Chapter 11

MUTAGENS AND THEIR MODE OF ACTION

A. INTRODUCTION :

In the progressive evolution, the organisms have attained two sets of genetic material in the form of two sets of chromosomes. Alternation of generation which embodies two major events, syngamy and reduction is the very basis of evolution. Syngamy restores by means of fusion of male and female gametes two sets of chromosomes which may carry like or unlike genes from the maternal and the paternal side. The restored diploid sets of chromosomes in the cell proliferates by multiplication or by mitotic division of a cell during when the genetic information restored in the chromosomes is truely distributed in the resulting daughter cells. This process leads to the cell lineage. The organism comes to reproduction with diploid set of chromosomes and enters in to meiotic cell division, which is the mean of reduction division. During this stage the maternal and paternal chromosomes pair, exchange genetic information and separate. Thus the resulting products are unidentical to each other with respect to genetic information for meiotic pairing and crossing over enables the reshafling of genetic information. In primitive organism the proliferation of haploid stage occurs where only one set of chromosomes exists. In the process of evolution the nature has seem to have disfavoured it for loss of genetic information provides no means of restoring it as there is no duplicate set.

During the process of cell division there is a chance for the organisms to undergo change which can readily manifested if such changes are favoured by nature. It is now known the most heriditary information of cell is carried by DNA. In growing or adult cells most DNA resides in chromosomes of the nucleus. However, a small fraction (0.1 to 10 %) is found in plastids and mitochondria (Ernst Freese, 1971). The cell lineage effected by mitotic division confires on cell the constant amount of chromosomal DNA per cell. The knowledge of DNA structure and duplication in conjunction with chemistry of DNA duplication makes it possible to explain many mutagenic effects in molecular terms. The DNA doublehelix consists of two strands of polydeoxy nucleotides which are held together by hydrogen bonds between complementary bases A-T and G-C and by Van der Waals forces between adjacent bases that are stacked parallel and one above the other along the axis. During duplication DNA unwinds by rotations in the opposite direction. To maintain a reasonable rate of rotation in the viscous cellular millieu swivel points are introduced by DNAse that cuts one of the two strands at certain places. Each of the separated strand is copied which apparently requires the pairing (by hydrogen bonding) of complementary bases. Two new double strands are produced each contains one old and one new strand. At some time later the swivel gaps are closed again by ligase (Earnst Freese, 1971).

It is established that genetic information for the proteins is carried by the bases. The changes in the base sequence leads to the change in the genetic code and eventually in the protein which may be menifested by the organisms as genetic change or mutation, such type of change can also be induced artificially has amply been demonstrated by many workers. The mechanism of alteration at molecular level, the causes and type of alterations have extensively been surveyed, studied and published in series of books by Hollaender (1971).

B. CAUSES AND TYPES OF CHANGES OF HERIDITARY MATERIAL :

1. General causes of genetic alterations :

Chromosomes are large molecular structures which undergo complex reactions during duplication, segregation and differentiation. Many agents such as high energy radiations, unsaturated chemicals, enzymes are known to bring about change or modification in structure of DNA (Hollaender, 1971).

According to Earnst Freese (1971) such agents bring about changes as follows :

Physical	Mechanical tearing apart of DNA.
	Cutting by ionizing radiations.
	Nondisjunction of chromosomes by high temperature.
	Cutting by 32p decay.

Chemical	Alteration or removal of DNA bases. Incorporation of altered bases. Intercalation of oligocyclic aromatic compounds. Alteration of DNA backbone.
Enzymes	Production of chemicals affecting DNA. Mistakes of the DNA replicating system. Alteration of DNA replicating system. Mistake in recombination or repair.

---

2. Types of genetic alterations :

An alteration in the genetic information may be lethal to the cell or all of its early progeny or it may produce any one of three major types of heriditary alterations : (a) a change in ploidy, (b) a recombination of existent information or (c) a mutation of such information.

Ploidy implies that a nucleus contains, in place of the normal set of 2 n chromosomes, 3 n, 4 n etc. Polyploidy can be caused by colchicine or alkaloids, which prevent spindle and cell plate formation and thus chromosome segregation.

Recombination is the reciprocal exchange of information between two homologous DNA molecules or chromosomes. Recombination

can be induced by agents which induce inactivating DNA alterations. It can also lead to greatly altered or even nonviable progeny.

A mutation is any heriditary alteration in the information content or in the distribution of the heriditary material in an organism or cell other than ploidy or recombination change. A mutation may lead to morphological changes or to biochemical requirements. It may either produce an altered phenotype or lethal effect.

Very large chromosome mutations can be observed cytologically in approprite organisms. They consist of deletion, insertion or inversions of chromosome pieces (Fig.2.1). If a mutant behaves as if only one biochemical function has been altered and if it can recombine with all other mutants, it is called a point mutation. A point mutation is not precisely defined in terms of nucleotide pairs affected. However the term is generally quite in usage (Earnst Freese). 1971

C. PRIMARY DNA ALTERATIONS AND THEIR GENOTYPIC AND PHENOTYPIC CONSEQUENCES :

Earnst Freese recognised chemical or enzymatic DNA alterations in to three types : (1) Nonheriditary, (2) Mutagenic, (3) Inactivation of DNA alterations (Fig.2.2).

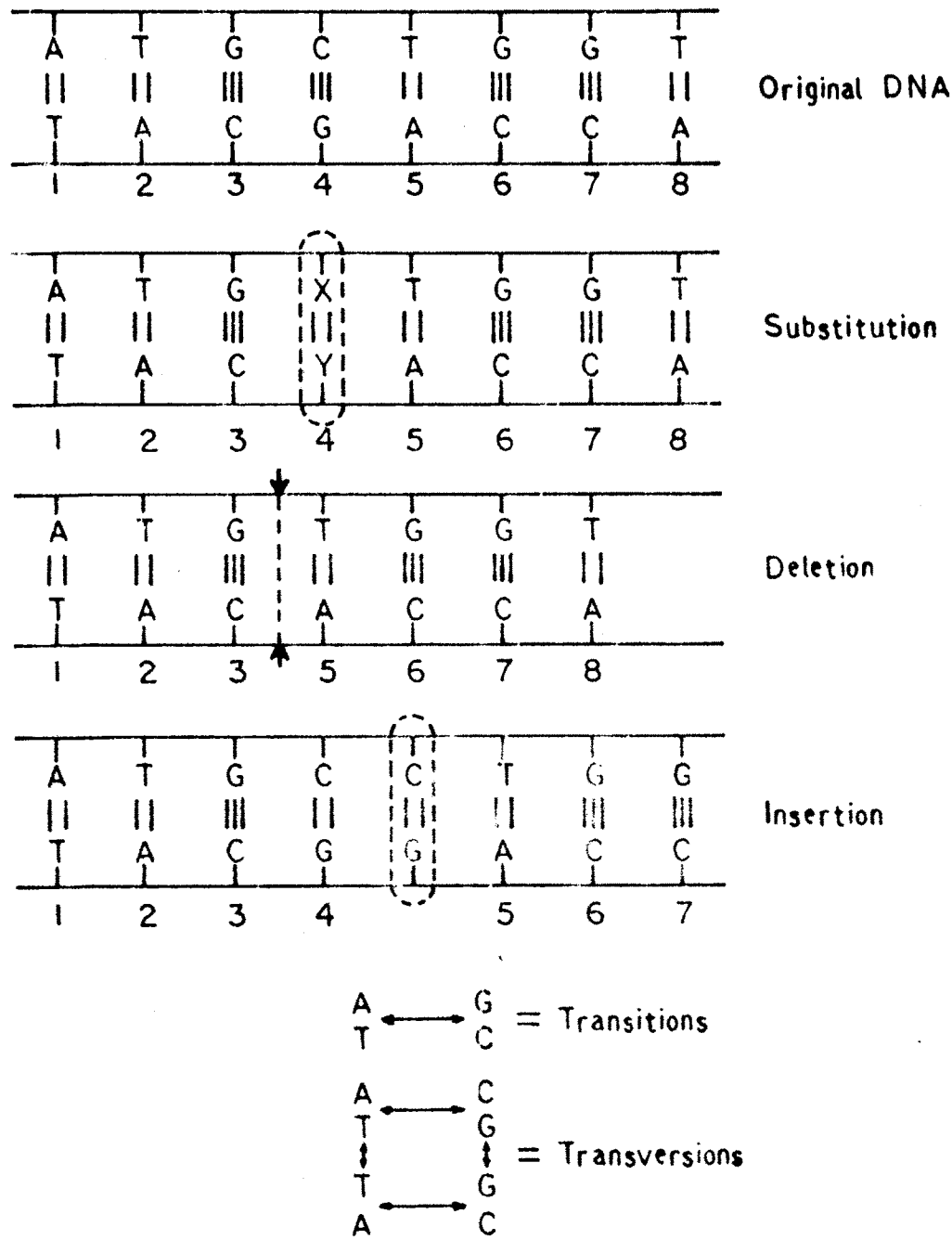


Fig. 2·1 UPPER: TYPES OF POINT MUTATIONS. LOWER: TYPES OF BASE-PAIR SUBSTITUTIONS.

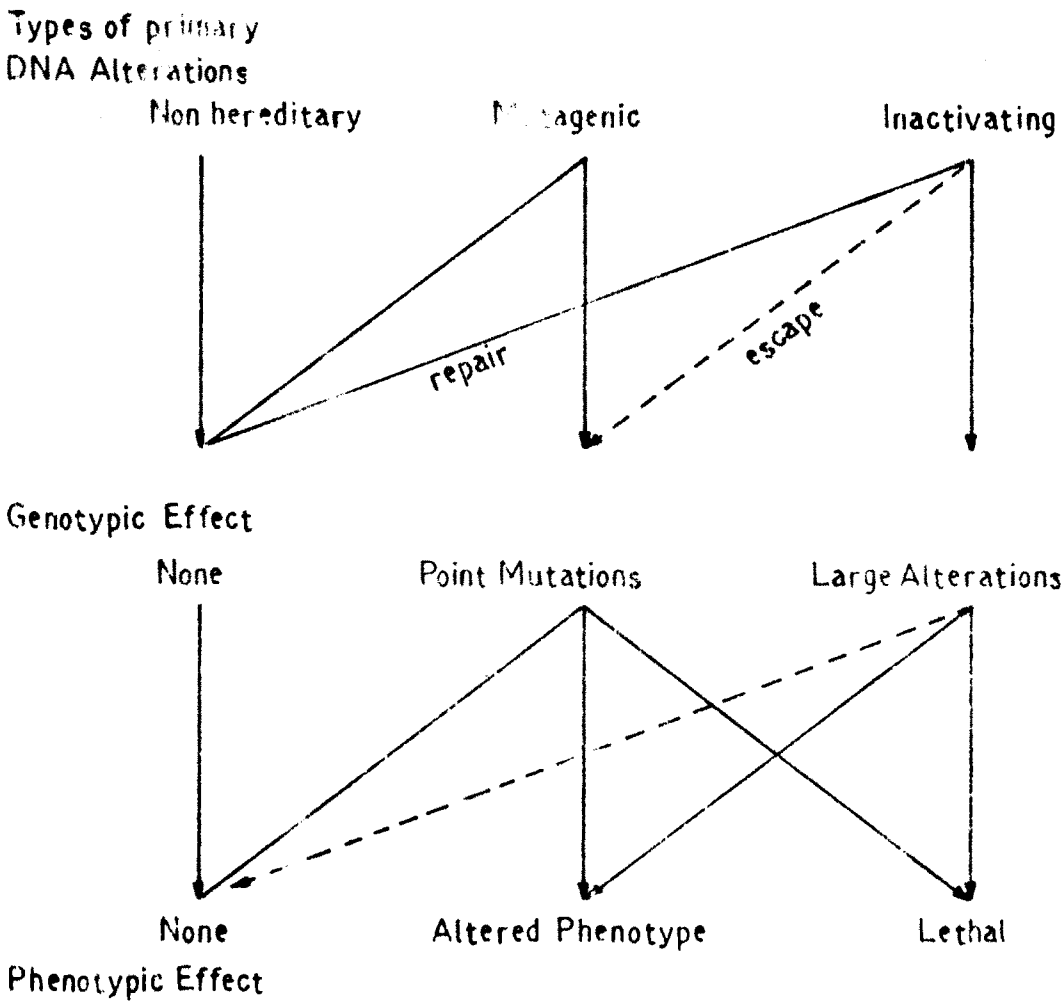


Fig.2·2 TYPES OF PRIMARY DNA ALTERATIONS AND THEIR POSSIBLE GENOTYPIC AND PHENOTYPIC CONSEQUENCES.

1. Nonheriditary DNA alterations :

Nonheriditary DNA alterations prevent the duplication of DNA nor induce changes in the DNA information (mutation). They usually involve the chemical or enzymatic modifications of the DNA bases at sites that do not interfere with base pairing, such as methylation or hydroxymethylation of pyrimidines of 5 position.

2. Mutagenic DNA alterations

Mutagenic DNA alterations also do not prevent the duplication of DNA but usually give rise to the change of one or a few nucleotide pairs (point mutations) in some of the progeny DNA. They do not induce large chromosomal alterations. Mutagenic alterations either consist of minor base modifications, which alter the specificity of hydrogen bonding to complementary bases or they are caused by base-pairing mistakes induced by some agents during DNA duplication. The resulting point mutation in DNA (genotypic effect) may produce no phenotypic (undetectable mutation) or a phenotypic change (detectable mutation), or it may be lethal to the cell if it alters a vital gene.

3. Inactivating DNA alterations

Inactivating DNA alterations prevent DNA duplication across the altered site, unless they have been eliminated by

repair, Inhibition of DNA synthesis has been demonstrated for many chemicals e.g., alkylating agents (Yamamoto et al., 1966; Iyer & Szybalski, 1963; Tanaka, 1965). That this replication block is caused by a reaction with DNA itself is inferred by the experimental demonstration on bacteriophage. When they were treated outside the cell with alkylating agents they are often inactivated (Garnst Freese, 1971). Agents inducing DNA alterations also induce chromosome breaks and chromosome mutations possibly as a consequence of the block in DNA synthesis. Thus both mutagenic and inactivating DNA alterations induce mutations at the molecular level (Fig. 2.3).

D. THE EFFECT OF DIFFERENT AGENTS ON DNA AND CHROMOSOMES :

Many chemicals as well as ionizing and UV radiations produce mutagenic effects. One can distinguish agents that mutate only replicating DNA (base analogs, intercalating agents) and others that alter resting DNA and exert their effect even when they are removed before DNA duplication is allowed to proceed (alkylating and radical-producing agents).

Since, there are large number of unsaturated or unstable chemical compounds and several other physical agents which cause mutations, the scope of mechanism of action of mutagens will be restricted to ionizing radiations and alkylating agents for in the present investigation comparative

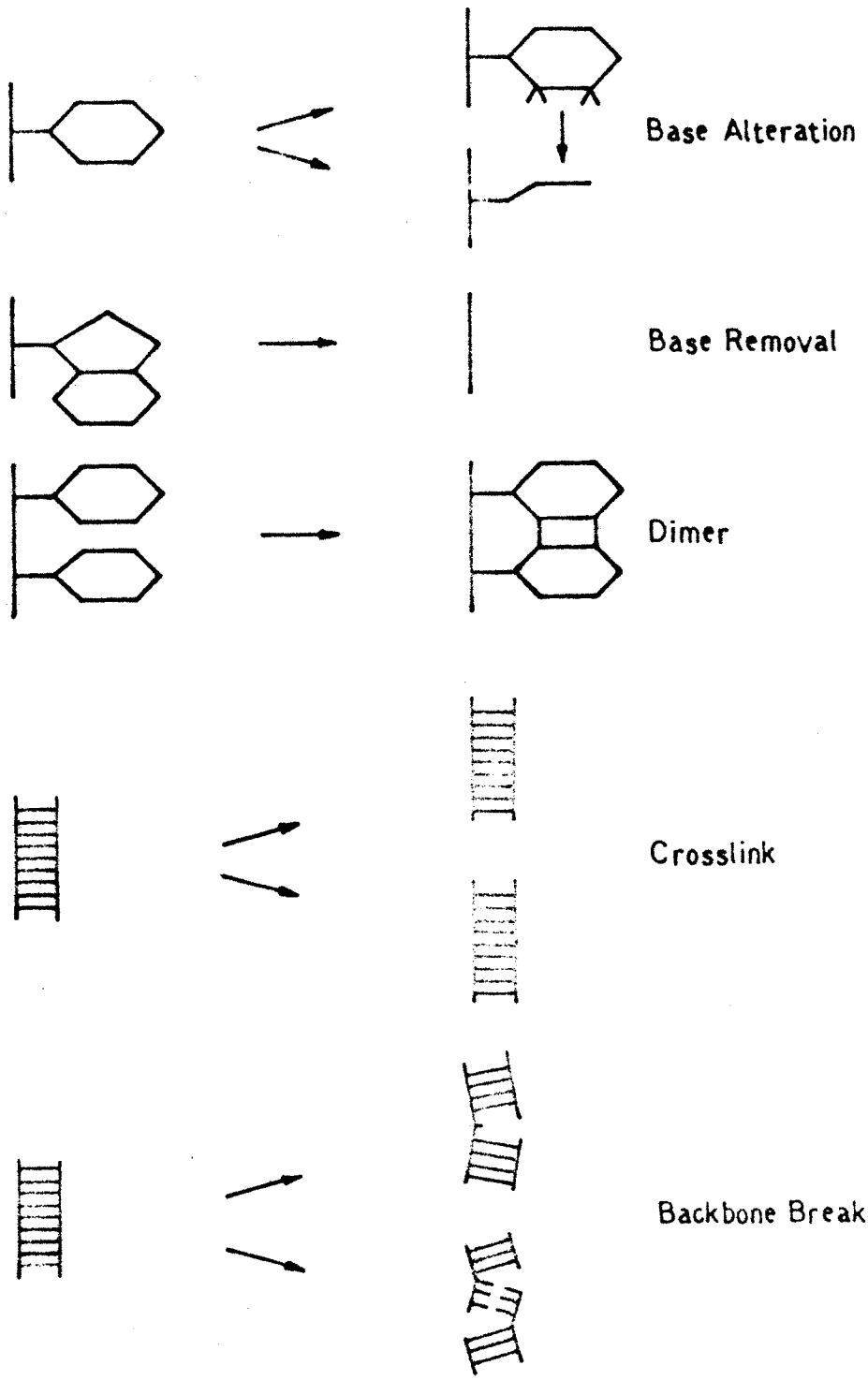


Fig.2·3 INACTIVATING DNA ALTERATIONS.

study of effect of these agents on the physiological parameters has been studied.

1. Ionizing radiations

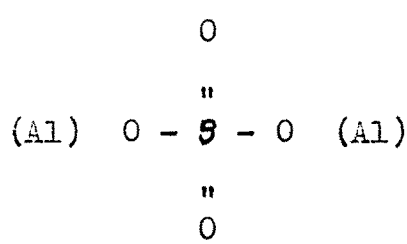
α, β, γ or X-rays are usually treated as if they produced DNA or chromosome alterations by directly hitting the heriditary material. According to Hollaender (1971) alterations in dehydrated cells, such as seeds or spores may be more dependent on direct DNA hits but these events occur at a much lower frequency. Ionizing radiations knock single electrons cut of molecule or they excite molecules by radiation transfer to decompose in to two radicals. The liberated electron is trapped by water to form the hydrated electron e^-_{99}, which acts like other radicals. Any of these radicals can react with water. If they encounter the biradial oxygen (.O-O.), they tend to react with it preferentially to form peroxy radicals (R-O-O.), which in turn can react with another radical to produce the chemically more stable peroxide.

Lethal or chromosome breaking effect of X-ray is greatly increased in the presence of oxygen. X-radiation of DNA in the presence of oxygen produces breakdown products of DNA bases and causes backbone linkage. Some of the reaction products of pyrimidines are hydroxy-hydroperoxy bases and T (Thymine) derivatives are stable; where as the C (Cytosine) derivatives decompose in to 5-hydroxycytosine or isobarbituric

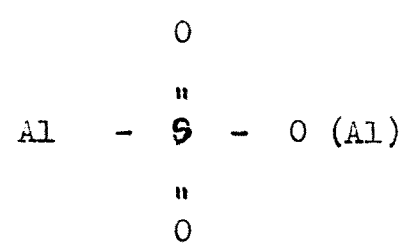
acid. Both the base alterations and the backbone breakage constitute inactivating DNA alterations, which apparently are caused to a large extent by radical reactions.

2. Alkylating agents

Alkylating agents include a large number of molecules that carry one or two or more of the alkylating groups and are consequently called mono-, di-, or polyfunctional alkylating agents. If the alkylating groups in a polyfunctional alkylating agent are covalently linked, they can crosslink DNA strands. Even mono functional agents are occasionally known to cause cross-linking. This is because alkylation causes break in DNA backbone. The broken ends react to cross-link.



Diethyl sulfate (DES)



Ethyl methane sulfonate (EMS)

Thus alkylating agents are known to induce point mutations (mainly transitions), chromosome breaks, and chromosome mutations.

Hollaender tried to explain as to how point mutation is induced by alkylating agents. According to him point mutagenic effect, in which mainly transition forms are

observed, apparently is caused by the alkylation of G (Guanine) at the 7 - position (Fig.2.4) chemically G is the preferable alkylated base in double stranded DNA, where as the alkylation of A or C is minor. Eventually the alkylated G (or A) hydrolyzes from DNA and the backbone of the unstable depurated DNA breaks. Following the discovery of the alkylation of G and its liberation from DNA, this effect has been considered the major mechanism by which alkylating agents cause a DNA backbone breakage.

The removal of G from DNA is clearly lethal, because depurination of transforming DNA by low pH inactivates DNA.

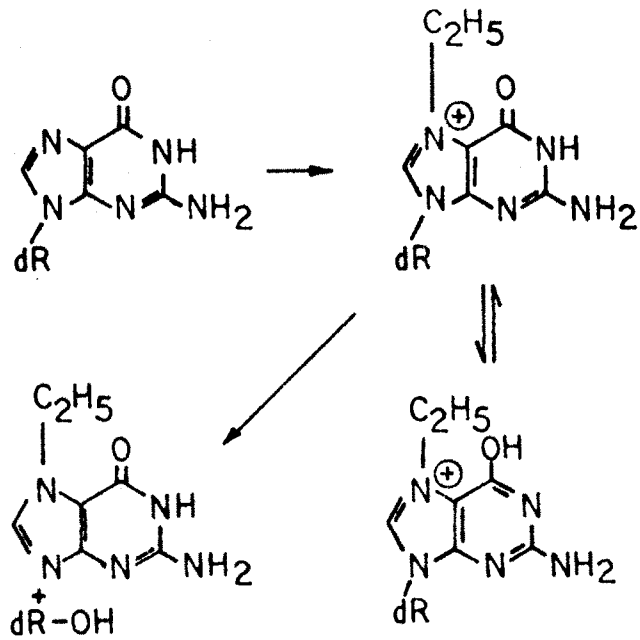


Fig.2·4 ALKYLATION (ETHYLATION) OF GUANINE (AT THE 7N POSITION) AND RESULTING TAUTOMERIC SHIFT (LOWER RIGHT) AND DEPURINATION.