# Mutagenic agents and the mechanisms of mutation

The probability of a mutation occurring spontaneously in a single bacterial gene is, on the average, about 1 in 1 million. In a bacterial culture containing $10^8$ to $10^9$ cells per millimeter, some genetic variation among the progeny is inevitable. Most spontaneous mutations are products of rare errors in DNA duplication.

**Physical agents:**

- Non ionizing radiation (UV light).
- Ionizing radiation (X-rays and gamma, beta and alpha rays).

**Chemical agents** (Natural and unnatural "synthetic")**:**

- Base analogues.
- DNA-modifying agents.
- Intercalating agents.

**Physical agents:**

- **Non ionizing radiation (UV light):**

Ultraviolet (UV) light with wave lengths around 260nm damages the DNA of microorganisms. It triggers the permanent bonds between thymine bases that lie adjacent to each other in the same DNA strand. These thymine dimmers acquire an abnormal configuration that impairs DNA replication and transcription. **The amount of damage is proportional to dose and length of exposure. At high doses, UV light is lethal to cells and can be used as a germicidal agent**.

Ultraviolet (UV) light with wave length 260nm induces mutations **not** only in bacteria but in the superficial cells of human as well. Sunlight contains mutagenic ultraviolet light, and overexposure to sunlight is associated with an increased incidence of **skin cancer**. In addition, looking directly into UV light may severely damage the retina of the eye and cause blindness. Ultraviolet light with wavelengths of 280 nm, however, is neither germicidal nor harmful to humans. This "black light" merely induces fluorescence and does not cause cellular or ocular damage.

Ultraviolet (UV) light has ability to form Thymine dimer or Cytosine dimer, sometimes Cytosine-Thymine dimer, this bond cause pulling the nitrogenous bases to each other, then causes distortion of DNA helix and hence lead to cause problem in advances of replication fork.

- **Ionizing radiation:**

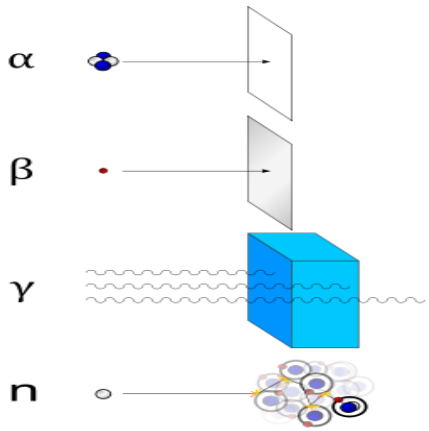
In all cases, the action of ionizing radiations on living cells is basically the same. These radiations are all highly penetrating. **They consist of particles or high energetic photons that penetrate cells and, as they do so, knock electrons of the cellular atoms in their path out of orbit. This results in the formation of ions, hence the name ionizing radiations.** Their effects are quite different from those of the less energetic ultraviolet, which is a non-ionizing radiation.

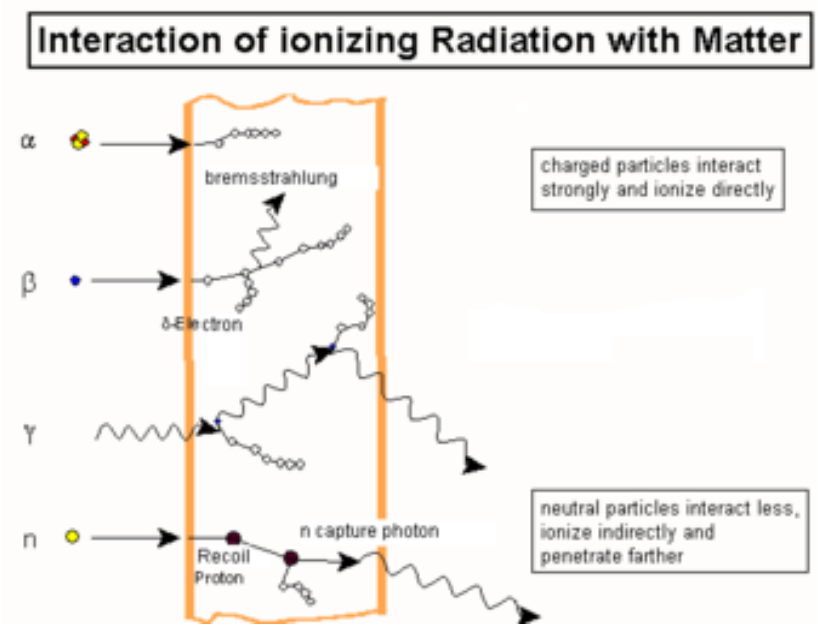
Ionizing of a chemical compound inside the cell whether it be DNA or whatever, may result in a chemical transformation of the compound. In addition, highly reactive substances such as free radicals are formed that they react with DNA bases and cause gene mutations. On the other hand, DNA may be directly altered to produce gene mutations.

Ionizing radiations cause both gene mutations and chromosome breaks. **In general rate of production of point mutations is directly proportional to the dose**.

In addition to the differential sensitivity of different tissues and stages of the cell cycle, the effect of ionizing radiations is influenced by chemical factors. For example, the concentration of oxygen in the cell is of great importance. **The higher of oxygen pressure the greater the damage done by equal doses of radiation**.

**Types of radiation**



## Chemical mutagen:

### Nitrous acid:

Convert the **amino** group of nitrogenous base to **keto** group. So it alters the nitrogenous base to other and as a result base pairing also change as well. The reaction by which nitrous acid act called **oxidative deamination**. By deamination of bases many chemical mutagens act by altering base-pairing.

Cytosine ⟶ Uracil

Adenine ⟶ Hypoxanthine (has ability to bind with cytosine) (H----C)

Guanine ⟶ Xanthine (has ability to bind with cytosine) (X----C)

Nitrous acid is a very potent mutagen that acts directly on either replicating or non-replicating DNA by oxidation or deamination of the bases that contain amino groups (adenine, guanine and cytosine). Conversion of the amino groups to keto groups changes the hydrogen bonding potential of the bases.

Adenine is deaminated to hypoxanthine, which then pairs preferentially with cytosine in the place of thymine. Cytosine is deaminated to uracil, which now pairs with adenine in the place of guanine. Deamination of guanine has zero effect, as deaminated guanine also pairs with cytosine.

Since the deamination of adenine leads to AT. GC transition and the deamination of cytosine results in GC. AT transitions, nitrous acid induces transitions in both directions. Nitrous acid also causes interstrand cross-linking of DNA. The DNA strands fail to separate and there is no DNA duplication, which is lethal or deleterious.

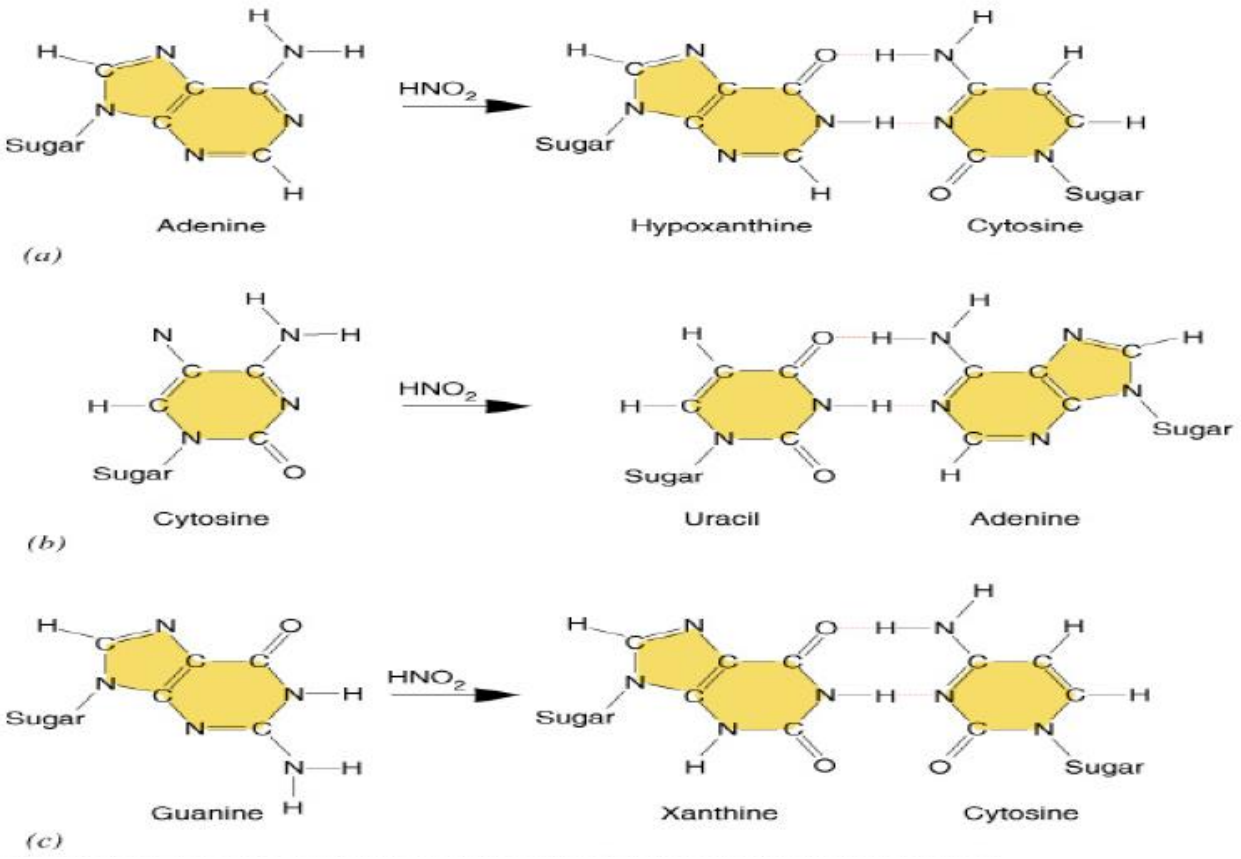
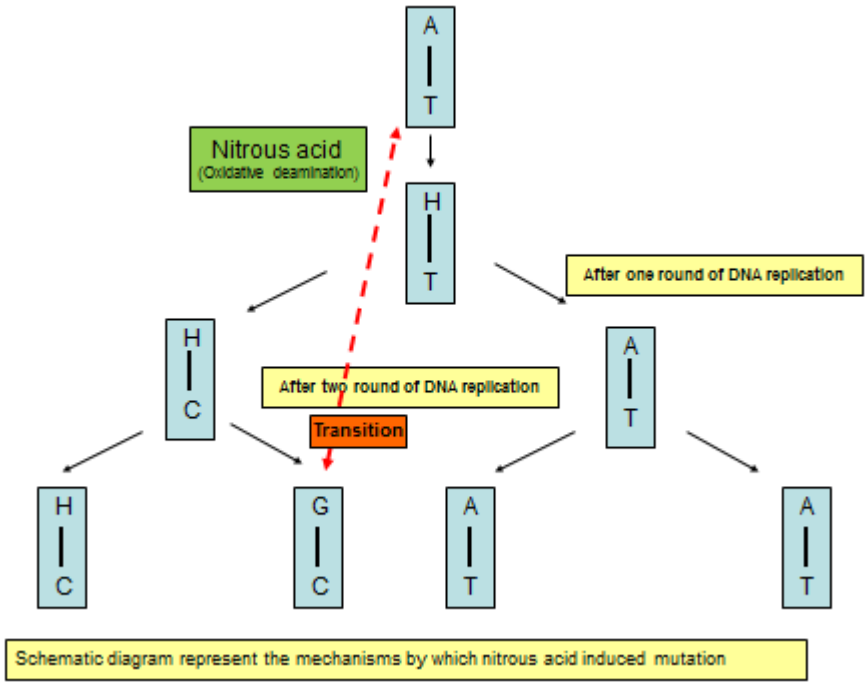
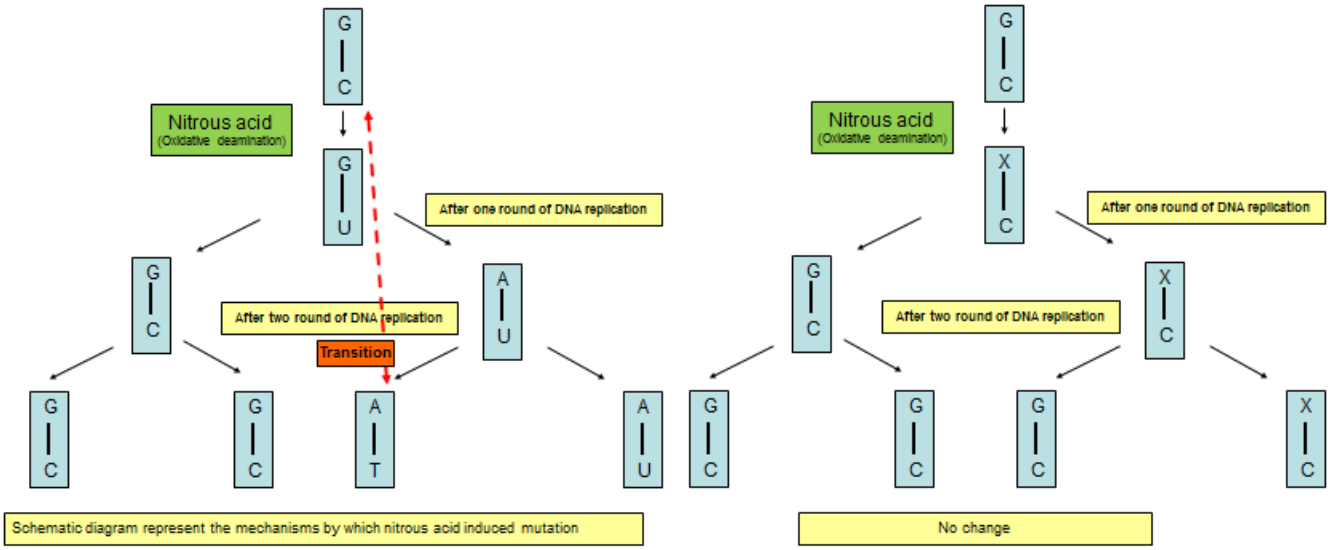



**Figure:** Nitrous acid induces mutations by oxidative deamination of the bases in DNA. Nitrous acid converts ***(a)*** adenine to hypoxanthine, causing A:T⟶G:C transitions; ***(b)*** cytosine to uracil, causing G:C ⟶A:T transitions; and ***(c)*** guanine to xanthine, which is not mutagenic. Together, the effects of nitrous acid on adenine and cytosine explain its ability to induce transitions in both directions, A:T ⟶ G:C. 






## Hydroxylamine:

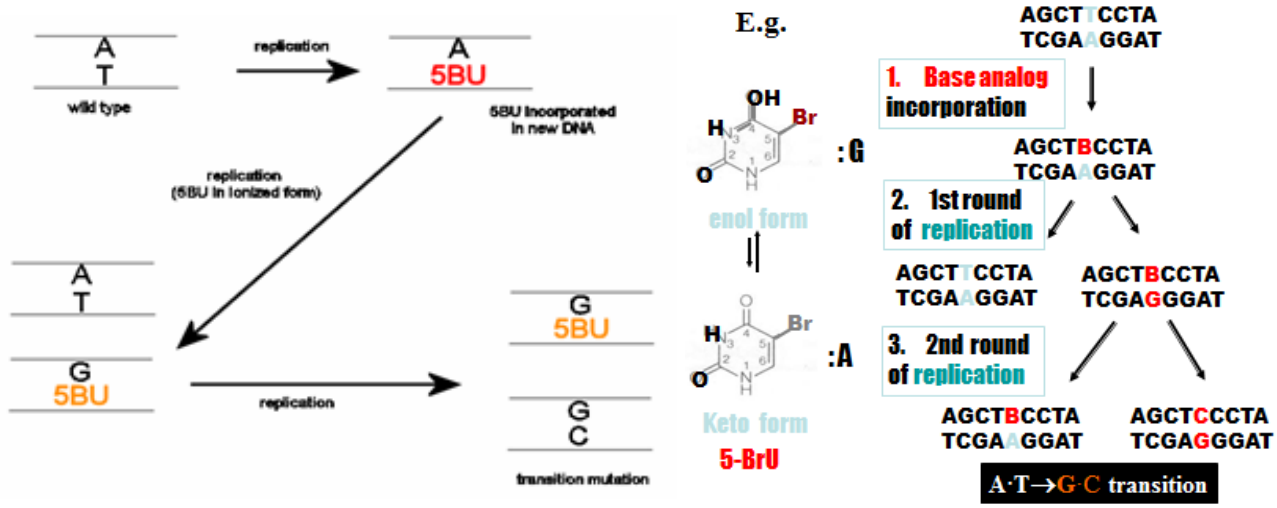
Hydroxylamine apparently reacts with **cytosine** and convert it to an altered base, which form hydrogen bonds readily with **adenine** rather than **guanine**. As a result, it causes transitions of the type G≡C to A=T. **Hydroxylamine has the same effects of nitrous acid.**

This has also been used in the past by biologists to introduce random mutations by switching base pairs from A to G, or from C to T. This is to probe functional areas of genes to elucidate what happens if their functions are broken. Nowadays other mutagens are used. Hydroxylamine can also be used to highly selectively cleave asparaginyl-glycine peptide bonds in peptides and proteins. It also bonds to and permanently disables (poisons) heme-containing enzymes. It is used as an irreversible inhibitor of the oxygen-evolving complex of photosynthesis on account of its similar structure to water.

## Base analog mutagen

A base analog is a chemical that can substitute for a normal nucleobase in nucleic acids.

A common example would be 5-bromouracil (5BU), the abnormal base found in the mutagenic nucleotide analog BrdU. When a nucleotide containing 5-bromouracil is incorporated into the DNA, it is most likely to pair with adenine; however, it can spontaneously shift into another isomer which pairs with a different nucleobase, guanine. If this happens during DNA replication, a guanine will be inserted opposite the base analog, and in the next DNA replication, that guanine will pair with a cytosine. This results in a change in one base pair of DNA, specifically a transition mutation.



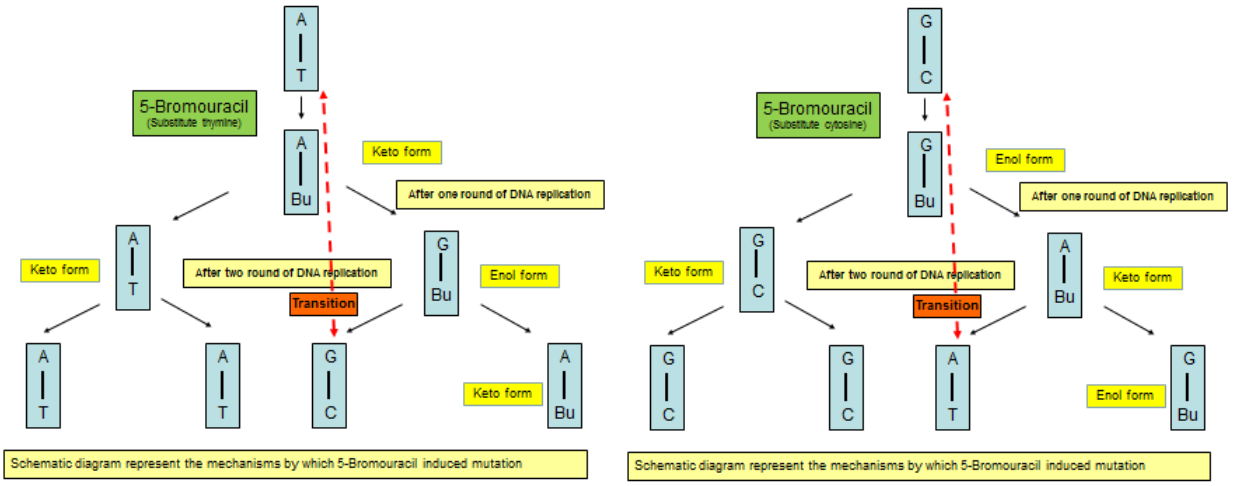

The stable, unrepaired base with altered base pairing properties in the DNA is fixed to a mutation during DNA replication.

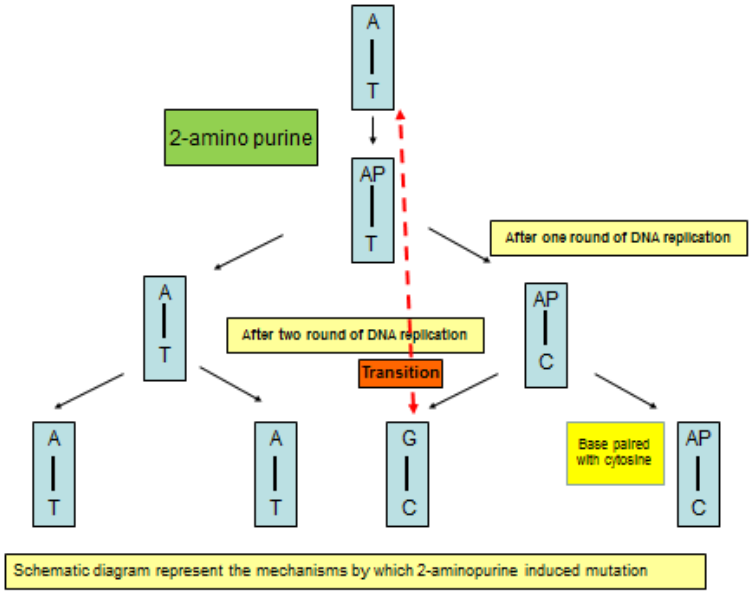
**5-Bromouracil, 2-aminopurine**

Can be build into DNA structure during polymerization.

5-Bromouracil → Keto form (substitute thymine)

5-Bromouracil → Enol form (substitute cytosine)



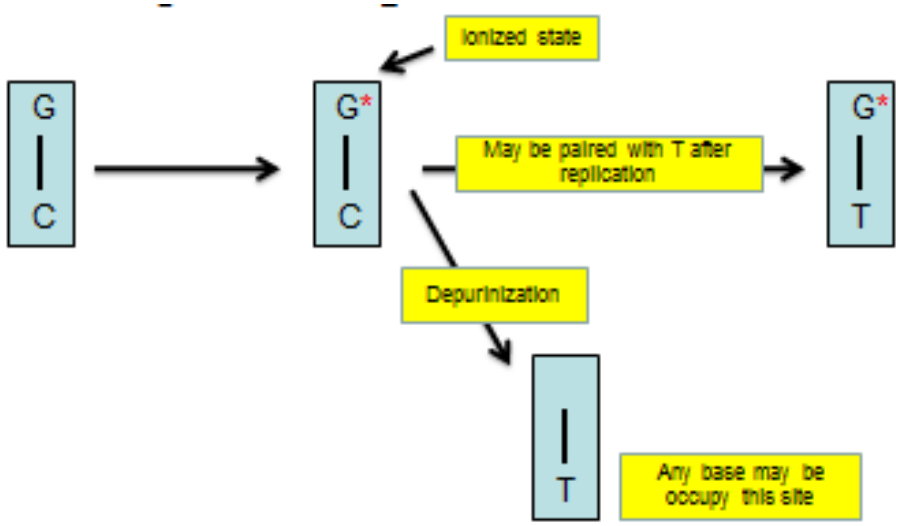



## Alkylating agents:

Alkylating agents are electrophilic chemicals (depurinizing agents) which readily add alkyl (e.g. methyl) groups to various positions on nucleic acids distinct from those methylated by normal methylating enzymes such as **methylmethane sulfonate (MMS)**, **ethyl-ethane sulfonate (EES), ethyl-methane sulfonate (EMS)** and **ethylnitrosourea**.

Specifically, guanine may be stripped from the chain, and, as a result, upon replication any one of four possible bases may occupy the position opposite the gap. The following replication may then result in a **transition** or **transversion**, a type of mutation in which a pyramidine is replaced by a purine or vice versa.

**The mechanism of Alkylating agent involves the addition of alkyl group (R) to the purine ring of the base.**




After the addition of R-group to guanine, then this base has ability to pair with thymine.

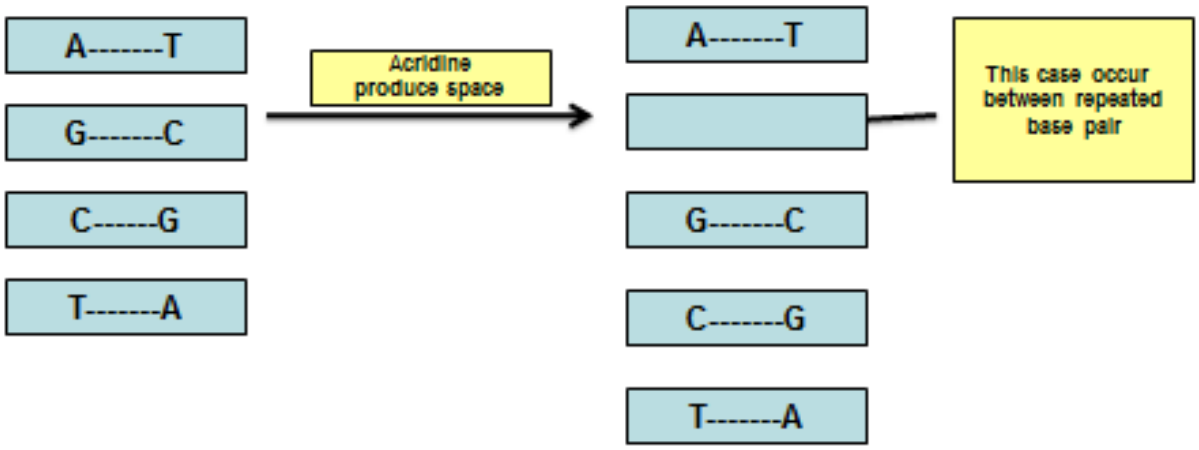
After alkalyzation the $C^1$ of deoxyribose which binds with nitrogen base by N-glycosidic bond became liable which lead to depurinization.

## Acridine

**Acridine**, $C_{13}H_9N$, is an organic compound and a nitrogen heterocycle. Acridine is also used to describe compounds containing the $C_{13}N$ tricycle. (Example: acridine orange, proflavin).

Acridine is structurally related to anthracene with one of the central CH groups is replaced by nitrogen. Acridine, a colorless solid, was first isolated from coal tar. It is a raw material used for the production of dyes and some valuable drugs. Many acridines, such as proflavine, also have antiseptic properties. Acridine and related derivatives bind to DNA and RNA due to their abilities to intercalate. Acridine orange (3,6-dimethylaminoacridine) is a nucleic acid-selective metachromatic stain useful for cell cycle determination. Acridarsine is formally derived from acridine by replacing the nitrogen atom with one of arsenic, and acridophosphine by replacing it with one of phosphorus.

Acridine bind to the DNA by process called intercalation and cause the base pair to move apart from each other, so that the space is generated and this space can accommodate (fit) a base pair because the dimension of acridine molecule are identical to the dimensional of base pair.




- Any base pair can be inserted in the space generated by acridine.
- All amino acids downstream of mutation are change.
- This type of mutation is irreversible.
- The repair system cannot detect this type of mutation.