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NAAC ACCREDITED 'A' GRADE

Topic: Mutation

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MUTATION

Inheritance is based on genes that are faithfully transmitted from parents to offspring during reproduction. Mechanisms have evolved to facilitate the faithful transmission of genetic material from generation to generation. Nevertheless, 'mistakes' or changes in the genetic material do occur. Such **sudden heritable changes in the genetic are called mutation.**

An organism exhibiting a novel phenotype as a result of the presence of a mutation is called a **mutant**. In a broad sense , such genotypic changes include changes in the chromosome number (polyploidy) or changes in chromosome structure (chromosomal aberration) as well as changes in individual genes. The first category of change is termed **genomic mutation** (mutation at the level of the genome).The second category of change is termed **chromosomal mutation** (mutation at the level of the chromosome). The third category is known as **gene mutation** (mutation at the level of the gene). Many gene mutations involve change in a single base pair (for example the substitution of a base pair by another or addition/deletion of base pairs). Since is is so localized it is often termed '**point mutation**'.

Somatic and Germinal Mutation

Somatic mutation occurs in a body cell or somatic cell. All cells that arise by division of this cell carries the mutation. It is not passed down to the next generation. Cancer is caused by a somatic mutation which occurs when the cell loses it's control over cell division and starts dividing uncontrollably giving rise to a tumor tissue. However, since it is a somatic mutation it is not transmitted to the next generation.

Germinal mutation occurs in the germ cells. Their effect may be expressed immediately in the progeny. If the mutation is recessive, their effect is suppressed in diploids by the dominant gene on the other homologue. Germinal mutations may occur at any stage of the reproductive cycle. If they occur in the gametes, only a single member of the progeny is affected. If on the other hand, mutation occurs in the germ mother cells, all the gametes receive the mutant gene and all the progeny carry the mutation.

Forward and Back Mutation

Mutation of a **wild type gene to a mutant** one is called forward mutation.

Mutation from a **mutant gene to the wild type** is referred to as back mutation or reverse mutation.

Conditional Lethal Mutation

These mutations are **lethal under certain condition called restrictive condition** and **viable under other conditions called permissive conditions**.. These mutations are of three types:

Auxotrophic Mutants-They are unable to synthesize an essential metabolite (amino acid, vitamin, purine, pyrimidine etc.) which are synthesized by the wild type. They survive only when the metabolite is supplemented in the medium (permissive condition).

Temperature Sensitive Mutants-These mutants survive under particular temperature range (permissive condition) and not above or below it.

Suppressor Sensitive Mutants-Sometimes a second mutation in a different location in the genome suppresses the effect of the original mutation (suppressor mutations). These mutants survive in presence of the suppressor(permissive condition).

Phenotypic Effects of Mutation

Mutations must normally cause some detectable phenotypic changes for their presence to be recognized. The effects of mutation is sometimes so minor an alteration that they can be detected only by genetic or biochemical techniques. On the other hand, the change be an observable **change in morphology** and in extreme cases it my result in **lethality**.

A gene consists of a sequence of nucleotides coding for a particular polypeptide chain. Any mutation within a given gene will produce a new form (allele) of that gene. Because of the degeneracy of the genetic code (many amino acids are coded by more thn one codon), some base pair changes do not change the protein product encoded by the gene. Therefore, when a mutated codon or the changed codon codes for the same amino acid as the original one, the amino acid and the polypeptide is unaltered, so the mutation is a **silent mutation**. Silent mutation also occurs when there is a change in the amino acid but it is not sufficient to modify the function of the protein appreciably.

A **mis-sense mutation** occurs when a change in one base pair gives rise to a new amino acid in a polypeptide chain thus altering its function. For example, human hemoglobin is composed of four polypeptide chains (two identical α chains and two identical β chains) plus a heme group. If the sixth amino acid from the amino terminal of the β chain of hemoglobin changes from glutamic acid (negatively charged amino acid) to valine (neutral amino acid), the alpha chains remaining normal a remarkable phenotypic change results and sickle cell hemoglobin is produced. It changes the morphology of the RBC, they become sickle-shaped and clog the blood vessels cutting off oxygen supply to the tissues and in many cases leads to death. When analysed, it was found that a change in a single base was responsible for this mutation.

Normal hemoglobin

DNA---------GAG---------

---------CTC---------

↓ Transcription

RNA----------GAG---------

↓ Translation

PP AA_1---------glu----------AA_{146}

Sickle cell hemoglobin

----------GTG----------

----------CAC----------

↓ Transcription

----------GUG----------

↓ Translation

AA_1----------val---------- AA_{146}

Non-sense mutation-Out of the 64 codons in the genetic code,61 code for amino acids, while 3 are responsible for the termination of transcription. These 3 codons are UAA, UAG and UGA. They are also called non-sense codons. Any mutation resulting in the formation of a termination codon abruptly terminates transcription forming an incomplete polypeptide chain. Therefore this type of mutation is called non-sense mutation. For example, if the codon UAC (which codes for Tyrosine) undergoes a base substitution from C to G , forming UAG (termination codon), the polypeptide chain synthesis stops abruptly, leading to the formation of an incomplete chain which is biologically inactive and deleterious.

Frame Shift Mutation-When bases are inserted into a DNA chain or deleted from it, a new sequence of codons or a new reading frame result from the point of insertion or deletion. These new codons code for a completely new sequence of amino acids and the protein becomes non functional. For eg. From the sequence CAC GAC CAC GAC CAC GAC if C in the 7^{th} position is deleted the sequence becomes CAC GAC ACG ACC ACG AC.. Similarly insertion of G in the same position produces the sequence CAC GAC GCA CGA CCA CGA C…both results are deleterious.

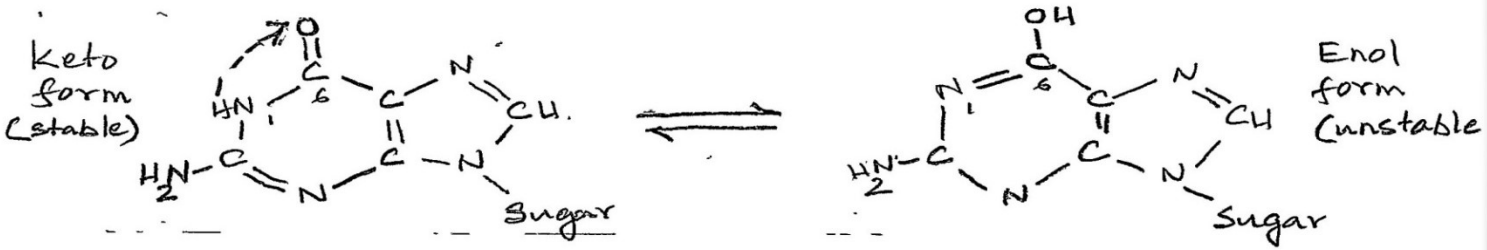
The Molecular basis of Mutation

When Watson and Crick described the double helix structure of DNA and proposed its semi-conservative replication based on specific base pairing to explain the faithful transmission of genetic information from one generation to the next, they also proposed a mechanism to explain spontaneous mutations. Watson and Crick pointed out that the structure of bases in DNA are not static. **Hydrogen atoms can move from one position in a purine or pyrimidine to another position**, for example from an amino group to a ring nitrogen. Such chemical fluctuations are called **tautomeric shifts**. Although rare, these tautomeric shifts may be of considerable importance as they alter the base pairing potential of the DNA bases. Normally Adenine (A) always pairs with Thymine (T) and Guanine (G) with Cytosine (C). However, the more stable keto form of Thymine and Guanine and the more stable amino form of Adenine and Cytosine may infrequently undergo tautomerization to the less stable enol and imino forms respectively. The bases are expected to stay in their less stable forms for very short periods of time before returning to the stable forms. However, if the base existed in this rare form at the moment that it was being replicated or being incorporated into a nascent DNA chain, a mutation might occur. When in their rare form, they can form A-C and G-T base pairs. The net effect of such an event and the subsequent replication required to segregate the mismatched base pair, is an AT-GC or a GC-AT base pair substitution.

Transition-It Is a type of mutation which involves the substitution of a purine base by another purine base or the substitution of a pyrimidine base by another pyrimidine base. Four types of transitions are possible.

Transversion-It Is a type of mutation which involves the substitution of a purine base by a pyrimidine base and vice versa. Eight types of trasversions are possible.

Tautomeric Shifts in the four bases of DNA



Physical Mutagens

Physical mutagens include different types of radiations. That part of the electromagnetic spectrum containing wavelengths shorter and of higher energy than visible light (wave lengths <0.1µm) can be sub-divided into **ionizing radiation** (X rays, Y rays and cosmic rays) and **non-ionizing radiations** (UV rays). Ionizing radiations can penetrate living tissues and in the process of penetrating, these **high energy rays collide with atoms and cause the release of electrons, leaving positively charged free radicals or ions.** These ions in turn collide with molecules causing release of further electrons. The net result is that a 'core' of ions is formed along the track of each high energy ray. **Ultraviolet rays having lower energy** penetrate only the surface layer of cells in multicellular organisms and do not induce ionizations. They **dissipate their energy to atoms that they encounter, raising the electrons in the outer orbitals to higher energy levels , a state referred to as excited state.** Molecules containing atoms either in the ionic forms or excited forms are chemically more reactive than those containing atoms in their normal stable state. **The increased reactivity of atoms present in DNA molecules is the basis of mutagenic effect of mutation.**

Effect of Ionizing Radiations

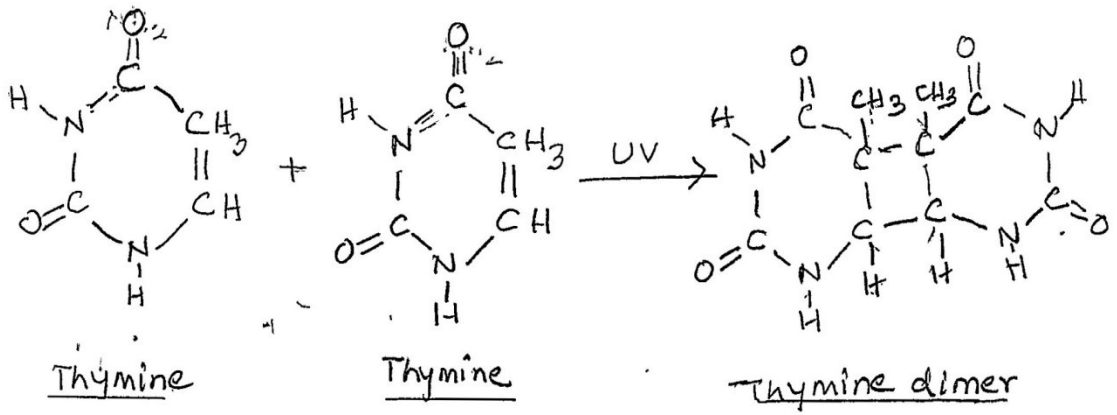
X rays and most other forms of ionizing radiations are quantitated in Roentgen units which is measured in terms of the number of ionizations/unit volume under a standard set of conditions. The dosage of radiation in Roentgen units does not involve a time scale. The same dosage may be obtained by a low intensity of irradiation over a long period of time or a high intensity of irradiation for a short period of time. **The frequency of induced point mutations is directly proportional to the dosage of irradiation**.

Radiation damage is more marked in rapidly dividing cells rather than non-dividing cells. Chromosomal aberrations were induced about 60 times more frequently at metaphase than at interphase. **Oxygen tension and temperature change, when associated with irradiation, also may significantly alter the frequency of mutations.** Low oxygen tension decreases mutations. Ionizing radiations also induce various kinds of gross changes in chromosome structure like deletions, duplications, inversions and translocations.

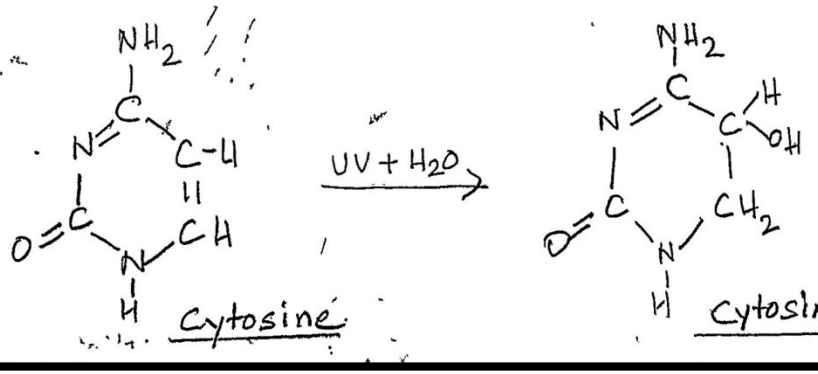
Effect of Non-Ionizing Radiation

Ultraviolet (UV) rays are non-ionizing radiations as they do not possess sufficient energy to induce ionizations. They are however readily absorbed by certain substances like purines and pyrimidines, which then enter a more reactive or excited state. Due to their low energy they are able to penetrate into only the surface layer of cells in multicellular organisms. Nevertheless they act as potent mutagens in unicellular organisms. **The maximum absorption of UV by DNA is at a wavelength of 254 nm, maximum mutagenicity is also observed at 254 nm.** This suggests that UV induced mutagenesis is mediated directly by absorption of UV by purines and pyrimidines. *In vitro* studies indicate that the pyrimidines (especially Thymine) absorb strongly at 254 nm and become very reactive. **The main products formed by the action of UV on pyrimidines is the formation of pyrimidine dimers and pyrimidine hydrates.** Thymine dimerization is the major effect of UV irradiation on DNA. Thymine dimers distort the DNA double helix and interfere with the replication process. Gaps are formed in the complementary strand opposite to the dimers during replication as DNA polymerqse cannot use the distorted strand as template. Cytosine under the influence of UV forms cytosine hydrate..

THE MAJOR EFFECTS OF UV IRRADIATION



Thymine + Thymine → Thymine dimer



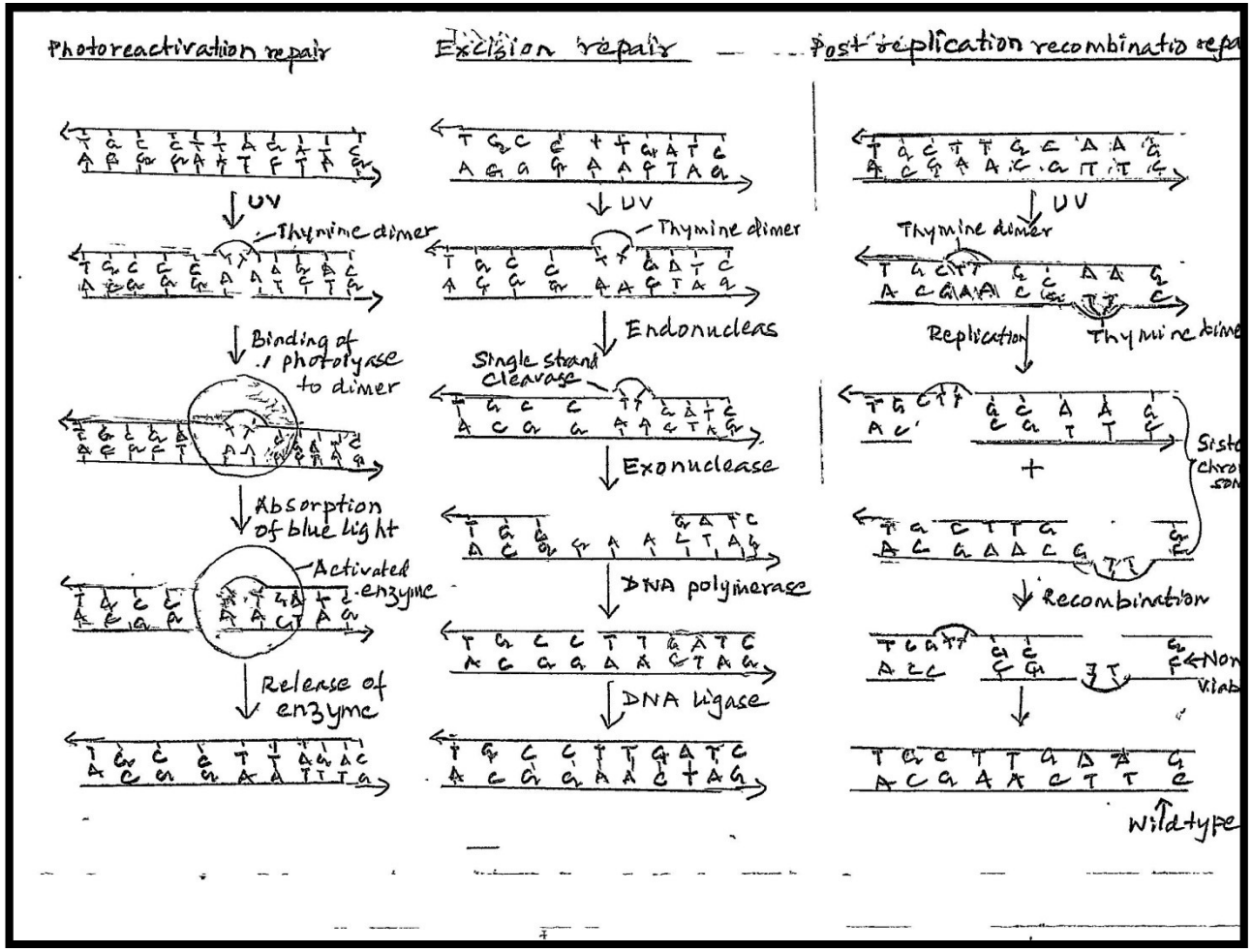
Cytosine → Cytosine hydrate

DNA Repair Mechanisms

Several repair mechanisms help to keep mutagenic effects at a tolerable level. *Ecoli* has developed 3 different repair mechanisms against thymine dimerization.

- **Photoreactivation repair**: This involves an enzyme that splits the thymine dimers directly without removal of nucleotides. This enzyme binds to thymine dimers present in DNA in the dark, but it cannot catalyze cleavage of the bonds joining the thymine molecules without energy derived from visible light specially blue light. The enzyme is also active on cytosine dimers. Thus, in order to cause mutations using UV the treatment has to be carried out in the dark to maximize the effect.
- **Excision repair**: This involves a series of enzyme catalysed steps. At first endonuclease recognises thymine dimers and cleaves the phosphodiester backbone of DNA at or near the dimer. In the second step by the 5'→3' exonuclease activity of DNA polymerase-I a small segment adjacent to the cut including the dimer is removed. In the third step DNA polymerase-I fills in the gap using the complementary strand as template. Then DNA ligase catalyses covalent closure of the phosphodiester bond.
- **Post replication recombination repair**:When DNA containing thymine dimers replicate, gaps are formed in the nascent complementary strand opposite the dimers as DNA polymerase cannot use the distorted strand as template. Replication is reinitiated at secondary sites beyond the dimer. The progeny helix has dimers in one strand and gaps in the other. Recombination between these result in dimers and gaps in one chromosome and another functional chromosome without any damage.

DNA repair mechanisms



Chemical Mutagens

A large variety of chemicals have mutagenic effects of which some have very specific effects. The first chemical mutagen discovered was mustard gas (sulphur mustard). Chemical mutagens can be classified into two groups:

- Those that act only on replicating DNA like Base analogues and acridine dyes.
- Those that act on both replicating and non-replicating DNA like alkylating agents and nitrous acid

Base analogues: These chemicals have a structure very similar to that of the DNA bases. As a result these bases are metabolized and incorporated into DNA instead of the bases during the replication process. However, they are sufficiently different to increase the rate of mispairing and cause mutation. The two most common base analogues are 5-bromouracil (5-BU) and 2-aminopurine.(2-AP).

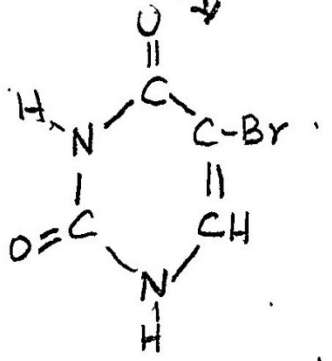
The base analogue 5BU is a base analogue of the pyrimidine thymine. It is different from thymine in having a bromine group at the 5 carbon position instead of a methyl group. The presence of bromine changes the charge distribution and increases the potentiality to get tautomerized from keto to enol state as compared to thymine. 5-BU in its more stable keto form pairs with adenine and in its rare enol form it pairs with guanine. Therefore, the nature of mutation depends on whether 5-BU enters into a DNA chain in its keto or enol form. If 5-BU is in its rare enol form at the time of entry into a nascent DNA chain it will be incorporated opposite guanine in the template strand and cause GC→AT transition. If however, 5-BU enters into the DNA chain in its stable keto form, it is incorporated opposite adenine and then undergoes tautomeric shift to is enol form during subsequent replication, it will cause AT→GC transition. Thusthe mutagenic effect of 5-BU is bidirectional.

The purine analogue 2-AP induces mutation in a similar manner.

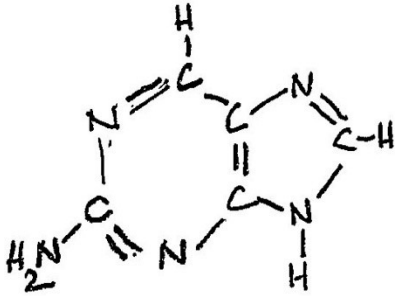
Acridine Dyes: Positively charged acridine dyes like proflavin and acridine orange enter into the DNA double helix and get intercalated or sandwiched in between the bases. This results in the distortion of the double helix forming ‘kinks’ in the molecule and breaks occur from this weak point resulting in deletion and addition of bases thus bringing about frame shift mutation

Different Chemical Mutagens

Base Analogue

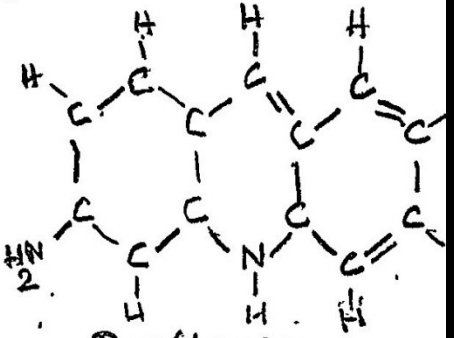


5-Bromouracil



2 Aminopurine

Acridine Dyes



Proflavin

Alkylating Agents

$Cl-CH_2-CH_2-S-CH_2-CH_2-Cl$

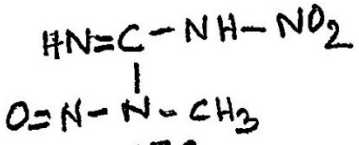
Mustard gas or sulphur mustard

$CH_3-CH_2-O-SO_2-CH_3$

EMS

$CH_3-CH_2-O-SO_2-CH_2-CH_3$

EES

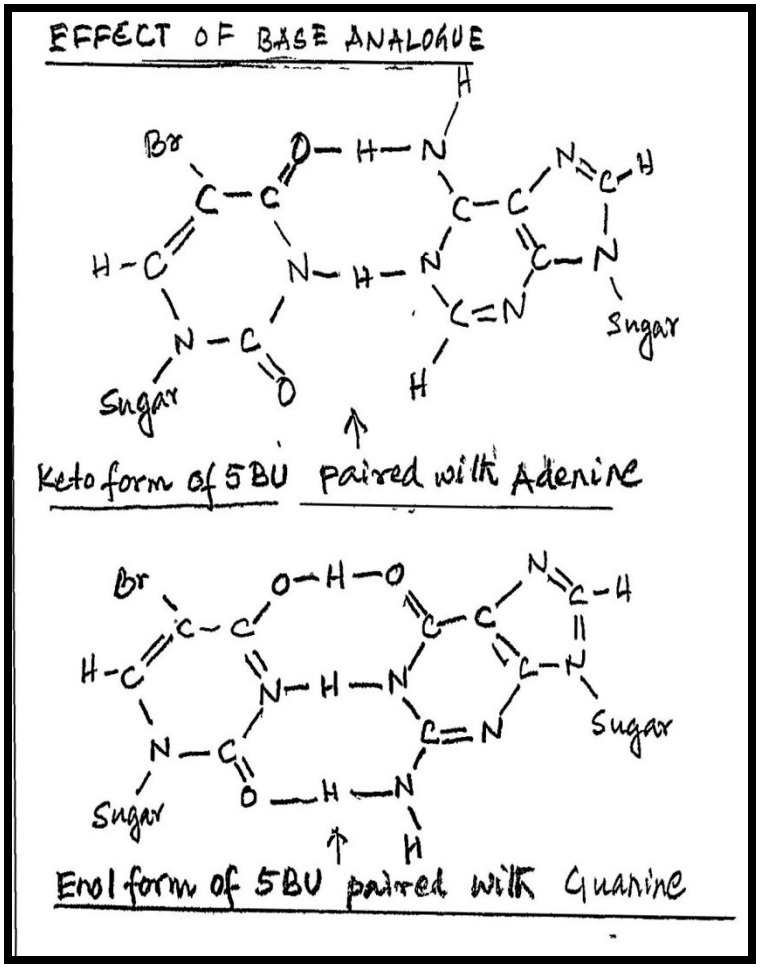


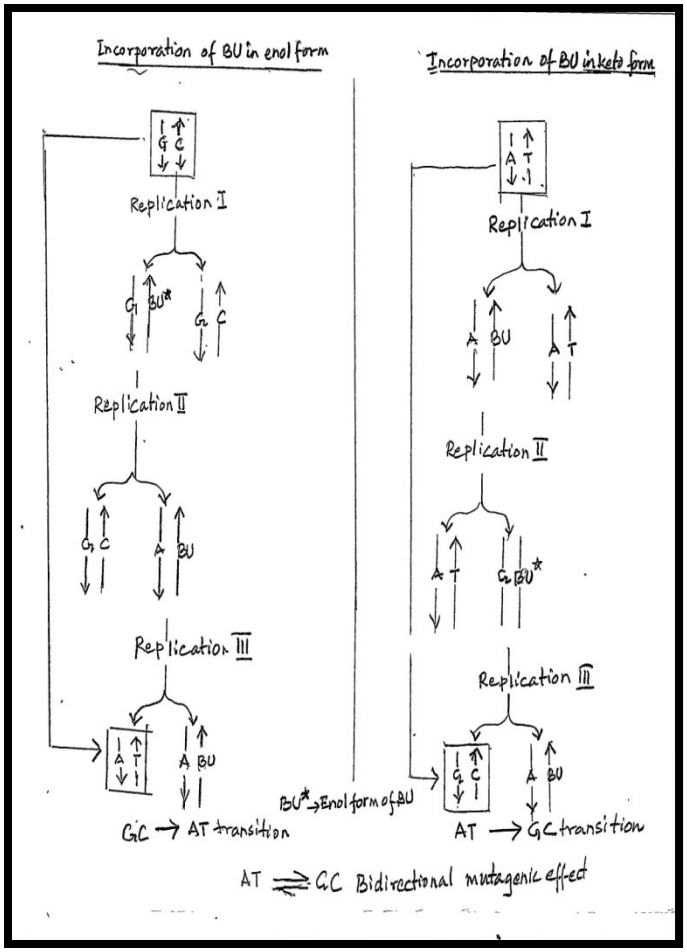
NTG

Nitrous Acid

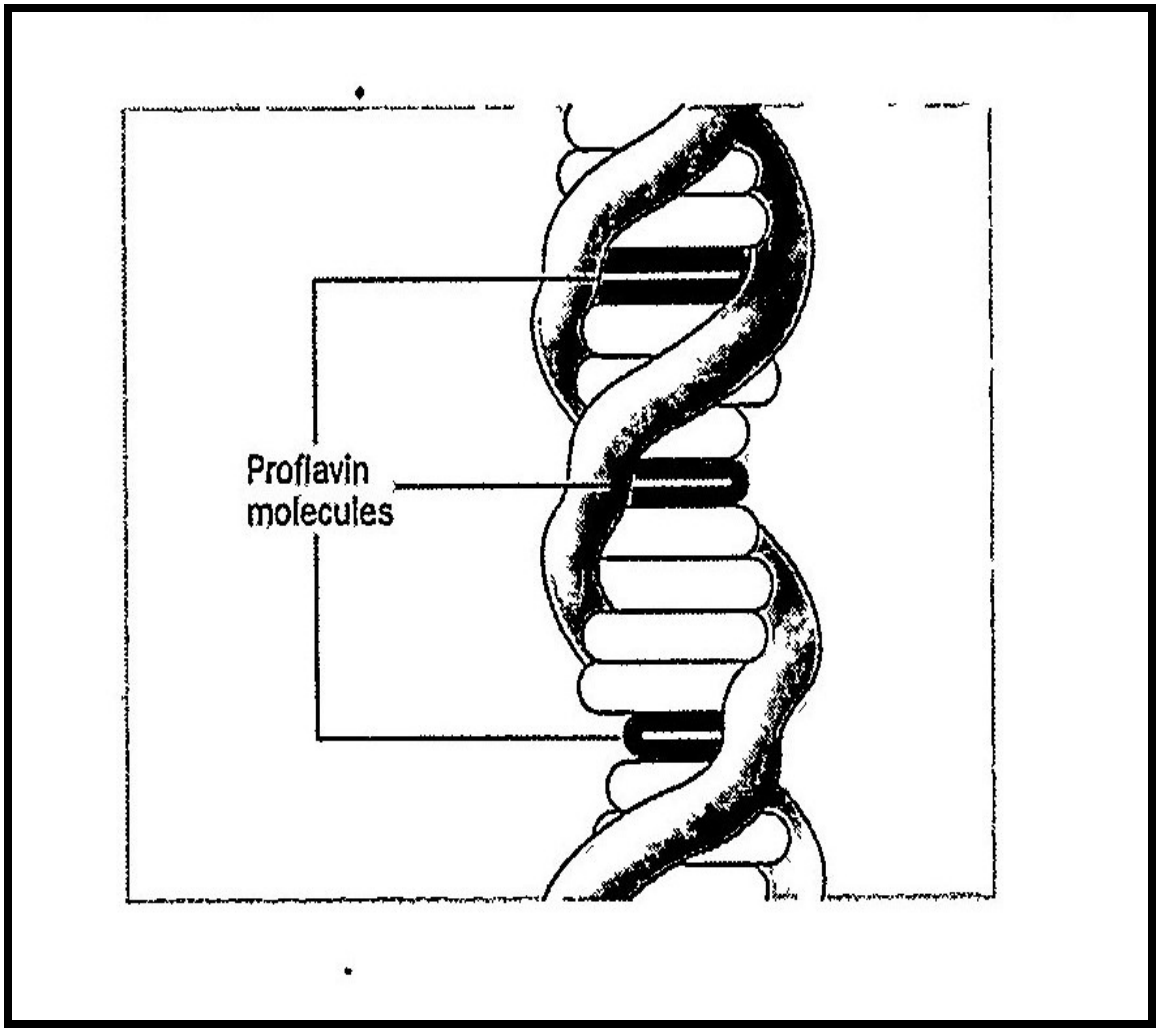
HNO_2

Effect of 5-Bromouracil





Intercalation of Proflavin into the DNA double helix



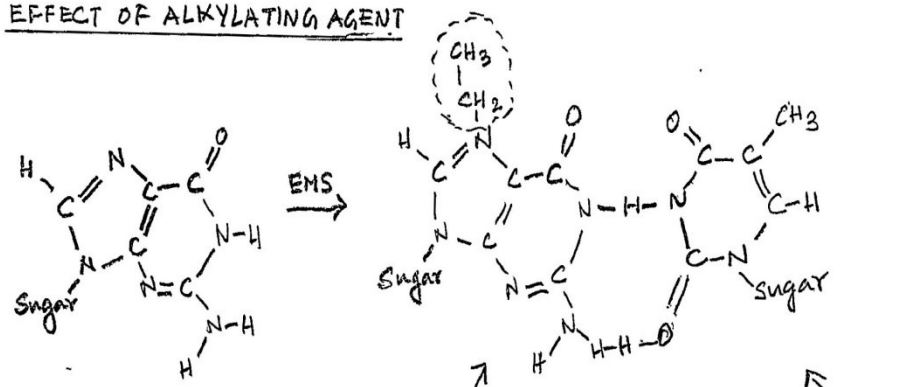
Taken from Principles of Genetics by Gardner,

Simmons & Snustad

Alkylating agents: This group of chemicals include nitrogen and sulphur mustards, ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS) , nitrosoguanidine (NTG) etc. These compound cause mutagenesis mainly by the transfer of alkyl groups to the bases of DNA , thus altering their pairing potential. The bases undergo mis-pairing. Wrong bases get incorporated into DNA causing mutation. For example EMS causes ethylation at the 7-N position or 5-O position of guanine. 7-Ethylguanine pairs with thymine instead of cytosine causing GC → AT transition. Some difunctional alkylating agents with two reactive alkyl groups cross link DNA molecules and induce chromosome breaks and different types of chromosomal aberrations. Therefore alkylating agents are less specific in their effects

.**Nitrous Acid**: It is a very potent mutagen. It causes mutation by oxidative deamination of bases having an amino group like adenine, guanine and cytosine. Adenine is deaminated to hypoxanthine which pairs with cytosine instead of guanine, thus causing AT →GC transition. Cytosine is deaminated to uracil which pairs with adenine thus causing GC →AT transition. Guanine is deaminated to xanthine but xanthine also pairs with cytosine so there is no direct mutation.

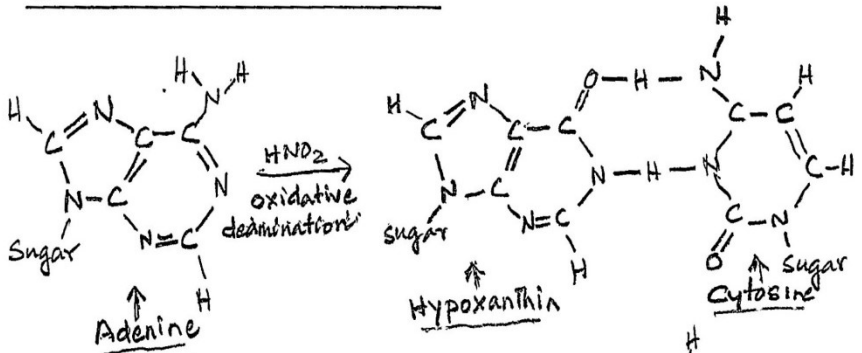
EFFECT OF ALKYLATING AGENT



Guanine

7-ethyl guanine paired with thymine

EFFECT OF NITROUS ACID



Adenine

Hypoxanthin

Cytosine

cytosine

Uracil

Adenine

Importance of Mutation

- **Role in Evolution**: Mutation is the major source of genetic variation; it provides raw material for evolution. Without mutation all genes would exist In only one form, allele would not exist. Different organisms would not be able to evolve and adapt to environmental changes.
- **Application in Plant Breeding**: Several important mutants have been obtained in different crops. For example, in wheat several useful mutations like branched ears, lodging resistance, amber seed colour and awned spikelet were utilized in plant breeding. In rice, several high yielding elite varieties like Reimei, Japonica, Indica have been obtained through mutations. In legumes, Hans pea, Ranjan lentil etc are mutants developed in India.
- **Increased Antibiotic Production**: Another important application of mutation is to increase the level of production of antibiotics like Penicillin from species of *Penicillium*.
- **Application in Horticulture**: In tissue culture several somaclonal mutants have been used to obtain improved horticultural varieties.