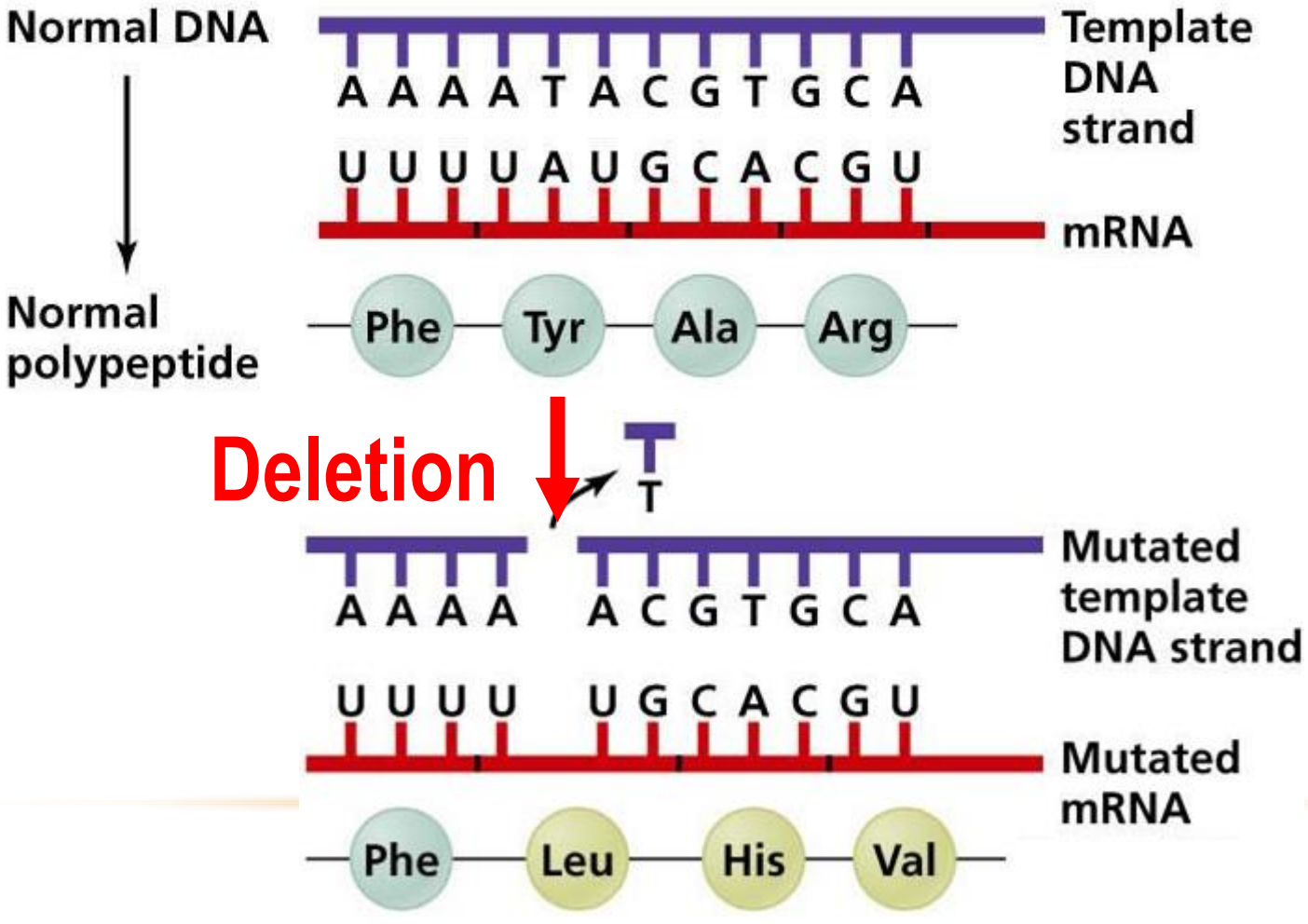
Nonsense Mutation: change to Stop Codon



Frameshift insertion: major change in AAs



Frameshift deletion: major change in AAs



Mutations In Protein-coding Gene

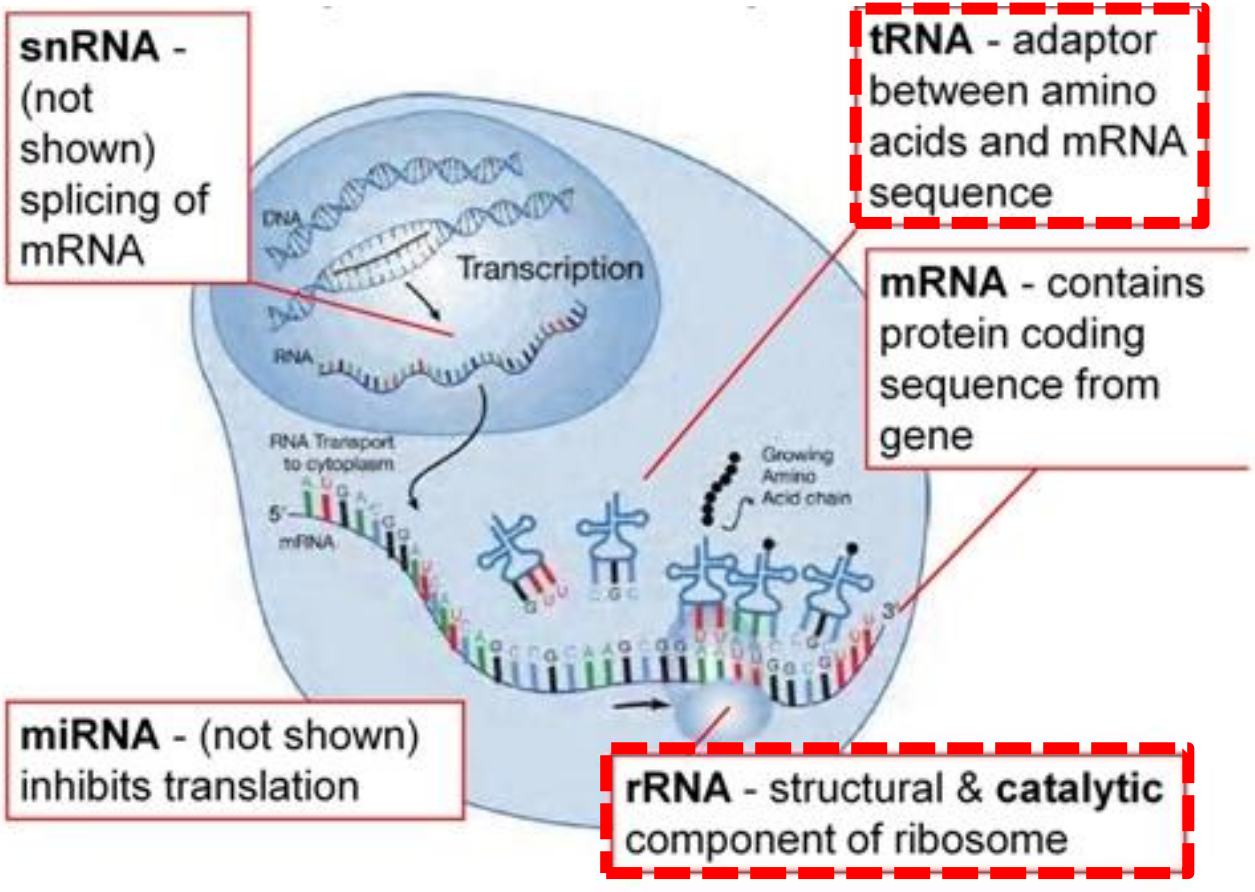
Type of Mutation	*Change in DNA*	*Example*
Forward Mutations		
None	None	甲硫氨酸 苏氨酸 丝氨酸 脯氨酸 赖氨酸 甘氨酸 5′-A-T-G-A-C-C-T-C-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Pro - Lys - Gly
Silent 沉默突变	Base substitution	5′-A-T-G-A-C-A-T-C-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Pro - Lys - Gly
Missense 错义突变	Base substitution	5′-A-T-G-A-C-C-T-G-C-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Cys - Pro - Lys - Gly
Nonsense 无义突变		半胱氨酸 5′-A-T-G-A-C-C-T-C-C-C-C-G-T-A-A-G-G-G-3′ Met - Thr - Ser - Pro - STOP!
Frameshift 移码突变	Insertion/deletion	5′-A-T-G-A-C-C-T-C-C-G-C-C-G-A-A-A-G-G-G-3′ Met - Thr - Ser - Ala - Glu - Arg

Effect caused by Mutation

- Mutations in protein-coding gene
- Mutations in noncoding RNA gene
- Mutations in transcription regulatory region

- **Mutations in tRNA and rRNA genes**

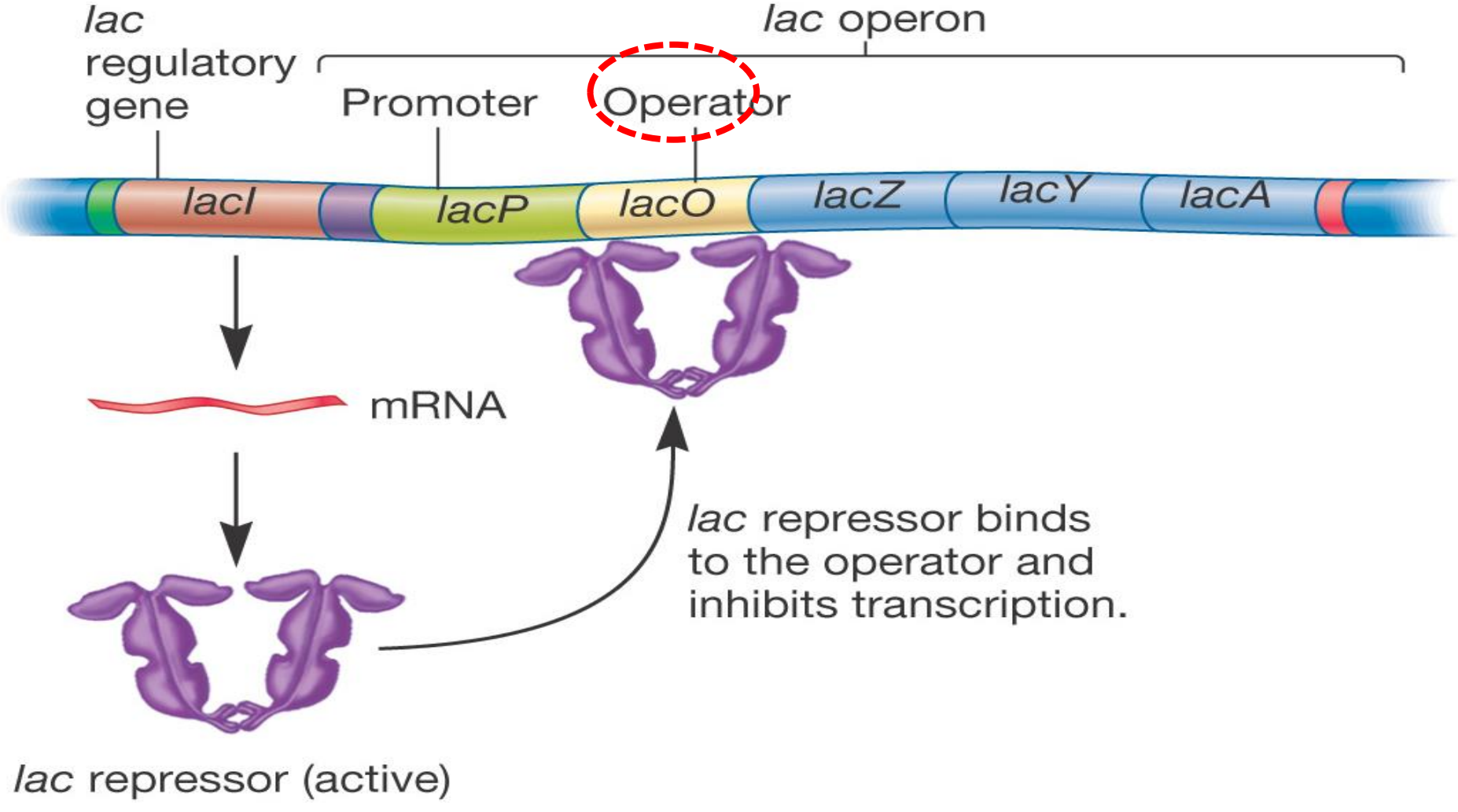
Protein synthesis is affected/disrupted



Mutations in Regulatory Sequences

- *lac* operon mutants
  - many of these mutations map in the operator site and produce altered operator sequences not recognized by repressor
  - operon is turned on and constitutively transcribed, causing β-galactosidase synthesized

Mutations in Regulatory Sequences



厦门大学
生|命|科|学|学|院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

MICROBIOLOGY

Chapter 12

12.2 Detection and Isolation of Mutants
突变株的检测和分离

1850s “Invisible factor”

1928 Bacteria transformation

1943 DNA is genetic material

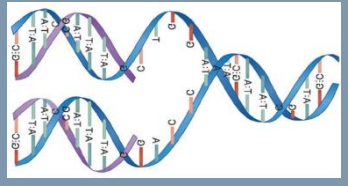
1953 Double Helix Structure of DNA strand

In 1950s, most biologists thought:

Genes are Stable Units,
DNA is stable in nucleotide order/sequence.

DNA突变的来源：自发突变

细菌细胞

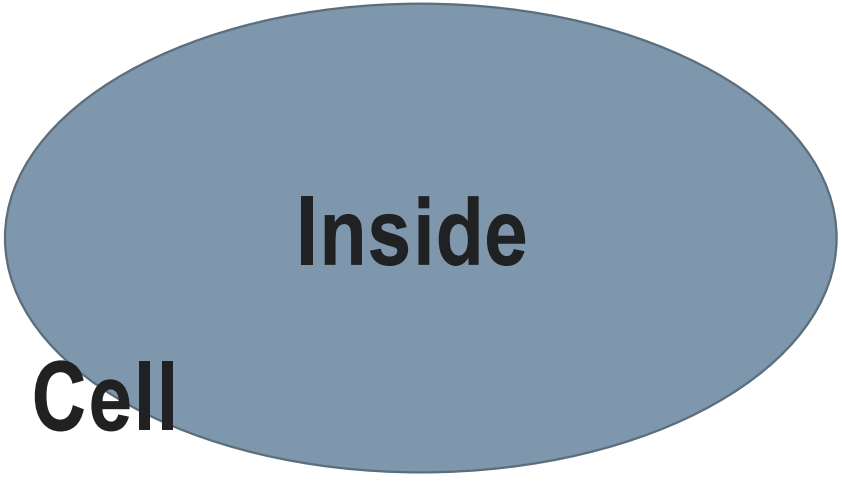


基因组DNA复制过程中的自身错误

突变率：

$$\frac{\text{1 mutant cell}}{10^{7} \text{ to } 10^{11} \text{ normal cells}}$$

Induced Mutations（诱变）

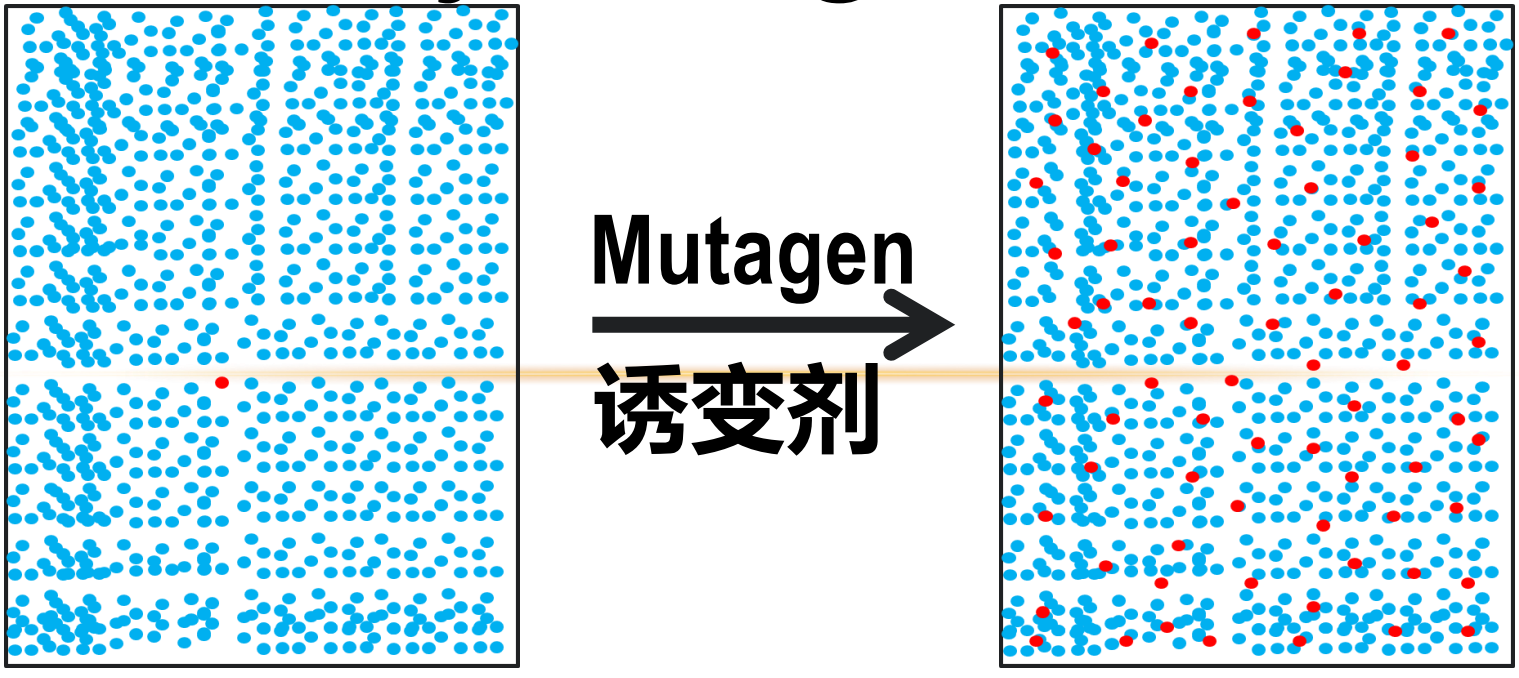


Outside

Chemically

Physically

Increase mutation frequency by mutagen



1 mutant

10^7 to 10^{11} cells

1 mutant

10^3 to 10^6 cells

Phenotype change cause by DNA mutation

☐ Morphological mutations

- Colony shape, size, and color
- Bacterial morphology, cell structure and organelle

☐ Biochemical mutations

Bacterial biochemistry, catabolism and anabolism

- Auxotrophic mutant 营养缺陷型突变
- Resistant mutant 抗性突变

Cultured *E.coli* Colony in Agar Plate

Bacteria dish

Colony

Phenotype change cause by DNA mutation

☐ Morphological mutations

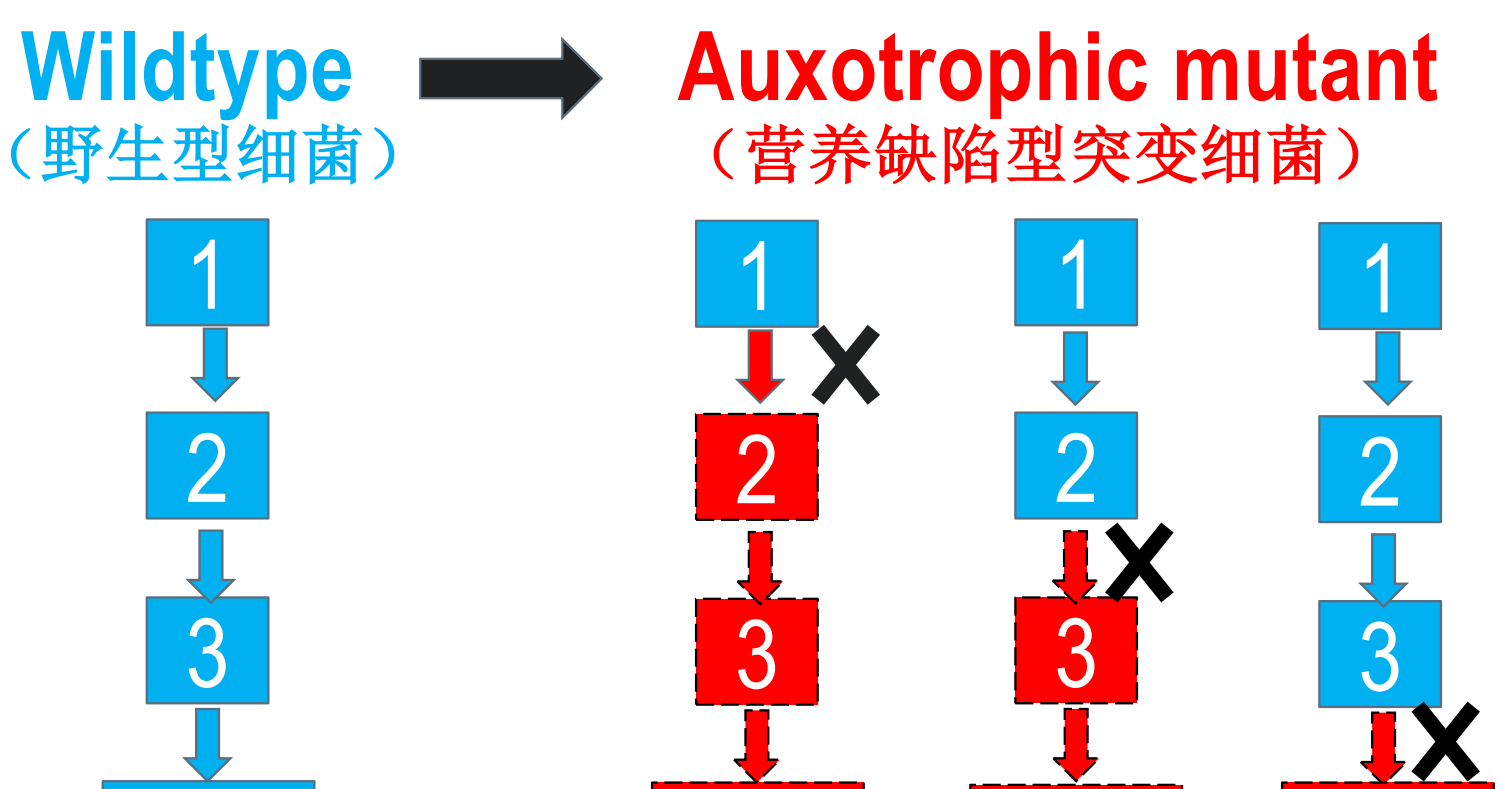
- Colony shape, size, and color
- Bacterial morphology, cell structure and organelle

☐ Biochemical mutations

Bacterial biochemistry, catabolism and anabolism

- **Auxotrophic mutant** 营养缺陷型突变
- **Resistant mutant** 抗性突变

Auxotrophic Mutant



必须营养组分：氨基酸或核苷酸 *Lys-*：赖氨酸营养缺陷型

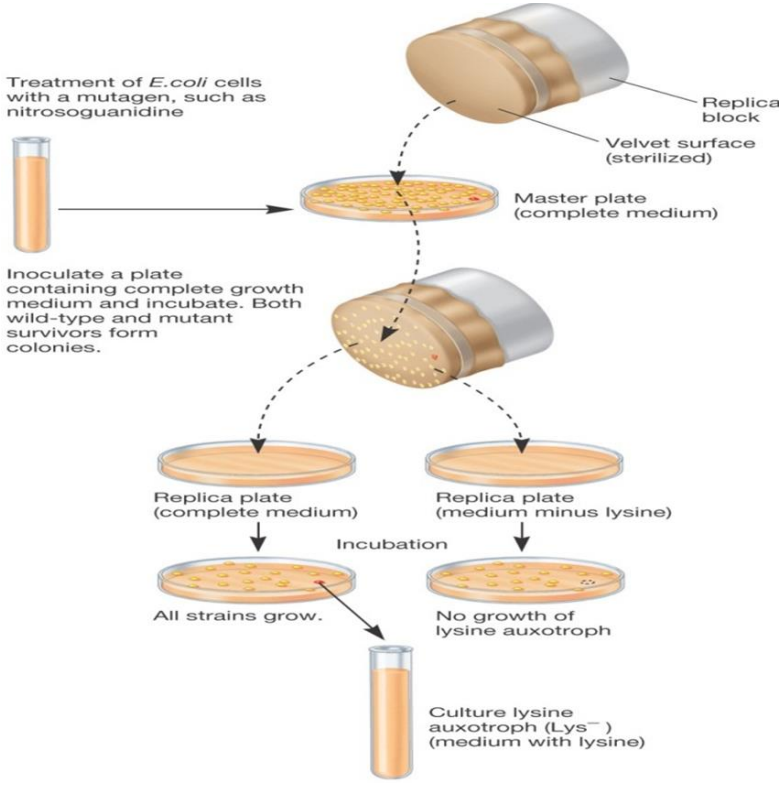
Auxotrophic Mutant

举例：*Lys-*/赖氨酸营养缺陷型突变细菌

- Wildtype is capable to synthesize the lysine, which is essential for growth.
- *Lys-* is unable to synthesize lysine
- *Lys-* is unable to grow on medium lacking lysine.
- Lys- grows only when exogenous lysine is provided

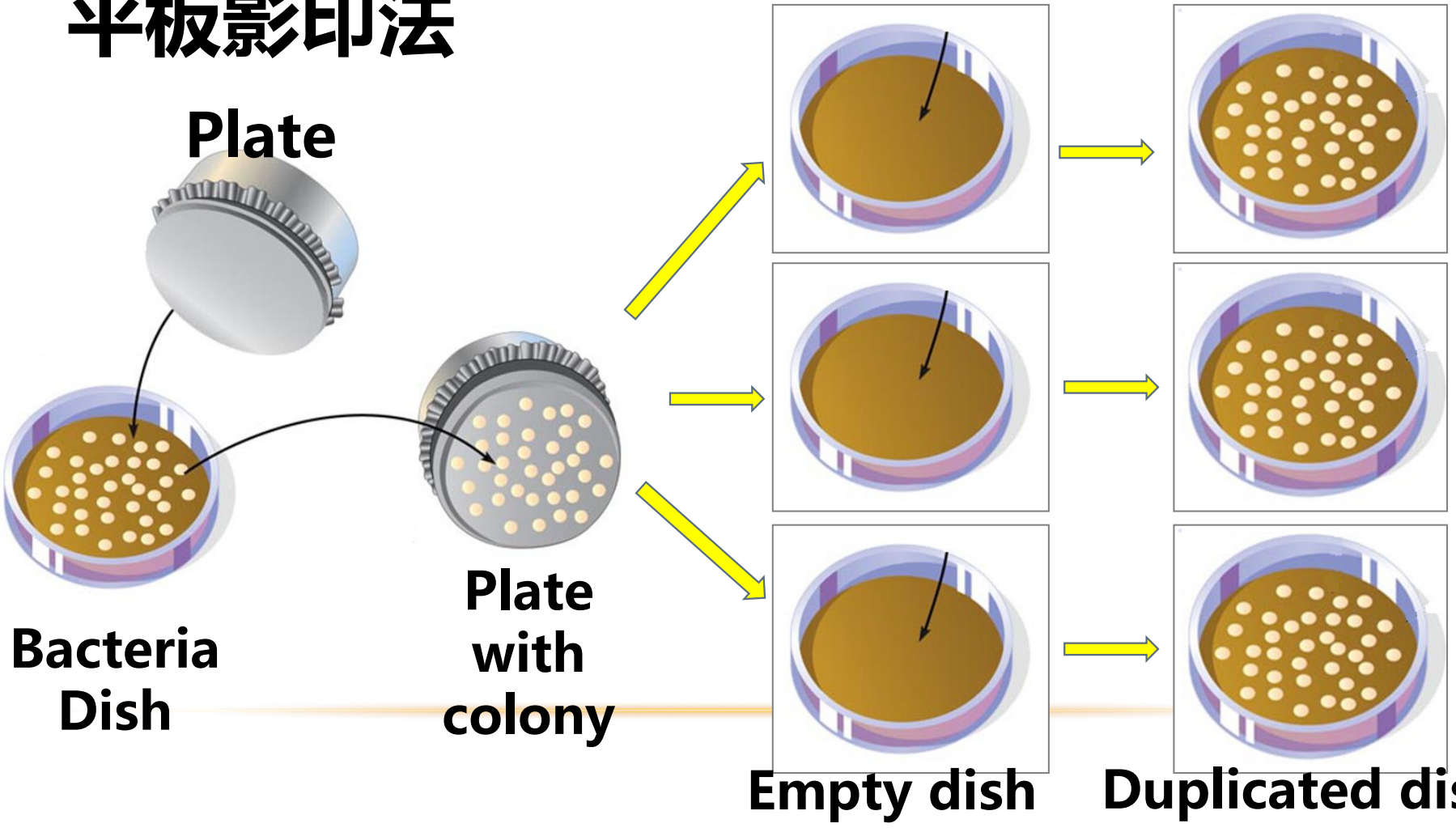
How to isolate the mutant bacteria “invisible” ?

Replica Plating 平板影印法

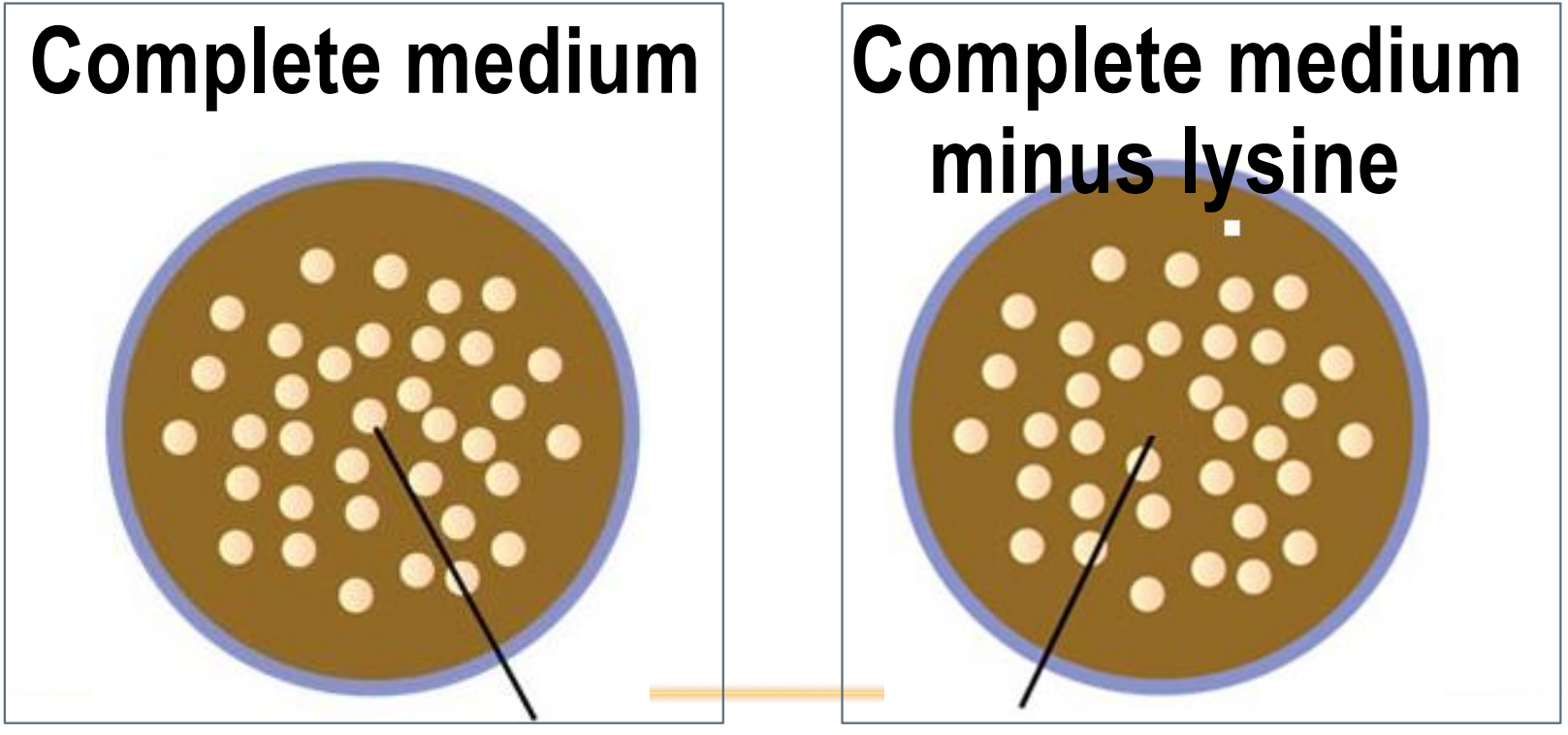


Lederberg, Joshua; **Lederberg, Esther** (1952)."Replica plating and indirect selection of bacterial mutants". Journal of Bacteriology 63(3): 399–406. PMID 14927572.

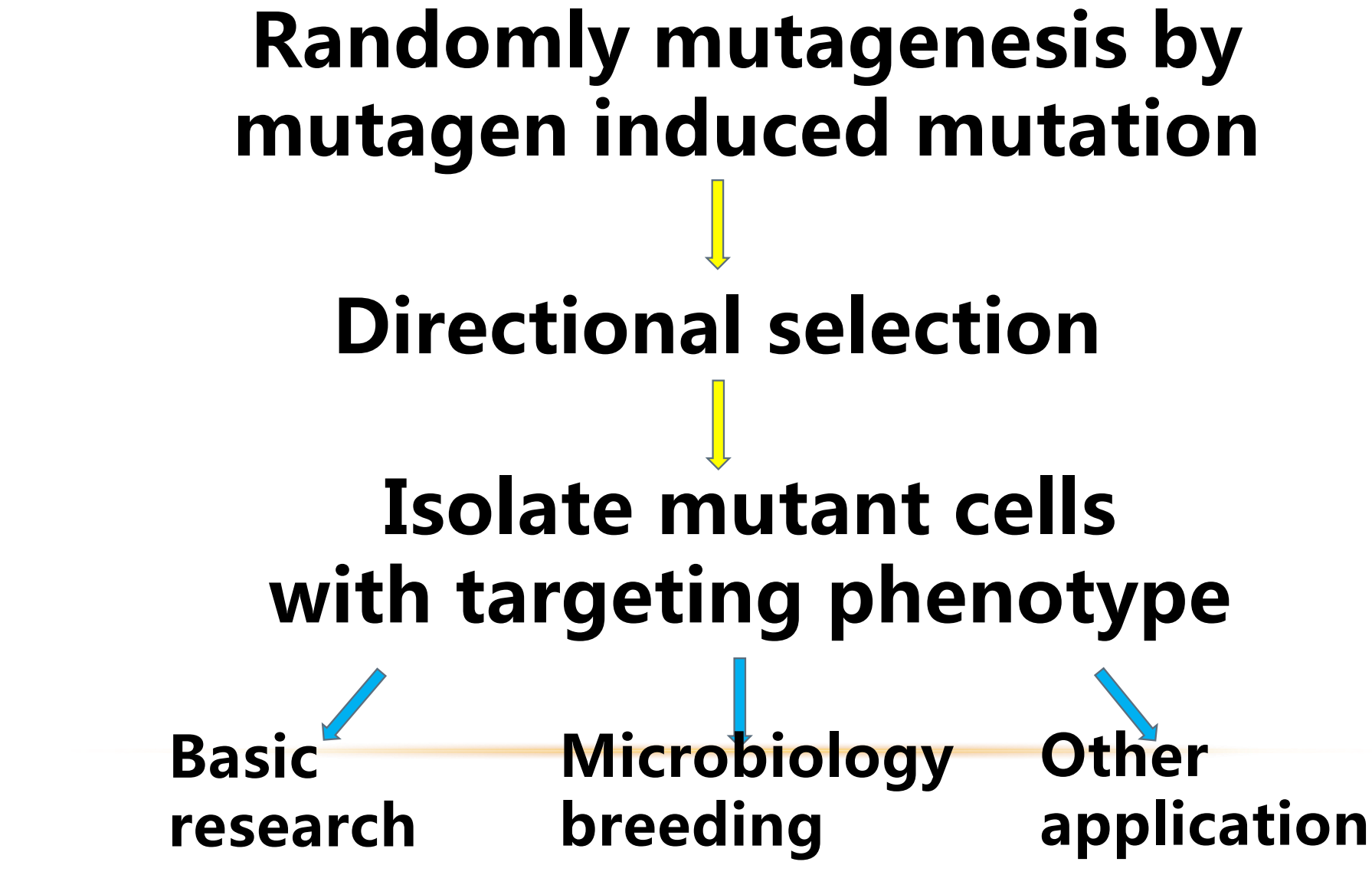
Replica Plating: Copying Bacteria Colony
平板影印法



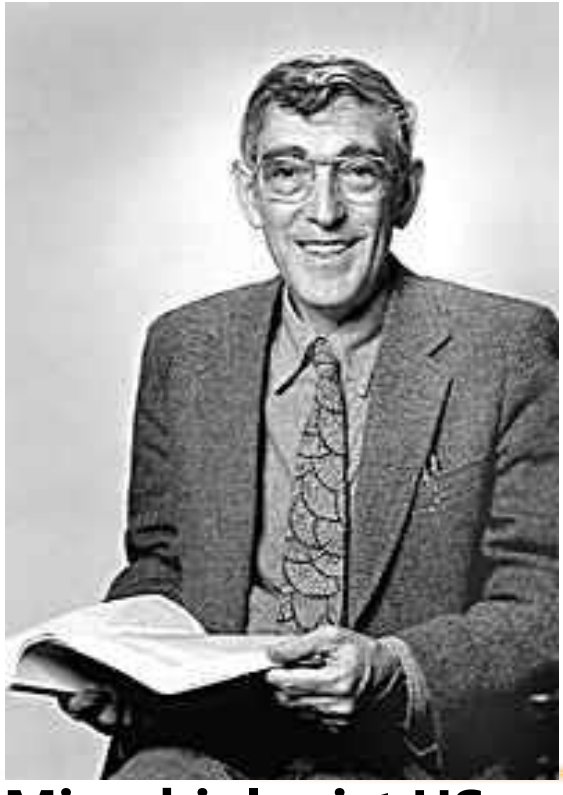
Directional screening to isolate invisible mutant cell



***Lys*-auxotrophic Mutant**


Randomly mutagenesis by mutagen induced mutation
Directional selection
Isolate mutant cells with targeting phenotype
Basic research
Microbiology breeding
Other application

Bruce Ames: Inventor of “The Ames Test”



Microbiologist US

1928---now

https://en.wikipedia.org/wiki/Bruce_Ames

https://en.wikipedia.org/wiki/Ames_test

1973年---美国科学院院院刊*PNAS*

An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens

一种改进的细菌测试系统，
用于化学诱变剂和致癌物的判定和分类

Key

Ames Test

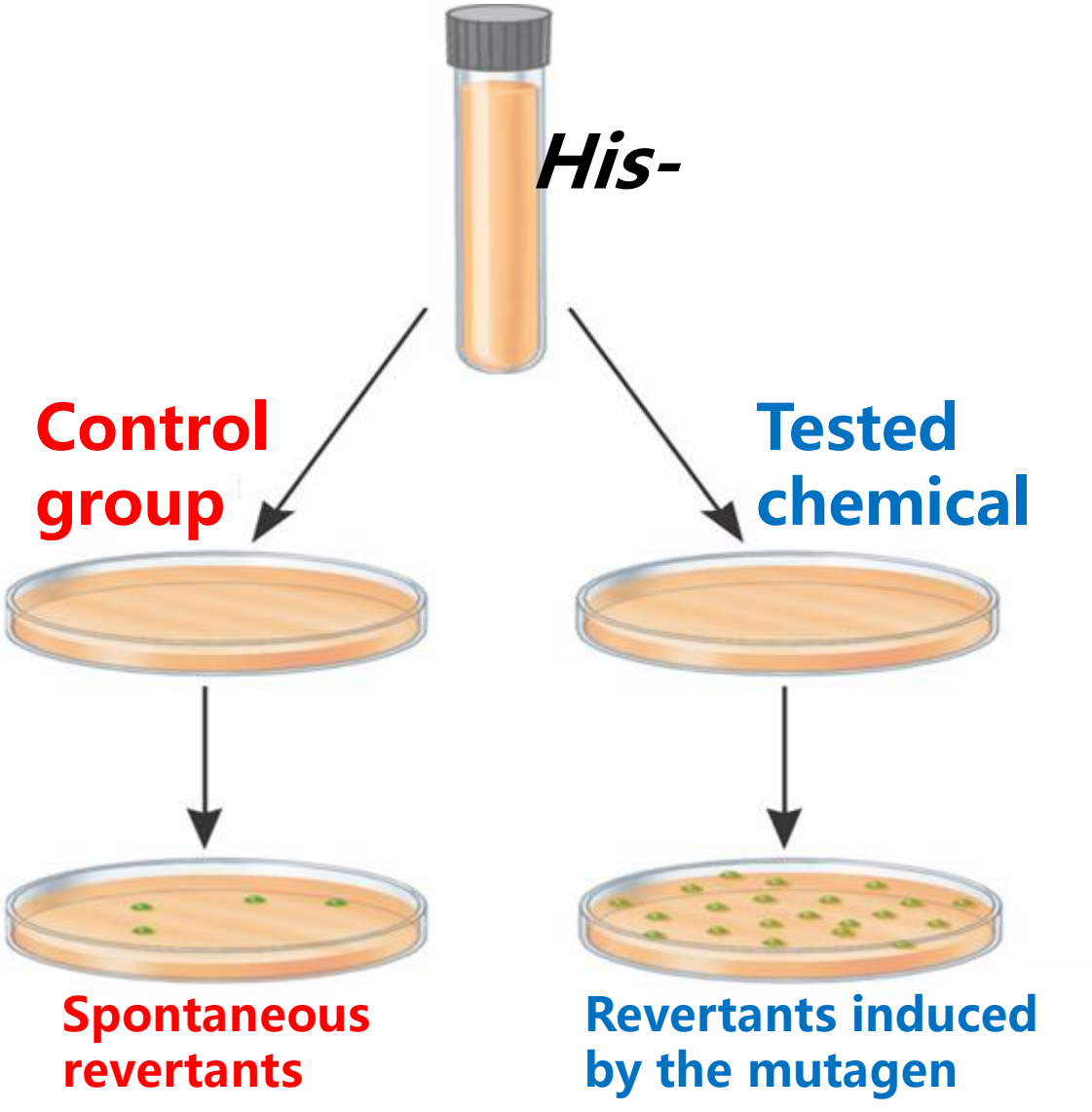
- Indicator organism is a mutant strain of *Salmonella typhimurium* that has lost the ability to synthesize histidine.鼠伤寒沙门氏菌/组氨酸
- This mutant is highly susceptible to back-mutation
  - Mutation in histidine biosynthesis operon (histidine auxotrophs)
  - Mutation make cell wall more permeable to test chemical
  - Defective in the ability to repair DNA correctly
- Reversion rate in presence of suspected carcinogen > reversion rate in absence of suspected carcinogen， then, agent is a mutagen, and may be carcinogen。

Strain: Histidine auxotrophic *Salmonella*

营养缺陷型沙门氏菌

Medium with minimal histidine

Revertant bacteria *His+*



Ames Test

Spontaneous revertants

Revertants induced by the mutagen

If: ■ < ■ **Then: mutagen** ✓

If: ■ = ■ **Then: mutagen** ✗

"A system for easily and cheaply testing the mutagenicity of chemicals using microbiology"

1975年---Bruce Ames---美国科学院院院刊*PNAS*

Hair Dyes Are Mutagenic:

Identification of a Variety of Mutagenic Ingredients

染发剂配方中化合物的诱变能力分析

结论：测定的18种染发剂配方化合物，12种显示强诱变能力。

厦门大学
生|命|科|学|学|院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

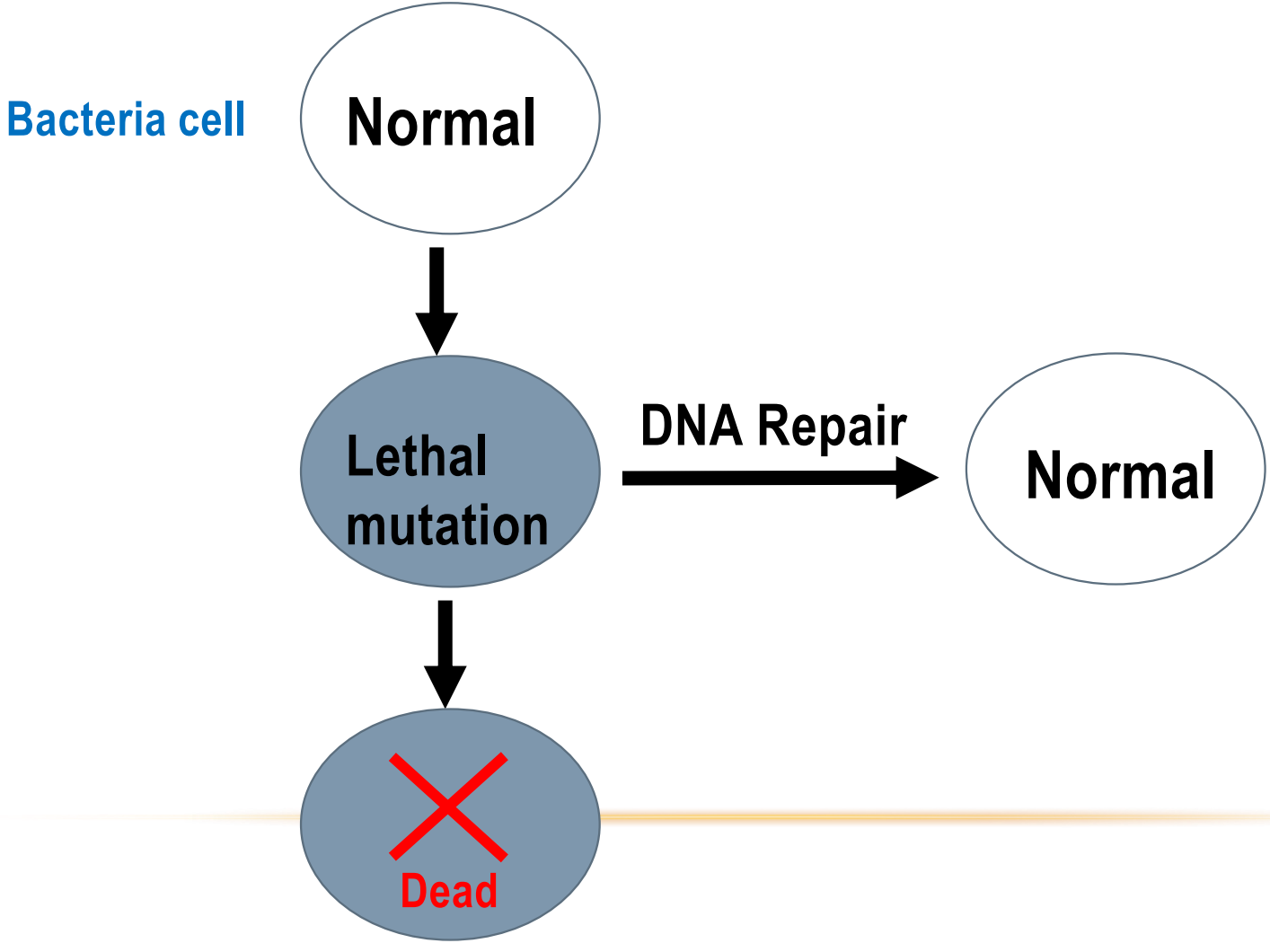
MICROBIOLOGY

Chapter 12

12.3 Bacterial DNA Repair

细菌DNA损伤修复

DNA Repair and Bacteria Surviving

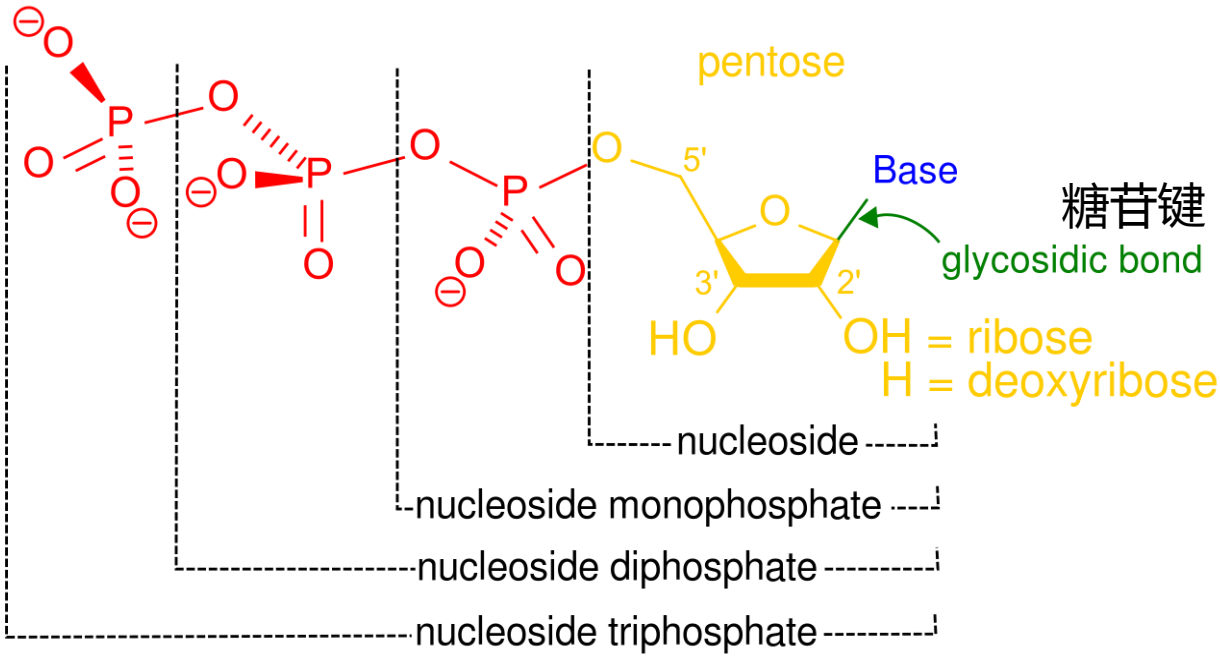


DNA Repair in Bacteria

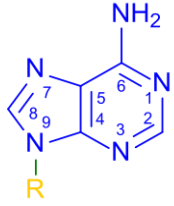
- Proofreading
- Mismatch Repair
- Excision Repair
- Direct Repair
- Recombinational Repair

Base, Nucleoside, Nucleotide

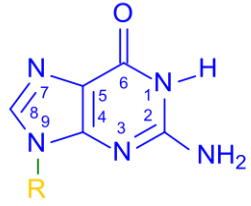
碱基　　核苷　　核苷酸



Purines

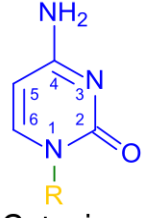


Adenine

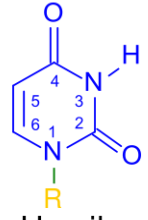


Guanine

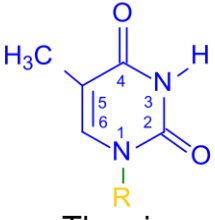
Pyrimidines



Cytosine



Uracil

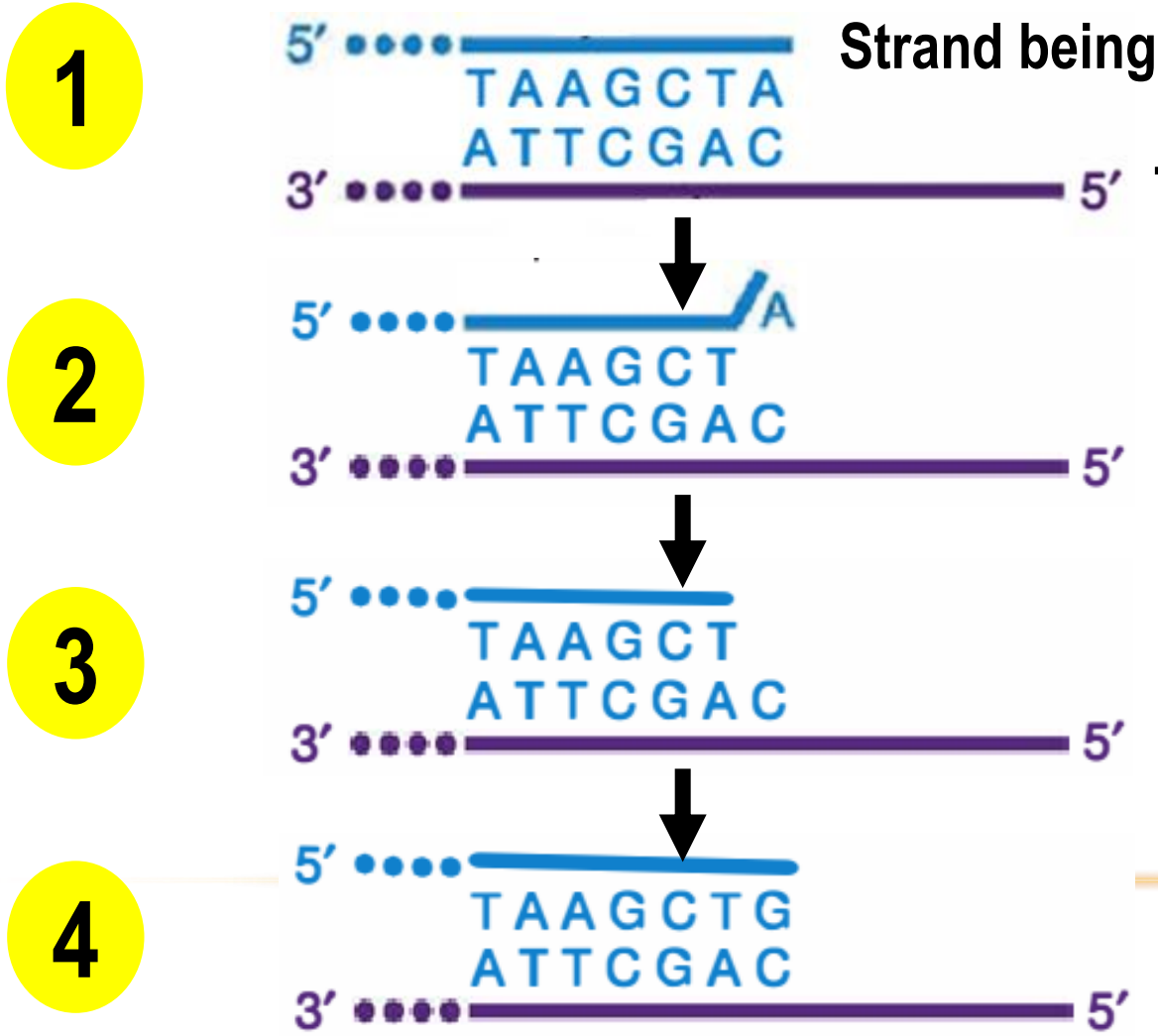


Thymine

腺嘌呤 A---- 胸腺嘧啶 T

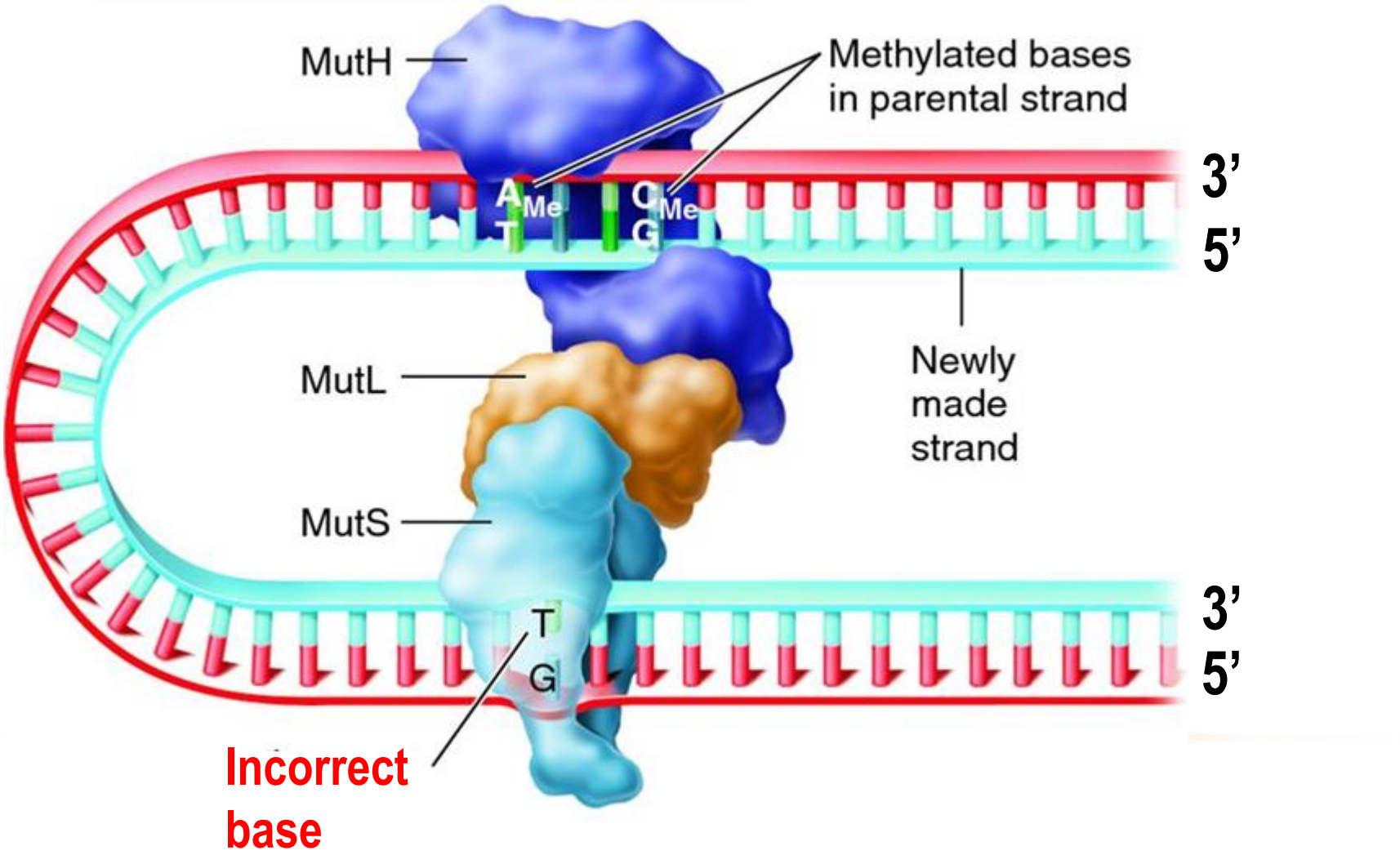
胞嘧啶 C---- 鸟嘌呤　G

Proofreading: The First Line of Defense

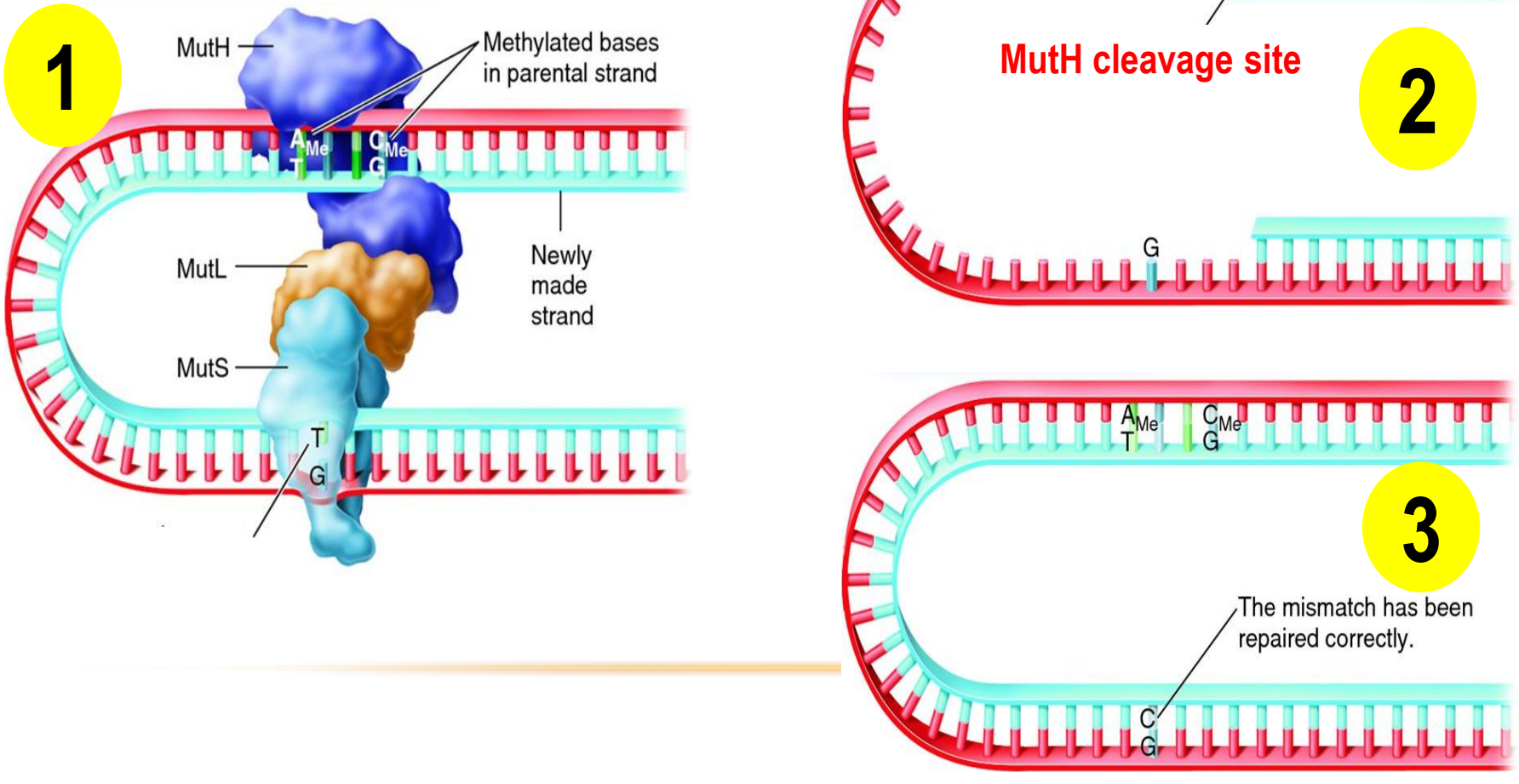


DNA polymerase:
3’ to 5’ exonuclease activity

MethI-Directed Mismatch Repair in E.coli



MethI-Directed Mismatch Repair in E.coli

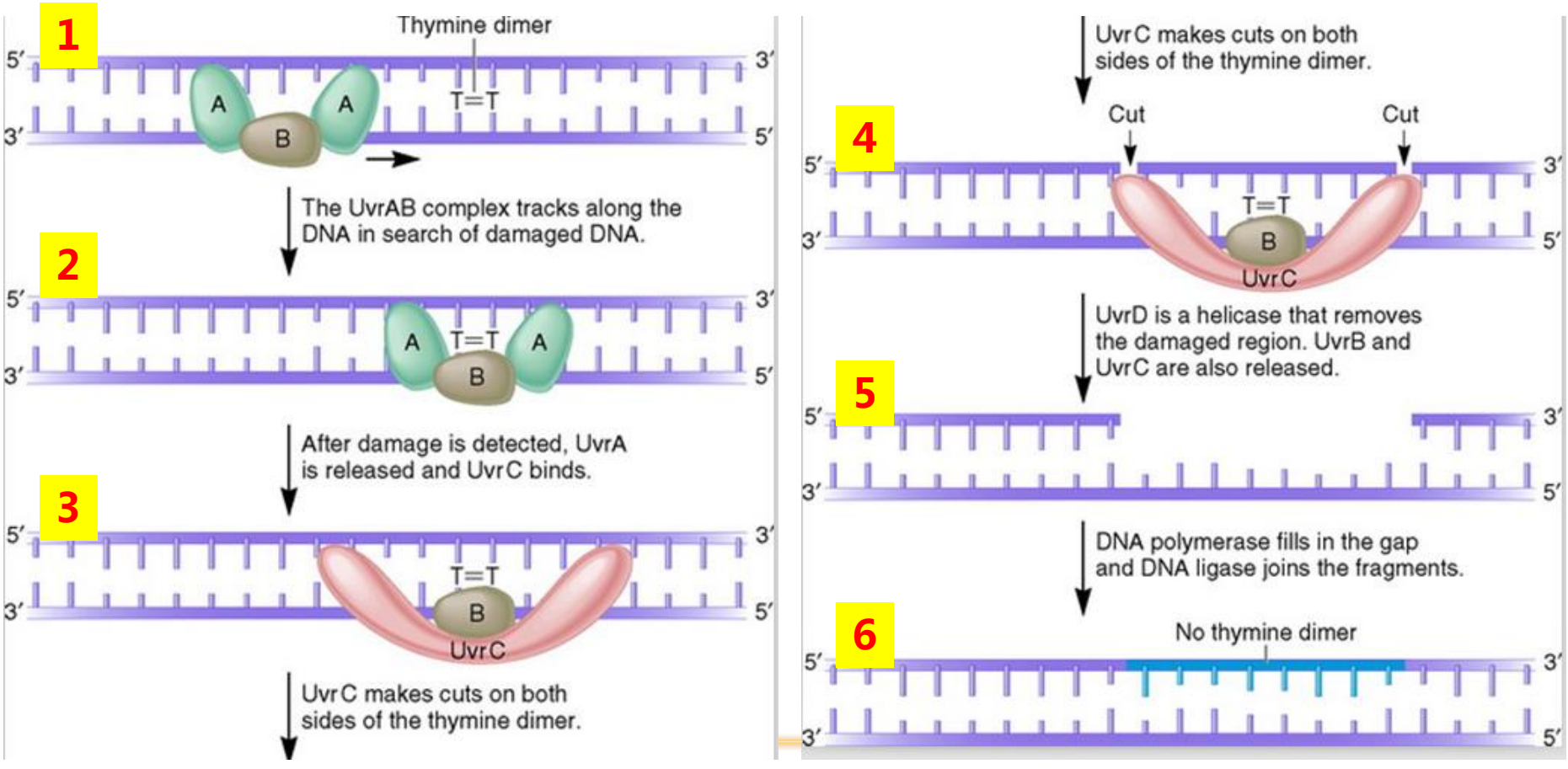


Excision Repair

Corrects damage that cause distortion in the double helix

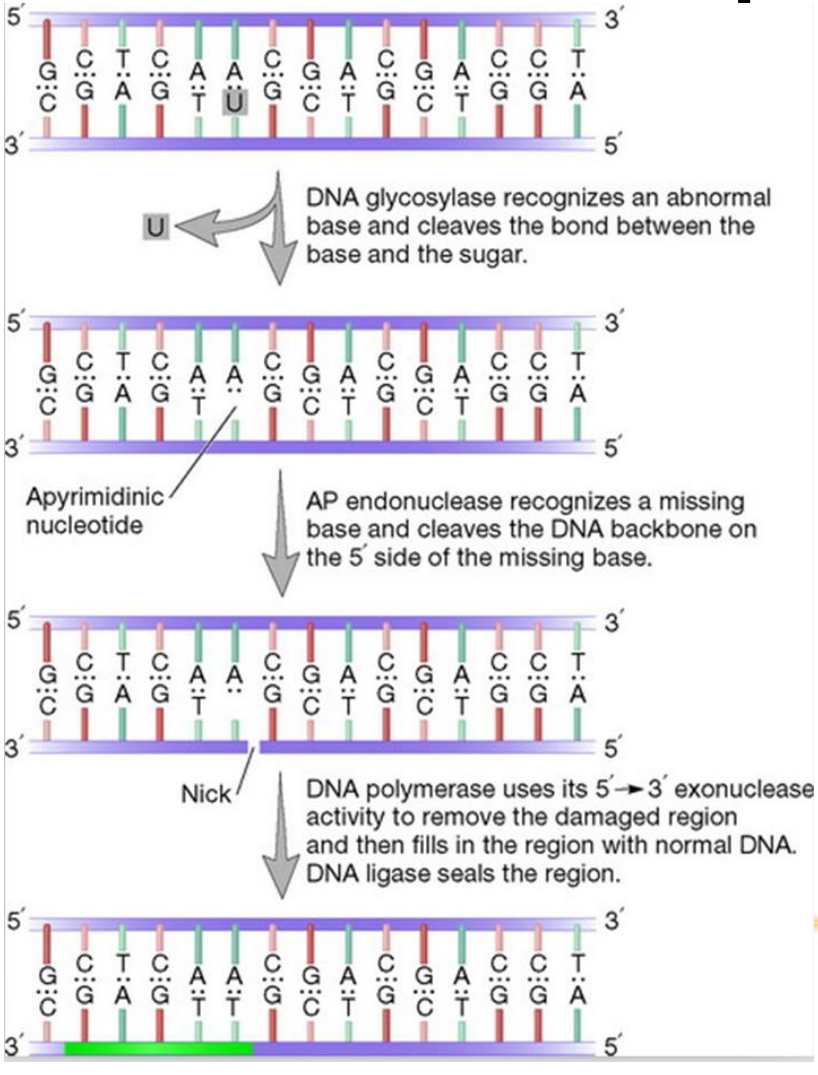
- **Nucleotide excision repair**
- **Base excision repair**

Nucleotide Excision Repair In *E. coli*



Thymine 胸腺嘧啶

Base Excision Repair In *E. coli*

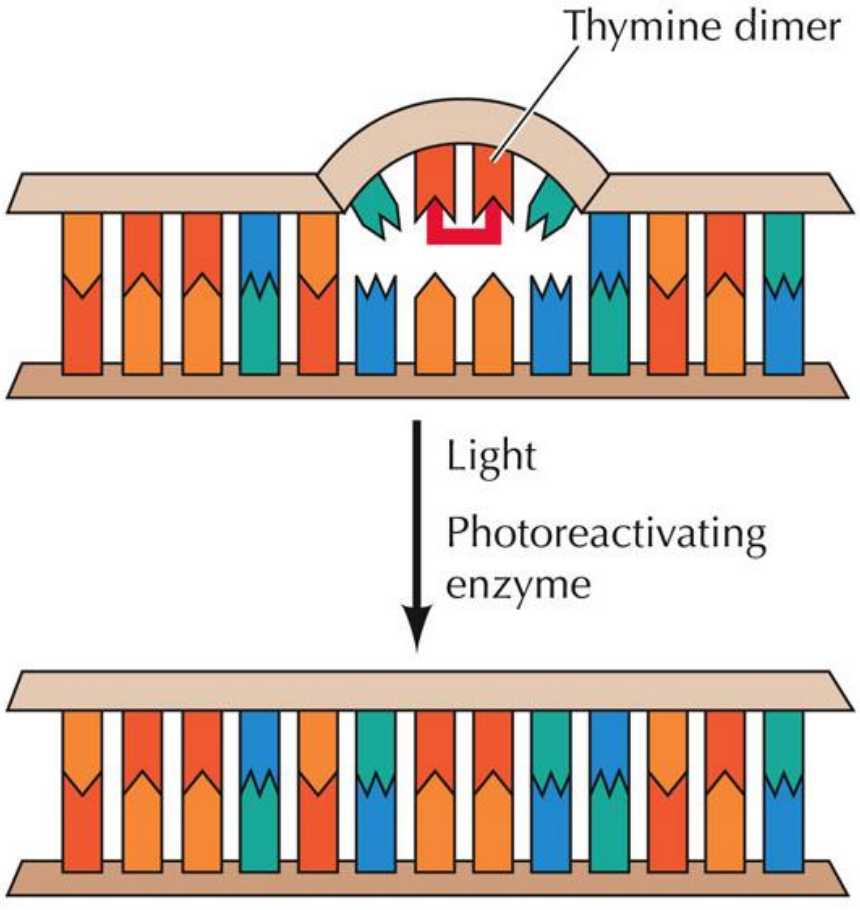


Uracil glycosylase
尿嘧啶糖基化酶
水解尿嘧啶和糖基之间的糖苷键

endonuclease
内切酶

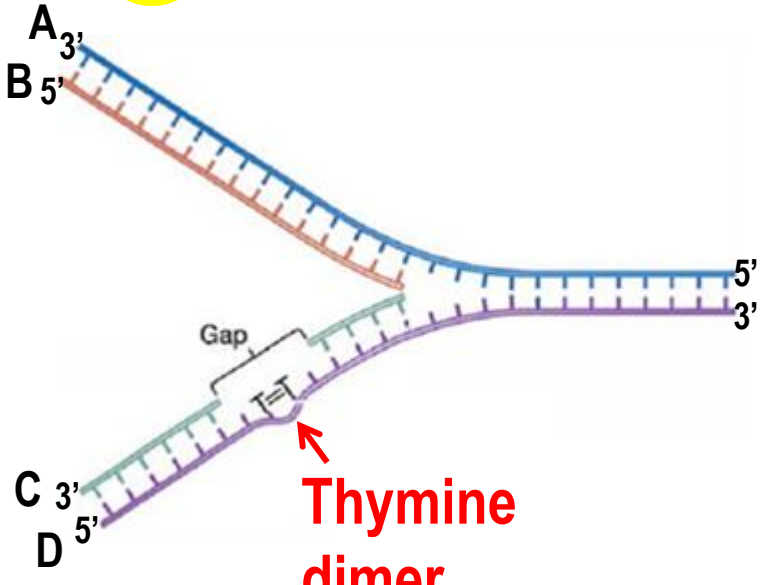
腺嘌呤 A-----胸腺嘧啶 T
胞嘧啶 C-----鸟嘌呤 G

Direct Repair

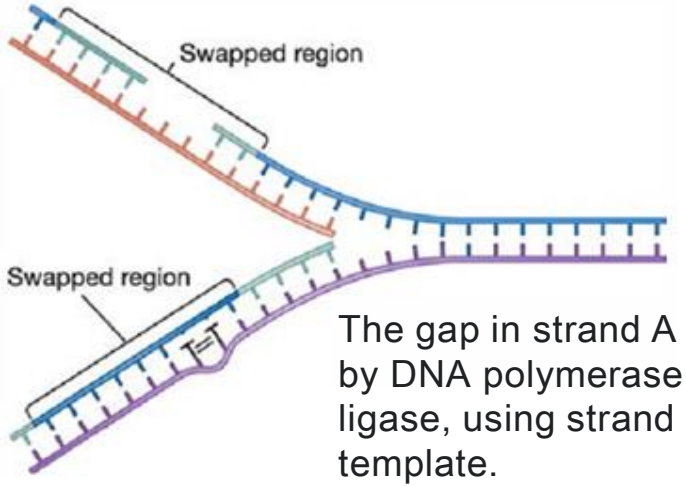


Recombinational Repair

1

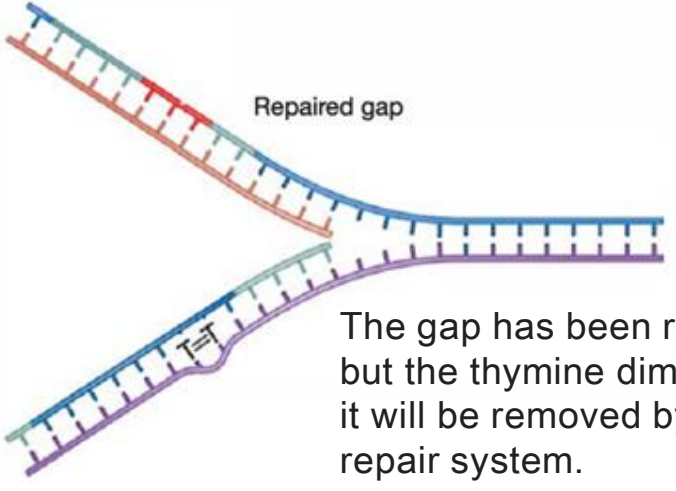


By recombination, a region in strand A is swapped for the same region in strand C.



2

The gap in strand A is filled in by DNA polymerase and DNA ligase, using strand B as a template.



3

The gap has been repaired, but the thymine dimer remains, it will be removed by another repair system.

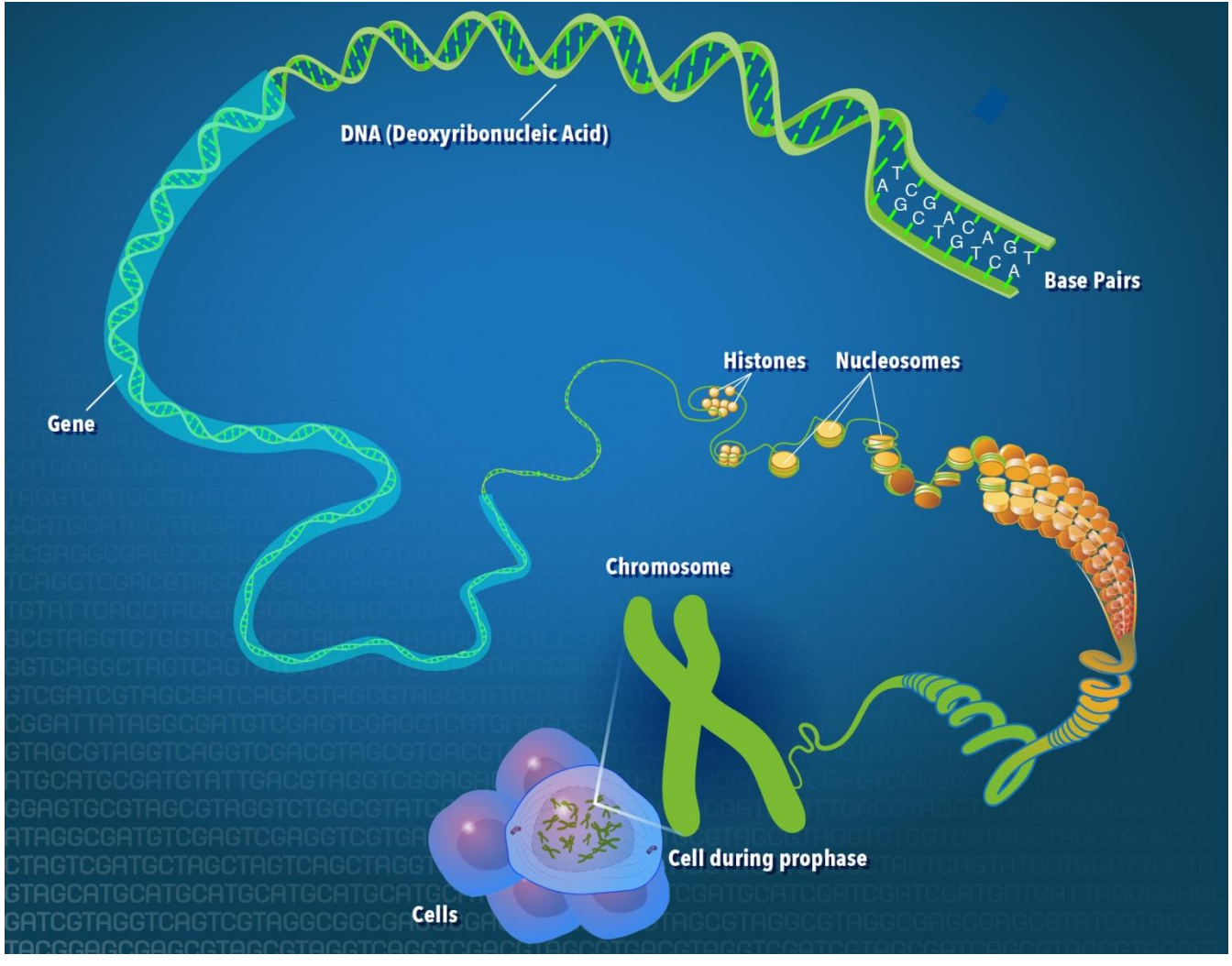
厦门大学
生|命|科|学|学|院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

MICROBIOLOGY

Chapter 12

12.4 Transposable Elements
转座元件

More and More Genomes Sequenced



Transposon 转座子

- Jumping Gene
- Mobile genetic elements
- Transposable elements

McClintock, Maize, and “Jumping Gene”

Barbara McClintock

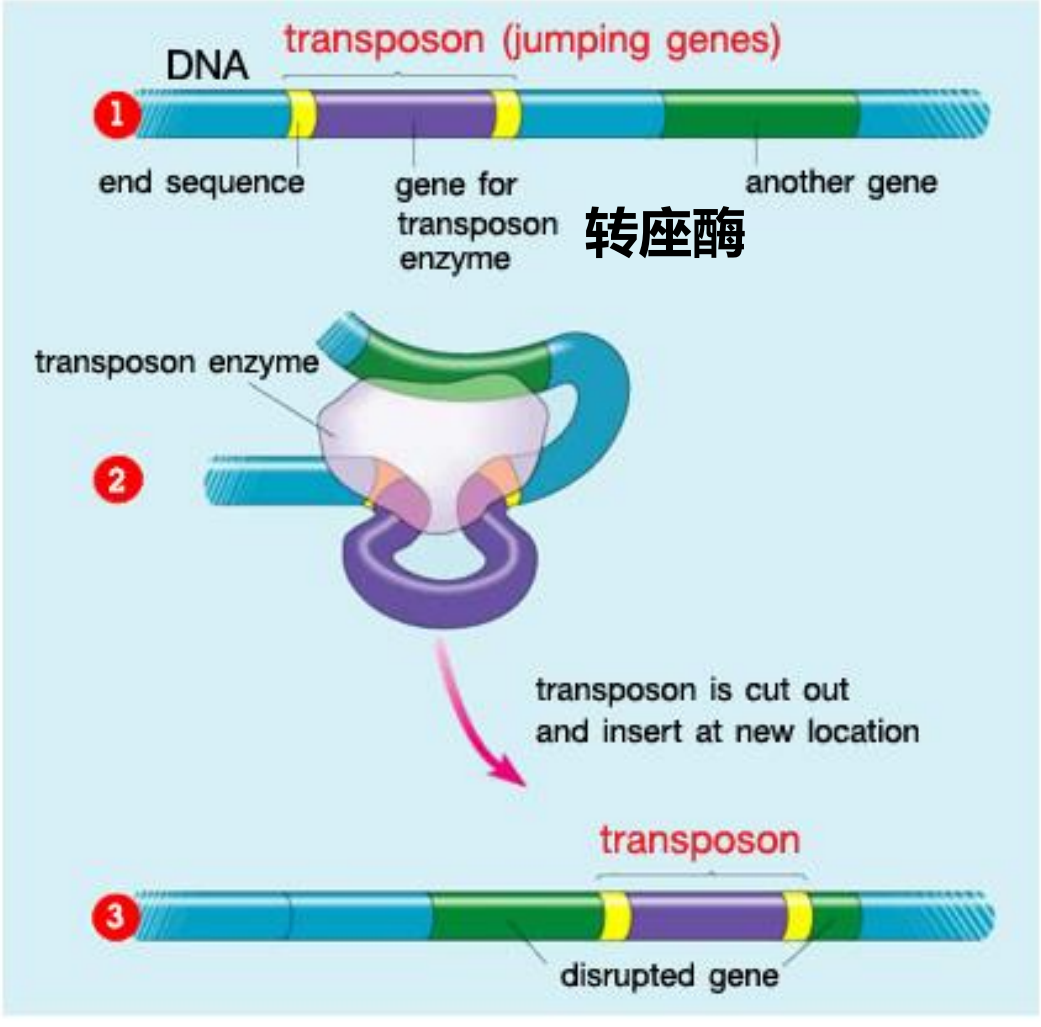
Creighton, H. B., & McClintock, B. A correlation of cytological and genetical crossing-over in *Zea mays*. *Proceedings of the National Academy of Sciences* **17**, 492–497 (1931)

Transposition 转座

The movement of a mobile genetic element (Transposon) in the genome.

Transposable elements（Transposon）

- Segments of DNA that move about the genome in a process called transposition **转座**
- Can be integrated into different sites in the chromosome



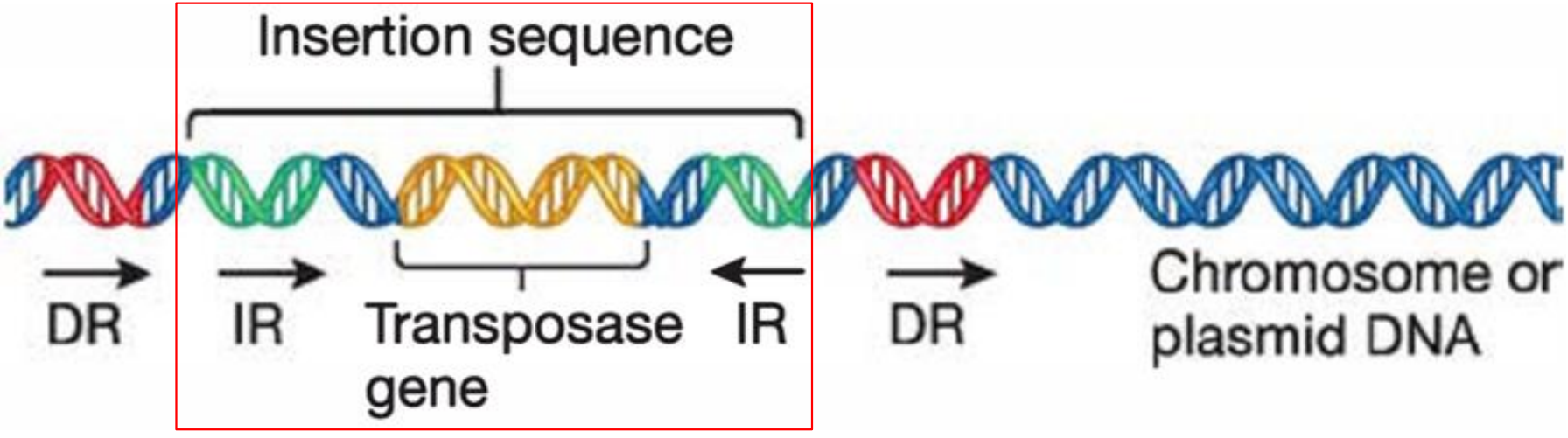
Classification of Transposable element

- Insertion Sequence
- Composite transposon
- Unit transposon

Insertion Sequences (IS)

- The simplest transposable elements.
- Short sequence of DNA around 750 to 1600 bp in length.
- Contain only the gene encoding the enzyme transposase, and it is bounded at both ends by inverted repeats(IR) in reversed orientation.
- Transposase is required for transposition and accurately recognizing the end of IS.

Insertion Sequences (IS)



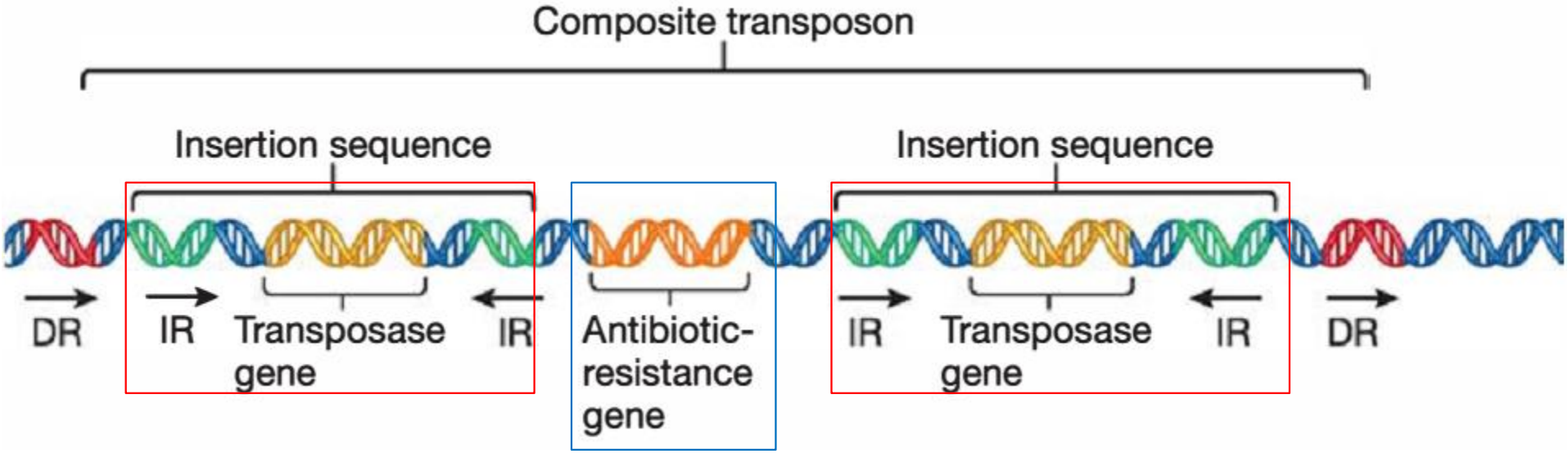
IR: inverted repeat
DR: direct repeat in the host DNA

Classification of Transposable element

- Insertion Sequence
- Composite transposon
  复合转座子
- Unit transposon

} Transposable elements which contain genes other than transposase for transposition

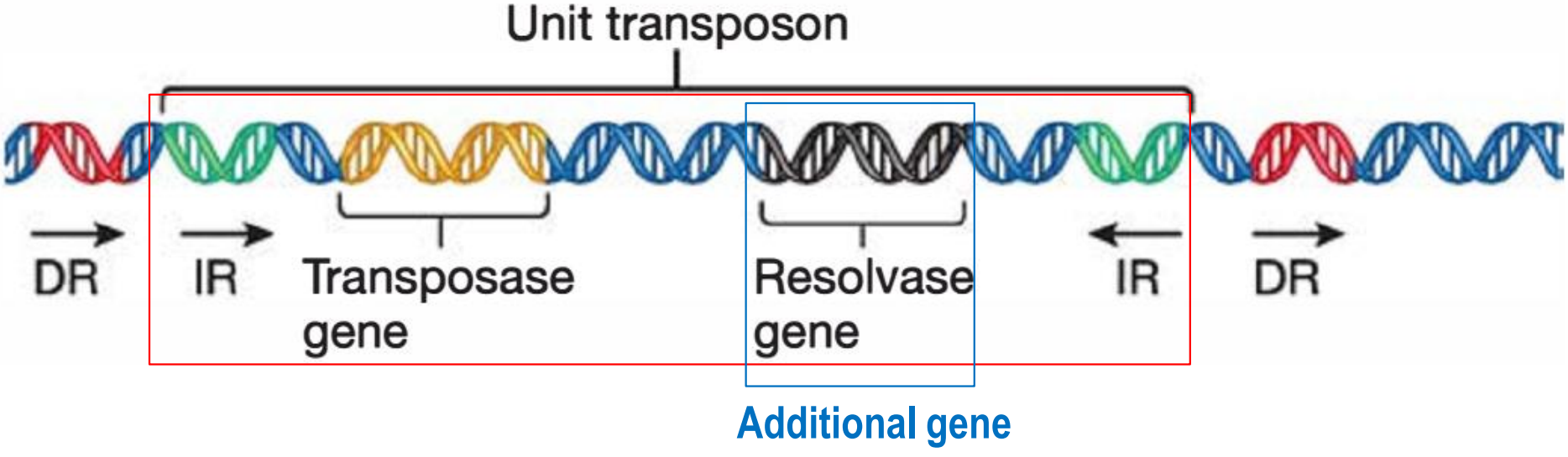
Composite transposon



Additional gene

IR: inverted repeat
DR: direct repeat in the host DNA

Composite transposon



IR: inverted repeat
DR: direct repeat in the host DNA

Properties Of Selected Composite Transposon

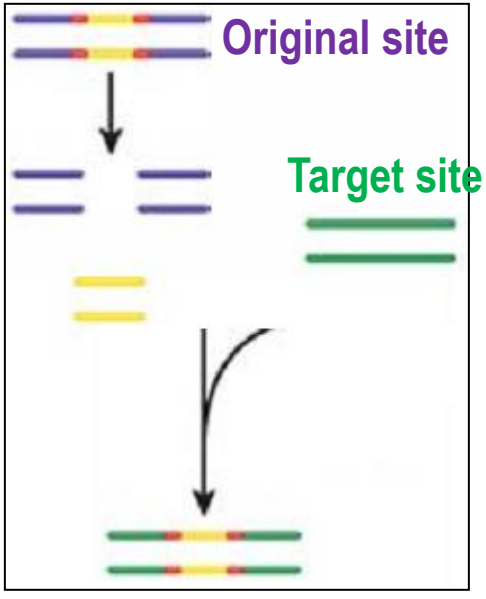
Table 14.4 The Properties of Selected Transposons

Transposon	*Length (bp)*	*Terminal Repeat Length*	*Terminal Module*	*Genetic Markers*
Tn*3*	4,957	38		Ampicillin resistance
Tn*501*	8,200	38		Mercury resistance
Tn*1681*	2,061		IS*1*	Heat-stable enterotoxin
Tn*2901*	11,000		IS*1*	Arginine biosynthesis

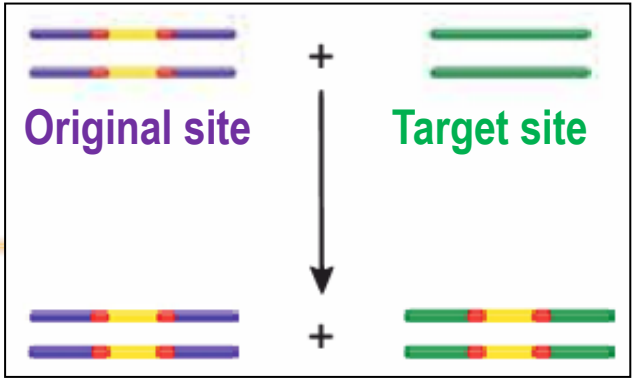
Transposon: Tn

Transposition in two mechanism

- Simple Transposition

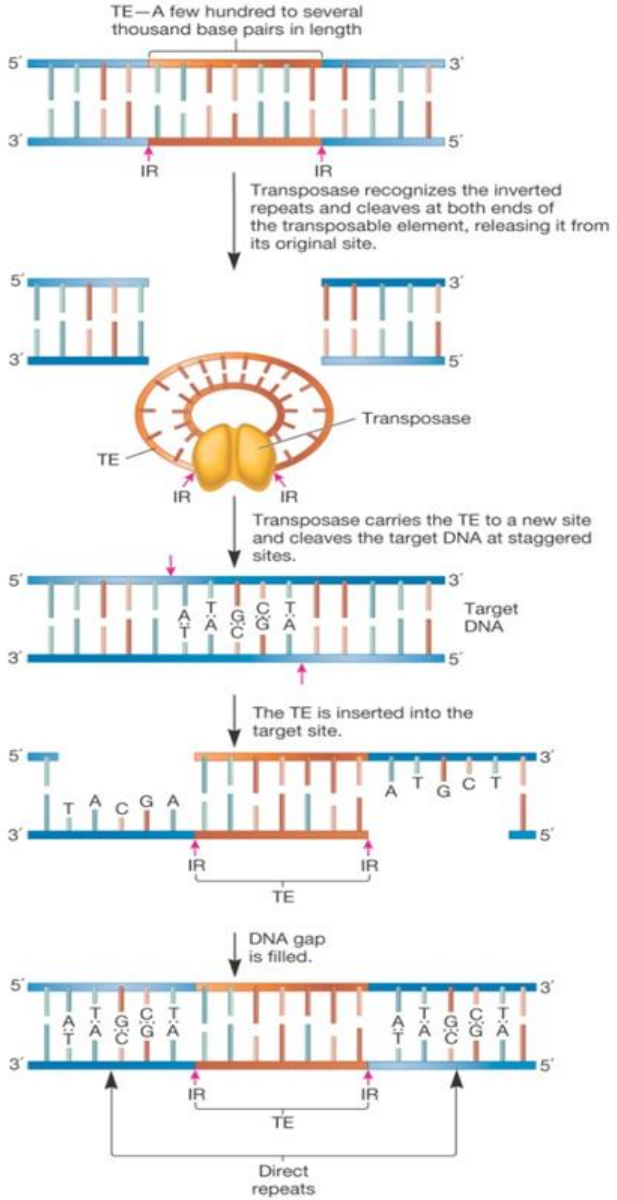


- Replicative Transposition



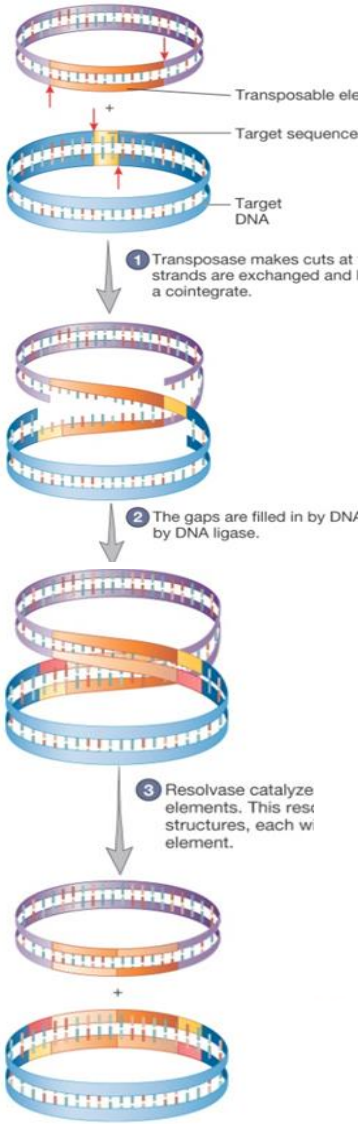
Simple Transposition

- Transposase catalyze excision of the transposon.
- Cleavage of new target site and ligation of cleaved transposon into this site.
- “Cut-and-Paste” transposition



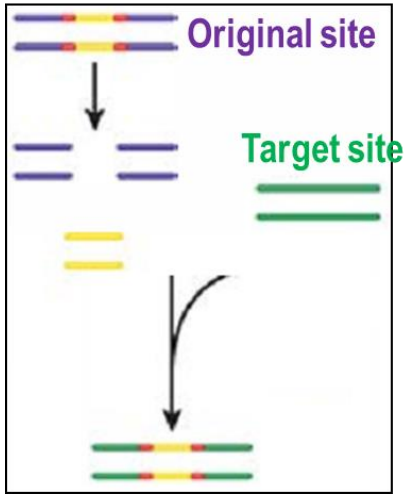
Replicative transposition

- Enzymes required:
  - Transposase 转座酶
  - Resolvase 解离酶
- Original transposon remains at parental DNA site in the genome.
- Another copy is inserted into the new target site.



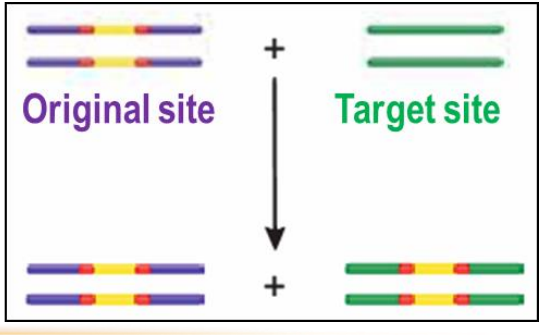
- Simple Transposition

"Cut-and-paste"



- Replicative Transposition

"Copy, Paste, and Double"



The Nobel Prize in Physiology or Medicine 1983

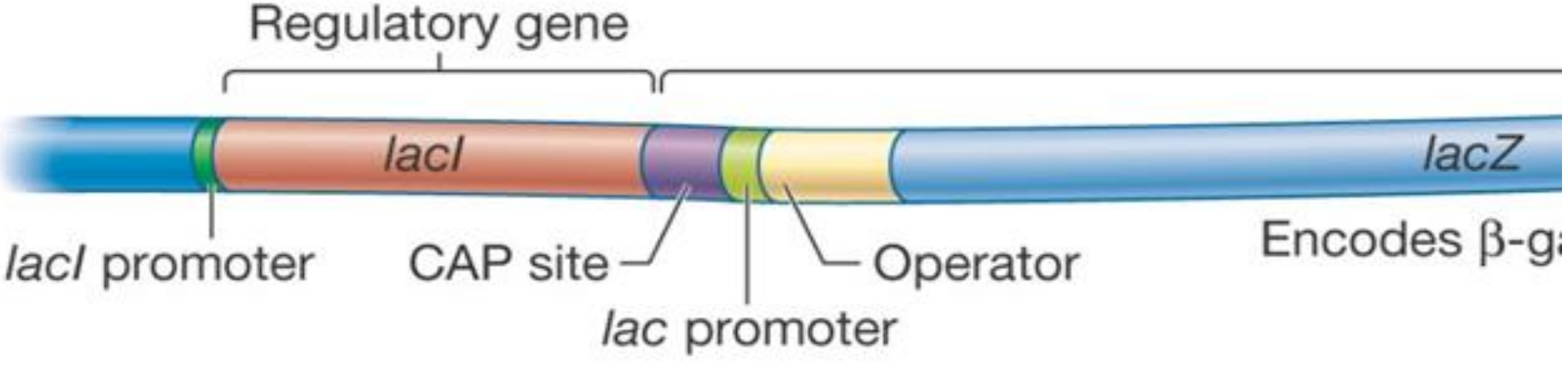
Barbara McClintock

"for her discovery of mobile genetic elements"

Question

Mutations caused by randomly Transposon insertion

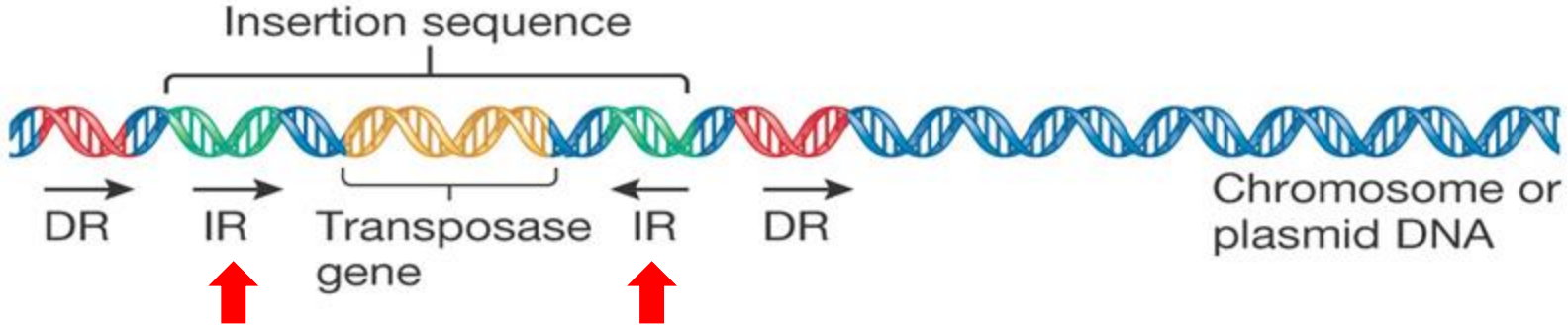
Mutations caused by randomly Transposon insertion



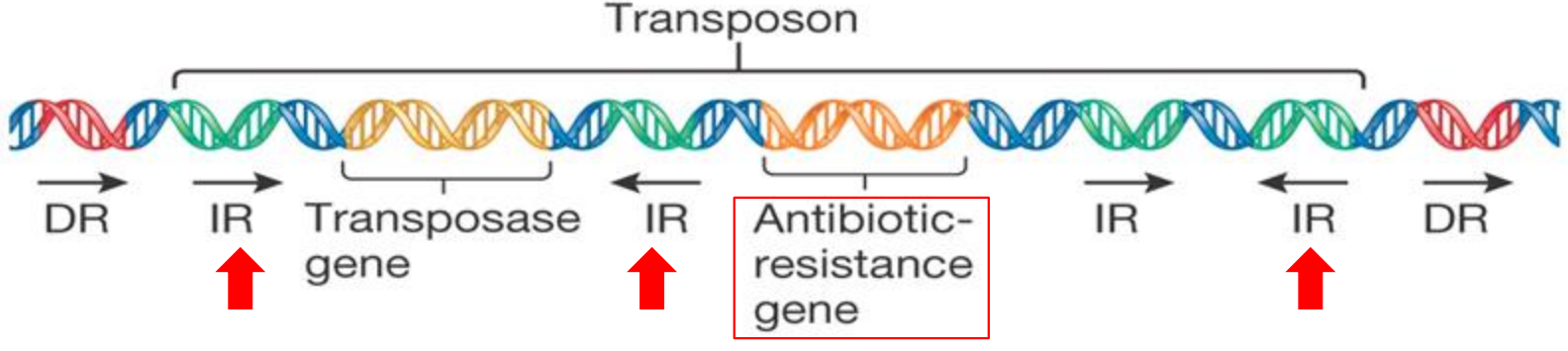
Question

Gene amplification by Composite Transposon insertion

Gene amplification by Composite Transposon insertion



(a)



(b)

厦门大学
生|命|科|学|学|院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

MICROBIOLOGY

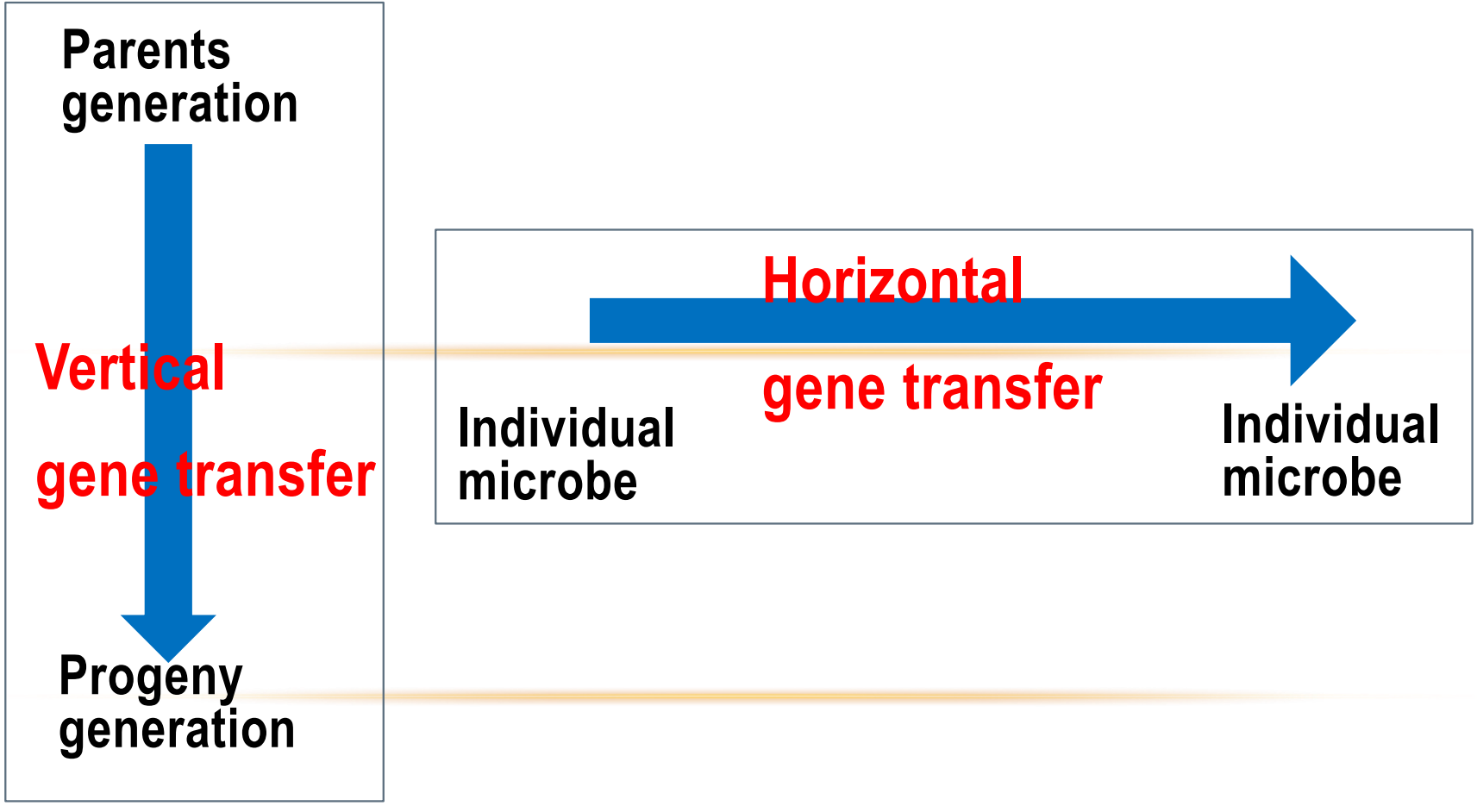
Chapter 12

12.5 DNA Transformation in Bacteria
细菌DNA转化

Genetic Variability Coming from….

- **DNA mutation**
- **DNA Recombination**

Genetic Variability Coming from….

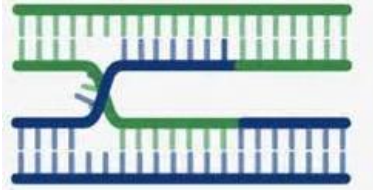


DNA Recombination

The process in which one or more DNA molecules are rearranged or combined to generate a DNA molecule with new nucleotide sequence (Recombinant).

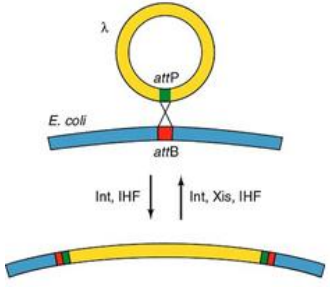
Recombination at the Molecular Level

- **Homologous recombination**



  - Most common recombination events
  - A reciprocal exchange between a pair of DNA molecule with similar nucleotide sequence (long regions of sequence homology)
  - Occur anywhere on the chromosome

- **Site-specific recombination**
  - Integration of viral genomes into specific site of host chromosome



- **Transposition**
  - Gene jumping inside the genome of microbe

Horizontal Gene Transfer (HGT) in *bacteria* and *archaea*

- HGT differs from vertical gene transfer
  - Transfer of genes from one independent, mature organism to another, often creating a stable recombinant having characteristics of both donor and recipient.
- Genes can be transferred to the microbe between the same or different species
- Important in evolution of many microbe species

Three Mechanisms of HGT In Bacteria

1. Transformation 转化
2. Conjugation 结合
3. Transduction 转导

DNA Transformation in Bacteria

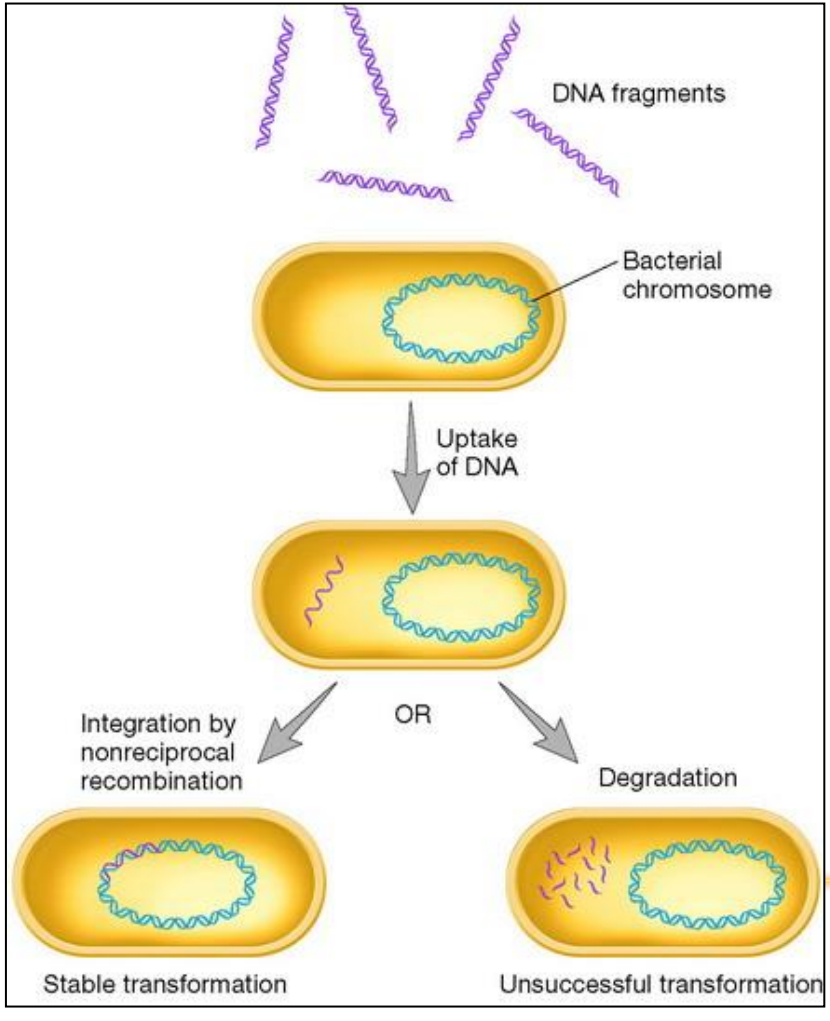
Question：

How the bacteria (*S.pneumoniae*) take up the exogenous DNA?

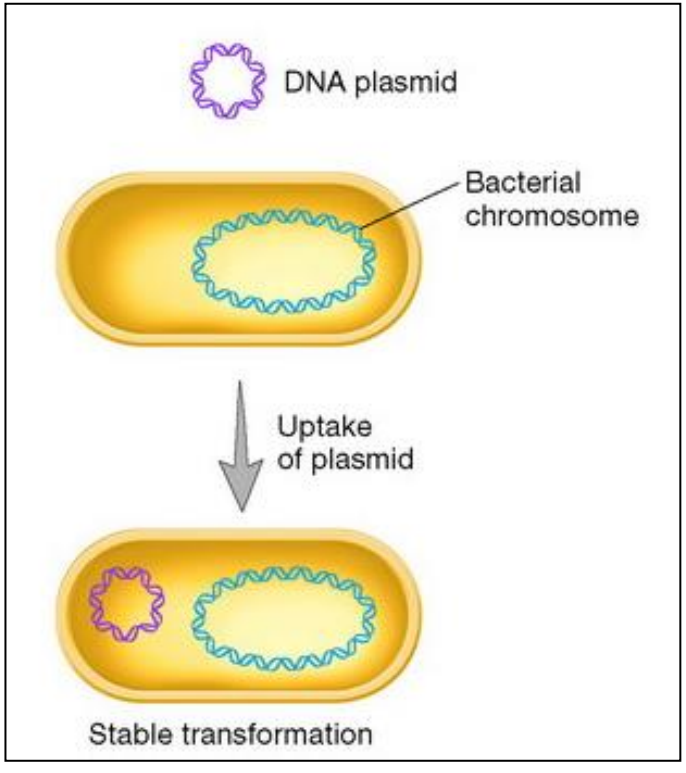
Bacteria Transformation

- Uptake of naked DNA by a bacteria competent cell from the surroundings.
- Competent cell: A cell that is able to take up DNA and be transformed, which the DNA can be bound to the cell and taken inside.
- Natural transformation occurs in certain genera microbes

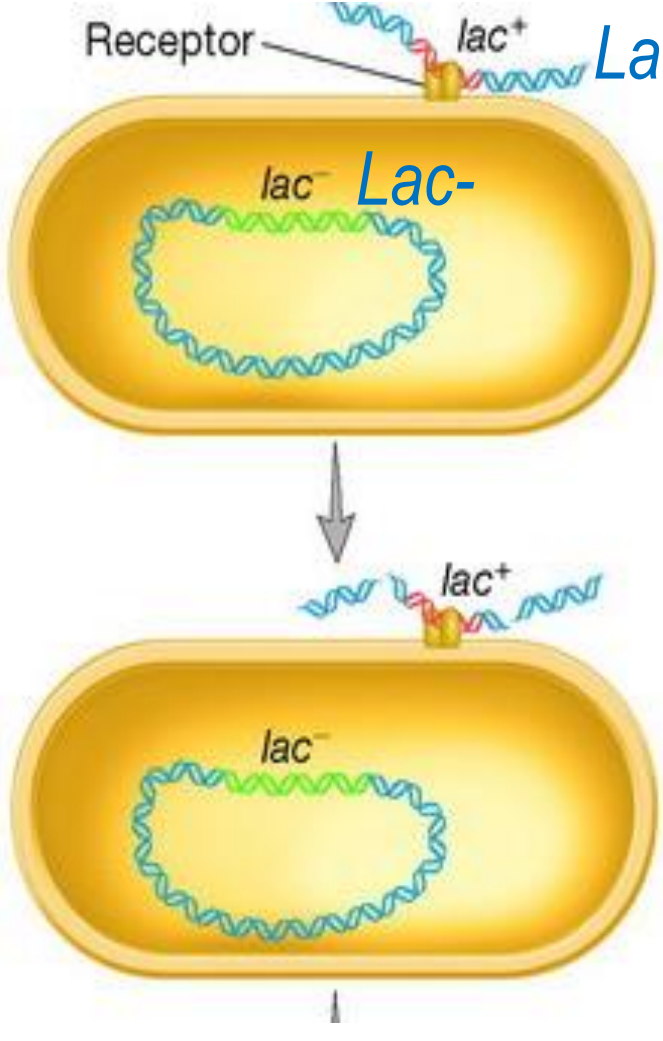
DNA fragment



DNA plasmid



Transformation in *S.pneumoniae*



1

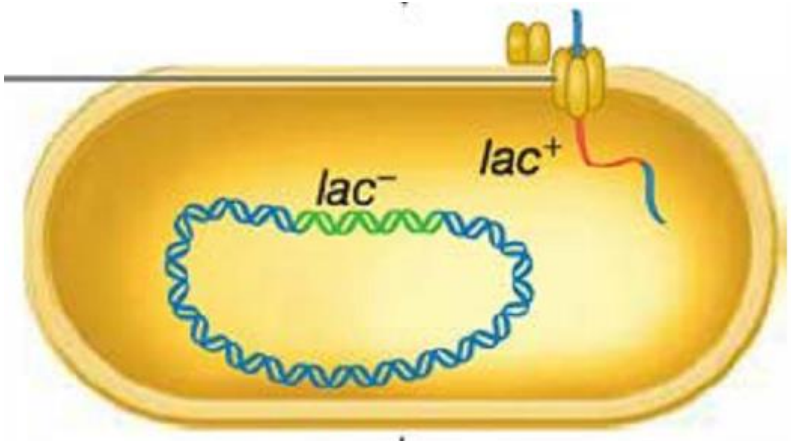
DNA fragment binds to a cell surface receptor

2

An extracellular endonuclease cuts the DNA into small fragments

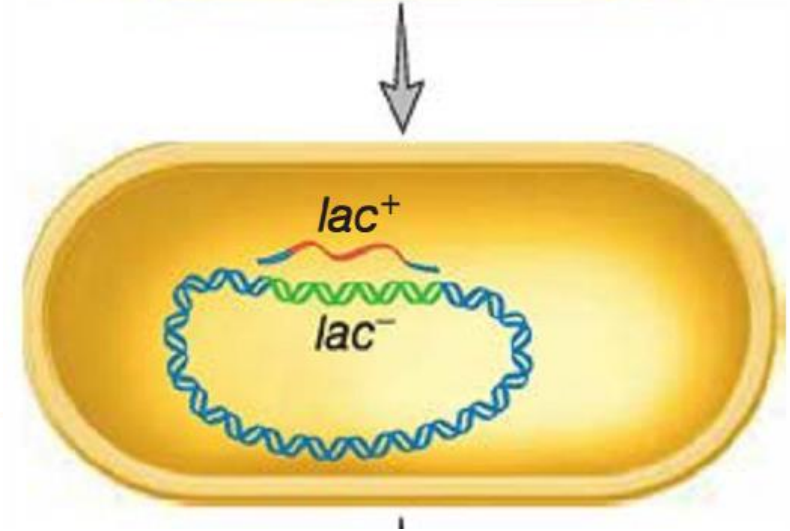
Transformation in *S.pneumoniae*

3



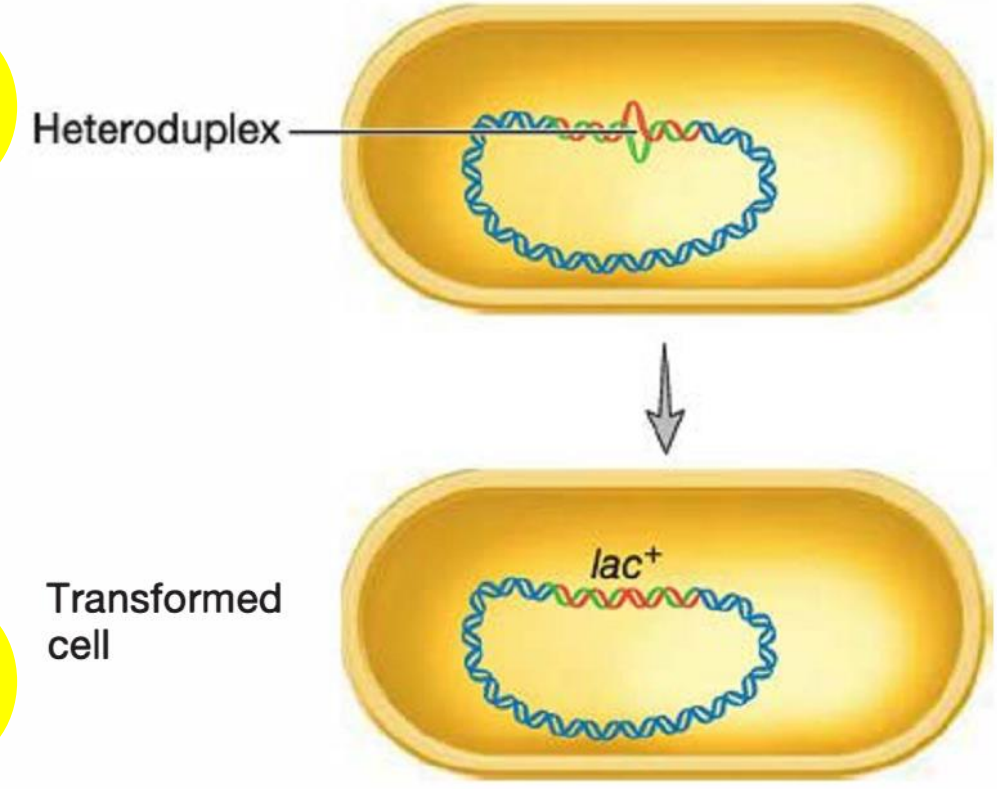
One strand is degraded and a single strand is transported into the cell.

4



The DNA strand aligns itself with a homologous region on the recipient cell chromosome

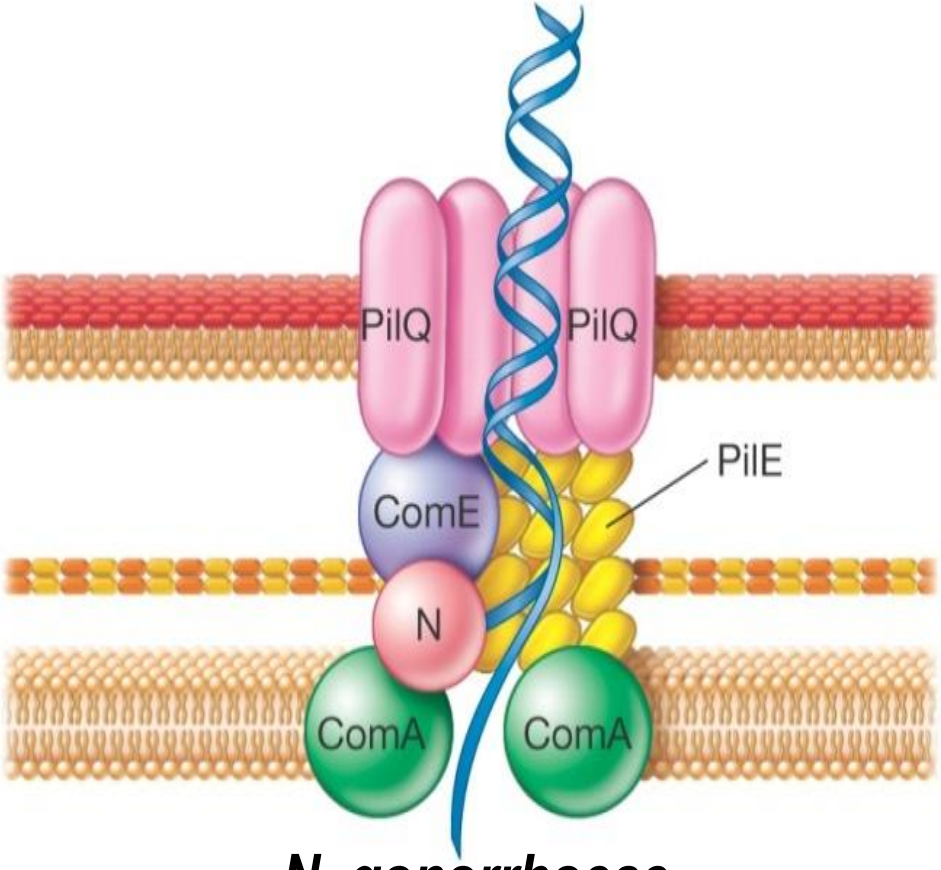
Transformation in *S.pneumoniae*



The DNA strand is incorporated in the chromosome via HR

The heteroduplex DNA is repaired in a way that changes lac- strand to create a lac+ gene.

Protein machinery for DNA transformation



N. gonorrhoeae
淋球菌

- **PilQ** aids in movement across outer membrane.
- **Pilin complex (PilE)** moves DNA across periplasm and peptidoglycan.
- **ComE** is DNA binding protein.
- **N** is the nuclease that degrades one strand before DNA enters the cytoplasm.
- **ComA** forms transmembrane channel for strand entering.

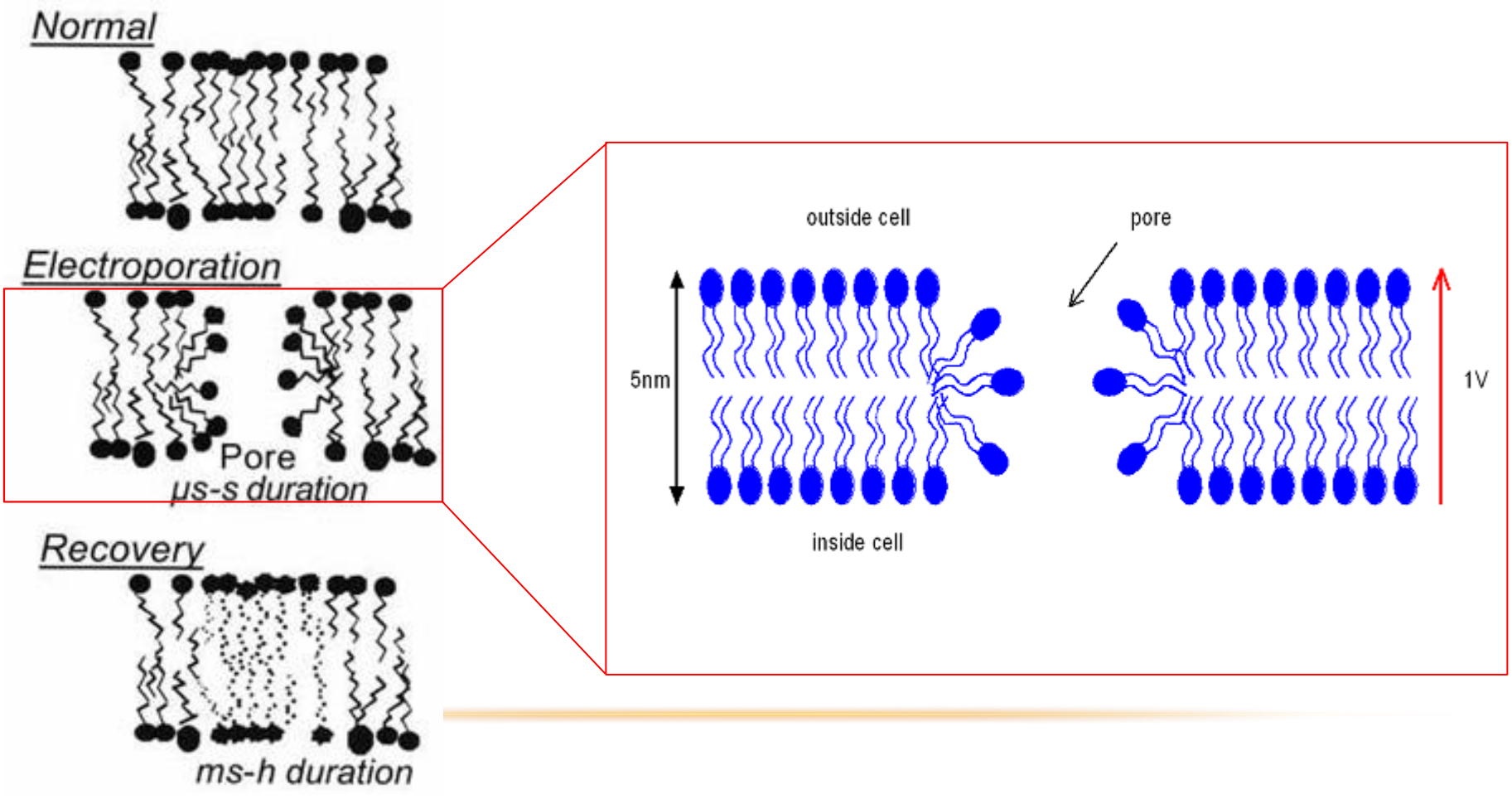
Bacteria Transformation

- Uptake of naked DNA by a bacteria competent cell from the surroundings.
- Competent cell: A cell that is able to take up DNA and be transformed, which the DNA can be bound to the cell and taken inside.
- **Natural transformation occurs in certain genera microbes**

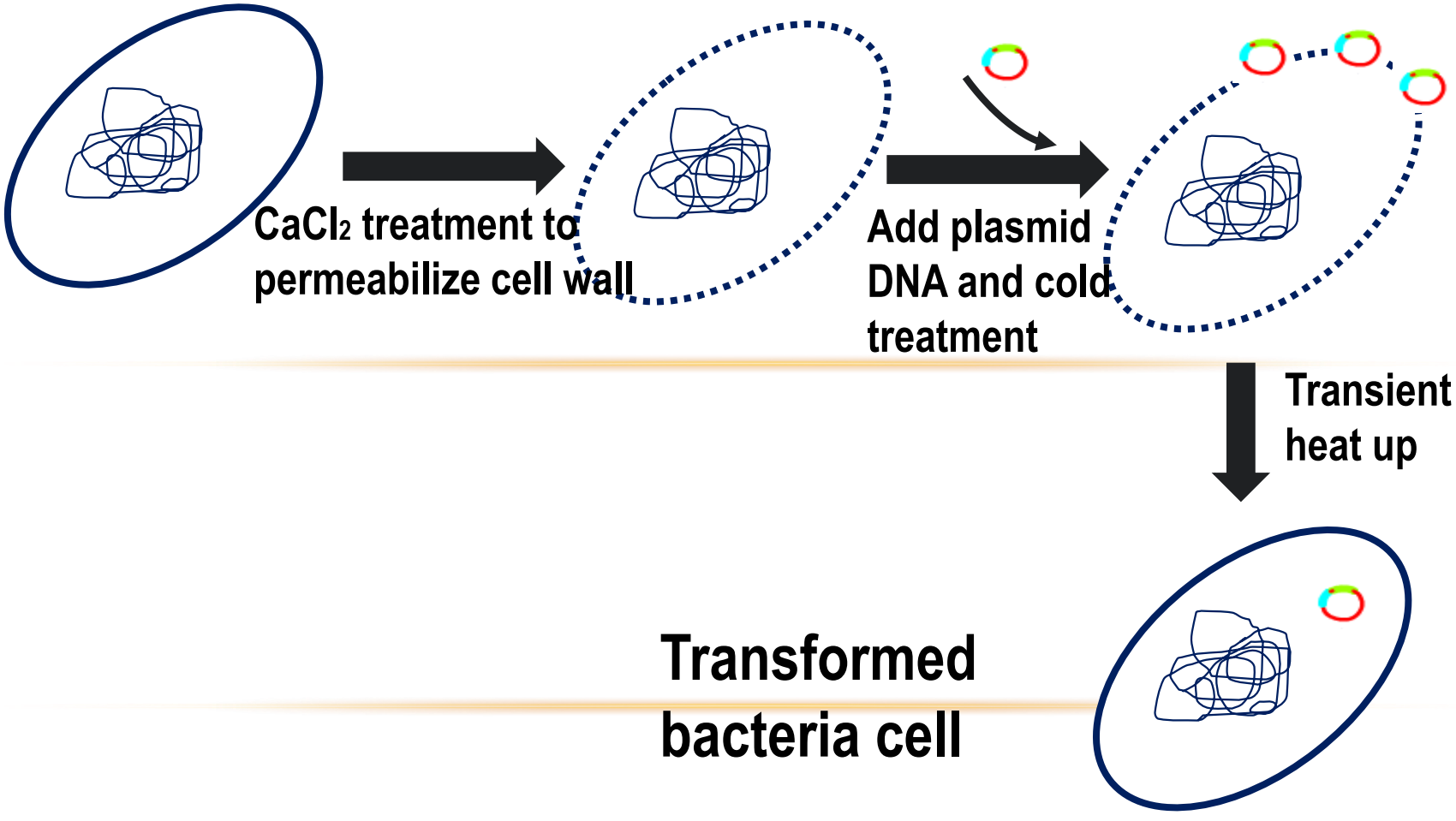
Transformation in non-natural competent cell, like *E.coli*

- Common used in recombinant DNA technology
- Artificial Competent Cell by certain treatment
  - Exposure to Calcium Chloride ($CaCl_2$)
  - Electrical Shock / Electroporation

DNA Transformation by Electroporation



$CaCl_2$ treatment for Competent Cell



厦门大学
生|命|科|学|学|院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

MICROBIOLOGY

Chapter 12

12.6 Bacteria Conjugation
细菌接合

Three Mechanisms of HGT In Bacteria

1. Transformation
2. Conjugation
3. Transduction

Bacterial Conjugation 细菌接合

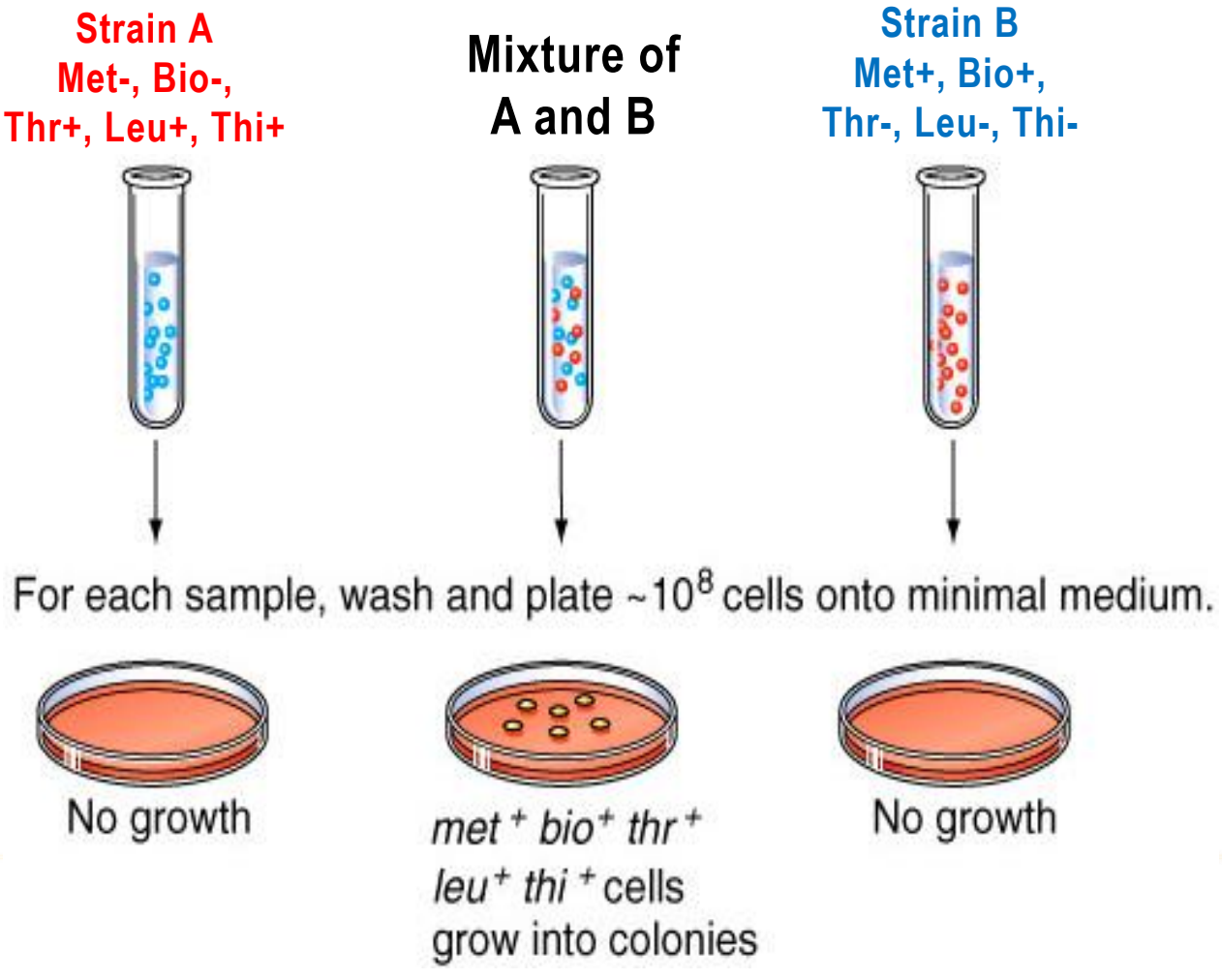
Joshua Lederberg & Edward Tatum 1946

6 mouths
1958

Edward Tatum
1909---1975

Joshua Lederberg
1925---2008

Gene Transfer Occurs

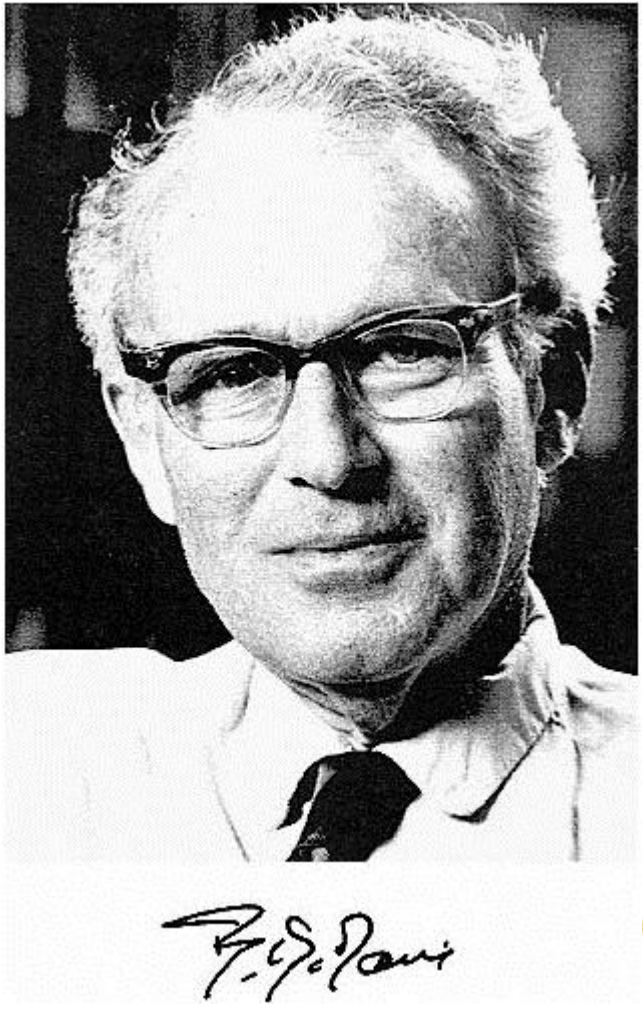


Met:蛋氨酸
Bio:生物素
Thr:苏氨酸
Leu:亮氨酸
Thi: 硫铵素

Observing by Joshua Lederberg & Edward Tatum (1946) indicated that the genes transferred between these two auxotrophic mutant strains A and B,

But how? Two possible way:

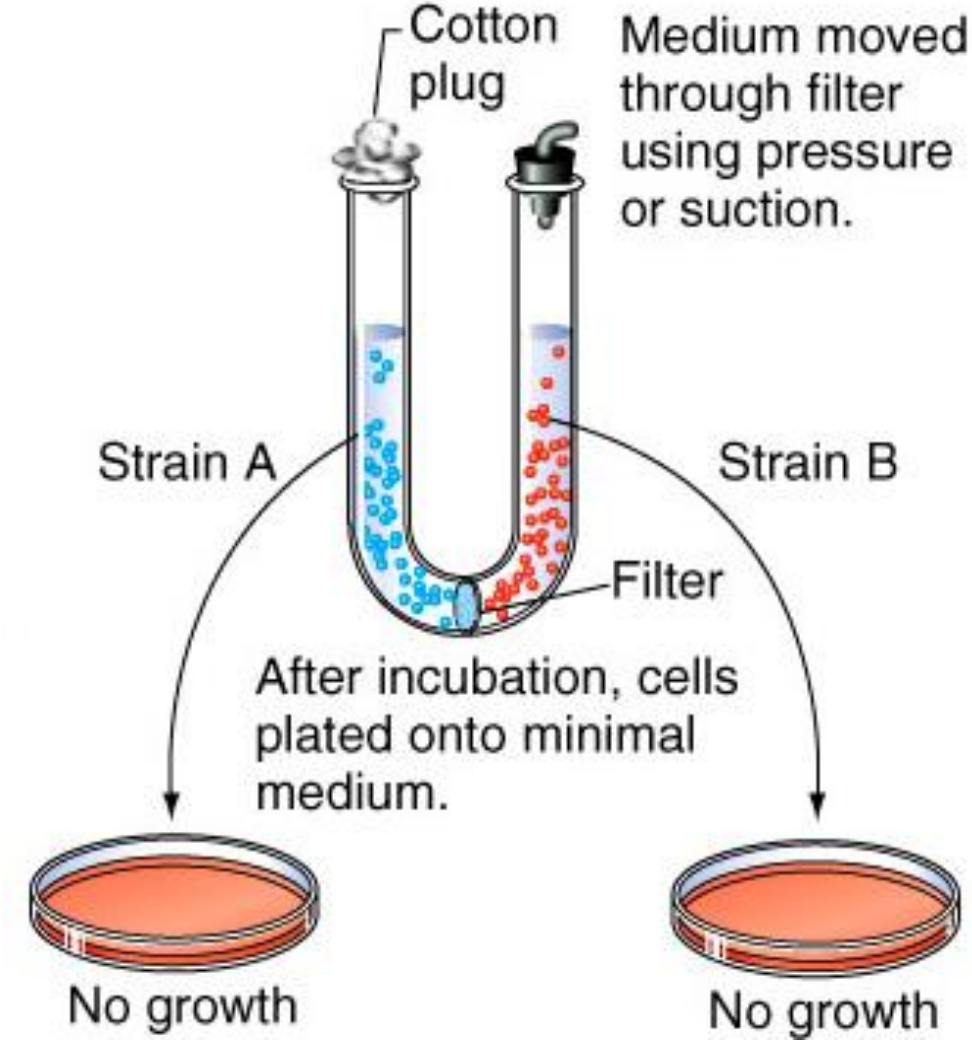
- Cell-cell physical contact ?
- Substance secreted by cell?



Bernard Davis

American microbiologist,1916–1994

Gene Transfer Require Cell to Cell Contact

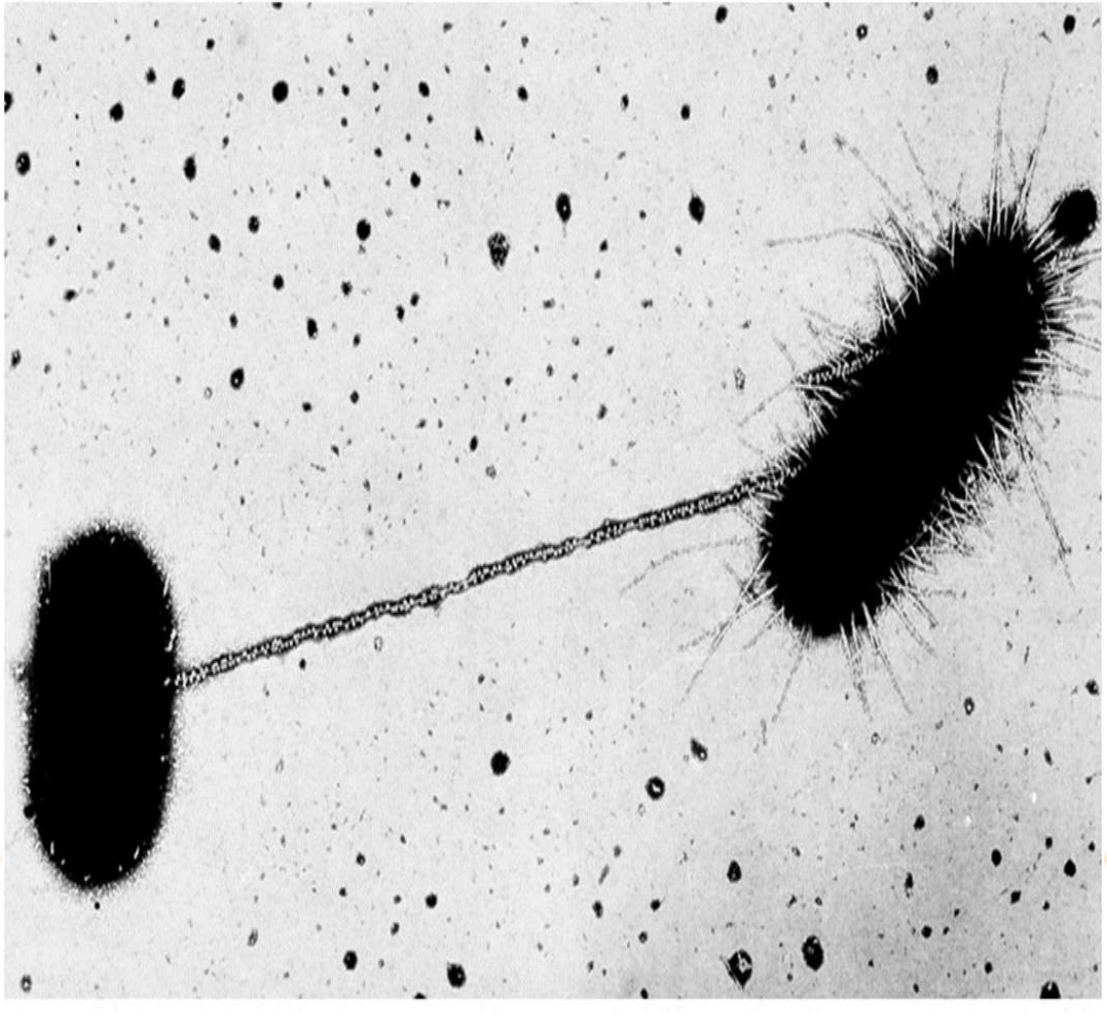


- Cell-cell physical contact ?
- Substance secreted by cell ? ✗

Bernard Davis, 1950

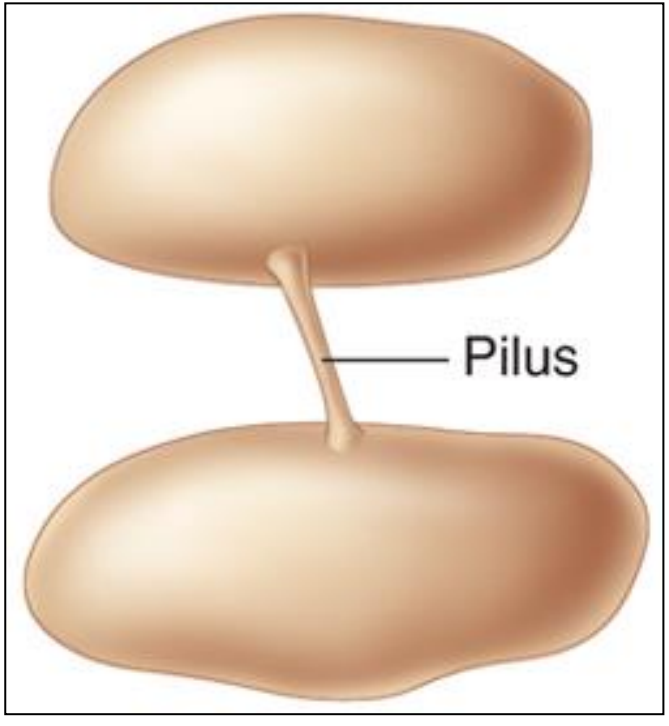
F Pilus Mediated Cell-to-cell Conjugation

性菌毛

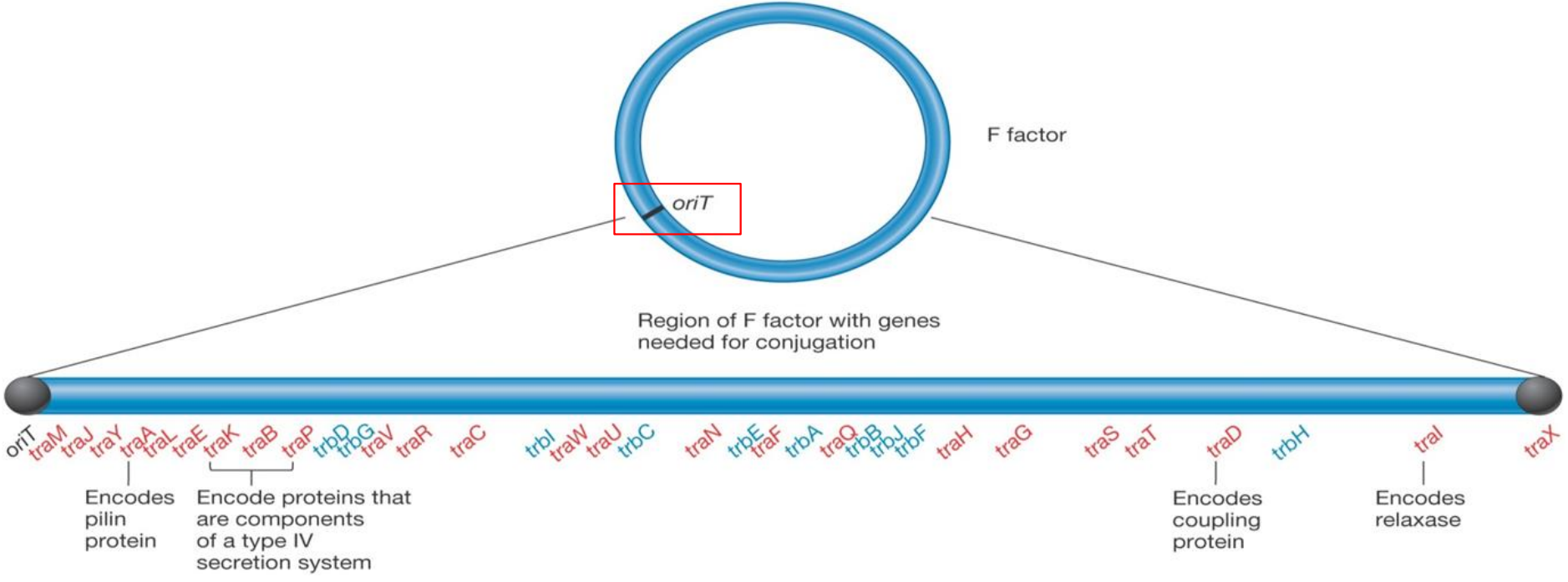


Bacterial Conjugation

- Depends on direct cell to cell contact mediated by the F pilus 性菌毛
- Unidirectional DNA transfer from donor to recipient cell
- The type of plasmid in conjugation



Fertility Factor (F Factor) for Conjugation
致育因子质粒



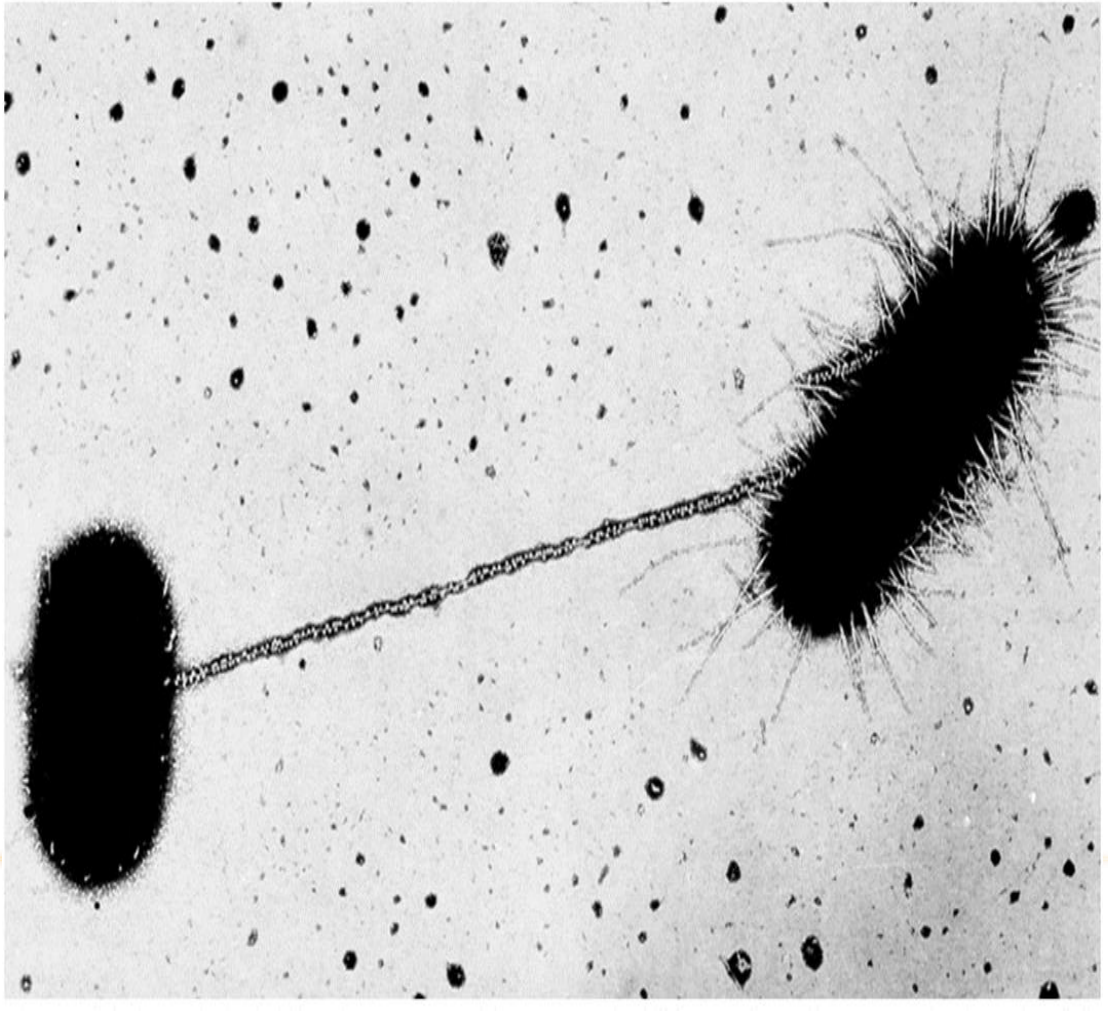
- 100 kb in length;
- tra operon, >28 gene contained;
- IS element for plasmid integration to host chromosome

Bacterial Fertility factor plasmid

- F factor contains the genes encoding proteins for building the sex pilus
  - F^+ cell attach to F^- cell for DNA transfer during bacterial conjugation
- Insertion Sequences (IS) in F factor assists in plasmid integration to host genome

F Pilus Mediated Cell-to-cell Conjugation

性菌毛



Recipient cell without pilus

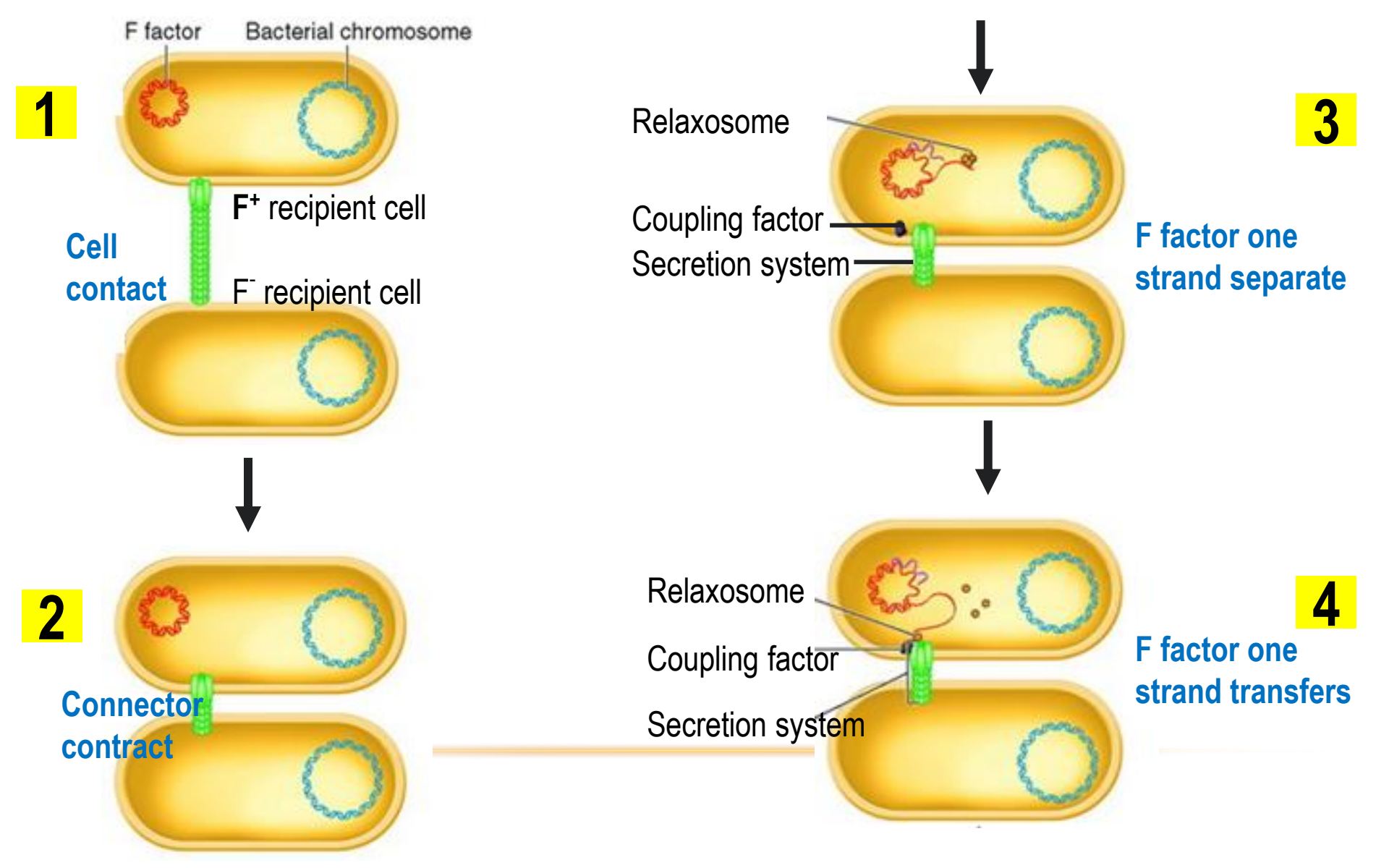
Donor cell with pilus

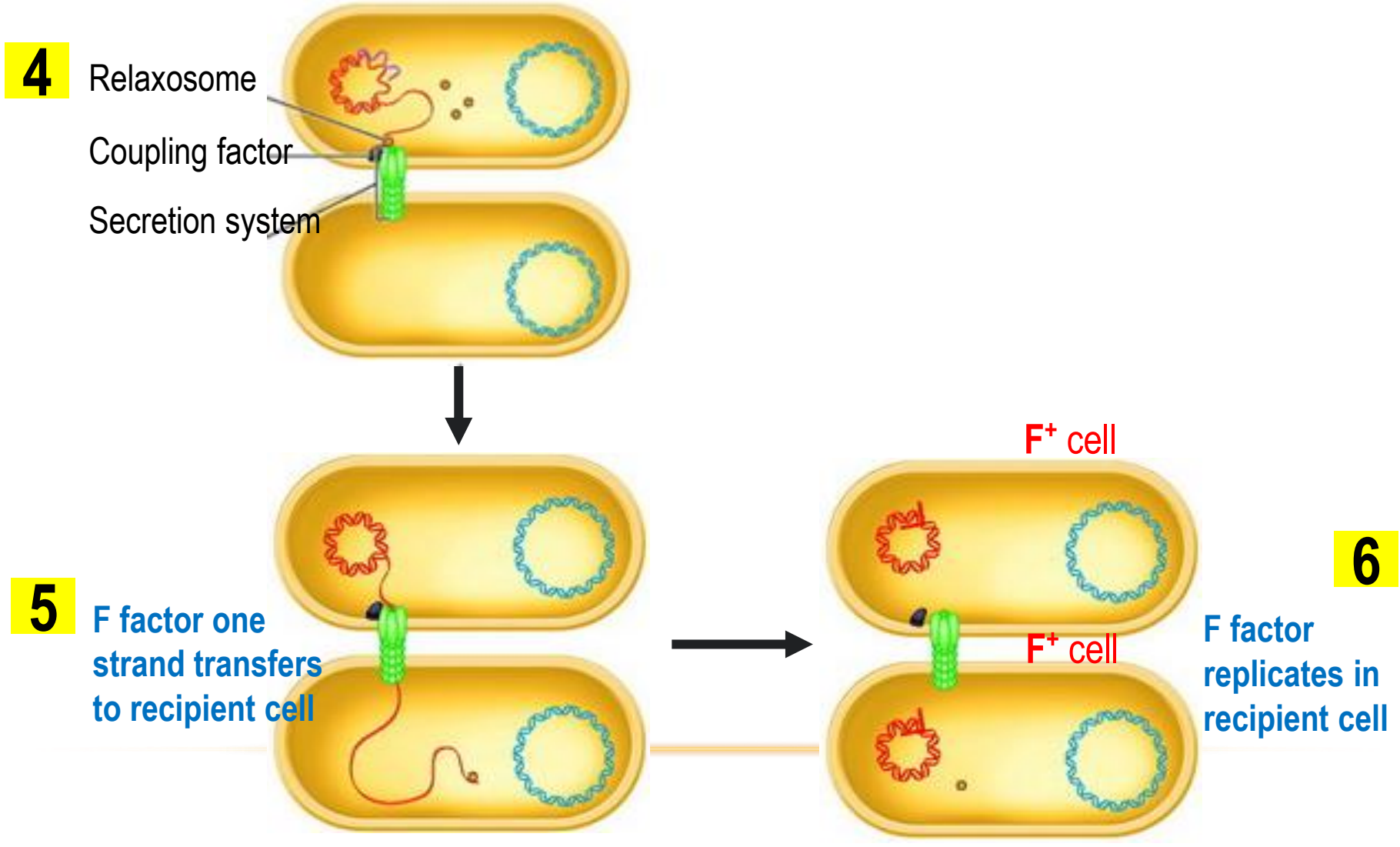
Three types of Bacterial Conjugation

- F^+ X F^- mating
- Hfr Conjugation
- F' Conjugation

Bacterial Conjugation

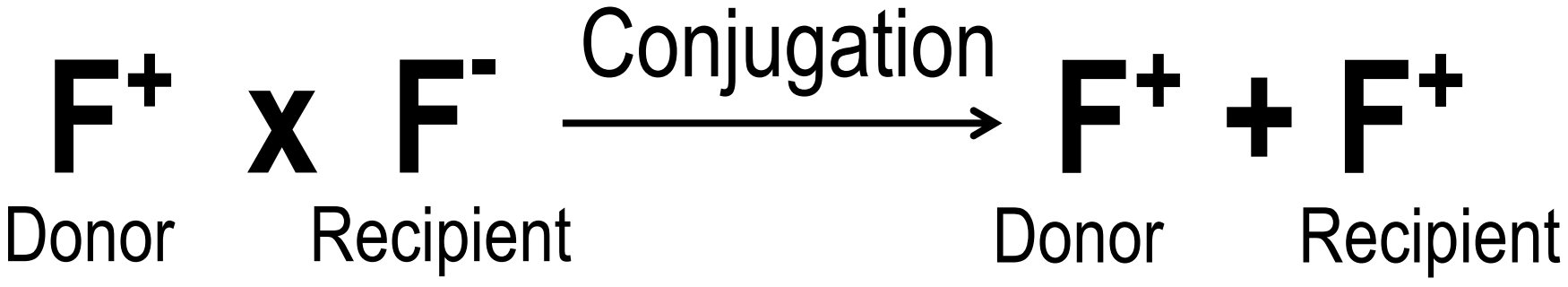
- F^+ X F^- mating
- Hfr Conjugation
- F' Conjugation


F factor
Bacterial chromosome
1
F+ recipient cell
Cell contact
F- recipient cell
2
Connector contract
Relaxosome
Coupling factor
Secretion system
3
F factor one strand separate
Relaxosome
Coupling factor
Secretion system
4
F factor one strand transfers


4
Relaxosome
Coupling factor
Secretion system
5
F factor one strand transfers to recipient cell
F+ cell
F+ cell
6
F factor replicates in recipient cell

F^+ X F^- mating

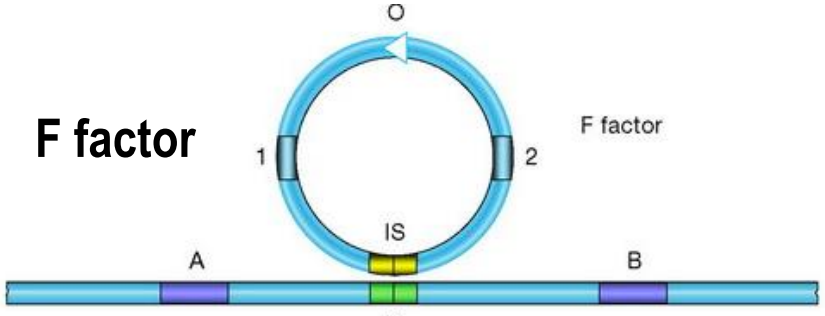
- One copy of the F factor is transferred to the recipient cell and does not integrate into the host chromosome.
- F factor plasmid is replicated by rolling circle mechanism.
- Donor cell chromosome DNA is not transferred.


F+ x F-
Conjugation
F+ + F+
Donor
Recipient
Donor
Recipient

Bacterial Conjugation

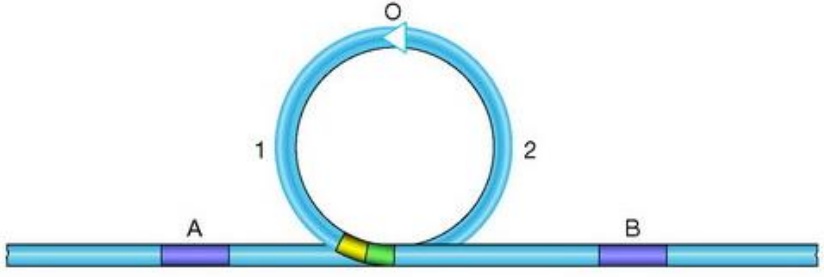
- F^+ X F^- mating
- **Hfr Conjugation**
- F` Conjugation

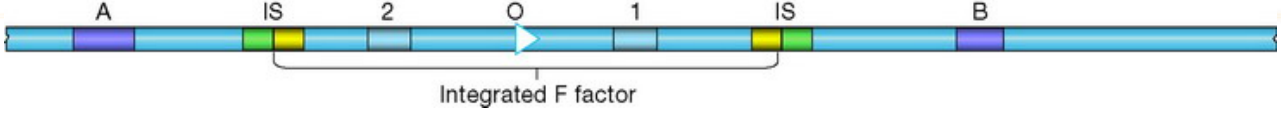
F factor Integration to Host Chromosome



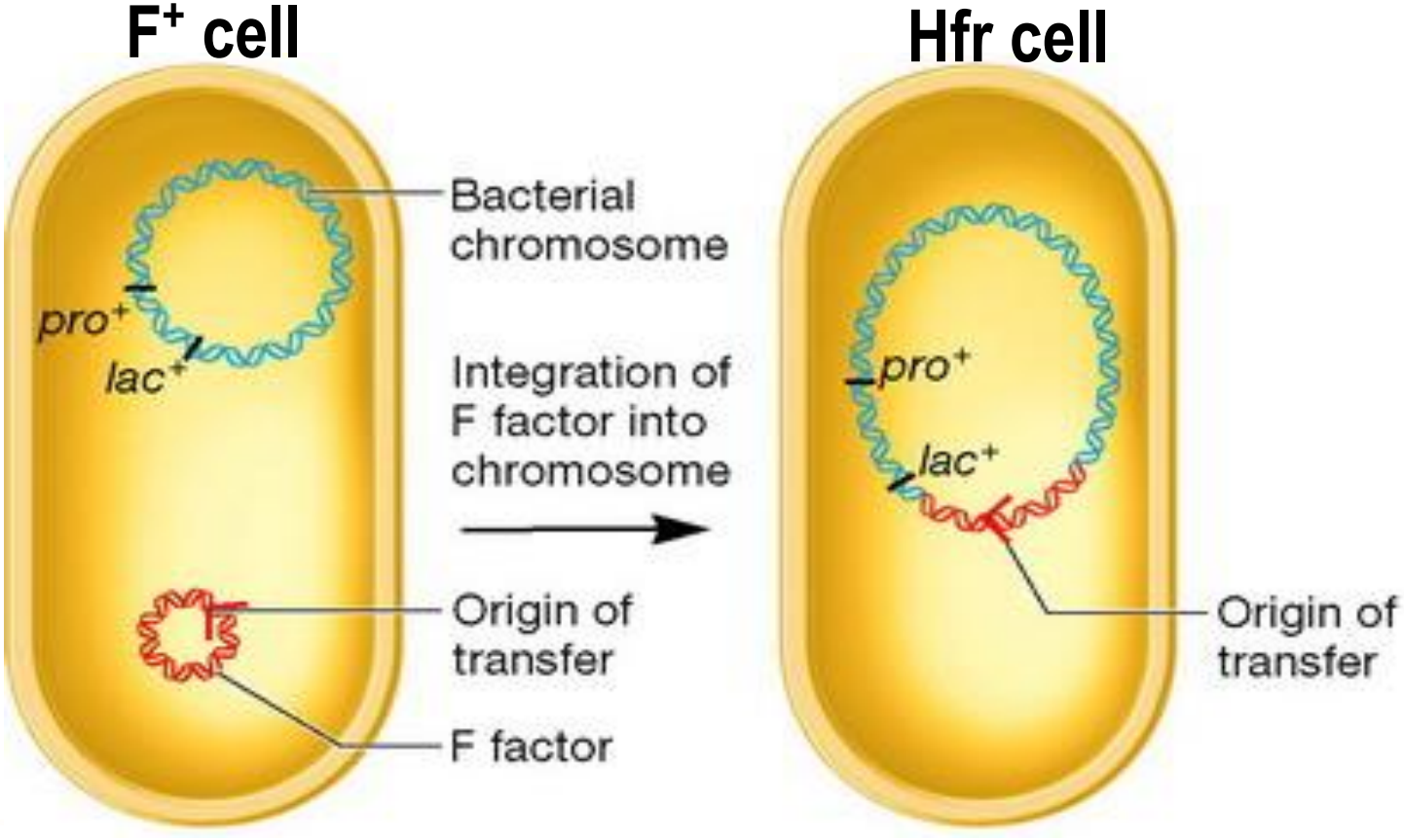
Integration ⇅ Disintegration

Insertion Sequences (IS) in F factor assists in plasmid integration to host genome.

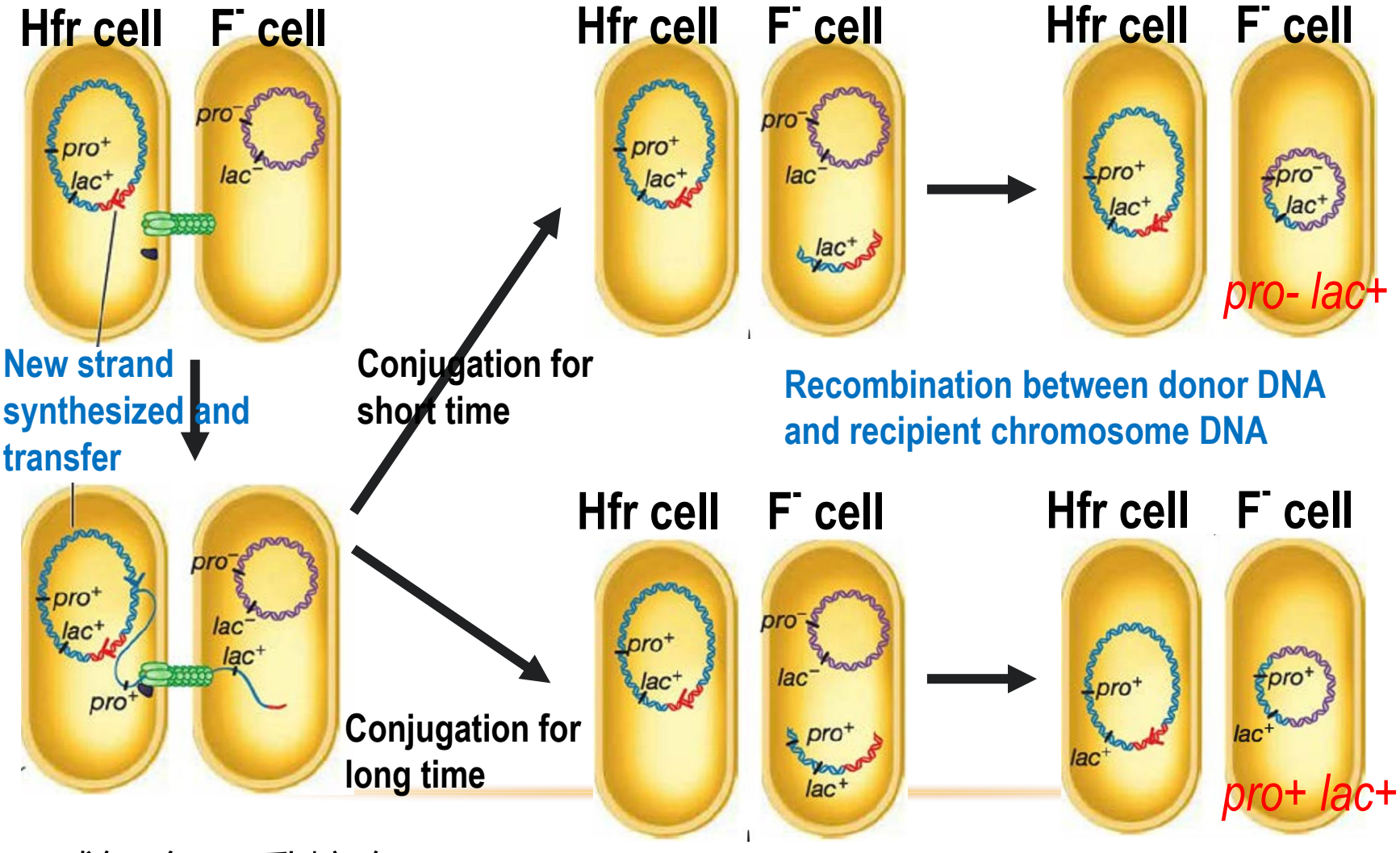




F factor Integration to host Chromosome



pro: 脯氨酸　*lac*: 乳糖酶

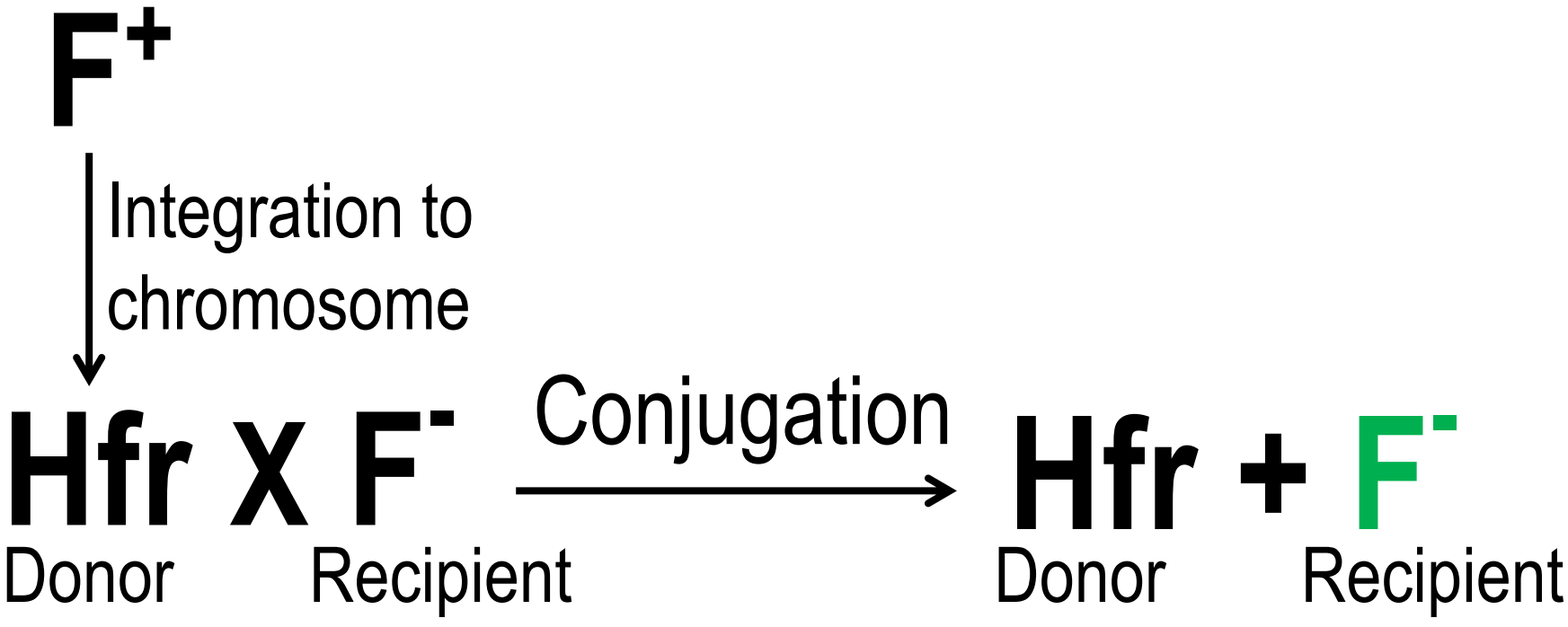


pro: 脯氨酸 *lac*: 乳糖酶

Blue: donor chromosome DNA, Red: F factor DNA, Purple: recipient chromosome

Hfr Conjugation

- High-frequency recombination(Hfr) – donor's fertility plasmid has been integrated into the donor bacterial chromosome.
- When conjugation occurs, a portion of the chromosome and a portion of the fertility plasmid are transferred to the recipient cell.
- Because the cell contact lasts for limited time, a complete copy of the F factor is usually not transferred.

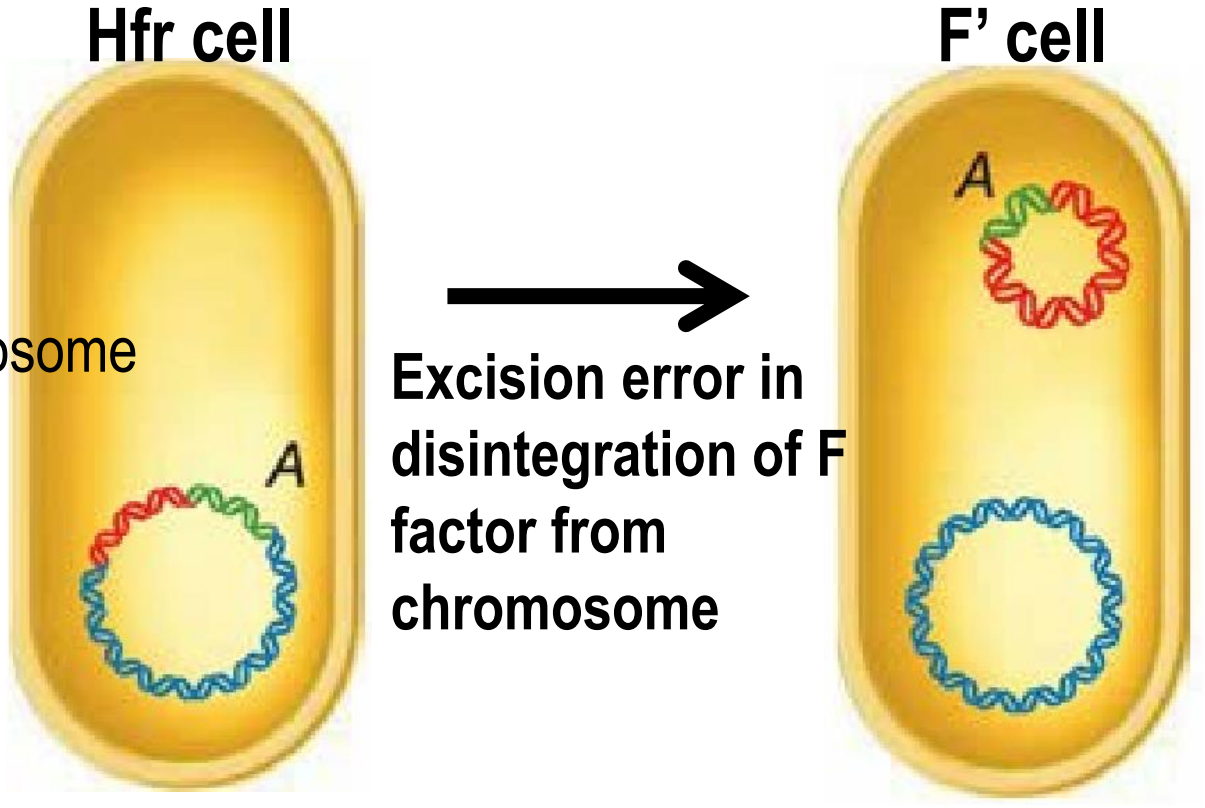


F⁻： Recipient cell transferred with some plasmid genes and some donor chromosomal genes

Bacterial Conjugation

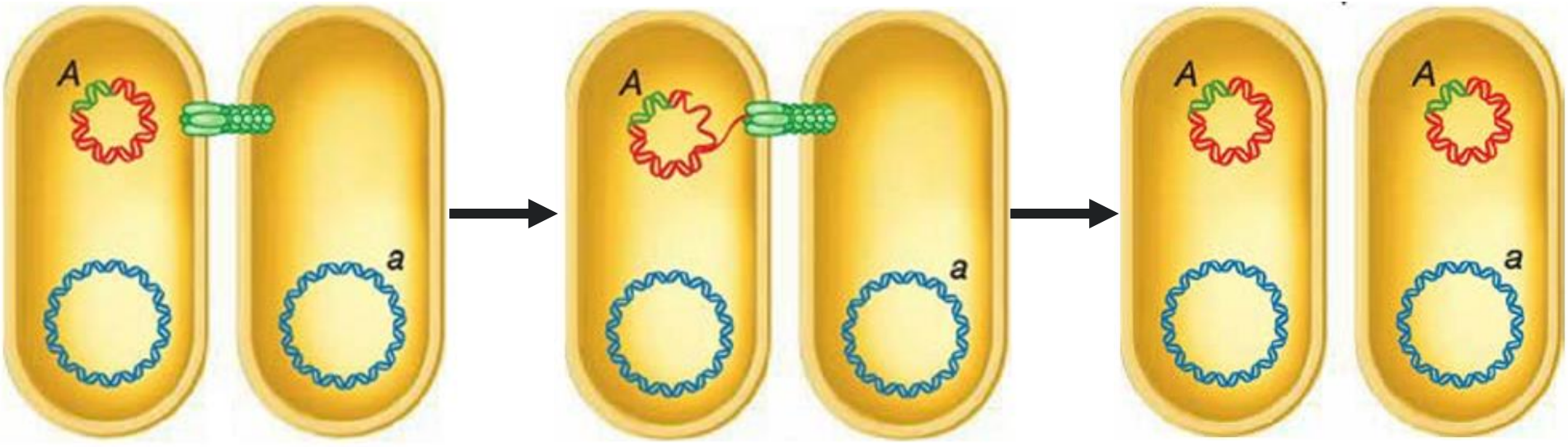
- F^+ X F^- mating
- Hfr Conjugation
- **F' Conjugation 性导**

F factor Disintegration from chromosome



Blue: chromosome DNA, Red: F factor DNA, Green: a chromosome gene

F’ Conjugation



Cell contact

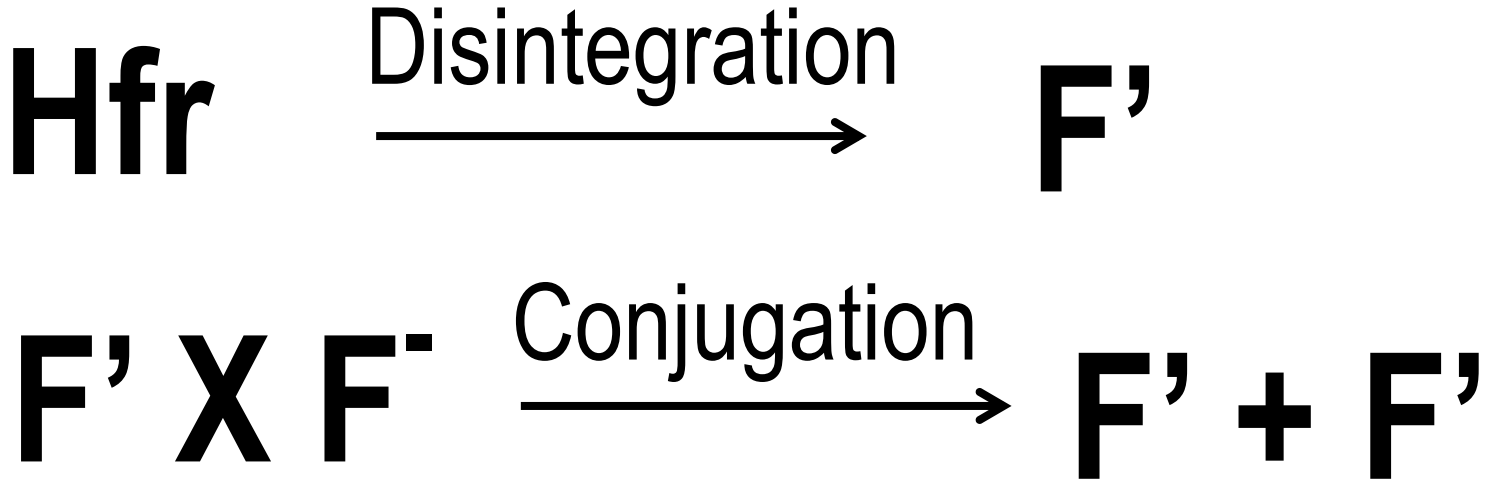
New strand synthesize and transfer

F’ factor replication

Blue: chromosome DNA, Red: F factor DNA, Green: a chromosome gene

F’ Conjugation 性导

- Integrated F factor leaves the host chromosome incorrectly, forms F’ factor.
- Part of original F factor is left in the host chromosome.
- Some genes from host chromosome moved along with the disintegrated F factor, becoming part of the F’ factor.
- These genes can be transferred to the recipient cell by F’ conjugation.


Hfr
Disintegration
F’
F’ X F-
Conjugation
F’ + F’

Bacterial Conjugation

- F^+ X F^- mating
- Hfr Conjugation
- F' Conjugation

厦门大学
生|命|科|学|学|院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

MICROBIOLOGY

Chapter 12

12.7 Transduction in Bacteria
细菌转导

Three Mechanisms of HGT In Bacteria

1. Transformation 转化
2. Conjugation 接合
3. Transduction 转导

Background

- 1946, Joshua Lederberg discovered the Bacterial Conjugation in *E.coli.*

- 1950, Bernard David proved the conjugation requires Cell-to-Cell contact in *E.coli*.

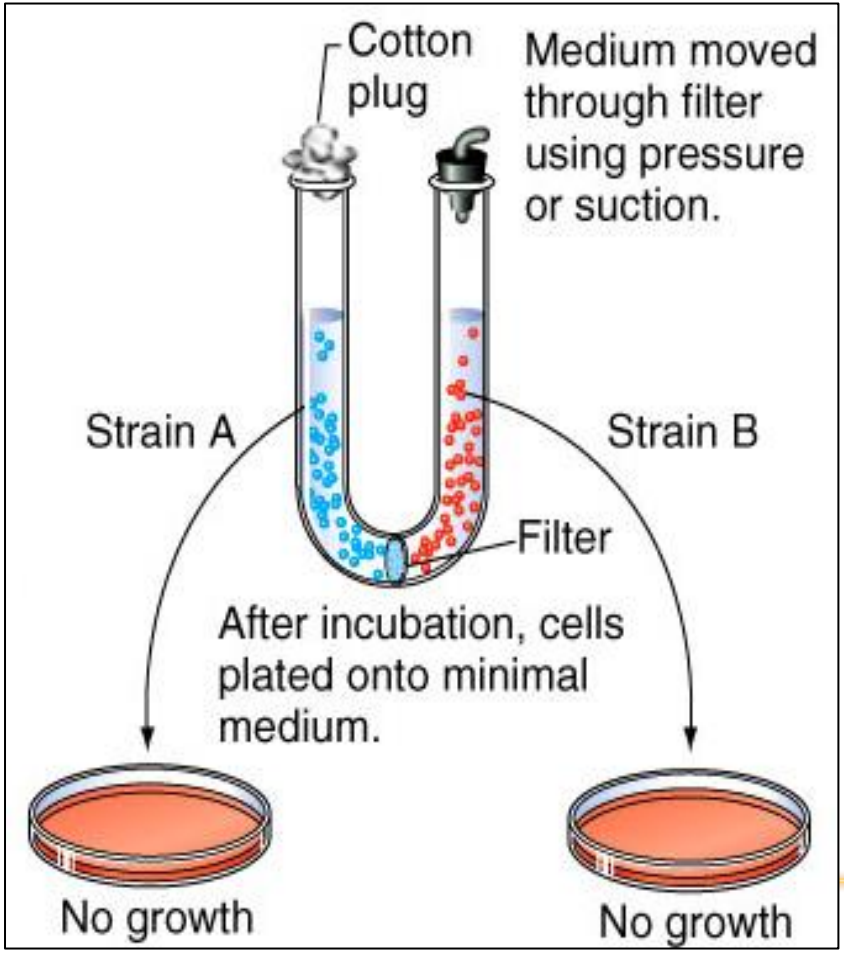
- 1951, Joshua Lederberg and Norton Zinder tested the conjugation phenomenon in more bacteria, but found something unexpected in *Salmonella typhimurium*.

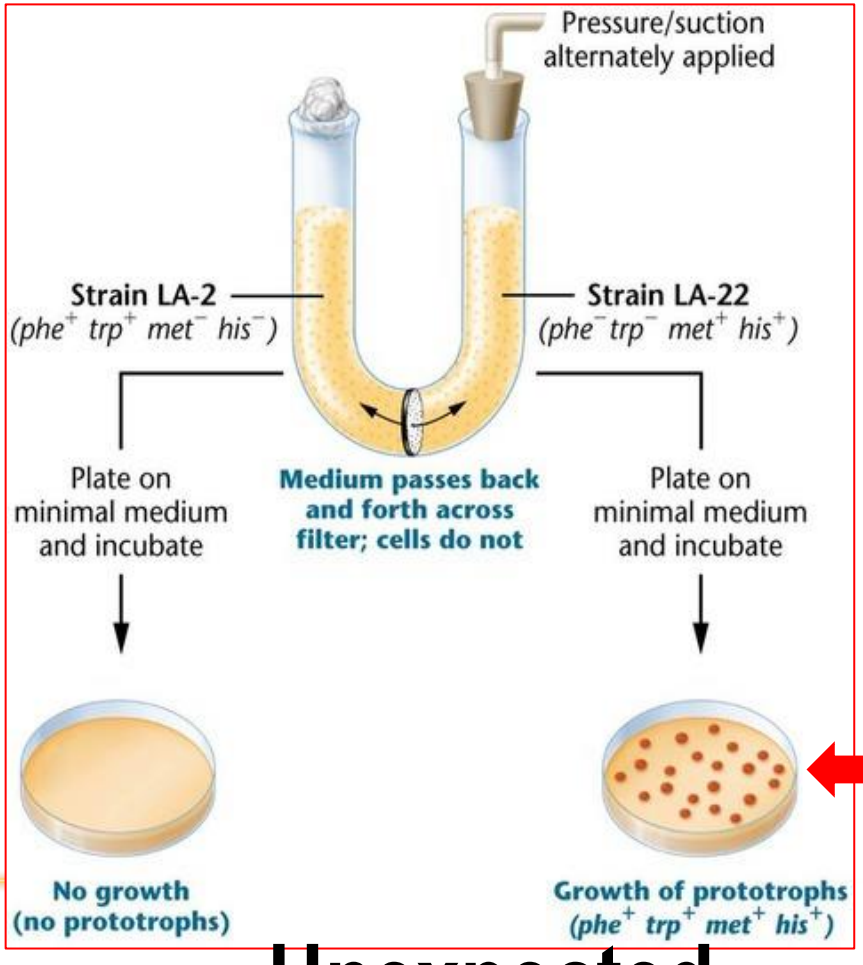
鼠伤寒沙门氏菌

鼠伤寒沙门氏菌

E.coli

Salmonella typhimuriu





Unexpected

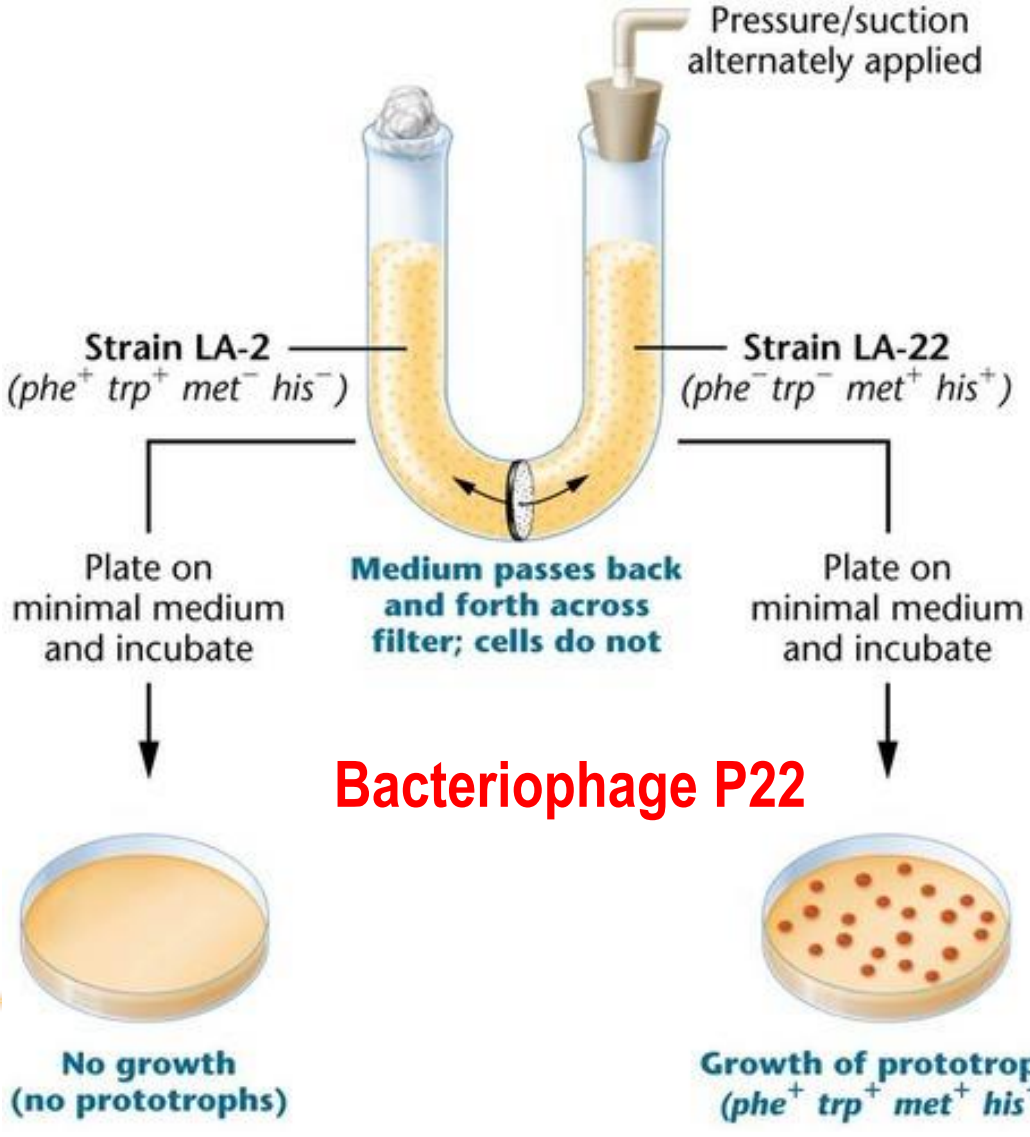
A new type of gene transfer mechanism in *Salmonella typhimurium*

- Lederberg, J.; Lederberg, E. M.; Zinder, N. D.; Lively, E. R. (1951). "Recombination analysis of bacterial heredity". Cold Spring Harbor symposia on quantitative biology 16: 413–443. PMID 14942753.

- Zinder, N. D.; Lederberg, J. (1952). "Genetic Exchange in Salmonella". Journal of bacteriology 64 (5): 679–699. PMC 169409. PMID 12999698.

Joshua Lederberg
Experiment
1951

Salmonella typhimurium
鼠伤寒沙门氏菌



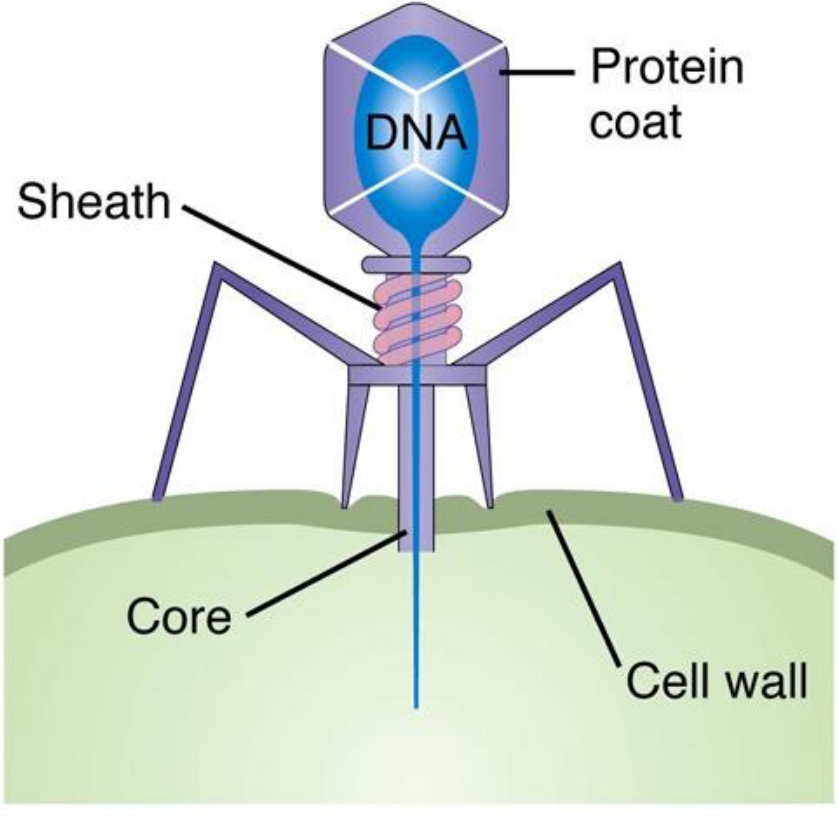
phe: 苯丙氨酸
trp:
met：蛋氨酸
his：组氨酸

原养型

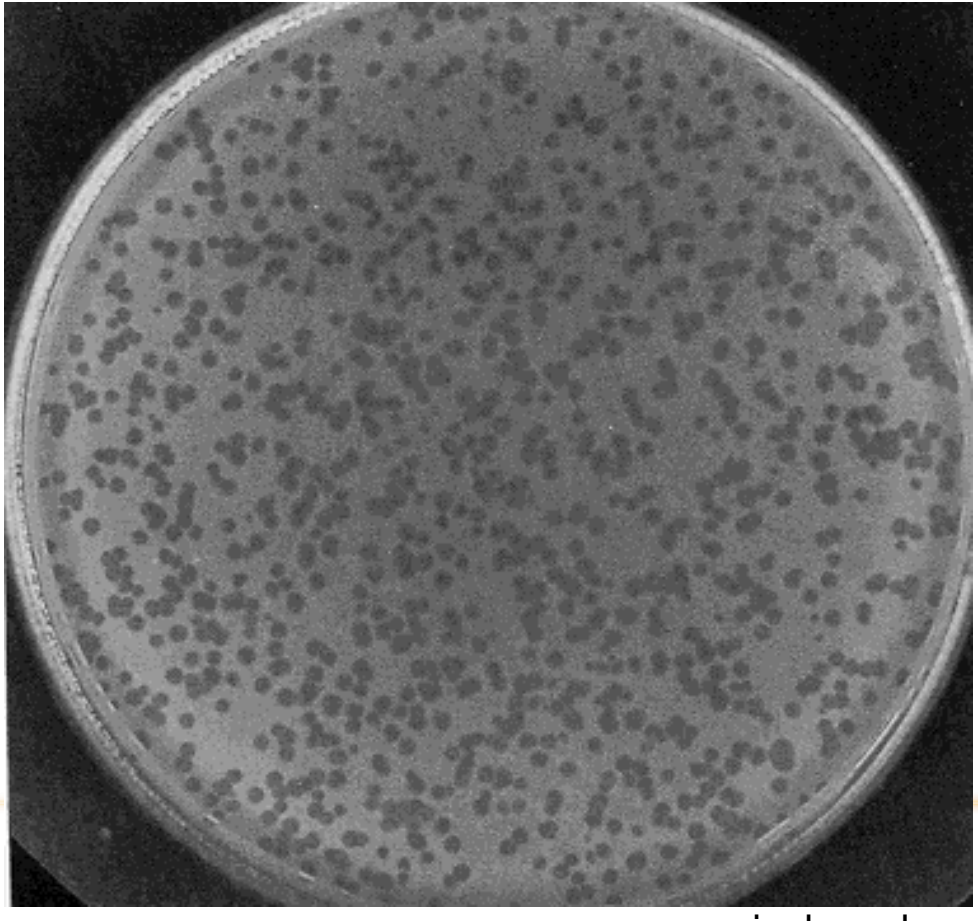
Transduction in Bacteria

Gene transfer using bacteria virus (bacteriophage) as a carrier of DNA from a donor cell to a recipient cell.

Bacteria Virus (Bacteriophage)

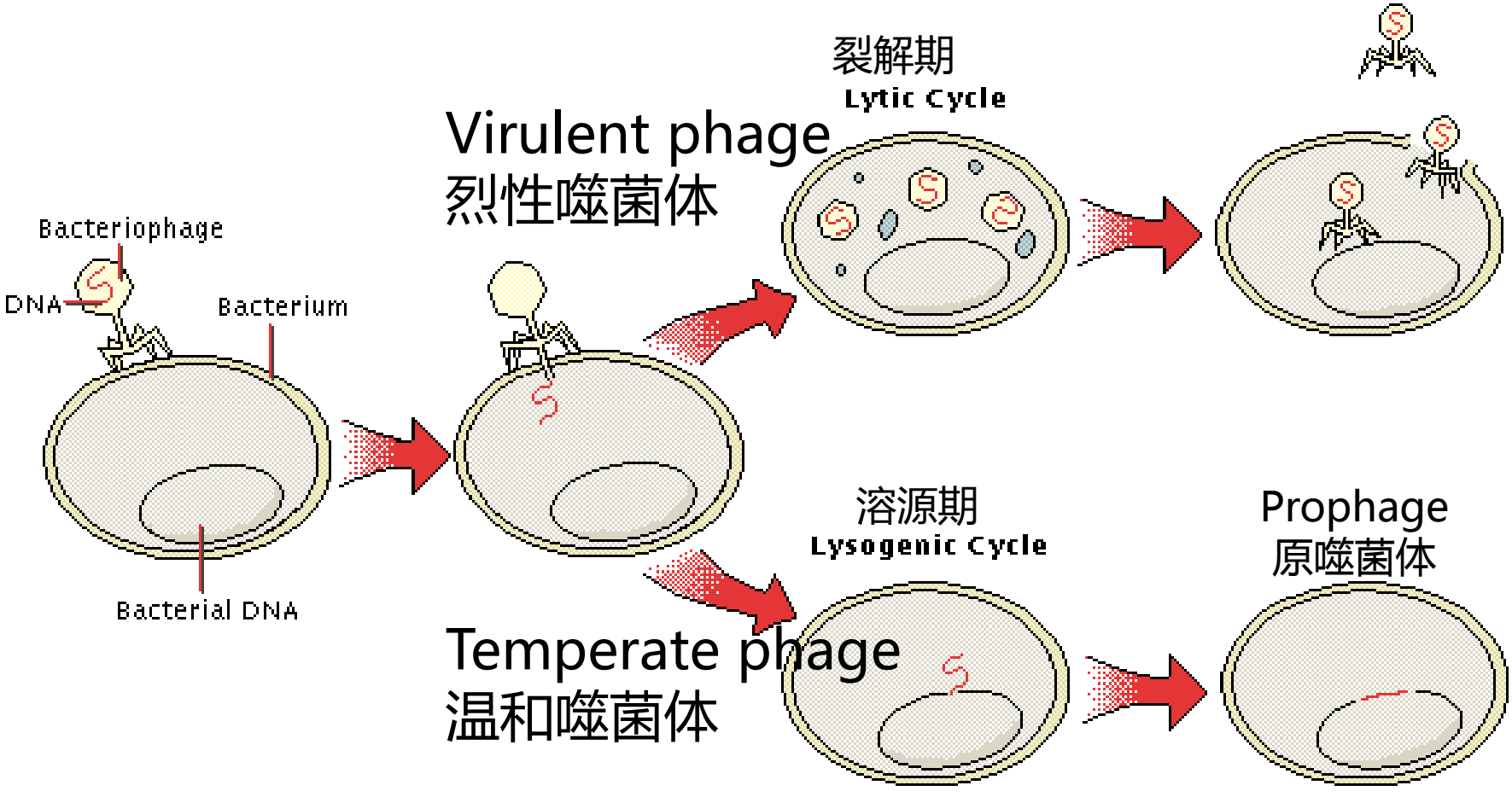


Circular Lysis Zones (Plaques) where bacteria were killed by bacteriophage infection



sci.sdsu.edu

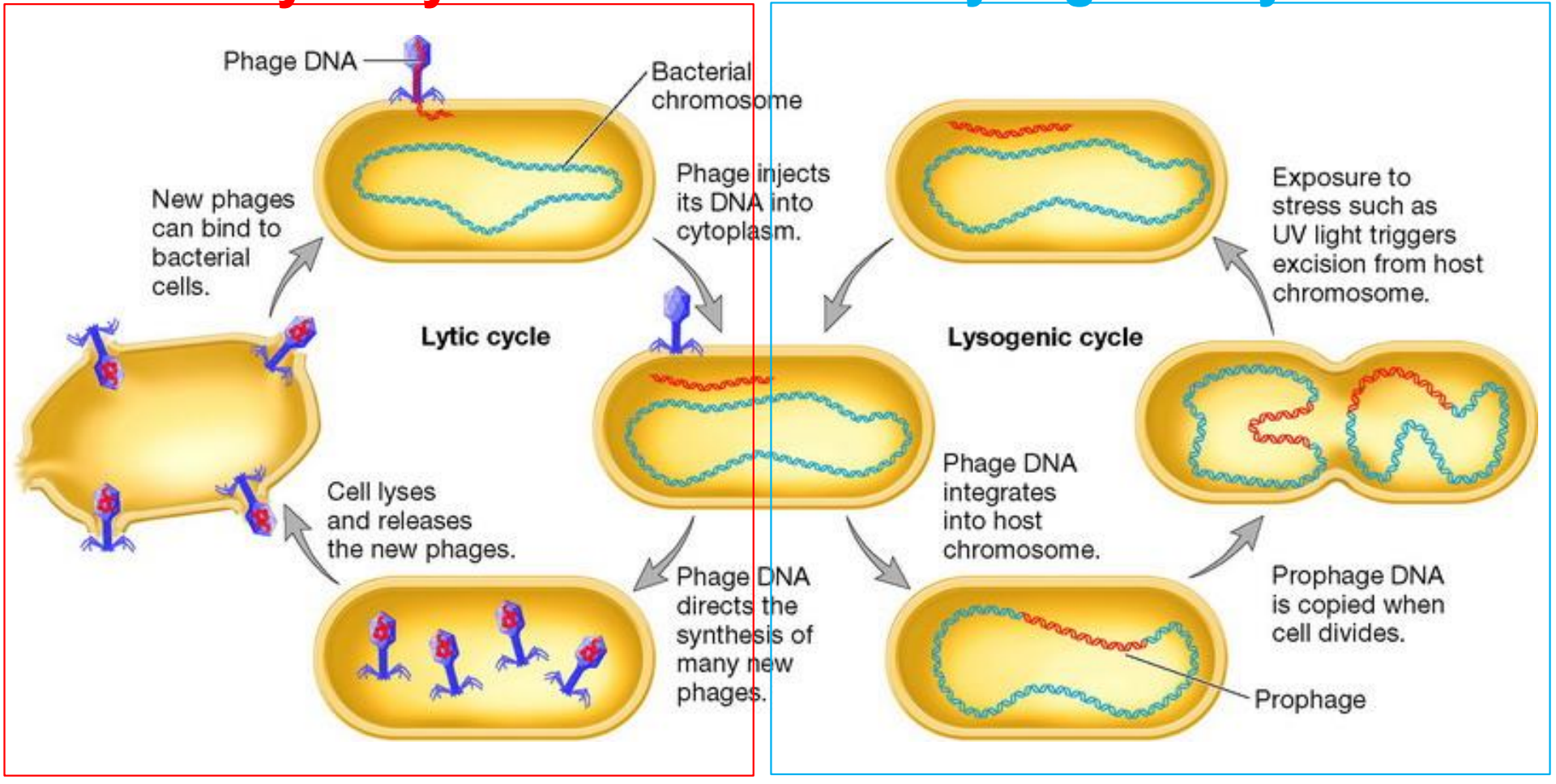
Virulent and Temperate Bacteriophage



Lytic and Lysogenic Cycles of Temperate Phage

Lytic cycle

Lysogenic cycle



Generalized and Specialized Transduction

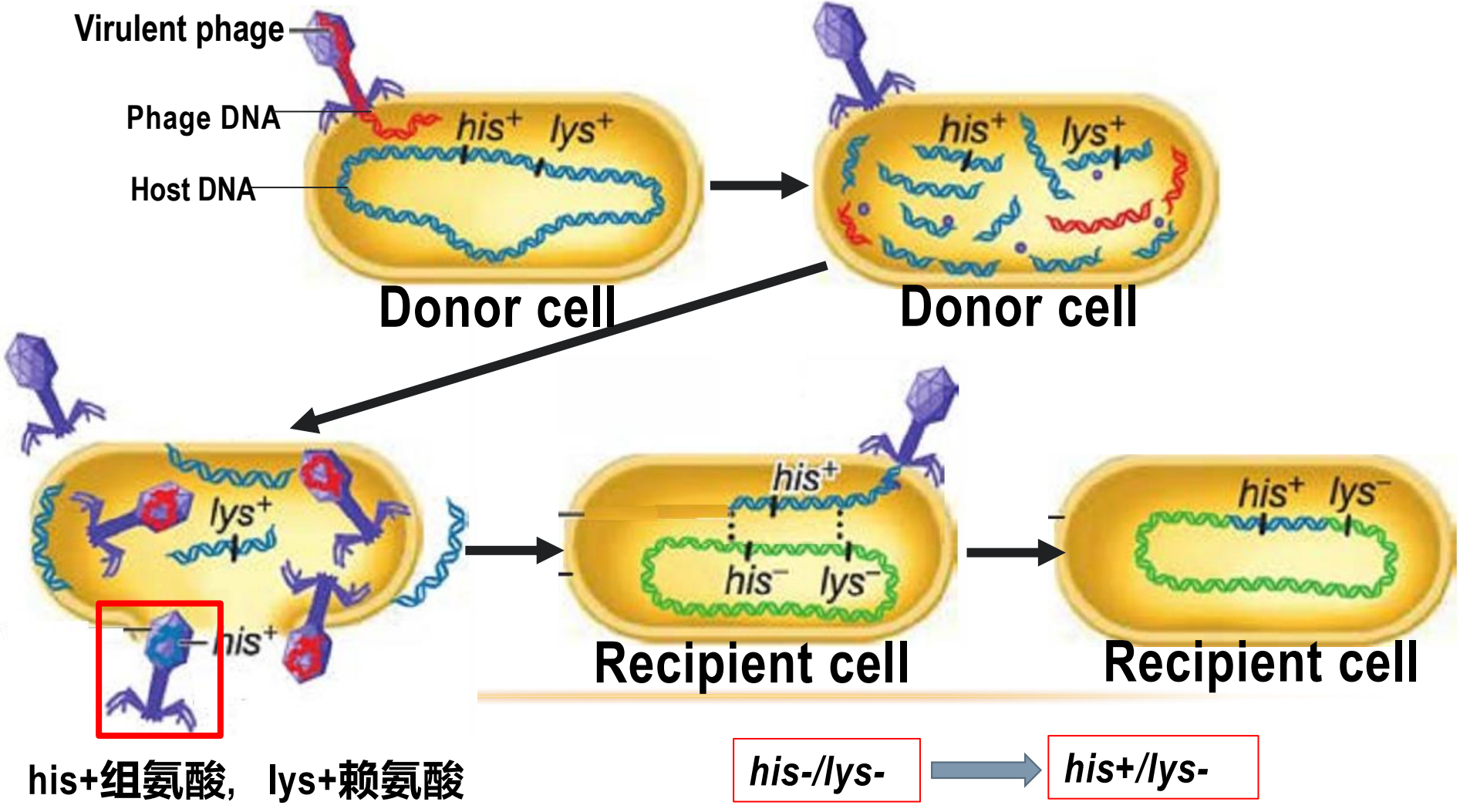
- **Generalized transduction　普遍性转导**

Random DNA fragments of hydrolyzed host chromosome are picked up by the bacteriophage during assembly; any gene could be transferred to the recipient via transduction in this way

- **Specialized transduction　局限性转导**

Specific part of the host genome is regularly incorporated into the virus (Temperate phage) and transmitted to the recipient.

Generalized transduction



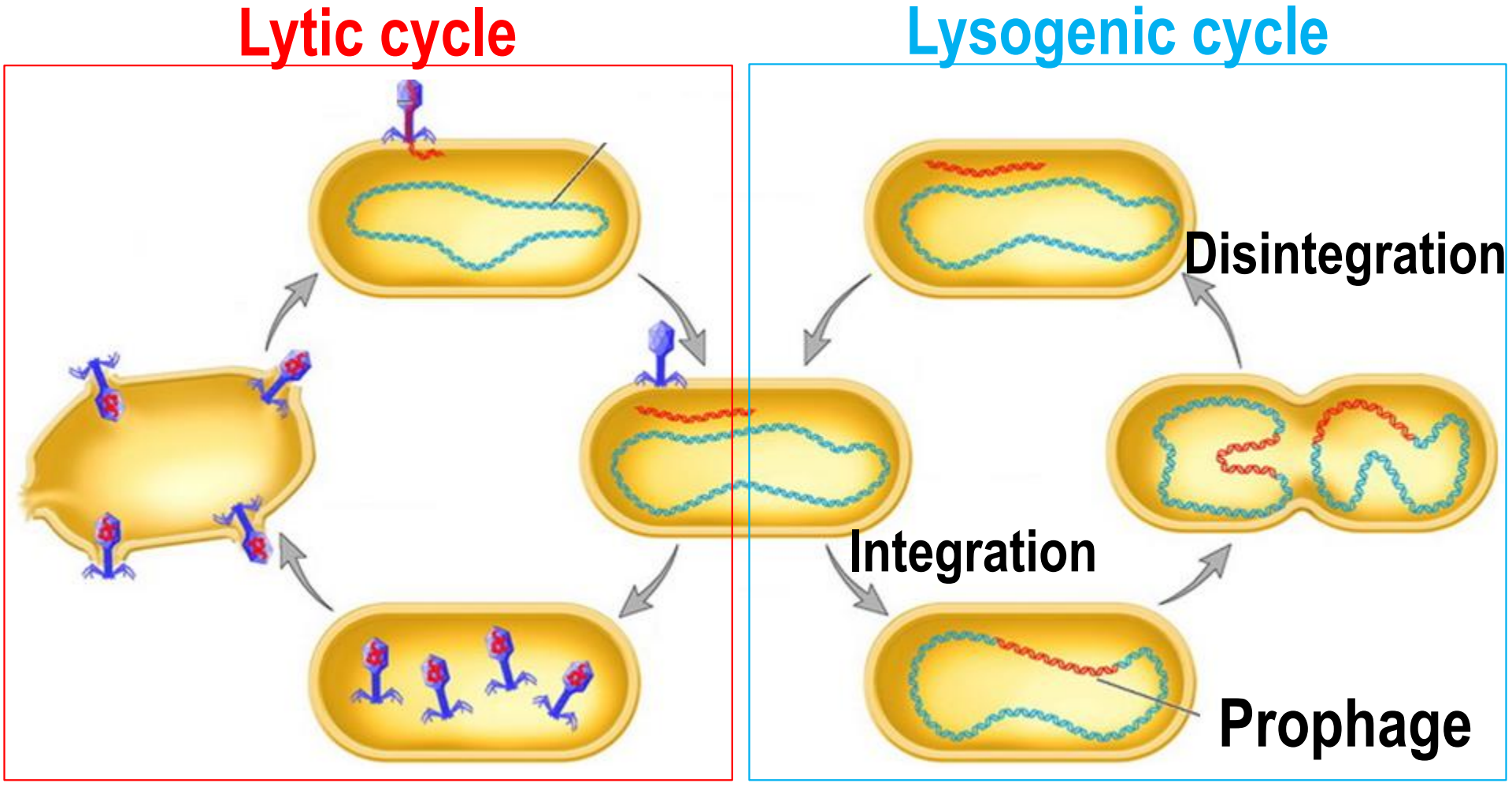
his+组氨酸， lys+赖氨酸

his-/lys- → his+/lys-

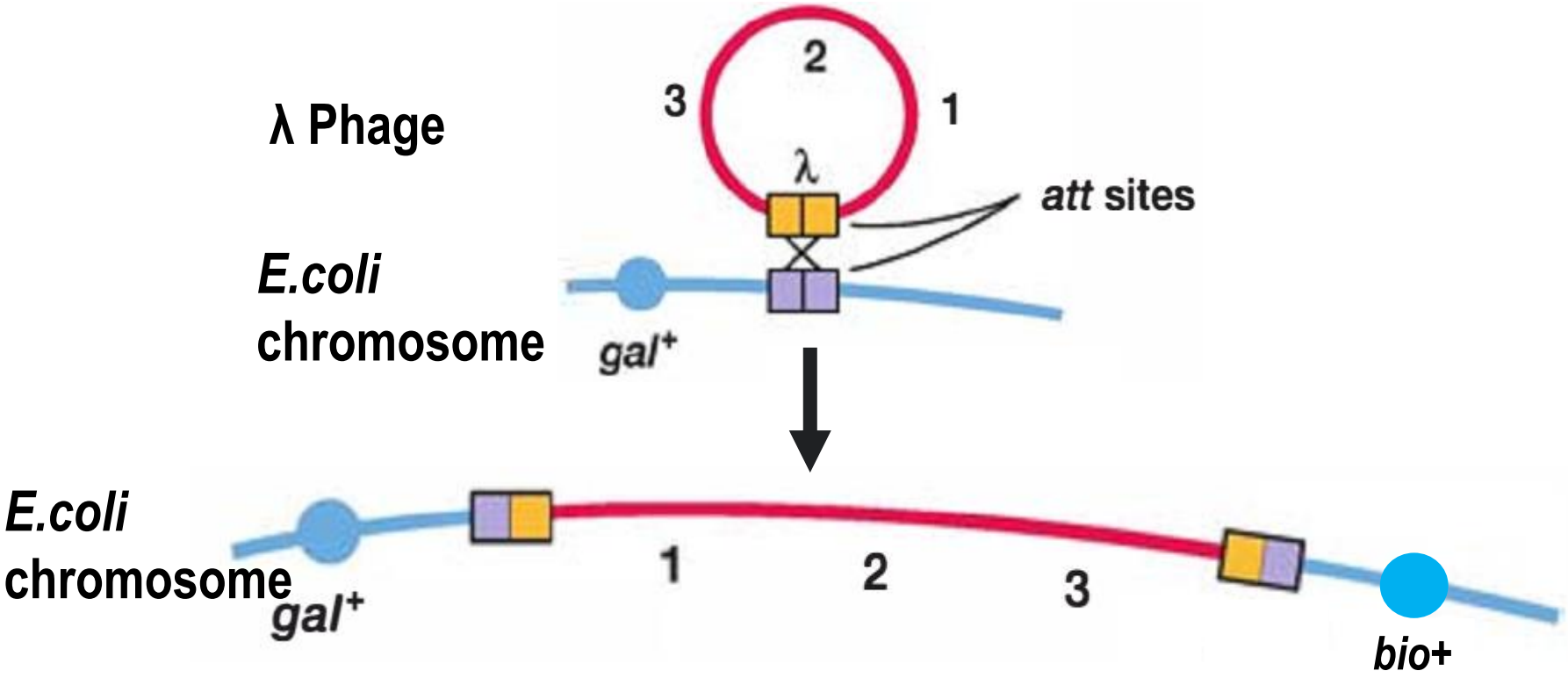
Generalized transduction

- Occurs during lytic cycle of virulent phage or some temperate phage.
- During virus particle assembly, fragments of host chromosome DNA mistakenly packaged into phage-generating transducing particle
- Any part of bacterial genome DNA can be transferred to recipient cell。

Prophage: Phage DNA integrated to host chromosome

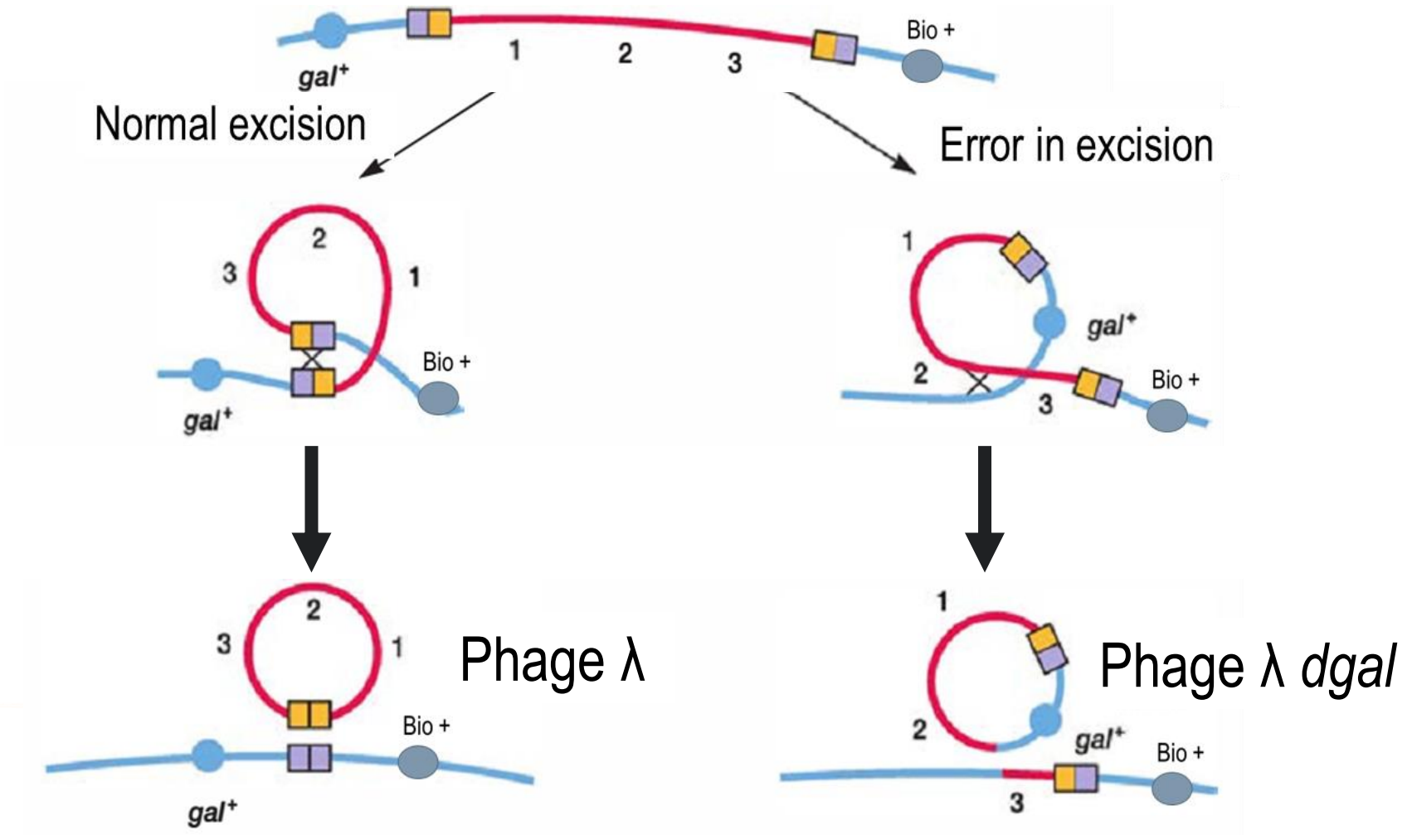


λ Phage Integrates at Specific Site in *E.coli* Chromosome

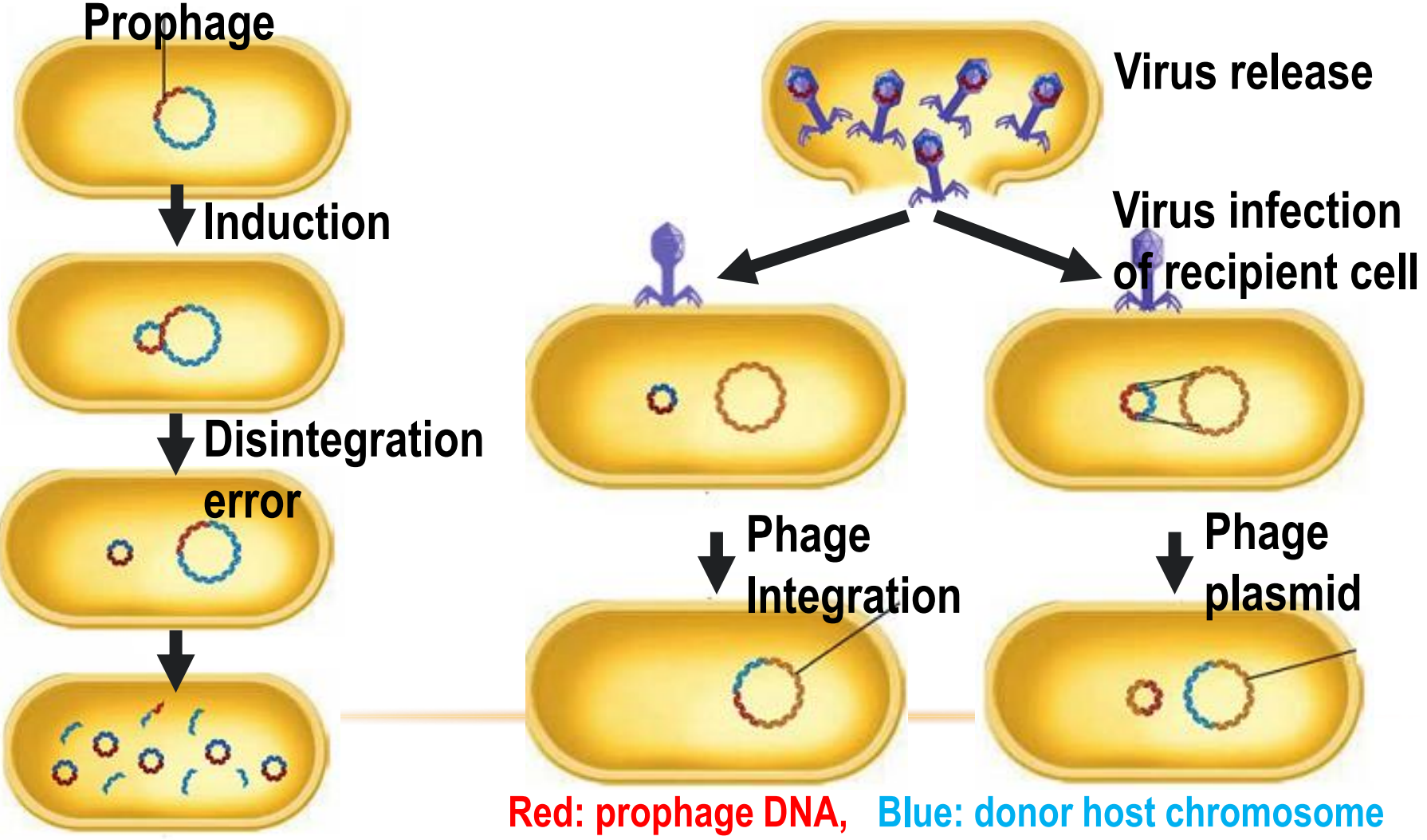


Site-specific recombination takes place between a specific attachment site on the circular λ phage DNA and a specific region on the bacterial chromosome between the *gal* and *bio* genes.

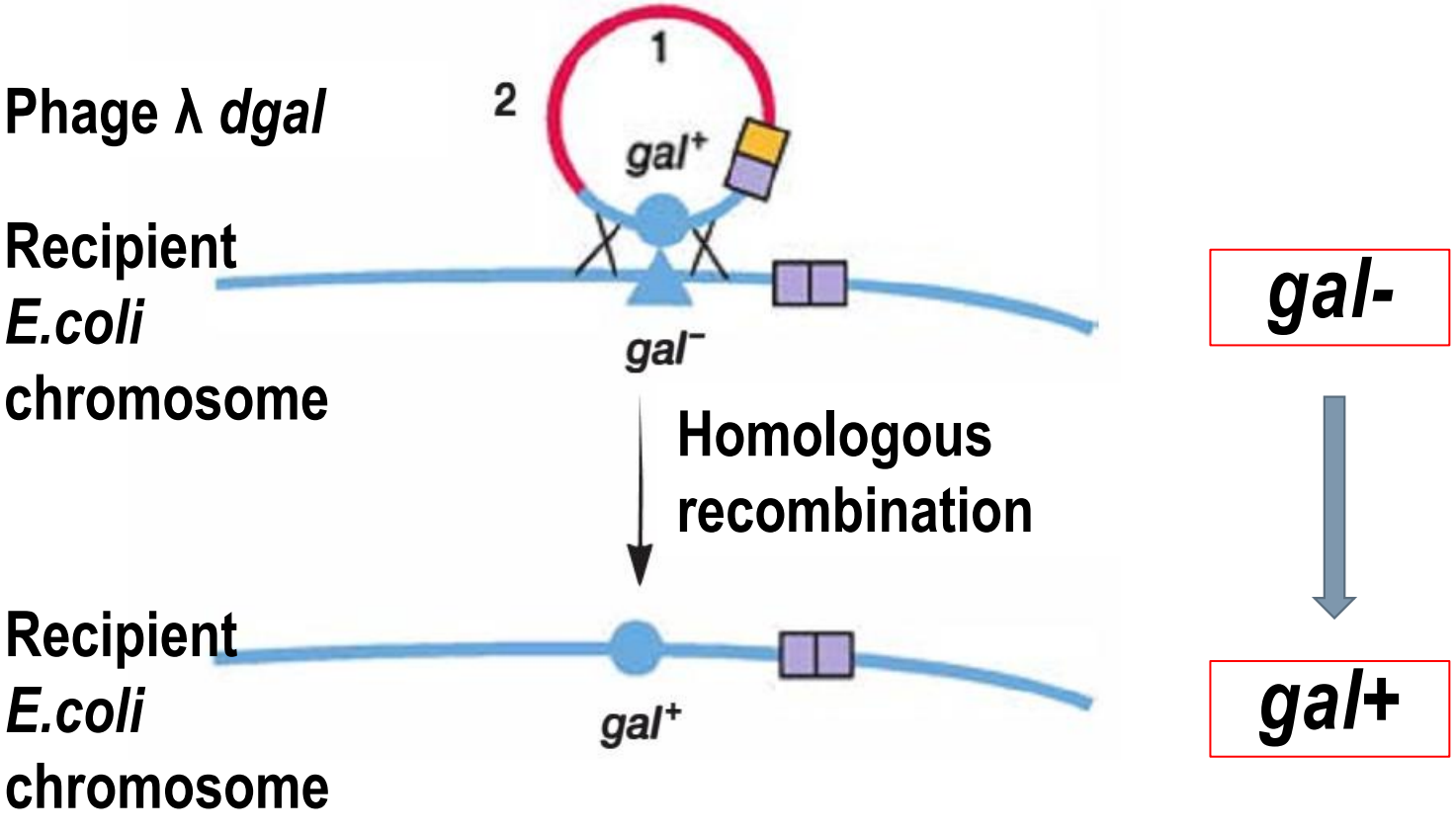
Prophage excision from host genome



Specialized Transduction by a Temperate Phage



Host Gene Transfer via Specialized Transduction

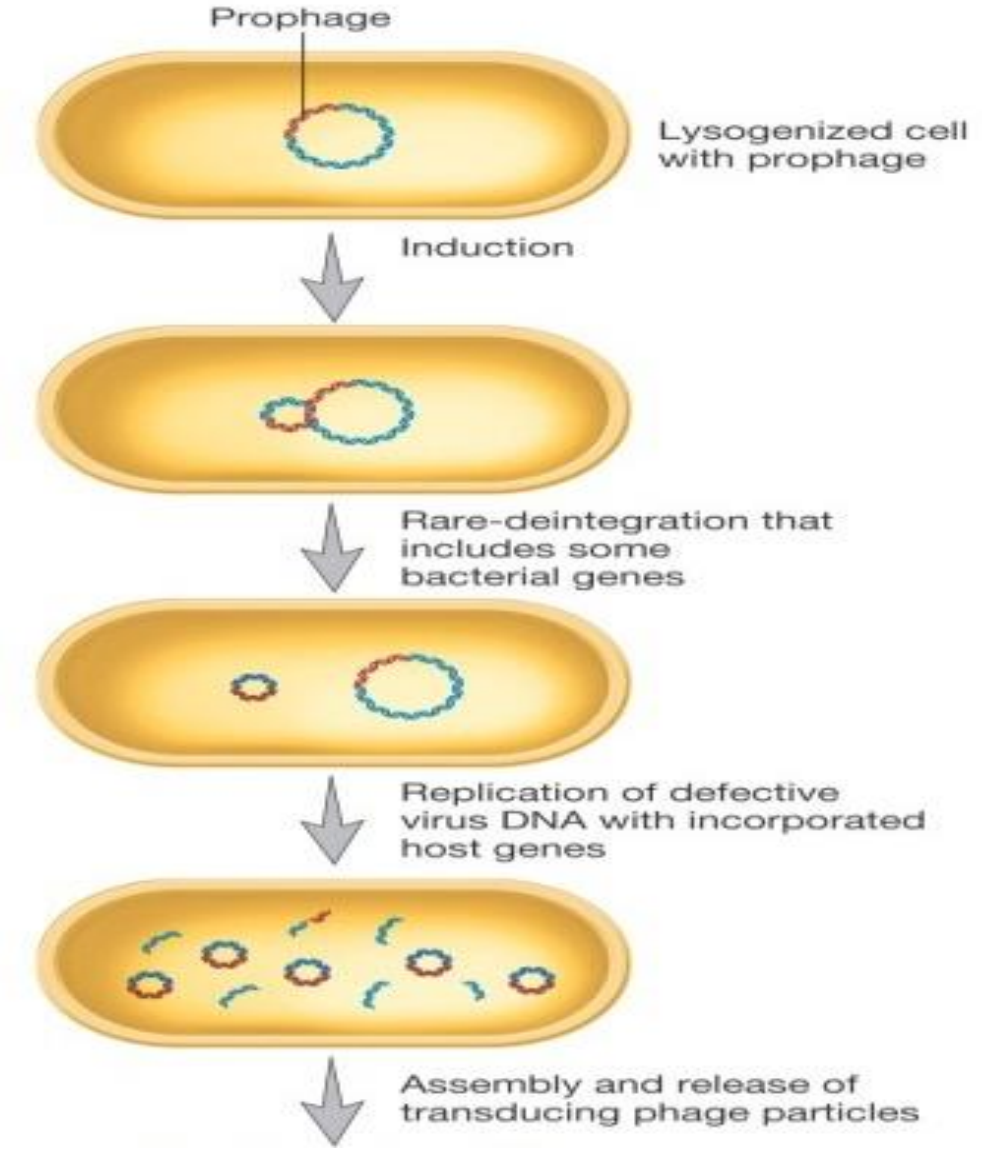


Specialized Transduction

- Carried out only by temperate phages that have established lysogeny.
- Occurs when prophage is incorrectly excised from host chromosome.
- Only specific portion of bacterial genome is transferred via transducing virus particle

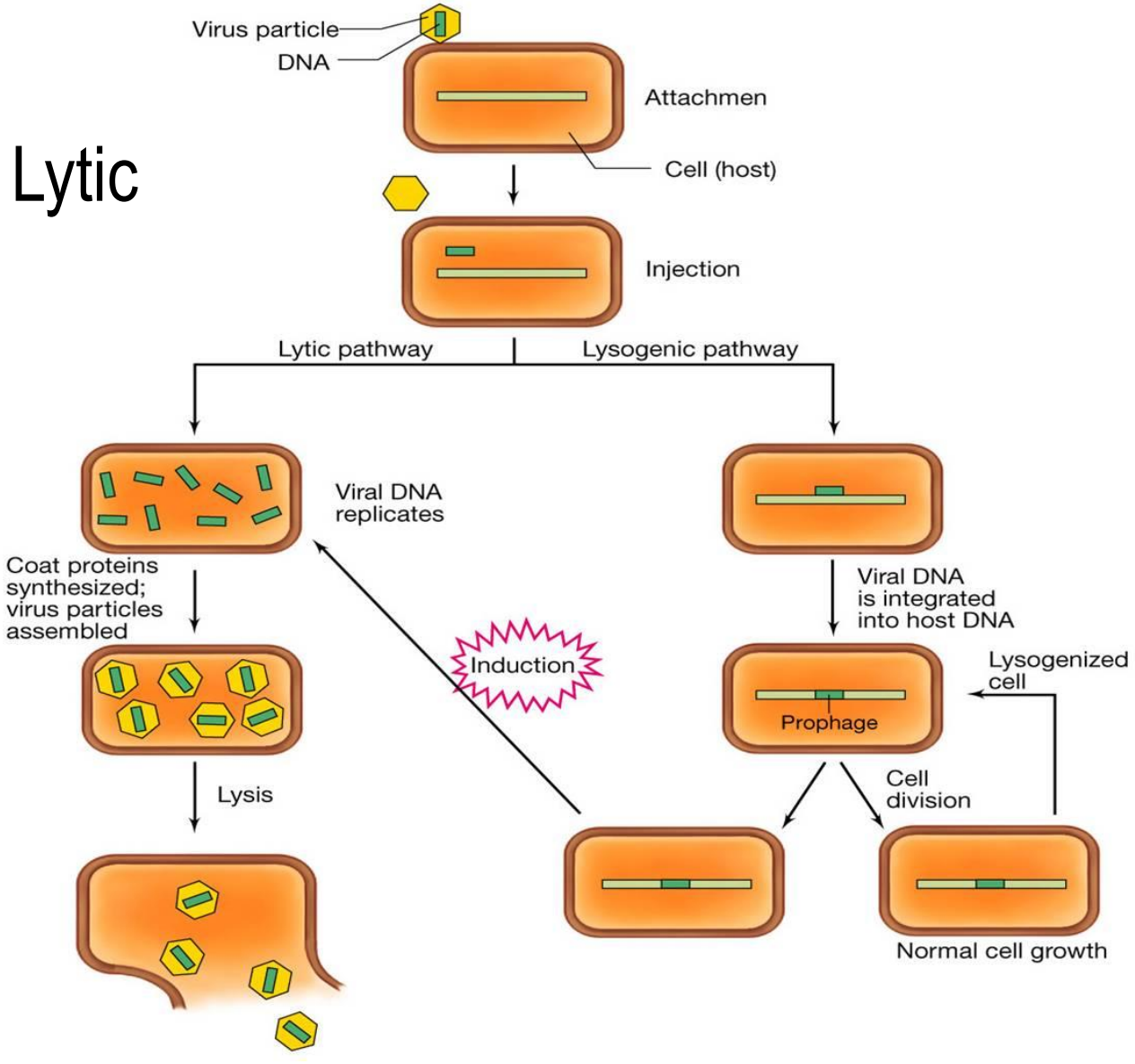
Question?

What triggers the disintegration of prophage from host chromosome?



Induction (诱导)
from Lysogenic to Lytic
By UV radiation

Danger signal



Three Mechanisms of HGT In Bacteria

1. Transformation 转化
2. Conjugation 结合
3. Transduction 转导

Three Mechanisms of HGT In Bacteria