Chapter 3 DNA damage, repair and recombination

1. Mutation

Mutations are **permanent** (永久的), **heritable** (可遗传的) **alterations** in the **base sequence** of the genetic material, DNA or RNA.

Mutation
- Point mutation
- Insertions or deletions 插入/缺失

1.1 Point mutation (点突变)

A point mutation is a type of mutation that causes a single base change in DNA.

1.1.1 Transition (转换)

One purine (or pyrimidine) is replaced by the other purine (or pyrimidine). A↔G T↔C

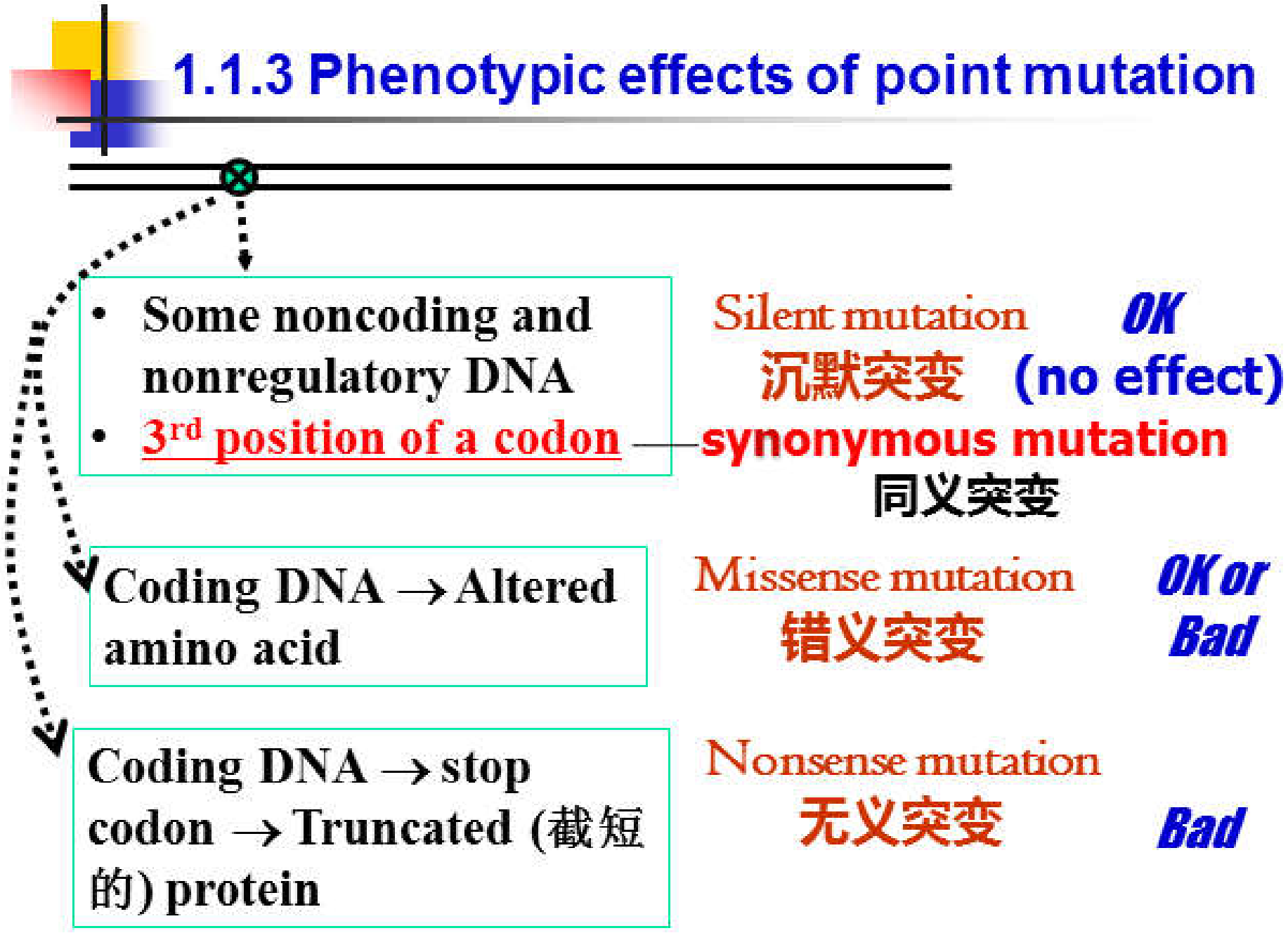
1.1.2 Transversion (颠换)

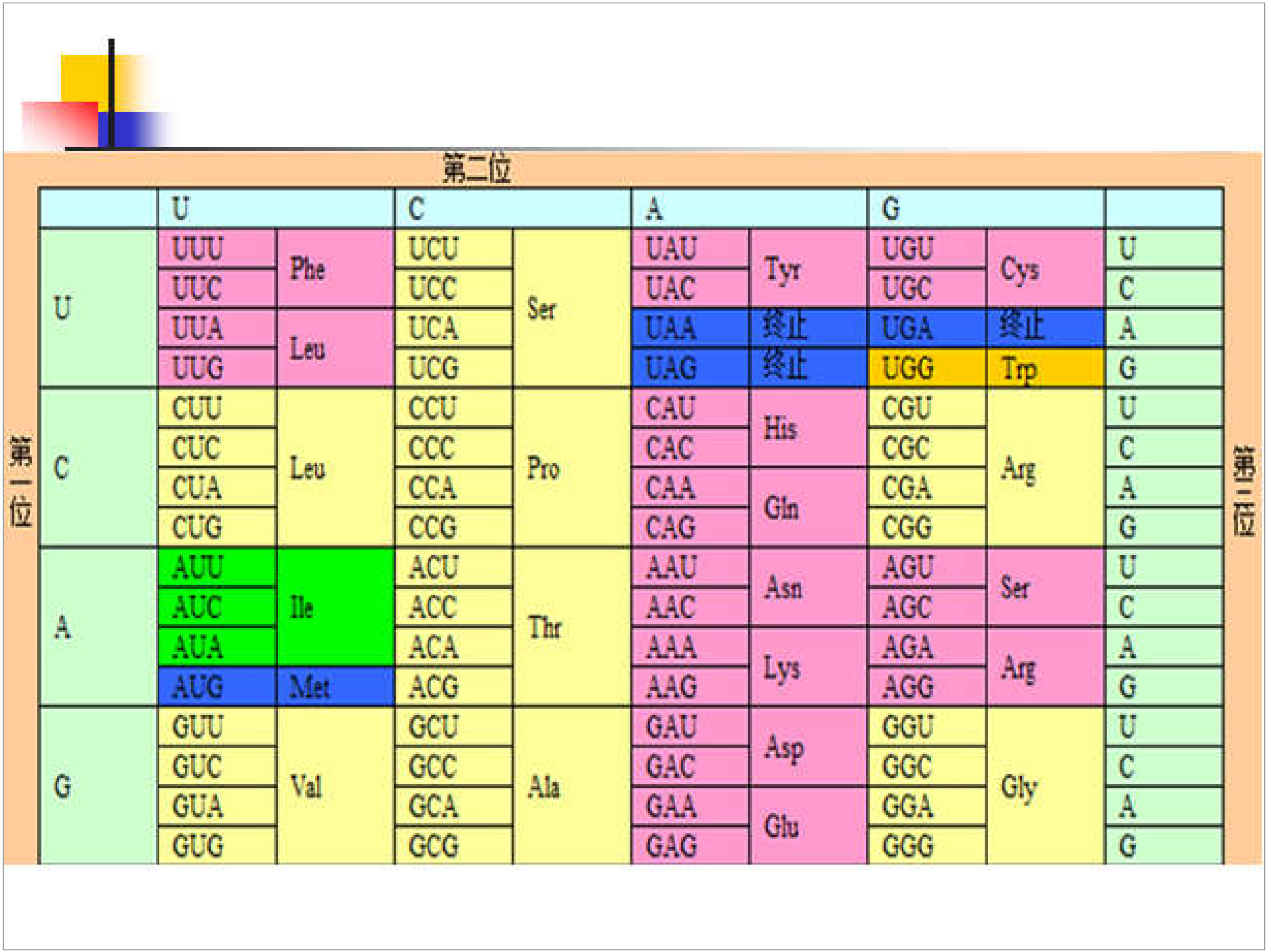
A purine is replaced by a pyrimidine or vice versa (反之亦然).

A ↔T or C, T ↔ A or G, G ↔T or C, C ↔ A or G

Transition mutations are about ten times more common than transversions.

1.1.3 Phenotypic effects of point mutation





1.2 Frame-shift mutation (移码突变)

A **frame-shift mutation** is a genetic mutation caused by **insertions (插入) or deletions (缺失)** of one or more nucleotides in a DNA sequence that **is not divisible (整除) by three.**

The translated protein sequence to the C-terminal side of the mutation is completely changed.

1.3 Consequences of mutation

1.3.1. Cell death

Mutations seriously affect DNA replication or transcription.

1.3.2 Tumorigenesis (癌变)

Mutations affect the processes of cell growth and apoptosis (凋亡).

1.3.3 Genetic polymorphisms (遗传多态性)

The accumulation of many silent and other non-lethal mutations in populations produces genetic polymorphisms – acceptable variations in the normal DNA and protein sequences.

2. DNA damage

DNA damage or **DNA lesion** is an **alteration** of the **normal** chemical or physical **structure** of the DNA.

2.1 Consequences of DNA damage

2.1.1 Mutation

- Loss of base pairing or altered base pairing.
- If such a lesion was allowed to remain in the DNA, a mutation could become fixed in the DNA.

2.1.2 Cell death

- Lethal mutation
- The altered bases produce a physical distortion (扭曲变形) → Blocks replication and/or transcription, then causing cell death.

2.2 Factors that cause DNA damage

内因

Spontaneous (自发的) DNA damages in cells

- Spontaneous errors in DNA replication
- Spontaneous changes in bases
- Intracellular metabolites (细胞内代谢物) e.g. reactive oxygen species (ROS, 活性氧) such as superoxide ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot OH$)

外因

External (外部) physical, chemical, biological factors --Mutagens (诱变剂)

2.2.1 Spontaneous errors in DNA replication

(1) Base mismatch during replication

Spontaneous mutation rate（自发突变率）is very low; one error per 10^{10} base (*E. coli*).

Replication fidelity is the high accuracy that preserve the meaning of the genetic information from one generation to the next.

复制忠实性/保真度是指维持遗传信息从上一代传至下一代的准确性。

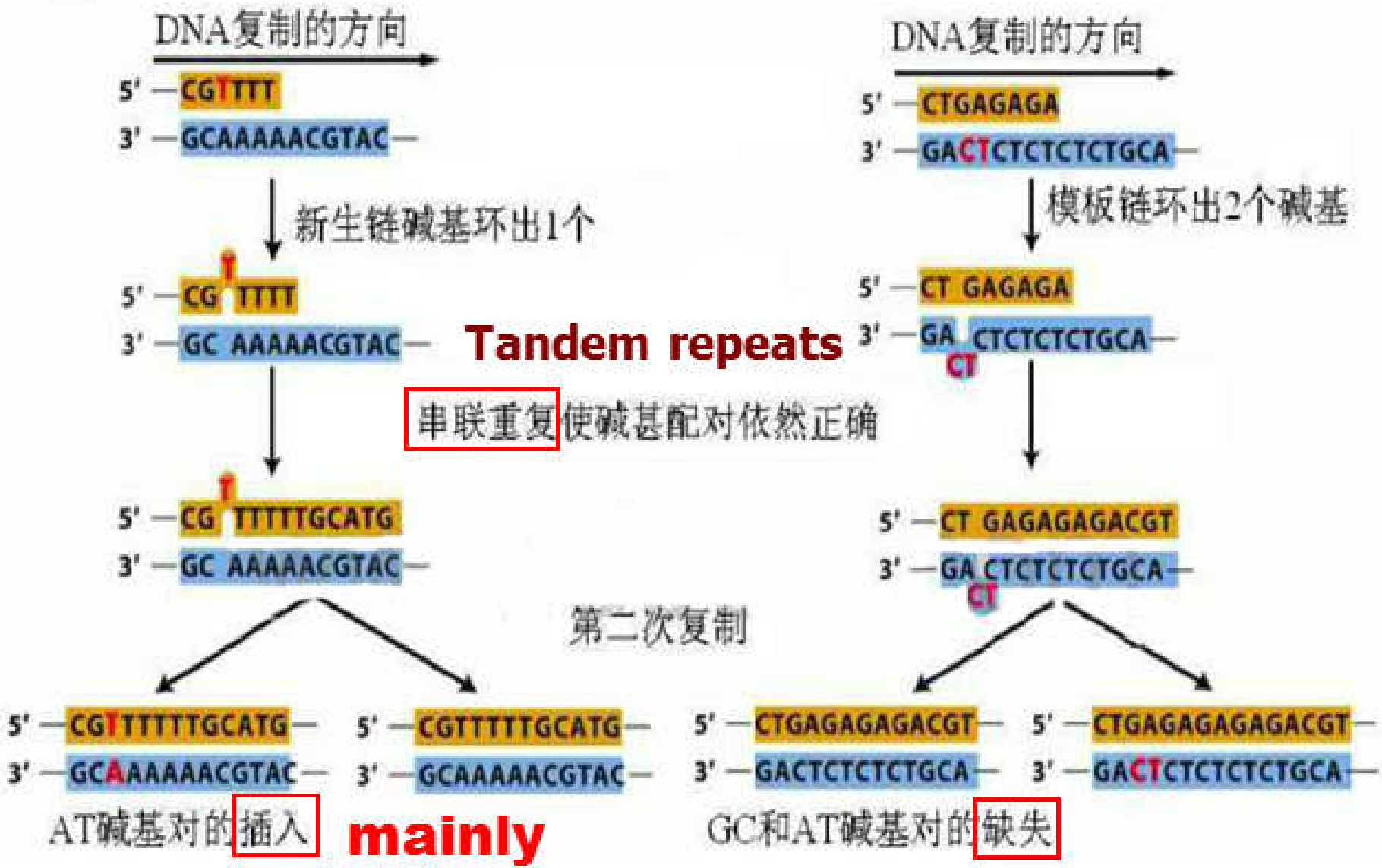
Molecular mechanisms that keep the replication fidelity
复制忠实性的分子机制

- DNA polymerase selectivity
- 3' → 5' proofreading exonuclease
- RNA priming
- Mismatch repair (错配修复)

(2) Replication slippage (复制打滑)

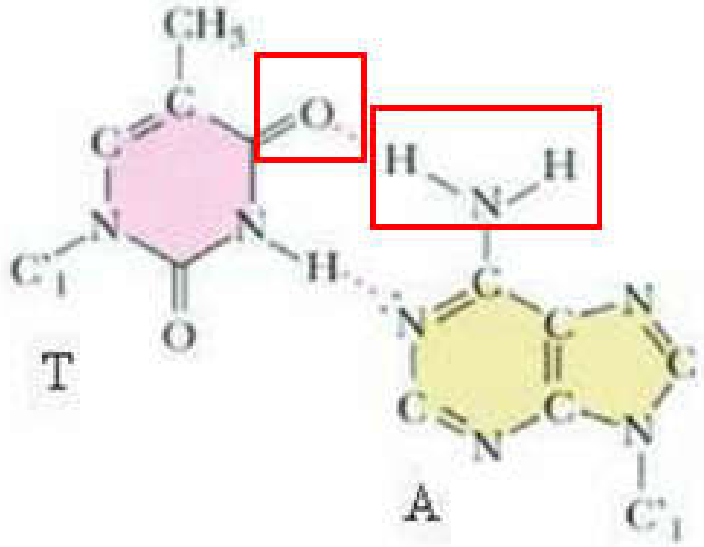
在DNA复制时，有时会出现模板链或新生链碱基的环出(looping out)现象，被称为**复制打滑**。

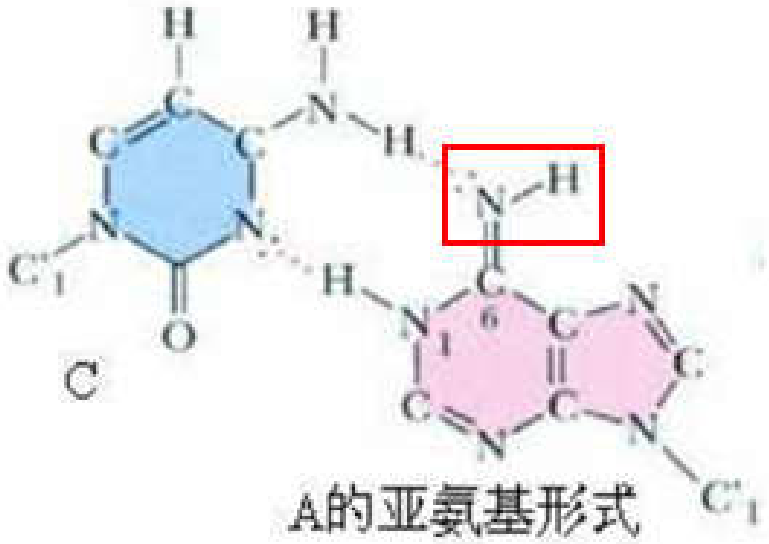
这种错误易发生在模板上有碱基**串联重复**的部位。结果是碱基的**插入或缺失**。


DNA复制的方向
5′ CGTTTT
3′ GCAAAAACGTAC
新生链碱基环出1个
5′ CG T TTT
3′ GC AAAAACGTAC
5′ CG T TTTTGCATG
3′ GC AAAAACGTAC
Tandem repeats
串联重复使碱基配对依然正确
第二次复制
5′ CGTTTTTTGCATG
3′ GCAAAAAACGTAC
5′ CGTTTTTGCATG
3′ GCAAAAACGTAC
AT碱基对的插入
mainly
DNA复制的方向
5′ CTGAGAGA
3′ GACTCTCTCTCTGCA
模板链环出2个碱基
5′ CT GAGAGA
3′ GA CTCTCTCTGCA
CT
5′ CT GAGAGAGACGT
3′ GA CTCTCTCTGCA
CT
5′ CTGAGAGAGACGT
3′ GACTCTCTCTGCA
5′ CTGAGAGAGAGACGT
3′ GACTCTCTCTCTGCA
GC和AT碱基对的缺失
Frame-shift mutation

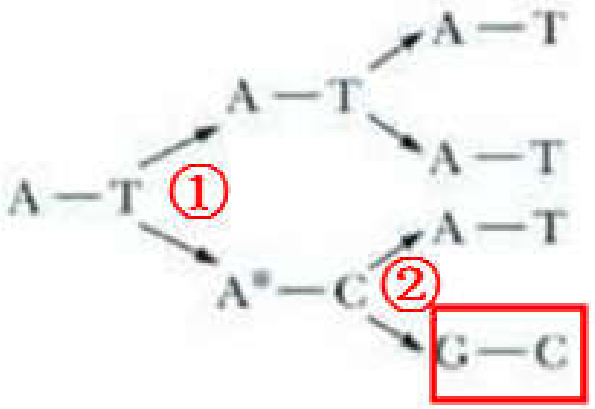
2.2.2 Spontaneous changes in bases

(1) Tautomerism (互变异构)

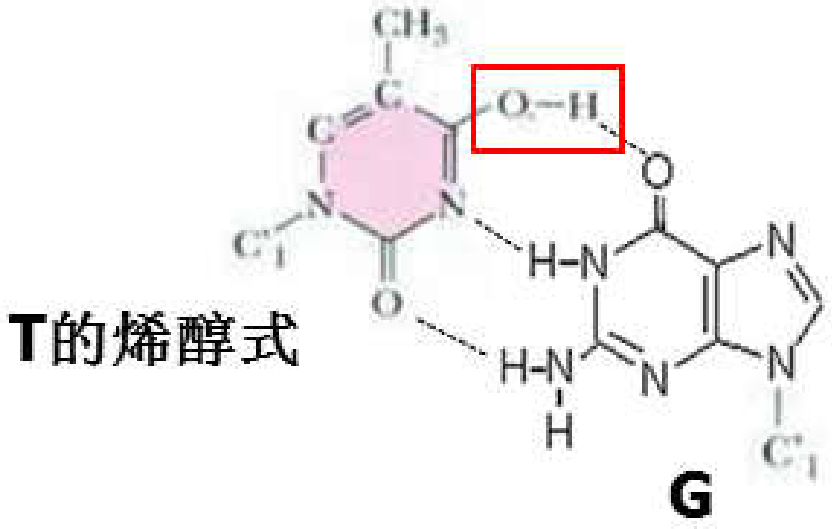


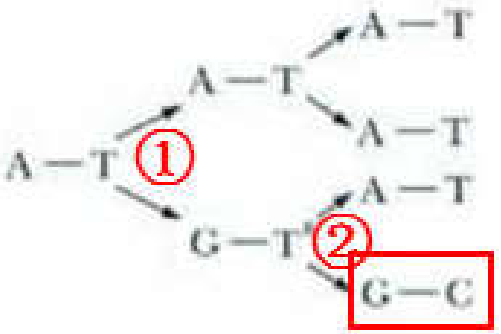


Fixed after a second round replication.



Transition





Direct mutagenesis results from the presence of a stable, unrepaired base with altered base pairing properties in the DNA.

直接诱变是由于DNA上存在稳定的、未被修复的碱基导致碱基配对性质的改变。

(2) Deamination (脱氨基)

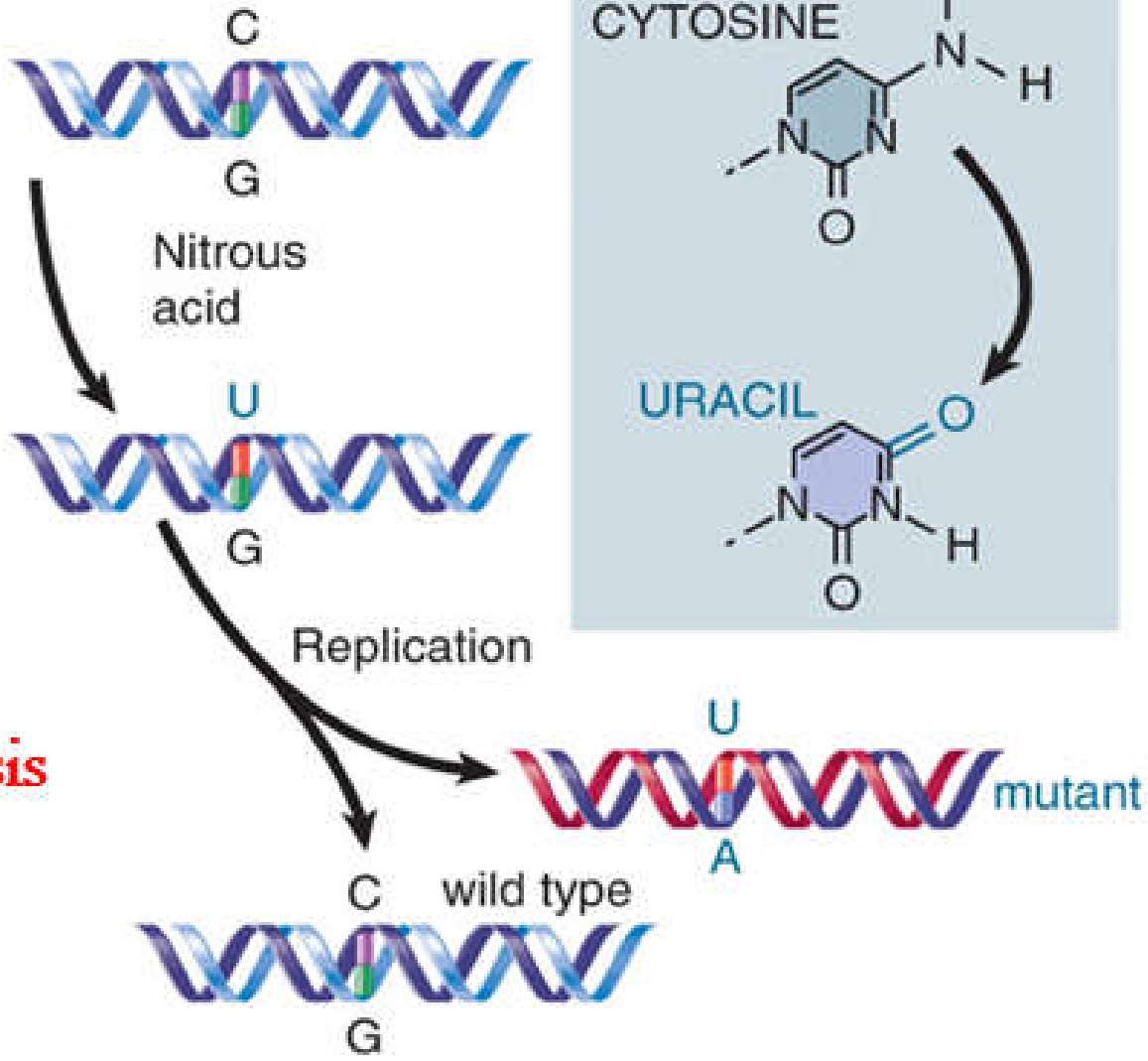
- **C→U**

  U-A

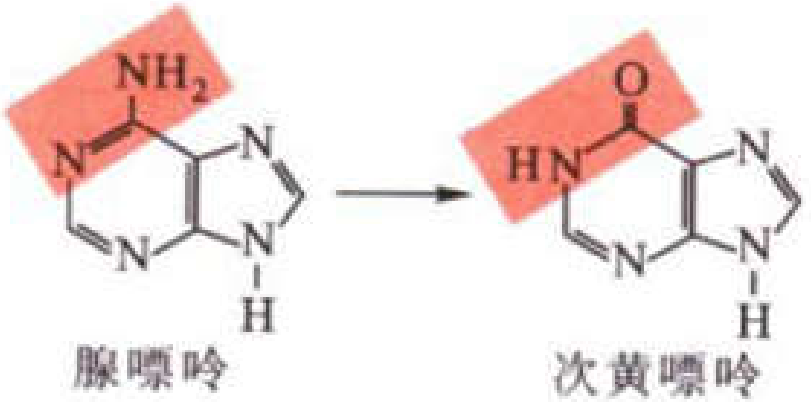
  C/G→T/A

  Transition

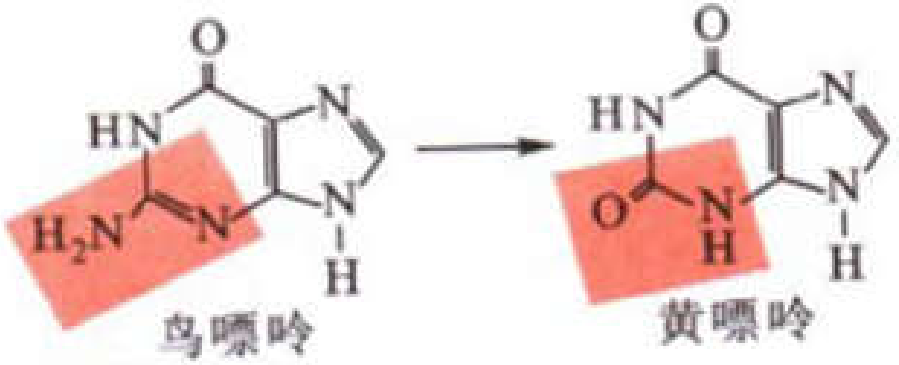
Direct mutagenesis



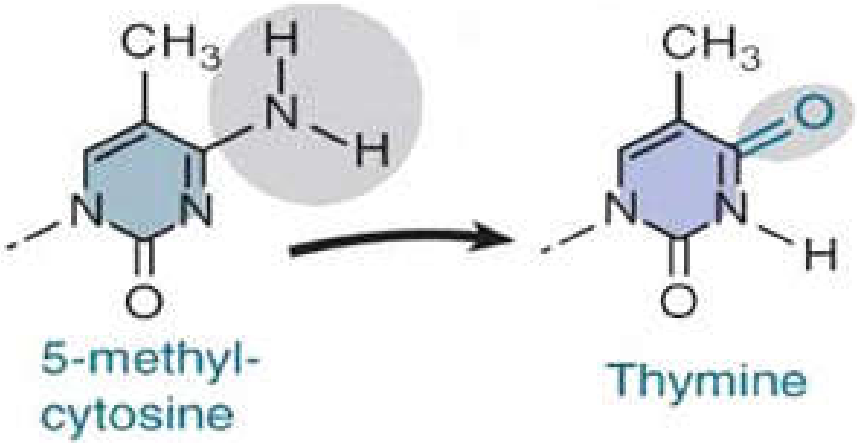
- **A→I (次黄嘌呤)**
  I-C、A
  A/T→G/C or T/A
  Transition or transversion



- **G→X (黄嘌呤)**
  X-C　　No effect

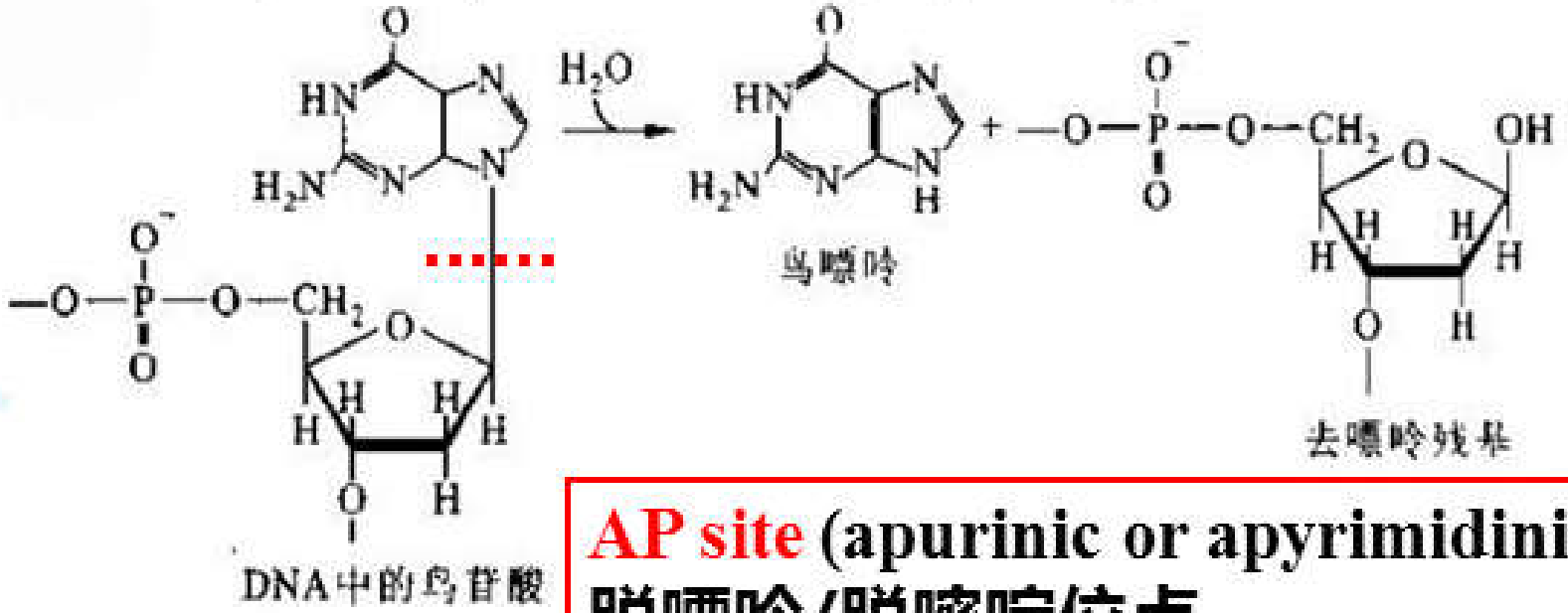


- **5′-methyl cytosine**
  5′-甲基胞嘧啶 → T
  C*/G →T/A
  Transition



(3) Loss of bases (碱基丢失)

- **Depurination (脱嘌呤): cleavage of the N-glycosylic bond (糖苷键) C1'-N9 by hydrolytic reaction.**



AP site (apurinic or apyrimidinic site)
脱嘌呤/脱嘧啶位点

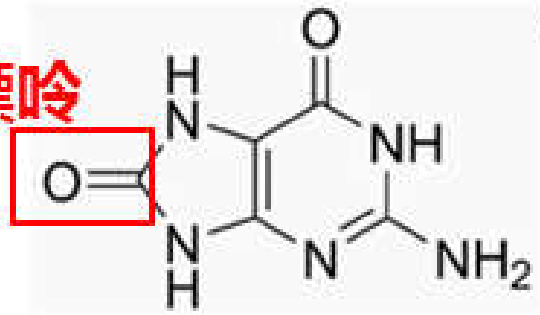
- **Depyrimidination (脱嘧啶): cleavage of the N-glycosylic bond C1'-N1. Very low frequency.**
- **Non-coding lesion →丢失遗传信息.**

2.2.3 Oxidative damage (氧化损伤) by ROS

- **ROS oxidize DNA bases**, such damage occurs spontaneously in cells but is increased by some exogenous agents.

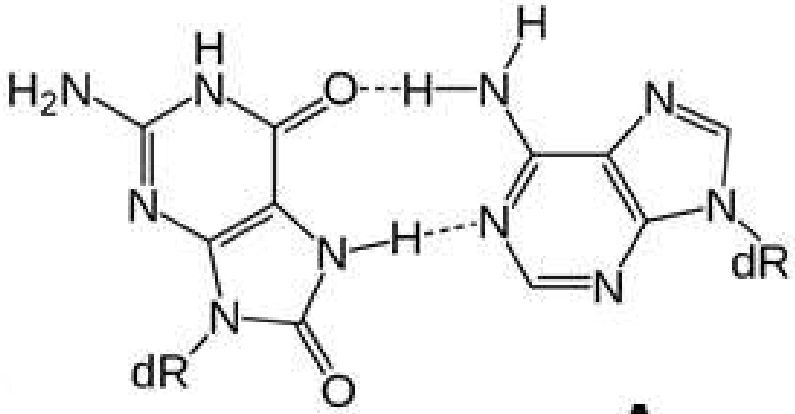
8-Oxoguanine

8-氧代(羟基)鸟嘌呤



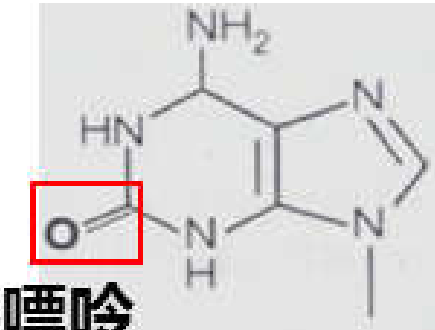
G/C → T/A

Transversion

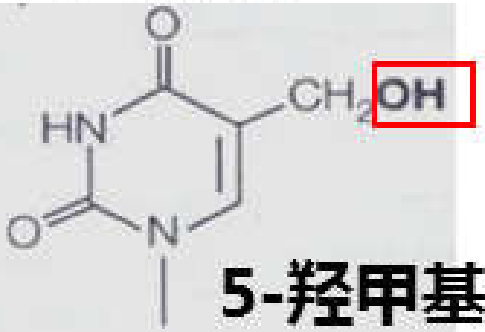


8-OxoG A

Direct mutagenesis



2-氧代腺嘌呤



5-羟甲基尿嘧啶

2-Oxoadenine 5-Hydroxymethyluracil

2.2.4 External factors

Mutagens（诱变剂）change the genetic material of an organism and thus increase the frequency of mutations above the natural background level.

→ **Spontaneous mutation rate**

As many mutations cause cancer, mutagens are therefore also likely to be carcinogens (致癌物).

诱变剂分类

- Physical mutagens
  - Ionizing radiation (电离辐射)
  - Ultraviolet (UV, 紫外线)
- Chemical mutagens
  - Base analogs (类似物)
  - Alkylating agents (烷化剂)
  - Intercalating agents (嵌入剂)
  - Nitrite (亚硝酸盐)
- Biological mutagens
  - Virus
  - Bacteria

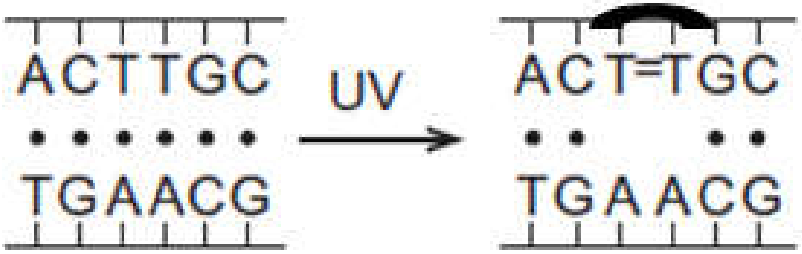
(1) Physical mutagens

① Ionizing radiations (电离辐射)

- **Ionizing radiations** are electromagnetic waves < 100 nm) such as X-rays and γ-rays.
- Cause extensive chemical alterations to DNA: **strand breaks** and **base/sugar destruction**
  链的断裂、碱基和戊糖的损伤
- 直接损伤：辐射的能量直接造成
- 间接损伤：自由基（ROS）

② Ultraviolet (UV, 紫外线)

- **UV** are absorbed strongly by bases, producing **pyrimidine dimers (嘧啶二聚体)** from adjacent (相邻的) pyrimidine bases.



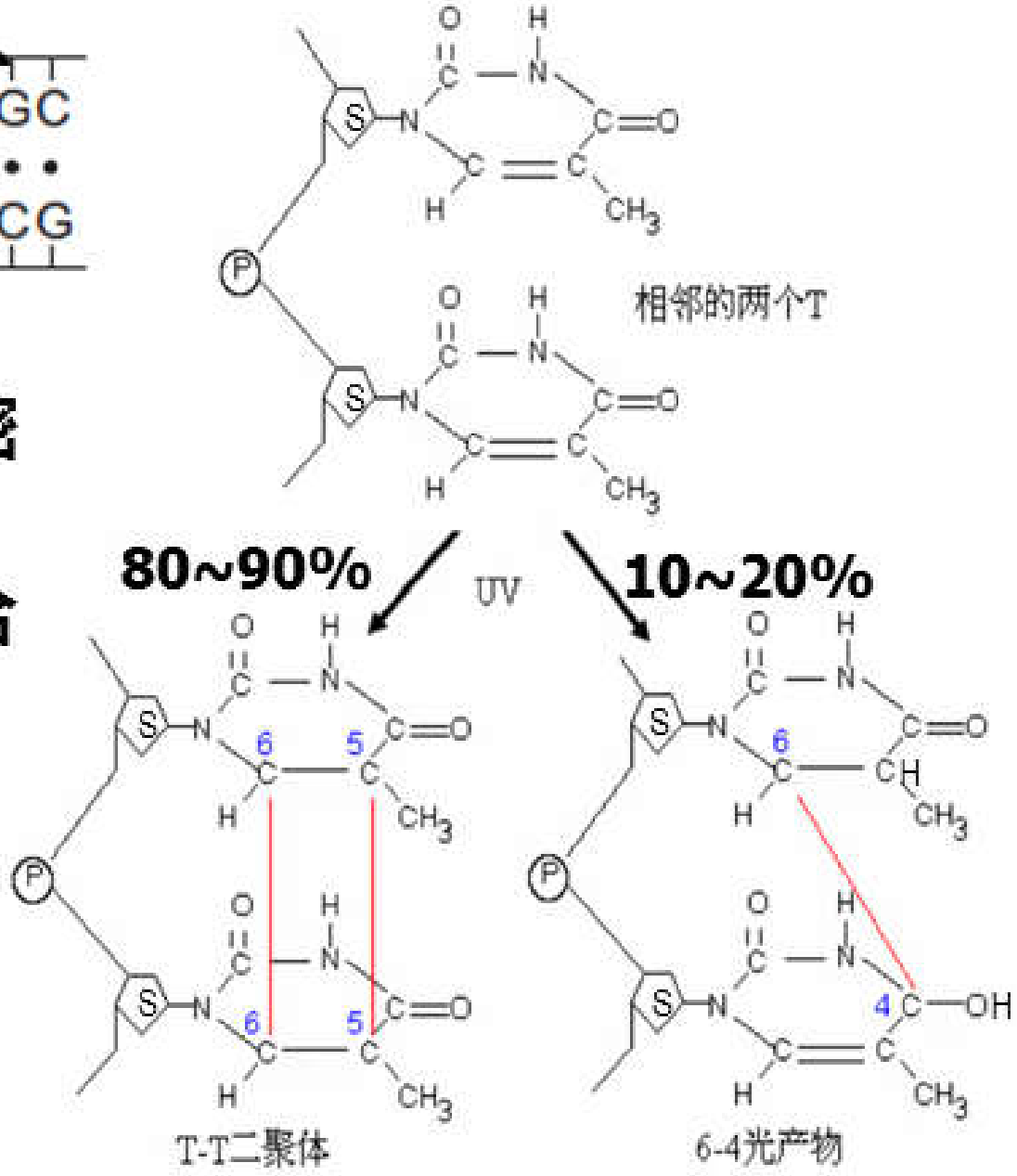
Cyclobutane pyrimidine dimers (CPDs, 环丁烷嘧啶二聚体)

Bulky adduct (聚化加合物) 导致大块损伤

不能配对

阻断DNA复制与转录

Indirect mutagenesis is induced by error-prone repair.

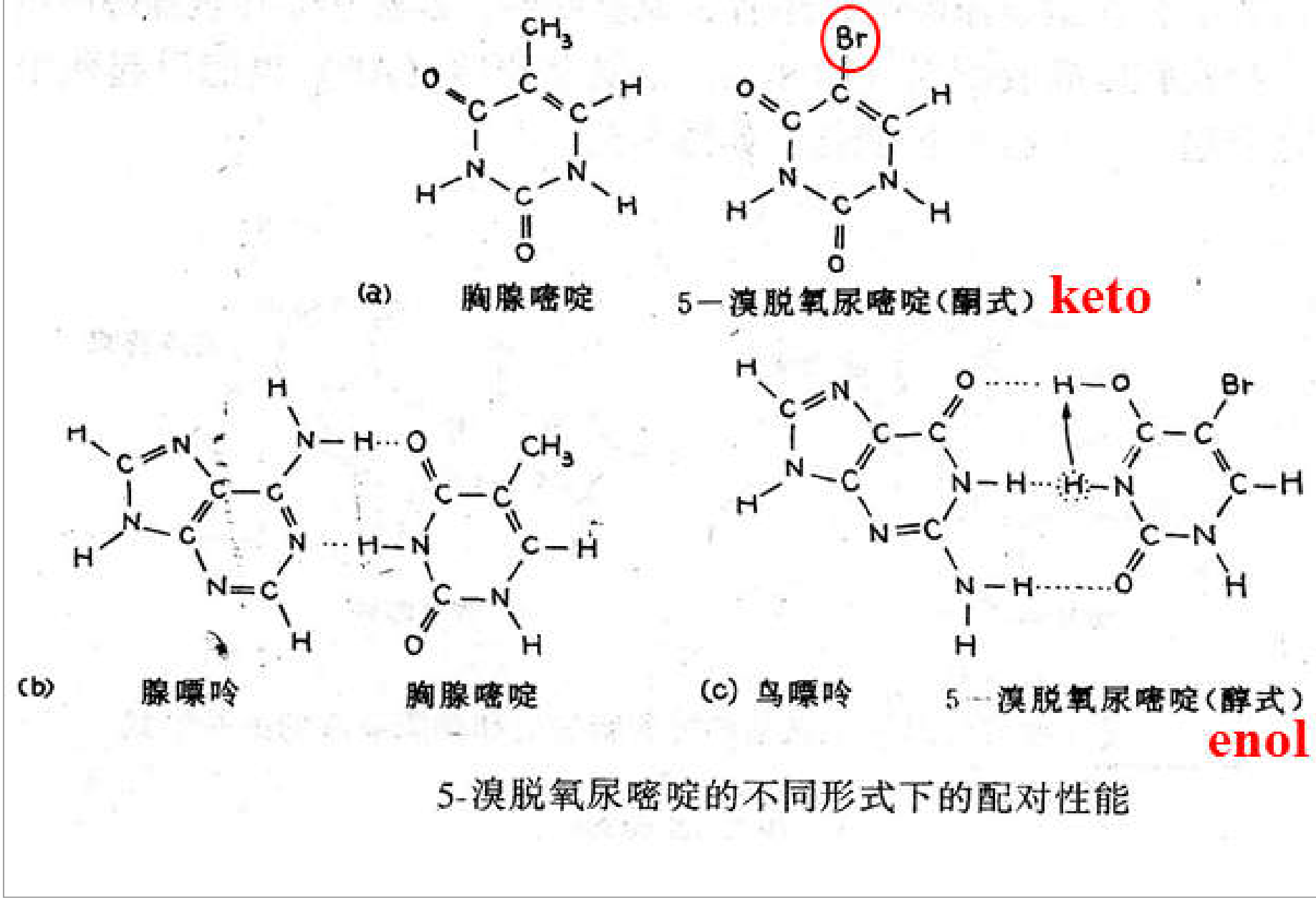


(2) Chemical mutagens

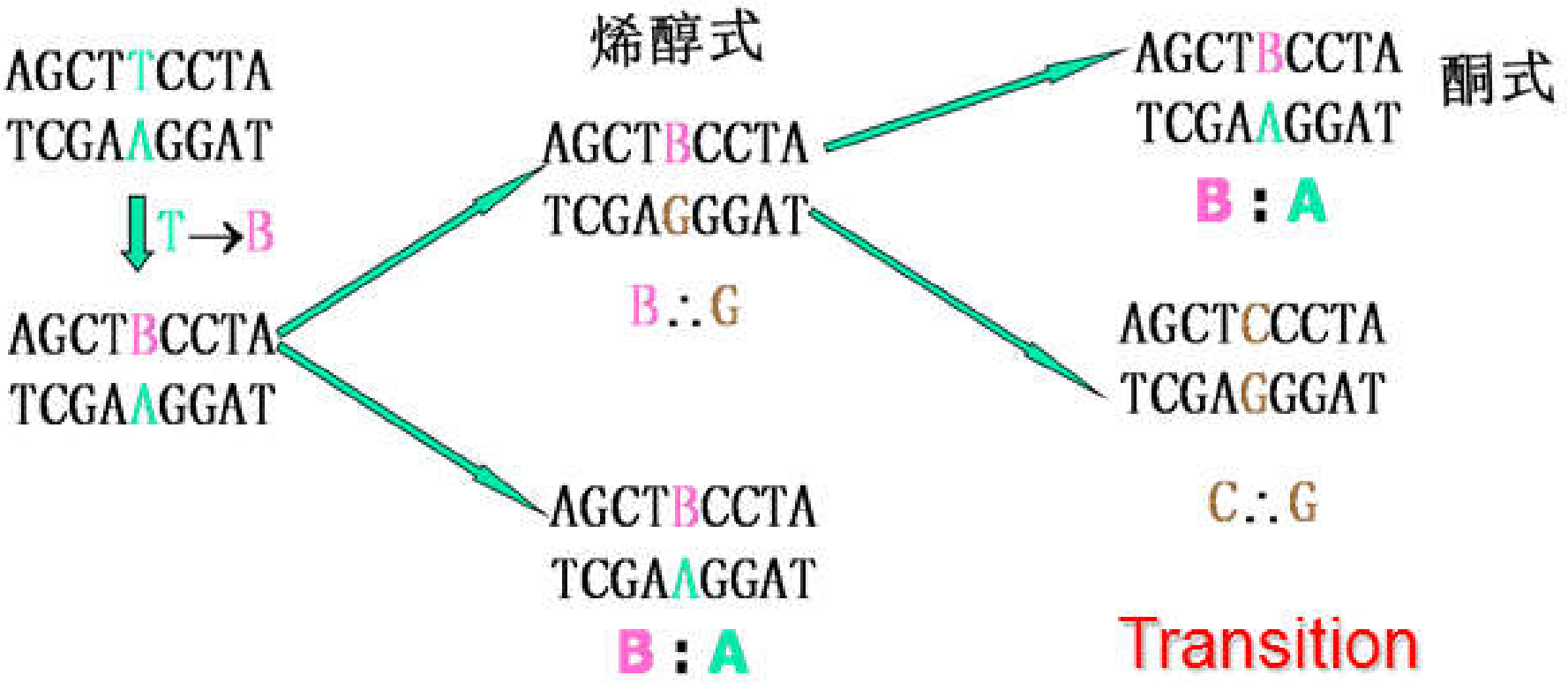
① Base analogs (碱基类似物)

- **Base analogs** are **derivatives** (衍生物) of the normal bases with **altered base pairing properties** and can **substitute** for DNA bases during replication.

- e.g. 5-溴尿嘧啶（5-BU）-T类似物
  5-氟尿嘧啶（5-FU）-T类似物
  2-氨基嘌呤（2-AP）-A类似物。

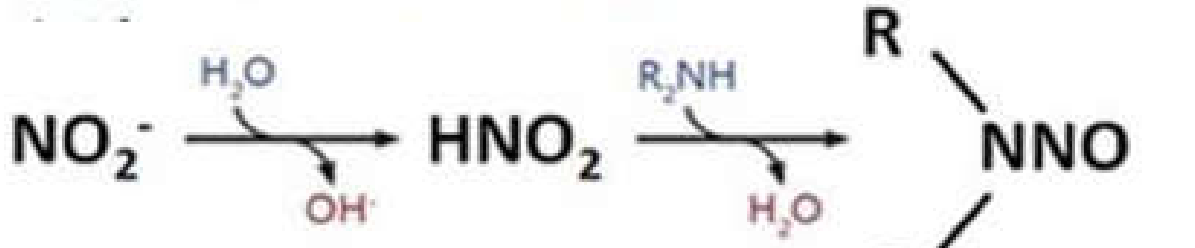


5-溴脱氧尿嘧啶的不同形式下的配对性能



Direct mutagenesis

② Nitrite (亚硝酸盐)



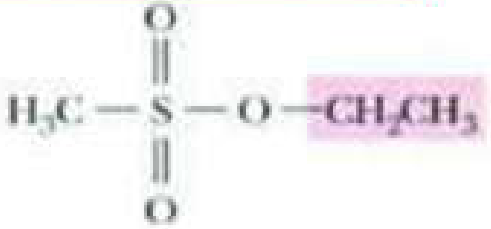
Nitrite
亚硝酸盐
↓
Deamination
脱氨基

Nitrosamine
亚硝胺
↓
Alkylation
烷化作用

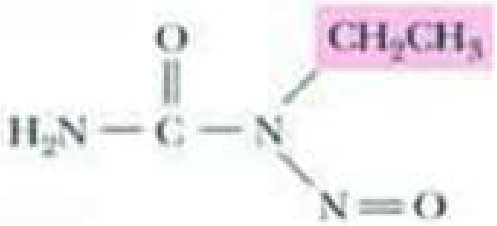
③ Alkylating agents (烷化剂)

- **Alkylating agents** are electrophilic (亲电子的) chemicals which are able to **transfer alkyl groups** to bases or the backbone phosphate groups of DNA.

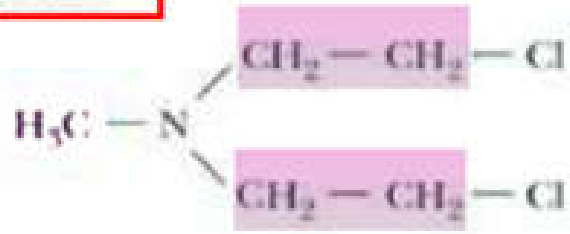
烷基硫酸盐化合物



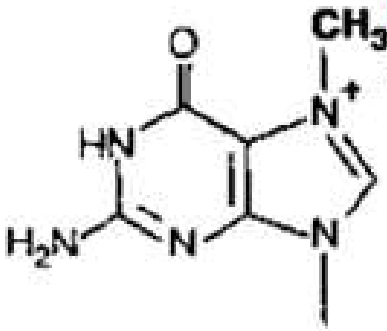
亚硝基脲化合物



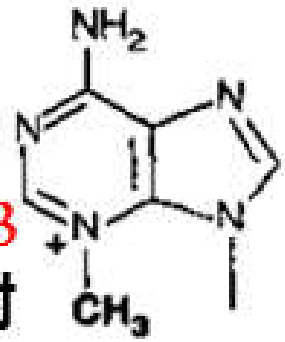
氮芥



常见烷化碱基

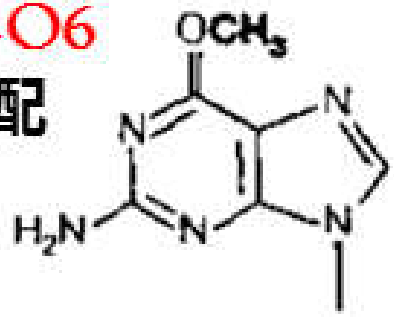


G-N7 一般无害



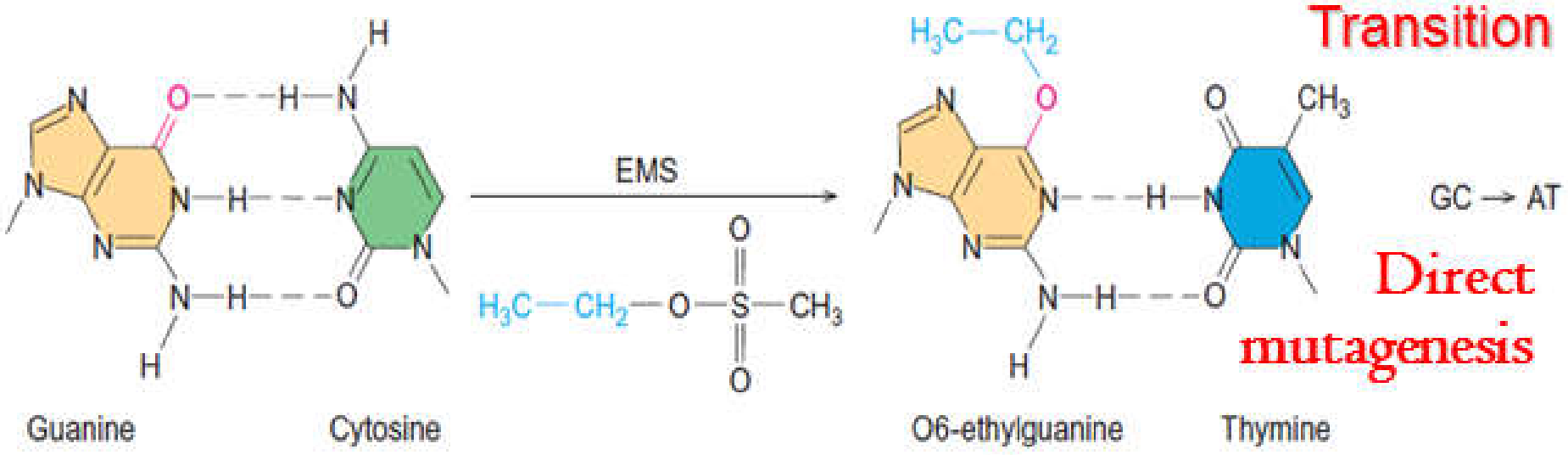
A-N3 不能配对

G-O6 错配



7-甲基鸟嘌呤　　3-甲基腺嘌呤　　O^6-甲基鸟嘌呤

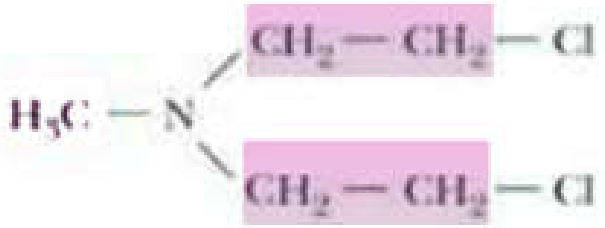
- **Alkylated bases may be mispared with other bases.**



Indirect mutagenesis ← Unstable → Loss of base

- **May cause DNA crosslinking and bulky adducts.**

e.g. 氮芥类（双功能烷化剂）



Arylating agents (芳基化剂)

苯并芘

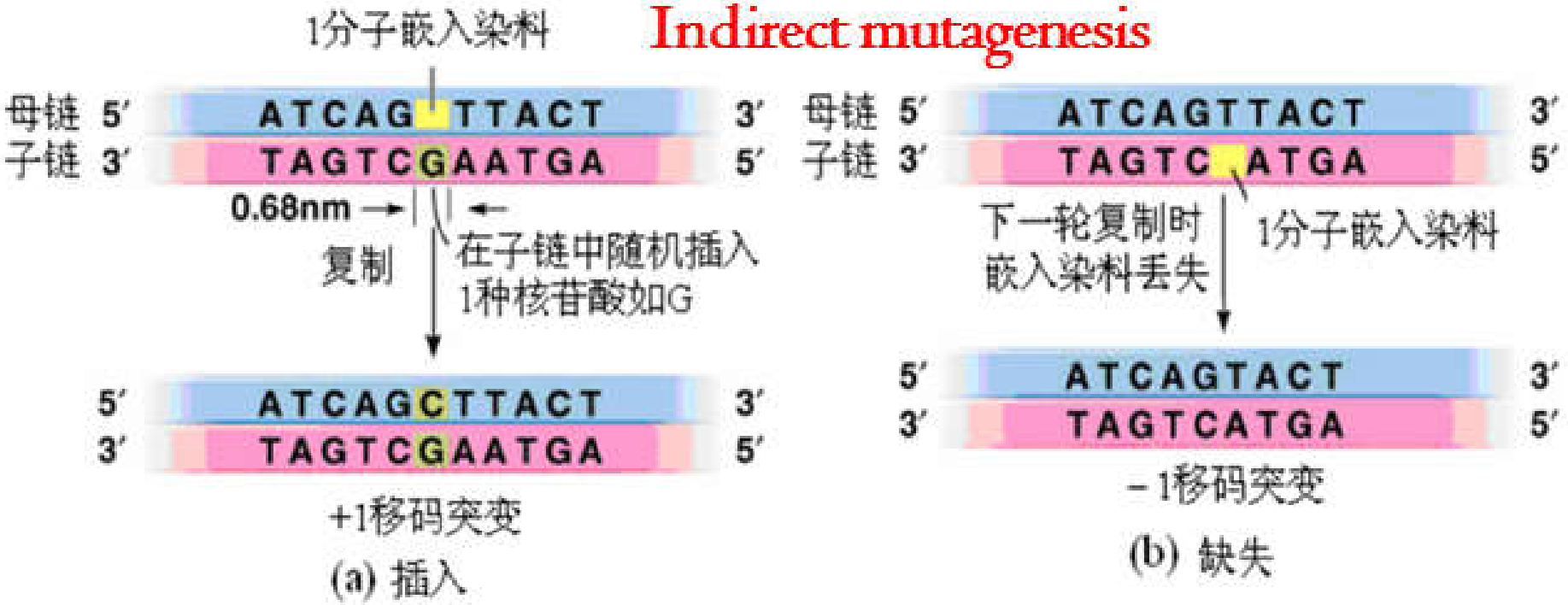
鸟嘌呤加合物

Indirect mutagenesis

Bulky adduct

④ Intercalators (嵌入剂)

- **Intercalating agents** are molecules that may insert between bases in DNA, **causing insertion or deletion** during replication. e.g. EB (溴化乙锭), 吖啶橙. 【平面多环芳烃】



(3) Biological mutagens

- **Virus DNA may be inserted into the genome and disrupts genetic function.**
- **Some bacteria such as *Helicobacter pylori* (幽门螺杆菌) cause inflammation (炎症) during which ROS are produced, causing DNA damage and reducing efficiency of DNA repair systems, thereby increasing mutation.**