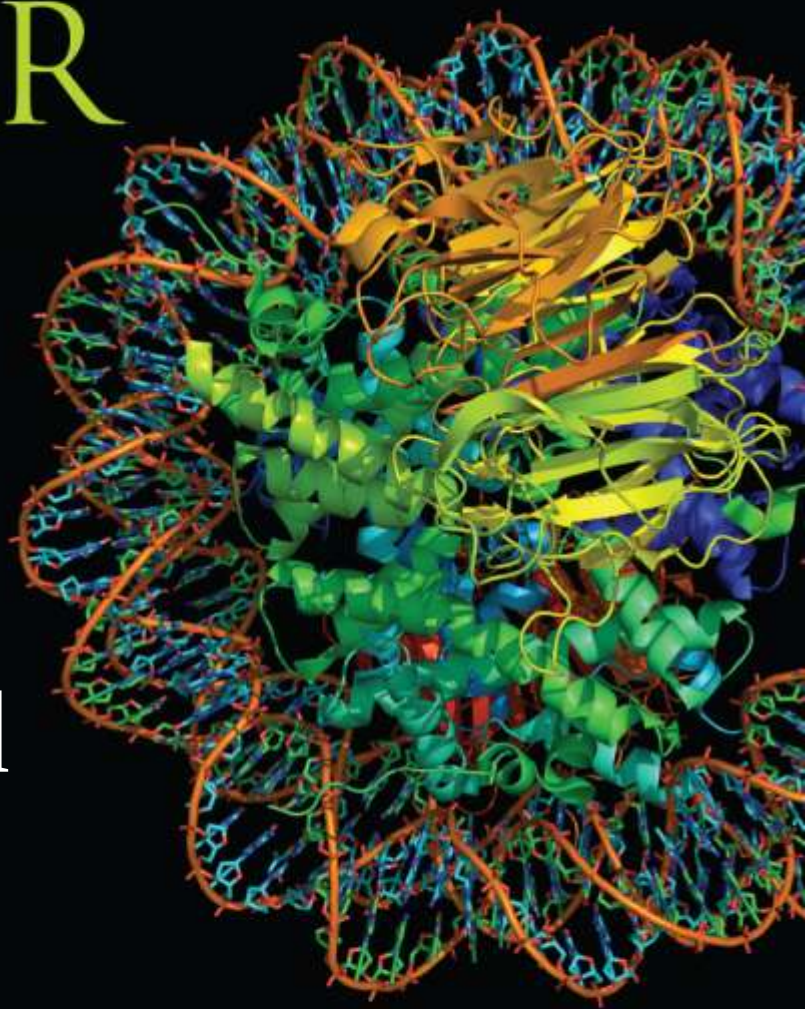
Principles of

MOLECULAR BIOLOGY

BURTON E. TROPP



Chapter 9
DNA Damage and Repair

DNA Damage and Repair

- DNA damage is a common occurrence
  - Cells require a restoration process
    - **DNA repair**
- DNA damage can come from
  - **Endogenous agents -** formed inside the cell
  - **Exogenous agents -** come from the surrounding environment

Radiation Damage

- Exposure to high energy electromagnetic radiation can cause considerable DNA damage
  - UV Light
  - Gamma Rays
  - X-Rays

Radiation Damage

- 2 major types of pyrimidine dimers account for nearly all UV-induced damage
  - **Cyclobutane pyrimidine dimers**
    - Most common are thymine-thymine dimers
  - **The (6-4) photoproduct**
    - causes a major distortion in B-DNA

Radiation Damage

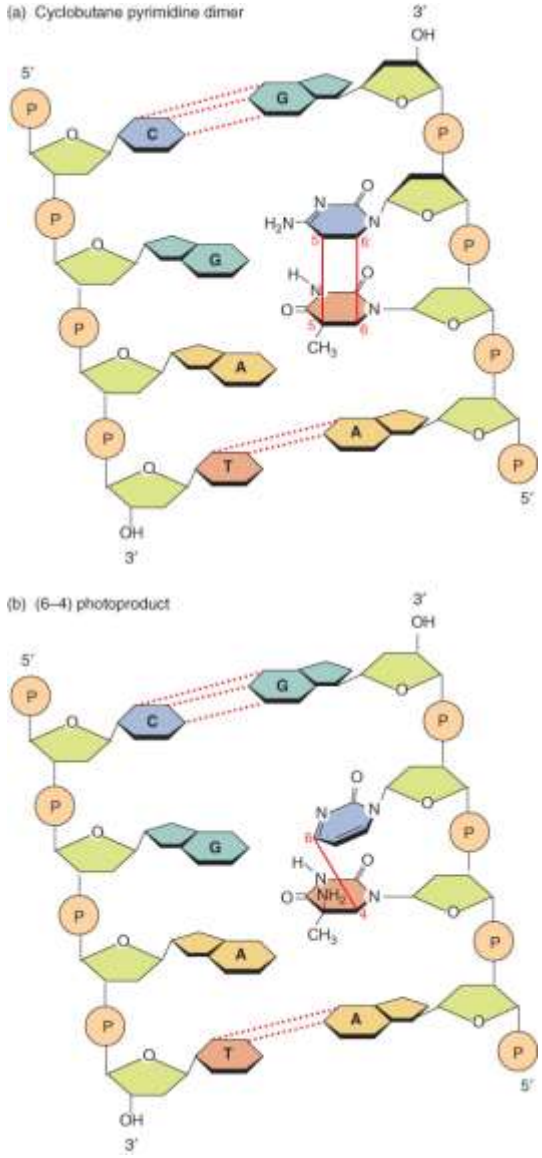


Figure 09.01: Ultraviolet light promoted cyclobutane pyrimidine dimer and (6-4) photoproduct formation.

(Adapted from Friedberg, E. C., et al. 2005. DNA Repair and Mutagenesis (2nd ed). ASM Press.)

Radiation Damage

X-rays and gamma rays cause many different types of DNA damage

- **Direct damage**
  - DNA or water tightly bound to it absorb the radiation
- **Indirect damage**
  - Water molecules surrounding DNA absorb the radiation and generate reactive species (free radicals)

Radiation Damage

- Lesions may be isolated or clustered
  - Clustered lesions
    - Double-stranded breaks can cause a variety of chromosomal aberrations
      - Translocations
      - Inversions

Radiation Damage

- ≈65% of the DNA damage caused by x-rays and γ-rays is due to indirect effects
  - Formation of 3 highly reactive chemical species
    - $H2O^{\bullet+}$ (water radical cation)
    - •OH (hydroxide radical)
    - O_2^- (superoxide)

DNA Instability in Water

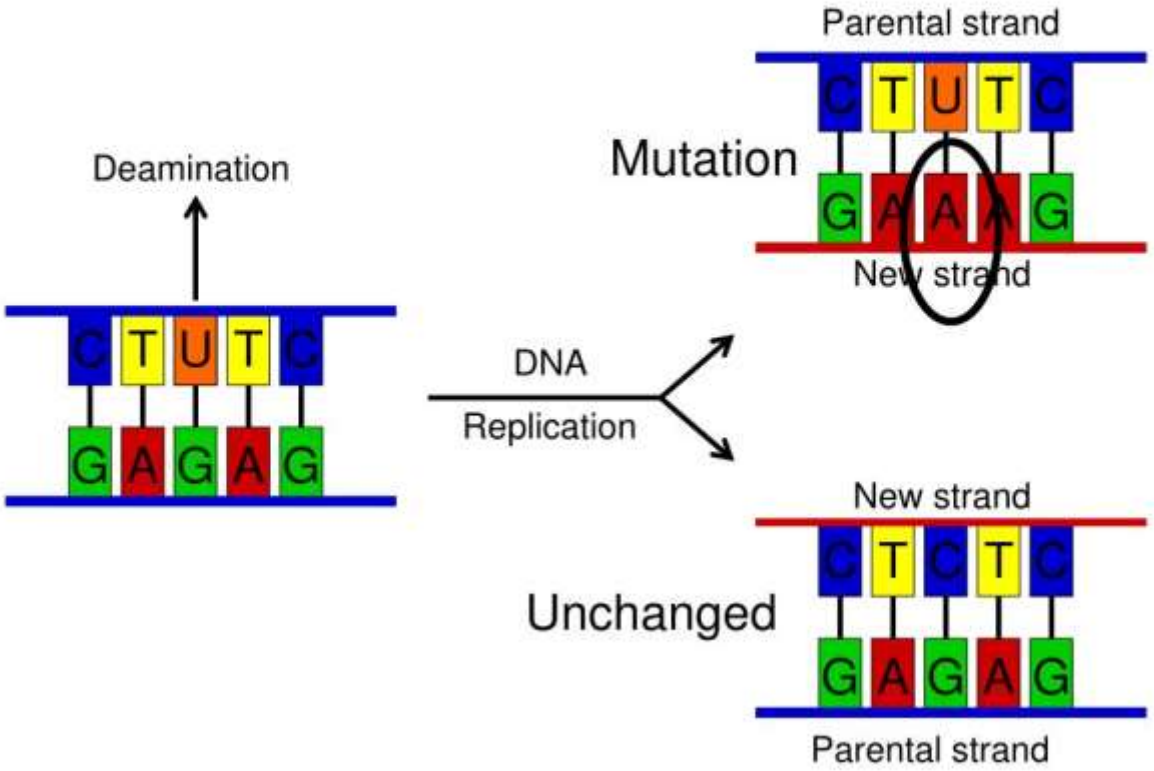
DNA is damaged by hydrolytic cleavage reactions

- DNA has 3 kinds of bonds sensitive to hydrolytic cleavage
  1. Phosphodiester bonds
  2. N-glycosyl bonds
  3. Bonds linking amine groups to the rings in C,A and G (deamination)

DNA Instability in Water

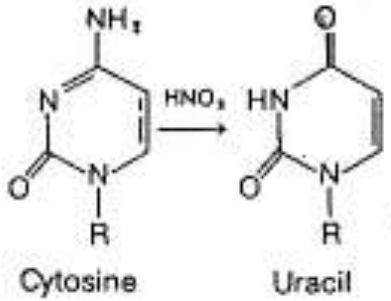
- Phosphodiester bond breakage is rare and probably not significant
- N-glycosyl bond cleavage forms an **abasic site**
  - Loss of information (no base identifier)
- **Deamination**
  - Gives rise to
    - Transition mutations
    - Transversion mutations

FAILURE TO REPAIR A DEAMINATED BASE = A POINT MUTATION

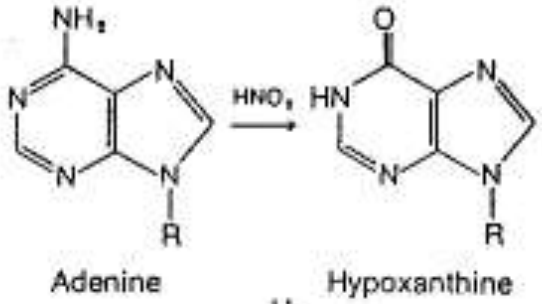


Chemical mutagens

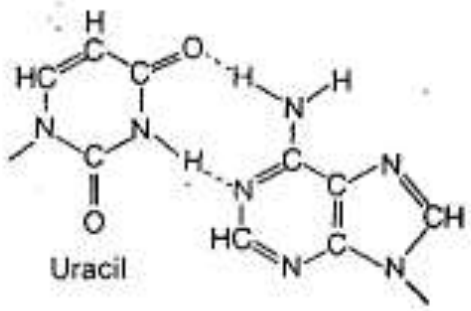
Deamination by nitrous acid



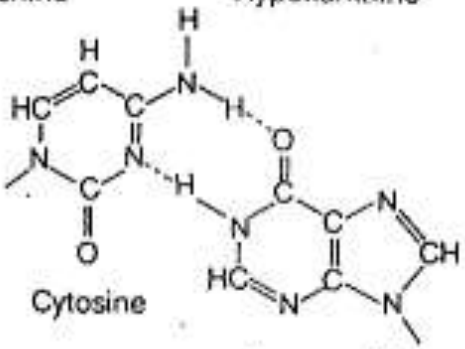
Cytosine Uracil



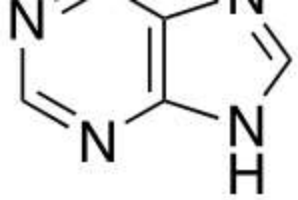
Adenine Hypoxanthine



Uracil Adenine

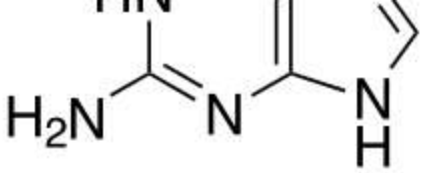


Cytosine Hypoxanthine



adenine

purines



guanine

A

Transitions

G

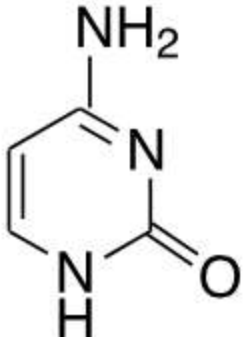
Transversions

Transversions

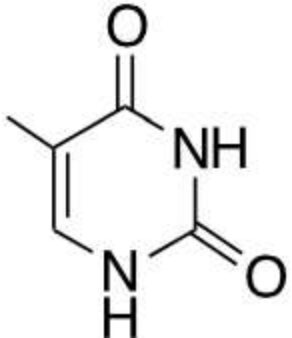
C

Transitions

T



pyrimidines



DNA Instability in Water

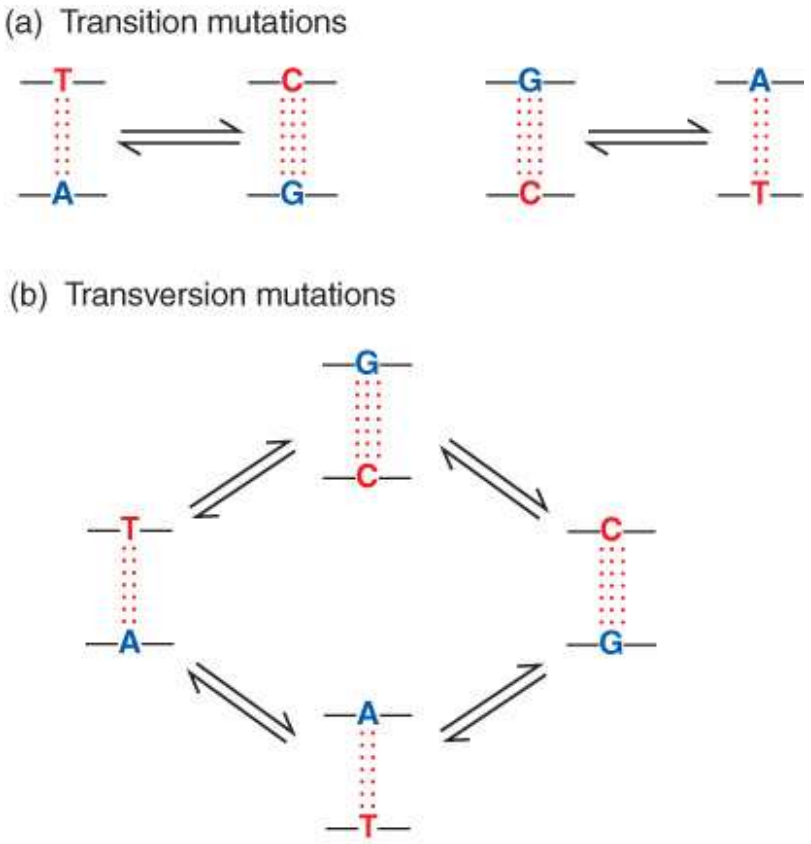


Figure 09.05: Transition and transversion mutations. (a) A transition mutation. (b) A transversion mutation.

Oxidative Damage

Reactive oxygen species damage DNA

- •OH (hydroxide radical)
  - Generated by ionizing radiation or cellular H_2O_2
  - 8-oxyguanine: oxoG-A >T-A transversion
  - Can produce cytotoxic mutations: thymine glycol > inhibit replication

Alkylation Damage by Monoadduct Formation

- DNA is readily attacked by electron seeking chemicals termed electrophiles
  - Alkylating agents: electrophiles that transfer methyl, ethyl or larger alkyl groups to DNA
    - The product formed is called an adduct

Alkylation Damage by Monoadduct Formation

- Many environmental agents become active alkylating agents after they have been metabolized
  - Commonly have 2 or more fused aromatic rings
  - Only damage DNA after being metabolized
  - Requires Cytochrome P450

Chemical Cross-Linking Agents

Many alkylating agents have 2 reactive sites and can form intrastrand or interstrand cross-links

- Interstrand crosslinks prevent strand separation and are lethal
- Crosslinking agents are often used as chemotheraputics
  - Nitrogen Mustard Gas
  - Cisplatin

Chemical Cross-Linking Agents

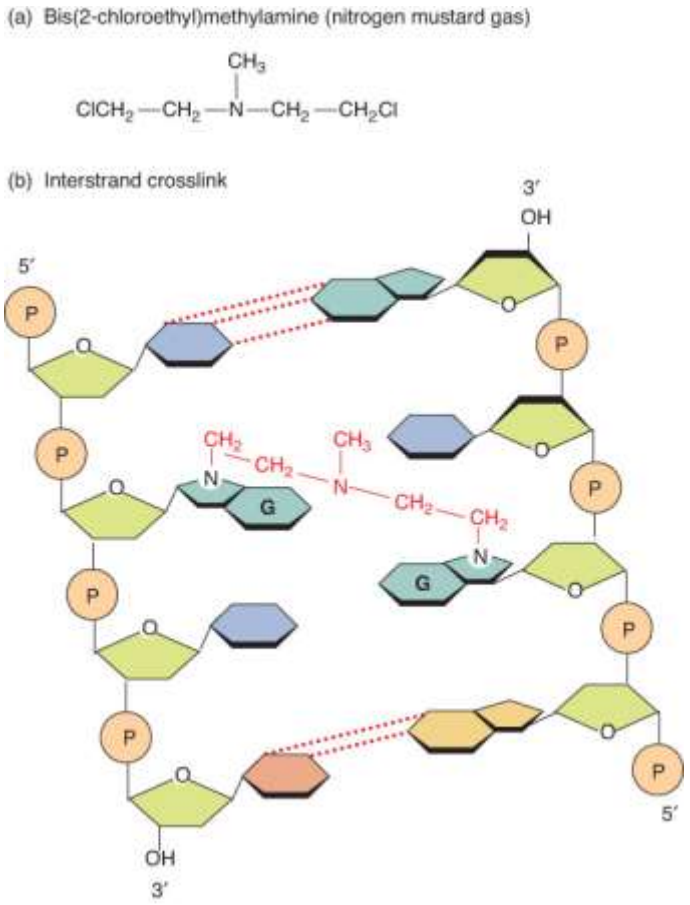


Figure 09.13: Nitrogen mustard gas, an agent that causes crosslink formation.

(Part b adapted from Friedberg, E. C., et al. 2005. DNA Repair and Mutagenesis [2nd ed]. ASM Press.)

Direct Reversal of Damage

Photolyase reverses damage caused by cyclobutane pyrimidine dimer formation

- Early observations suggested that UV damage in bacterial DNA could be repaired by exposure to visible light **- photoreactivation**
  - UV irradiation induces cyclobutane dimer formation
  - Photoreactivation reverses this
  - Energy provided by blue light (350-450nm)
    - Cyclobutane pyrimidine dimer photolyase

Direct Reversal of Damage

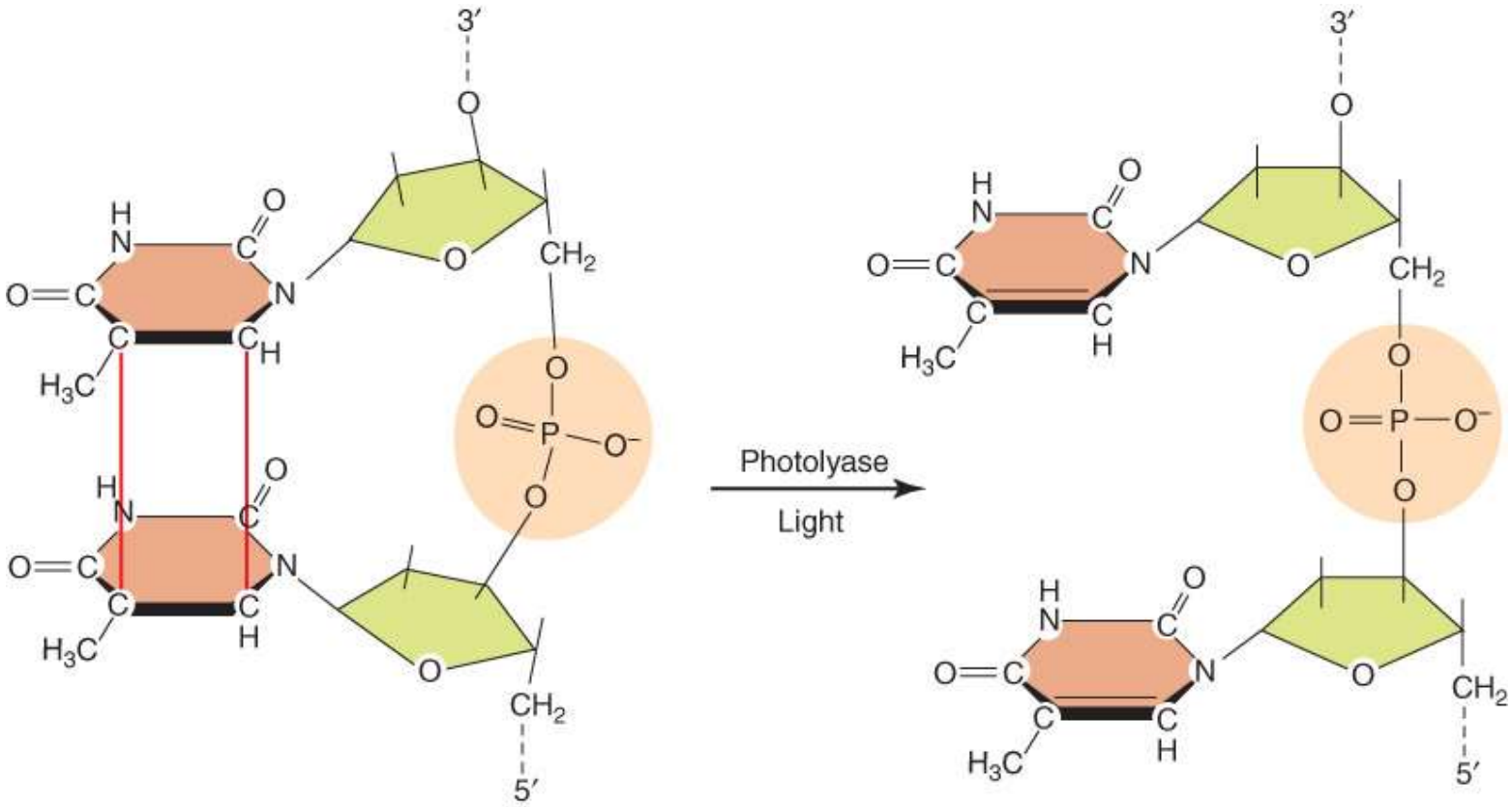


Figure 09.14: Photolyase catalyzes a light driven reaction that disrupts the cyclobutane ring in cyclobutane pyrimidine dimers, reversing the effect of UV irradiation.

Direct Reversal of Damage

(6-4) **photolyase** catalyzes the reaction shown here

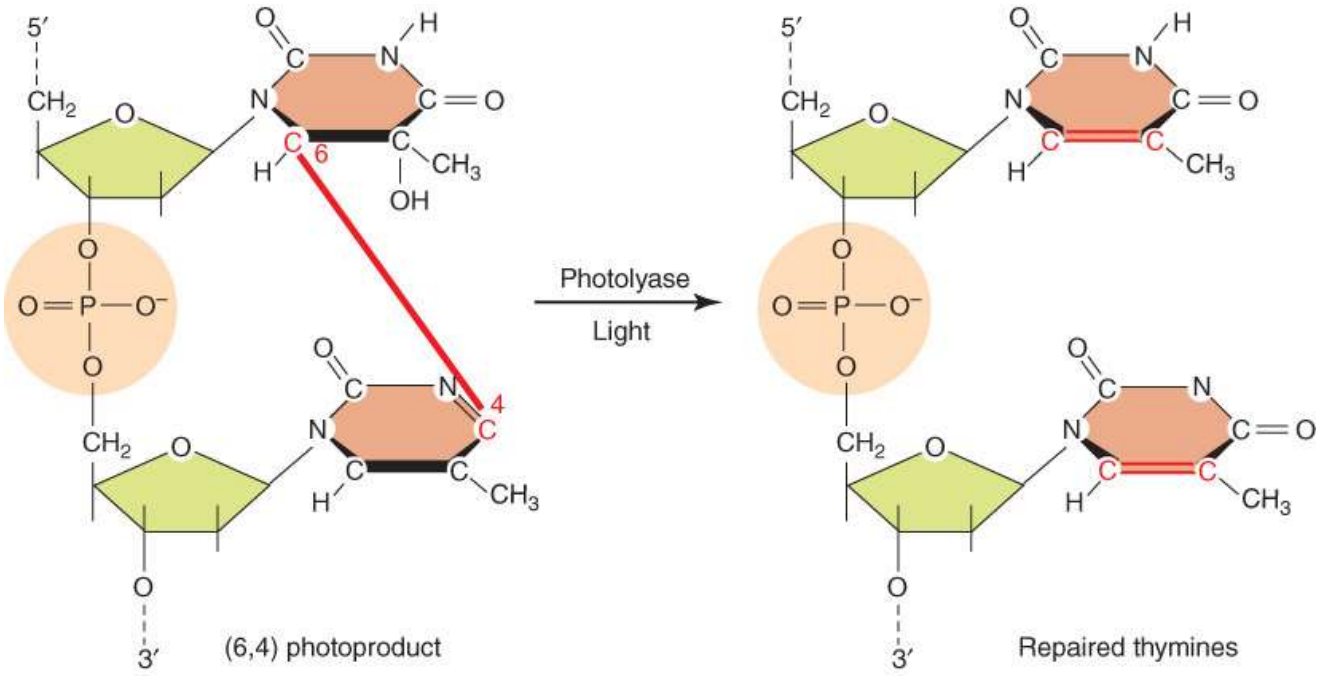


Figure 09.15: Reaction catalyzed by the (6-4) photolyase.

Direct Reversal of Damage

- Another means of direct damage reversal is **dealkylation**
  - **O^6-alkylguanine DNA alkyltransferase I** can remove methyl groups attached to O-6 in guanine
  - Enzyme loses activity after acting only 1 time
    - Suicide enzyme
    - Methylation of the enzyme converts it to a transcriptional activator of itself and other DNA repair systems
  - Human alkylguanine DNA alkyltransferase is of great interest in tumor cell biology

Base Excision and Repair

The base excision and repair pathway removes and replaces damaged or inappropriate bases

- Damage that cannot be repaired by a single enzyme reversal must rely on a multistep pathway **- base excision repair**

Base Excision and Repair

Base excision derives its name from the 1st step of N-glycosyl bond cleavage that forms an abasic site

- Cells must use different enzymes
  - Some are monofunctional DNA glycosylases
  - Others have additional AP lyase function that cleaves the bond between the sugar and the phosphate 3’ to the damaged site

Base Excision Repair

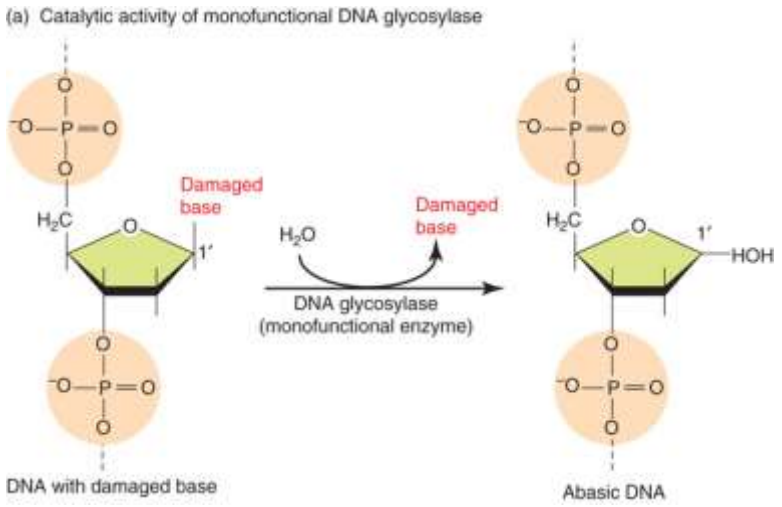


Figure 09.17A: Monofunctional and bifunctional DNA glycosylases. (a) Monofunctional DNA glycosylases excise a damaged base.

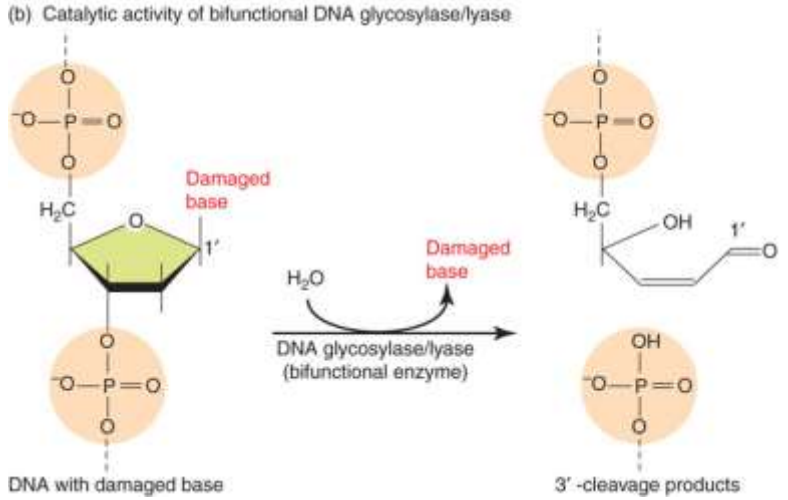


Figure 09.17B: Monofunctional and bifunctional DNA glycosylases. (b) Bifunctional DNA glycosylases also have an AP lyase activity.

Base Excision Repair

Base excision pathway can be divided into 2 stages

1. Base excision and chain cleavage
   - DNA glycosylase excises the damaged base
   - AP endonuclease hydrolyzes the phosphodiester bond
2. Nucleotide replacement and ligation
   - 2 subpathways
     - Short patch repair
     - Long patch repair

Base Excision Repair

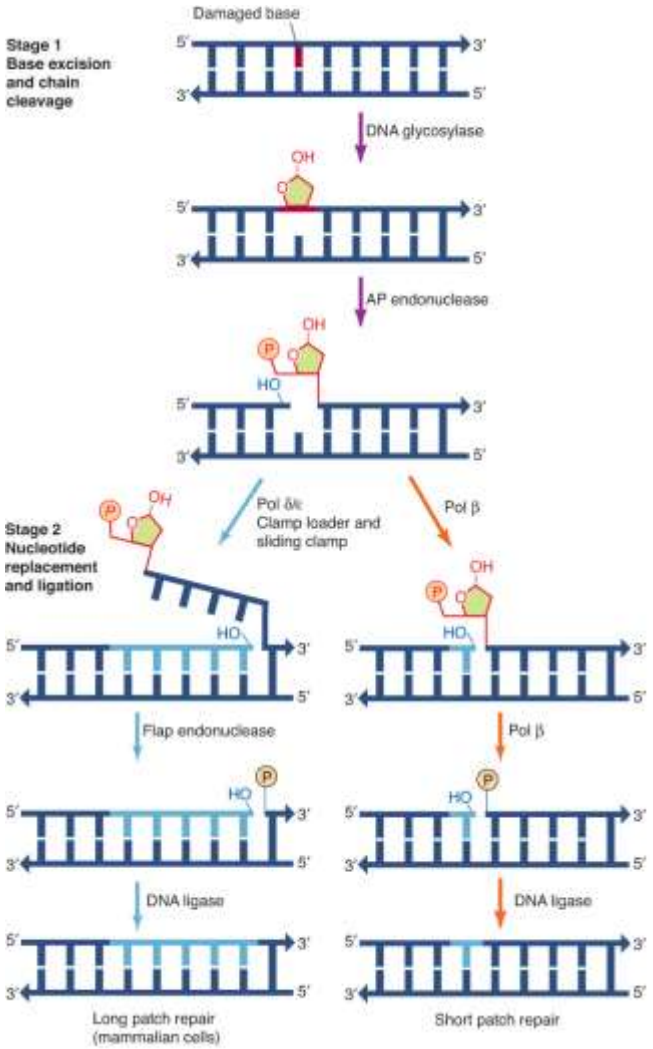


Figure 09.18: Base excision repair in eukaryotes starting with a monofunctional DNA glycosylase.

(Adapted from Schaerer, O. D. 2003. Angew Chem Int Ed Engl 34:2946–2974.)

Base Excision Repair

- **Short patch repair**
  - Replaces only one nucleotide
  - Uses DNA polymerase β
  - DNA ligase completes the repair
- **Long patch repair**
  - Replaces 2 or more nucleotides
  - Uses DNA polymerase δ or ε
  - Flap endonuclease cleaves the displaced strand
  - DNA ligase seals the nick

Nucleotide Excision Repair

Nucleotide excision repair removes bulky adducts from DNA by excising an oligonucleotide bearing the lesion

- Damage recognition
- Cutting DNA on each side of the lesion
- Excision of the oligonucleotide
- Synthesis of new DNA using undamaged strand as template
- Ligation of the remaining nick

Nucleotide Excision Repair

- Eukaryotes have a similar repair system
- Individuals with a defect in the pathway suffer from xeroderma pigmentosum

Nucleotide Excision Repair

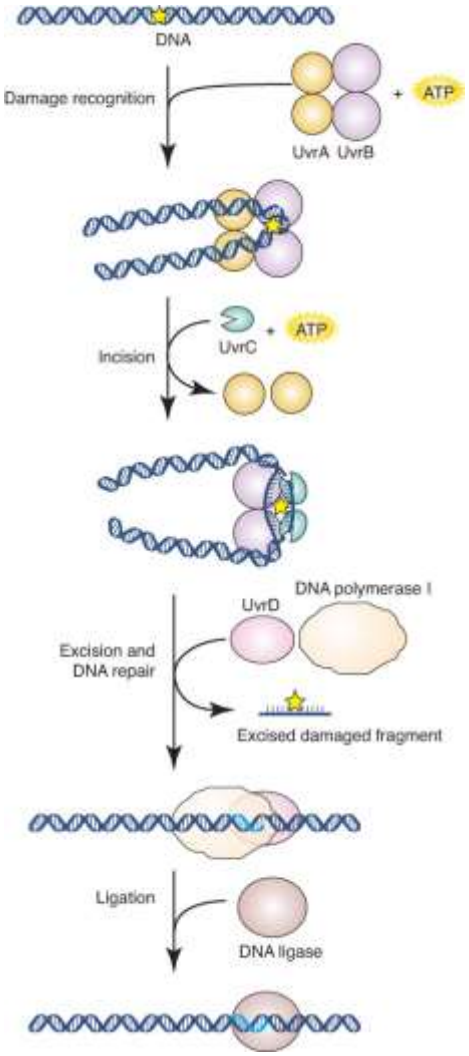


Figure 09.19: Bacterial nucleotide excision repair pathway.

Mismatch Repair

The DNA mismatch repair system removes mismatches and short insertions or deletions that are present in the DNA

- DNA replication is very accurate
  - DNA polymerase introduces 1 mispair in 10^5 nucleotides
  - 3’→5’ proofreading exonuclease increases fidelity to 1 mispair in 10^7 nucleotides
- This level would still result in a high mutation rate
- Slippage can also occur in repeat sequences

Mismatch Repair

E. Coli mismatch repair systems

- Differs from Gram positive bacteria and eukaryotes
- Mismatch repair system can distinguish the newly synthesized
  - Only the parental strand has methyl groups attached to the sequence GATC
  - Un-methylated (newly synthesized) DNA with a mismatch can be cut at GATC

Mismatch Repair

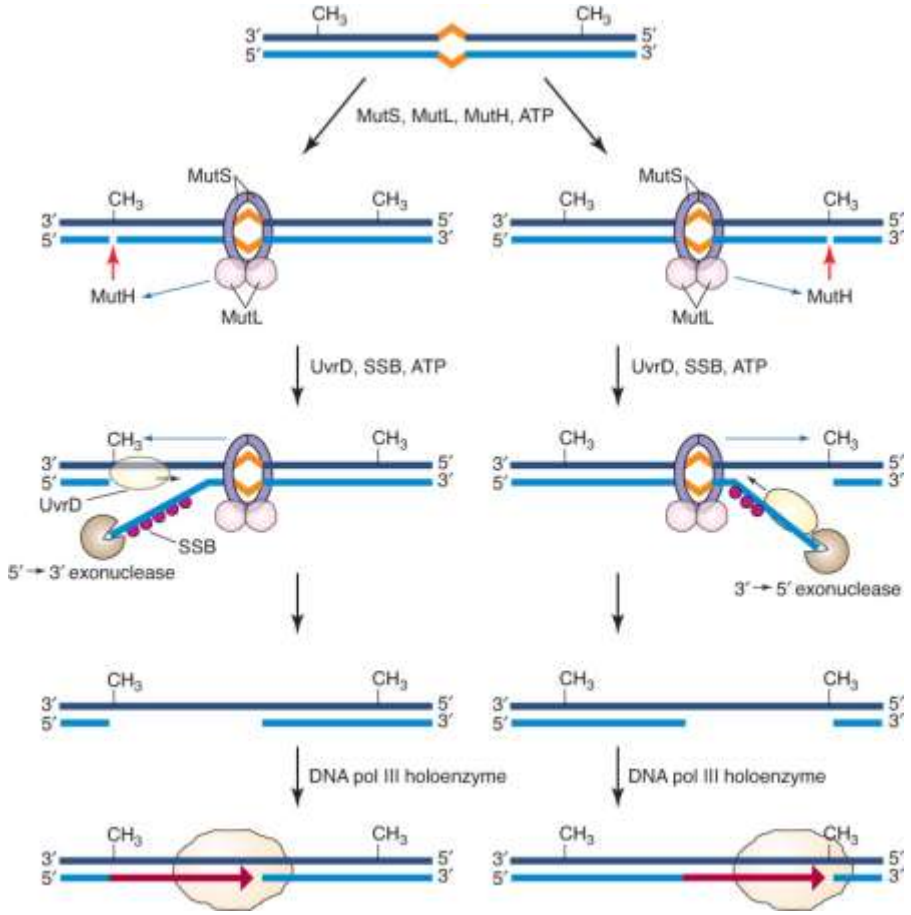


Figure 09.20: E. coli mismatch repair system. The newly synthesized DNA strand (light blue) with a mismatch (orange) is transiently unmethylated at GATC sites.

Adapted from Lyer, R. R., et al. 2006. Chem Rev 106:302–323.)

Mismatch Repair

- DNA mismatch activates MutS·MutL ATP complex
  - Stimulates the MutH endonuclease
  - MutH endonuclease cleaves the nearest unmethylated GATC and exonucleases digest the nicked strand
  - Resulting gap is filled in by DNA Pol III holoenzyme

Mismatch Repair

Eukaryotes have a similar system

- The MutS homolog has endonuclease activity
  - Lagging strand is recognized because of Okazaki fragments
  - Leading strand recognition is not understood yet