

3. DNA repair

Nobelpriset i kemi 2015 The Nobel Prize in Chemistry 2015

 **Nobelpriset i kemi 2015** KUNGL. VETENSKAPS AKADEMIEIN THE ROYAL SWEDISH ACADEMY OF SCIENCES



Tomas Lindahl
Francis Crick Institute and
Clare Hall Laboratory,
Hertfordshire, UK

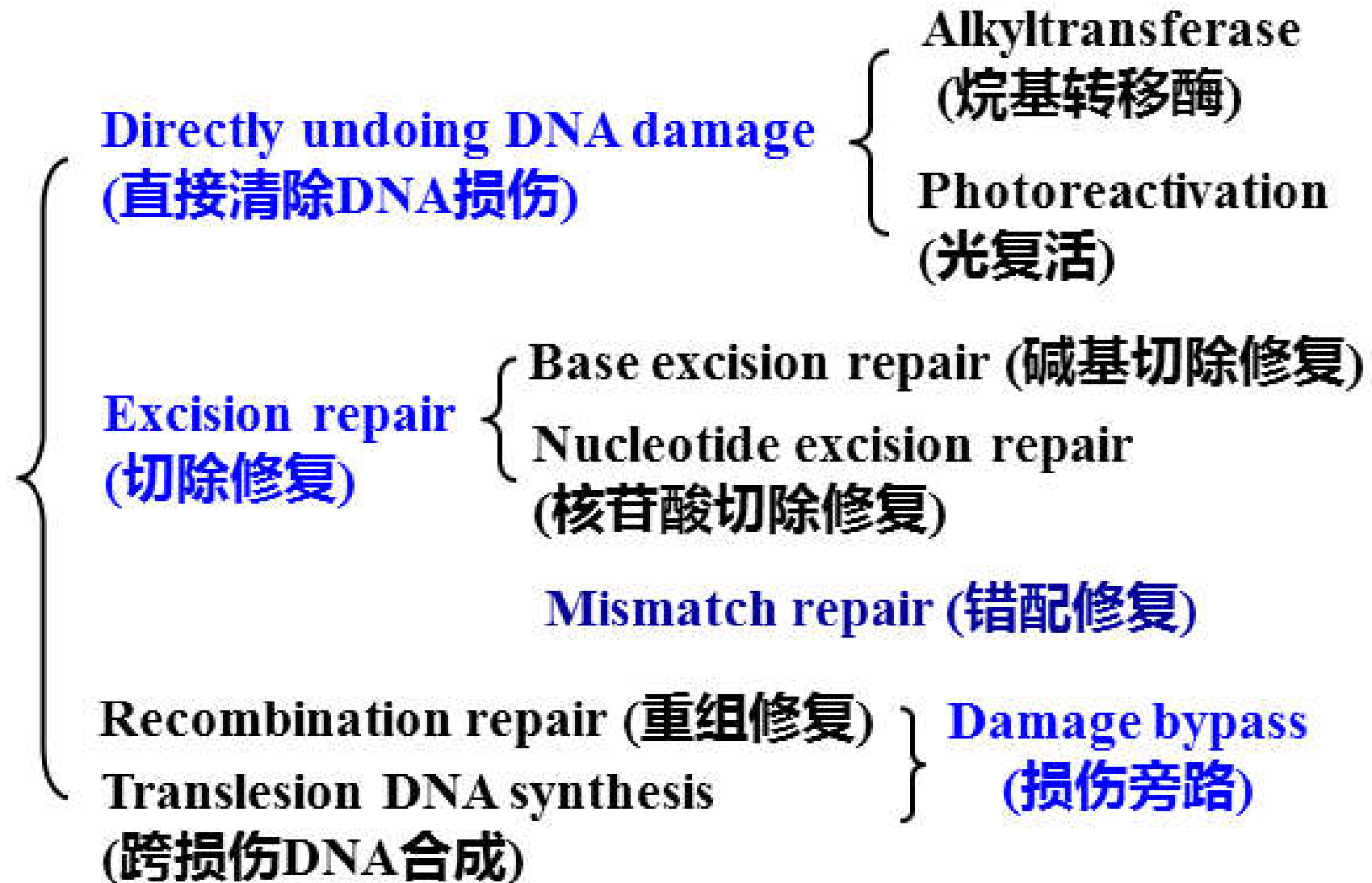


Paul Modrich
Howard Hughes Medical
Institute and Duke University
School of Medicine, Durham,
NC, USA



Aziz Sancar
University of North Carolina,
Chapel Hill, NC, USA

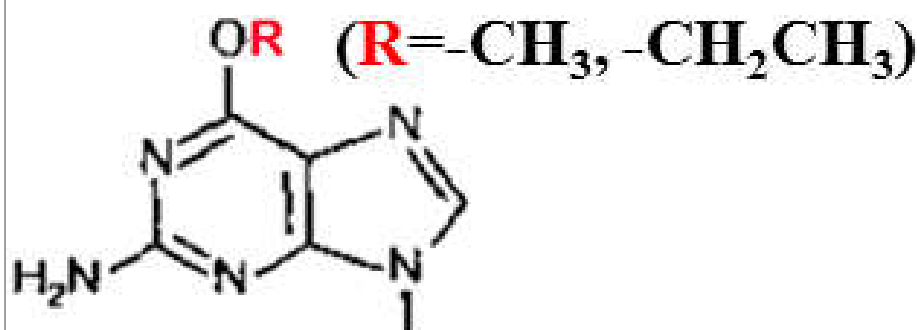
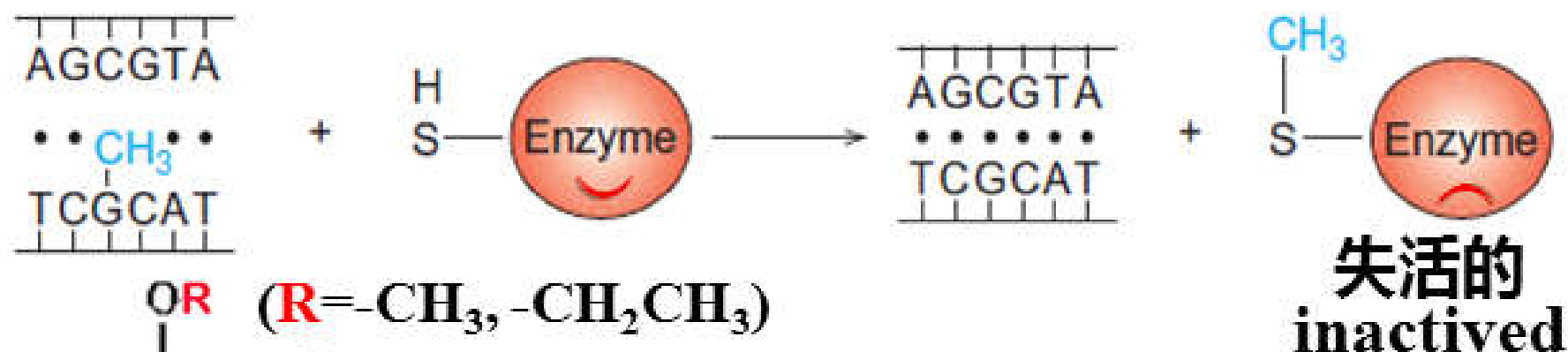
"för mekanistiska studier av DNA-reparation"
"for mechanistic studies of DNA repair"



3.1 Directly undoing DNA damage

Restore (恢复) damaged DNA to its original, undamaged state. **【Error-free direct reversal】**

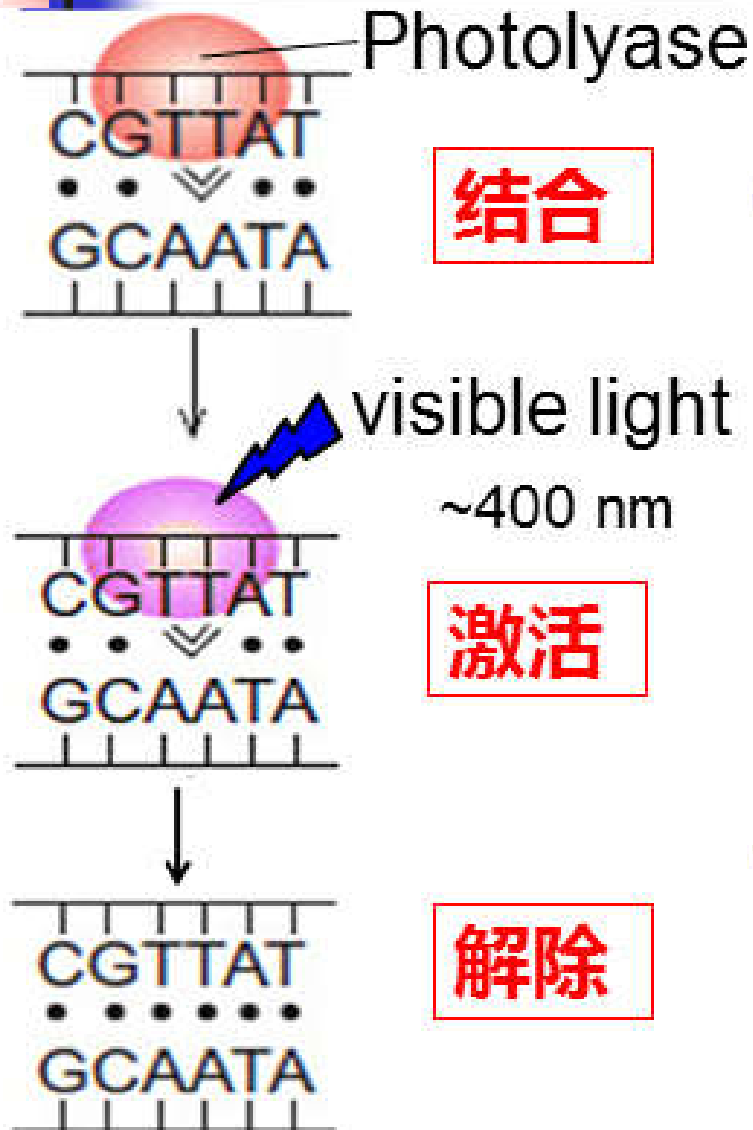
3.1.1 Alkyltransferase (烷基转移酶)



O⁶-烷基鸟嘌呤(alkylguanine)

Each alkyltransferase can only be used once.

3.1.2 Photoreactivation (光复活)



- In the presence of visible light, the DNA **photolyases** (光裂合酶, photoreactivating enzymes, 光复活酶) can resolve (分解) pyrimidine dimers into monomer.
- It is **highly specific** and only repair pyrimidine dimers formed by UV.

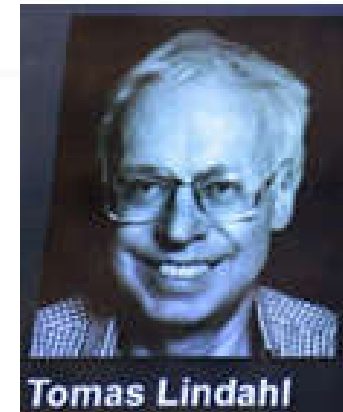


超净台在用紫外灯照射杀菌时，哪种条件下效果好？明亮or黑暗？

Higher mammals, including humans, do not have photolyase...

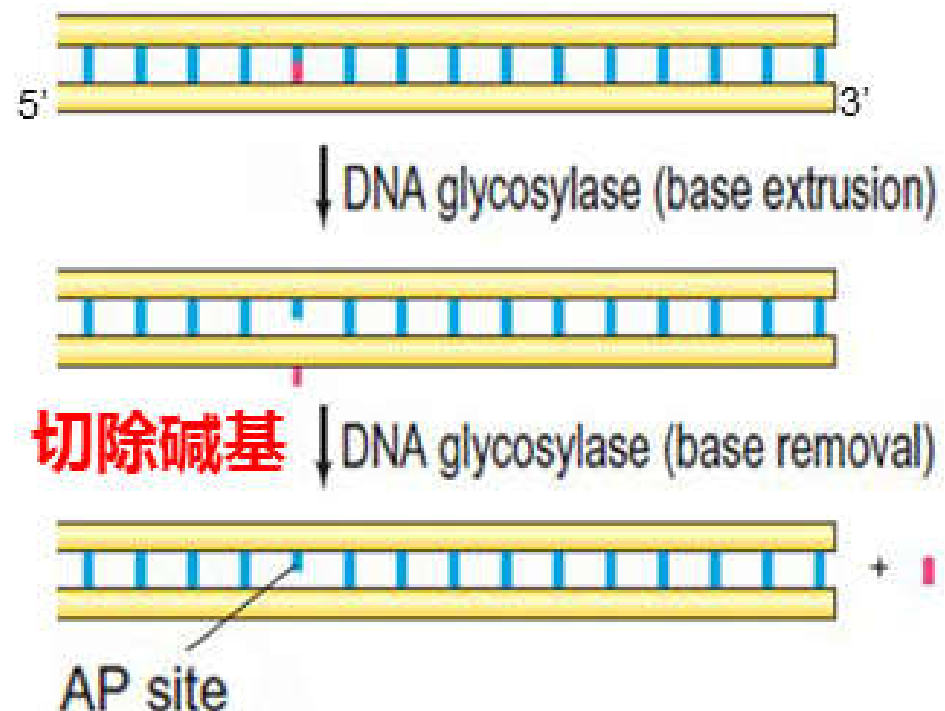
3.2 Excision repair 【Error-free】

{ Base excision repair (BER) →
Nucleotide excision repair (NER)



3.2.1 BER

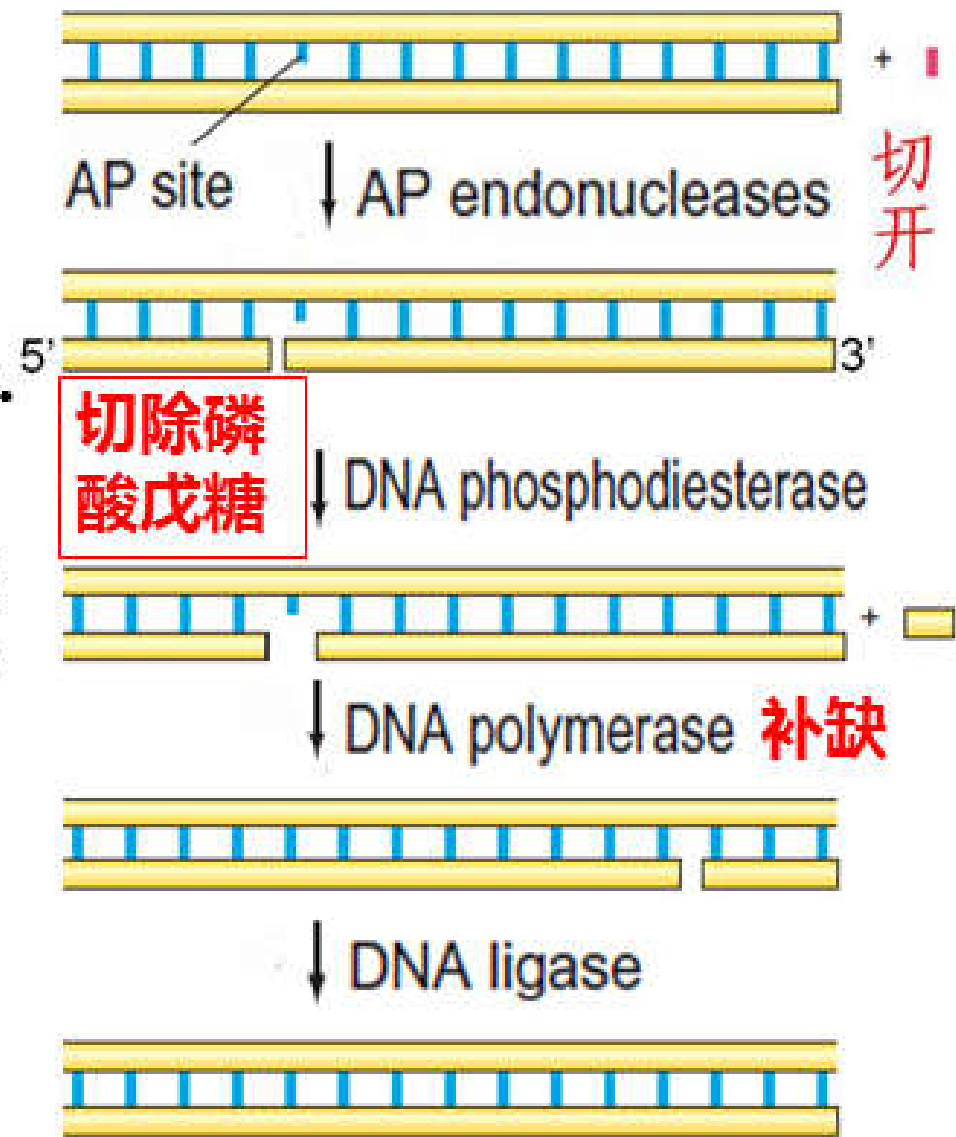
(1) A damaged base is recognized by **DNA glycosylase** (糖基化酶/糖苷酶), which **breaks the glycosidic bond** (糖苷键). This leaves an **AP site**.

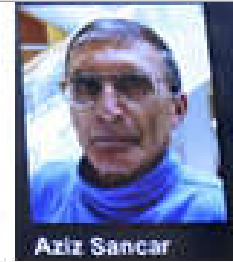


(2) AP site is recognized by an **AP endonuclease** (**APE**, 核酸内切酶) that cuts the DNA strand on the 5'-side of the AP site.

(3) **Exonuclease** or **DNA phosphodiesterase** (磷酸二酯酶) removes the AP sugar phosphate.

(4) **DNAPol I** (β) performs repair synthesis. **DNA ligase** seals the remaining nick.



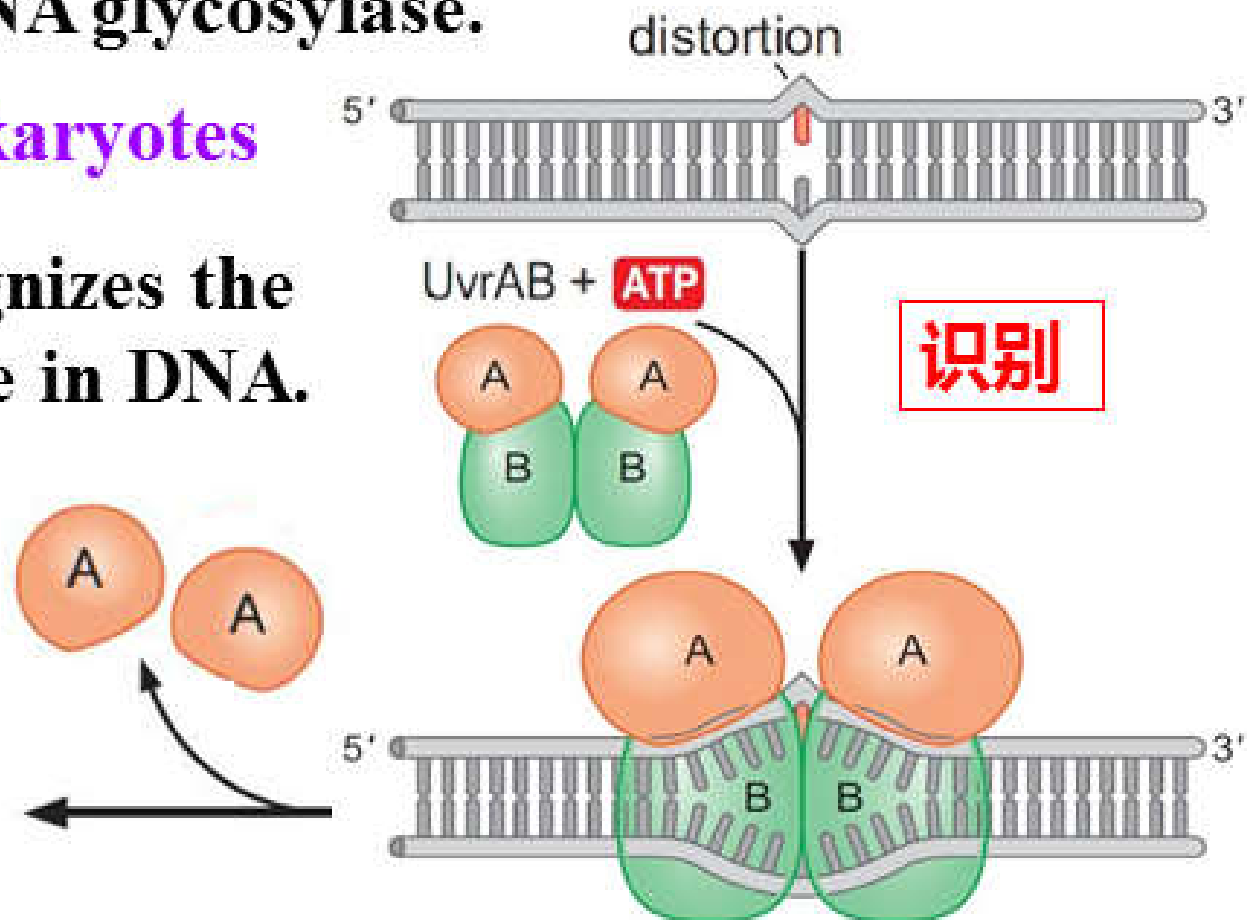


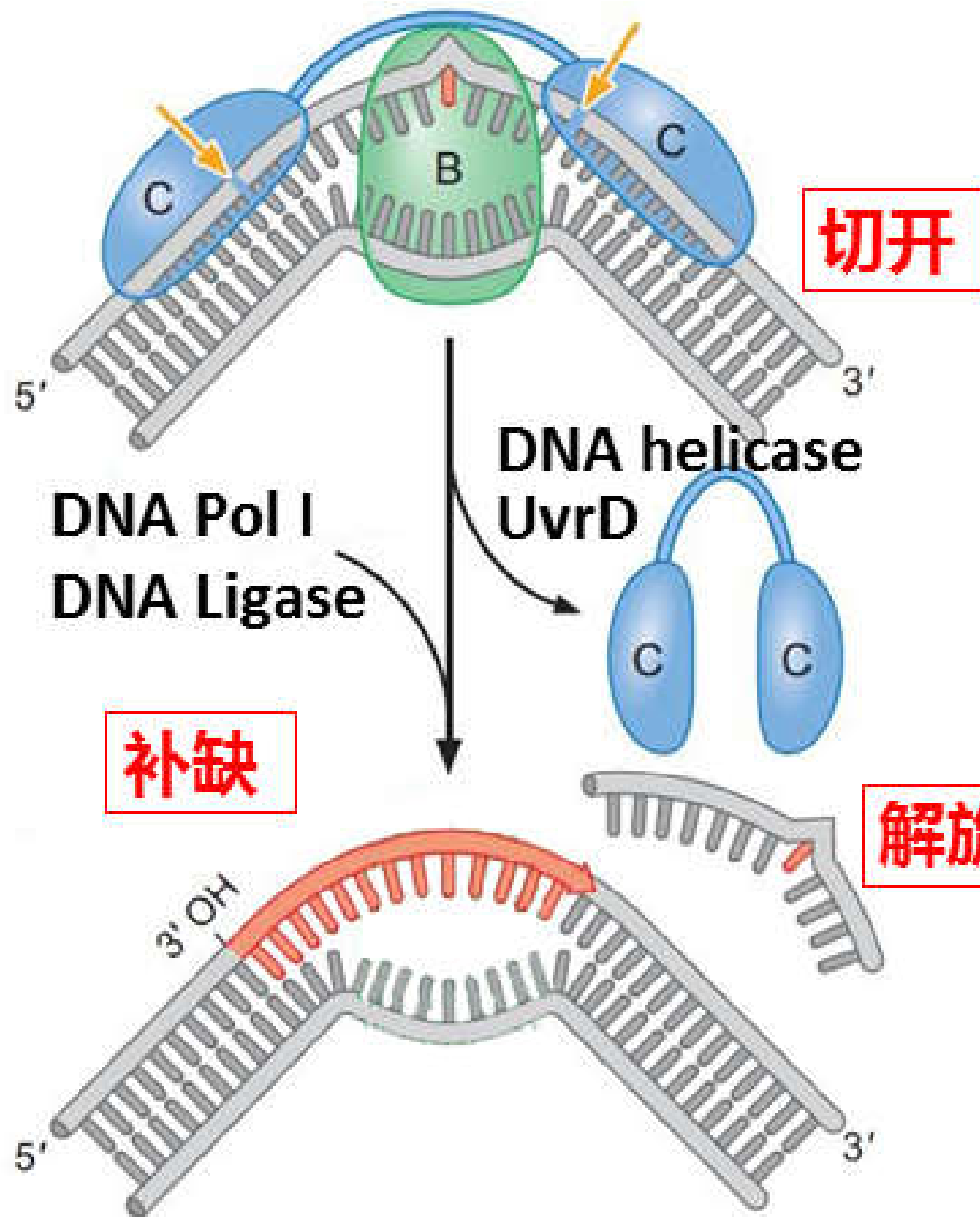
3.2.2 NER

Bulky base damage, including pyrimidine dimers, can be removed directly by NER, **without** help from a DNA glycosylase.

(1) NER in prokaryotes

- ① **UvrAB** recognizes the distortion site in DNA. Then UvrA dissociates from the complex of UvrAB.

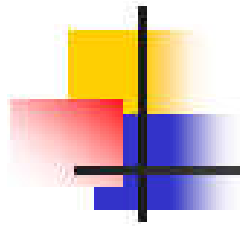




② **Excinuclease (切除核酸酶) UvrC** cuts the DNA on both side of damaged site.

③ **DNA helicase UvrD** releases the fragment of oligonucleotide that is 12-13 nt long.

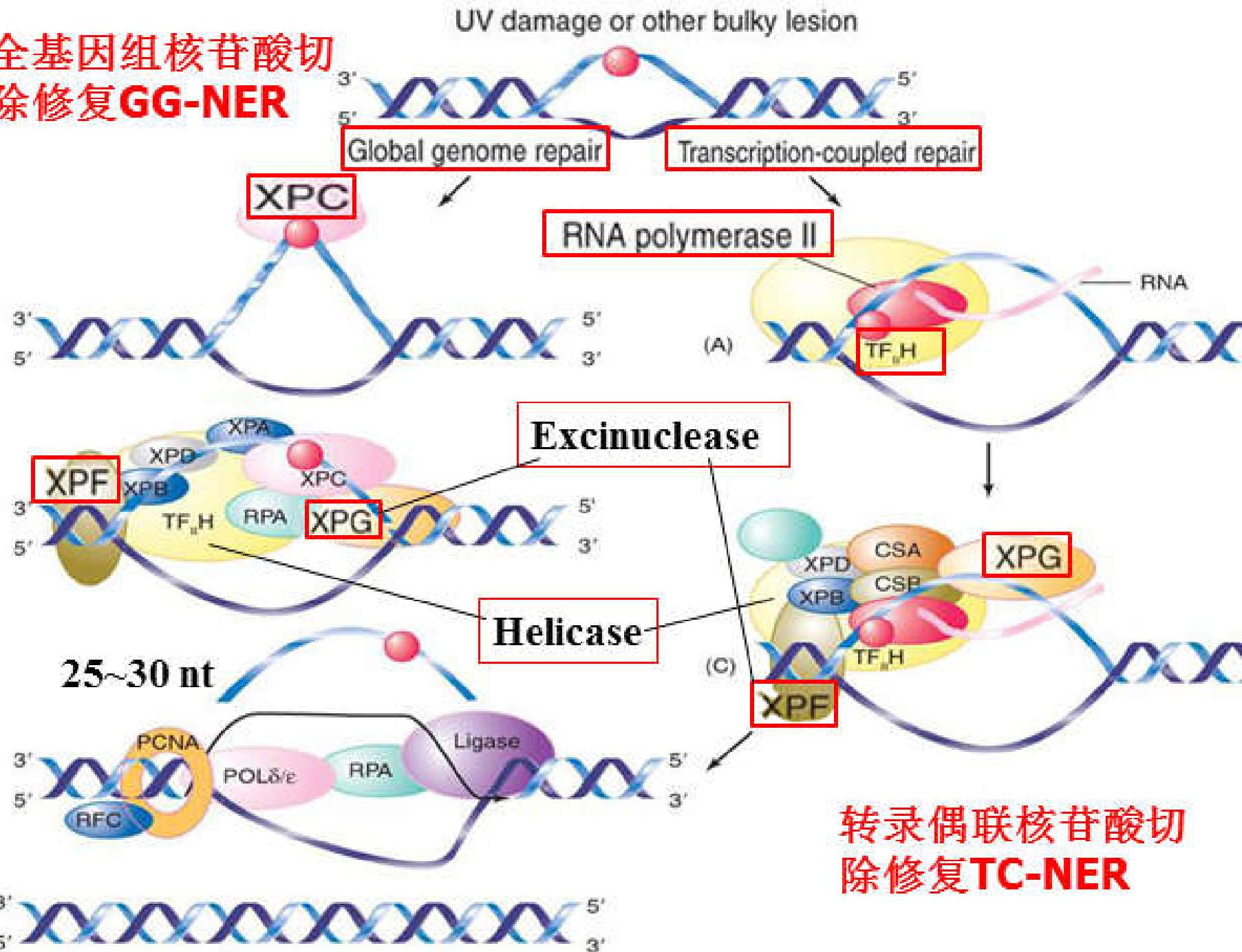
④ **Seal gap.**



(2) NER in eukaryotes

- **Xeroderma pigmentosum (XP, 着色性干皮病)**, a sun-sensitive skin cancer-prone disorder, is caused by a **deficiency in NER** (mutations in several NER genes).
- Numerous proteins, including **XP products** and the transcription factor **TF II H**, are involved in eukaryotic NER.

全基因组核苷酸切除修复GG-NER

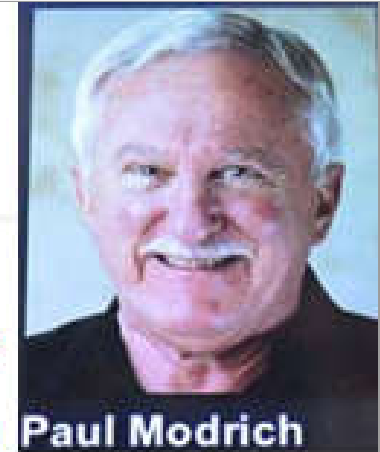


转录偶联核苷酸切除修复TC-NER

- Global genome nucleotide excision repair (**GG-NER**) recognizes damage anywhere in the genome.
- Transcriptionally active genes are preferentially repaired via transcription-coupled nucleotide excision repair (**TC-NER**).
- GG-NER and TC-NER differ in their mechanisms of **damage recognition (XPC vs. RNA polymerase II)**.
- TF II H has helicase activity.
- **XPF and XPG are excinuclease (切除核酸酶)** in eukaryotic NER.

Cockayne syndrome are defective in TC-NER, but are not cancer-prone.

3.3 Mismatch repair

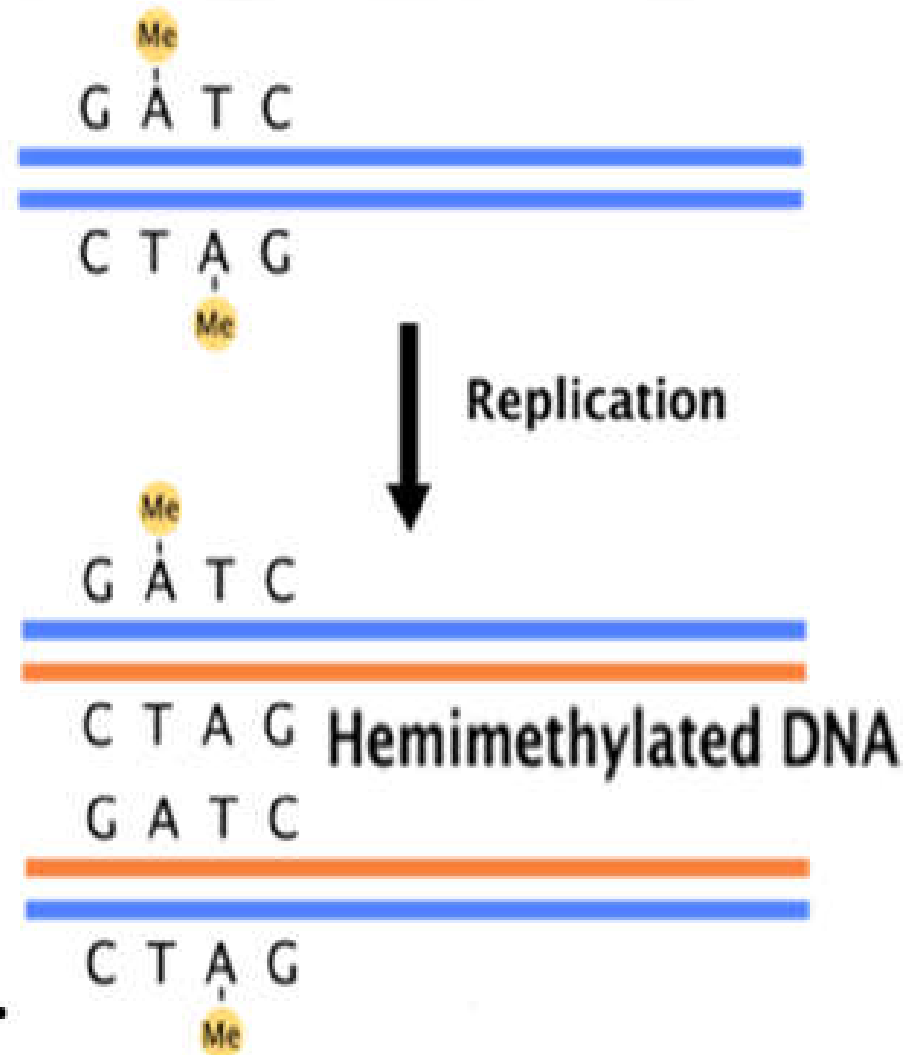


- **Mismatch repair** deals with any **base mispairs produced during replication** and that have **escaped proofreading**. It is a specialized form of excision repair.

错配修复是切除修复的一种特殊形式，用来修复在**复制中错配并漏过校正**的任何碱基。

- The wrong base is in the daughter strand. Mismatch repair system must have a way of **distinguishing the parental strand and daughter strand** after the replication fork has passed.

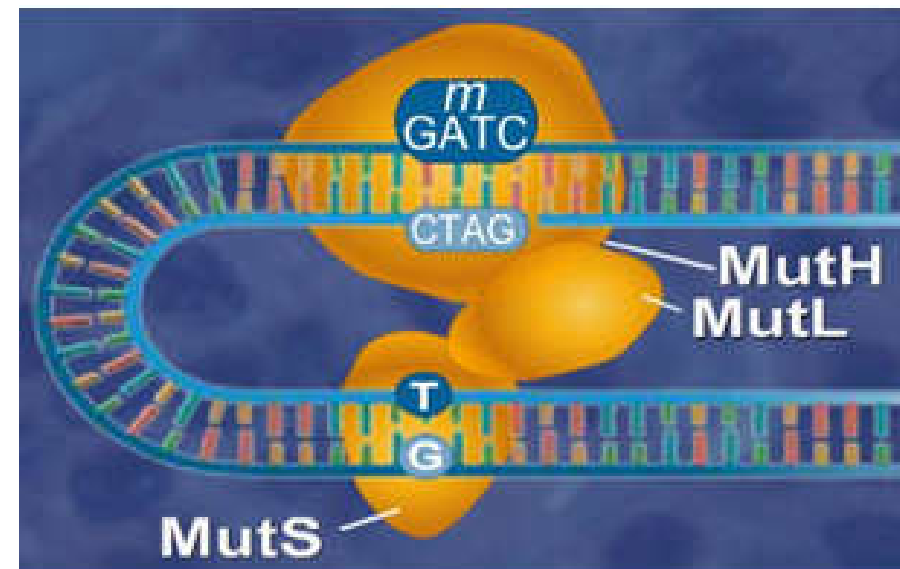
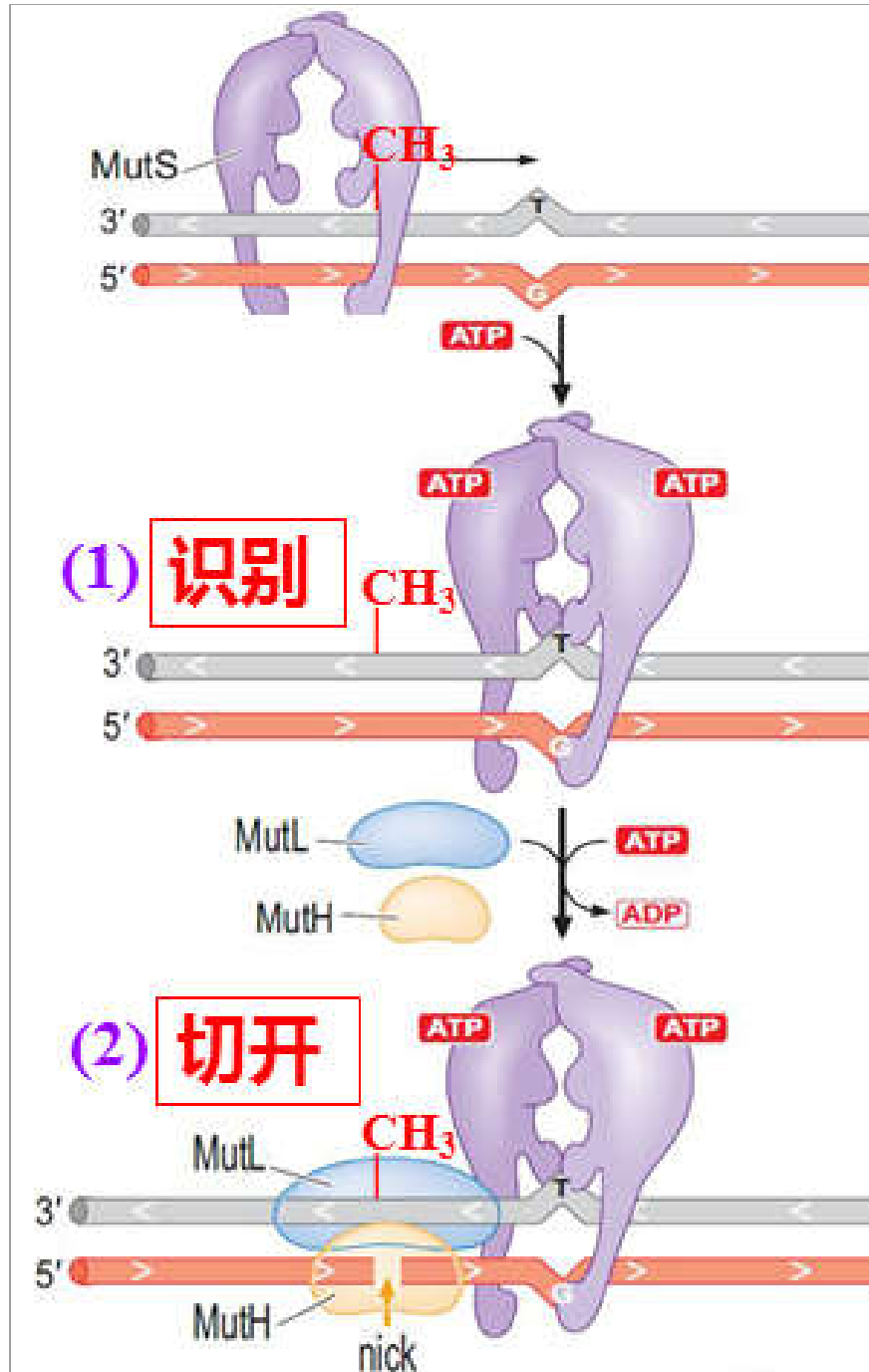
- In *E. coli*, the parental strand has **identification tags - methylated adenines** that distinguish it from the daughter strand.
- The *mut* genes code for a mismatch repair system that deals with mismatched base pairs.



错配修复 (MMR) 过程

(1) **MutS** recognizes a base mismatch along with ATP.

(2) **MutH endonuclease** nicks the daughter strand at a nearby GATC site with the help of **MutL**.



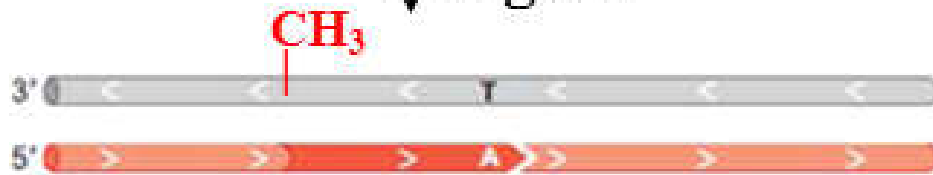
(3) 切除

Helicase
Exonuclease



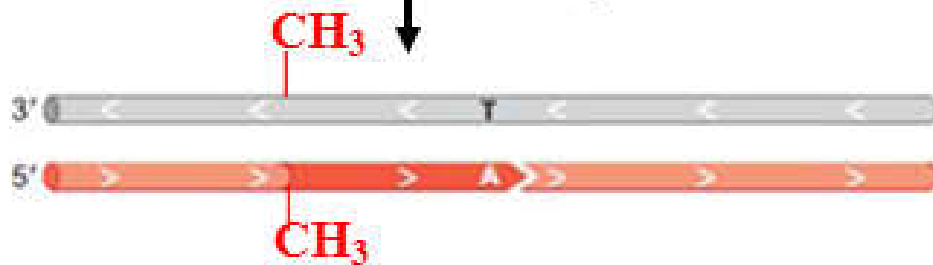
(4) 补缺

DNA Pol III Ligase



(5) 甲基化

Methyltransferase



(3) Exonuclease
removes the DNA
fragment including
the incorrect
nucleotide with the
help of DNA
helicase.

(4) Seal the gap (DNA Pol III and ligase)

(5) GATC methylation

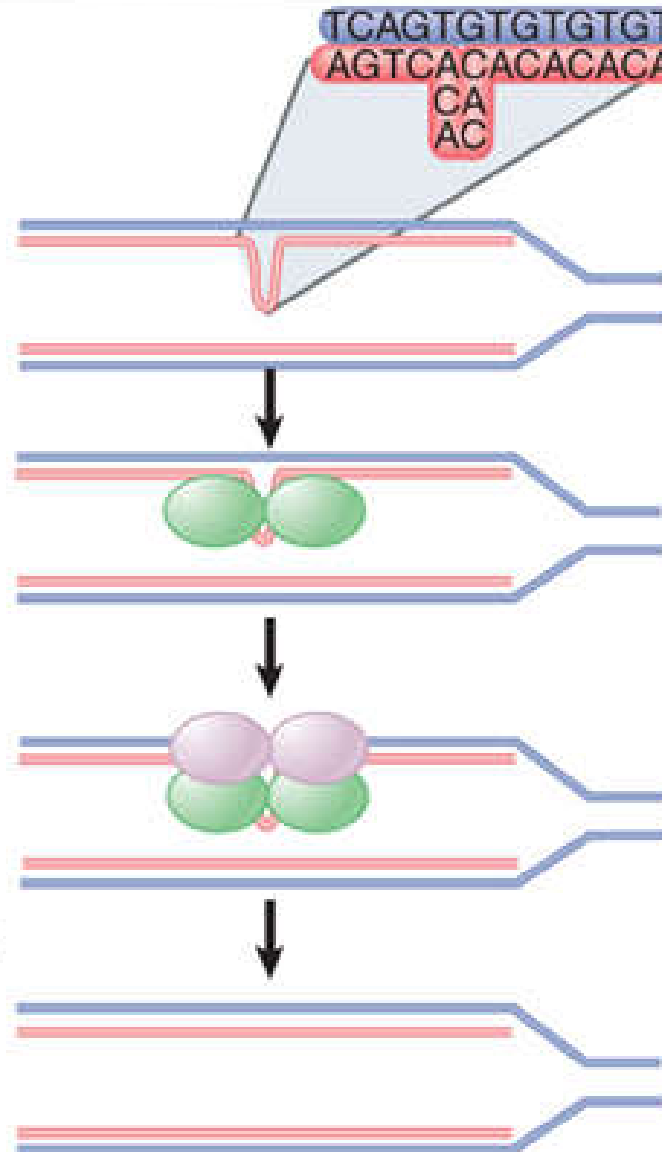
MutS/MutL repair replication slippages

Replication slippage generates a single-strand loop

MutS binds to the mismatch

MutL binds

Mismatch is removed by exonuclease, helicase, DNA polymerase, and ligase

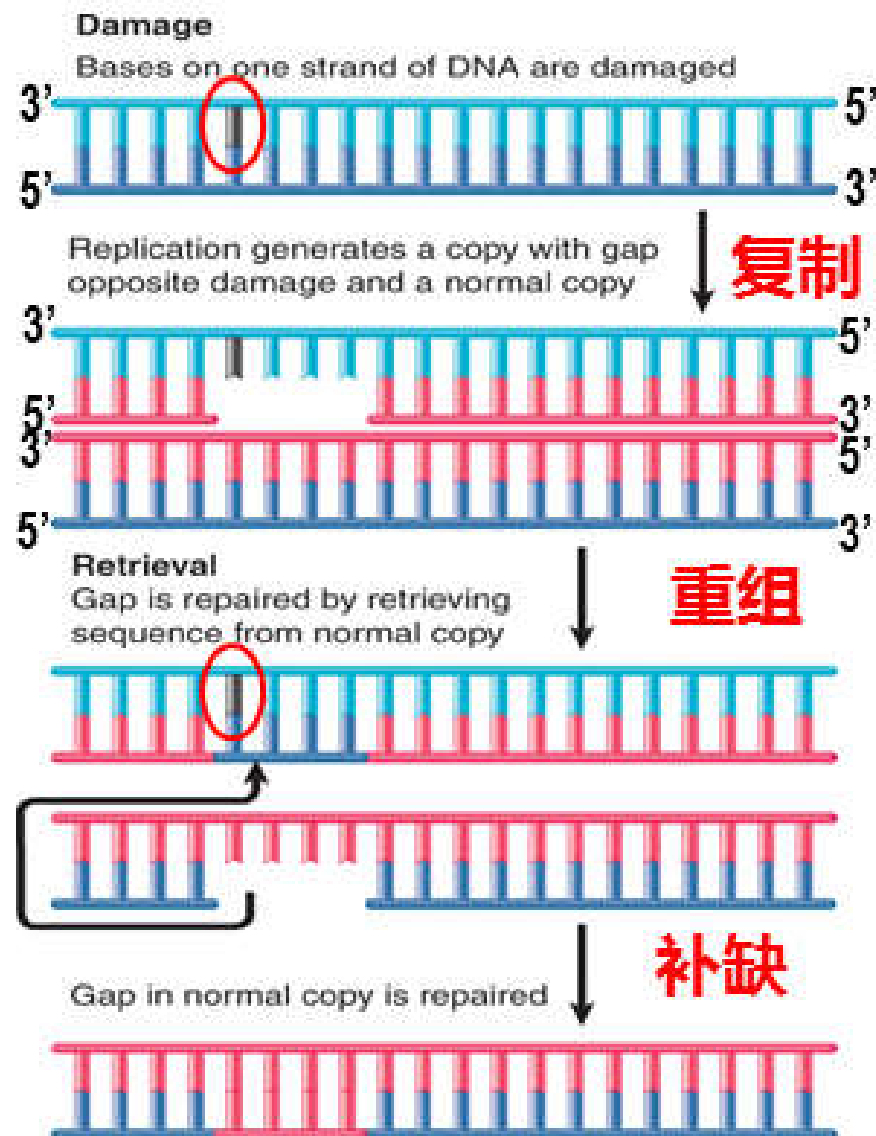


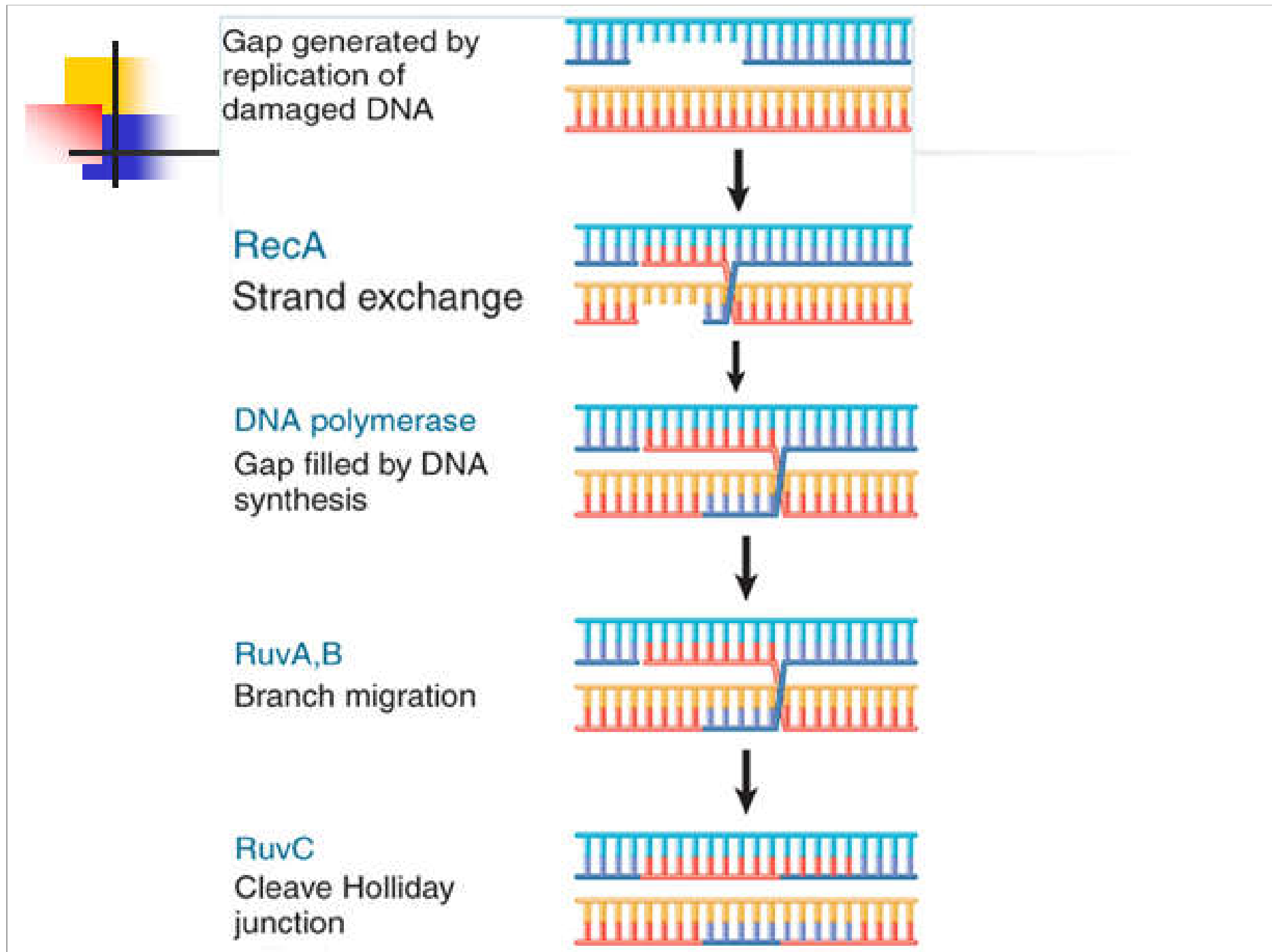
- **Eukaryotic MutS/L systems repair mismatches and insertion/deletion loops.**

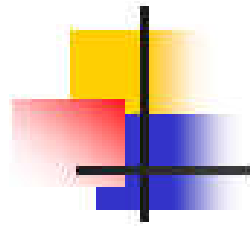
- **Mismatch repair deficiency**
→ Hereditary nonpolyposis carcinoma of colon (HNPCC, 遗传性非息肉结肠癌).

3.4 Recombination repair

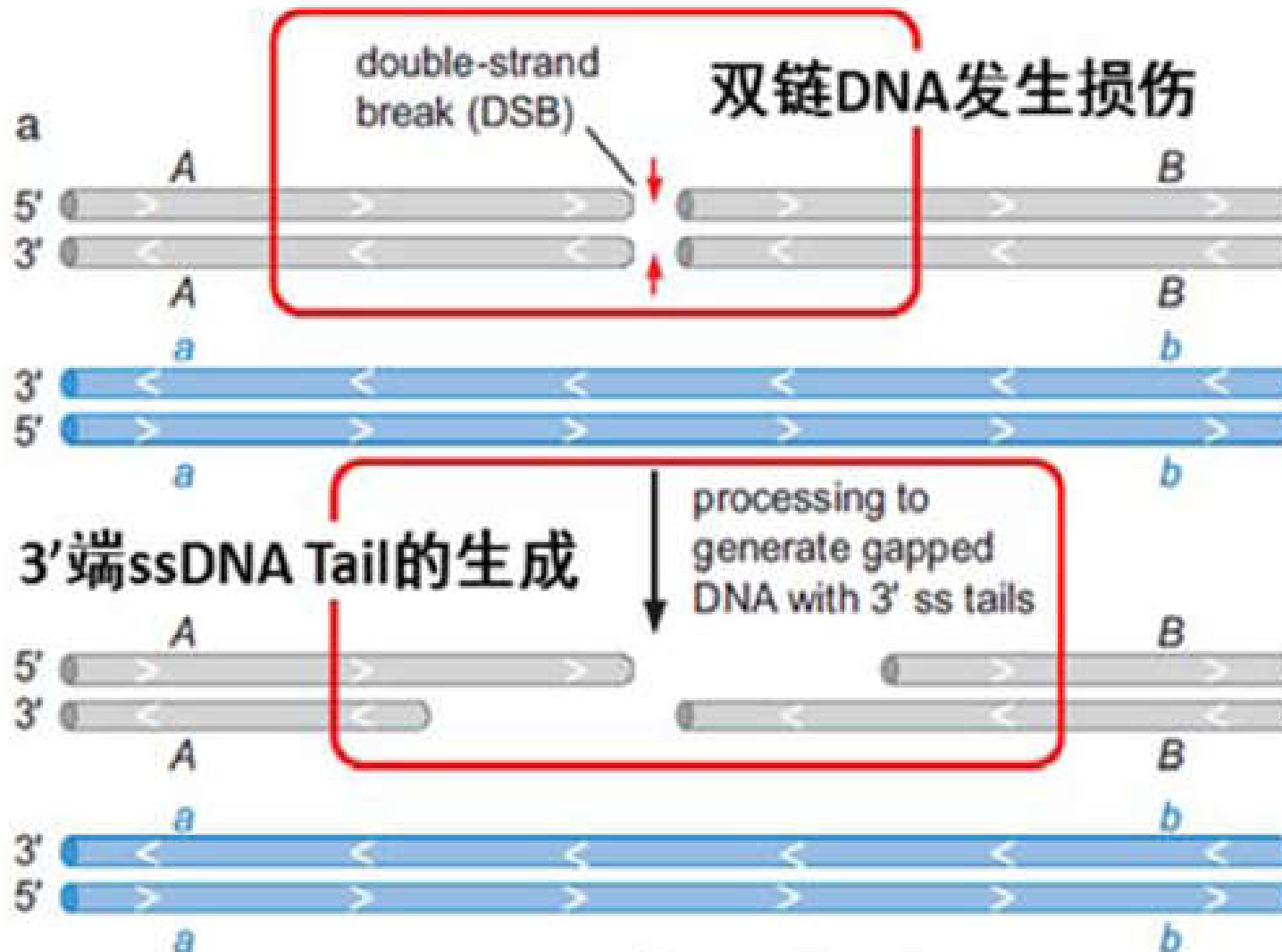
- **Recombination repair** is also called **postreplication repair** (复制后修复).
- The recombination occurs between the gapped strand and its **homolog** (同源) on the other daughter DNA duplex (双链).
- This solves the gap problem but leaves the **original damage unrepaired**. 并非完全校正

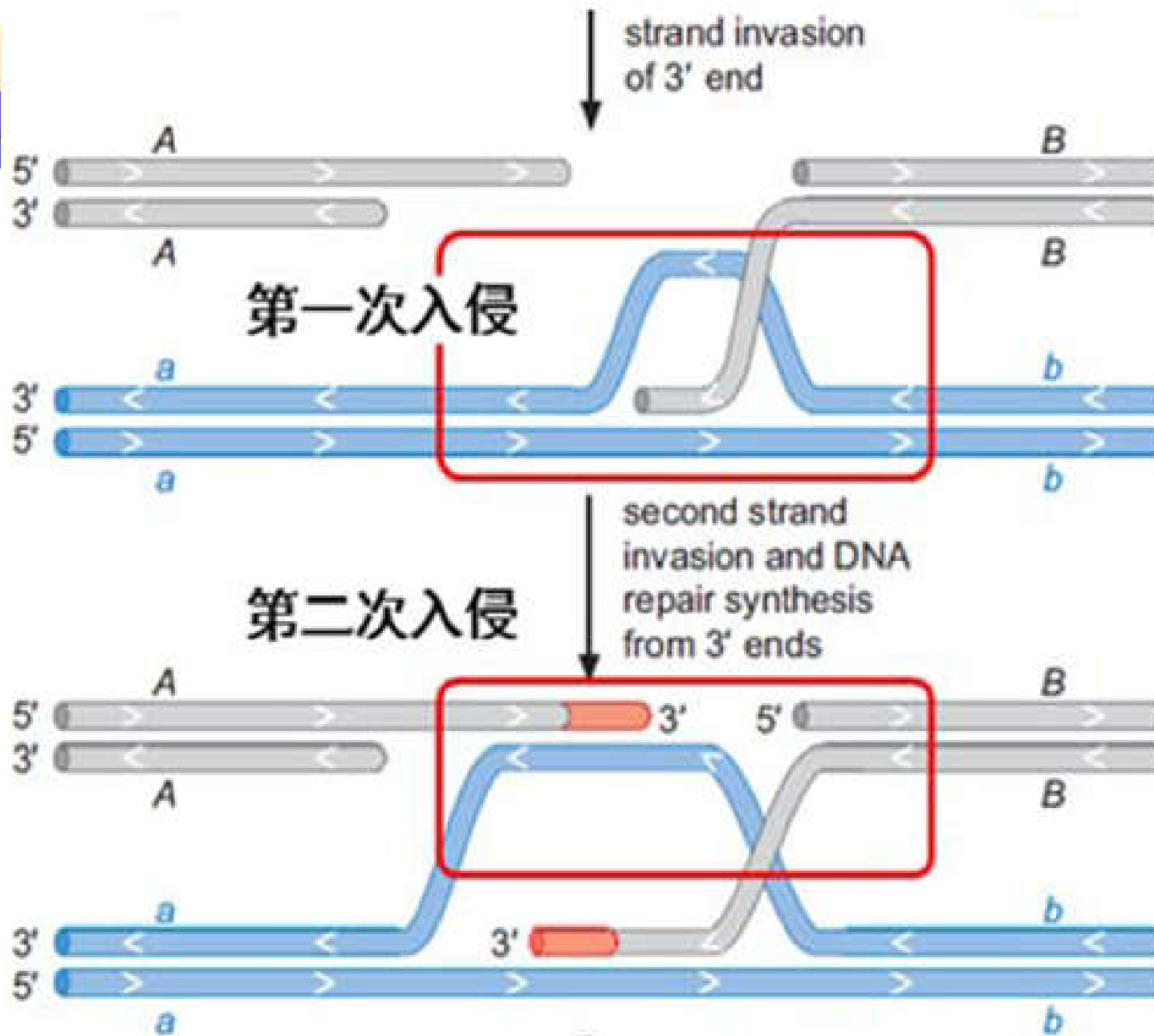
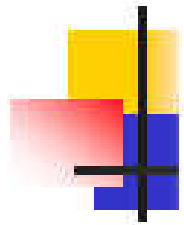


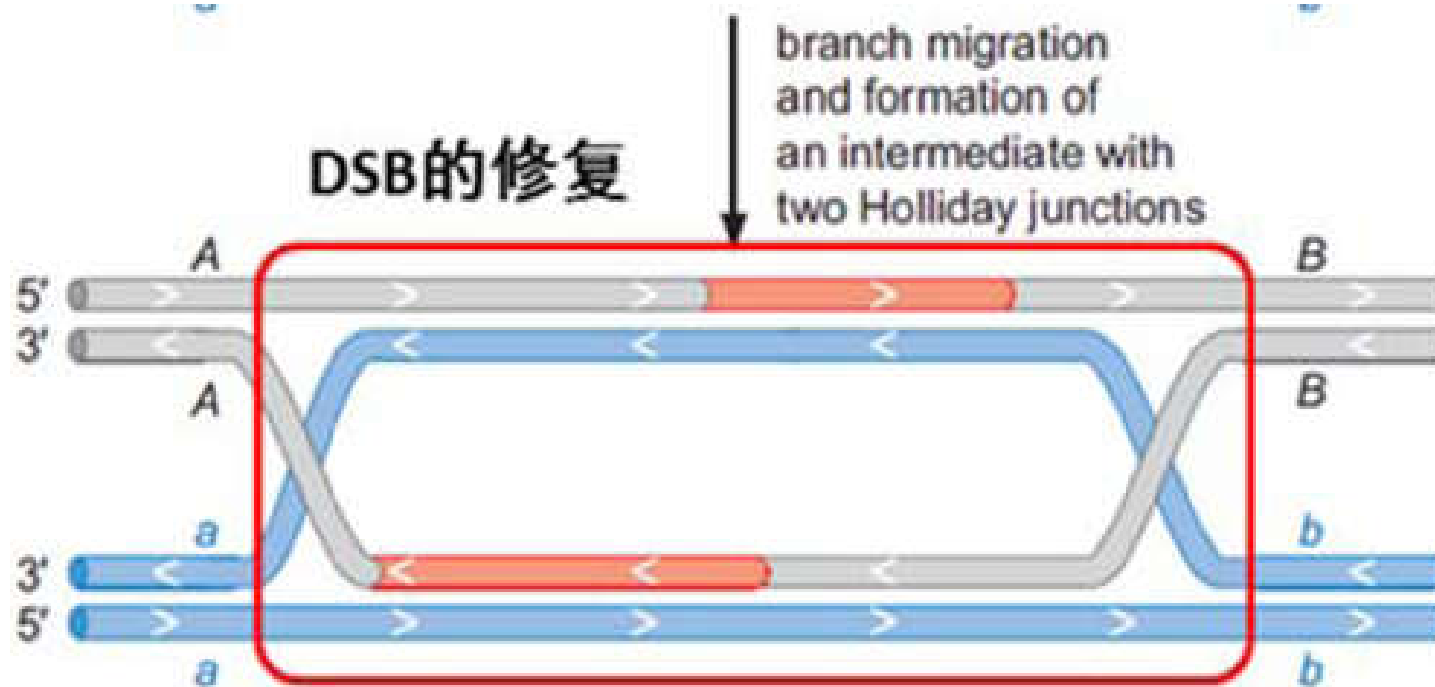
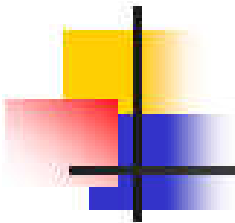




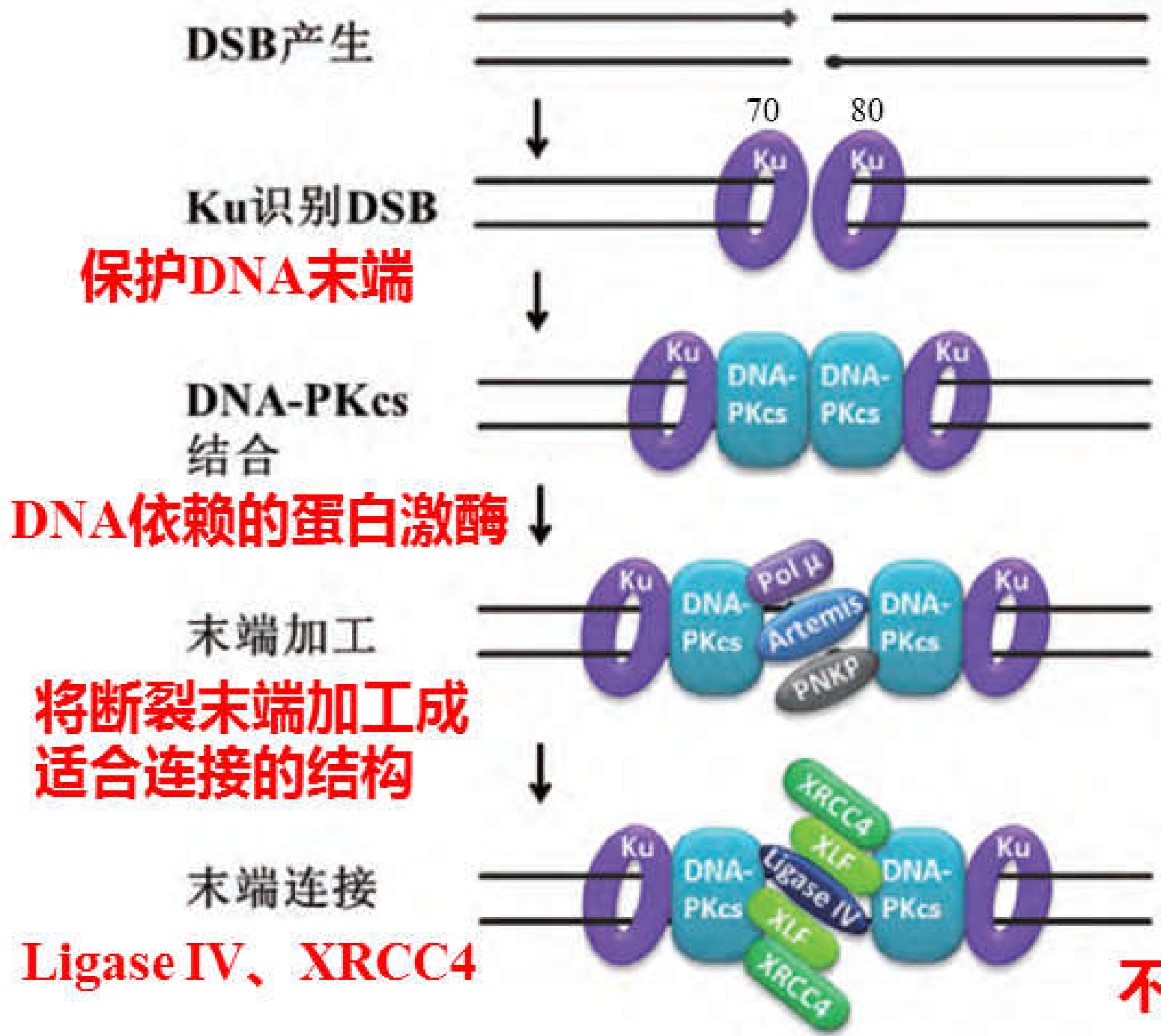
Recombination repairs double-strand break in DNA (DSB)







Nonhomologous end-joining (非同源末端连接, NHEJ) repairs DSB



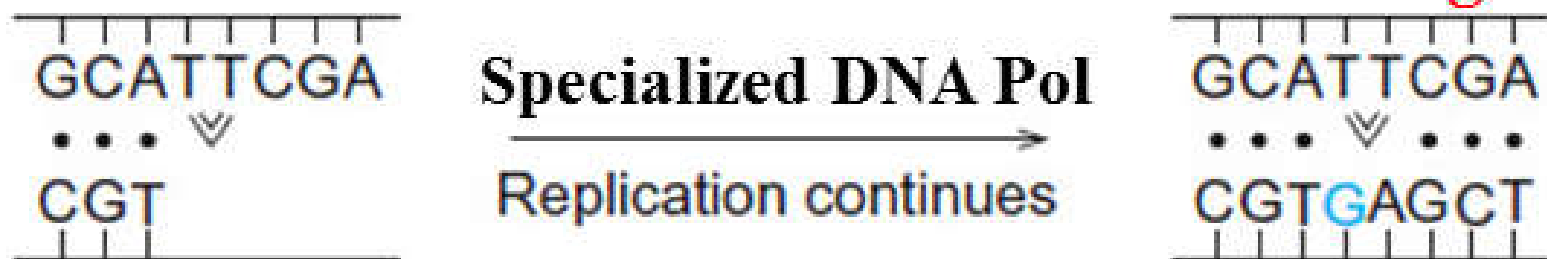
不够精确

3.5 Translesion DNA synthesis

- **Translesion DNA synthesis (跨损伤DNA合成):**
Insertion of nucleotide opposite the unrepaired lesion regardless of the original sequence to **maintain integrity** during DNA replication.

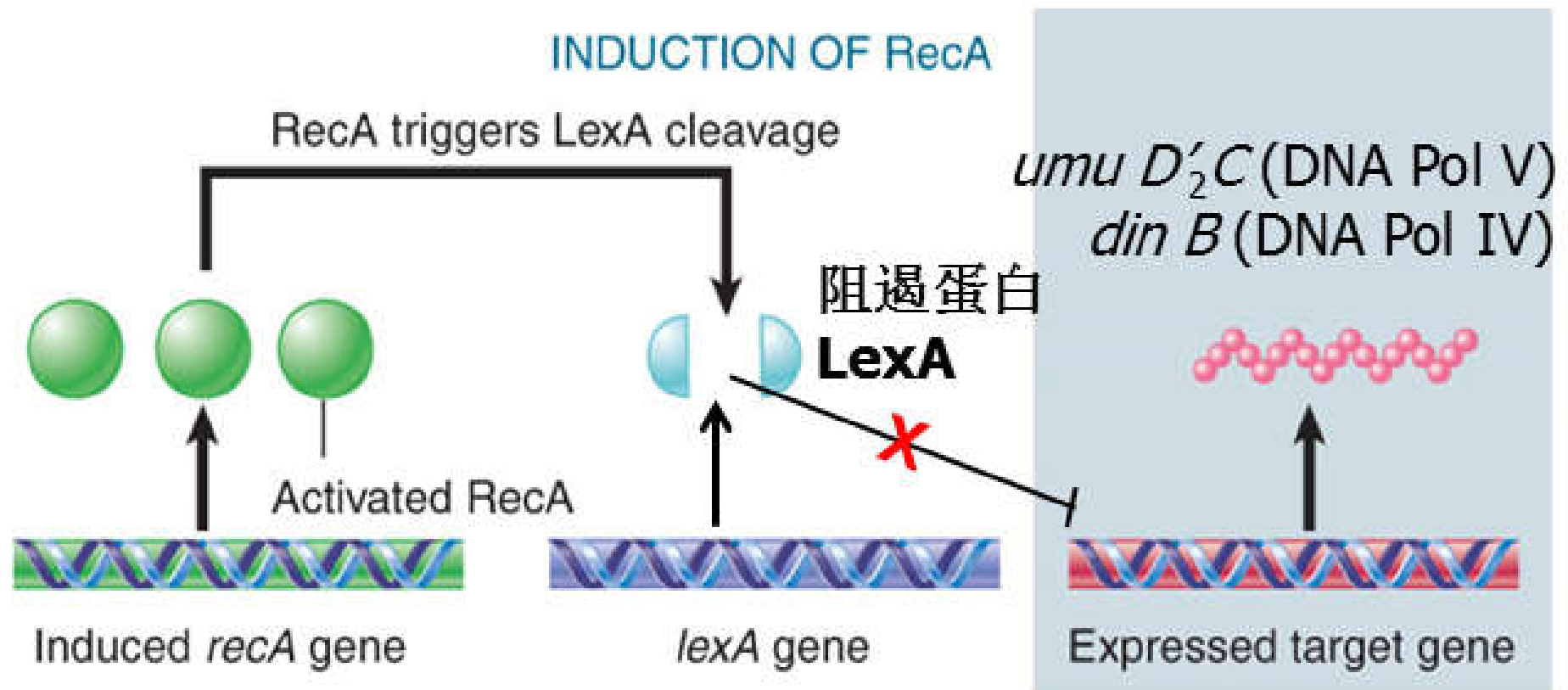
DNA合成时，不考虑原始DNA序列，在损伤对应的位点上插入核苷酸以保持DNA复制链的**完整性**

Indirect mutagenesis



真核：DNA Pol η (eta) DNA Pol ζ (zeta)

- In prokaryotes, translesion DNA synthesis is part of the SOS response to DNA damage.
- DNA受到严重损伤、细胞处于危急状态时所诱导的一种DNA修复方式——SOS repair。修复结果只能维持基因组的完整性，提高细胞的生存率，但留下的错误较多，所以又称为error-prone (易错/倾错性) repair。



修复途径	修复对象	参与修复的关键酶/蛋白
光复活	嘧啶二聚体	光裂合酶/光复活酶
碱基切除修复	受损的碱基	DNA糖基化酶, AP核酸内切酶, 外切酶
核苷酸切除修复	嘧啶二聚体等大块损伤(DNA螺旋结构的改变)	<i>E.coli</i> UvrABCD ; 人XP蛋白(XPC、XPF、XPG等), TFIIH, TC-NER中RNA Pol II
错配修复	复制中的碱基错配、打滑	MutS/L系统(<i>E.coli</i> 中有MutH内切核酸酶)
重组修复	损伤DNA复制后子链gap, 双链断裂	重组相关蛋白(RecA等)
非同源末端连接	双链断裂	Ku蛋白等
跨损伤DNA合成	大范围非编码损伤和大块损伤来不及修复	其他类型DNA聚合酶

总结：DNA损伤、突变与修复的关系

