DNA Stability

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DNA damage

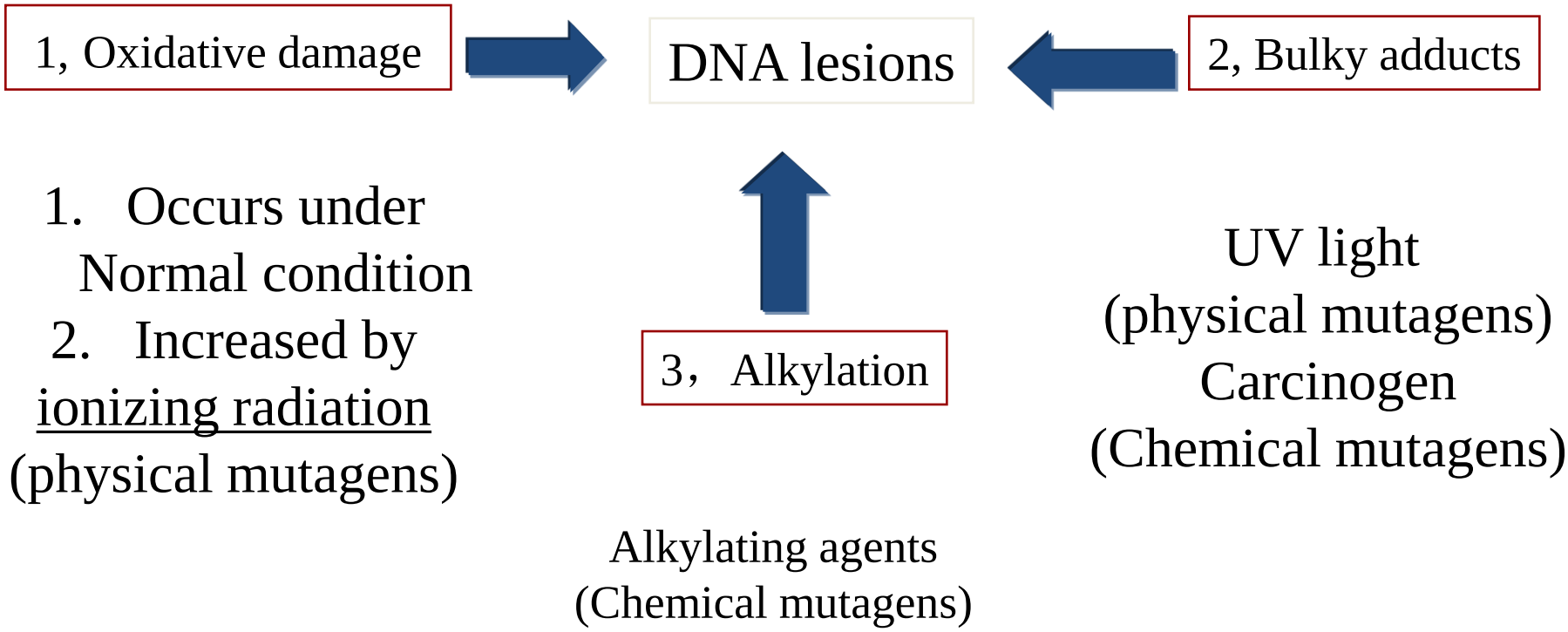

1, Oxidative damage
DNA lesions
2, Bulky adducts
1. Occurs under Normal condition
2. Increased by ionizing radiation
(physical mutagens)
3，Alkylation
Alkylating agents
(Chemical mutagens)
UV light
(physical mutagens)
Carcinogen
(Chemical mutagens)

Fig. 19.7 Production of a mutation as a result of a mismatch caused by wobble base pairing

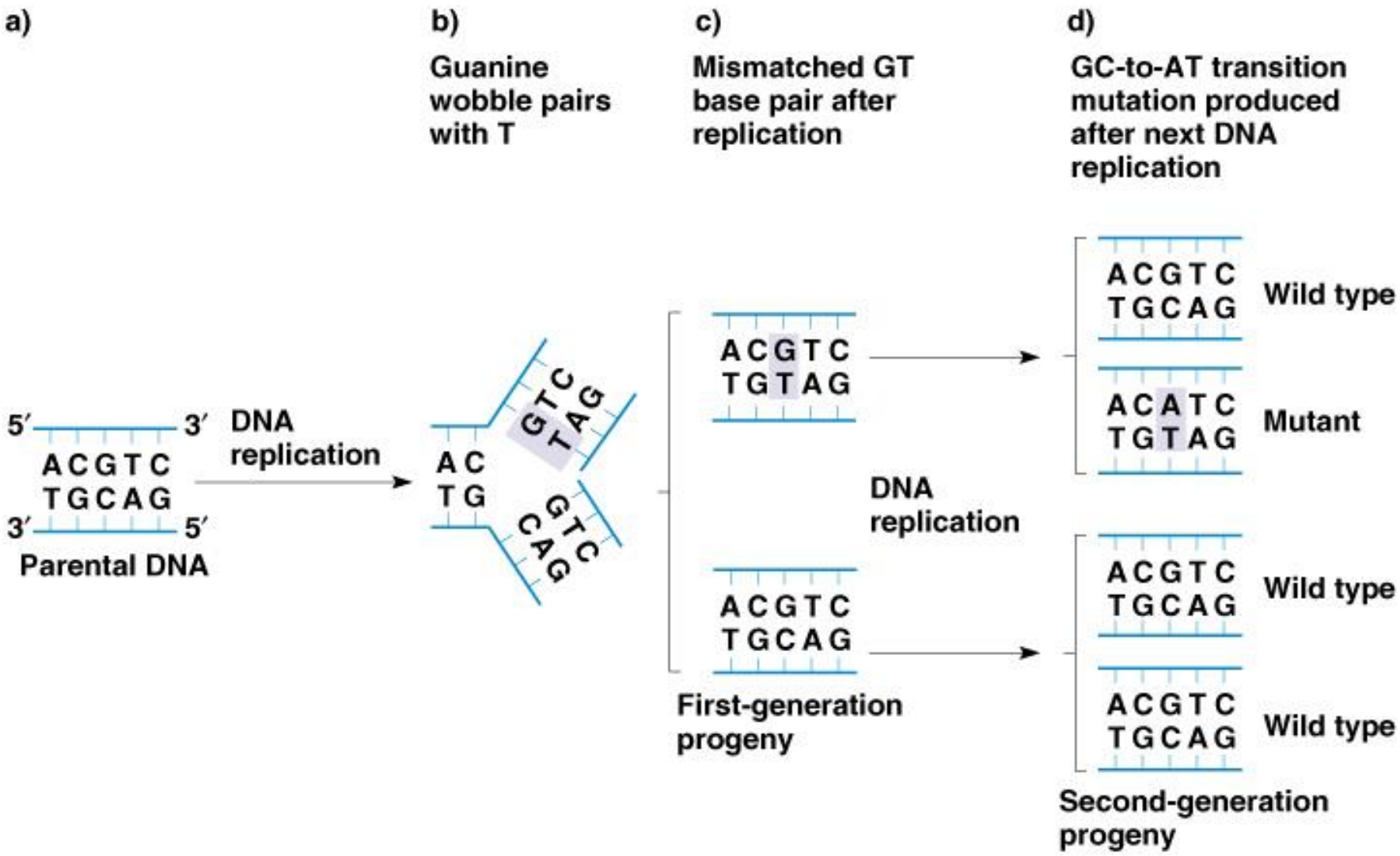


Fig. 19.8 Spontaneous generation of addition and deletion mutants by DNA looping-out errors during replication

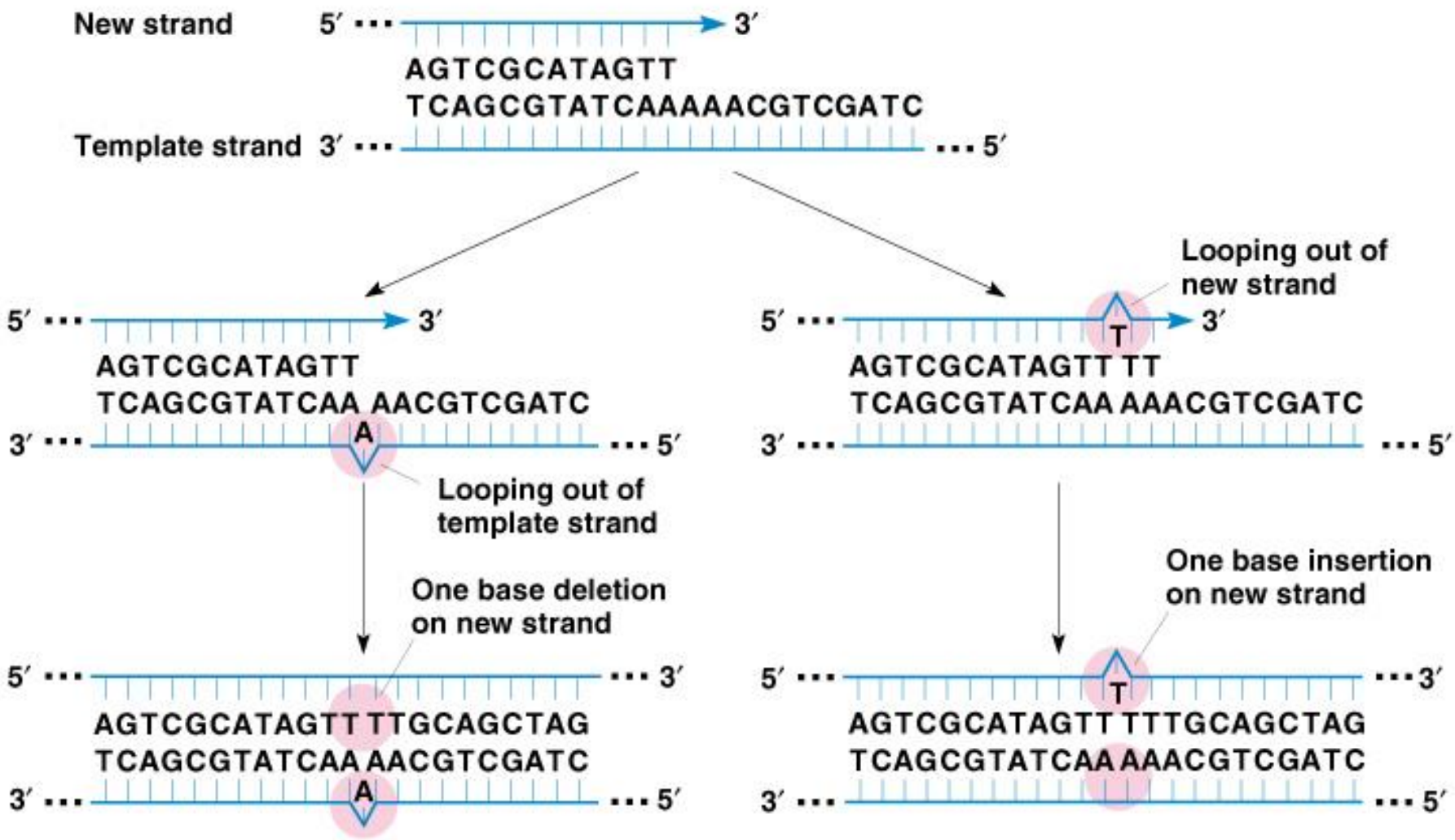
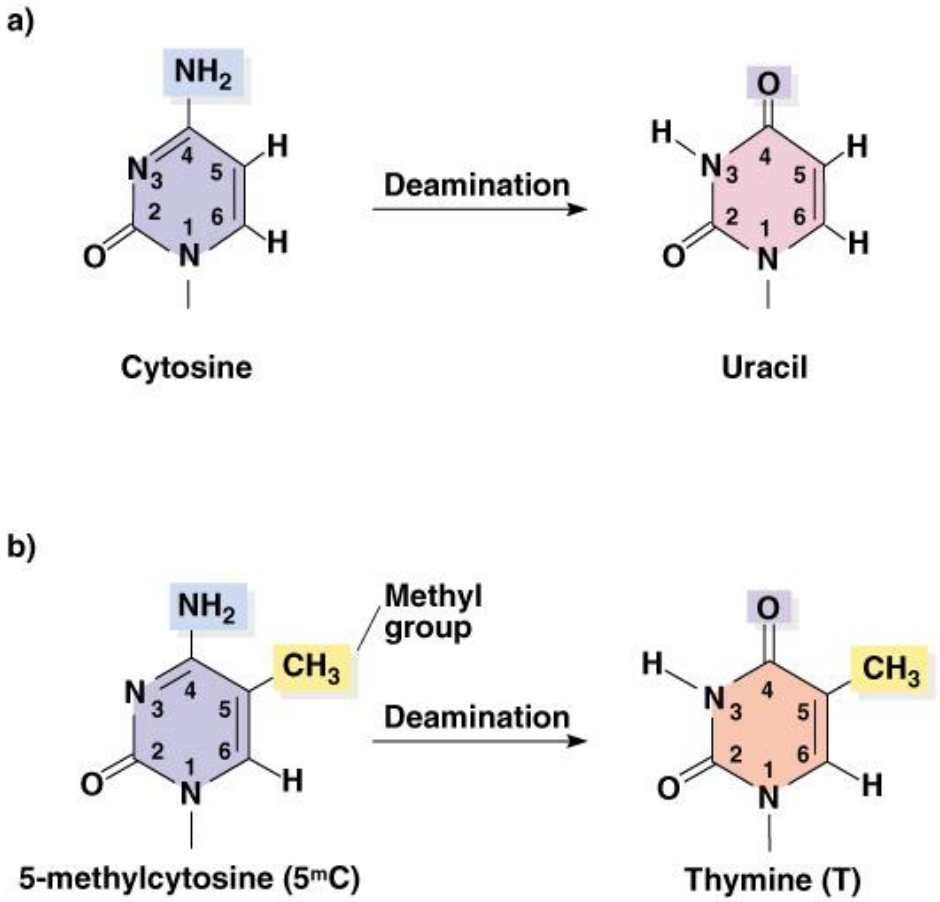


Fig. 19.9 Deamination of cytosine to uracil (a); deamination of 5-methylcytosine to thymine



Deamination removes an amino group from a base (e.g., cytosine to uradil) (Figure 19.9).

i. Uracil is an abnormal base in DNA, and it will usually be repaired.

ii. If uracil is not replaced, it will pair with an A during replication, resulting in a CG-to-TA transition.

Both prokaryotic and eukaryotic DNA have small amounts of 5 methylcytosine (5^mC) in place of the normal C.

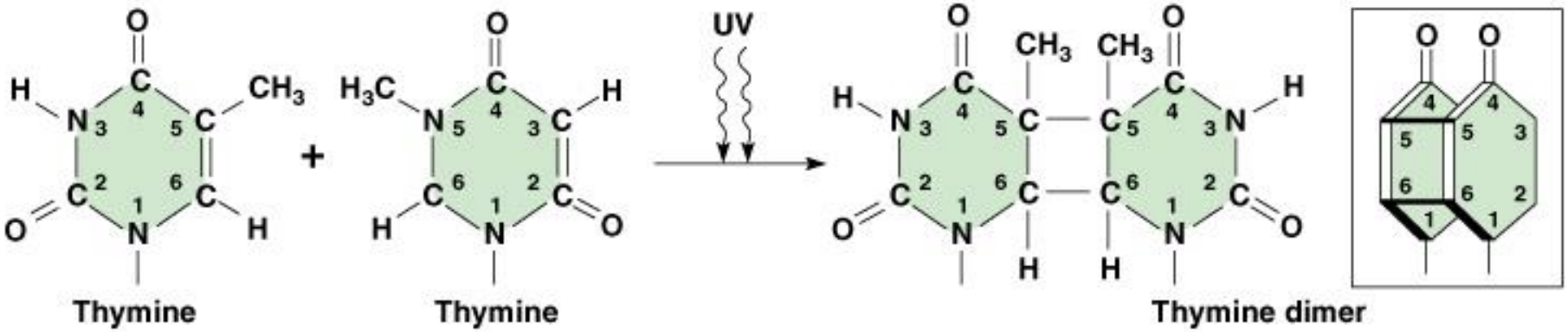
(1) Deamination of 5^mC produces T.

(2) T is a normal nucleotide in DNA, so it is not detected by repair mechanisms.

(3) Deamination of 5^mC results in CG-to-TA transitions.

(4) Locations of 5^mC in the chromosome are often detected as mutational hot spots

Fig. 19.11 Production of thymine dimers by ultraviolet light irradiation



Ultraviolet (UV) causes photochemical changes in the DNA.

i. **UV has lower-energy wavelengths than X rays, and so has limited penetrating power.**

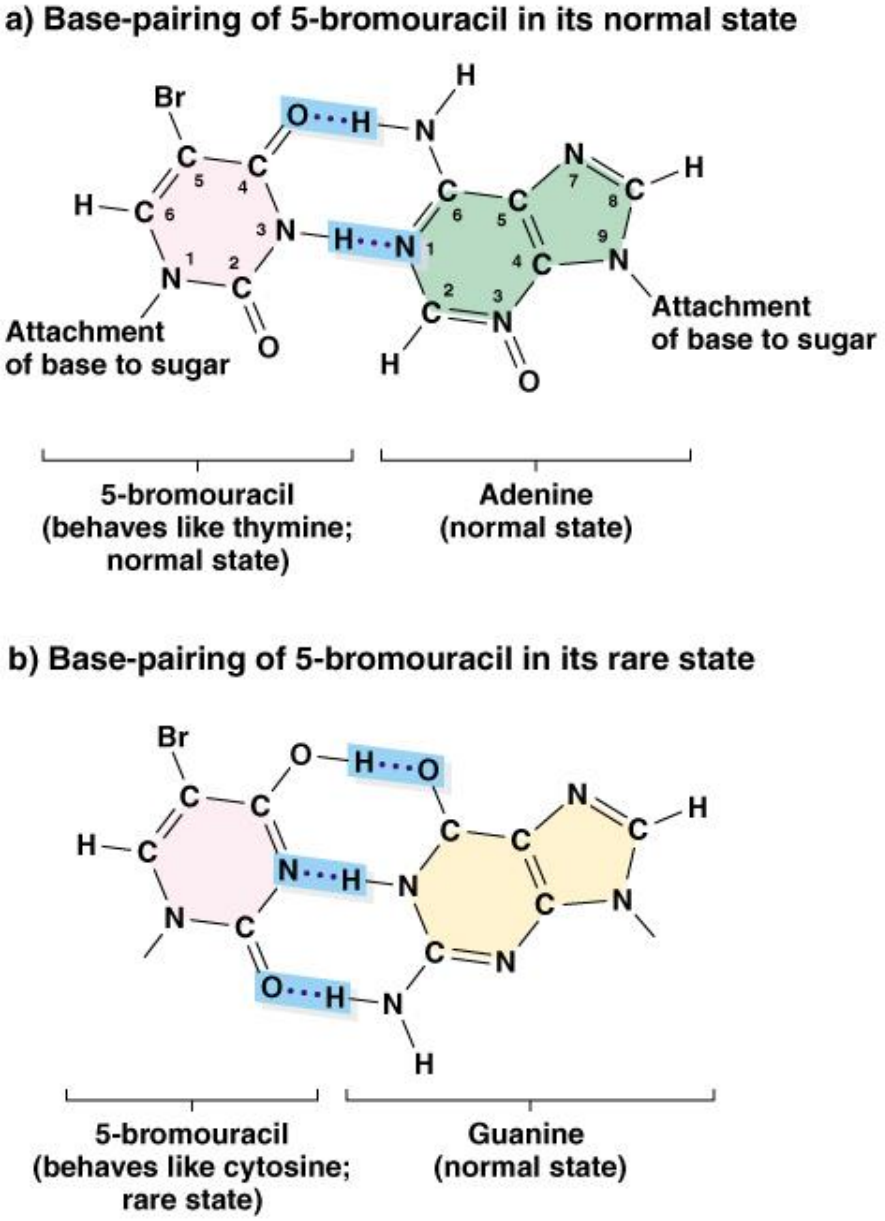
ii. **However, UV in the 254–260 nm range is strongly absorbed by purines and pyrimidines, forming abnormal chemical bonds.**

(1) A common effect is dimer formation between adjacent pyrimidines, commonly thymines (designated T^T) (Figure 19.11).

(2) C^C, C^T and T^C dimers also occur, but at lower frequency. Any pyrimidine dimer can cause problems during DNA replication.

(3) Most pyrimidine dimers are repaired, because they produce a bulge in the DNA helix. If enough are unrepaired, cell death may result.

Fig. 19.12a, b Mutagenic effects of the base analog 5-bromouracil (5BU)



iii. 5-bromouradil (5BU) is an example. 5BU has a bromine residue instead of the methyl group of thymine (Figure 19.12).

(1) Normally 5BU resembles thymine, pairs with adenine and is incorporated into DNA during replication.

(2) In its rare state, 5BU pairs only with guanine, resulting in a TA-to-CG transition mutation.

(3) If 5BU is incorporated in its rare form, the switch to its normal state results in a CG-to-TA transition.

Fig. 19.12c Mutagenic effects of the base analog 5-bromouracil (5BU)

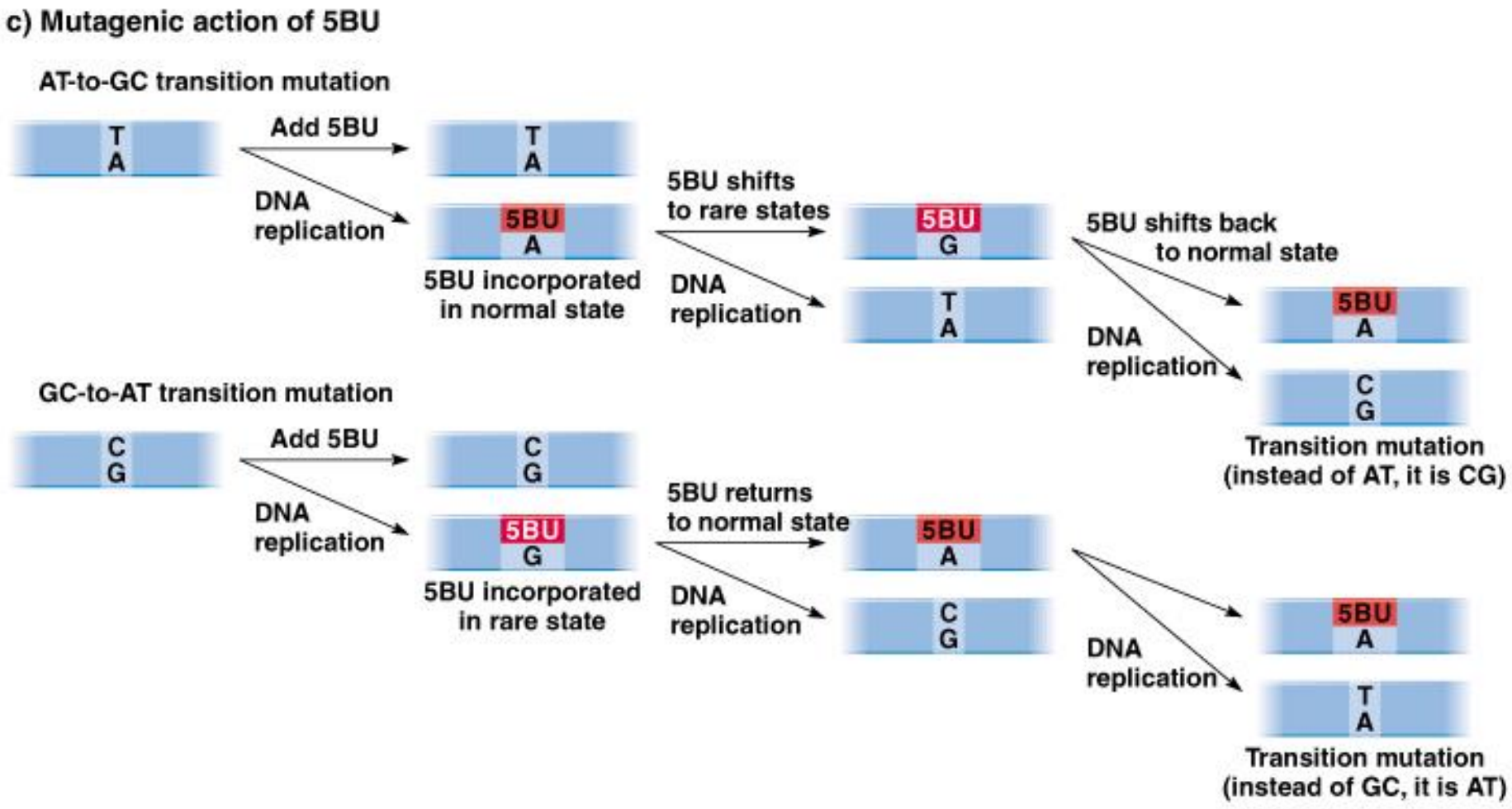
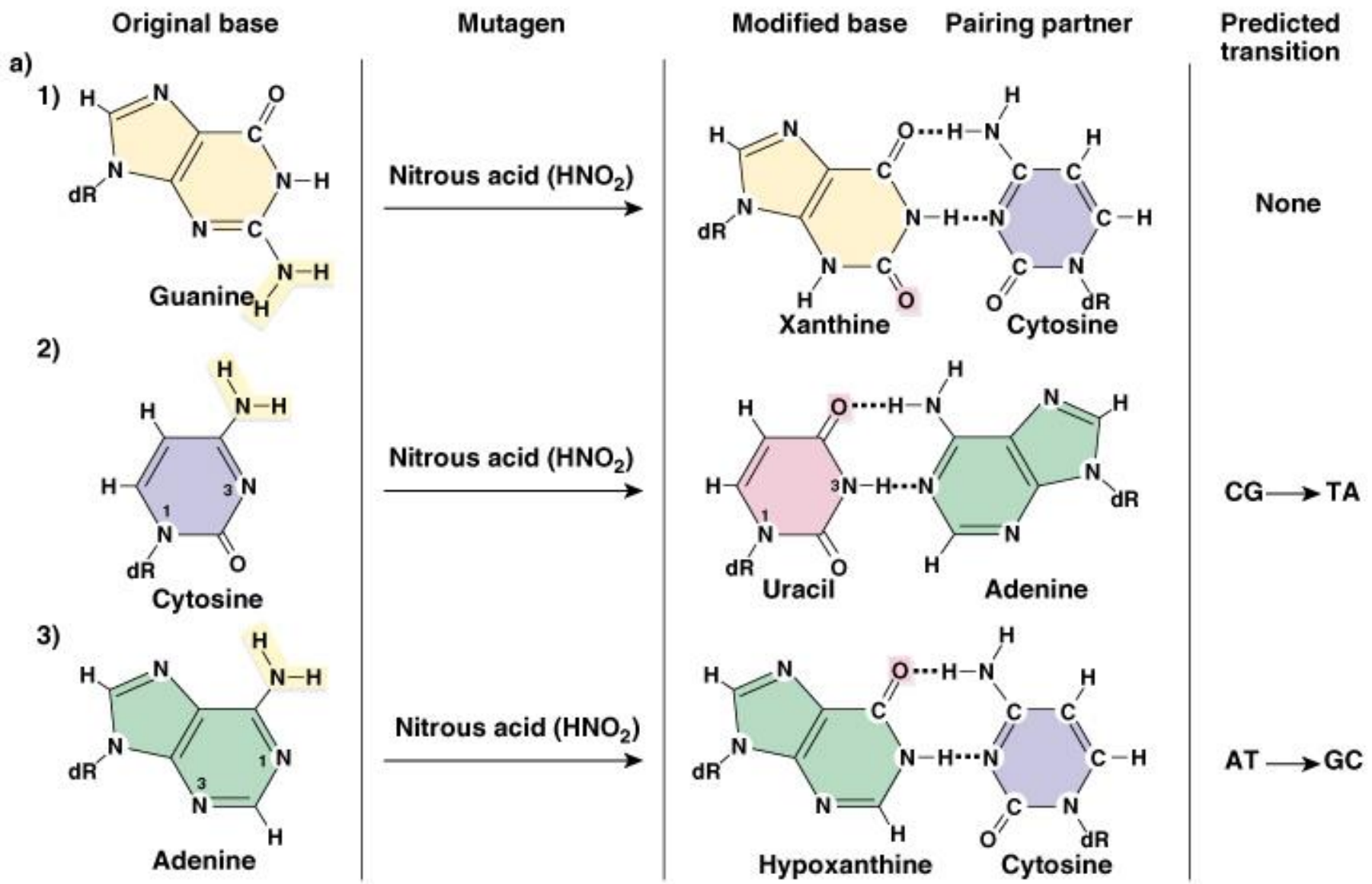
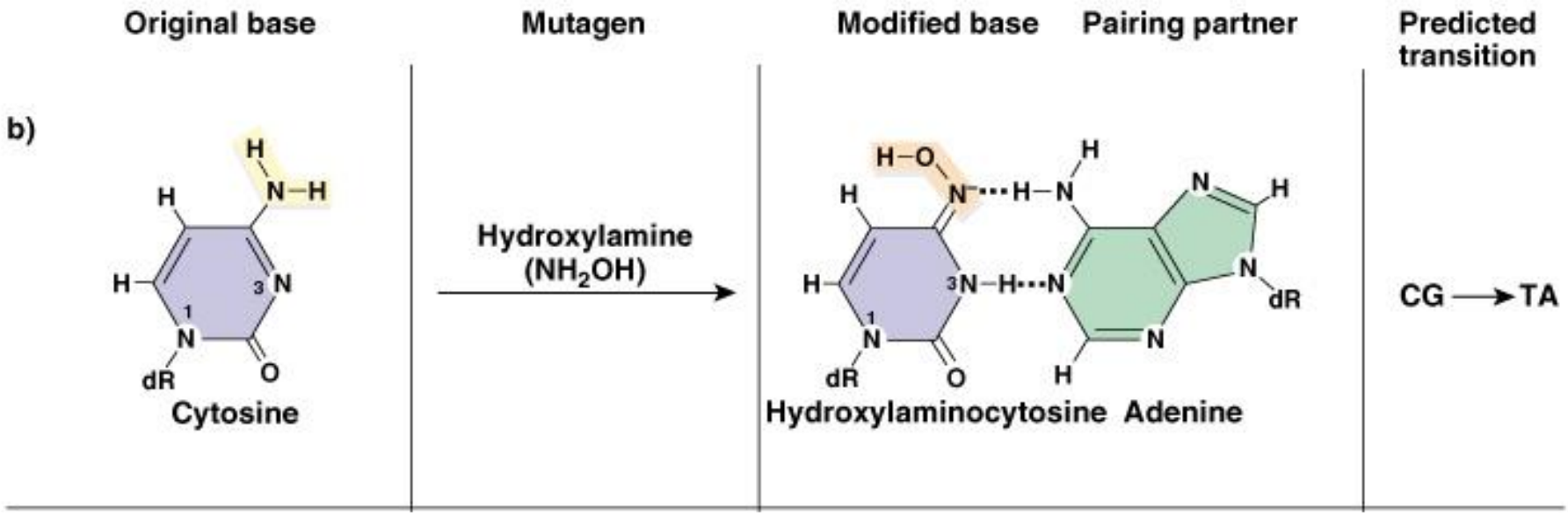


Fig. 19.13a Action of three base-modifying agents





Hydroxylating agents include hydroxylamine (NH_2OH).

(1) NH_2OH specifically modifies C with a hydroxyl group (OH), so that it pairs only with A instead of with G.

(2) NH_2OH produces only CG-to-TA transitions, and so revertants do not occur with a second treatment.

(3) NH_2OH mutants, however, can be reverted by agents that do cause TA-to-CG transitions (e.g., 5BU and HNO_2).

Fig. 19.13b, c Action of three base-modifying agents

- **Allcylating agents are a diverse group that add ailcyl groups to bases. Usually alkylation occurs at the 6-oxygen of G, producing O^6-allcylguanine.**
  - (1) An example is methylmethane sulfonate (MMS), which methylates G to produce O^6-alkyl G.
  - (2) O^6-aIkylG pairs with T rather than C, causing GC-to-AT transitions.

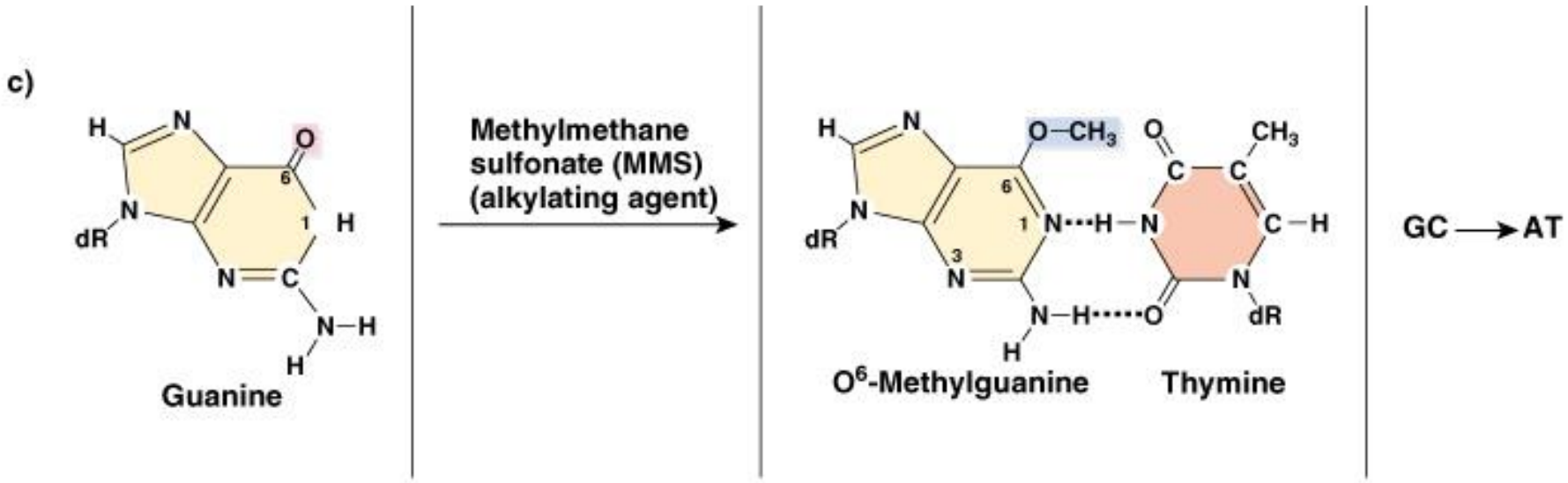
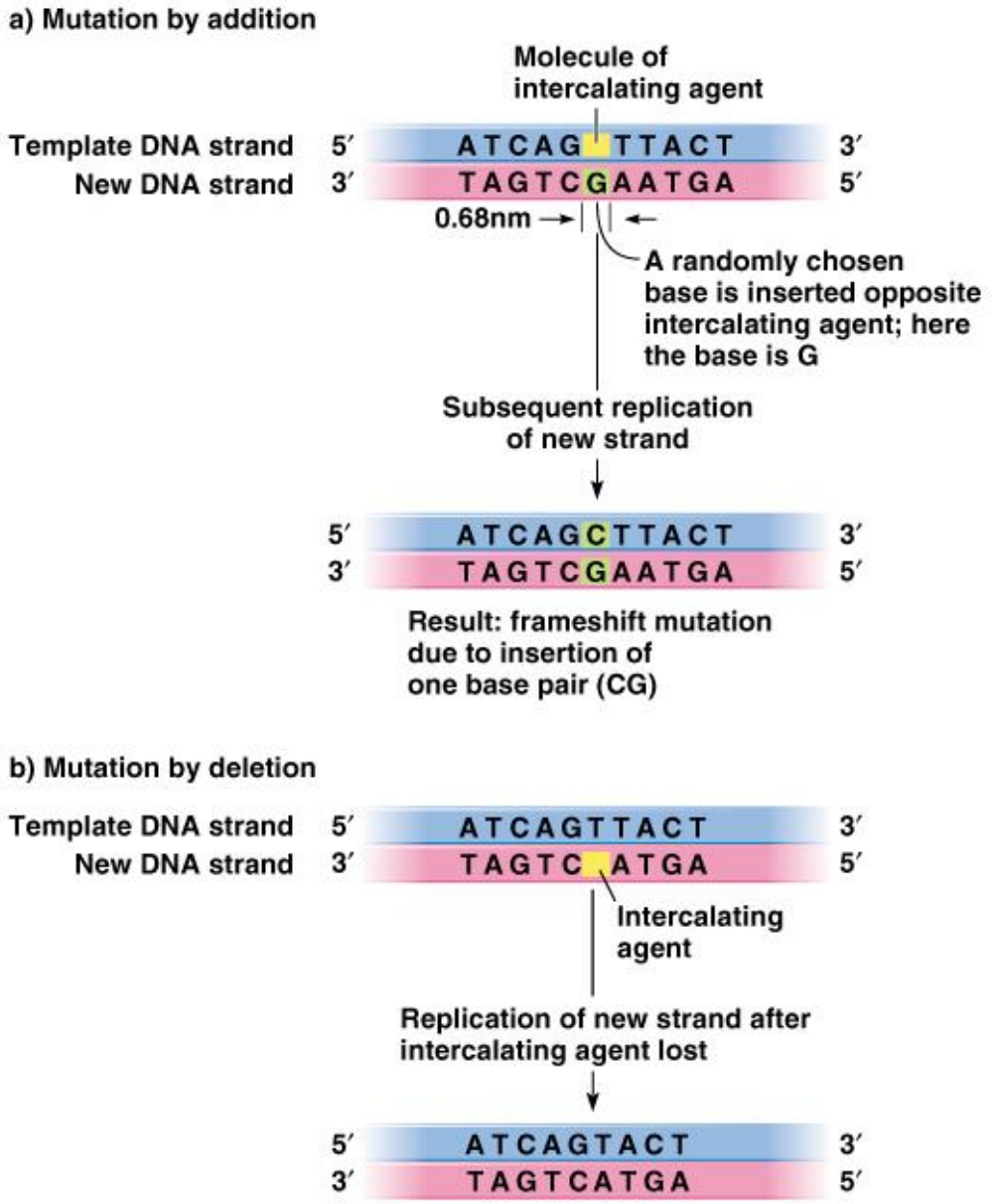


Fig. 19.14 Intercalating mutations



DNA repair mechanisms:

Enzyme-based repair mechanisms prevent and repair mutations and damage to DNA in prokaryotes and eukaryotes.

Types of mechanisms

DNA polymerase proofreading –

DNA polymerase proofreading corrects most of the incorrect nucleotide insertions that occur during DNA synthesis, which stalls until the wrong nucleotide is replaced with a correct one.

a. The role of 3'-to-5' exonuclease activity is illustrated by mutator mutations in *E. coli*, which confer a much higher mutation rate on the cells that carry them.

b. The mutD gene, encoding the e subunit of DNA polymerase III, is an example. Cells mutant in mutD are defective in proofreading.

Photoreactivation (also called light repair) – UV-induced pyrimidine dimers are repaired using photoreactivation (light repair).

a. Near UV light (320–370 nm) activates photolyase (product of the phr gene) to split the dimer.

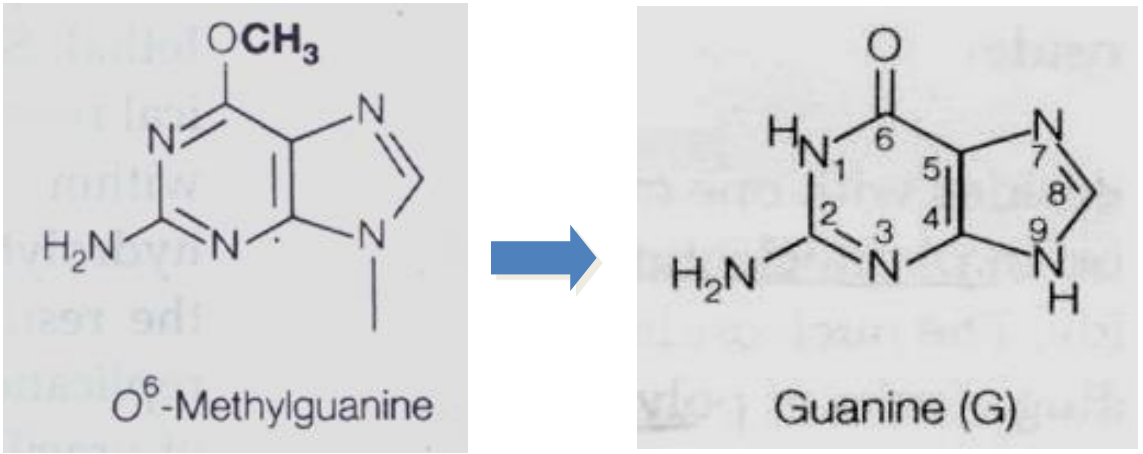
b. Photolyases are found in prokaryotes and simple eukaryotes, but not in humans.

- Demethylating DNA repair enzymes

Damage by alkylation (usually methyl or ethyl groups) can be removed by specific DNA repair enzymes.

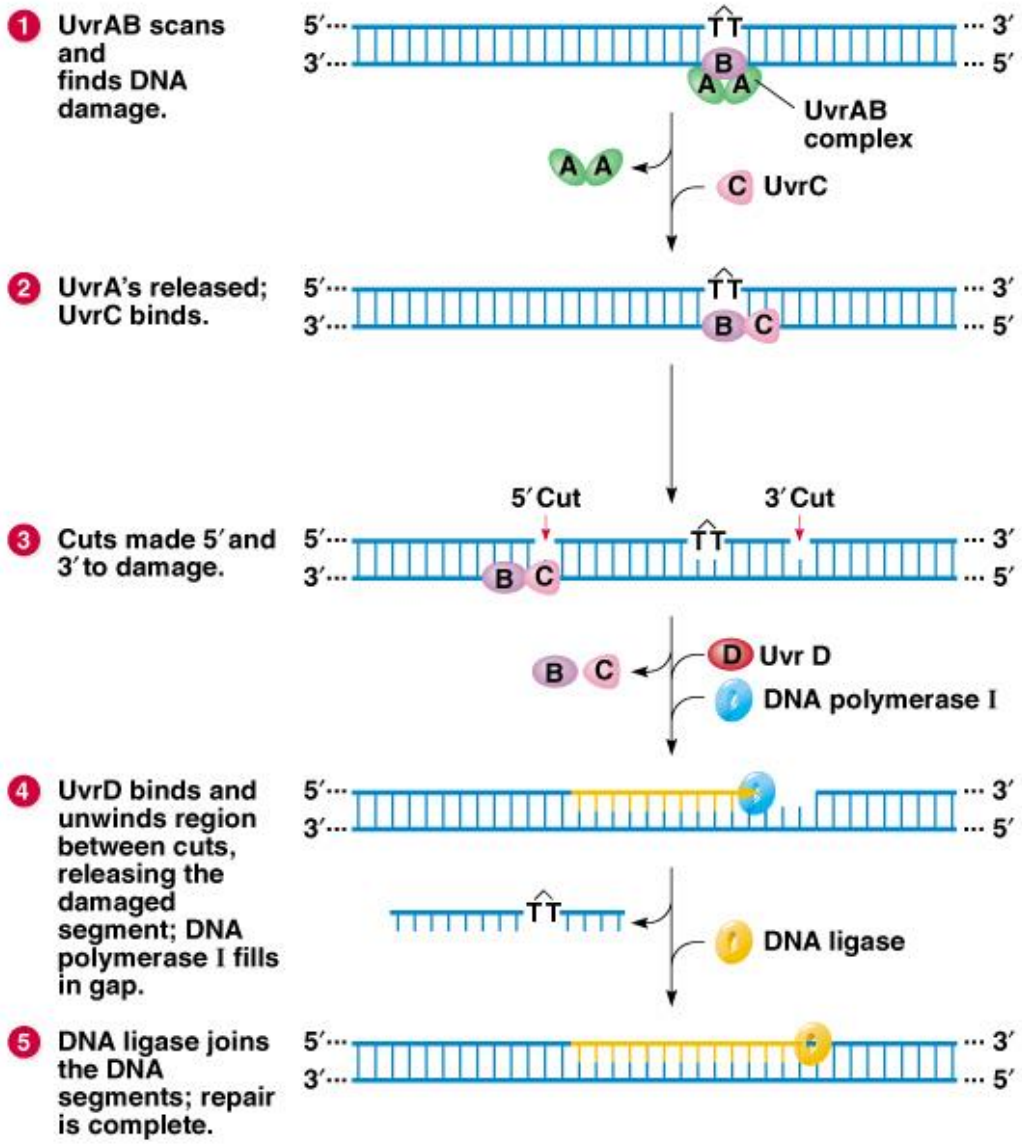
a. For example, O^6-methylguanine methyltransferase (from the ada gene) recognizes O^6-methylguanine in DNA, and removes the methyl group.

b. Demethylation restores the base to its original form.



Direct reversal of a lesion and is error-free

Fig. 7.16 Nucleotide excision repair (NER) of pyrimidine dimmer and other damage-induced distortions of DNA



It was discovered in 1964. It is called dark repair, the excision repair system, or the nucleotide excision repair (NER) system.

a. In *E. coli*, NER corrects pyrimidine dimers and other damage-induced distortions of the DNA helix.

b. The proteins required are UvrA, UvrB, UvrC and UvrD (encoded by genes of the same name) (Figure 19.17).

c. A complex of two UvrA and one UvrB proteins slides along the DNA. When it encounters a helix distortion, the UvrA subunits dissociate, and a UvrC binds the UvrB at the lesion.

d. When UvrBC forms, the UvrC cuts 4–5 nucleotides from the lesion on the 3' side, and eight nucleotides away on the 5' side. Then UvrB is released and UvrD binds the 5' cut end.

e. UvrD is a helicase that unwinds the region between the cuts, releasing the short ssDNA, while DNA polymerase I fills the gap and DNA ligase seals the backbone.

f. In yeast and mammalian systems, about 12 genes encode proteins involved in excision repair.

Base excision repair

1. modified bases are recognized by relatively specific DNA glycosylases which cleave the N-glycosylic bond between the altered base and the sugar), leaving an apurinic or apyrimidinic (AP) site.
2. An AP endonuclease then cleaves the DNA at this site and a gap may be created by further exonuclease activity. The gap is generally larger in NER and can be as small as one nucleotide in BER.
3. The gap is filled by DNA polymerase I and sealed by ligase

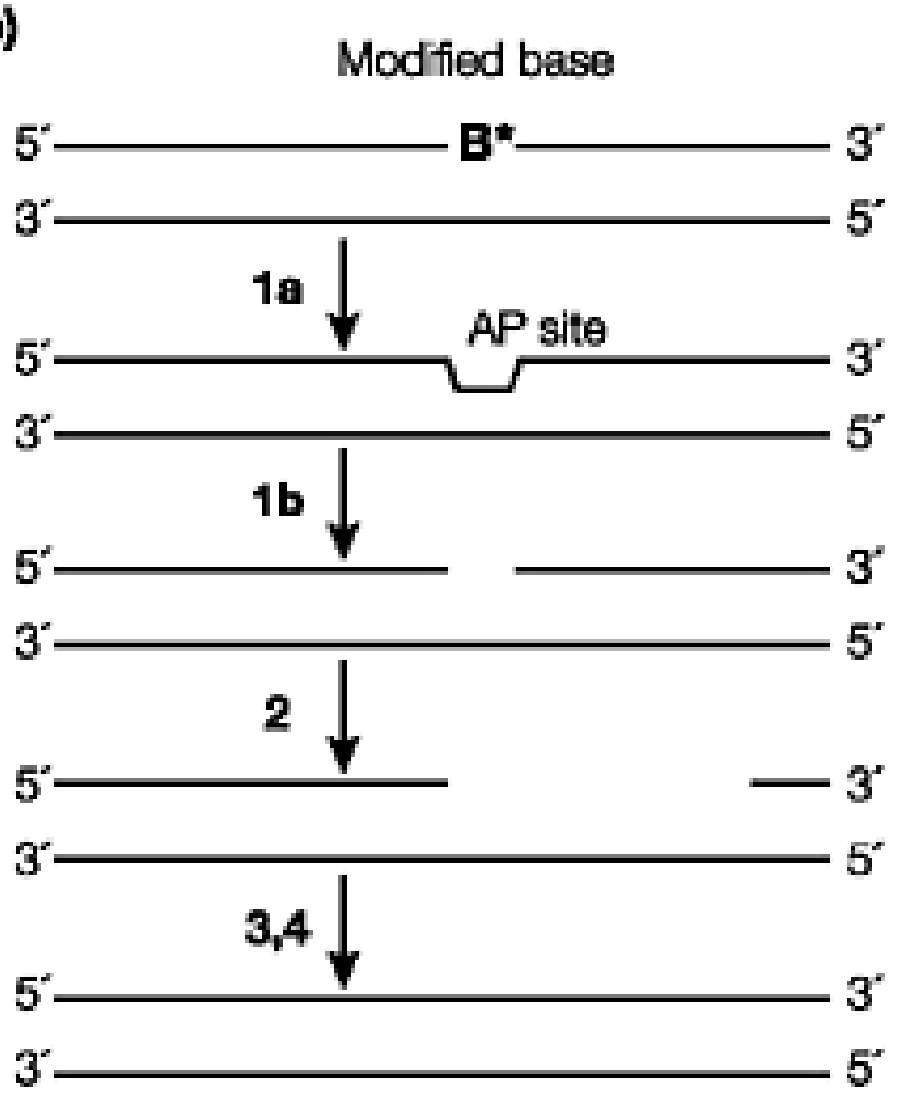
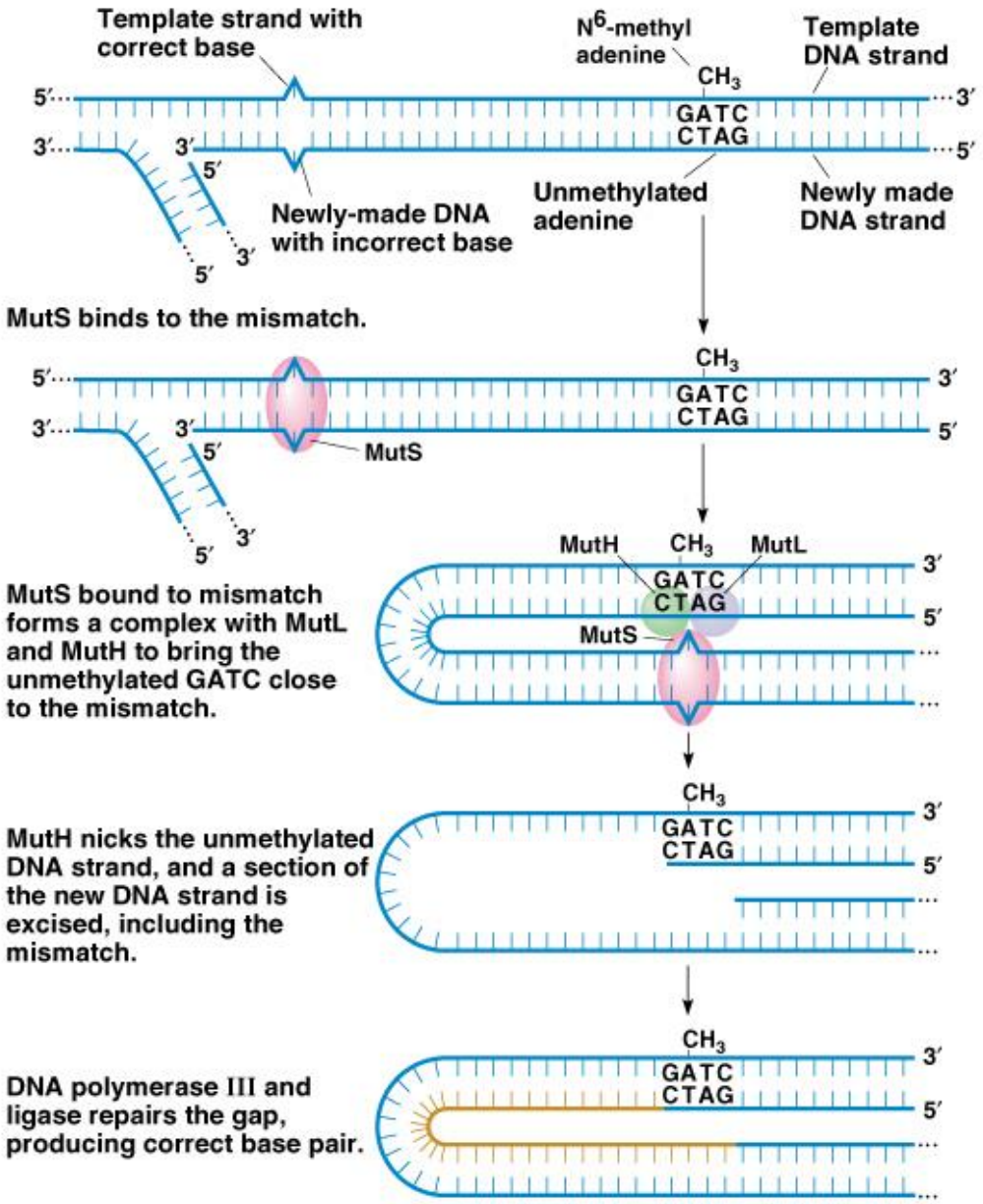


Fig. 7.17 Mechanism of mismatch correction repair

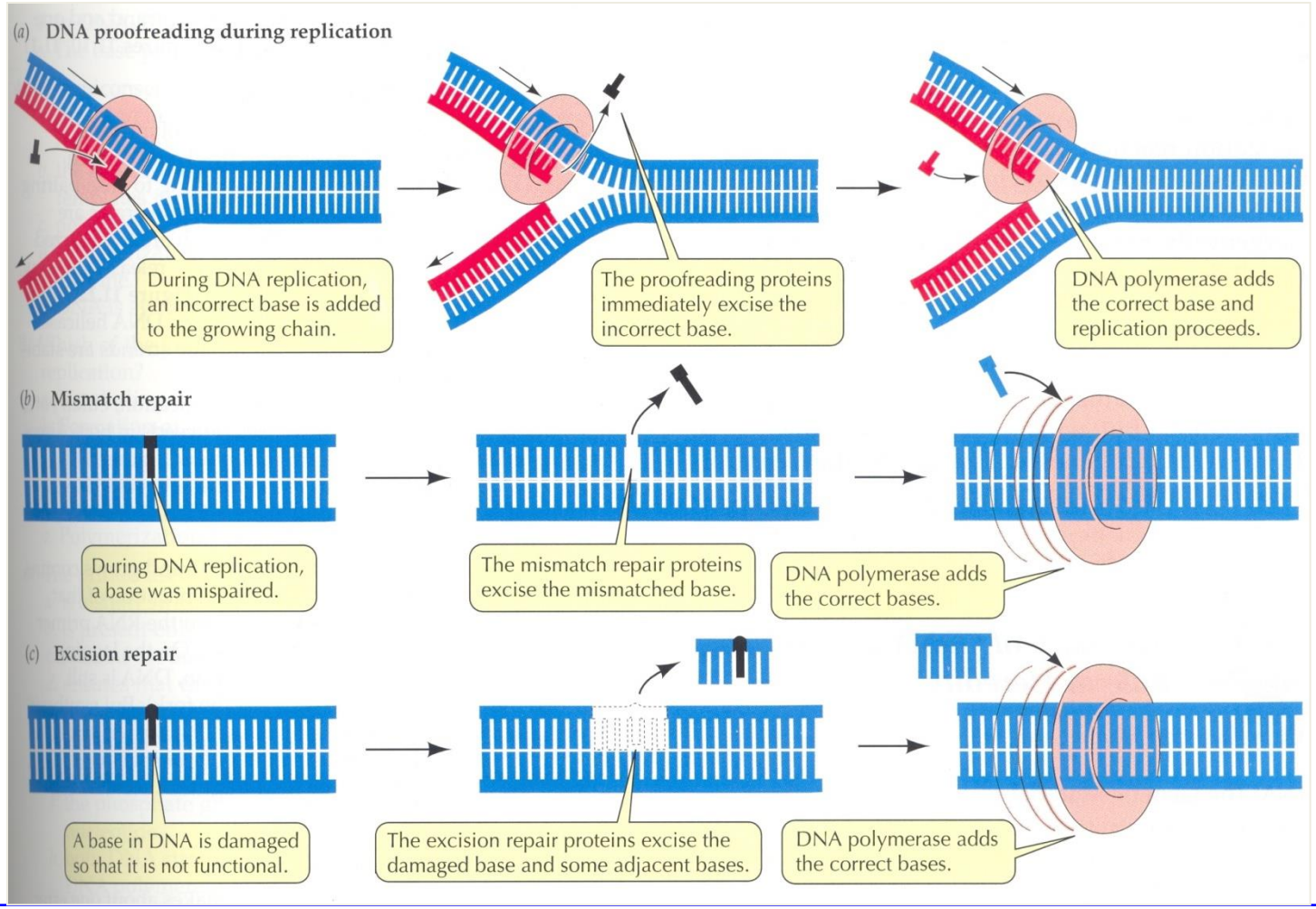


a. In *E. coli*, initial stages involve products of the *mutS*, *mutL* and *mutH* genes

i. MutS binds the mismatch, and determines which is the new strand by its lack of methylation.

ii. MutL and MutH bind unmethylated GATC sequences (site of methylation in *E. coli*) and bring the GATC close to the mismatch by binding MutS.

iii. MutH then nicks the unmethylated GATC site, the mismatch is removed by an exonuclease and the gap is repaired by DNA polymerase III and ligase.

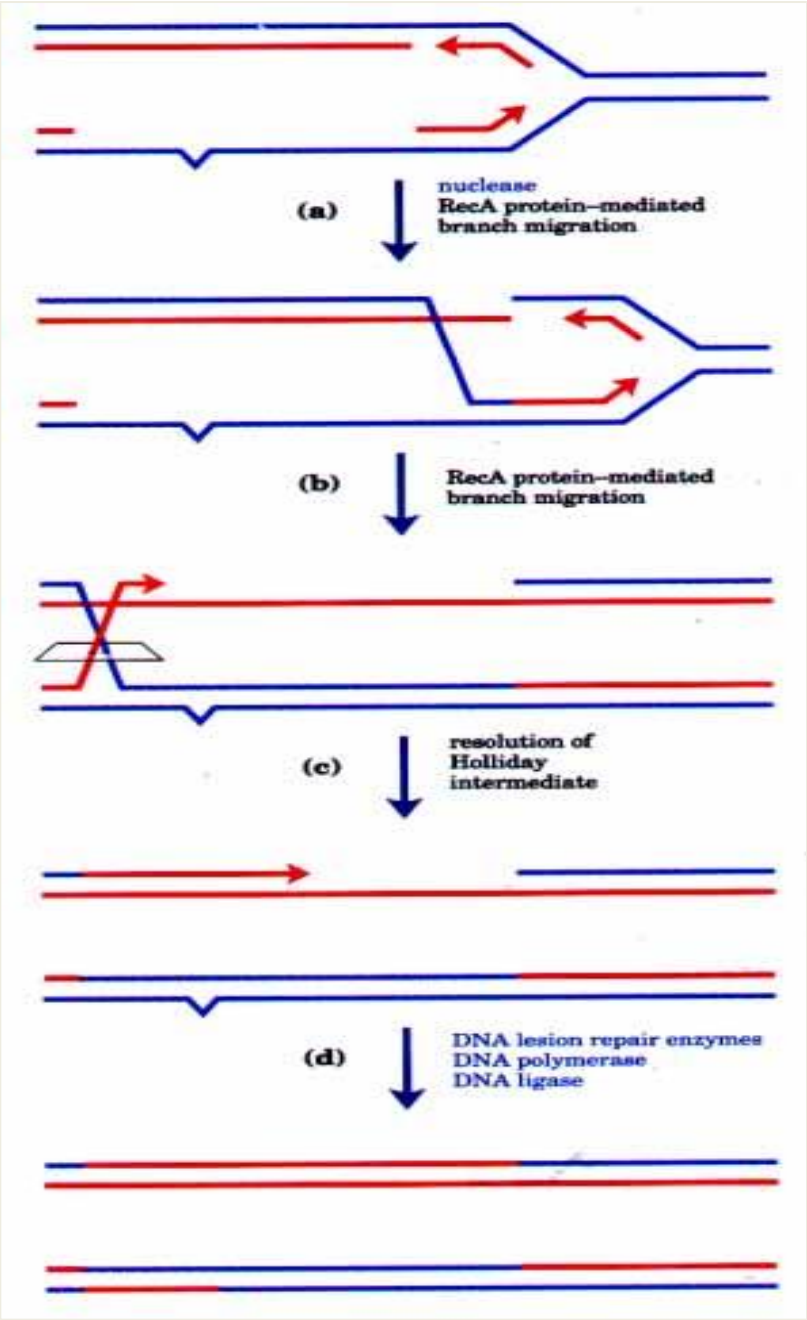


DNA Repair Mechanisms

The proteins of DNA replication also play roles in the life-preserving repair mechanisms, helping to ensure the extract replication of template DNA.

Recombination based DNA repair at replication fork, also called post-replication repair

a. Replication encounter a DNA lesion
b. Skip the lesion & re-initiate on the other side of the lesion
c. Fill the daughter strand gap by replacing it with the corresponding section from the parental sister strand by recombination
d. The original lesion can be removed later by normal excision repair.



Acknowledgement

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- Thanks are due to all those original contributors and entities whose pictures used for making this presentation.

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