DNA Damage & Repair

Nebras Melhem

Introduction

- Most cells have only one or two sets of genomic DNA.
- Damaged proteins and RNA molecules can be quickly replaced by using information encoded in the DNA, but DNA molecules themselves are irreplaceable.
- Maintaining the integrity of the information in DNA is a cellular imperative, supported by an elaborate set of DNA repair systems.
- DNA can become damaged by a variety of processes, some spontaneous, others catalyzed by environmental agents.
- Replication itself can very occasionally damage the information content in DNA when polymerase errors create mismatched base pairs (such as G paired with T).

Introduction

- The genomic DNA in a typical mammalian cell accumulates many thousands of lesions during a 24-hour period. However, as a result of DNA repair, fewer than 1 in 1,000 become a mutation.

- DNA is a relatively stable molecule, but in the absence of repair systems, the cumulative effect of many infrequent but damaging reactions would make life impossible.

Introduction

- The number and diversity of repair systems reflect both the importance of DNA repair to cell survival and the diverse sources of DNA damage.
- Some common types of lesions, such as pyrimidine dimers, can be repaired by several distinct systems.
- Nearly 200 genes in the human genome encode proteins dedicated to DNA repair. In many cases, the loss of function of one of these proteins results in genomic instability and an increased occurrence of oncogenesis.

Introduction

- Accurate DNA repair is possible largely because the DNA molecule consists of two complementary strands.
- Damaged DNA in one strand can be removed and replaced, without introducing mutations, by using the undamaged complementary strand as a template.
- We consider here the principal types of repair systems, beginning with those that repair the rare nucleotide mismatches that are left behind by replication.

Mechanisms of DNA repair

1. Mismatch repair
2. Base excision repair
3. Nucleotide excision repair
4. Double strand break repair

The defective region in one strand can be repaired relying on the complementary information stored in the unaffected strand.

Mismatch repair

- The mismatches are nearly always corrected to reflect the information in the old (template) strand, which the repair system can distinguish from the newly synthesized strand by the presence of methyl group tags on the template DNA.

- The methyl-directed mismatch repair system of E. coli efficiently repairs mismatches up to 1,000 bp from a hemimethylated GATC sequence.

Mismatch repair

- In bacteria, strand discrimination is based on the action of Dam methylase, methylates DNA at the N^6 position of all adenines within (5')GATC sequences.

- Immediately after passage of the replication fork, there is a short period (a few seconds or minutes) during which the template strand is methylated but the newly synthesized strand is not.

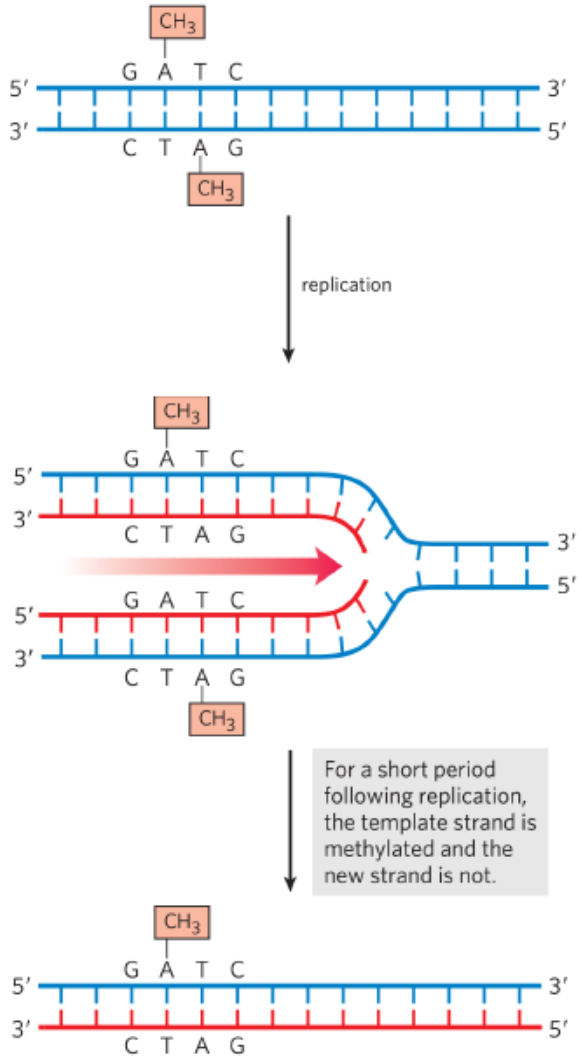
Mismatch repair

- The transient unmethylated state of GATC sequences in the newly synthesized strand permits the new strand to be distinguished from the template strand.

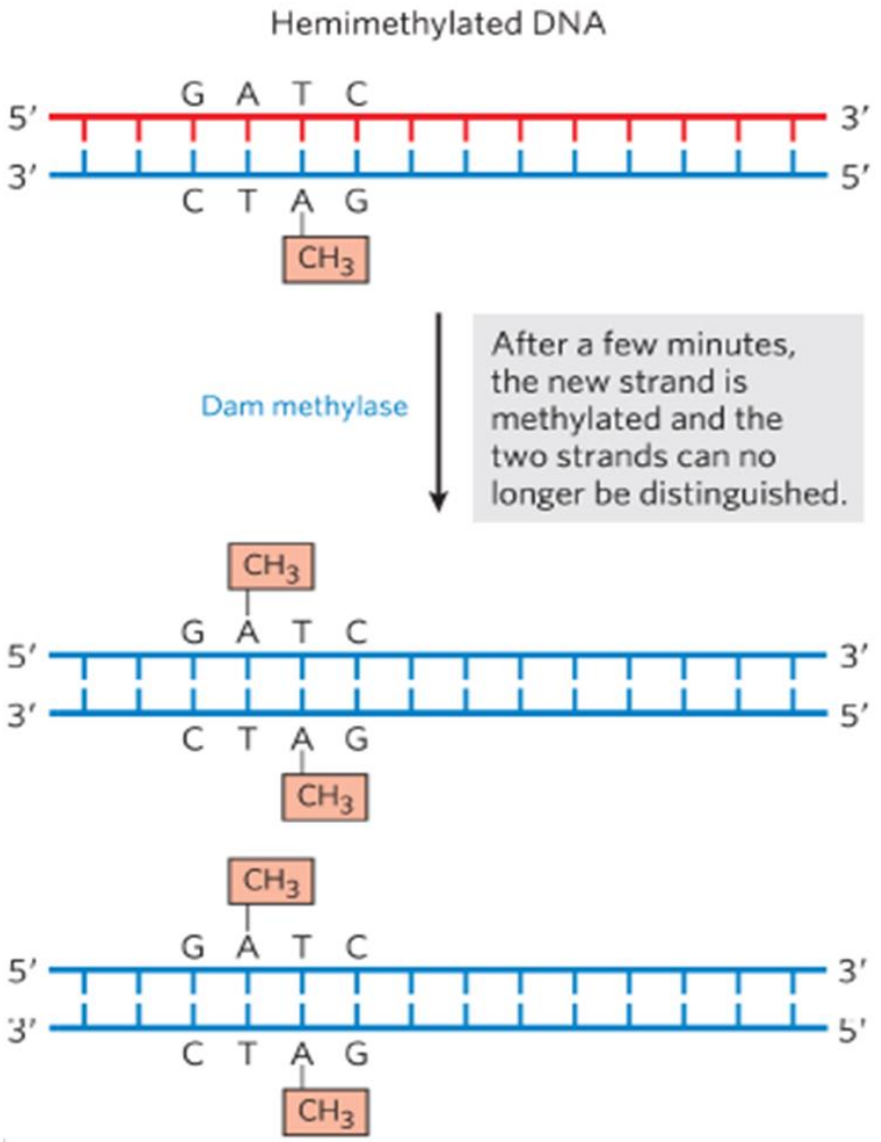
- Replication mismatches in the vicinity of a hemimethylated GATC sequence are then repaired according to the information in the methylated parent (template) strand.

Mismatch repair

- An endonuclease cuts the strand containing the mutation at GATC site adjacent to the defective site.
- An exonuclease then digests this strand from the site of the cut through the mutation, removing the mismatch area.
- A repair DNA polymerase then fills the gap and the DNA ligase seals the nick in the DNA.

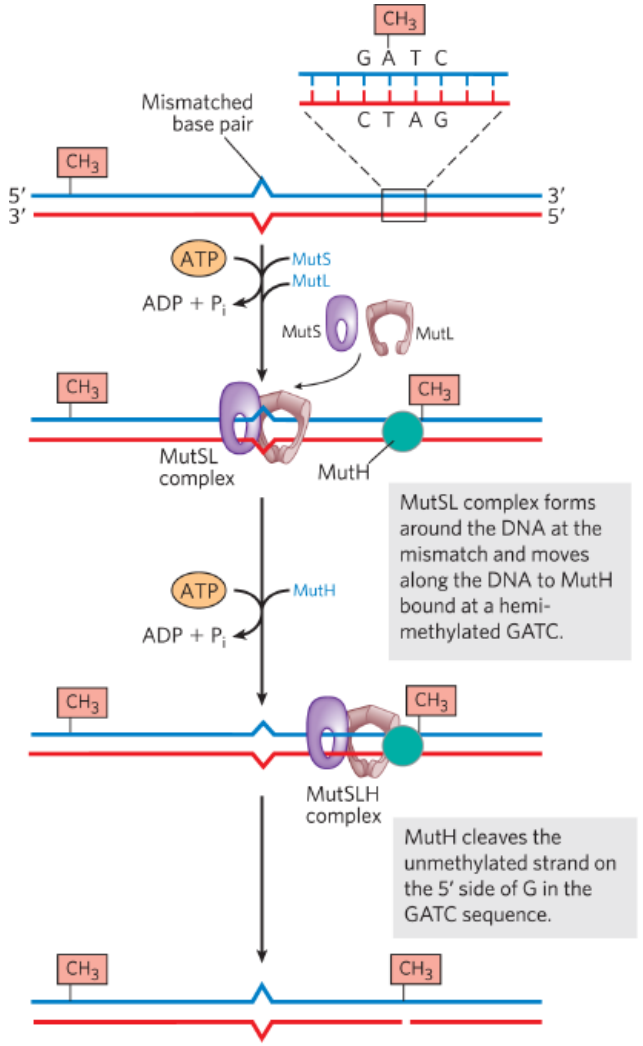


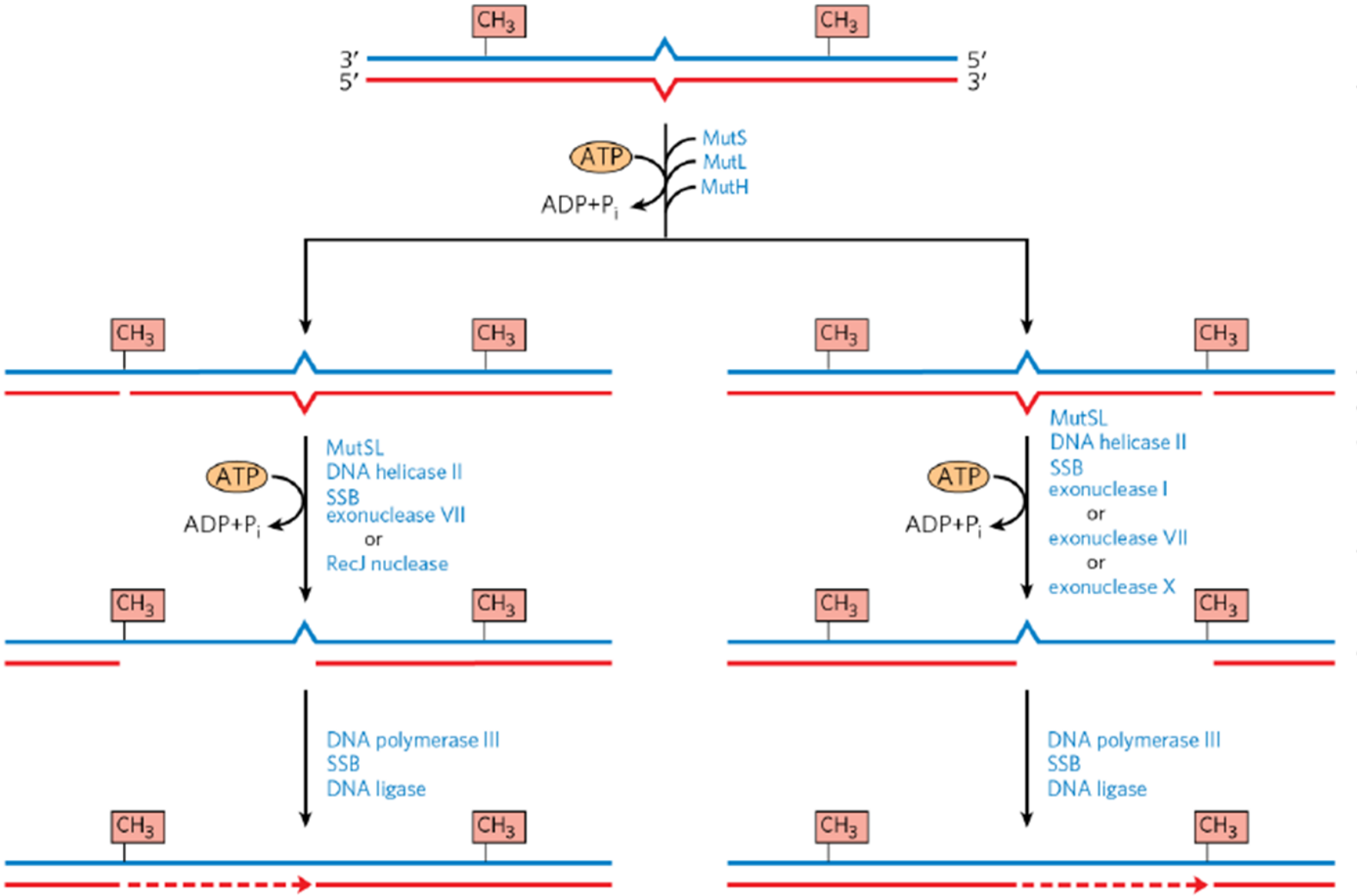
Hemimethylated DNA


Hemimethylated DNA
G A T C
5′
3′
3′
5′
C T A G
CH3
Dam methylase
After a few minutes, the new strand is methylated and the two strands can no longer be distinguished.
CH3
G A T C
5′
3′
3′
5′
C T A G
CH3
CH3
G A T C
5′
3′
3′
5′
C T A G
CH3

Mismatch repair

- **MutS** scans the DNA and forms a clamplike complex upon encountering a lesion. The complex binds to all mismatched base pairs.
- **MutL** protein forms a complex with MutS protein, and the MutSL complex slides along the DNA to find a hemimethylated GATC sequence.
- **MutH** binds to MutL, and the MutSLH complex moves in either direction at random along the DNA.
- MutH has a site-specific endonuclease activity that is inactive until the complex encounters a hemimethylated GATC sequence.
- At this site, MutH catalyzes cleavage of the unmethylated strand on the 5' side of the G in GATC, which marks the strand for repair.


CH3
G A T C
C T A G
Mismatched base pair
CH3
5′
3′
3′
5′
ATP
ADP + Pi
MutS
MutL
MutS
MutL
CH3
CH3
MutSL complex
MutH
MutSL complex forms around the DNA at the mismatch and moves along the DNA to MutH bound at a hemi-methylated GATC.
ATP
ADP + Pi
MutH
CH3
CH3
MutSLH complex
MutH cleaves the unmethylated strand on the 5′ side of G in the GATC sequence.
CH3
CH3


CH3
CH3
3′
5′
5′
3′
ATP
ADP+Pi
MutS
MutL
MutH
CH3
CH3
CH3
CH3
ATP
ADP+Pi
MutSL
DNA helicase II
SSB
exonuclease VII
or
RecJ nuclease
ATP
ADP+Pi
MutSL
DNA helicase II
SSB
exonuclease I
or
exonuclease VII
or
exonuclease X
CH3
CH3
CH3
CH3
CH3
CH3
CH3
CH3
DNA polymerase III
SSB
DNA ligase
DNA polymerase III
SSB
DNA ligase

Mismatch repair

- When the mismatch is on the 5' side of the cleavage site, the unmethylated strand is unwound and degraded in the 3′ → 5′ direction from the cleavage site through the mismatch, and this segment is replaced with new DNA.

- This process requires the combined action of DNA helicase II (also called UvrD helicase), SSB, exonuclease I or exonuclease X (both of which degrade strands of DNA in the 3′ → 5′ direction) or exonuclease VII (which degrades single-stranded DNA in either direction), DNA polymerase III, and DNA ligase.

Enzymes/proteins	Type of damage
Mismatch repair	
Dam methylase MutH, MutL, MutS proteins DNA helicase II SSB DNA polymerase III Exonuclease I Exonuclease VII RecJ nuclease Exonuclease X DNA ligase	Mismatches
Base-excision repair	
DNA glycosylases AP endonucleases DNA polymerase I DNA ligase	Abnormal bases (uracil, hypoxanthine, xanthine); alkylated bases; in some other organisms, pyrimidine dimers
Nucleotide-excision repair	
ABC excinuclease DNA polymerase I DNA ligase	DNA lesions that cause large structural change (e.g., pyrimidine dimers)
Direct repair	
DNA photolyases	Pyrimidine dimers
O^6-Methylguanine-DNA methyltransferase	O^6-Methylguanine
AlkB protein	1-Methylguanine, 3-methylcytosine

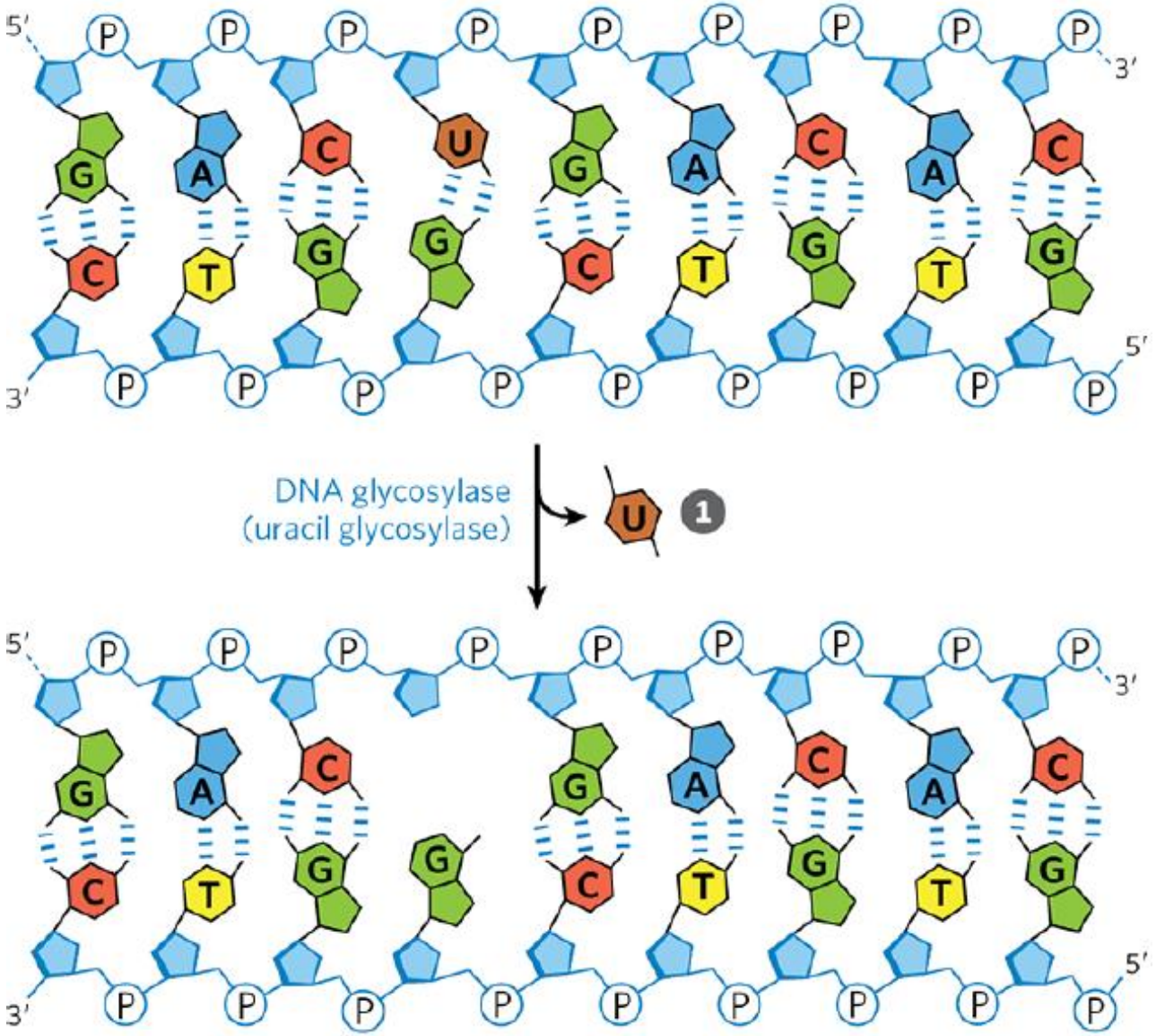
Base-Excision Repair

- Every cell has a class of enzymes called ***DNA glycosylases*** that recognize particularly common DNA lesions and remove the affected base by cleaving the **N-glycosyl bond**.
  - Lesions such as the products of cytosine and adenine deamination.

- **First step** involves only the removal of the base rather than an entire nucleotide.

- The cleavage creates an **apurinic** or **apyrimidinic** site in the DNA, commonly referred to as an AP site or abasic site.

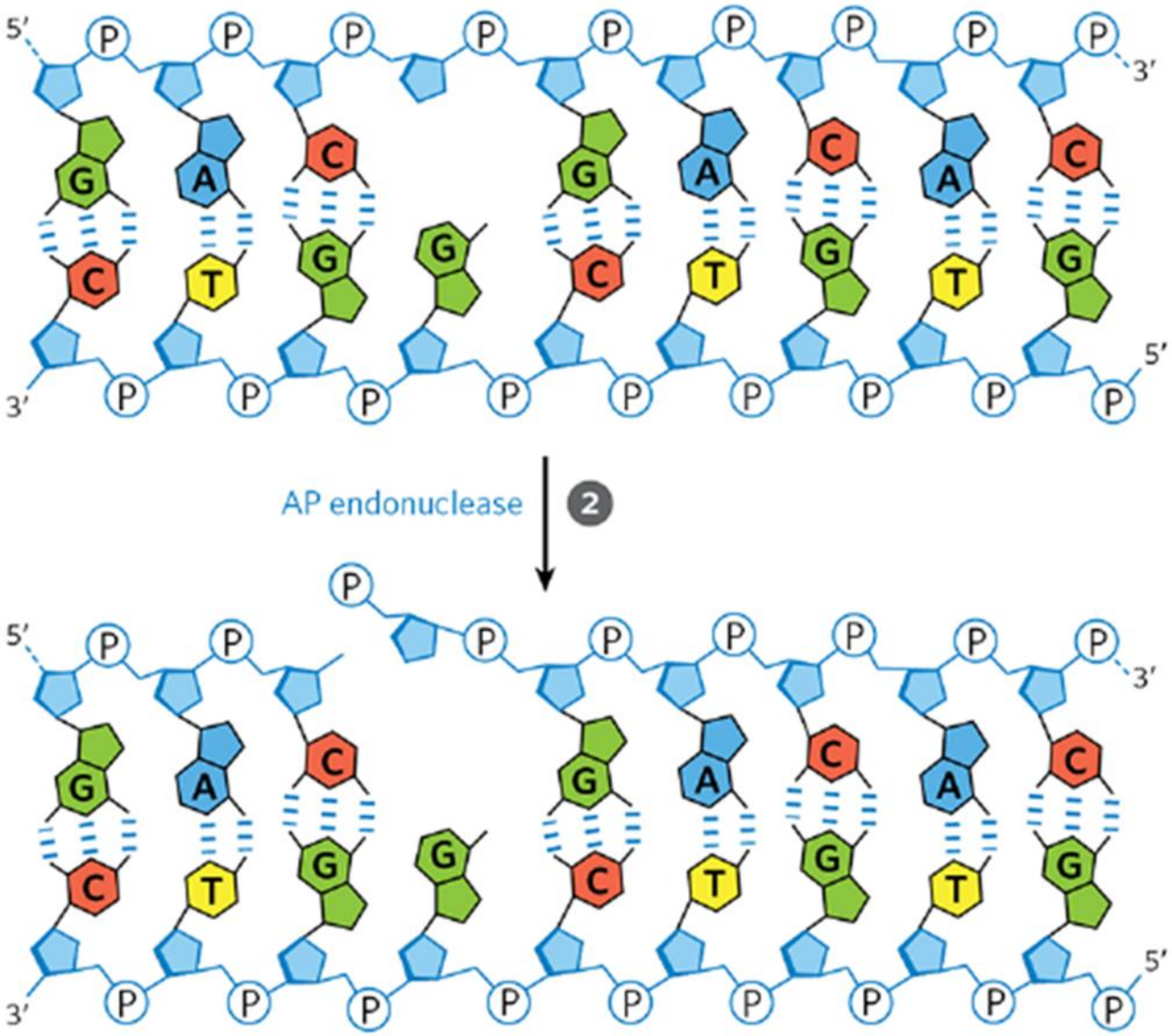
- Each DNA glycosylase is generally specific for one type of lesion.


5′
3′
P
G
A
C
U
T
DNA glycosylase
(uracil glycosylase)
1

Base-Excision Repair

The second step:

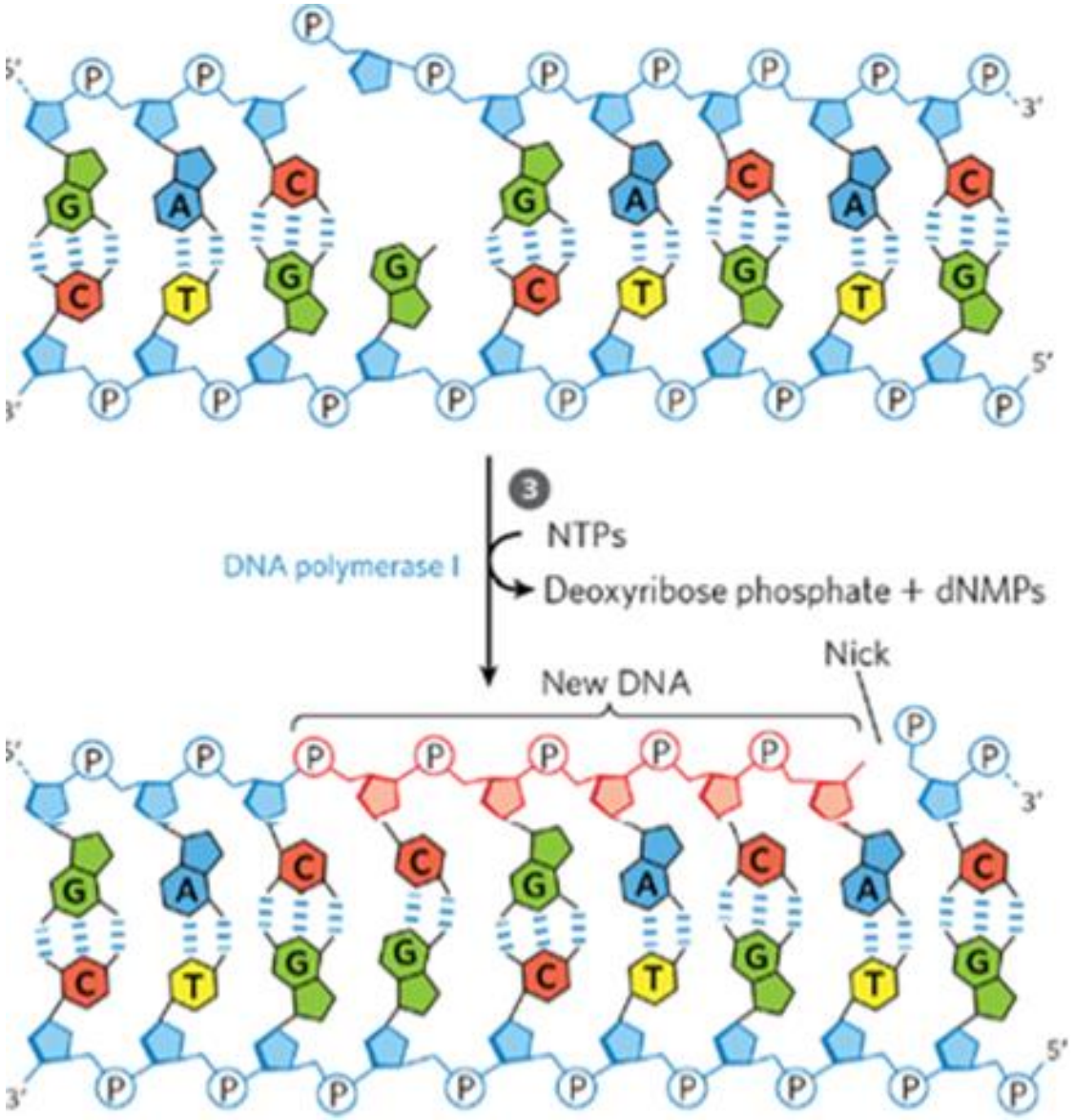
- Once an AP site has been formed by a DNA glycosylase, another type of enzyme must repair it.
- The deoxyribose 5'-phosphate left behind is removed and replaced with a new nucleotide.
- This process begins with one of the AP endonucleases, enzymes that cut the DNA strand containing the AP site.
- The position of the incision relative to the AP site (5' or 3′ to the site) depends on the type of AP endonuclease.


5′
3′
P
G
A
C
T
AP endonuclease
2

Base-Excision Repair

The third step:

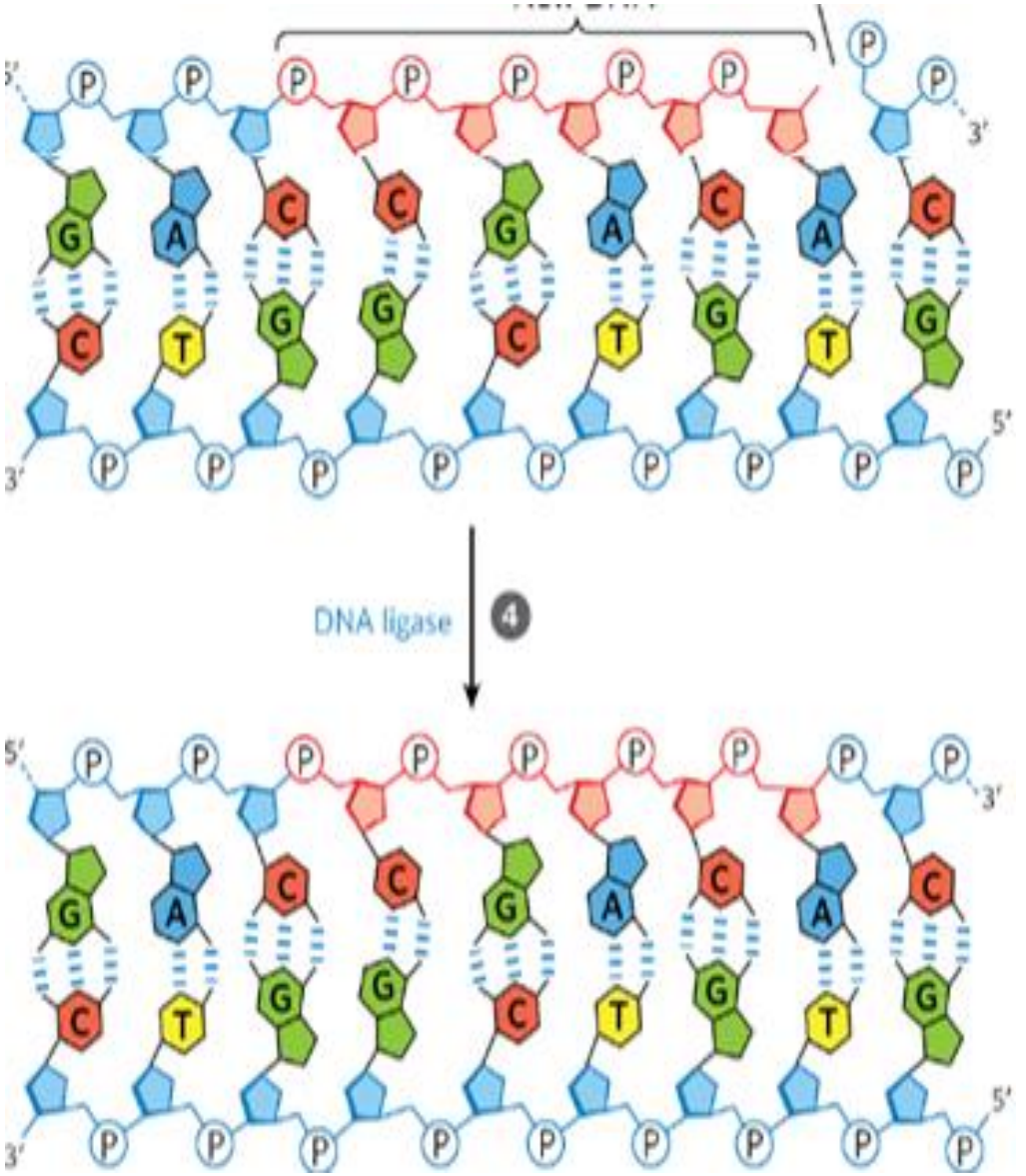
DNA polymerase I initiates repair synthesis from the free 3' hydroxyl at the nick, removing (with its 5′ → 3′ exonuclease activity) and replacing a portion of the damaged strand.


3
NTPs
DNA polymerase I
Deoxyribose phosphate + dNMPs
Nick
New DNA

Base-Excision Repair

The fourth step:

- The nick remaining after DNA polymerase I has dissociated is sealed by DNA ligase.


DNA ligase
4

Nucleotide-Excision Repair

- DNA lesions that cause large distortions in the helical structure of DNA generally are repaired by the **nucleotide-excision system**; a repair pathway critical to the survival of all free-living organisms.

- This mechanism is used to replace several damaged bases, up to 30 bases.

Nucleotide-Excision Repair

- In nucleotide-excision repair, a multisubunit enzyme **(excinuclease)** hydrolyzes **two phosphodiester bonds**, one on either side of the distortion caused by the lesion.

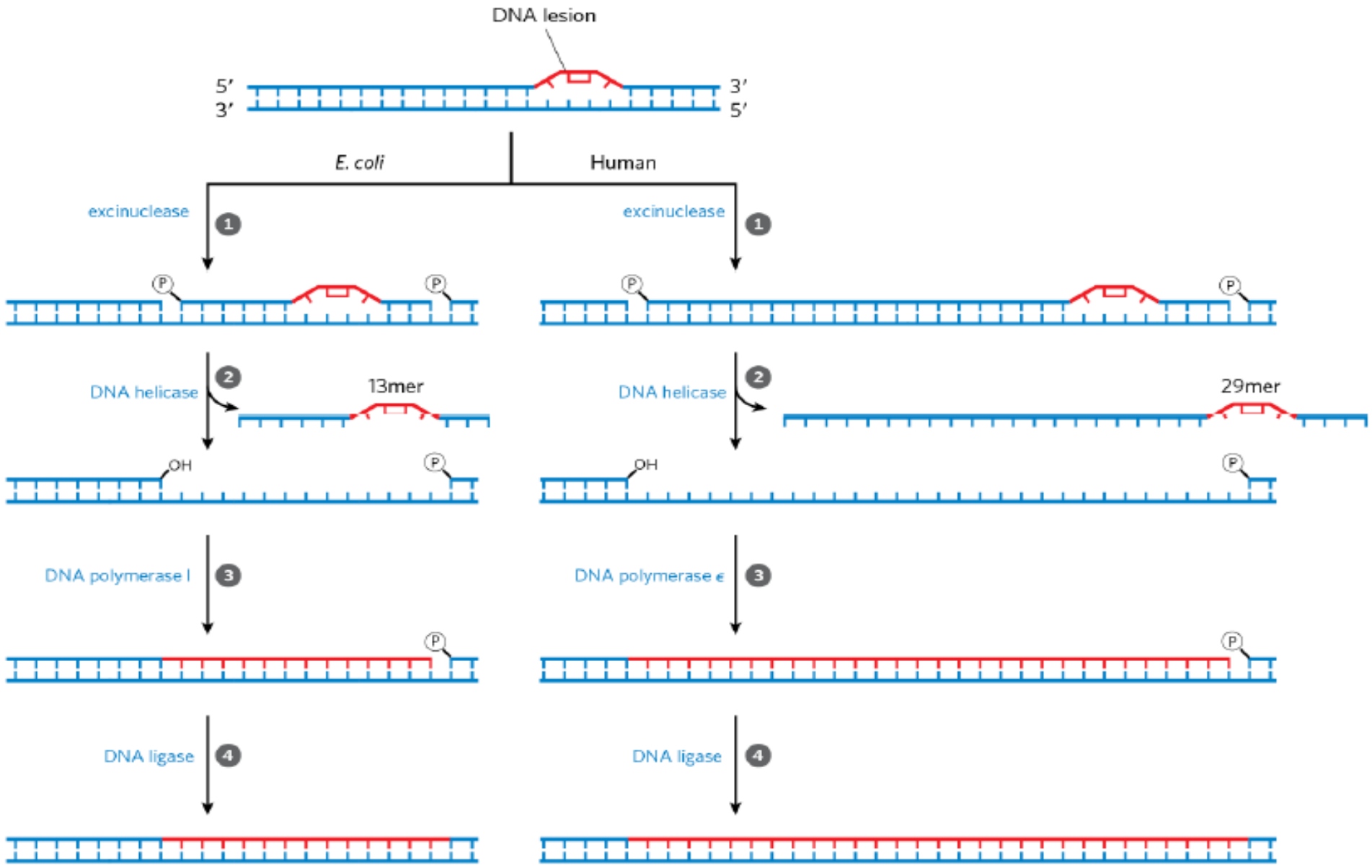
- In humans and other eukaryotes, the enzyme system hydrolyzes **the sixth phosphodiester bond on the 3' side** and the **twenty-second phosphodiester bond on the 5' side**, producing a fragment of 27 to 29 nucleotides.

- The DNA segment — of 13 nucleotides or 29 nucleotides — is removed with the aid of **a helicase**.

Nucleotide-Excision Repair

- Following the dual incision, **the excised oligonucleotides are released** from the duplex and **the resulting gap is filled** — by DNA polymerase I in E. coli and DNA polymerase ε in humans.

- **DNA ligase** seals the nick.


DNA lesion
5′
3′
3′
5′
E. coli
Human
excinuclease
1
excinuclease
1
P
P
P
P
DNA helicase
2
13mer
DNA helicase
2
29mer
OH
P
OH
P
DNA polymerase I
3
DNA polymerase ϵ
3
P
P
DNA ligase
4
DNA ligase
4

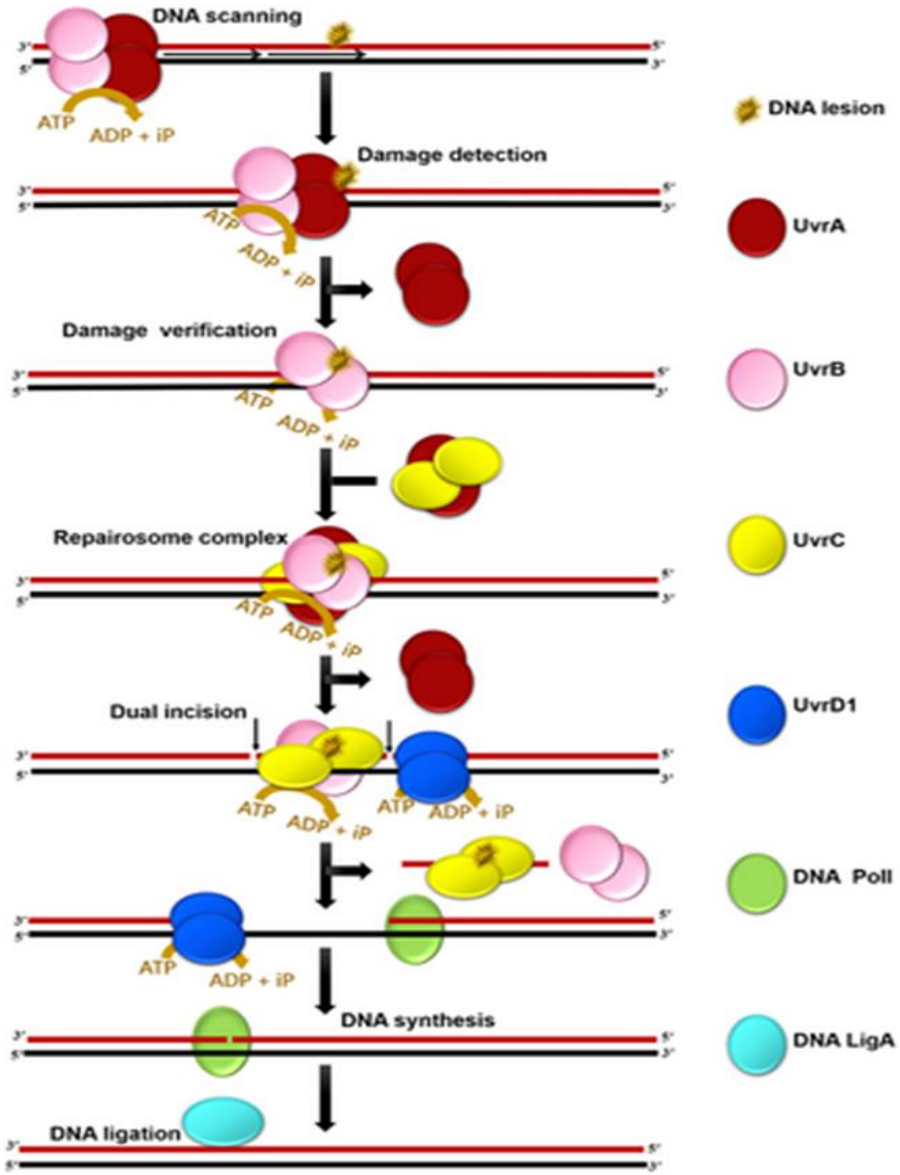
Nucleotide-Excision Repair

The key enzymatic complex is the **ABC excinuclease**, which has three protein components, UvrA, UvrB, and UvrC.

- Uvr stands for **ultraviolet light repair** of pyrimidine dimers and can be used to repair other types of damage (exposure to cigarette smoke).

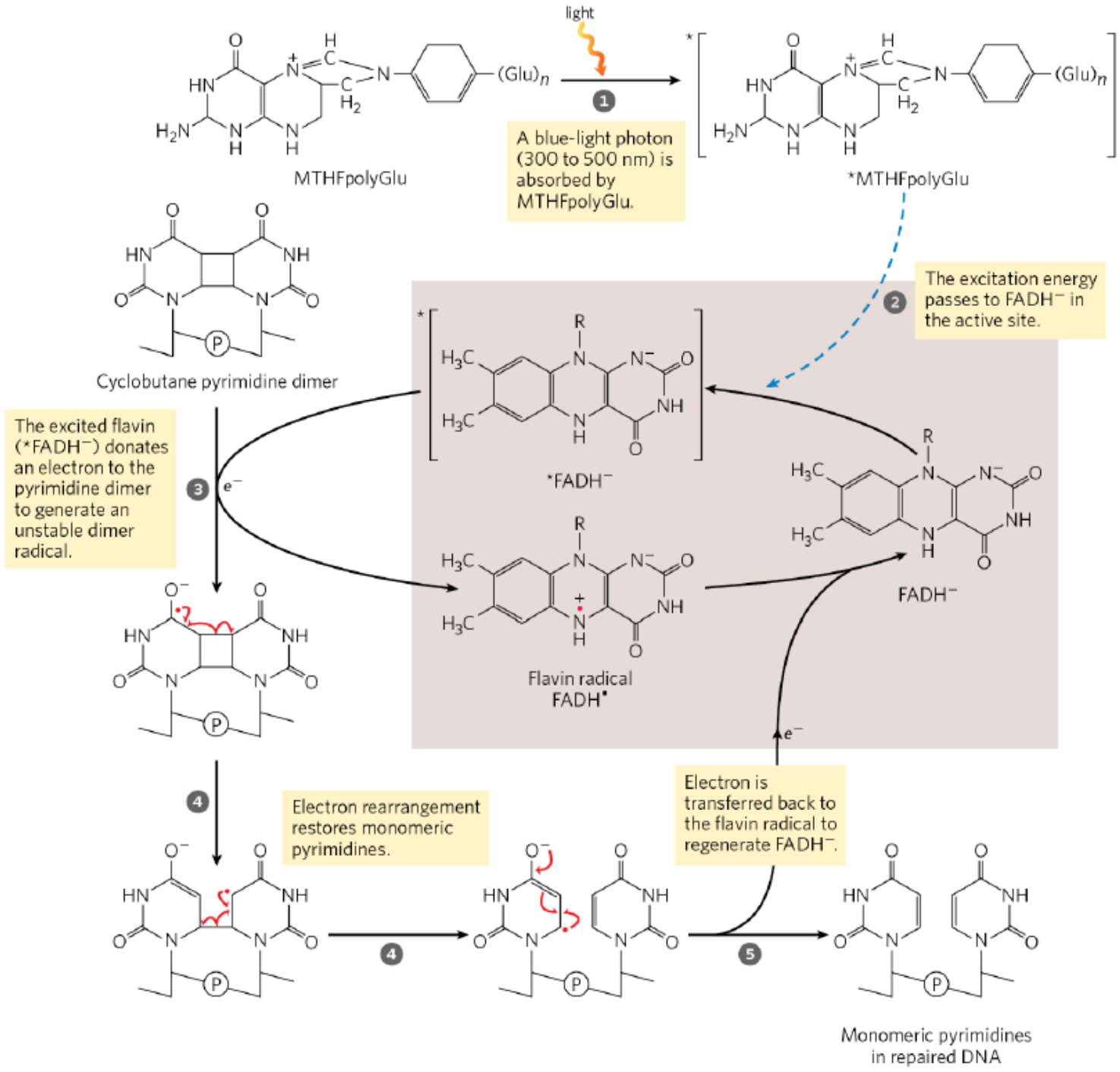
The term “excinuclease” is used to describe the unique capacity of this enzyme complex to catalyze two specific endonucleolytic cleavages, distinguishing this activity from that of standard endonucleases.

1. The dimeric UvrA protein (an ATPase) scans the DNA and binds to the site of a lesion.
2. A **UvrB protein can bind to UvrA** either before or after an encounter with the lesion.
3. At the lesion, the **UvrA dimer dissociates**, leaving a tight UvrB-DNA complex.
4. **UvrC protein then binds to UvrB**, and UvrB makes an incision at the fifth phosphodiester bond on the 3' side of the lesion.
5. This is followed by a UvrC-mediated incision at the eighth phosphodiester bond on the 5' side.
6. The resulting fragment, consisting of 12 to 13 nucleotides, **is removed by UvrD helicase.**
7. The short gap thus created is filled in by DNA polymerase I and DNA ligase.


DNA scanning
ATP
ADP + iP
Damage detection
Damage verification
Repairosome complex
Dual incision
DNA synthesis
DNA ligation
DNA lesion
UvrA
UvrB
UvrC
UvrD1
DNA PolI
DNA LigA

Direct Repair

- Several types of damage are repaired without removing a base or nucleotide.
- The best-characterized example is **direct photoreactivation of cyclobutane pyrimidine dimers**, a reaction promoted by DNA photolyases. Pyrimidine dimers result from a UV-induced reaction.
- Photolyases use energy derived from absorbed light to reverse the damage. Photolyases generally contain two cofactors that serve as light-absorbing agents, or chromophores: in all organisms, one is **FADH2**; in E. coli and yeast, the other is a **folate**.


light
MTHFpolyGlu
A blue-light photon (300 to 500 nm) is absorbed by MTHFpolyGlu.
*MTHFpolyGlu
The excitation energy passes to FADH⁻ in the active site.
Cyclobutane pyrimidine dimer
The excited flavin (*FADH⁻) donates an electron to the pyrimidine dimer to generate an unstable dimer radical.
*FADH⁻
FADH⁻
Flavin radical FADH•
Electron rearrangement restores monomeric pyrimidines.
Electron is transferred back to the flavin radical to regenerate FADH⁻.
Monomeric pyrimidines in repaired DNA

Diseases caused by defective DNA repair

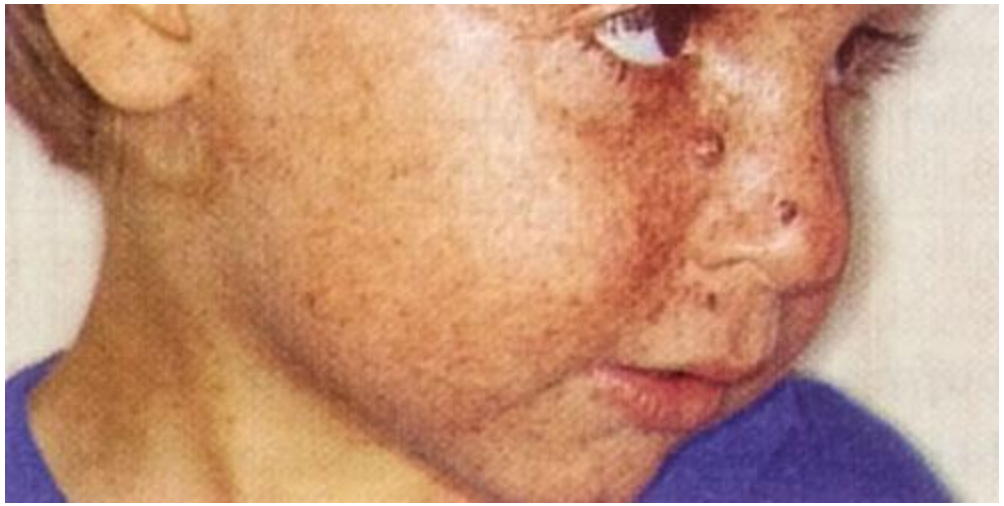
Hereditary non polyposis colon cancer (HNPCC)

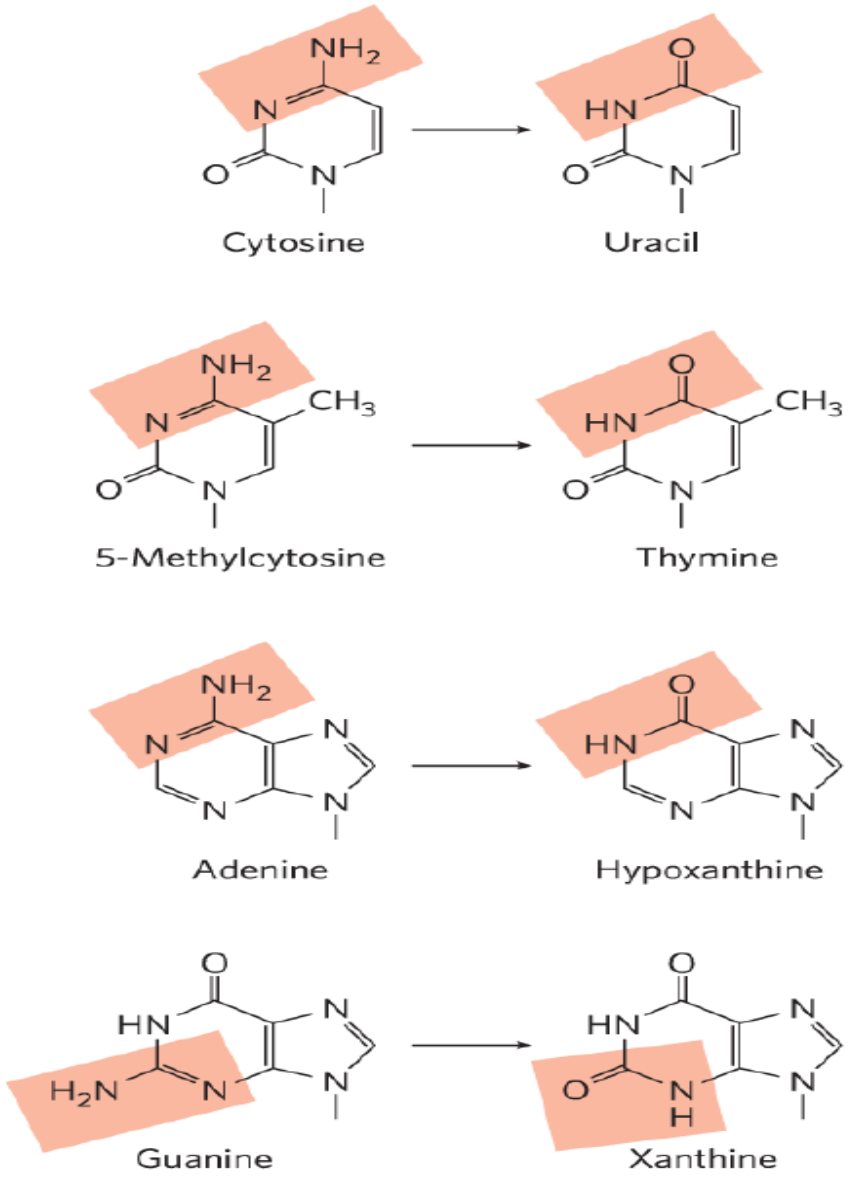
- This is one of the most common inherited cancers. It results from a mutation in a gene involved in mismatch repair.

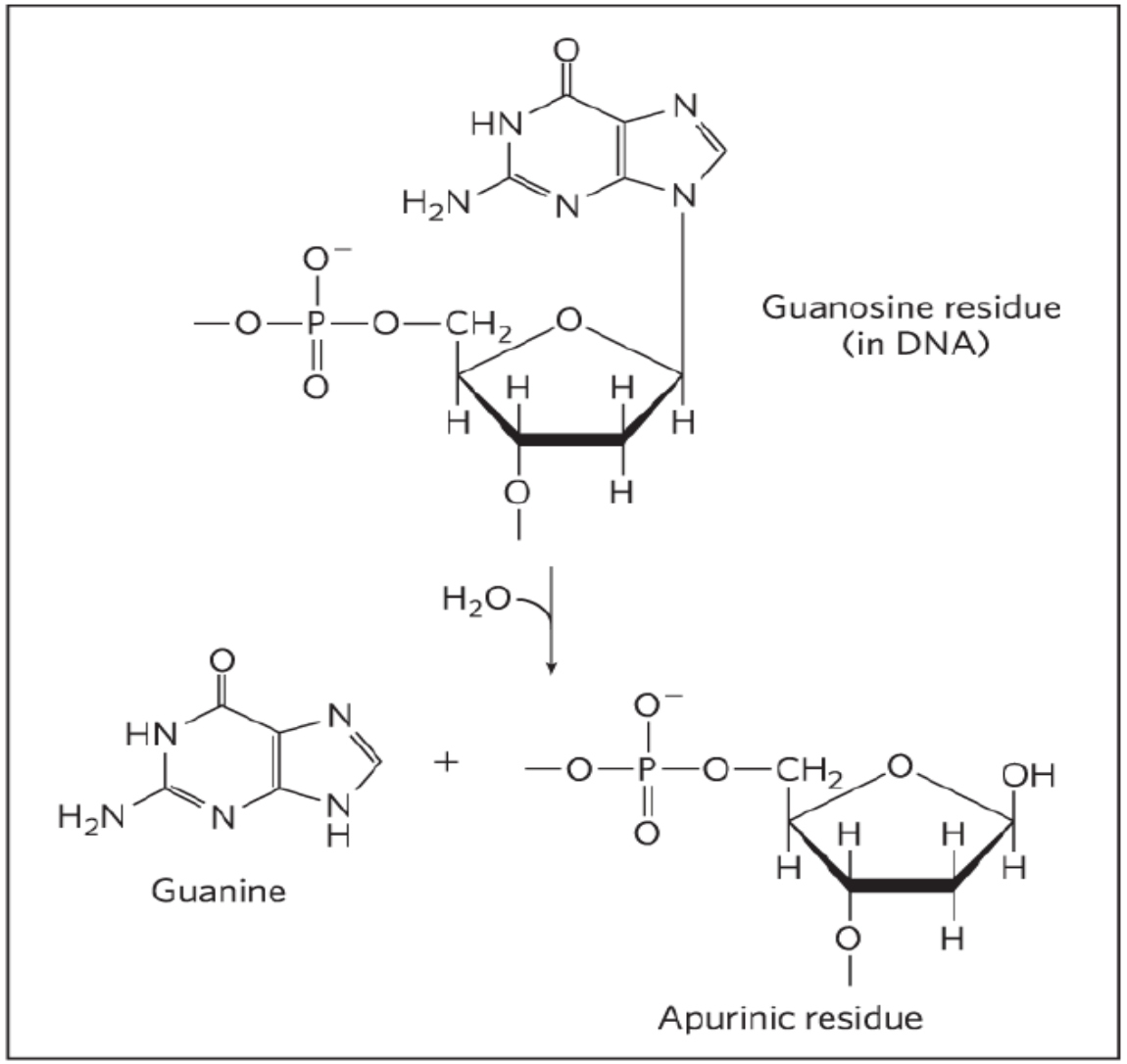
Xeroderma pigmentosum

- This is an autosomal recessive disease characterized by **sensitivity to ultraviolet light**, leading to skin damage, pigmentation, and multiple skin cancers It is caused by defective thymine-thymine dimer repair (nucleotide excision repair).

Xeroderma pigmentosum

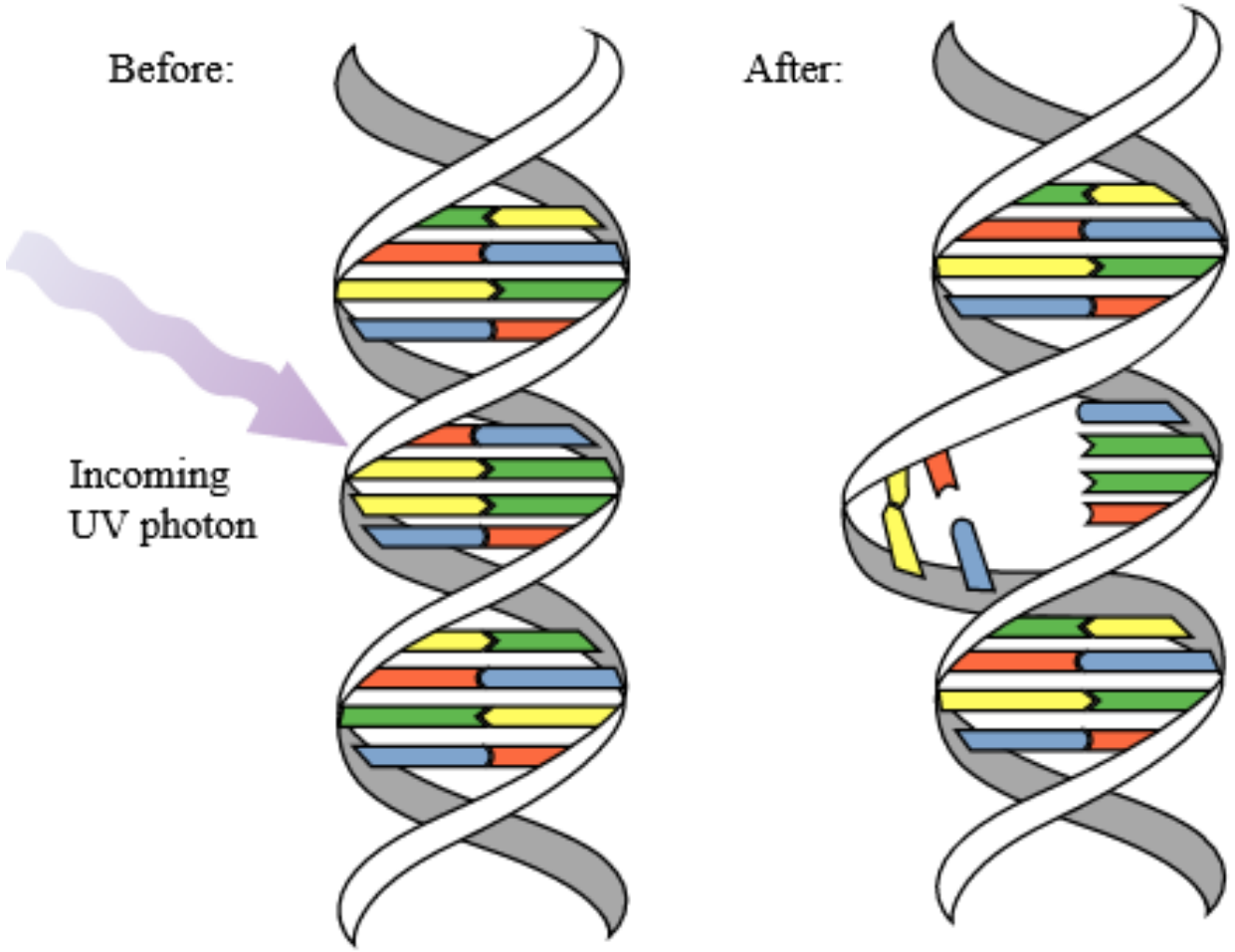


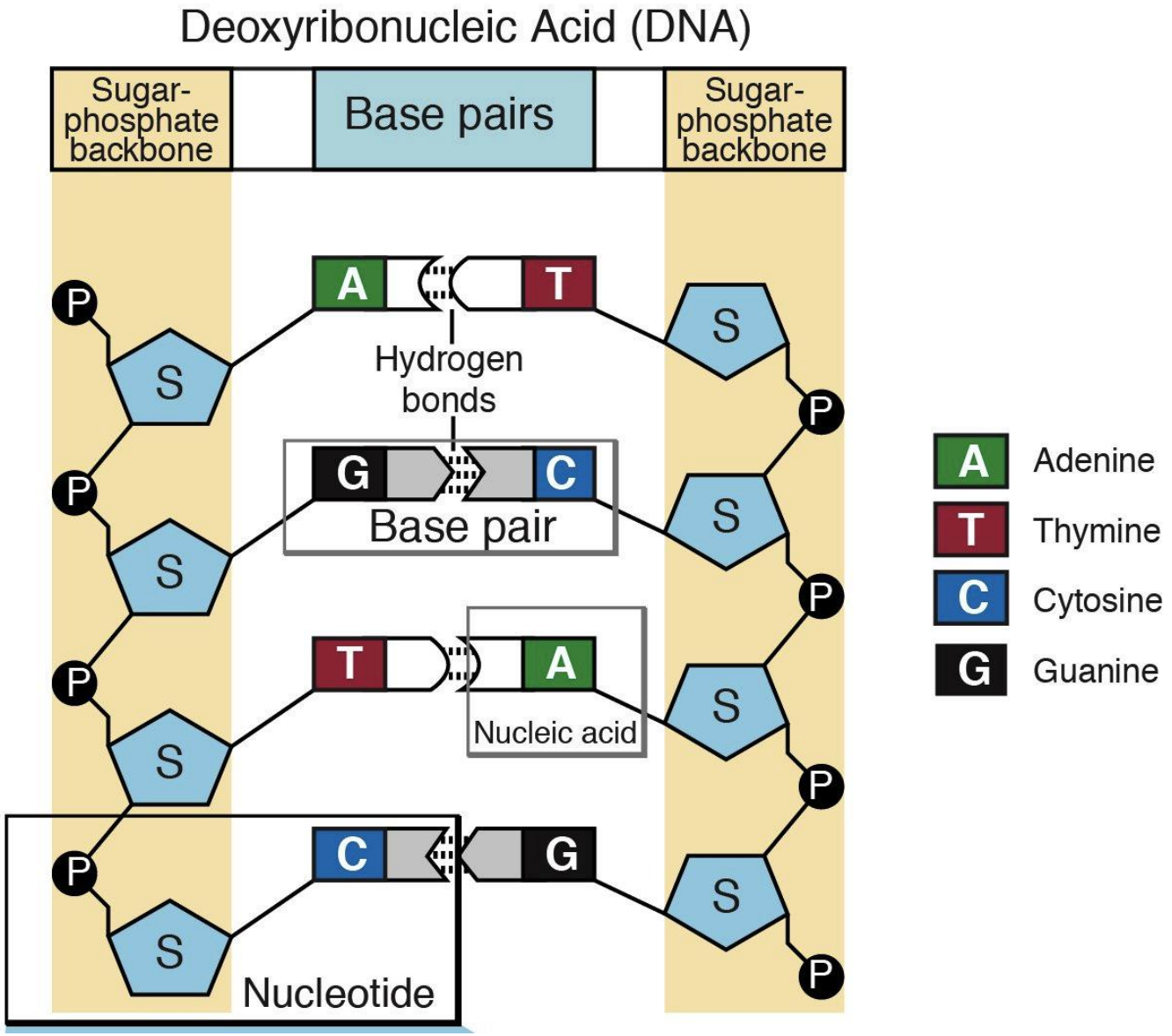

NH2
N
O
N
Cytosine
O
HN
O
N
Uracil
NH2
N
CH3
O
N
5-Methylcytosine
O
HN
CH3
O
N
Thymine
NH2
N
N
N
Adenine
O
HN
N
N
Hypoxanthine
O
HN
N
H2N
N
Guanine
O
HN
N
O
N
H
Xanthine



(b) Depurination

Pyrimidine dimers




Deoxyribonucleic Acid (DNA)
Sugar-phosphate backbone
Base pairs
Sugar-phosphate backbone
A
T
Hydrogen bonds
G
C
Base pair
T
A
Nucleic acid
C
G
Nucleotide
P
S
Adenine
Thymine
Cytosine
Guanine