Nucleic Acid Metabolism

Contents

Section 1	Nucleic Acid Catabolism
Section 2	Nucleic Acid Biosynthesis
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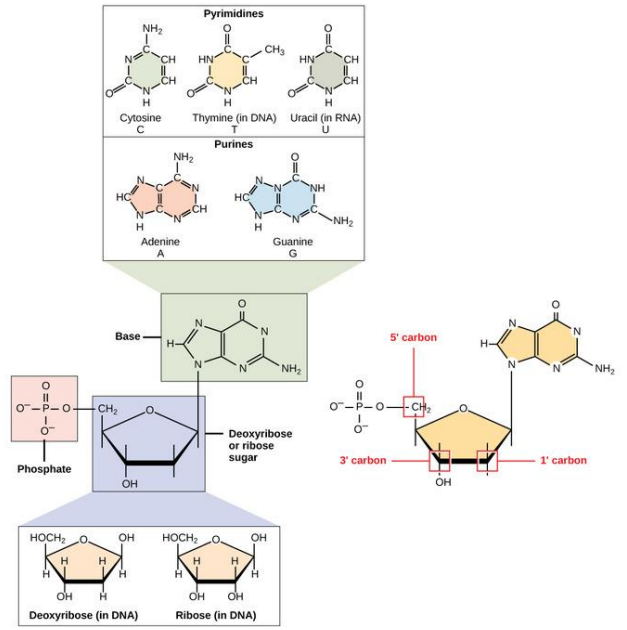
Nucleic Acid Catabolism



Nucleic acid

↓ Nucleases（核酸酶）

Nucleotides（核苷酸）

↓ Nucleotidases / phosphatases（核苷酸酶 / 磷酸酶）

Phosphate ← → **Nucleosides（核苷）**

↓ Nucleoside phosphorylases（核苷磷酸化酶）

ribose-1-phosphate（核糖-1-磷酸） ← → **Nitrogenous bases（含氮碱基）**

↓

carbohydrate metabolism

Degradation or salvage?（降解或补救合成？）

Nucleic Acid Catabolism

Nucleases（核酸酶）

I. Classified by substrate（按底物分类）

- **DNases（DNA酶）**: degrade DNA（降解 DNA）
- **RNases（RNA酶）**: degrade RNA（降解 RNA）

II. Classified by mode of action（按作用方式分类）

- **Exonucleases（外切核酸酶）：**Remove nucleotides one at a time from the ends of nucleic acid chains
- **Endonucleases（内切核酸酶）：**Cleave phosphodiester bonds within a nucleic acid chain
- **Restriction endonucleases（限制性内切酶）：**A special class of endonucleases that recognize specific DNA sequences

Nucleic Acid Catabolism

Restriction endonucleases

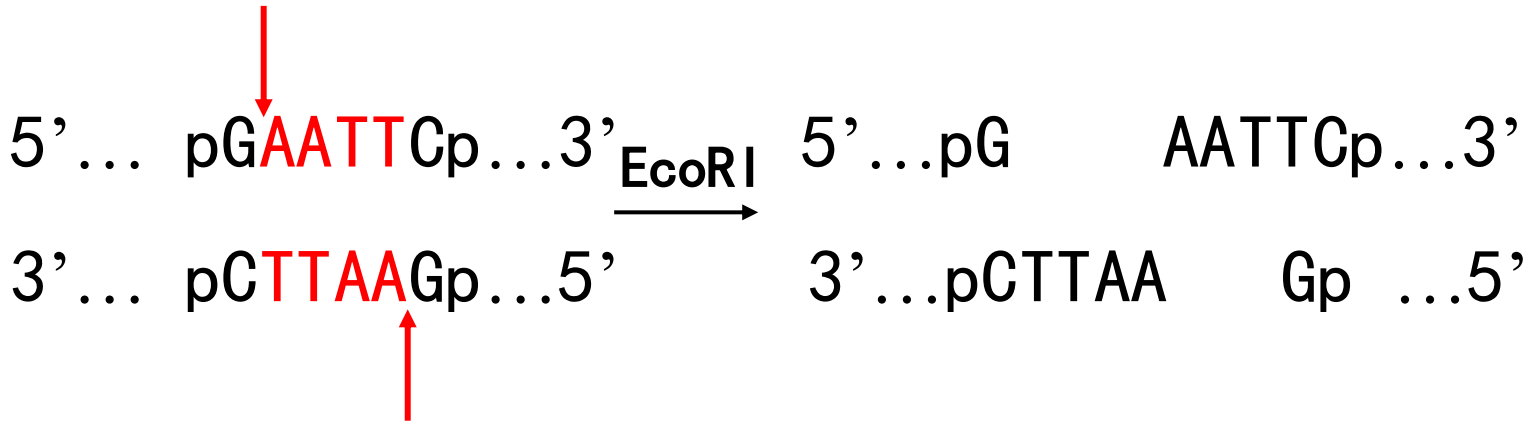
Restriction endonucleases are bacterial enzymes that recognize specific sequences in **double-stranded DNA（双链 DNA）** and cleave the DNA at or near these recognition sites. They are part of bacterial **defense systems** against foreign DNA, such as bacteriophage DNA（噬菌体 DNA）.

- **Restriction enzymes show high sequence specificity（高度序列特异性）.**
- **They recognize specific DNA sequences, usually 4–8 base pairs（4–8 bp） long.**
- **Many recognition sequences are palindromic sequences（回文序列）.**
- **Cleavage may generate:**
  - ✓ **Sticky ends / cohesive ends（黏性末端）**
  - ✓ **Blunt ends（平末端）**

Nucleic Acid Catabolism

Restriction endonucleases

Correct explanation of palindrome（回文结构）： A palindromic DNA sequence（回文 DNA 序列） reads the same in the 5′→3′ direction on both complementary strands.



This produces 5′ sticky ends（5′ 黏性末端）：5′-AATT

Nucleic Acid Catabolism

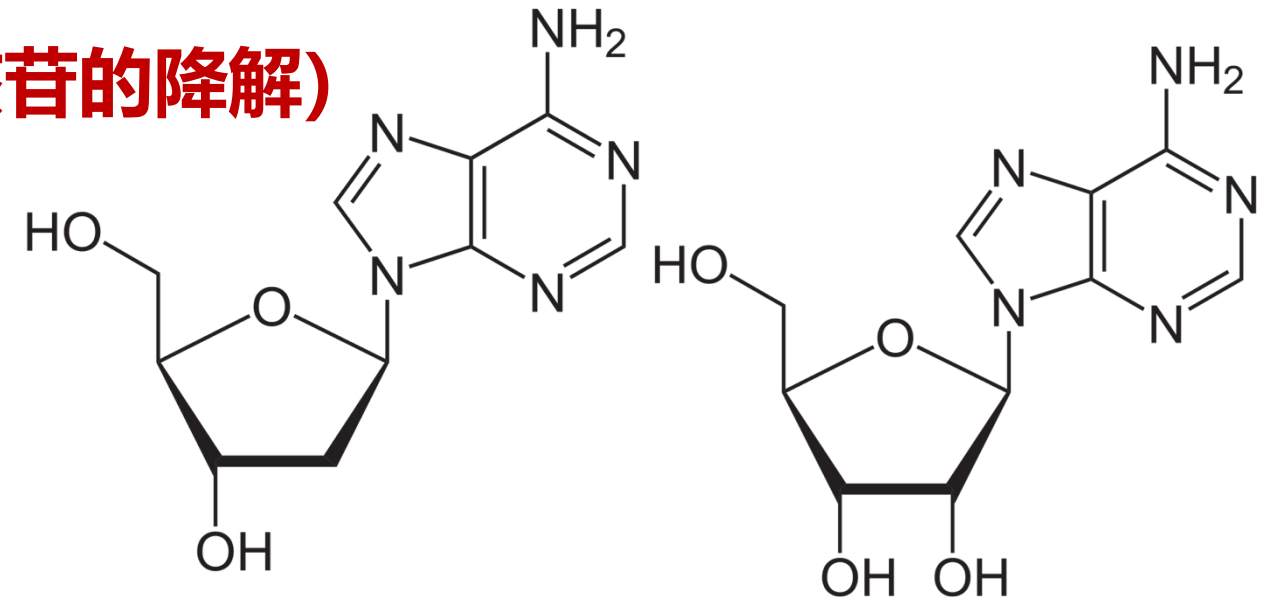
Degradation of Nucleotides（核苷酸降解）



Feature	Nucleotidases	Phosphatases
Specificity	High (Nucleotide-specific)	Broad (General organic molecules)
Primary Target	5' or 3' phosphate on sugars	Various phosphate esters
Main Function	Nucleotide catabolism & Signaling	General metabolism & Regulation
Example	5'-Nucleotidase	Alkaline Phosphatase (ALP)

Nucleic Acid Catabolism

Degradation of Nucleosides（核苷的降解）



1. Phosphorolysis（磷酸解）

Catalyzed by **nucleoside phosphorylase（核苷磷酸化酶）**：

Nucleoside（核苷） + Pi（无机磷酸） → Base + Ribose-1-phosphate（核糖-1-磷酸）

- ❖ **Occurs widely in organisms（广泛存在于生物体内）**
- ❖ **Important in nucleoside degradation and salvage metabolism（补救代谢）**

Nucleic Acid Catabolism

Degradation of Nucleosides（核苷的降解）

2. Hydrolysis（水解）

Catalyzed by nucleosidase / nucleoside hydrolase（核苷水解酶）：

Nucleoside（核苷） + H_2O → Base（碱基） + Ribose（核糖）

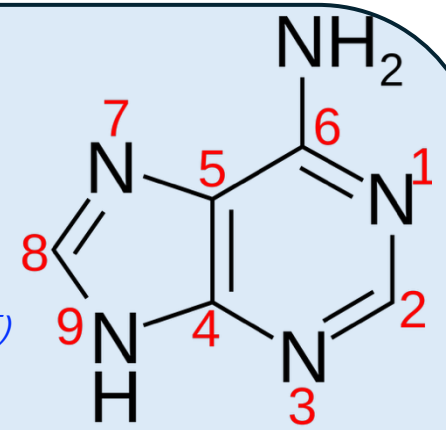
- **Common in plants and microorganisms（常见于植物和微生物）**
- **Acts mainly on ribonucleosides（核糖核苷）**

Nucleic Acid Catabolism

Degradation of Purines（嘌呤的降解）

Purine bases are degraded through deamination（脱氨） and oxidation（氧化） reactions.

Adenine（腺嘌呤）



deamination（脱氨）

Hypoxanthine（次黄嘌呤）

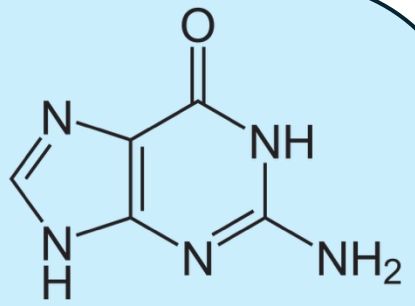
xanthine oxidase / xanthine oxidoreductase
（黄嘌呤氧化酶 / 黄嘌呤氧化还原酶）

Xanthine（黄嘌呤）

xanthine oxidase（黄嘌呤氧化酶）

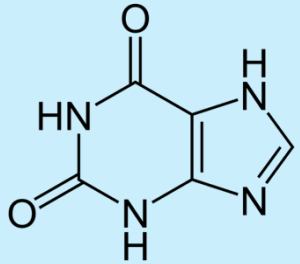
Uric acid（尿酸）

Guanine（鸟嘌呤）



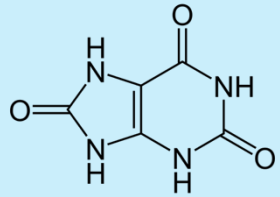
xanthine oxidase（黄嘌呤氧化酶）

Xanthine（黄嘌呤）



guanine deaminase（鸟嘌呤脱氨酶）

Uric acid（尿酸）



Nucleic Acid Catabolism

Degradation of Purines（嘌呤的降解）

Fate of uric acid（尿酸的去向）

Humans and higher primates（人类和高等灵长类）

- Final product: **uric acid（尿酸）**
- Humans lack functional **uricase / urate oxidase（尿酸氧化酶）**
- Therefore, uric acid is excreted mainly in urine.

Most other mammals（多数其他哺乳动物）

- Uric acid is further oxidized to **allantoin（尿囊素）**
- Enzyme: **uricase / urate oxidase（尿酸氧化酶）**

Some lower organisms（某些低等生物）

- Allantoin may be further degraded to:
  allantoic acid（尿囊酸） → **urea（尿素）** → CO_2 + NH_3

Nucleic Acid Catabolism

Degradation of Purines（嘌呤的降解)

Gout: A Disorder of Purine Metabolism（痛风：嘌呤代谢紊乱)

- Acute gout: sudden severe joint pain, swelling, warmth, and redness. （急性关节剧痛、发热、发红)
- Common site: big toe joint, but ankle, knee, wrist, and fingers may also be involved.
- Chronic gout: recurrent attacks may cause tophi, joint stiffness, deformity, and kidney complications.
- Risk is higher in men and postmenopausal women; estrogen(雌激素) partly protects premenopausal women by promoting uric acid excretion.



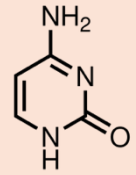




Nucleic Acid Catabolism

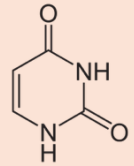
Degradation of Pyrimidines（嘧啶的降解）

Cytosine（胞嘧啶）



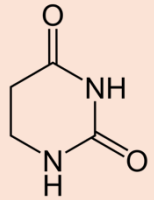
deamination（脱氨）

Uracil（尿嘧啶）

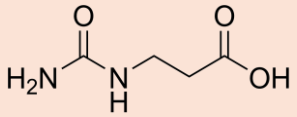


dihydropyrimidine dehydrogenase
（二氢嘧啶脱氢酶）

Dihydrouracil（二氢尿嘧啶）

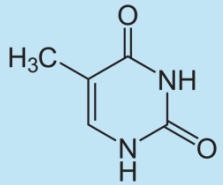


β-ureidopropionate（β-脲基丙酸）



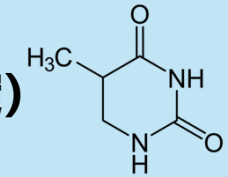
β-alanine（β-丙氨酸） + CO_2 + NH_3

Thymine（胸腺嘧啶）

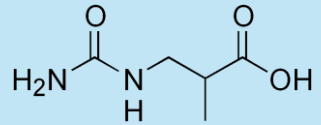


dihydropyrimidine dehydrogenase
（二氢嘧啶脱氢酶）

Dihydrothymine（二氢胸腺嘧啶）



β-ureidoisobutyrate（β-脲基异丁酸）



β-aminoisobutyrate（β-氨基异丁酸） +
CO_2 + NH_3

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Section 2	Nucleic Acid Biosynthesis
Section 3	Applications: Genetic Engineering

Nucleic Acid Biosynthesis

Nucleotides are synthesized by two major pathways:

1. *De novo* synthesis（从头合成途径）

- Nucleotides are synthesized from small precursor molecules.
- This pathway is especially important in rapidly dividing cells.
- It is the major pathway in many tissues, but its contribution varies.

2. Salvage pathway（补救合成途径）

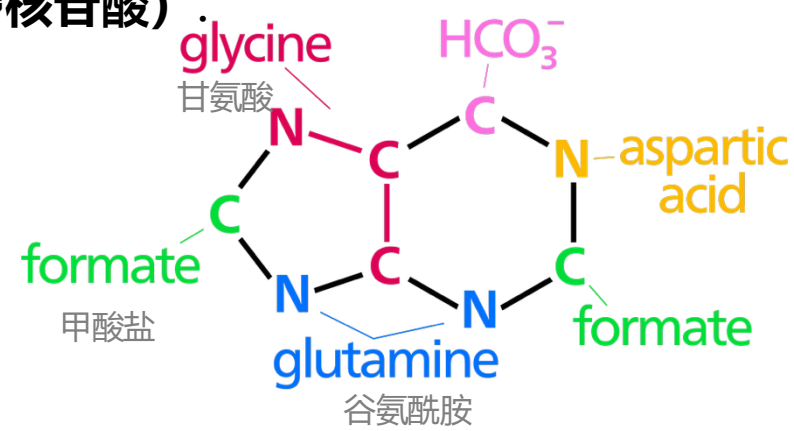
- Free bases and nucleosides are recycled to form nucleotides.
- Saves energy compared with de novo synthesis.
- It is especially important in tissues with limited de novo synthesis, such as the brain and bone marrow.

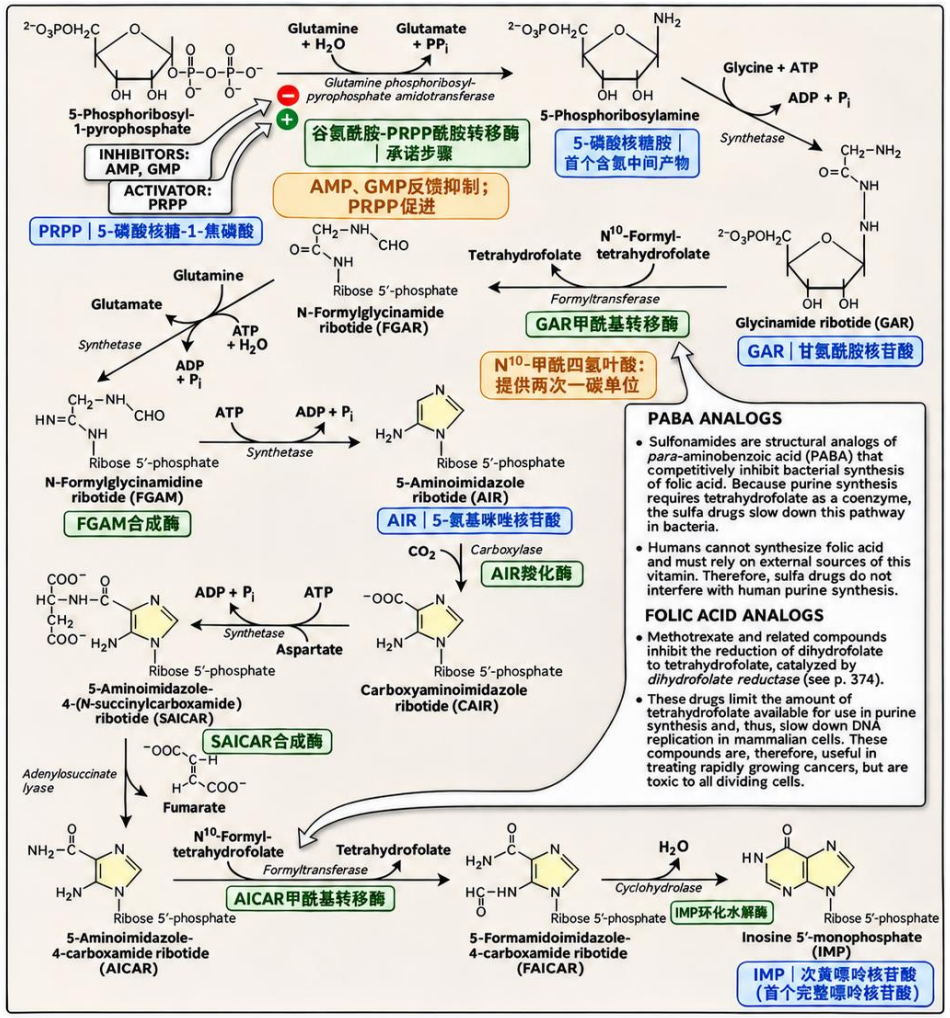
Nucleic Acid Biosynthesis

De Novo Synthesis of Ribonucleotides（核糖核苷酸的从头合成）

Purine Ribonucleotides（嘌呤核糖核苷酸）

- Starts from **PRPP（5-phosphoribosyl-1-pyrophosphate，5-磷酸核糖-1-焦磷酸）**.
- The purine ring is assembled step by step **on the ribose phosphate backbone（在核糖磷酸骨架上）**.
- The first complete purine nucleotide is **IMP（inosine monophosphate，次黄嘌呤核苷酸）**.
- IMP is converted into:
  - ✓ **AMP（腺苷酸）**
  - ✓ **GMP（鸟苷酸）**



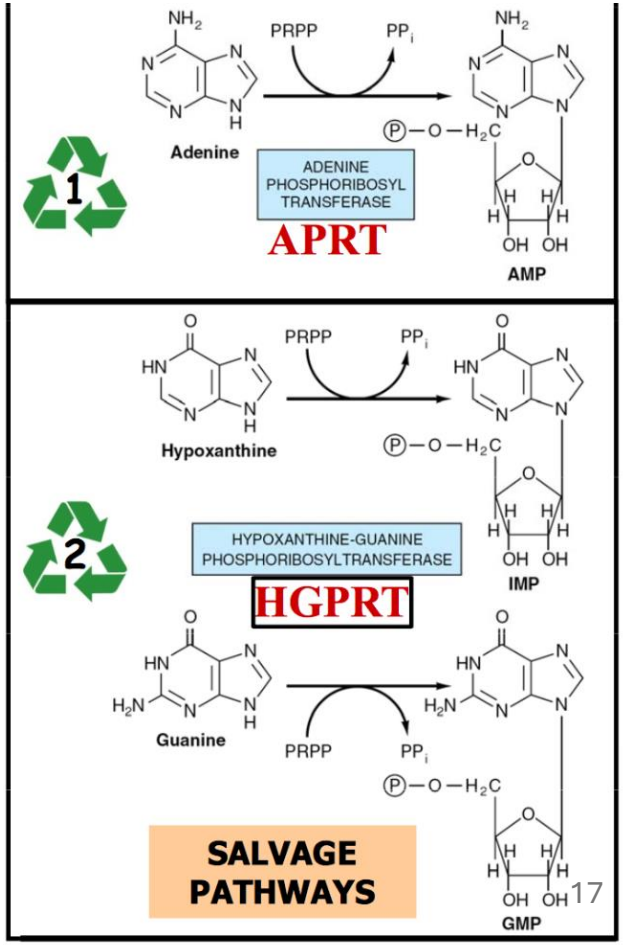


Nucleic Acid Biosynthesis

De Novo Synthesis of Ribonucleotides（核糖核苷酸的从头合成）

Purine Nucleotide Salvage Pathway（嘌呤核苷酸补救合成途径）

- **What:** Recycling of purine bases produced during nucleic acid breakdown.
- **How:** Free purine bases react with **PRPP（5-phosphoribosyl-1-pyrophosphate，5-磷酸核糖-1-焦磷酸）** to form nucleotides.
- **Key enzymes（关键酶）：**
  - ✓ **APRT（adenine phosphoribosyltransferase，腺嘌呤磷酸核糖转移酶）**
  - ✓ **HGPRT（hypoxanthine-guanine phosphoribosyltransferase，次黄嘌呤-鸟嘌呤磷酸核糖转移酶）**
- **Why:** Salvage synthesis uses less energy than de novo purine synthesis and helps maintain nucleotide pools.



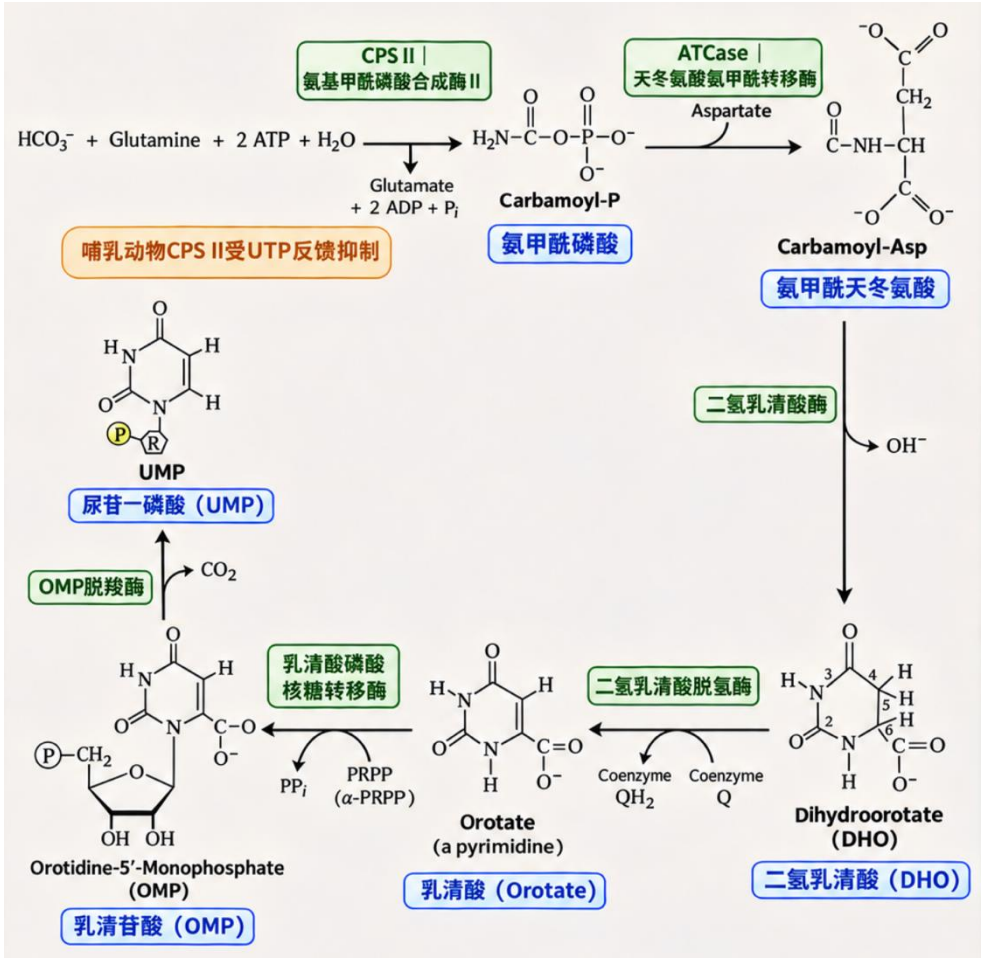
Nucleic Acid Biosynthesis

De Novo Synthesis of Ribonucleotides（核糖核苷酸的从头合成）

Pyrimidine Ribonucleotides（嘧啶核糖核苷酸）

- The pyrimidine ring is synthesized first from small precursors(前体).
- The ring intermediate **orotate（乳清酸）** is then attached to PRPP.
- The first pyrimidine nucleotide product is **OMP（orotidine monophosphate，乳清苷酸）**, which is decarboxylated to **UMP（uridine monophosphate，尿苷酸）**.
- UMP is the precursor for other pyrimidine nucleotides.

嘌呤先有糖，嘧啶先成环



Nucleic Acid Biosynthesis

De Novo Synthesis of Ribonucleotides（核糖核苷酸的从头合成）

Pyrimidine salvage pathway（嘧啶核苷酸补救合成途径）

Route 1: Direct phosphoribosylation（直接磷酸核糖转移）

Uracil（尿嘧啶） + PRPP → UMP（尿苷酸） + PPi

Enzyme: Uracil phosphoribosyltransferase, UPRT（尿嘧啶磷酸核糖转移酶）

Route 2: Via uridine（经尿苷途径）

Uracil（尿嘧啶） + ribose-1-phosphate（核糖-1-磷酸） ⇌ Uridine（尿苷） + Pi

Enzyme: Uridine phosphorylase（尿苷磷酸化酶）

Uridine（尿苷） + ATP → UMP（尿苷酸） + ADP

Enzyme: Uridine kinase / uridine-cytidine kinase（尿苷激酶 / 尿苷-胞苷激酶）

Nucleic Acid Biosynthesis

Biosynthesis of Deoxyribonucleotides（脱氧核糖核苷酸的生物合成）

Step 1: Formation of NDPs（NDP 的形成）

NMP + ATP → NDP + ADP

Enzyme: *nucleoside monophosphate kinase（核苷一磷酸激酶）*

Step 2: Reduction of NDPs to dNDPs（NDP 还原为 dNDP）

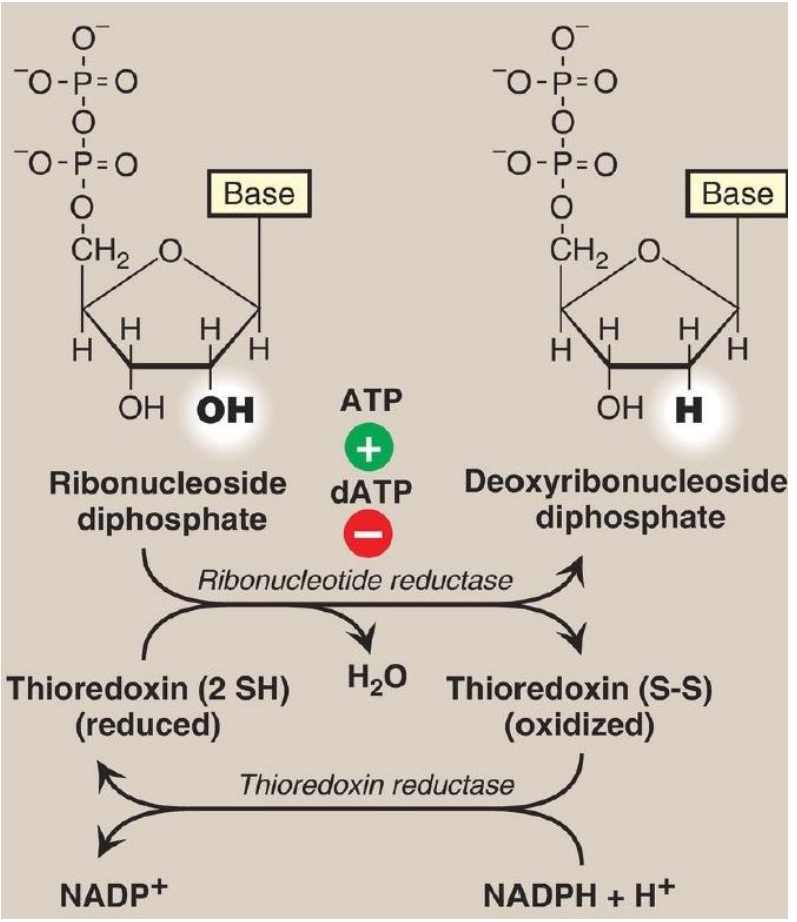
NDP + reduced thioredoxin → dNDP + H_2O + oxidized thioredoxin

Enzyme: *ribonucleotide reductase, RNR（核糖核苷酸还原酶）*

Step 3: Regeneration of reduced thioredoxin（还原型硫氧还蛋白的再生）

oxidized thioredoxin + NADPH + H^+ → reduced thioredoxin + $NADP^+$

Enzyme: *thioredoxin reductase（硫氧还蛋白还原酶）*



Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

- The major biological pathway for DNA synthesis is **DNA replication（DNA 复制）**.
- DNA replication produces an identical copy of the genome before cell division.
  （DNA 复制在细胞分裂前产生一份相同的基因组拷贝）
- It is a **template-directed polymerization reaction（模板指导的聚合反应）**.
- DNA synthesis requires:
  - ✓ DNA template（DNA 模板）
  - ✓ RNA primer（RNA 引物）
  - ✓ dNTPs: dATP, dGTP, dCTP, dTTP（脱氧核苷三磷酸）
  - ✓ DNA polymerase（DNA 聚合酶）
  - ✓ Mg^{2+} as a cofactor（Mg^{2+} 辅因子）

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

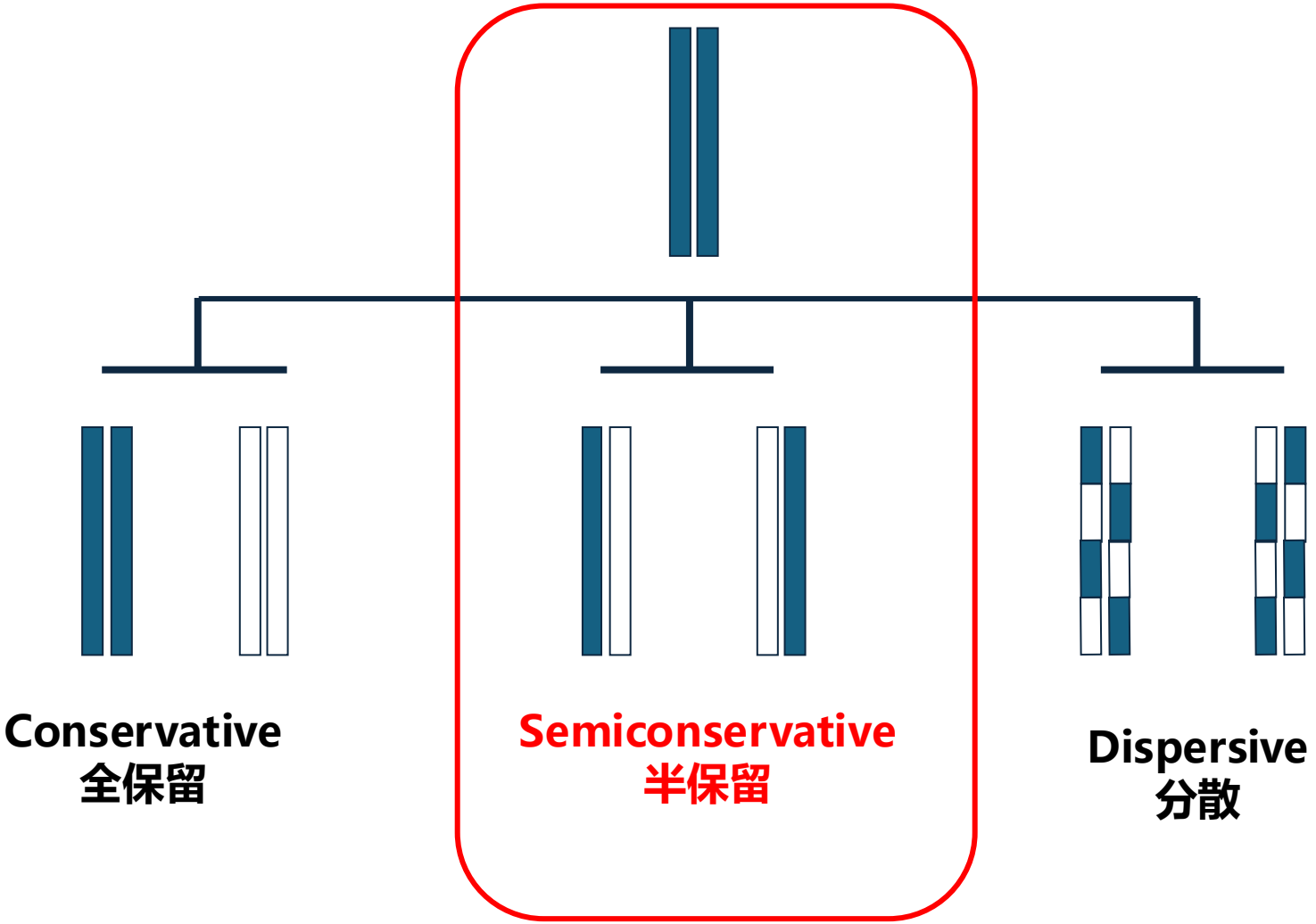
Basic Rules of DNA Replication

1. Semiconservative Replication（半保留复制）

- Each parental DNA strand serves as a template.（每条亲代 DNA 链作为模板）
- After replication, each daughter DNA molecule contains one parental strand and one newly synthesized strand.（复制后，每个子代 DNA 分子含有一条亲代链和一条新合成链）
- This mechanism ensures accurate transmission of genetic information.（保证遗传信息的准确传递）

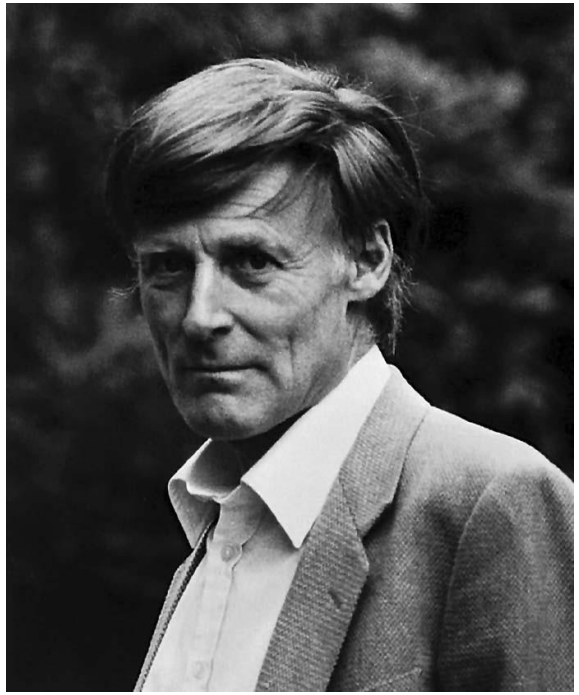
Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

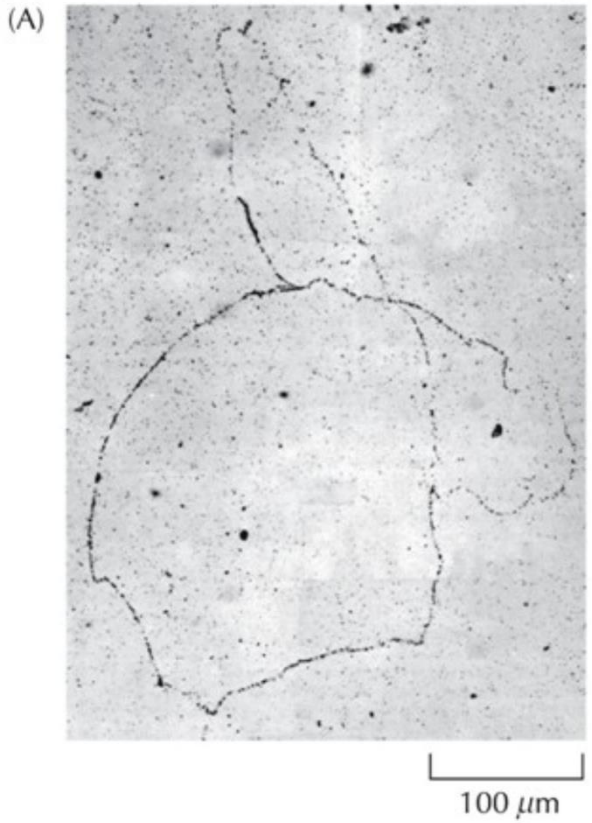


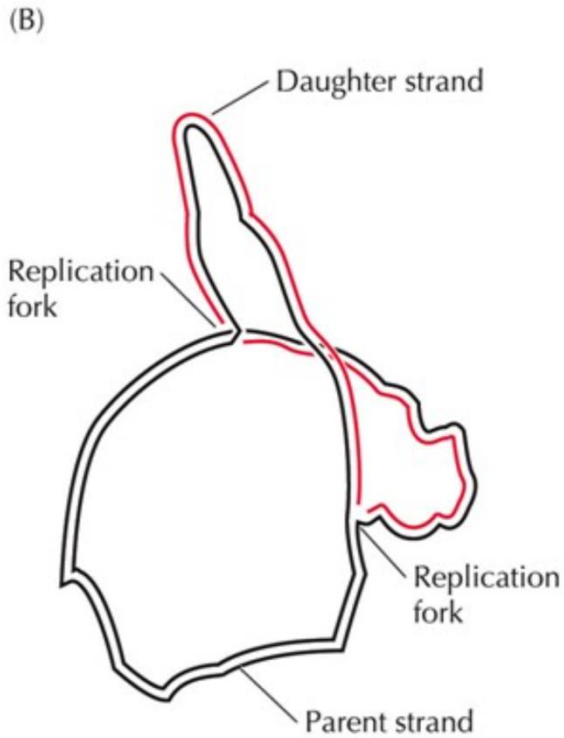
Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）



H. John F. Cairns (1922–2018)





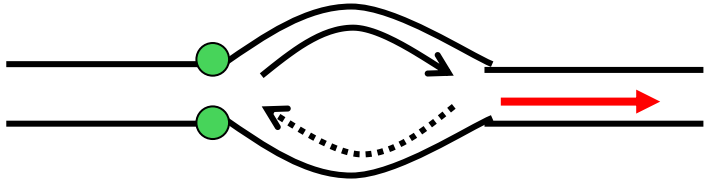
1963, John Cairns cultured E. coli cells for long periods on ^{3}H-thymidine (^{3}H-T), to make their entire cellular DNA radioactive.

Nucleic Acid Biosynthesis

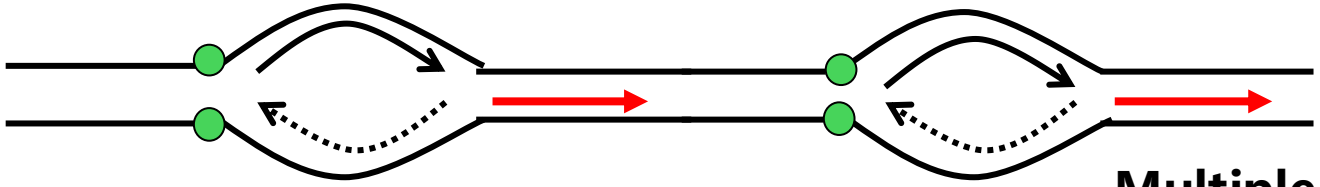
DNA Biosynthesis: DNA Replication（DNA复制）

Basic Rules of DNA Replication

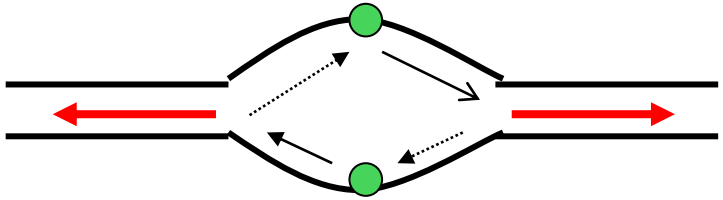
2. Replication Modes: Origin Number and Directionality（复制起点数目与方向性）



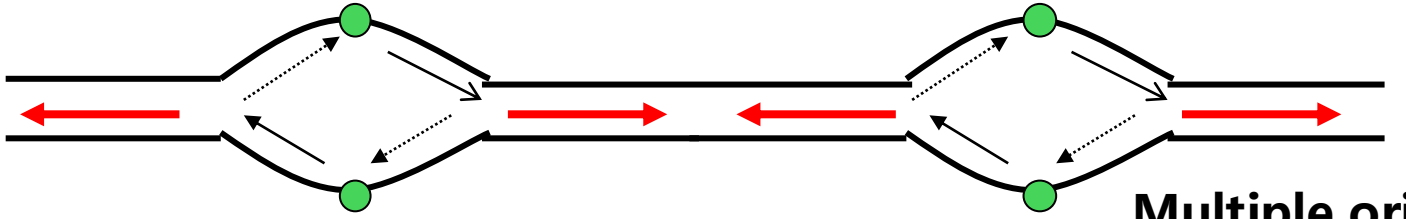
Single origin, unidirectional（单起点，单方向）



Multiple origins, unidirectional（多起点，单方向）



Single origin, bidirectional（单起点，双方向）



Multiple origins, bidirectional（多起点，双方向）

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Basic Rules of DNA Replication

2. Replication Modes: Origin Number and Directionality（复制起点数目与方向性）

Common examples:

- Bacteria: usually **single origin, bidirectional replication**（细菌通常为单起点、双方向复制）
- Eukaryotes: usually **multiple origins, bidirectional replication**（真核生物通常为多起点、双方向复制）

Replication fork（复制叉）：A Y-shaped structure formed when parental DNA strands are unwound and separated. DNA 复制过程中，亲代双链 DNA 被解旋并分开后形成的 Y 字形结构。

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Basic Rules of DNA Replication

3. DNA Replication Requires an RNA Primer

- In cells, DNA polymerase cannot start DNA synthesis *de novo*.（DNA 聚合酶不能从头开始合成 DNA）
- DNA polymerase can only extend from a pre-existing **3′-OH group（3′-羟基）**.
- Therefore, cellular DNA replication requires a short **RNA primer（RNA 引物）**, synthesized by **primase（引物酶）**.
- In contrast, PCR uses synthetic **DNA primers（DNA 引物）** for in vitro amplification.（PCR 使用人工合成的 DNA 引物进行体外扩增）

4. DNA Elongation Proceeds 5′→3′

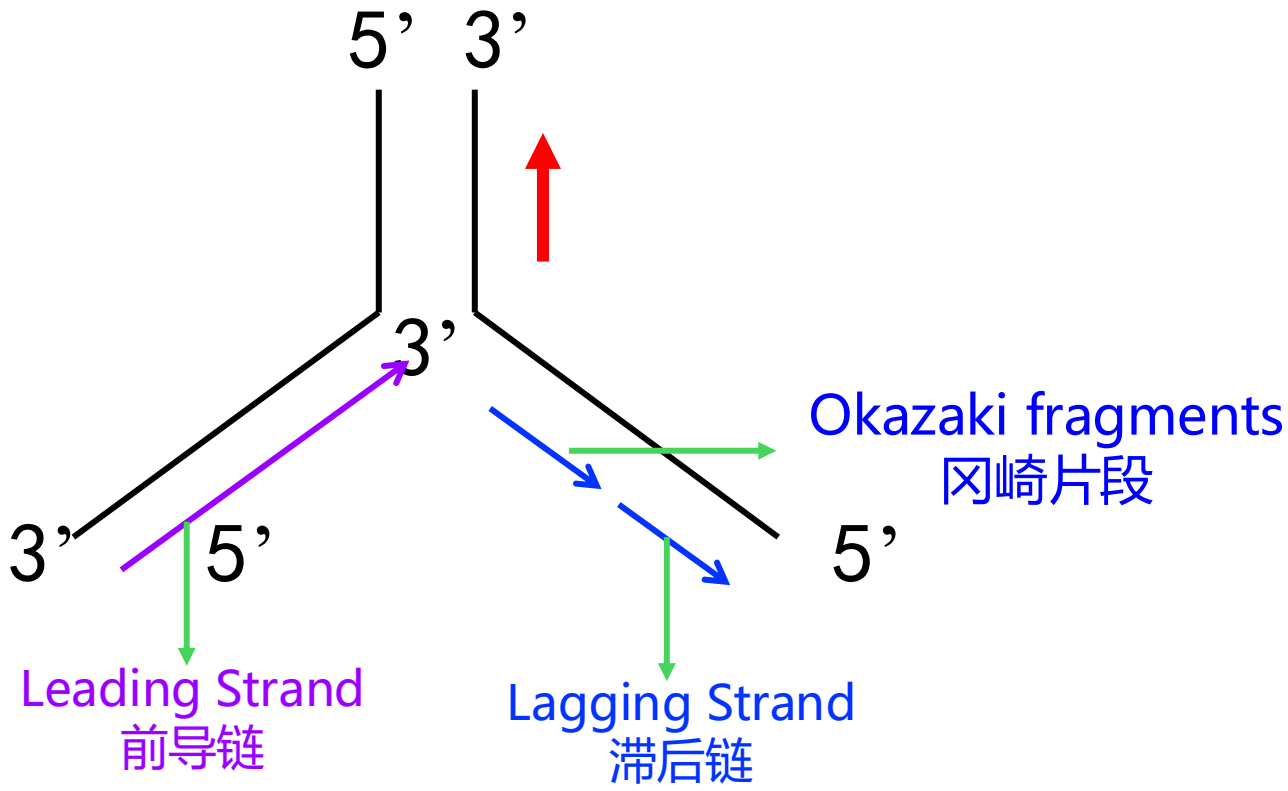
- New nucleotides are added to the **3′-OH end** of the growing DNA strand.
  （新的核苷酸加到正在延伸链的 3′-OH 端）
- The 3′-OH attacks the α-phosphate of an incoming dNTP, forming a **3′,5′-phosphodiester bond**（3′,5′-磷酸二酯键）.
- Therefore, the newly synthesized DNA strand grows in the 5′→3′ direction.

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Basic Rules of DNA Replication

5. Semidiscontinuous Replication（半不连续复制）



Definition

Because DNA polymerase can synthesize DNA only in the 5′→3′ direction, the leading strand is synthesized continuously, whereas the lagging strand is synthesized discontinuously as *Okazaki fragments*.

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Enzymes and Cofactors in DNA Replication（DNA 复制所需的酶和辅因子）

1. DNA Polymerase（DNA pol; DNA 聚合酶）

Full name: DNA-dependent DNA polymerase（依赖 DNA 的 DNA 聚合酶）

- **5′→3′ polymerase activity（5′→3′ 聚合酶活性）**
  - ✓ Adds dNTPs to the 3′-OH end of the growing DNA strand.
  - ✓ Responsible for DNA chain elongation.
- **3′→5′ exonuclease activity（3′→5′ 外切酶活性）** 能辨认错配的碱基对，并将其水解。
  - ✓ Removes incorrectly incorporated nucleotides.
  - ✓ Provides proofreading function.
- **5′→3′ exonuclease activity（5′→3′ 外切酶活性）** 能切除突变的 DNA片段。
  - ✓ Present in some DNA polymerases, such as ***E. coli*** **DNA polymerase I（大肠杆菌 DNA 聚合酶 I）**.
  - ✓ Removes RNA primers during lagging-strand synthesis.

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Enzymes and Cofactors in DNA Replication（DNA 复制所需的酶和辅因子）

1. DNA Polymerase（DNA pol; DNA 聚合酶）

Bacterial DNA polymerases I, II, and III（细菌 DNA 聚合酶 I、II、III）

DNA-pol I（DNA 聚合酶 I）

- Removing RNA primers（切除 RNA 引物）
- Filling gaps with DNA（填补 DNA 缺口）
- DNA repair（DNA 修复）

DNA Pol II（DNA 聚合酶 II）

- Mainly involved in DNA repair（主要参与 DNA 修复）
- Helps restart replication after DNA damage（帮助 DNA 损伤后的复制重新启动）

DNA Pol III（ DNA 聚合酶 III）

- The major replicative DNA polymerase in *E. coli*
- Responsible for rapid, highly processive DNA synthesis

The major bacterial replicative polymerase

Comparison of *E. coli* DNA Polymerases

	DNA Polymerase I	DNA Polymerase II	DNA Polymerase III
Subunit number	1	≥ 7	≥ 10
Relative activity（相对活性）	**1**	**0.05**	**50**
5′→3′ polymerase activity (5′→3′ 聚合作用)	+	+	+
3′→5′ exonuclease activity (3′→5′ 核酸外切酶活性)	+	+	+
5′→3′ exonuclease activity (5′→3′ 核酸外切酶活性)	+	–	–
Main function	Primer removal and repair（切除引物、修复）	Repair（修复）	Replication（复制）

Nucleic Acid Biosynthesis

1. DNA Polymerase（DNA pol; DNA 聚合酶）

Eukaryotic DNA Polymerases（真核细胞 DNA 聚合酶）

Polymerase	Main function（主要功能）	Key feature（特点）
Pol α	Initiates nuclear DNA replication（启动核 DNA 复制）	Primase-associated; makes RNA-DNA primer（与引物酶结合，合成 RNA-DNA 引物）
Pol δ	Lagging-strand synthesis（滞后链合成）	3′→5′ proofreading（校对活性）
Pol ε	Leading-strand synthesis（前导链合成）	3′→5′ proofreading; also repair/checkpoint roles（校对，也参与修复/检查点）
Pol β	DNA repair（DNA 修复）	Base excision repair（碱基切除修复）
Pol γ	Mitochondrial DNA replication（线粒体 DNA 复制）	Main mitochondrial DNA polymerase（线粒体主要 DNA 聚合酶）

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Enzymes and Cofactors in DNA Replication（DNA 复制所需的酶和辅因子）

2. Primase（引物酶）

Primase uses **DNA as the template** and **NTPs as substrates** to synthesize a short **RNA primer**. This RNA primer provides a free **3′-OH group（3′-OH 端）**, which allows DNA polymerase to begin DNA chain elongation.

Primase associates with other replication proteins to form the primosome（引发体）.

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Enzymes and Cofactors in DNA Replication（DNA 复制所需的酶和辅因子）

3. DNA Ligase（DNA 连接酶）

DNA ligase seals nicks in double-stranded DNA by forming a phosphodiester bond（磷酸二酯键） between the adjacent 3′-OH group（3′-羟基） and the 5′-phosphate group（5′-磷酸基）

a) DNA replication（DNA 复制）

- DNA ligase joins Okazaki fragments on the lagging strand. DNA 连接酶连接滞后链上的冈崎片段。

b) DNA repair and recombination（DNA 修复与重组）

- DNA ligase seals DNA nicks during repair, recombination, and splicing-like DNA joining events. DNA 连接酶在 DNA 修复、重组及 DNA 片段连接过程中封闭缺口。

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Enzymes and Cofactors in DNA Replication（DNA 复制所需的酶和辅因子）

4. DNA Helicase（DNA 解旋酶 / 解螺旋酶）

DNA helicase unwinds the DNA double helix by breaking the hydrogen bonds between complementary base pairs. DNA 解旋酶通过破坏互补碱基之间的氢键，使 DNA 双螺旋解开。

- ❖ Unwinds the DNA double helix（解开 DNA 双螺旋）
- ❖ Breaks hydrogen bonds between base pairs（破坏碱基对之间的氢键）
- ❖ Requires ATP hydrolysis（需要 ATP 水解供能）
- ❖ Generates single-stranded DNA templates for replication（形成可作为模板的单链 DNA）

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Enzymes and Cofactors in DNA Replication（DNA 复制所需的酶和辅因子）

5. Single-Stranded DNA-Binding Protein（SSB，单链 DNA 结合蛋白）

SSB binds to and stabilizes unwound single-stranded DNA to prevent the separated DNA strands from re-annealing and protects single-stranded DNA from nuclease degradation. 可防止 DNA 单链重新配对复性，并保护单链 DNA 不被核酸酶降解。

SSB prevents:

- ✓ **Re-annealing（复性） of DNA strands**
- ✓ **Formation of secondary structures（二级结构）**
- ✓ **Degradation by nucleases（核酸酶）**

Nucleic Acid Biosynthesis

DNA Biosynthesis: DNA Replication（DNA复制）

Enzymes and Cofactors in DNA Replication（DNA 复制所需的酶和辅因子）

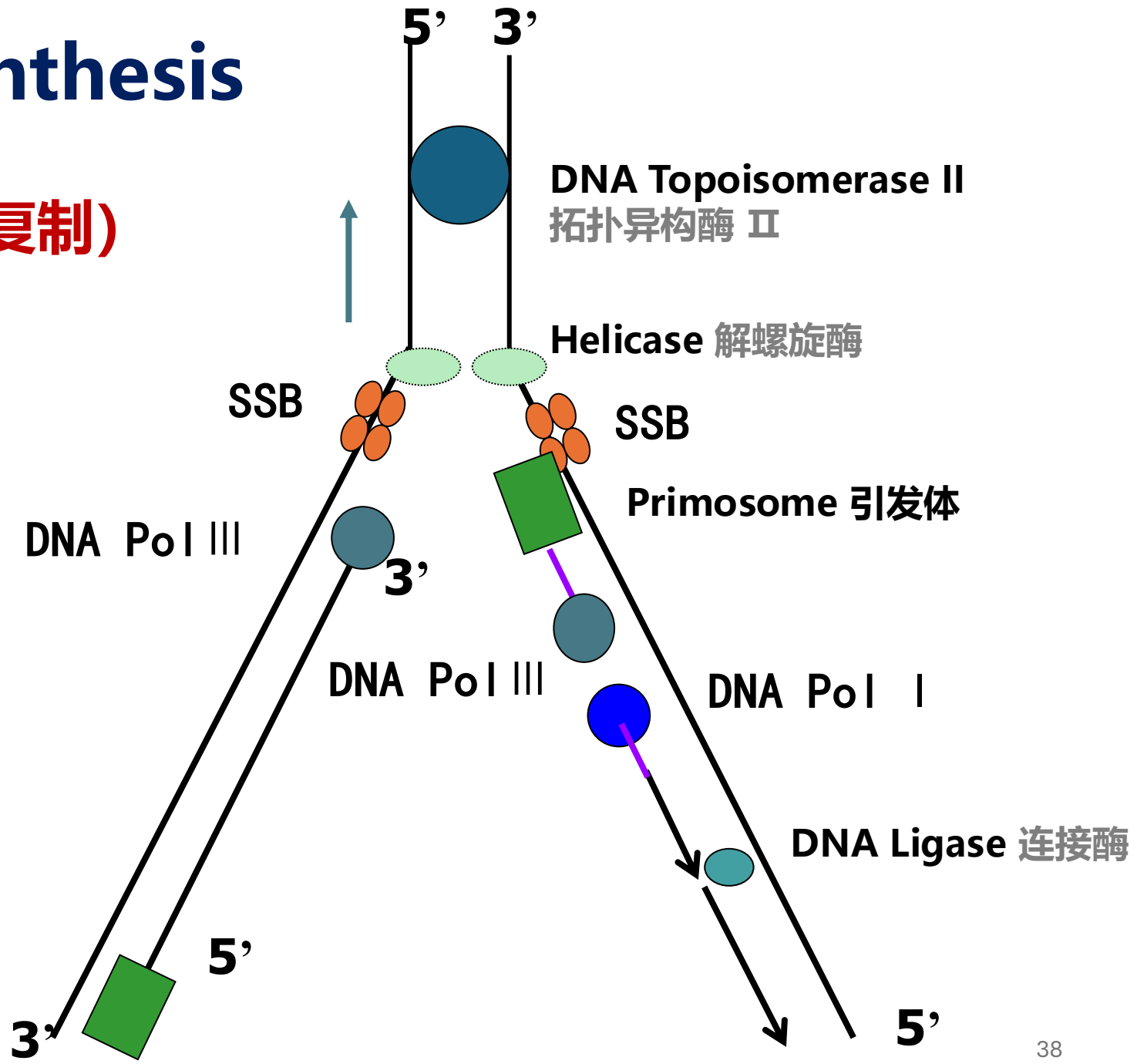
6. DNA Topoisomerase（拓扑异构酶）

Relieves positive supercoiling（正超螺旋） ahead of the replication fork（复制叉）.



Nucleic Acid Biosynthesis

DNA Replication（DNA复制）



Nucleic Acid Biosynthesis

DNA Replication in *E. coli*（大肠杆菌（原核生物）DNA复制）

Origin recognition（起始位点识别） → DNA unwinding（DNA解链） → RNA primer synthesis

DNA replication initiation must solve two problems:

1. **Open the DNA double helix into single strands DNA解开成单链，提供模板。**
2. **Synthesize a primer to provide a 3′-OH end 合成引物，提供3′-OH末端。**

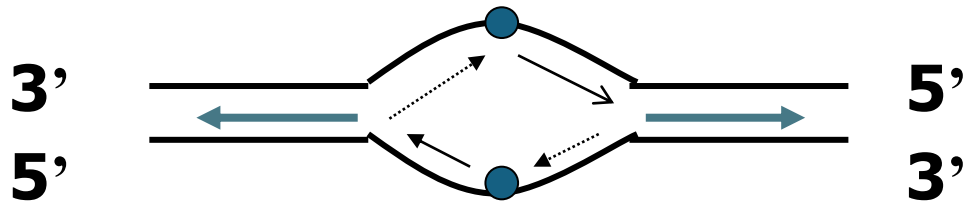
Nucleic Acid Biosynthesis

DNA Replication in E. coli （大肠杆菌（原核生物）DNA复制）

Replicon (复制子)

A replicon is a unit of DNA that is replicated from a single origin of replication.

- **Prokaryotic chromosomes usually have one origin, one replicon. 原核生物：一个复制起点，一个复制子。**
- **Eukaryotic chromosomes usually have many origins, many replicons. 真核生物：多个复制起点，多个复制子。**



This diagram shows **single-origin, bidirectional replication**.

Nucleic Acid Biosynthesis

DNA Replication in E. coli （大肠杆菌（原核生物）DNA复制）

Synthesis and Elongation of DNA Strands DNA 链的合成与延长

1. Leading strand synthesis（先导链的延伸）

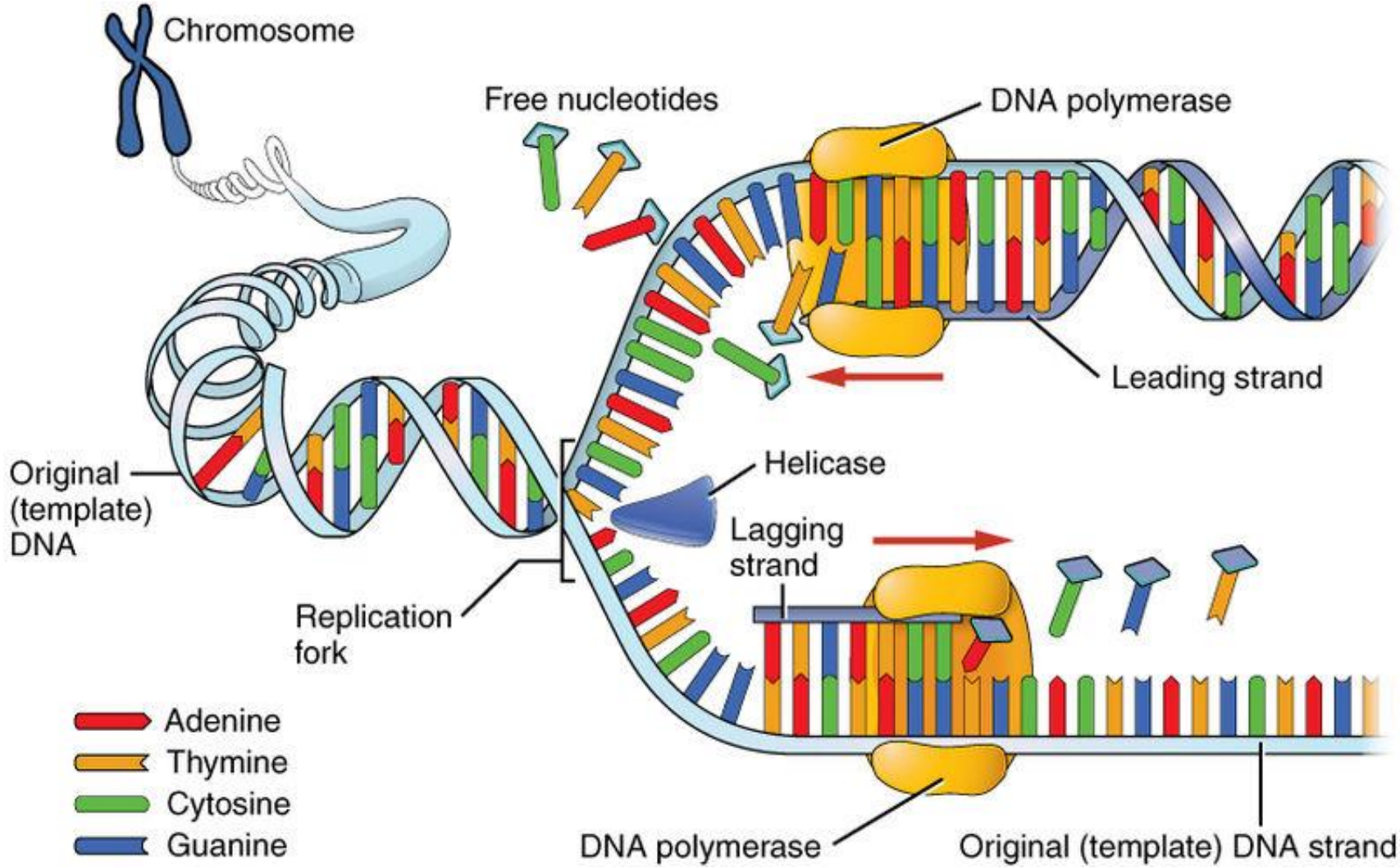
- ❖ DNA polymerase III reads the template strand（模板链）in the **3′→5′ direction** and synthesizes the new DNA strand in the **5′→3′ direction**.
- ❖ New dNTPs are added to the **3′-OH end（3′-OH末端）** of the growing strand.
- ❖ The leading strand is synthesized **continuously** toward the replication fork（复制叉）.

2. Lagging strand synthesis（滞后链的合成）

- ❖ The lagging strand is synthesized discontinuously as short Okazaki fragments（冈崎片段）.
- ❖ The steps are: Okazaki fragment formation → RNA primer removal → gap filling → DNA ligation

Nucleic Acid Biosynthesis

DNA Replication in E. coli （大肠杆菌（原核生物）DNA复制）



Nucleic Acid Biosynthesis

DNA Replication in E. coli （大肠杆菌（原核生物）DNA复制）

Termination of DNA Replication 复制的终止

- DNA synthesis ends when replication forks（复制叉）meet near the terminus region（终止区）.
- In *E. coli*, Ter sites（ter位点）and Tus protein（Tus蛋白）help regulate fork termination.
- After replication, each daughter DNA molecule contains one parental strand（亲代链）and one newly synthesized strand（新生链）.

Nucleic Acid Biosynthesis

DNA Replication in Eukaryotes (真核生物DNA复制)

1. Chromosomal DNA Replication 染色质DNA复制

	Prokaryotic Cells	Eukaryotic Cells
Replicons/复制子	**Usually 1 per chromosome**	**Many; often >1000**
Replication mode	**Single origin, bidirectional（单起点、双方向）**	**Multiple origins, bidirectional（多起点、双方向）**
Major DNA polymerases	**DNA Pol III for chain elongation; DNA Pol I removes RNA primers**	**DNA Pol α, δ, ε**
Okazaki fragments	**~1000–2000 nt**	**~100–200 nt**

Eukaryotic DNA replication is similar to bacterial DNA replication in its basic principles, but differs in genome organization, number of origins, and polymerase usage.

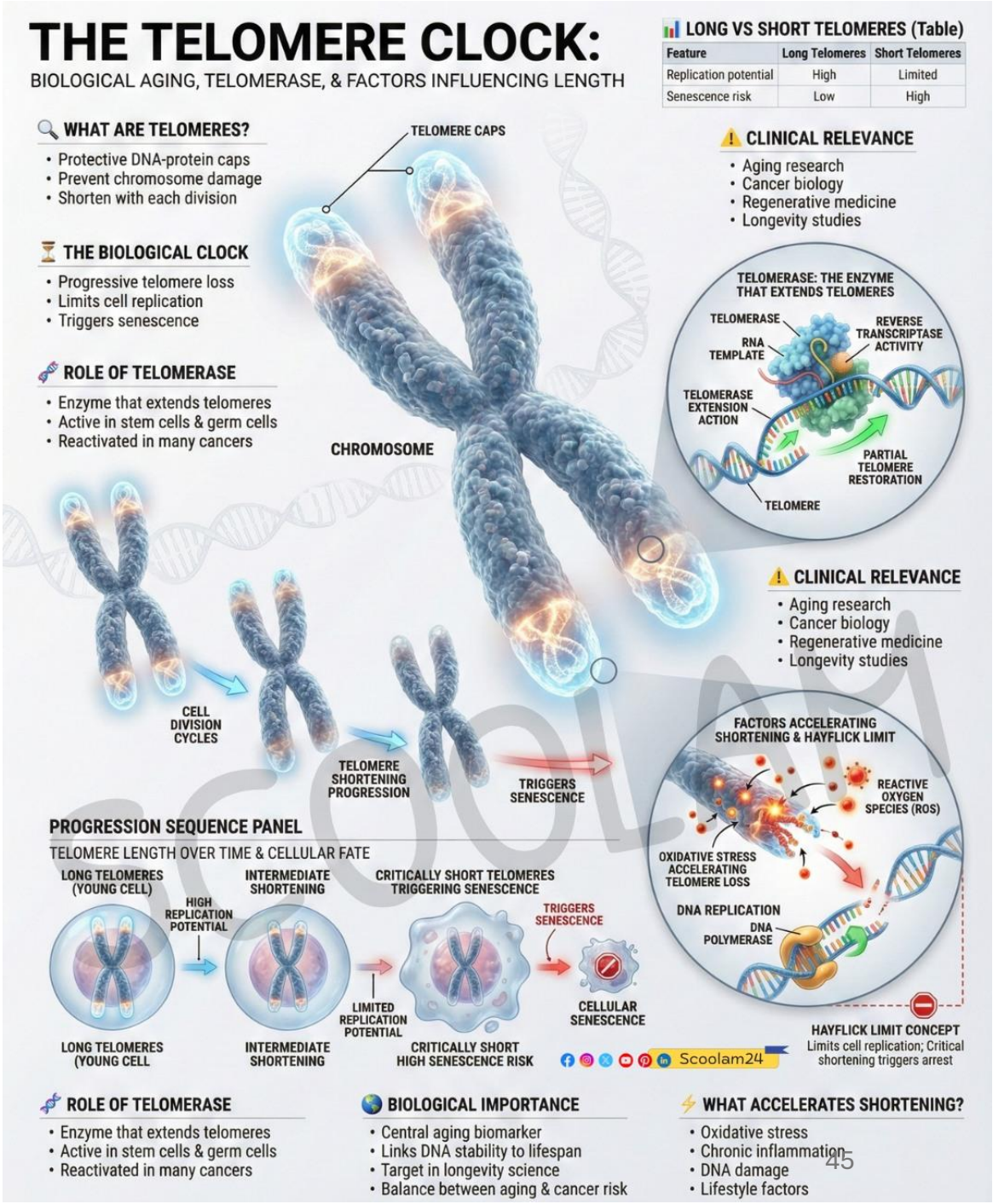
Nucleic Acid Biosynthesis

DNA Replication in Eukaryotes

2. Replication of Chromosome Ends

Telomeres（端粒） are repetitive DNA sequences located at the ends of eukaryotic chromosomes.

- **Functions:** Protect chromosome ends and maintain chromosome stability
- **Problem:** After removal of the terminal RNA primer, DNA polymerase cannot fill the last gap because no upstream **3'-OH** is available.
- **Consequence:** Chromosome ends may shorten with each round of replication.



Nucleic Acid Biosynthesis

DNA Replication in Eukaryotes

2. Replication of Chromosome Ends

- **Telomeres（端粒）** are maintained by **telomerase（端粒酶）**.
- Telomerase is a **ribonucleoprotein enzyme（核糖核蛋白酶）** with reverse transcriptase activity.

Telomerase contains:

1. **An RNA template（RNA模板）that provides the sequence information for telomeric DNA repeat synthesis.**
2. **A catalytic protein subunit（催化蛋白亚基） that synthesizes DNA using the RNA template.**

Nucleic Acid Biosynthesis

DNA Biosynthesis: Reverse transcription（逆转录）

Reverse transcription（逆转录） is the process in which DNA is synthesized using RNA as the template. This reaction is catalyzed by RNA-dependent DNA polymerase（依赖 RNA 的 DNA 聚合酶）, also called reverse transcriptase（逆转录酶）.

Reverse transcription occurs in:

1. Retroviruses（逆转录病毒）

For example, HIV uses reverse transcriptase to convert its RNA genome into DNA.

2. Normal animal cells（正常动物细胞）

For example, telomerase（端粒酶） uses an RNA template to extend telomeric DNA.

Nucleic Acid Biosynthesis

DNA Biosynthesis: Reverse transcription（逆转录）

- **Template: RNA template（RNA模板）**
- **Primer: tRNA primer（tRNA引物）**
- **Substrates: four dNTPs（四种 dNTP）**
- **Product: cDNA**

- **cDNA（complementary DNA，互补 DNA）** is a single-stranded DNA molecule synthesized using RNA as the template.
- In retroviruses, reverse transcriptase first synthesizes a DNA strand complementary to viral RNA, forming an **RNA–DNA hybrid（RNA-DNA杂交分子）**.

Nucleic Acid Biosynthesis

DNA Biosynthesis: Reverse transcription（逆转录）

Enzymatic Activities of Reverse Transcriptase 逆转录酶的多功能酶活性

1. RNA-dependent DNA polymerase activity 依赖 RNA 的 DNA 聚合酶活性

Uses RNA as the template to synthesize DNA, forming an RNA–DNA hybrid.

2. RNase H activity 核糖核酸酶 H 活性

Degrades the RNA strand in the RNA–DNA hybrid.

3. DNA-dependent DNA polymerase activity 依赖 DNA 的 DNA 聚合酶活性

Uses the newly synthesized DNA strand as the template to synthesize the complementary DNA strand, forming double-stranded DNA.

Nucleic Acid Biosynthesis

DNA Mutation

DNA mutation（DNA突变 / 基因突变） refers to a stable and heritable change in the nucleotide sequence of DNA. Mutations may occur during DNA replication, or may be induced by external or internal mutagens such as radiation, chemicals, oxidative damage, or errors in DNA repair.

Base substitution
碱基置换

One or more base pairs are replaced by anothers.

May be silent, missense, or nonsense depending on its effect on the encoded protein.

Insertion
插入

One or more nucleotide pairs are added into the DNA sequence.

If the number of inserted bases is not a multiple of three, the reading frame changes, causing a frameshift mutation（移码突变）.

Deletion
缺失

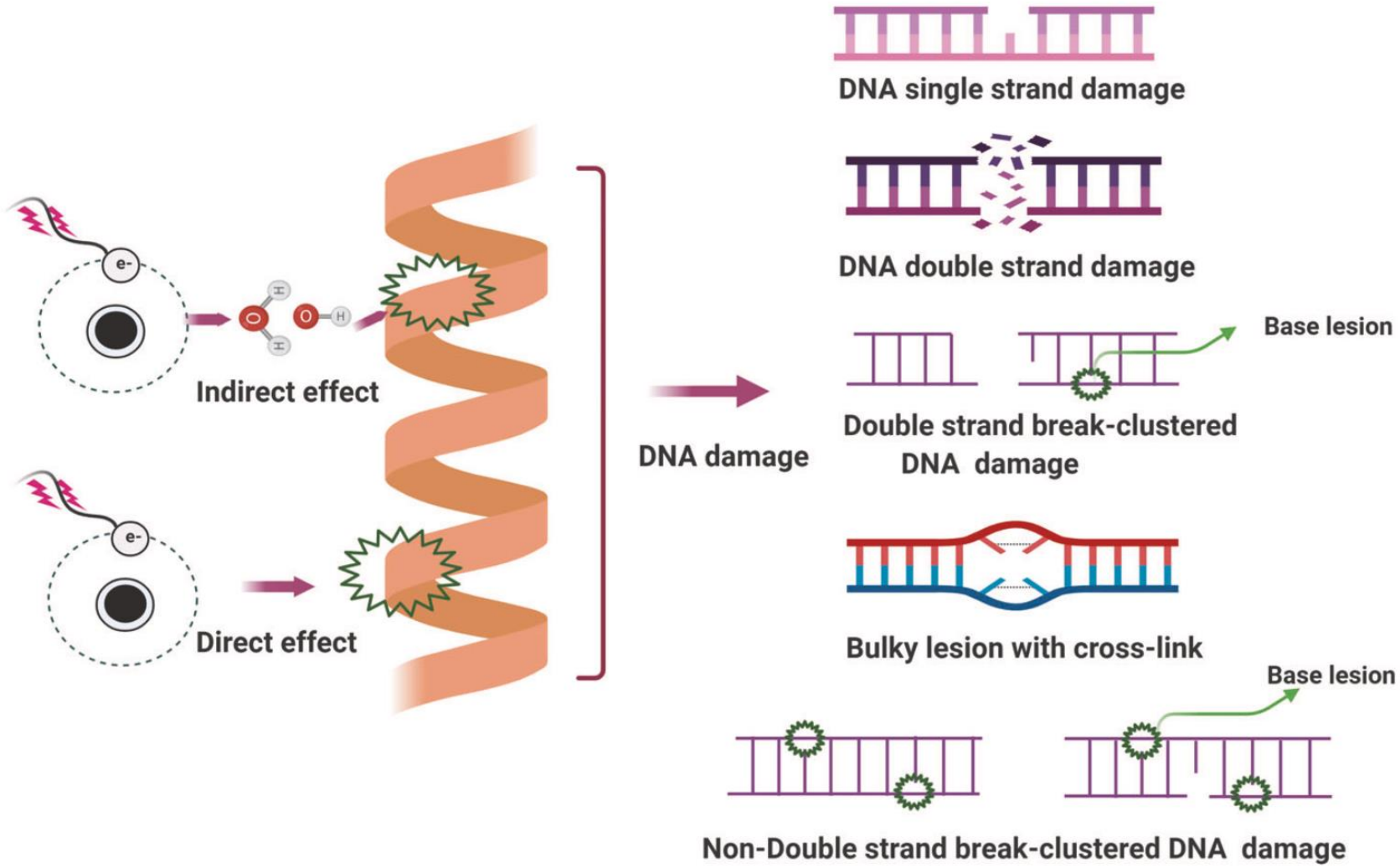
One or more nucleotide pairs are removed from the DNA sequence.

Like insertion, deletion may also cause a frameshift mutation if the number of deleted bases is not a multiple of three.

Nucleic Acid Biosynthesis

DNA Damage

DNA damage is any chemical or structural alteration of DNA, including strand breaks（链断裂）, abasic sites（无碱基位点）, modified bases（修饰碱基）, bulky lesions（大体积损伤）, and abnormal covalent crosslinks（异常共价交联）.



Nucleic Acid Biosynthesis

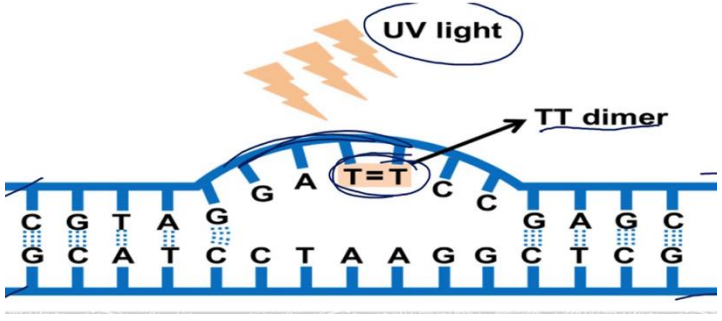
DNA Damage Repair

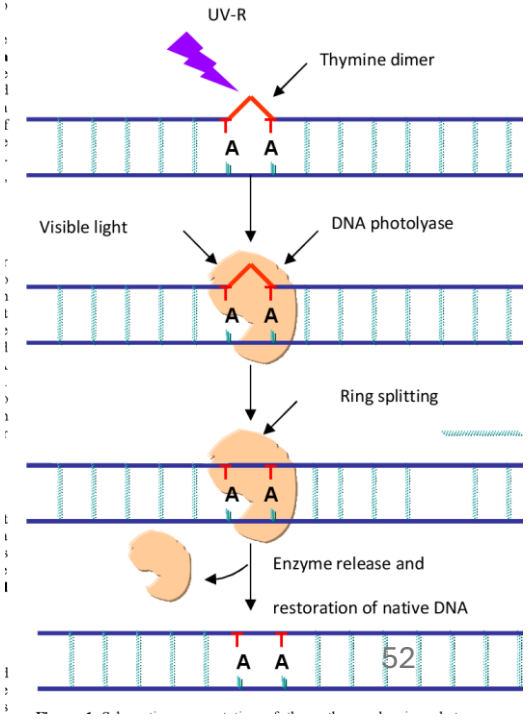
DNA is chemically stable, but it is continually damaged by environmental agents and endogenous metabolism. Common sources include UV radiation（紫外线）, ionizing radiation（电离辐射）, reactive oxygen species（活性氧, ROS）, alkylating chemicals（烷化剂）, and replication errors（复制错误）. To preserve genomic integrity（基因组完整性）, cells use multiple DNA repair pathways.

1. Direct Reversal

❖ **Photoreactivation（光复活修复）**

Photolyase（光裂合酶） uses visible light energy to split UV-induced pyrimidine dimers（嘧啶二聚体）, especially thymine dimers（胸腺嘧啶二聚体）.





Photolyase is absent in placental mammals, including humans.

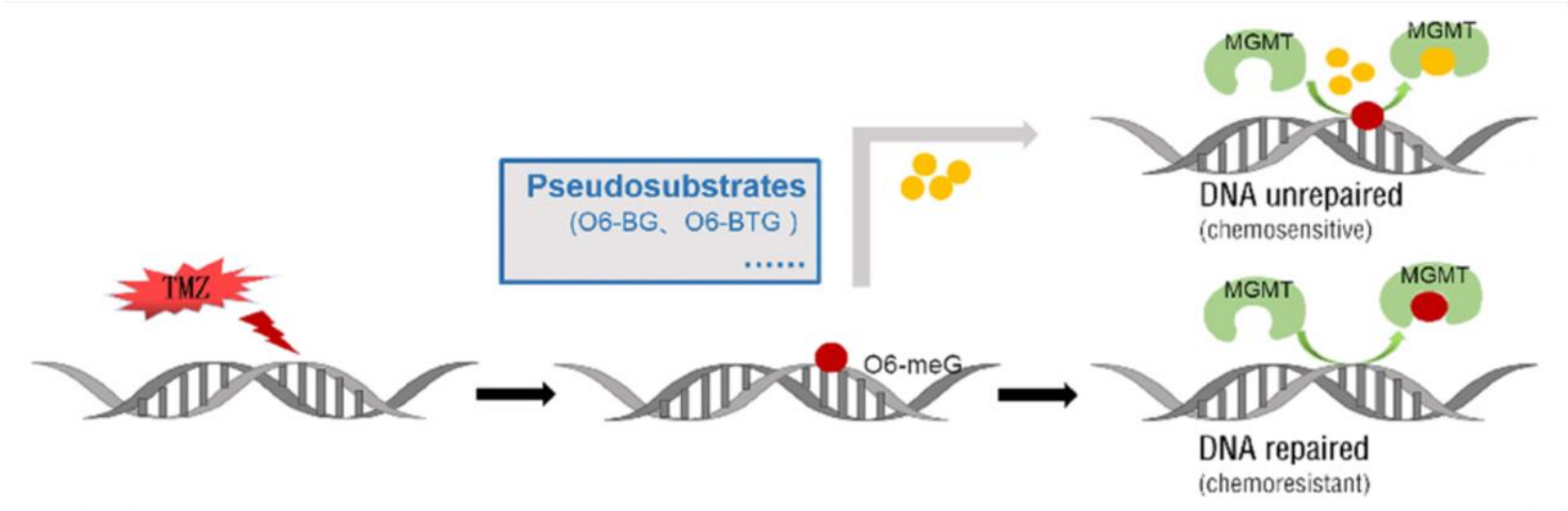
Nucleic Acid Biosynthesis

DNA Damage Repair

1. Direct Reversal

❖ **Methyltransferase repair（甲基转移酶修复）**

MGMT / O^6-methylguanine-DNA methyltransferase（O^6-甲基鸟嘌呤-DNA甲基转移酶） removes the methyl group from O^6-methylguanine（O^6-甲基鸟嘌呤）. This is a suicide enzyme（自杀酶） because one MGMT molecule repairs one lesion and is then inactivated.



Nucleic Acid Biosynthesis

DNA Damage Repair

2. Repair of Single-Strand DNA Damage 单链损伤修复

Most single-strand DNA damage can be repaired accurately because the undamaged complementary strand（互补链） provides the correct template.

❖ **Base Excision Repair, BER/ 碱基切除修复**

BER repairs small, non-bulky base lesions（小型碱基损伤）, such as oxidized, deaminated, or alkylated bases.

1. DNA glycosylase（DNA糖基化酶） recognizes the damaged base and removes it.This creates an AP site / abasic site（无嘌呤/无嘧啶位点；无碱基位点）.
2. AP endonuclease（AP内切酶） cuts the DNA backbone near the AP site.
3. DNA polymerase（DNA聚合酶） fills the missing nucleotide.
4. DNA ligase（DNA连接酶） seals the remaining nick（缺口）.

AP = apurinic/apyrimidinic site无嘌呤/无嘧啶位点，也叫 无碱基位点（abasic site）。

Nucleic Acid Biosynthesis

DNA Damage Repair

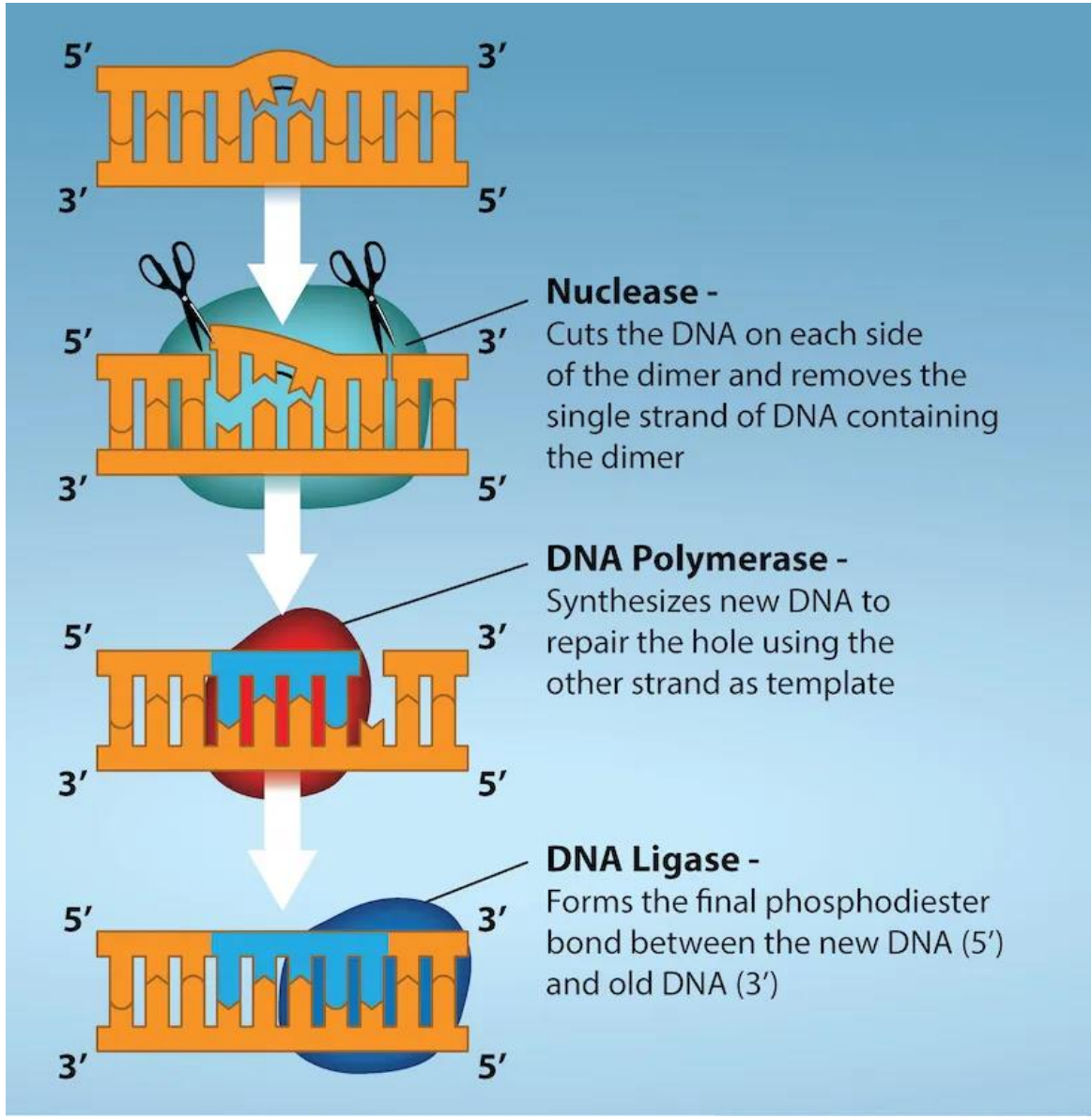
2. Repair of Single-Strand DNA Damage

❖ **Nucleotide Excision Repair, NER 核苷酸切除修复**

NER repairs bulky DNA lesions（大体积DNA损伤）that distort the DNA double helix.

Typical lesions include:

- ✓ UV-induced thymine dimers（胸腺嘧啶二聚体）
- ✓ 6-4 photoproducts（6-4光产物）
- ✓ large chemical adducts（大体积化学加合物）



Nucleic Acid Biosynthesis

DNA Damage Repair

2. Repair of Single-Strand DNA Damage

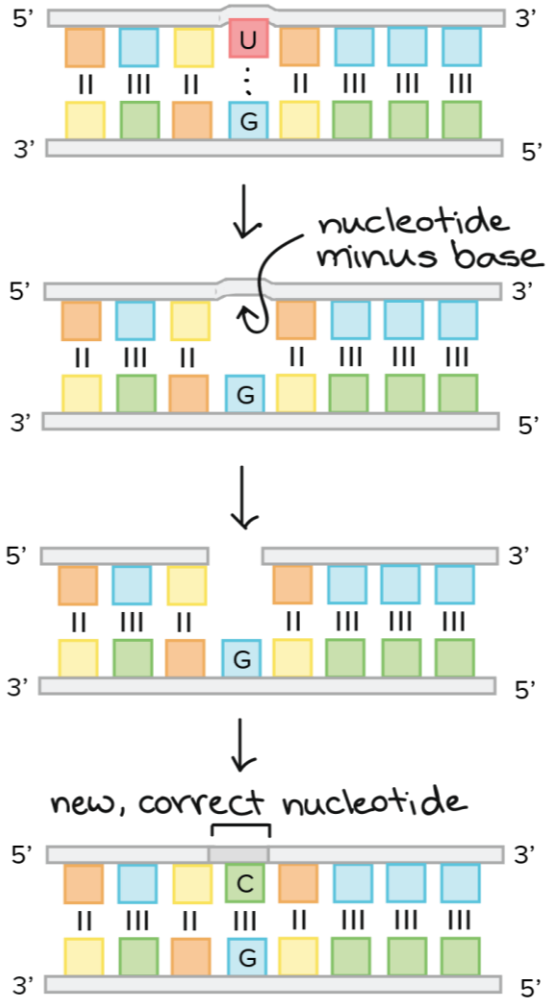
❖ **Mismatch Repair, MMR错配修复**

MMR corrects replication errors that escape DNA polymerase proofreading（校对）.

Typical targets include:

✓ base-base mismatches（碱基错配）, such as G–T

✓ small insertion/deletion loops（小片段插入/缺失环）



Deamination converts a cytosine base into a uracil.

The uracil is detected and removed, leaving a base-less nucleotide.

The base-less nucleotide is removed, leaving a small hole in the DNA backbone.

The hole is filled with the right base by a DNA polymerase, and the gap is sealed by a ligase.

Nucleic Acid Biosynthesis

DNA Damage Repair

3. Double-Strand Break Repair

Double-strand breaks, DSBs（DNA双链断裂） are highly dangerous because both DNA strands are broken. They may cause:chromosome rearrangements（染色体重排）,deletions（缺失）,translocations（易位） and cell death（细胞死亡）

Pathway	中文	Key feature	Fidelity
Non-Homologous End Joining, NHEJ	非同源末端连接	Broken DNA ends are processed and ligated directly	Error-prone（易出错）
Homologous Recombination, HR	同源重组修复	Uses a homologous template, usually sister chromatid	High fidelity（高保真）

Nucleic Acid Biosynthesis

RNA Biosynthesis

Nucleic Acid Biosynthesis

RNA Synthesis

RNA synthesis directed by a DNA template is called transcription(转录)

A transcription unit（转录单位） is a segment of DNA that is transcribed into one RNA molecule. It extends from the transcription start site（转录起始位点） to the transcription termination site（转录终止位点）.

A transcription unit may contain:

- One gene（一个基因） — common in eukaryotes
- Multiple genes（多个基因） — common in prokaryotic operons（操纵子）

Nucleic Acid Biosynthesis

RNA Synthesis

Asymmetric Transcription（不对称转录）

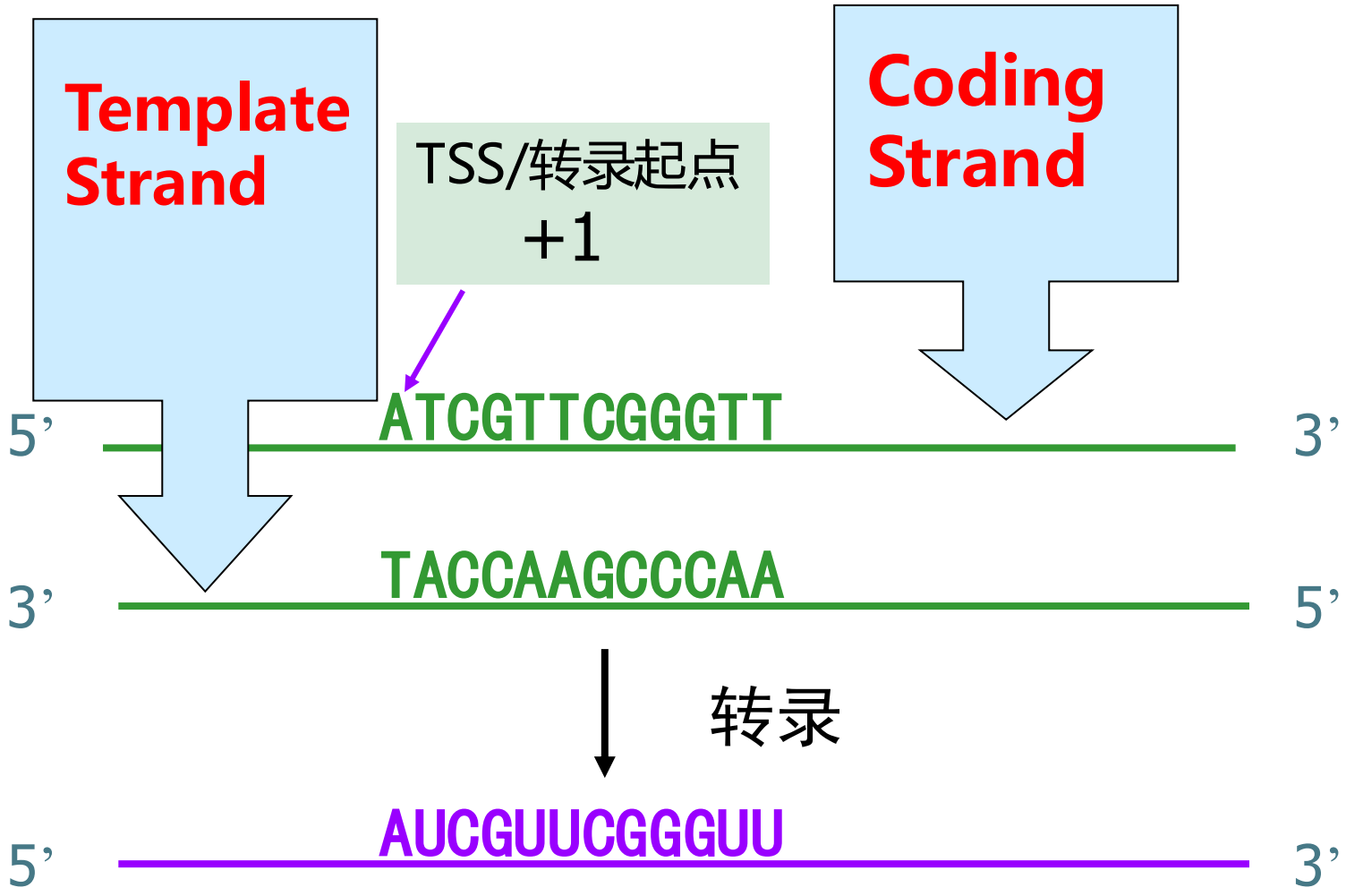
During transcription, **only one strand** of the DNA double helix serves as the template for RNA synthesis.

- **Template Strand**（模板链）:The DNA strand that is used by RNA polymerase as the template is called the template strand（模板链）. It is also called the: noncoding strand（非编码链）, antisense strand（反义链） or negative strand（负链）.
- RNA is synthesized complementary and antiparallel to this strand.

- **Coding Strand**（编码链）: The DNA strand that is not used as the template is called the coding strand（编码链）. It is also called the: sense strand（有义链）, or positive strand（正链）.
- The RNA sequence is almost identical to the coding strand, except that RNA contains U instead of T.

Nucleic Acid Biosynthesis

RNA Synthesis



Nucleic Acid Biosynthesis

RNA Synthesis

RNA Polymerase（RNA聚合酶）

1. Prokaryotic RNA Polymerase（原核生物RNA聚合酶）

$$\alpha_2\beta\beta'\sigma = \alpha_2\beta\beta' + \sigma + 2\,Zn^{2+}$$

Subunit	Main function
α	Enzyme assembly; interaction with regulatory proteins
β	Catalyzes phosphodiester bond formation during RNA elongation
β′	Binds DNA template
ω	Helps enzyme assembly and stability
σ factor	Recognizes **promoter sequences** and initiates transcription

Nucleic Acid Biosynthesis

RNA Synthesis

RNA Polymerase（RNA聚合酶）

2. Eukaryotic RNA Polymerases（真核生物RNA聚合酶）

Eukaryotic cells contain several nuclear RNA polymerases. The three major ones are RNA polymerase I, II, and III.

RNA polymerase	Location（分布）	RNA synthesized	Sensitivity to α-amanitin（α-鹅膏蕈碱）
RNA polymerase I	Nucleolus（核仁）	45S pre-rRNA → 18S, 5.8S, 28S rRNA	Insensitive（不敏感）
RNA polymerase II	Nucleoplasm（核质）	pre-mRNA; many snRNA and miRNA transcripts	Highly sensitive（高度敏感）
RNA polymerase III	Nucleoplasm（核质）	tRNA, 5S rRNA, U6 snRNA and other small RNAs	Moderately to highly sensitive（中到高度敏感）

Nucleic Acid Biosynthesis

RNA Synthesis

DNA Template Features for Transcription

In a DNA template, transcription-related sequence elements mainly include the promoter（启动子）, the transcription unit（转录单位）, and the terminator（终止子）.

❖ A **promoter（启动子）** is a specific DNA sequence that is recognized and bound by **RNA polymerase（RNA聚合酶）** and transcription factors.

It determines:

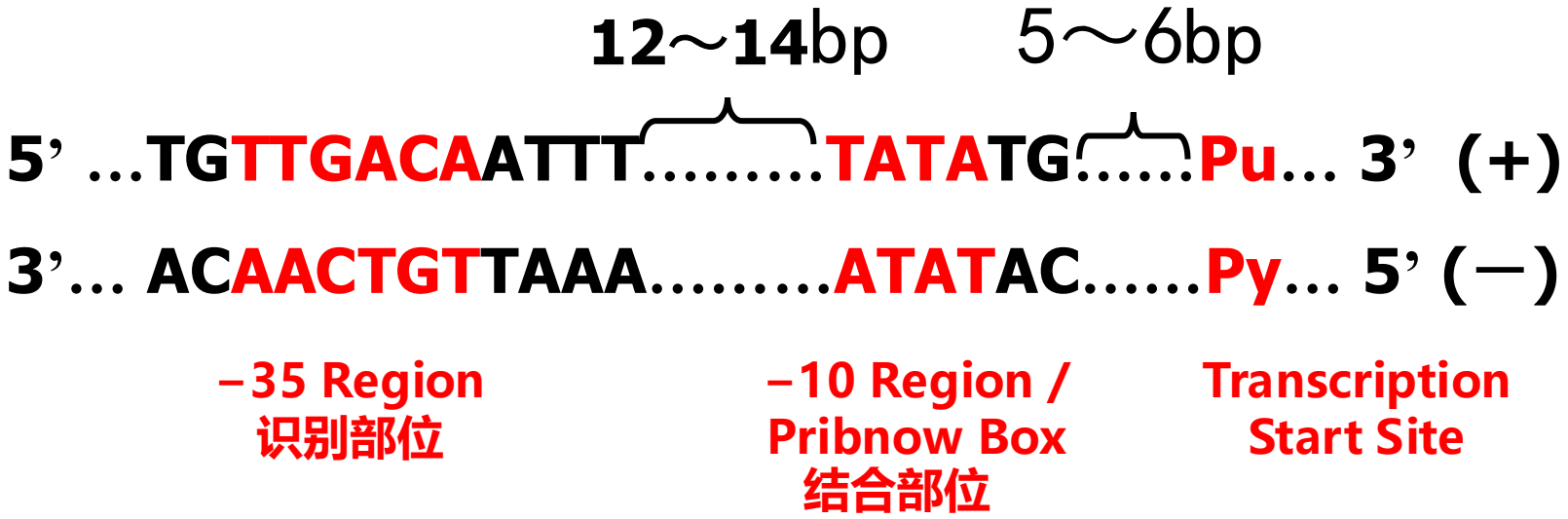
- ✓ **where transcription starts** → the **transcription start site, TSS（转录起始位点）**
- ✓ **which DNA strand is used as the template strand（模板链）**
- ✓ **the direction of transcription（转录方向）**
- ✓ **the efficiency of transcription initiation（转录起始效率）**

The promoter is usually located upstream（上游） of the transcription start site.

Nucleic Acid Biosynthesis

RNA Synthesis

Prokaryotic Promoter Structure/原核生物启动子结构



Recognized by the σ factor and helps RNA polymerase bind to the promoter.

A-T-rich region that is easily unwound, allowing formation of the open complex.

The nucleotide where RNA synthesis begins.

Nucleic Acid Biosynthesis

RNA Synthesis

DNA Template Features for Transcription

❖ A **transcription unit**（转录单位） is the DNA region that is transcribed into RNA. It extends from the transcription start site（转录起始位点） to the termination site（转录终止位点）. During transcription, RNA polymerase reads the template strand（模板链） in the 3′ → 5′ direction, while RNA is synthesized in the 5′ → 3′ direction.

❖ **A terminator**（终止子） is a DNA sequence that signals RNA polymerase to stop transcription.

- ✓ where transcription ends → the transcription termination site（转录终止位点）
- ✓ release of the RNA transcript（RNA转录产物释放）
- ✓ dissociation of RNA polymerase from DNA（RNA聚合酶从DNA上解离）

Nucleic Acid Biosynthesis

RNA Synthesis

Termination of RNA Transcription RNA转录的终止

ρ factor（ρ因子） is an auxiliary protein that helps bacterial RNA polymerase recognize certain transcription termination signals.

1. ρ-independent termination

The RNA forms a GC-rich hairpin structure（发夹结构）, followed by a U-rich sequence（连续U）. This causes RNA polymerase to pause and the RNA transcript to dissociate.

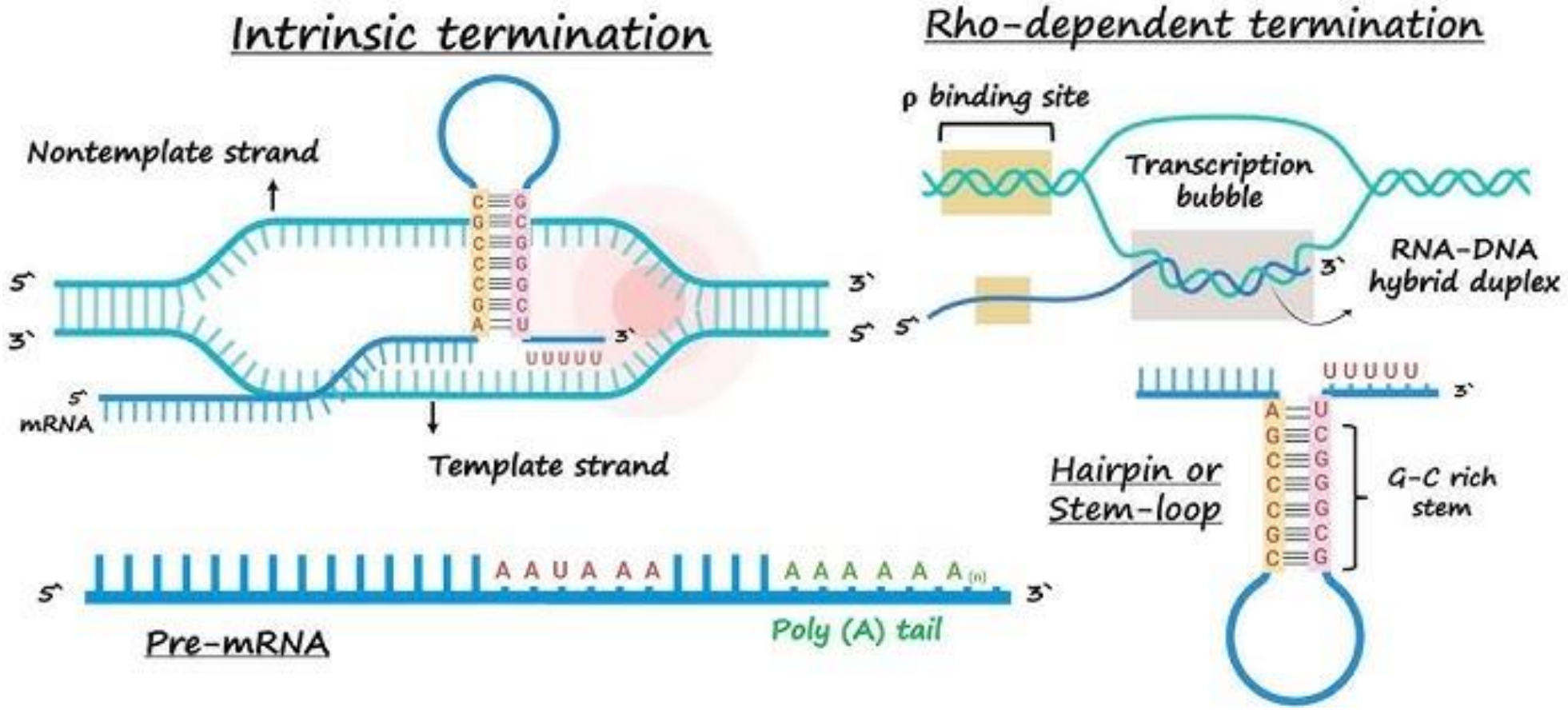
2. ρ-dependent termination

ρ factor（ρ因子）binds the RNA at a rut site（ρ利用位点）, moves along the RNA using ATP, and releases the RNA when RNA polymerase pauses.

Nucleic Acid Biosynthesis

RNA Synthesis

Termination of RNA Transcription RNA转录的终止



Nucleic Acid Biosynthesis

RNA Synthesis

RNA Transcription in *E. coli*

Initiation（起始）
RNA polymerase holoenzyme recognizes the promoter（启动子） through σ factor（σ因子） and locally unwinds DNA to form a transcription bubble（转录泡）.

Promoter clearance（启动子清除）
After several nucleotides are synthesized, σ factor usually dissociates, and the core enzyme continues transcription.

Elongation（延长）
RNA polymerase moves along the DNA template strand（模板链） and synthesizes RNA in the 5′ → 3′ direction. A short RNA–DNA hybrid（RNA-DNA杂合体） is maintained inside the transcription bubble.

Termination（终止）
When RNA polymerase reaches a terminator（终止子）, transcription stops. The RNA transcript, RNA polymerase, and DNA dissociate.

Nucleic Acid Biosynthesis

RNA Synthesis

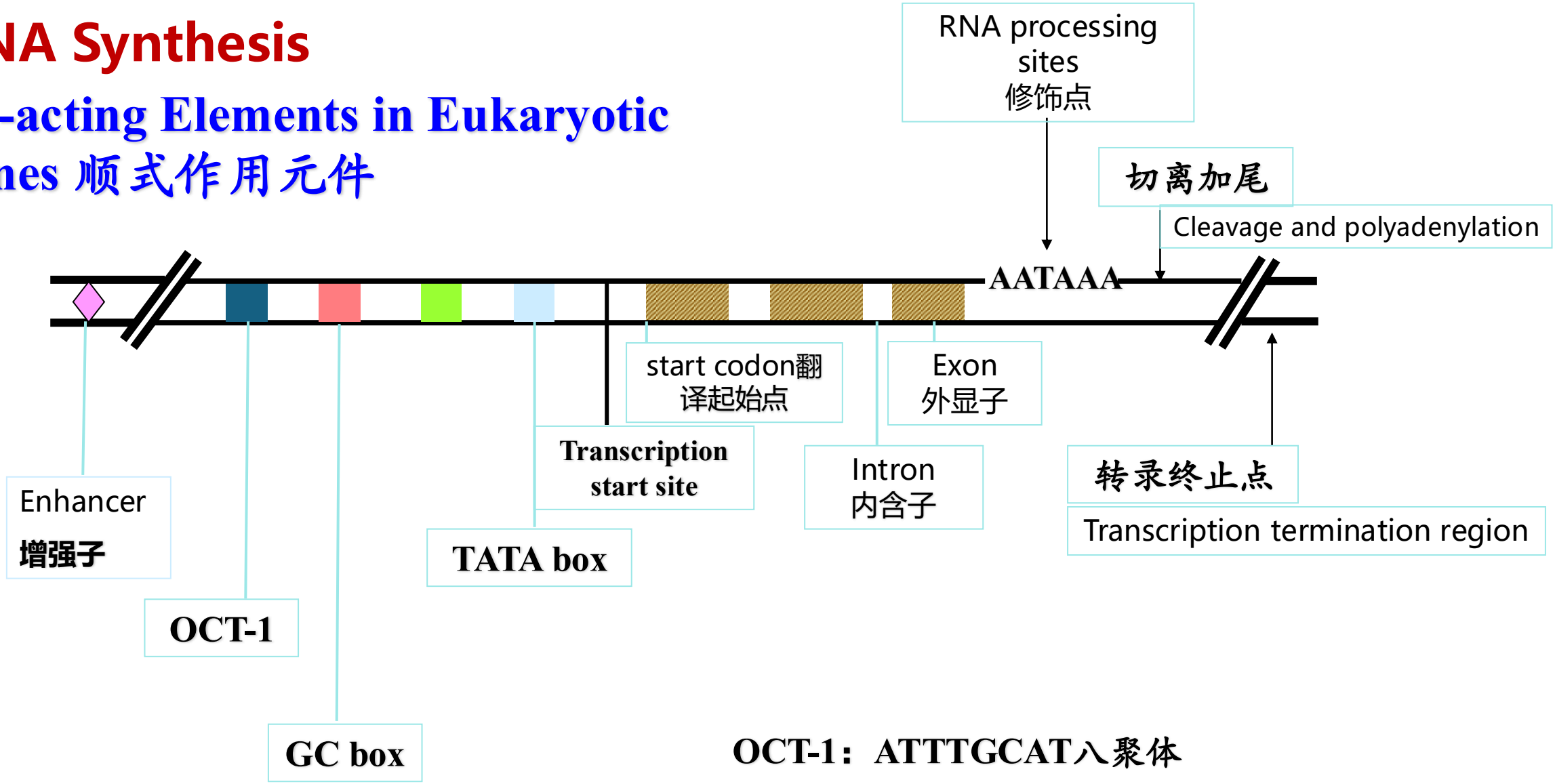
Transcription in Eukaryotes compared with Prokaryotes

1. **Separated sites（区室分隔）：** Transcription occurs in the nucleus（细胞核）, while translation occurs in the cytoplasm（细胞质）.
2. **Multiple RNA polymerases（多种RNA聚合酶）：** Eukaryotes contain RNA polymerase I, II, and III.
   RNA polymerase II synthesizes pre-mRNA（mRNA前体）.
3. **More complex promoters（启动子更复杂）：** Some promoters contain a TATA box（TATA盒） around −25 to −30 bp, but many are TATA-less.
4. **RNA processing after transcription（转录后加工）：** Pre-mRNA undergoes 5′ capping（5′端加帽）, splicing（剪接）, and polyadenylation（加poly(A)尾） before becoming mature mRNA.

Nucleic Acid Biosynthesis

RNA Synthesis

Cis-acting Elements in Eukaryotic Genes 顺式作用元件



OCT-1：ATTTGCAT八聚体

Nucleic Acid Biosynthesis

RNA Synthesis

Transcription Factors 转录因子

Transcription factors, TFs（转录因子） are trans-acting proteins（反式作用蛋白） that recognize and bind specific DNA regulatory sequences. They can bind either directly or indirectly to regulatory DNA regions located upstream, downstream, or near the transcription unit.

1. **Bind cis-acting elements（结合顺式作用元件）**

   Examples: promoter（启动子）, enhancer（增强子）, silencer（沉默子）.

2. **Recruit or regulate RNA polymerase（招募或调节RNA聚合酶）**

   They help RNA polymerase initiate transcription efficiently.

3. **Activate or repress transcription（激活或抑制转录）**

   Some transcription factors increase gene expression, while others reduce it.

Nucleic Acid Biosynthesis

RNA Synthesis

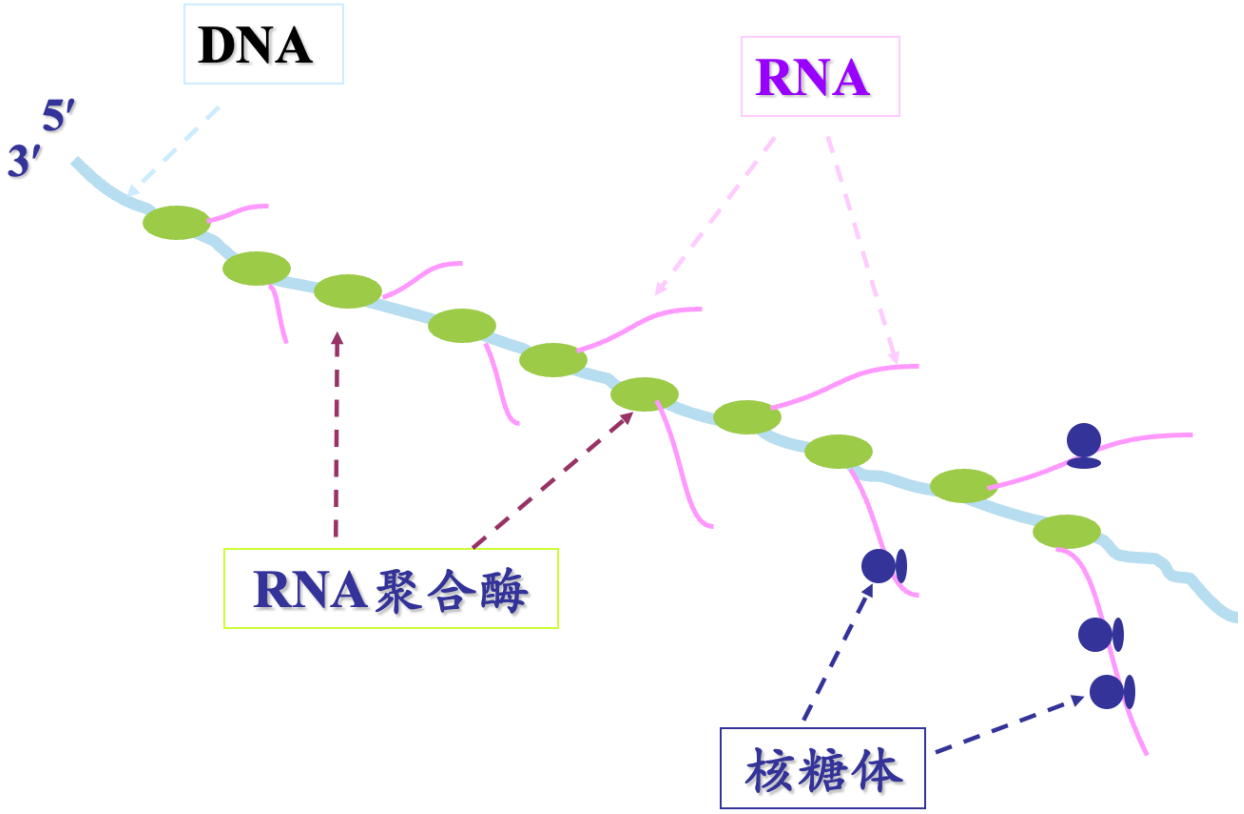
Concept	Nature	Example
Cis-acting element (顺式作用元件)	DNA sequence	TATA box, enhancer, GC box
Trans-acting factor (反式作用因子)	Protein or RNA factor	Transcription factor, RNA polymerase-associated factor

Nucleic Acid Biosynthesis

RNA Synthesis

Post-transcriptional Processing of RNA

In prokaryotes（原核生物）, mRNA usually undergoes little or no post-transcriptional processing. Because prokaryotes do not have a nucleus, transcription（转录） and translation （翻译） are spatially coupled and can occur almost simultaneously.



Nucleic Acid Biosynthesis

RNA Synthesis

Post-transcriptional Processing of Eukaryotic RNA

In eukaryotes, newly synthesized RNA transcripts usually require processing before they become functional molecules.

For mRNA, the primary transcript is called pre-mRNA（mRNA前体）, historically also called hnRNA（核内不均一RNA, heterogeneous nuclear RNA）.

Intron（内含子）: Non-coding sequence within a eukaryotic gene that is transcribed into pre-mRNA but removed during RNA splicing.

Exon（外显子）: Sequence retained in the mature RNA. In mRNA, exons usually contain coding regions, but they may also include untranslated regions, such as the 5′ UTR and 3′ UTR.

Nucleic Acid Biosynthesis

RNA Synthesis

Post-transcriptional Processing of Eukaryotic RNA

Eukaryotic pre-mRNA is converted into mature mRNA through three major processing steps:

5′ capping（5′端加帽）:A **7-methylguanosine cap（7-甲基鸟苷帽, m^7G cap）** is added to the 5′ end through a 5′–5′ triphosphate linkage.

3′ polyadenylation（3′端加poly(A)尾）: The 3′ end is cleaved, and a **poly(A) tail（poly(A)尾）** is added. In many mammalian mRNAs, the poly(A) tail is about **200–250 nucleotides** long.

RNA splicing（RNA剪接）: Introns（内含子） are removed, and exons（外显子） are joined together. Additional modification may occur, such as **RNA methylation（RNA甲基化）**, including modifications like **m^6A**.

Nucleic Acid Biosynthesis

RNA Synthesis

tRNA Precursors

1. **Removal of extra sequences at the 5′ and 3′ ends**

   The 5′ leader sequence is removed by **RNase P（核糖核酸酶P）**.
2. **Addition of CCA at the 3′ end**

   Many mature tRNAs receive a **CCA sequence（CCA序列）** at the 3′ end.

   This CCA is the amino acid attachment site.
3. **Base modification（碱基修饰）**

   Some bases are chemically modified, producing unusual bases such as:

   dihydrouridine（DHU, 二氢尿苷）, **pseudouridine（Ψ, 假尿苷）**, and **inosine（I, 次黄苷）**.
4. **Intron removal in some tRNAs**

   Some eukaryotic tRNA precursors contain introns that must be removed.

Nucleic Acid Biosynthesis

RNA Synthesis

rRNA Precursors

In bacteria / prokaryotes

A precursor transcript contains:

- **16S rRNA → small ribosomal subunit（30S小亚基)**
- **23S rRNA + 5S rRNA → large ribosomal subunit（50S大亚基)**

Together they form the **70S ribosome**.

In eukaryotes

The **45S pre-rRNA**is processed into:

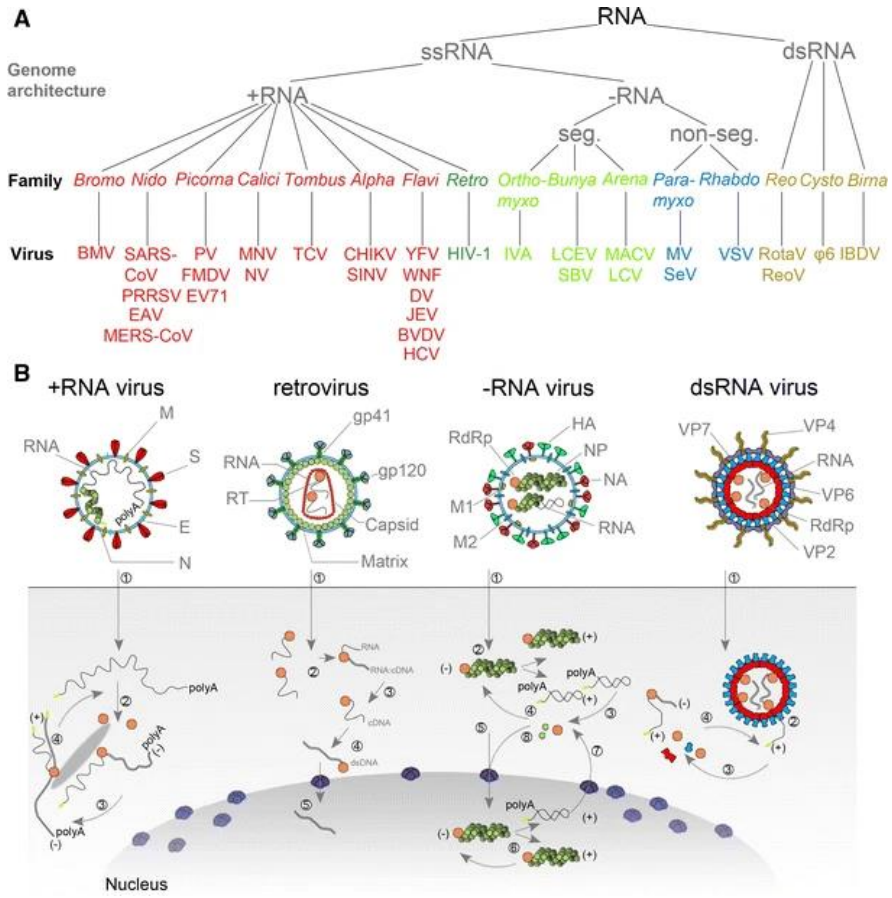
- **18S rRNA → small ribosomal subunit（40S小亚基)**
- **28S rRNA + 5.8S rRNA + 5S rRNA → large ribosomal subunit（60S大亚基)**

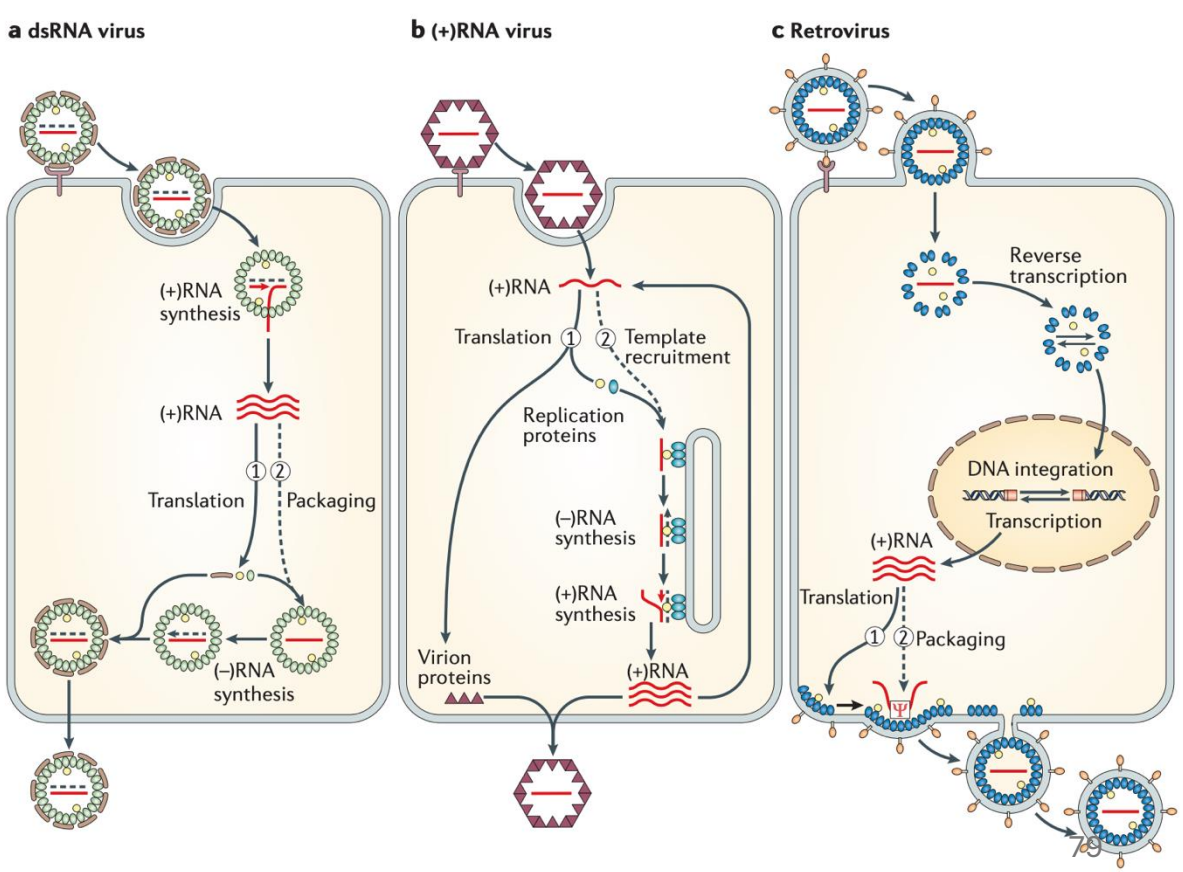
Together they form the **80S ribosome（80S核糖体）**.

Nucleic Acid Biosynthesis

RNA Synthesis

RNA Replication in RNA Viruses (自学)



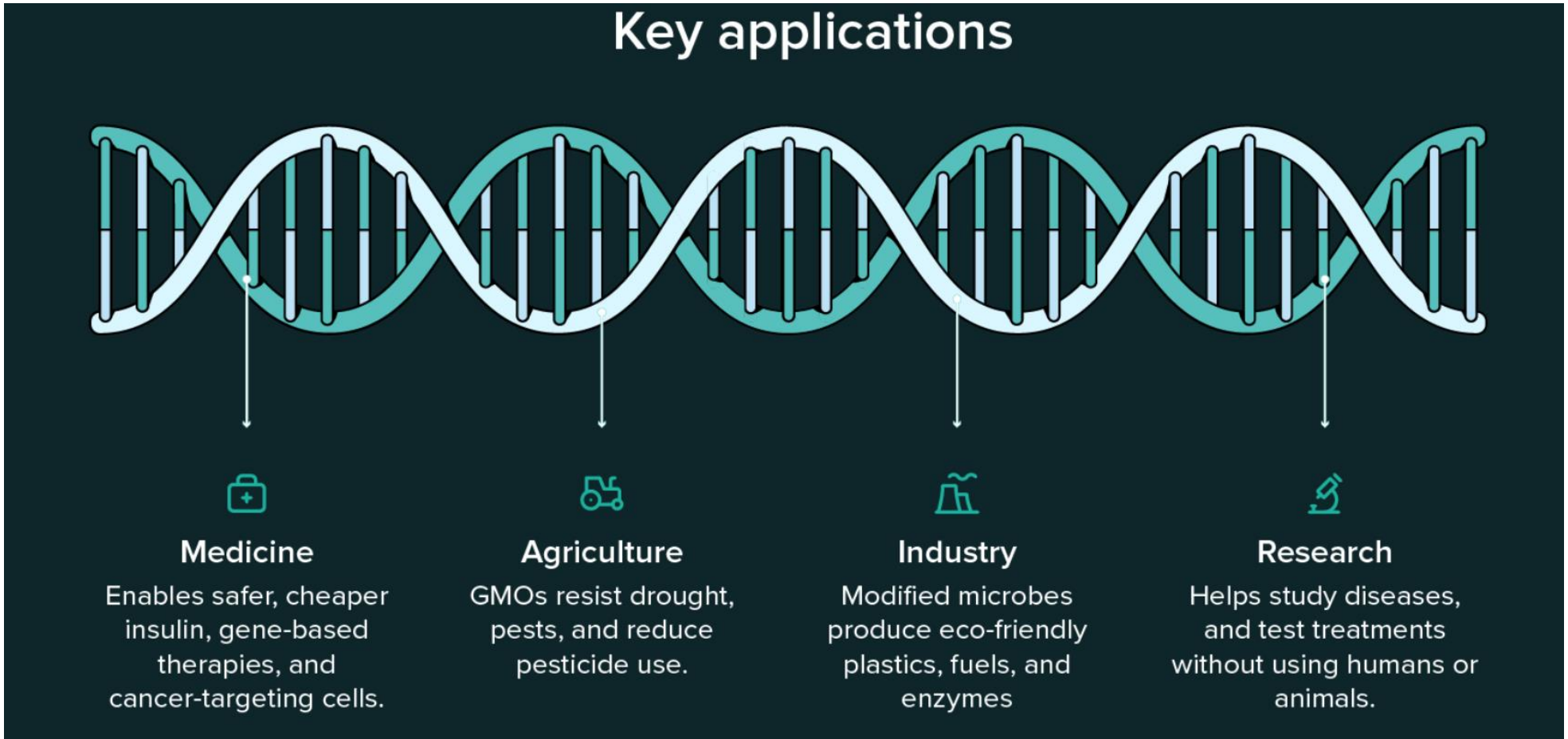


Contents

Section 1	Nucleic Acid Catabolism
Section 2	Nucleic Acid Biosynthesis
Section 3	**Applications: Genetic Engineering**

What is genetic engineering?

Genetic engineering（基因工程）is the deliberate modification of DNA to change the genetic information or gene expression of an organism, cell, or virus.



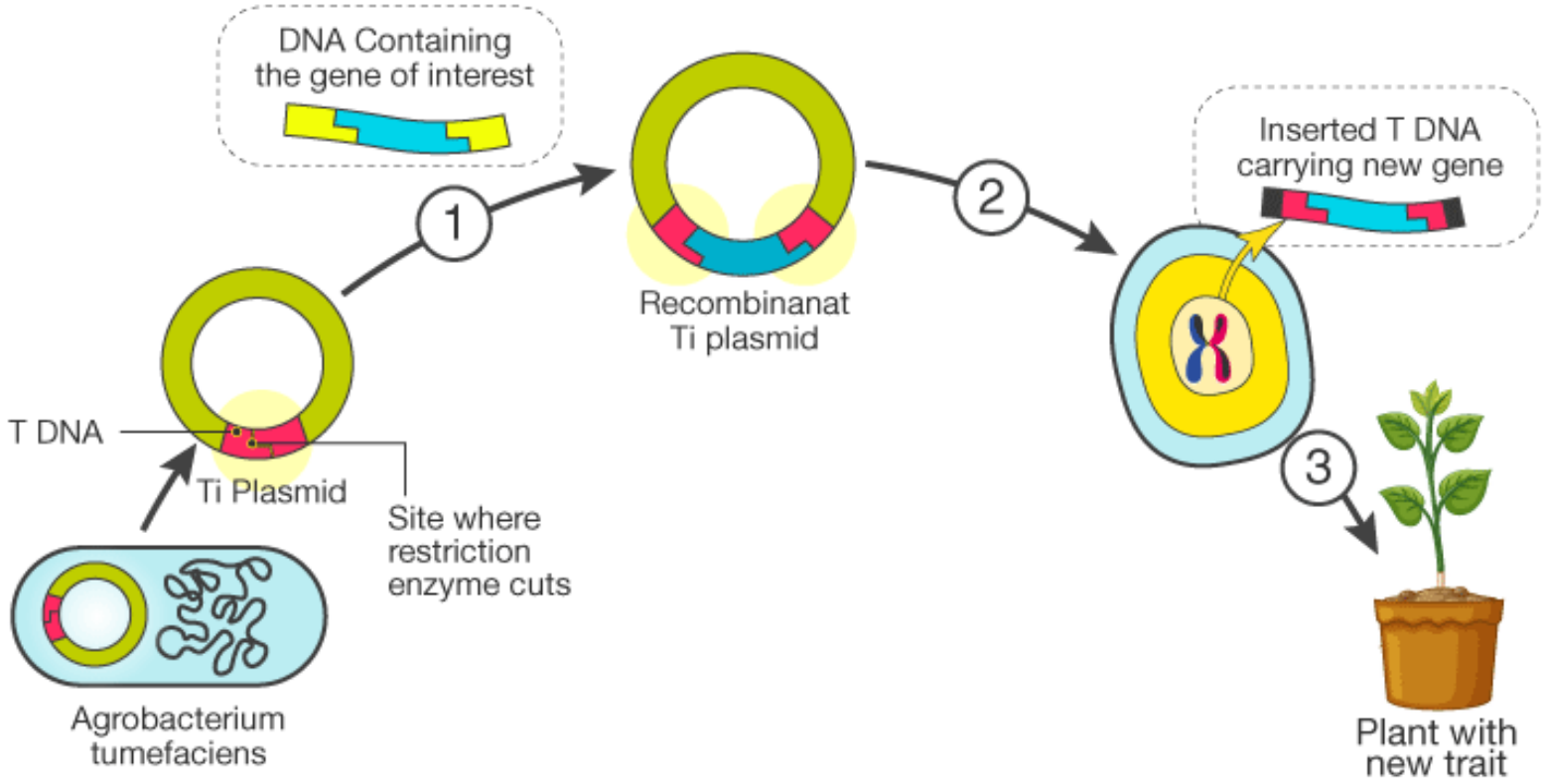
Genetic engineering technologies

Recombinant DNA Technology / 重组DNA

- DNA can be cut by restriction endonucleases（限制性内切酶）
- DNA fragments can be joined by DNA ligase（DNA连接酶）
- DNA from different sources can be assembled into recombinant DNA
- A vector（载体）, usually a plasmid, carries the target gene

Genetic engineering technologies

Recombinant DNA Technology / 重组DNA



1. Treat foreign DNA and plasmid with restriction enzyme and DNA ligase.
2. Introduce the recombinant plasmid into cultured plant cells.
3. Regenerate new plant from cultured cells.

Genetic engineering technologies

Recombinant DNA Technology / 重组DNA

Gene vectors（基因载体） are DNA molecules used to carry foreign DNA into host cells.

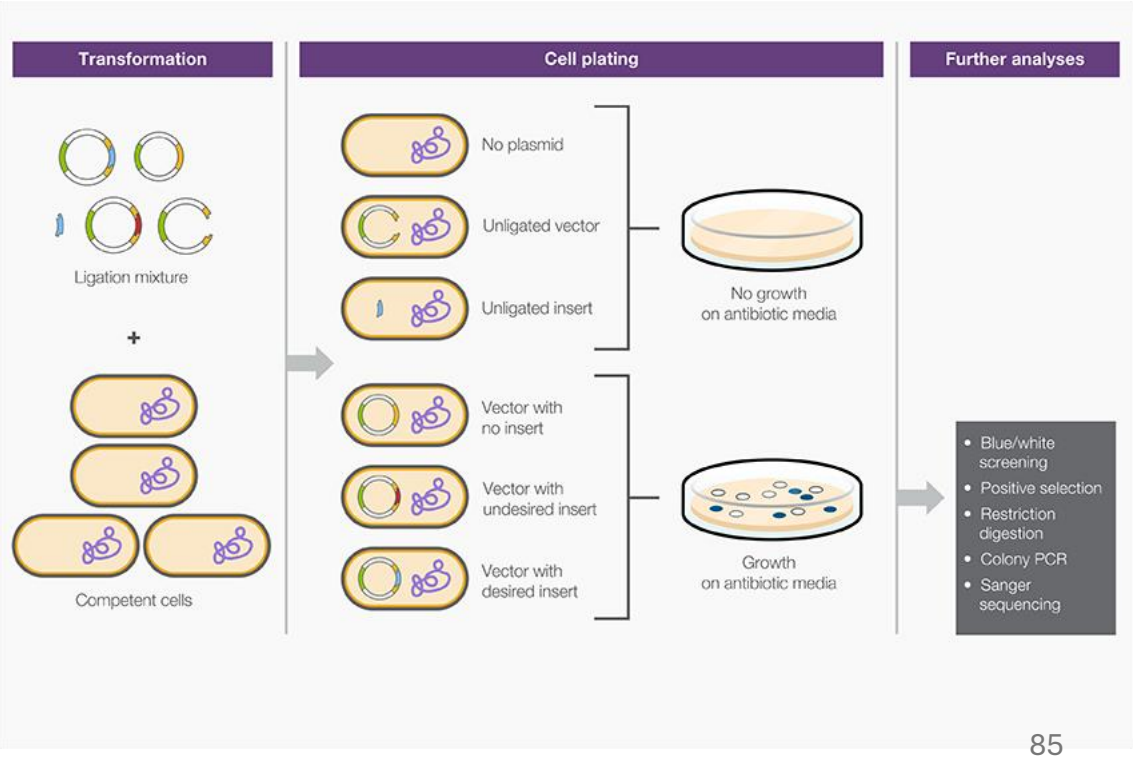
Vector type	Key feature	Example
Plasmid vector（质粒载体）	Small, circular, double-stranded DNA that replicates independently in bacteria	pBR322, pUC19
Viral vector（病毒载体）	Uses viral delivery mechanisms to introduce DNA into eukaryotic cells	adenovirus, lentivirus, AAV
Phage vector（噬菌体载体）	Derived from bacteriophages; useful for cloning larger DNA fragments	λ phage
Artificial chromosome（人工染色体）	Carries very large DNA fragments	BAC, YAC

Genetic engineering technologies

Gene Cloning / 基因克隆

target gene（目的基因） → vector（载体） → recombinant plasmid（重组质粒） → host cell（宿主细胞） → clone selection（克隆筛选）

- plasmid（质粒）
- origin of replication, ori（复制起点）
- selectable marker（筛选标记）
- multiple cloning site, MCS（多克隆位点）
- transformation（转化）



Genetic engineering technologies

Gene Expression

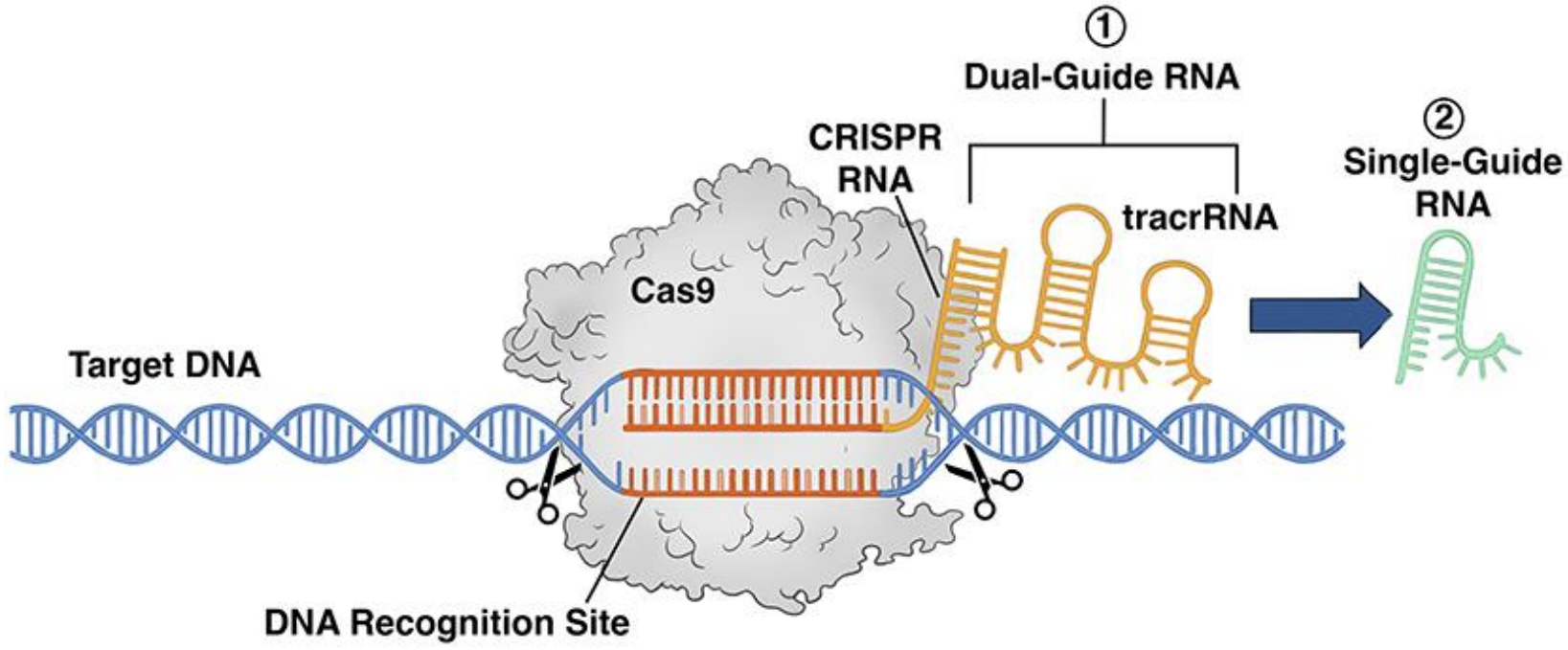
Gene expression is the process by which genetic information in DNA is used to produce a functional product, usually RNA or protein.

Component	Function
Expression vector（表达载体）	Carries the target gene and regulatory elements
Promoter（启动子）	Recruits RNA polymerase and controls transcription initiation
Coding sequence, CDS（编码序列）	DNA sequence that encodes the protein
Ribosome-binding site, RBS（核糖体结合位点）	Required for bacterial translation initiation
Kozak sequence（Kozak序列）	Helps translation initiation in eukaryotes
Terminator（终止子）	Signals transcription termination
Fusion tag（融合标签）	Helps protein purification or detection, e.g., His-tag

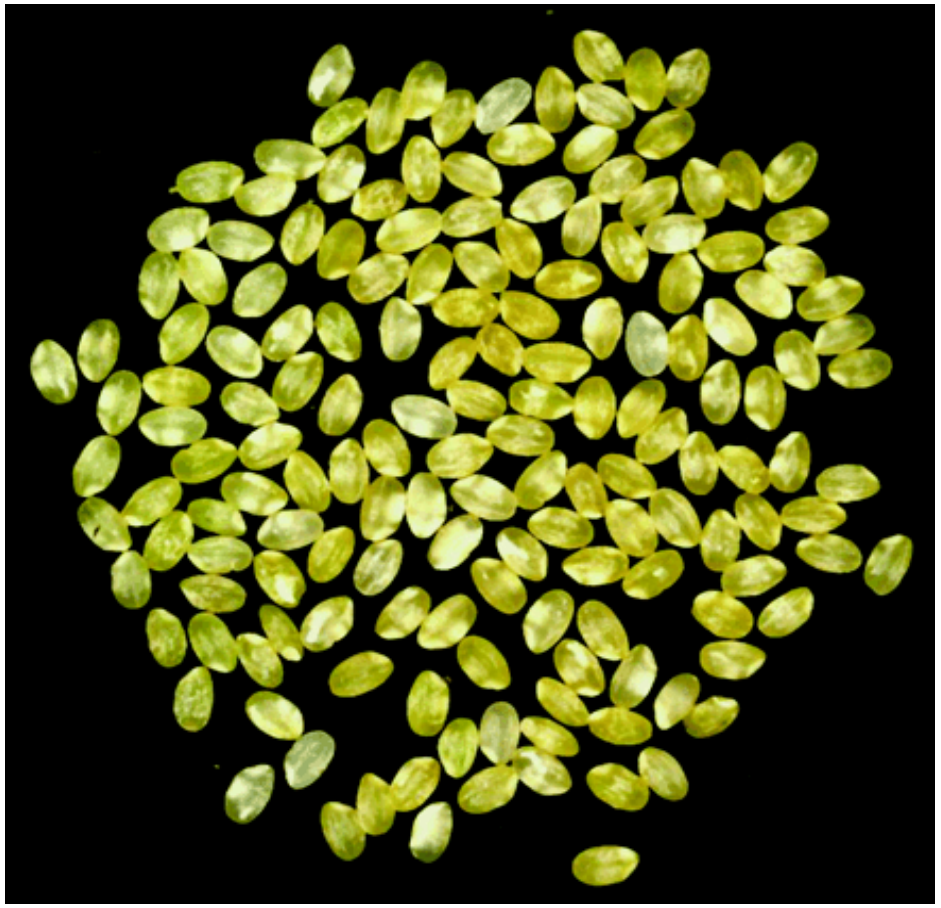
Genetic engineering technologies

Genome Editing / 基因组编辑

Genome editing refers to technologies that introduce targeted changes into genomic DNA. The most widely used system is CRISPR-Cas9, which uses a guide RNA to direct Cas9 nuclease to a specific DNA sequence.



Genetic Engineering Applications



黃金水稻(Golden Rice)

Provitamin A (β-Carotene) :

phytoene synthase,

phytoene desaturase,

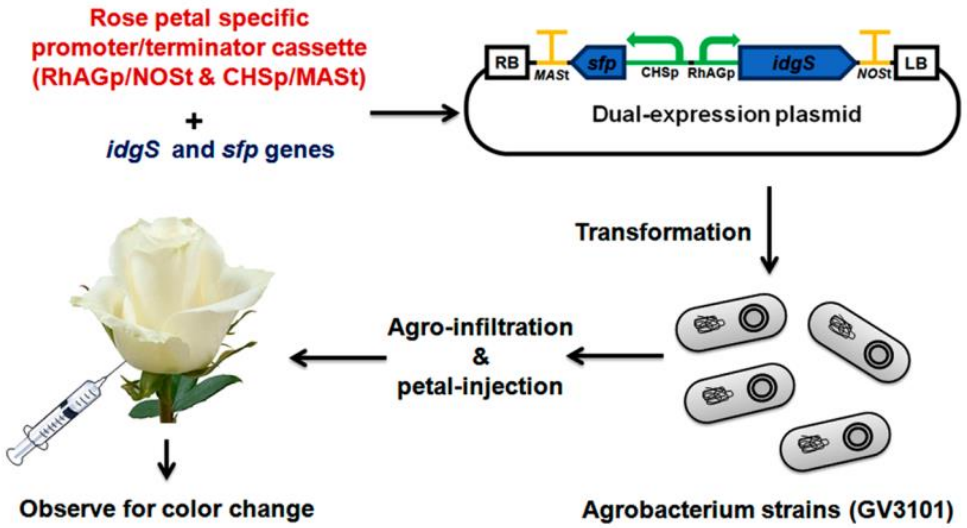
ξ-carotene desaturase,

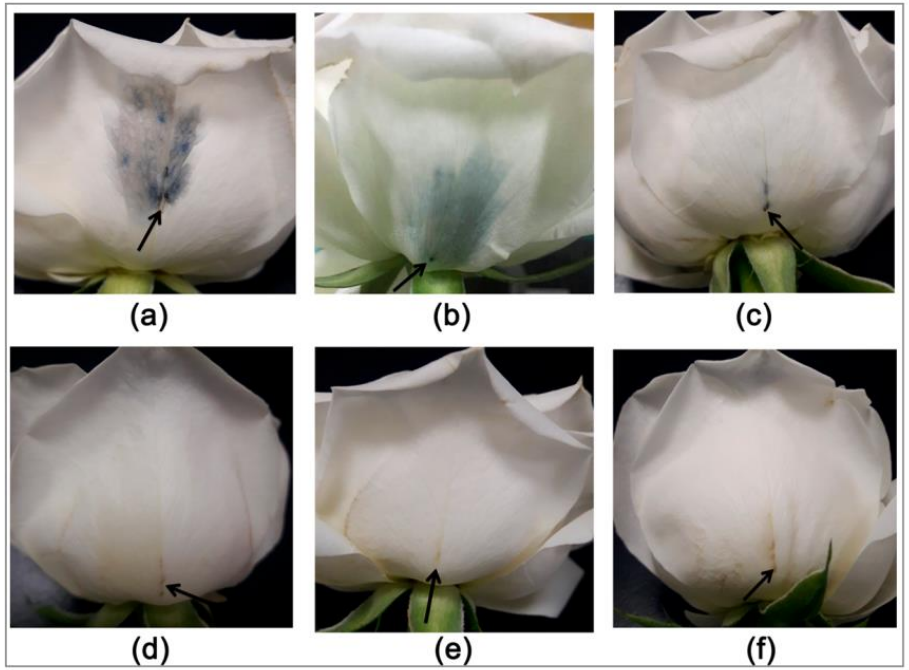
Lycopene cyclase

Genetic Engineering Applications



blue rose





天津大学：成功研发全球首枝蓝玫瑰

2018年10月17日09:29 | 来源：中国教育报

T$_T$ 小字号

在英语里，“蓝玫瑰”意味着无法实现的希望或不可能完成的任务，因为自然界中没有蓝玫瑰。不过如今，“蓝玫瑰”的含义可以改写了。天津大学药学院张雁教授研究团队与中国科学院大学陈义华教授团队合作，利用合成生物学技术成功研发出全球首枝蓝玫瑰。

近日，这篇题为《一种用于蓝玫瑰生产的非核糖体肽合成酶的克隆与表达》的文章发表在《美国化学会合成生物学》上，第一作者为天津大学药学院国际博士后安卡纳哈利·南加瓦。

人类有5000年玫瑰培育历史，却无法获得蓝色的玫瑰。张雁课题组通过构建双重表达质粒实现了这一目标，该质粒包括两个参与靛蓝合成的细菌基因。研究人员将该质粒转化进入农杆菌中，再将农杆菌注射到白玫瑰的花瓣中，注射12小时后，农杆菌在植物荷尔蒙乙酰丁香酮的诱导下把基因转移到玫瑰花瓣细胞基因组中，从而使玫瑰细胞表达合成深蓝色的酶。

全球首枝蓝玫瑰就这样生成了。据悉，下一步张雁团队计划与相关科研团队合作，利用遗传学技术，永久性地修改植物的基因组，从而获得稳定可遗传的蓝色玫瑰。（陈欣然）

（责编：方文宇(实习生)、熊旭）