Methods of inducing mutations and C / B technique

Mutagens

Mutations can be induced by anumber of agents; the agents capable of inducing mutations are called mutagens. Mutagen is a natural or human-made agent (physical or chemical) which can alter the structure or sequence of DNA. The different mutagenic agents may be classified into the following two broad groups:

1) Physical mutagens

2) Chemical mutagens

Physical mutagens

The different types of radiations having mutagenic properties are known as physical mutagens. The radiations may be a part of the electromagnetic spectrum having shorter wavelength and higher energy than visible light (eg: uv rays, X rays, gamma rays and cosmic rays) or may be particulate radiations produced by the decay of radio isotopes.

Radiations

Radiation was the first mutagenic agent known; its effects on genes were first reported in the 1920's. Radiation itself was discovered in 1890's: Roentgen discovered X-rays in 1895, Becquerel discovered radioactivity in 1896, and Marie and Pierre Curie discovered radioactive elements in 1898. These three discoveries and others led to the birth of atomic physics and our understanding of electromagnetic radiation.

Radiations are grouped into two classes depending on the kind of effects they have on the atoms in their path:

1. ionizing and

2. non – ionizing radiations

Non – ionizing radiations

Ultraviolet rays are the only non ionizing radiation with mutagenic properties. The wave length ranges from 100 – 3900 A^{o} And they are specifically absorbed by puriines and pyrimidines present in DNA. The maximum absorption of UV rays by DNA as well as by pyrimidines, particularly thymine occurs at the wavelength of 254 nm, which is also the most mutagenic wavelength of UV.

The mutagenic action of uv is the consequence of both its direct and indirect effects on DNA. The direct effect of uv on DNA is of two types: formation of (1) pyrimidine dimmers and pyrimidine hydrates.

Ionizing radiations

Ionizing radiations are so called because they cause ionization in the atoms present in their path. There are two types of ionizing radiations: (1) particulate and (2) non particulate radiations. Particulate radiations consist of high energy atomin particles generated due to radioactive decay. The non particulate ionizing radiations are represented by X rays and gamma rays which are high energy radiations composed of photons.

The genetic effects of radiations may be (1) direct or (2) indirect. The direct effect of radiations is produced due to ionizations directly in the DNA molecule, while their indirect effect is produced through ionizations ini molecules other than DNA and is believed to be mediated by free radical formation.

Sources of radiation

Natural sources of radiation produce so-called background radiation. These include cosmic rays from the sun and outer space, radioactive elements in soil and terrestrial products (wood, stone) and in the atmosphere (radon). One's exposure due to background radiation varies with geographic location.

In addition, humans have created artificial sources of radiation which contribute to our radiation exposure. Among these are medical testing (diagnostic X-rays and other procedures), nuclear testing and power plants, and various other products (TV's, smoke detectors, airport X-rays).

Chemical mutagens

The first report of mutagenic action of a chemical was in 1942 by Charlotte Auerbach, who showed that nitrogen mustard (component of poisonous mustard gas used in World Wars I and II) could cause mutations in cells. Since that time, many other mutagenic chemicals have been identified and there is a huge industry and government bureaucracy dedicated to finding them in food additives, industrial wastes, etc. It is possible to distinguish chemical mutagens by their modes of action; some of these cause mutations by mechanisms similar to those which arise spontaneously while others are more like radiation in their effects.

1. Base analogs

These chemicals structurally resemble purines and pyrimidines and may be incorporated into DNA in place of the normal bases during DNA replication:

- **bromouracil** (BU)--artificially created compound extensively used in research. Resembles thymine because it has Br in the 5 position instead of methyl group and has the same effect on its base pairing behavior as that of –CH 3 in the same position and therefore 5 BU behaves like thymine and usually pairs with adenine.
- **aminopurine** --adenine analog which can pair with T or (less well) with C; causes A:T to G:C or G:C to A:T transitions. Base analogs cause transitions, as do spontaneous tautomerization events.

2. Chemicals which alter structure and pairing properties of bases

There are many such mutagens; some well-known examples are:

- **nitrous acid**--formed by digestion of nitrites (preservatives) in foods. It causes C to U, meC to T, and A to hypoxanthine deaminations. Hypoxanthine in DNA pairs with C and causes transitions. Deamination by nitrous acid, like spontaneous deamination, causes transitions.
- **nitrosoguanidine, methyl methanesulfonate, ethyl methanesulfonate**-chemical mutagens that react with bases and add methyl or ethyl groups. Depending on the affected atom, the alkylated base may then degrade to yield a baseless site, which is mutagenic and recombinogenic, or mispair to result in mutations upon DNA replication.

3. Intercalating agents

Acridine orange, proflavin, ethidium bromide (used in labs as dyes and mutagens). All are flat, multiple ring molecules which interact with bases of DNA and insert between them. This insertion causes a "stretching" of the DNA duplex and the DNA polymerase is "fooled" into inserting an extra base opposite an intercalated molecule. The result is that intercalating agents cause frameshifts.

4. Agents altering DNA structure

This is used as a "catch-all" category which includes a variety of different kinds of agents. These may be:

- --large molecules which bind to bases in DNA and cause them to be noncoding--we refer to these as "bulky" lesions (eg. **NAAAF**)
- --agents causing intra- and inter-strand crosslinks (eg. **psoralens**--found in some vegetables and used in treatments of some skin conditions)
- --chemicals causing DNA strand breaks (eg. **peroxides**)

What these agents have in common is that they probably cause mutations not directly but by induction of mutagenic repair processes.

Detection of mutation

The occurrence of mutational event at the gene level is detected by the altercation it brings about in the phenotypic expression of one or more traits of the concerned organism. Therefore the efficiency of detection of mutations will depend largely on the availability of techniques for an easy and rapid scoring of the mutant phenotypes in very large populations. Scoring of some types of mutations in certain organisms is relatively easy. For example, mutations for antibiotic resistance in bacteria are simply detected by plating the bacterial cells on a medium containing a lethal concentration of the concerned antibiotic (selective medium); the colonies that develop on such a medium will be produced by cells resistant to the antibiotic. The medium lacking the antibiotic is called the non selective medium.

$$\text{Frequency of mutant cells (\%)} = \frac{\text{Number of colonies on the selective medium}}{\text{Number of colonies on the non selective medium}} \times 100$$

Detection of morphological mutations in eukaryotes requires examination of each individual of the population for the mutant phenotype; this is not only tedious requiring time, but is also a source of errors in the data. Therefore, elaborate procedures for mutation detection have been developed in some eukaryotes . eg; Drosophila, maize etc. These procedures employ specific markers to facilitate the identification of chromosomes from the treated or irradiated individuals. Clearly these techniques detect only germinal mutations. In drosophila, several specila genetic stocks have been constructed for the detection of lethal and visible mutations in X chromosomes and in autosomes; the two genetic stocks most commonly used for mutation detection in X chromosome are (1) CIB and (2) Attached X stocks.

C/B Technique

This method was invented by Muller and used for the unequivocal demonstration of mutagenic action of X rays. In this method, females containing one normal X-chromosome and another X-chromosome (C/B) containing extra 3 genes are used for the analysis. Out of the 3 extra genes, one gene suppresses crossover (c), the other is a recessive lethal (L) in heterozygous condition, and the last gene is semidominant marker, Bar (B) gene.

Females containing C/B chromosome are called as C/B stock drosophila. The normal males are exposed to mutagenic source for a fixed period and then mated to the C/B stock drosophila. Males containing C/B chromosome will die due to the effect of lethal genes, whereas norm ill males and females both normal and with C/B will survive.

Females with C/B chromosomes and identified by barred phenotype are selected and crossed to normal males. In this next generation 50% of males (which have received the C/B gene) will die.

If mutation has occurred in normal X chromosome then even the normal male (without C/B gene) will die. If no mutation has occurred all the other 50% of males will survive. The frequency of lethal mutations can be accurately scored in large samples. This technique is simple, rapid and there is little chance of an error in scoring. However, it is suitable for the scoring of sex linked recessive lethal only.

The attached X chromosome technique

This technique is based on attached – X females (X- XY) and is designed to study visible sex linked mutations in Drosophila. Mutagen treated males are mated with attached X females. The X- XX (super female) and YY progeny produced from such crosses do not survive; only X- XY (female) and XY (male) progeny are recovered. All male (XY) progeny receive their Y chromosomes from their attached X female parent, while their X chromosomes is contributed by their mutagen treated male parent. If a visible mutation was induced in the X chromosomes of any sperm produced by the mutagen treated male, it will be expressed in the male progeny. Therefore all the male progeny obtained from the cross are scored for visible mutations. The frequency of a visible mutation is expressed as the ratio

between the number of progeny males showing a mutation and the total number of males in the progeny.

Detection of mutations in plants

Techniques for the detection of mutations are relatively poorly developed for plant species. The following two approaches are generally adopted for this purpose. (1) In some species, eg; maize, strains homozygous for several recessive genes as well as for dominant alleles of these genes are available. In such cases seeds or plants of a strain homozygous for several dominant genes are treated with a mutagen. The plants (M1 generation) are crossed with a strain having the recessive forms of the traits governed by the concerned genes are counted and the frequency of mutation for a gene is estimated as follows:

$$\text{Mutation frequency (\%) for a gene} = \frac{\text{Number of plants having the recessive form of trait governed by the gene}}{\text{Total number of plants in the progeny}} \times 100$$

The plants showing the recessive form of such a trait will receive one recessive allele from the tester parent with the recessive traits, while the other recessive allele would have been produced due to mutation in the mutagen treated parent.