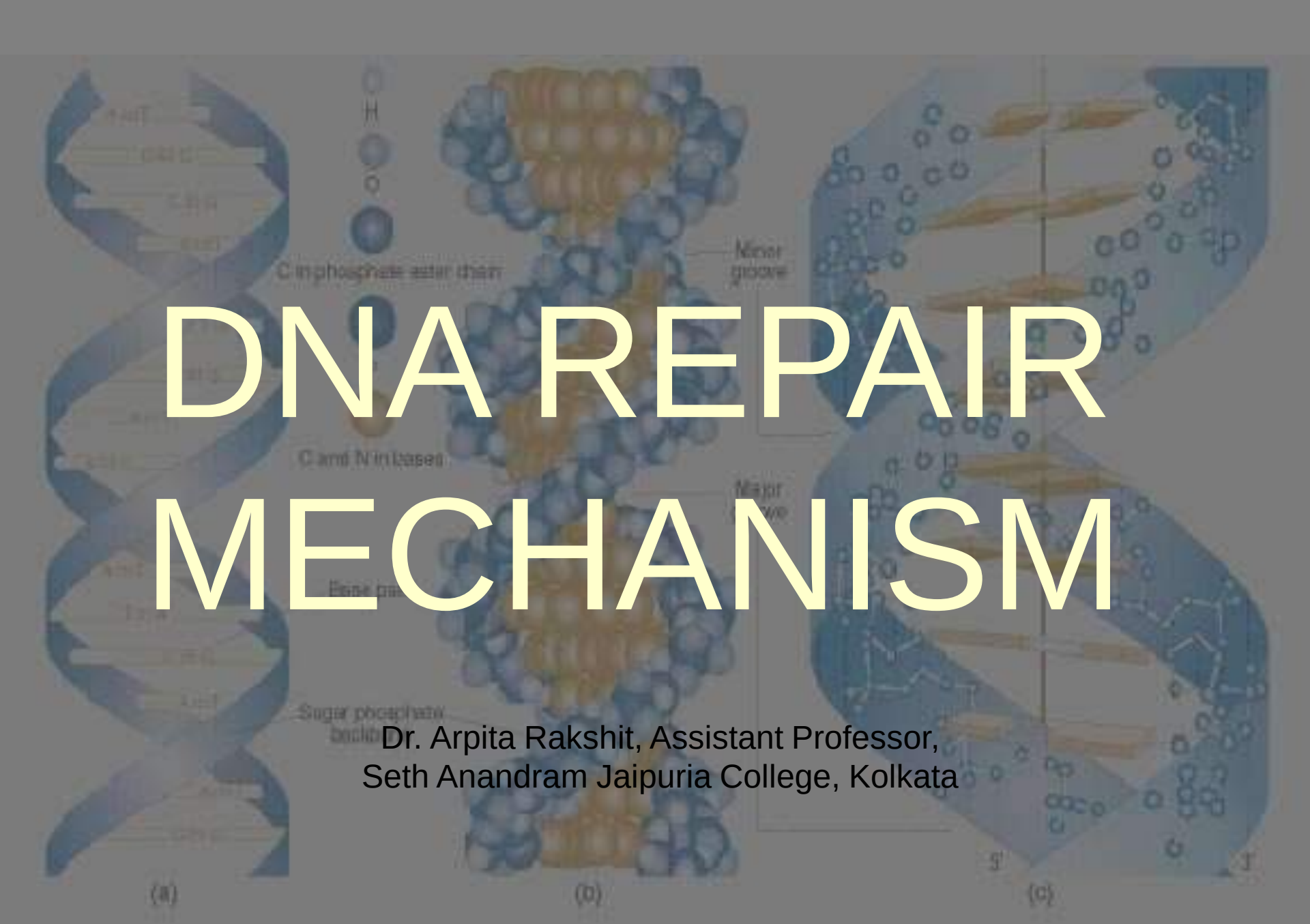


DNA REPAIR MECHANISM

Dr. Arpita Rakshit, Assistant Professor,
Seth Anandram Jaipuria College, Kolkata

- DNA is exposed to several types of chemicals and also subjected to chemical alterations by different agents.
- **CONSEQUENCE**
- DNA may undergo alterations
- Defect in the DNA replication mechanism may also cause alteration.
- Any alteration in DNA molecule need to be corrected to maintain the integrity of the genetic information encoded in it
- Cells have evolved an efficient DNA repair mechanism that function to maintain the integrity of the DNA molecules
- The recent publication of the human genome has already revealed 130 genes whose products participate in DNA repair. More will probably be identified soon.

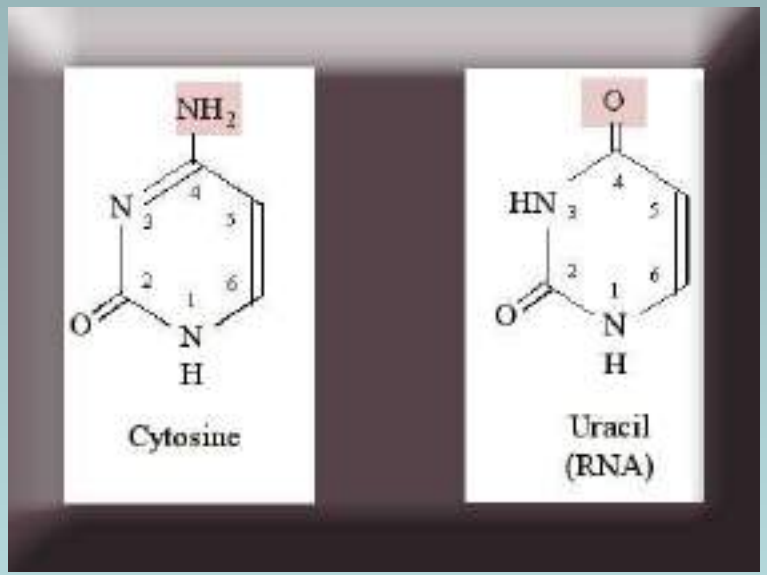
Agents that Damage DNA

- Certain wavelengths of **radiation**
  - **ionizing radiation** such as **gamma rays** and x-rays
  - **ultraviolet rays**, especially the **UV-C rays** (~260 nm) that are absorbed strongly by DNA but also the longer-wavelength UV-B that penetrates the ozone shield
- Highly-reactive **oxygen radicals**
- Chemicals in the **environment**
  - many hydrocarbons, including some found in cigarette smoke
  - some plant and microbial products, e.g. the **aflatoxins** produced in moldy peanuts
- Chemicals used in **chemotherapy**

Types of DNA Damage

1. **Covalent modification of all four of the bases in DNA at various positions**

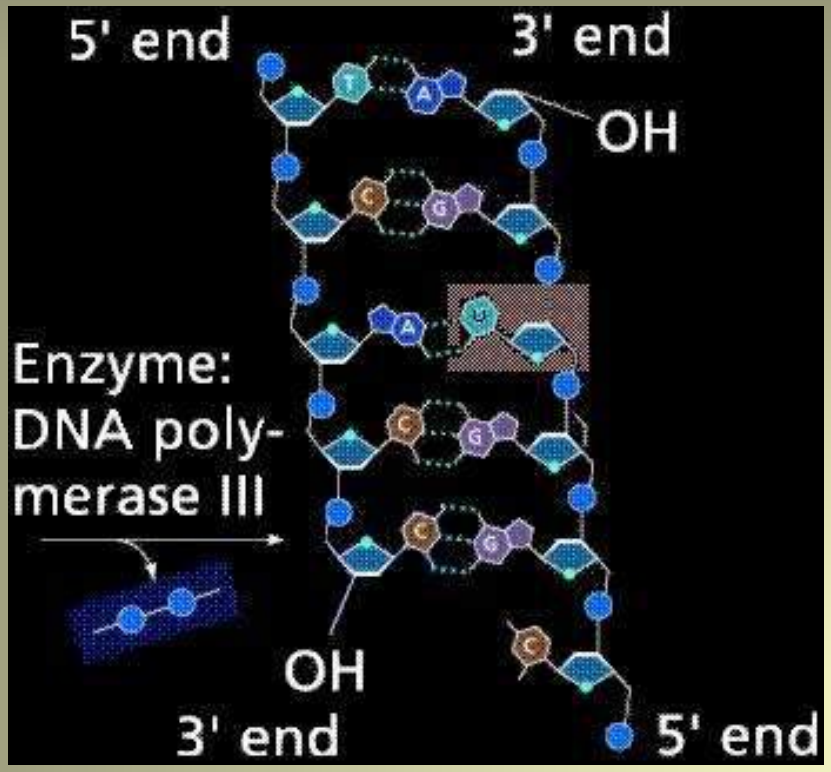
   - **most frequent is the loss of an amino group ("deamination") — resulting in a C being converted to a U.**



2. **Mismatches** : **incorporation of U for T**
3. **Breaks** in single or double strand – **caused by ionizing radiation or chemicals**
4. **Crosslinks** : **intra-strand or inter-strand**

2. Mismatch of nucleotide bases

Mismatches of the normal bases because of a failure of proofreading during DNA replication.

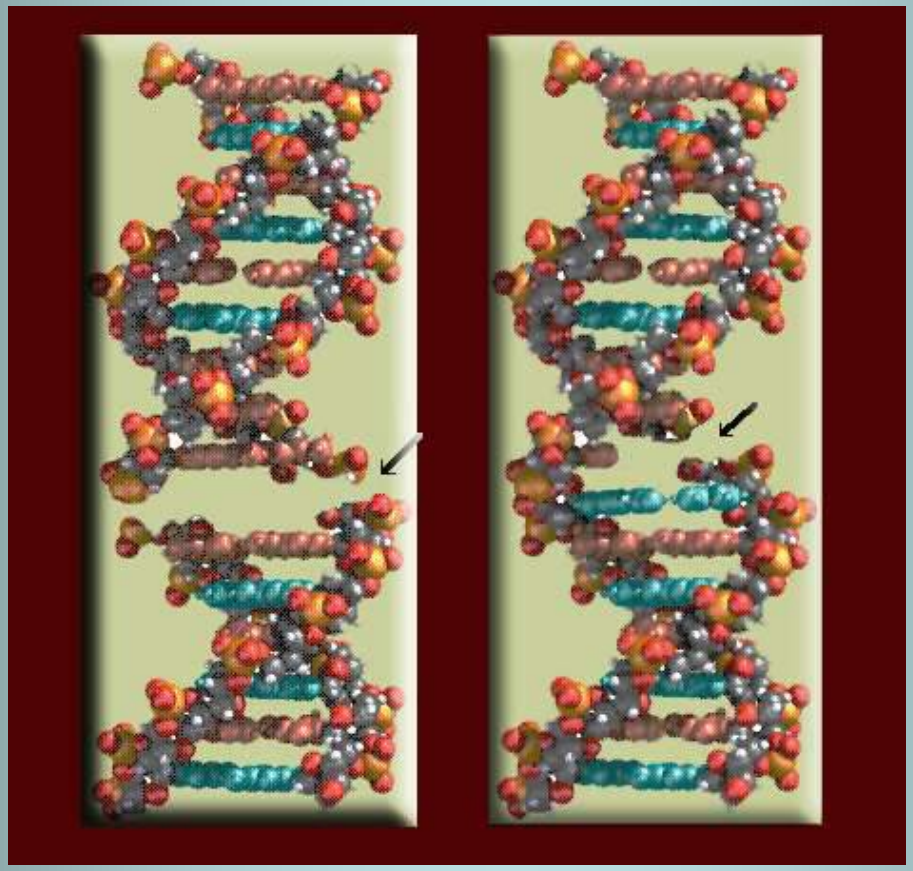


Common example: incorporation of the pyrimidine **U** (normally found only in RNA) instead of **T**.

3. Breaks in DNA strands

Breaks in the backbone:

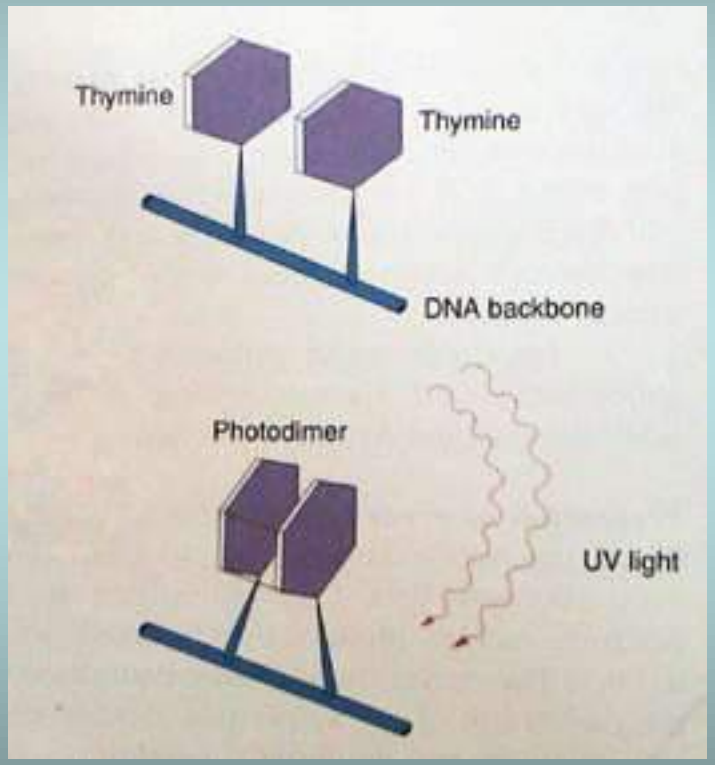
May be single stranded break, or **d**ouble-**s**tranded **b**reak



Ionizing radiation is a frequent cause, but some chemicals can also produce breaks

4. Crosslinks of DNA residues

Crosslinks: Covalent linkages can be formed between intrastrand or interstrand bases



Several chemotherapeutic drugs used against cancers crosslink DNA

Repairing Damaged Bases

Damaged or inappropriate bases can be repaired by several mechanisms:

a. Prevention of errors

b. Direct chemical reversal

c. Excision Repair **damaged base or bases are removed and then replaced with the correct ones in a localized burst of DNA synthesis. Three excision repair mechanism operate, each having specialized sets of enzymes**

i) Base Excision Repair (BER)

ii) Nucleotide Excision Repair (NER)

Iii) Mismatch Repair (MMR)

a. Prevention of Errors

Existence of enzyme system that neutralizes the potential damaging compounds

For example:

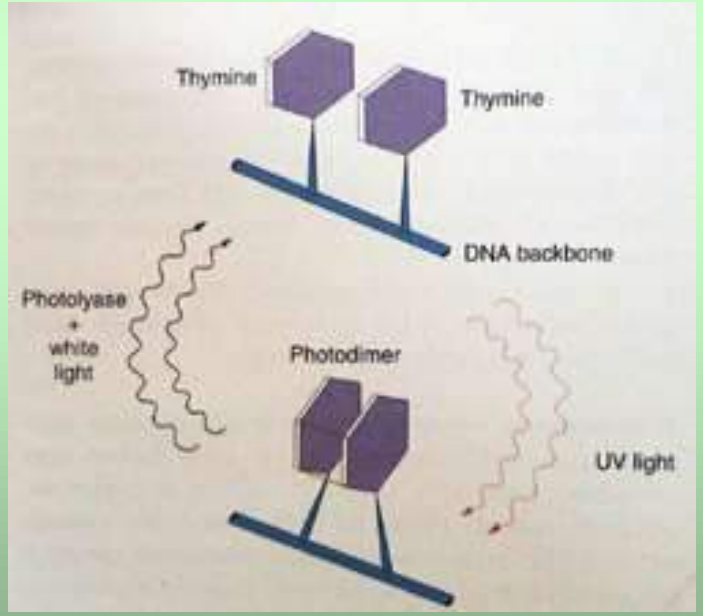
Superoxide dismutase catalyzes the conversion of superoxide radicals to hydrogen H_2O_2

Catalase enzyme then converts H_2O_2 to H_20

b. Direct Reversal of Base Damage

PHOTODIMERS INDUCED BY UV LIGHT

- **Photolyase**, an enzyme found in bacteria and lower eukaryotes can directly restore the original bases
  - Photolyase enzyme uses some visible light wave length to repair the damage.
  - Photolyase enzyme hence cannot operate in dark



SOME BUT NOT ALL DNA DAMAGES CAN BE DIRECTLY REPAIRED

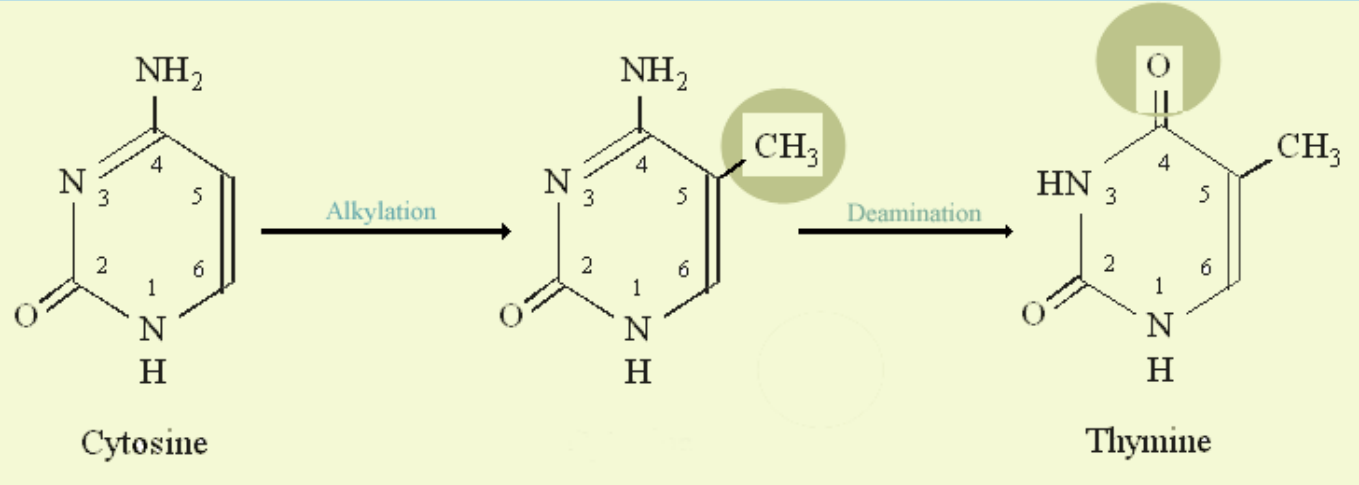
b. Direct Reversal of Base Damage

Transformation of Cytosin to Thyamine

Most frequent cause of point mutations in humans is the spontaneous addition of a methyl group (CH3-) (**alkylation**) to Cytosin followed by deamination to a Thymine residue

Reversal of Base Damage

Glycosylases, that remove the mismatched T restoring the correct C.



Drugs used in cancer chemotherapy induce alkylation. Removal of the CH3- group requires a protein coded by MGMT gene

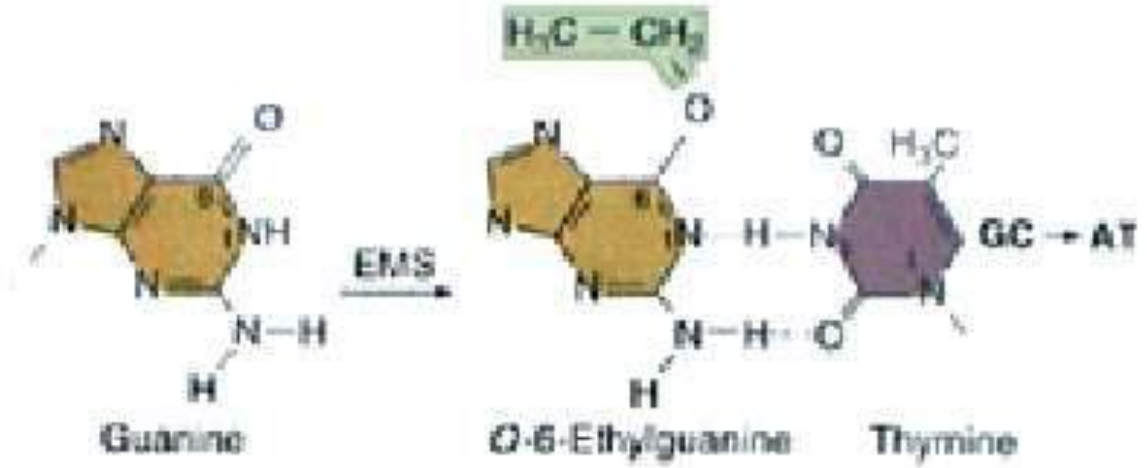
b. Direct Reversal of Base Damage

Alkylation of Guanine at any of the 0 to 6 position

Mutagens like nitrosoguanidine and ethyl methanesulfonate are responsible for the alkylation of guanosine residues.

Reversal of Base Damage

Alkyltransferases, directly reverses the lesions.



Alkyltransferases gets inactivated after removal of one methyl group

c. Excision Repair

Excision repair system is more of generalized type mechanism that is capable of correcting all sorts damage inflicted on the DNA by chemical or physical means.

Base Exicision Repair:

The steps involved are:

1. **Removal of the damaged base by a DNA glycosylase** (occur @ of 20000/per day 8 genes code for glycosylase, and each is responsible to take care of specific type of base damage)

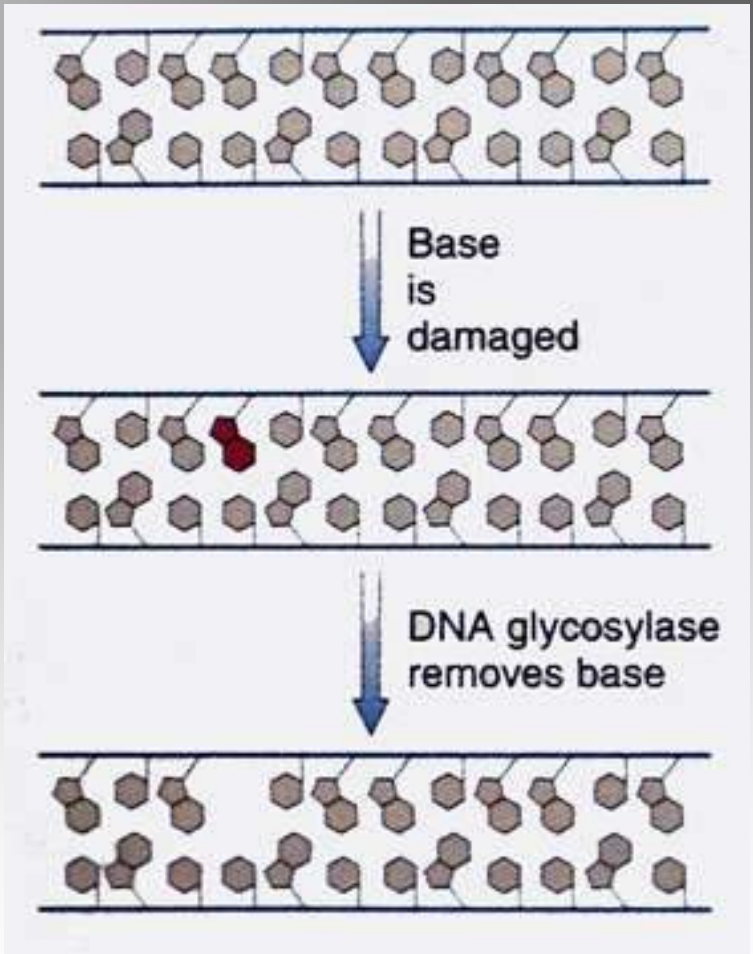
2. **The gap produced in the DNA backbone is filled by DNA polymerase beta** (This relies on one of at least 11 DNA polymerases encoded by our genes)

3. **The nick between the two strand is ligated by ligase**.

4. **In prokaryotes, 12-14 bases are removed while in eukaryotes, 27-29 bases are removed**

C-2: Nucleotide Excision Repair Mechanism

- **Some DNA alteration are so subtle that general excision repair system fail to detect the change.**
- **There exists Specific excision pathways that operate where the general excision repair mechanism fail to function**
- **DNA glycosylase detects the lesion and cleave N-glycosidic (base-sugar) bond and creates apurinic or apyriminidic site**
- **The resulting site is then repaired by site specific AP-endonuclease pathway**



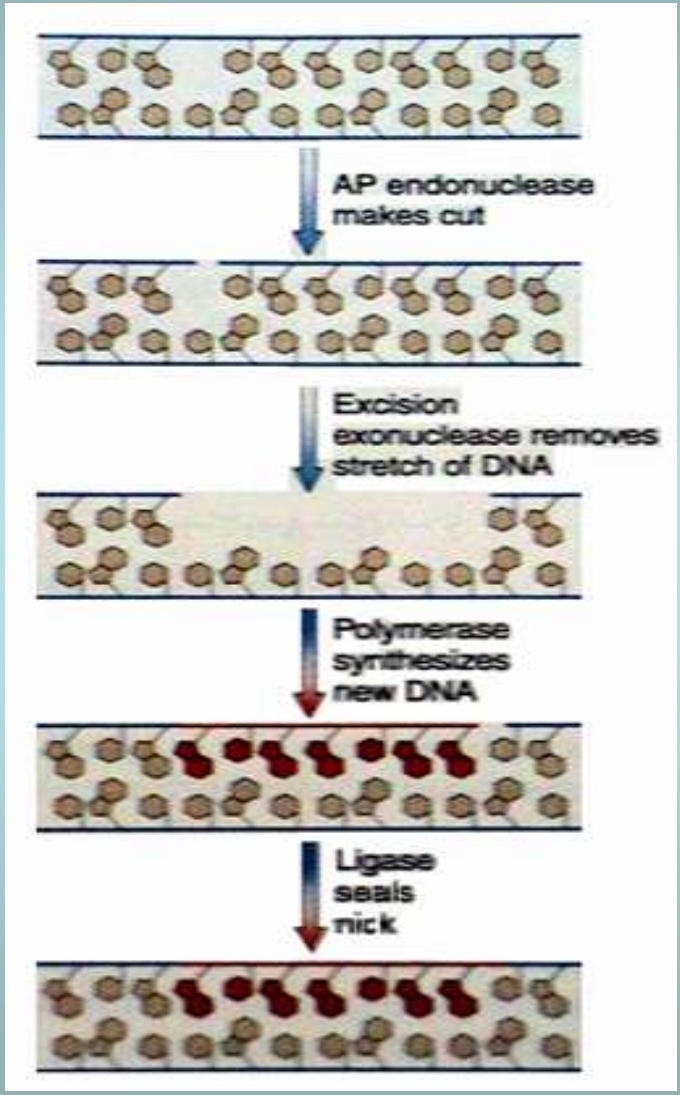
AP-endonucleases

Glycosyalase detects the alteration in DNA and creates apurinic or apyrimidinic sites (AP sites)

AP-endonucleases then introduces chain brakes by cleaving phosphodiester bonds at AP sites

This initiates the excision repair process mediated by three enzymes –

- an endonuclease
- DNA polymerase I
- DNA ligase



Excision Repair Mechanism

- The damage is recognized by one or more protein factors that assemble at the location.
- The DNA is unwound producing a "bubble". The enzyme system that does this is **TFIIH**
- Cuts are made on both the 3' side and the 5' side of the damaged area so the tract containing the damage can be removed.
- A fresh burst of DNA synthesis — using opposite strand as a template — fills in the correct nucleotides.
- The DNA polymerases responsible are designated polymerase **delta** and **epsilon**.
- A **DNA ligase** covalent binds the fresh piece into the backbone.

C-3: Mismatch Repair (MMR)

Although the DNA Replication mechanism is very efficient, mismatches of the **normal bases frequently occur during replication process.** Post DNA Replication, Mismatch repair mechanism operates to detect and repairs the mismatches.

Both repair mechanism operates

- base-excision repair (BER) and
- nucleotide-excision repair (NER)

A battery of enzymes functions during this process, and their main function is to:

- **Recognition** of a mismatch (requires several different proteins including one encoded by ***MSH2)***.
- **Excise** out the mismatch (requires several proteins, including one encoded by ***MLH1)***.
- Repair by substituting the correct bases.

C-3: Mismatch Repair (MMR)

HOW DOES THE MISMATCH REPAIR SYSTEM DECIDE WHICH ONE OF THE MISMATCHED BASE IS TO BE REPLACED BECAUSE BOTH THE BASES ARE NORMAL TO DNA

-mismatch is produced only on the newly synthesized strand and the base on the newly synthesized needs to be replaced.

HOW IS THE NEWLY SYNTHESIZED STRAND RECOGNIZED

-To distinguish the old template strand from the newly synthesized strand, the mismatch repair system takes advantage of a delay in the methylation of the following sequence, which normally occurs after replication

```
-5` - G – A – T – C – 3`
-3` - C – T – A – G – 5`
```

Adenine methylase produces **6-methyladenine** on each strand but takes several minutes to function at **GATC sites**. GATC sites therefore acts as a tag for the newly synthesized DNA

C-3: Mismatch Repair (MMR)

Once the mismatched site is identified, the mismatch-repair system corrects the error

The E. coli recA gene, one of the genes of the SOS bypass system also takes part in post-replication repair . Here the DNA replication system stalls at a UV photodimer or other blocking lesions and then restarts past the block, leaving a single stranded gap. The RecA product then takes part in recombinational repair, a process in which the gap is patched by DNA fro the sister molecule. This process is not error-prone.