DNA Damage by Mutagens

Introduction

Mutagens are agents that increase the rate of mutations above the spontaneous rate. Hermann Joseph Müller (1927) demonstrated that X-rays (an environmental agent) could induce mutations in Drosophila melanogaster using specially designed X-chromosome. Since Müller's pioneering work a large number of mutagens have been discovered that alter the structure of DNA in all organisms.

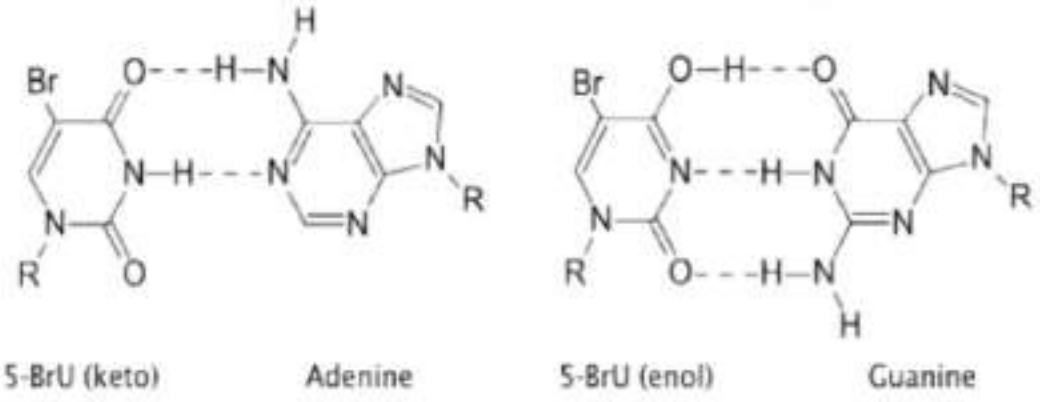
The first chemical shown to be mutagenic again in fruit flies was mustard gas by Charlotte Auerbach, a Jewish -German geneticist (immigrated from Nazi-Germany to Britain in 1933) and John Robson (1941). This and related chemicals were used as chemical weapons during World War I and so her work was kept confidential. She could finally publish her results in 1947-48. These compounds belong to a class of mutagens called alkylating agents. In addition some chemicals are not mutagenic unless processed by cellular enzymes.

Hermann J. Muller

Charlotte Auerbach

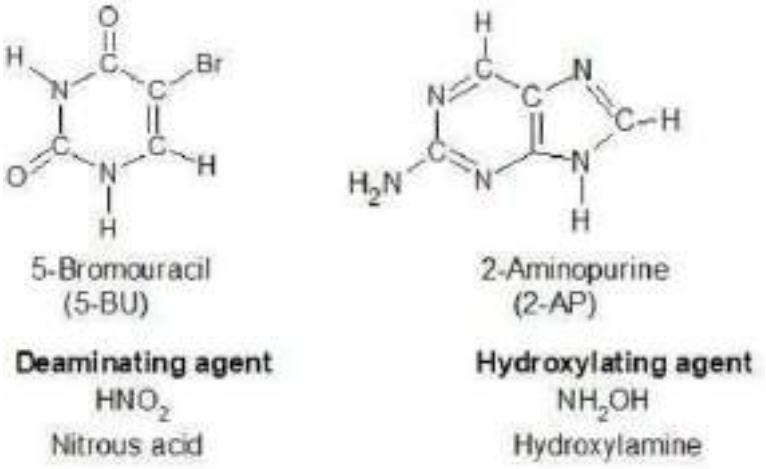
Major classes of chemical mutagens

Base analog is a substance that structurally resembles one of the four DNA bases so they compete for incorporating during DNA replication after conversion to the corresponding deoxynucleoside triphosphate. Examples include 5-bromouracil (5-BU; analog of thymine) and 2-amino purine (2-AP; analog of adenine). The presence of bromine at 5th position increases the frequency of tautomeric shifts (keto to enol). The enol form is less stable, preferentially mispairs with guanine causing G-C to A-T transition mutation (G-C → G-BU → A-BU → A-T. In a transition mutation a purine is changed to another purine or a pyrimidine to another pyrimidine. But if 5-BU was incorporated in its keto form opposite adenine and then undergoes a tautomeric shift, it will result in an A-T to G-C transition in the next round of replication. Due to its ability to cause transition mutation in both directions mutations caused induced by BU can also be reversed by it. 2-AP also acts in similar fashion to cause transitions in both directions, T-A←→C-G



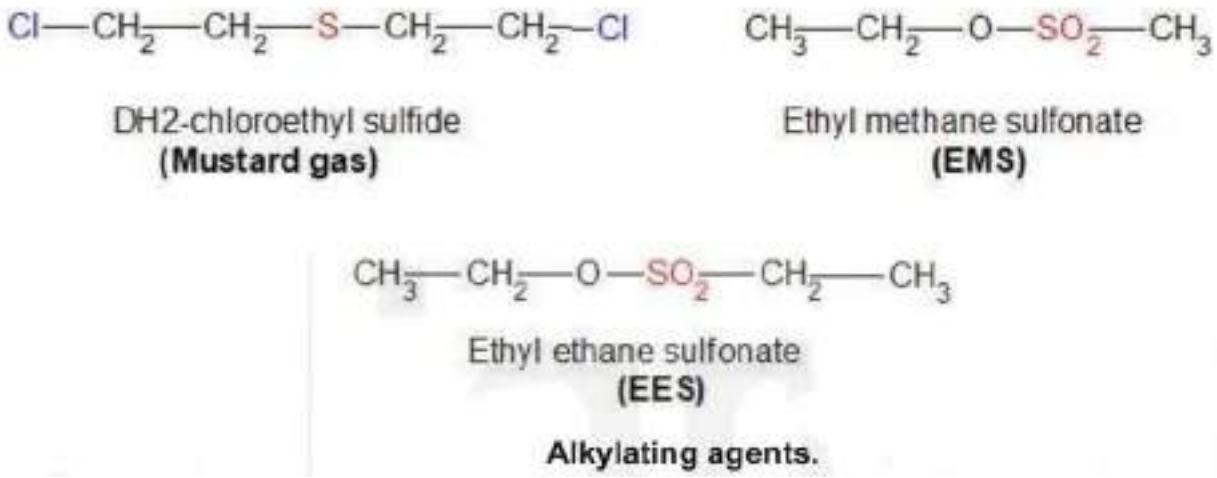
Base pairing with tautomers of 5-bromouracil (R-Sugar).

Oxidative deamination of bases can be induced by nitrous acid. It deaminates adenine to hypoxanthine, cytosine to uracil and guanine to xanthine; active on both replicating and non replicating DNA. Hypoxanthine pairs with cytosine, giving rise to A-T to G-C transition mutations. Similarly uracil pairs with adenine which is in the next round of replication produces G-C to A-C transitions. Xanthine pairs with cytosine as guanine.



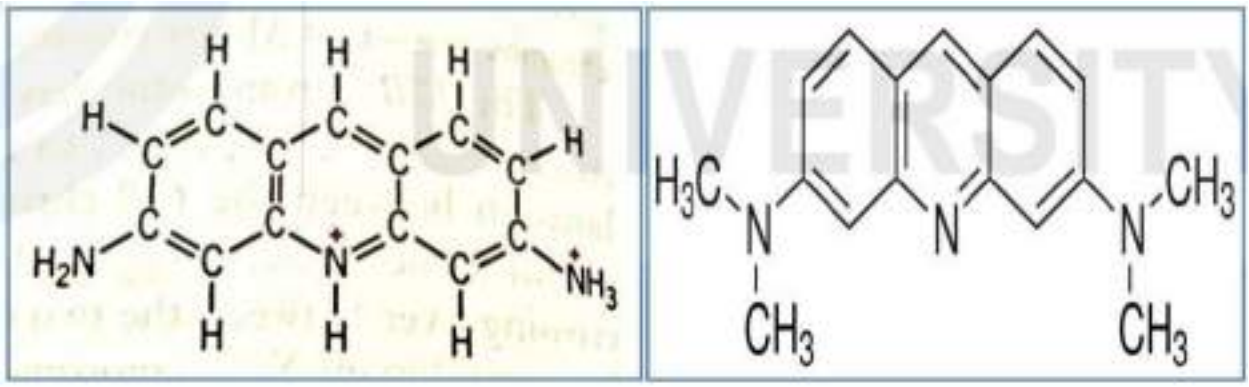
Chemical mutagens that cause base substitutions.

Alkylating agents methyl / ethyl methane sulphonate (MMS/EMS) and (nitrogen mustard) donate alkyl groups to DNA bases such as guanine (O^6 – ethyl and N-7-ethylguanine) and thymine (4-ethylthymine), resulting in mispairing, CG ←→ TA. They react less readily with adenine and cytosine. The mechanism of mutagenesis is complex as they can induce all types of mutations including transversion (Pu to Py & vice versa), cross linking of DNA strands (G-G cross links by nitrogen mustard) and chromosomal aberrations.



Alkylating agents.

Intercalating agents proflavin, acridine orange) are planar molecules that intercalate between stacked bases, distorting the structure of DNA. The replication of dye bound DNA lead to frame shift mutations in the coding regions, due to misalignment that results in addition / deletion of one / few base pairs, often not in multiples of three.



(a) Proflavin (2,8-Diamino acridine) (b) Acridine orange

Structure of intercalating dyes.

Effects

The phenotypic effect of mutations may be beneficial (enhance chances of survival and reproduction), deleterious (reduce fitness; increase susceptibility to disease or disorder) or neutral as they can occur anywhere in the genome. Functionally a base substitution in the coding region of a gene may result in the incorporation of a different amino acid **(missense mutation)** premature termination of protein synthesis **(non sense mutation)** or it will have no effect on amino acid sequence due to degeneracy of the genetic code or it affects non essential DNA **(silent mutation).** A missense mutation may either cause dramatic effect on protein function as in sickle cell anemia or it has minimal effect on protein function due to replacement with a chemically similar amino acid or one which has no direct effect on its function **(neutral mutation).**

A frameshift mutation is due to insertion or deletion of one / more nucleotides (not in multiples of three) in the coding region of a gene. All amino acids encoded following the mutation are altered. Some may cause premature termination as they encounter an in frame non sense codon. If additions / deletions occur in multiples of three, the protein can still be synthesised with few amino acids more / less, respectively. Therefore the effects are relatively less drastic.

Some mutations suppress the effect of another mutation. These mutations are **suppressor mutations**. They may occur within the original gene (intragenic) or in another gene (extragenic). In either case they are not reverse mutations because the original mutation still persists. Both types may suppress in several possible ways, for example a frameshift deletion in the original gene may be suppressed by a close by addition. Similarly an intergenic suppressor may work by expressing a mutant tRNA that inserts an amino acid even when it encounters a non sense codon.

Lethal mutations cause premature death of an organism. They are either dominant or recessive lethal. These genes encode for indispensible functions. There are some mutations that have a mutant phenotype only under restrictive conditions and wild type under permissive conditions (conditional Mutations). Mutations outside the coding region (UTRs, splice junctions, Promoter) may also influence gene function.