CHAPTER 7 LECTURE NOTES

I. Mutation Overview

A. Definitions

1. Mutation = a process that produces a gene or chromosome that differs from the wild type
2. Mutation = the gene or chromosome that results from a mutational process
3. a mutant is the organism or cell whose changed phenotype is attributed to a mutation

B. General Types

1. Gene mutation = the allele of a gene changes (this chapter)
2. Chromosome mutation = segments of chromosomes, whole chromosomes, or entire sets of chromosomes change (will be considered in Ch. 8 and 9)

C. What does wild type (wt) mean? Wild type is an arbitrary standard for what "normal" is for an organism. Please remember that what is considered wild type today may have been a mutant in the evolutionary past.

D. Direction of the mutation

1. Forward mutations are changes away from the wt
2. Reverse mutations (reversions) are changes from the mutant allele back to the wt allele

E. Mechanisms for gene mutation

1. Errors in DNA replication
2. Errors in DNA repair
3. Environmental mutagen causes DNA damage that is not repaired correctly
4. Transposons and insertion sequences (a mobile DNA elements that can move from one location in the chromosome to another; the element may "jump" into a gene thereby mutating it)

F. Why study gene mutation?

1. Variants in genes (which are caused by mutations) are needed to study the transmission of traits
2. Mutations can tell the researcher about the function of a gene product in a biological system
3. Mutations are the basis for cancer and other genetic diseases
4. Gene mutations serve as the source for most alleles in a population and is therefore the origin of genetic variation within a population
5. Mutations drive evolution: mutations are the raw material upon which natural selection acts.

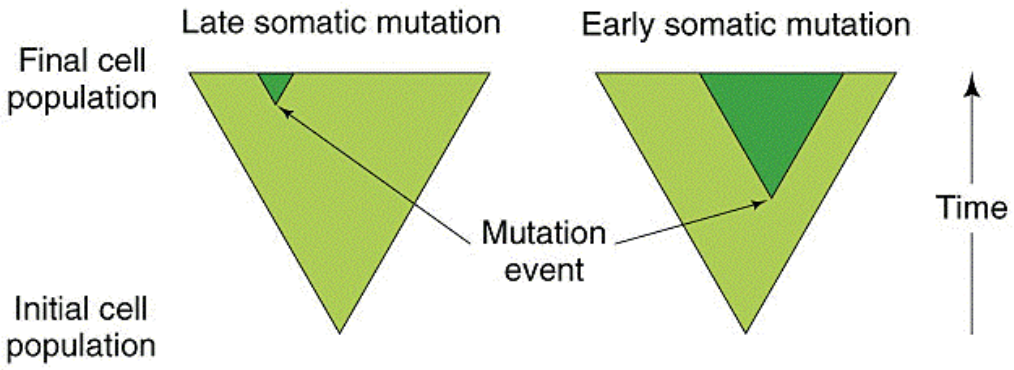
II. Classification of mutations

A. General info

1. Various schemes for classification depending upon which aspect of mutation is being examined
2. Classes are not mutually exclusive

B. Point of origin

1. Somatic mutations
   a) mutations that are in the somatic tissues of the body
   b) mutations are NOT transmitted to progeny
   c) the extent of the phenotypic effect depends upon whether the mutation is dominant or recessive (dominant mutations generally have a greater effect)
   d) the extent of the phenotypic effect depends upon whether it occurs early or late in development (early arising mutations have a greater effect)



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   e) sectoring phenotypes may be seen when the mutation occurs during embryonic development
   f) cancer caused by somatic mutations
2. Germinal mutations
   a) mutations that are in the germ tissues of the body
   b) mutations MAY BE transmitted to progeny
   c) dominant mutations are seen in first generation after the mutation occurs
   d) if a female gamete containing an X-linked mutation is fertilized, the males will show the mutant phenotype
   e) recessive mutations will only be seen upon the chance mating with an individual carrying the recessive allele too; thus, the recessive mutation may remain hidden for many generations

C. Phenotypic effects

1. Morphological mutations are mutations that affect the outwardly visible properties of an organism (i.e. curly ears in cats)
2. Lethal mutations are mutations that affect the viability of the organism (i.e. Manx cat).

3. Conditional mutations are mutations in which the mutant allele causes the mutant phenotype only in certain environments (called the restrictive condition). In the permissive condition, the phenotype is no longer mutant. (i.e. Siamese cat – mutant allele causes albino phenotype at the restrictive temperature of most of the cat body but not at the permissive temperature in the extremities where the body temperatures is lower).

4. Biochemical mutations are mutations that may not be visible or affect a specific morphological characteristic but may have a general affect on the ability to grow or proliferate.

a) Most microorganisms are prototrophs which means that they can grow on a simple growth medium including an energy source and inorganic salts. Biochemical mutations include those that affect proteins or enzymes required to grow on various nutrients or to synthesize various components. Thus, these mutations cause the microorganisms to become auxotrophs (they must be supplied with additional nutrients if they are to grow). For example, the bacterium *Escherichia coli* does NOT require the amino acid tryptophan for growth because they can synthesize tryptophan. However, there are *E. coli* mutants that have mutations in the *trp* genes. These mutants are auxotrophic for tryptophan, and tryptophan must be added to the nutrient medium for growth.

b) Humans can also have biochemical mutations (also called inborn errors in metabolism). Such examples include hemophilia, phenylketonuria, and galactosemia.

D. Loss of function vs. gain of function mutations

1. Loss of function mutations are those that destroy the function of the gene product. Many times in diploid organisms, these are recessive mutations because the other wild type allele still encodes a functional gene product. However, it is possible to have a dominant loss of function mutation in which the mutant gene product interferes with the activity of the gene product from the wild type allele.

a) Null mutation = loss of function mutation where gene product is completely inactive

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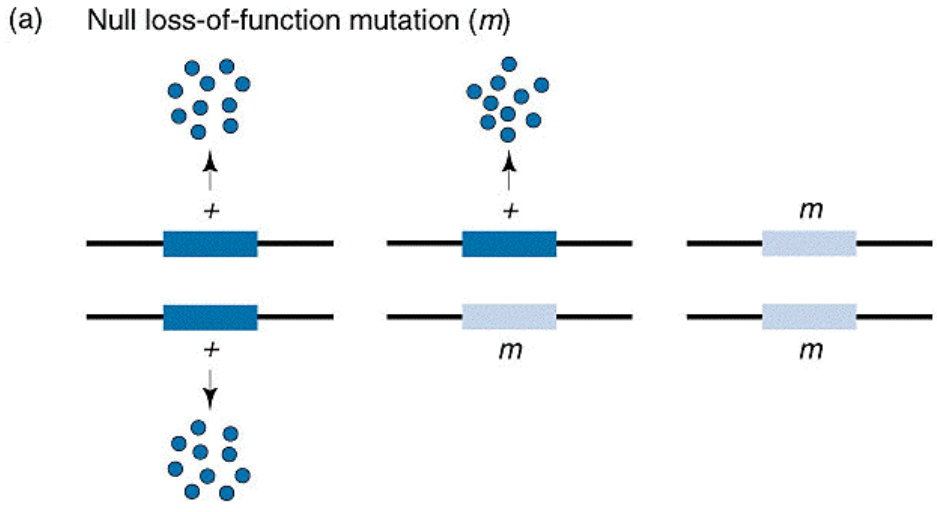
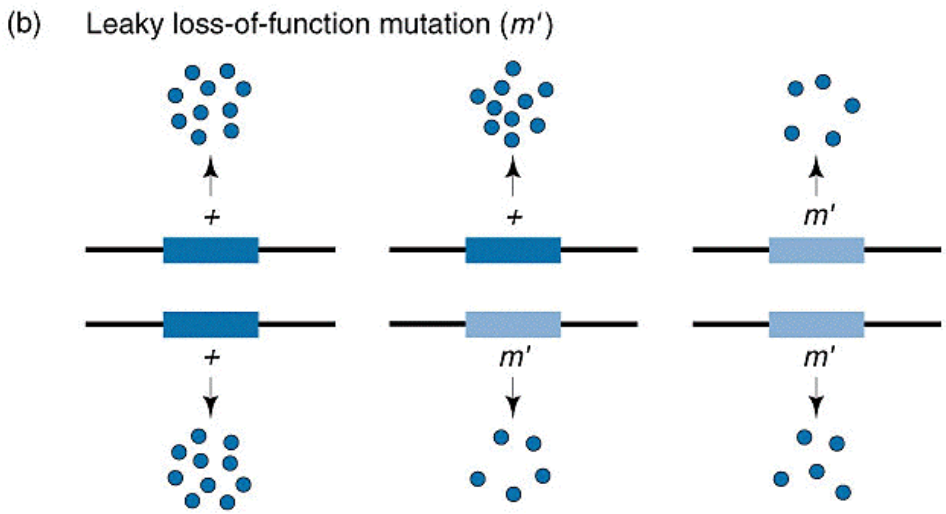


b) Leaky mutation = loss of function mutation where gene product is not completely inactive (partially active still)



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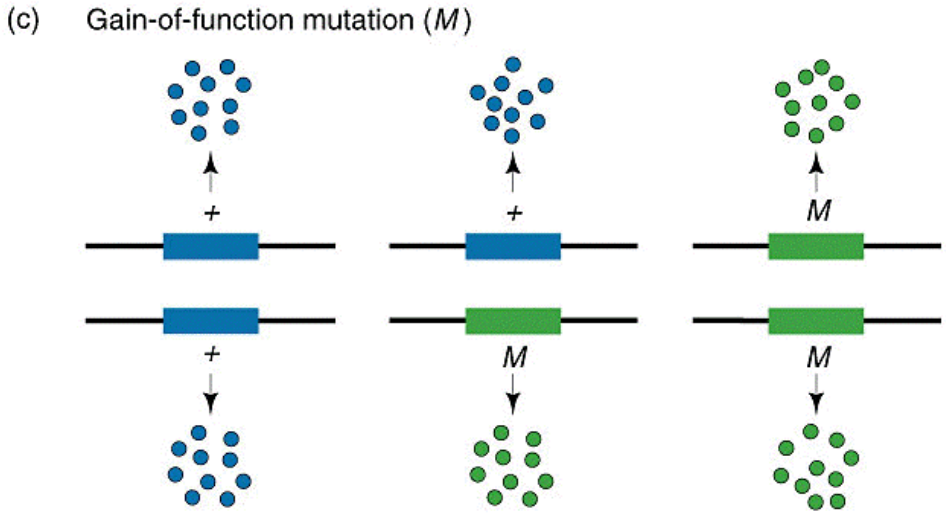
2. Gain of function mutations are those that produce a new function for the gene product. Gain of function mutations are dominant.



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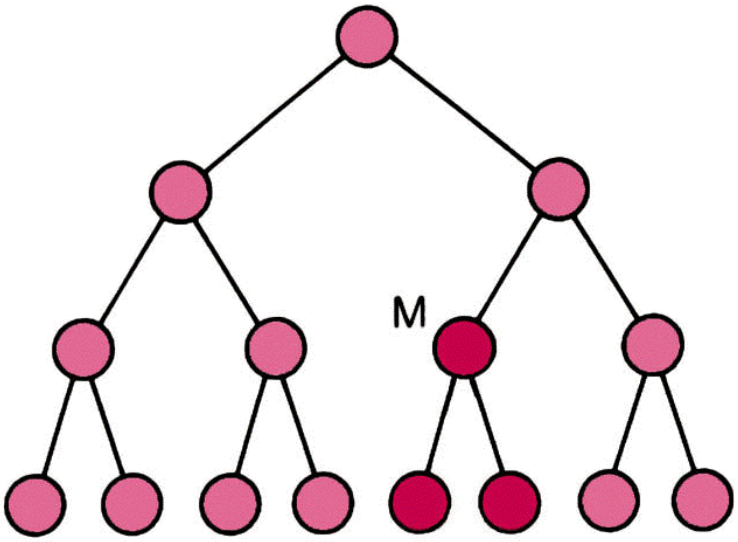
III. The occurrence of mutations

A. Frequencies of mutations

1. Mutation frequency = # of times mutation appears in the population / # of individuals in the population where a population can be bacterial cells, people, gametes
2. Mutation rate = # of mutations / unit time where unit time can be per cell division, cell generation



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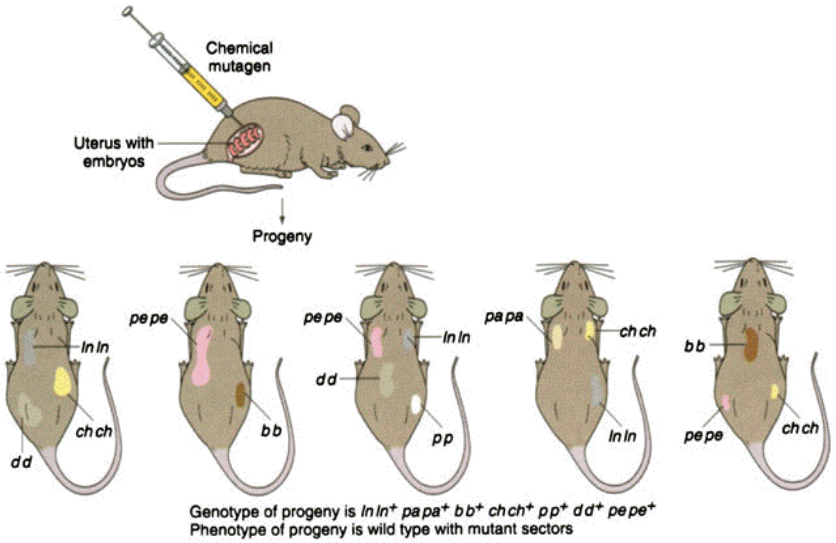
3. Mutations are relatively rare.
4. Different genes have different mutation frequencies (Table 7-1)
5. Different organisms have different overall mutation frequencies (Table 7-2)

B. Detection of mutations in humans

1. Detection of germinal dominant mutations by human pedigree analysis (shows up in the pedigree as the sudden appearance of a novel phenotype)
2. Detection of germinal recessive mutations are more difficult because they remain masked by the dominant allele until the union of two heterozygotes
3. Detection of germinal X-linked mutations arising in female gametes appear in some of the males in the generation after the mutation occurred.

C. Detection of mutations using the specific-locus test, a system for detecting recessive mutations in diploids. Heterozygote individual for gene(s)A that give phenotype A is crossed with a homozygous recessive individual for gene(s)a that gives phenotype a. The frequency of the mutant phenotype (a) is quantitated.



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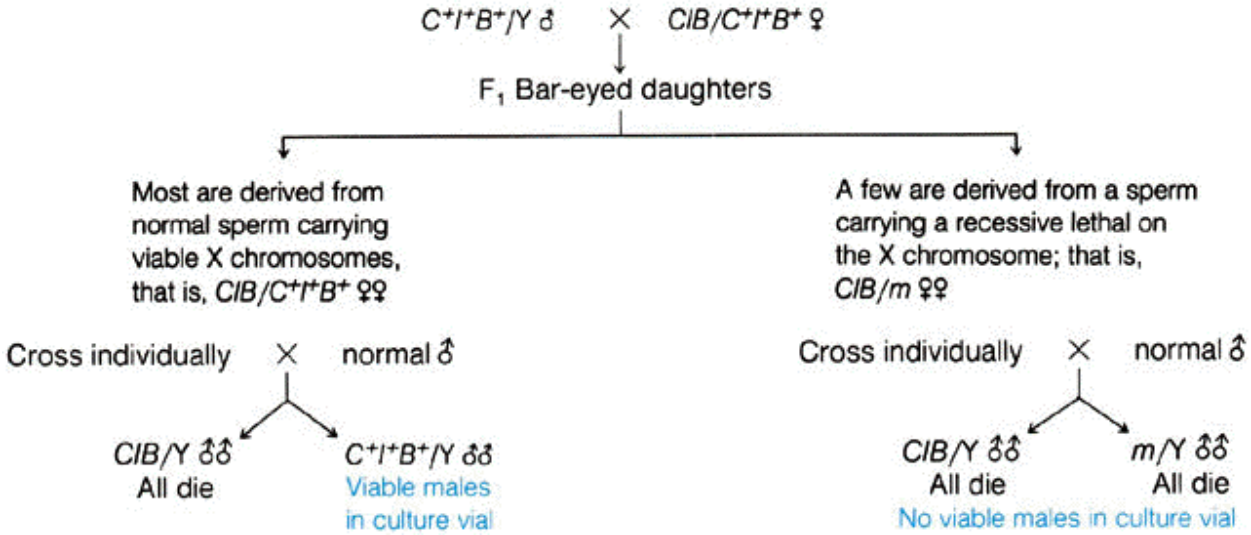
D. Detection of X-linked mutations in *Drosophila* using the ClB chromosome

The ClB chromosome is the X chromosome bearing the C allele which prevents crossover, the l allele which is a recessive lethal, and the Bar allele which is a dominant eye mutation.



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E. Detection of mutations in microorganisms

1. General info on microorganism growth and manipulation

a) Growth is rapid (some bacterial populations double every 30 minutes)

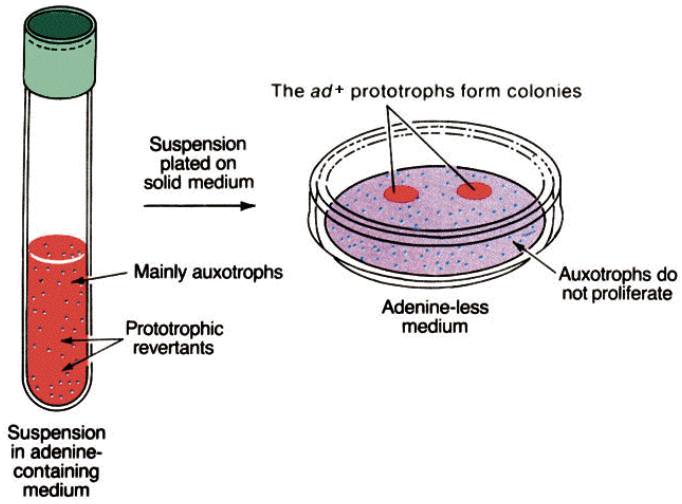
b) Growth can occur in liquid media to cell density of approx 10^9 bacteria/ml

c) Growth can occur on solid media (agar plate). Organisms are spread across a plate of solid media (plating). Single cells are deposited randomly and will grow into a colony of cells that is visible to the naked eye.

2. Microorganisms allow for the use of <u>selective systems</u> for mutation detection vs. the <u>screening systems</u> used for higher organisms. A selective system is one in which the experimenter can DEMAND that the only individuals that grow or survive are the ones that have the mutation of interest. On the other hand, a screening system is one in which the experimenter must examine each individual to see if it has the mutation of interest.

3. Microbial selective systems

a) Selection for reversion of an auxotroph to a prototroph: Plate 10^9 adenine auxotrophs on agar plate with no adenine. The only bacteria that grow are those that have a random mutation in the mutant *ad* gene that now reverts it back to the wild type allele.



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b) Selection for resistance to an environmental factor (bacteriophage, antibiotics).
Example, Luria-Delbruck experiment: Tested whether mutations arose in response to plating on selective media (in this case bacteriophage resistant mutants were selected for by plating on media containing bacteriophage which normally kill the bacterium). Two hypotheses: (a) physiological change where mutations arose **after** plating because the bacteria sensed the phage and altered themselves so that they may become resistant to the phage or (b) random mutation where mutations arose randomly **before** plating and all plating did was select for the resistant bacteria
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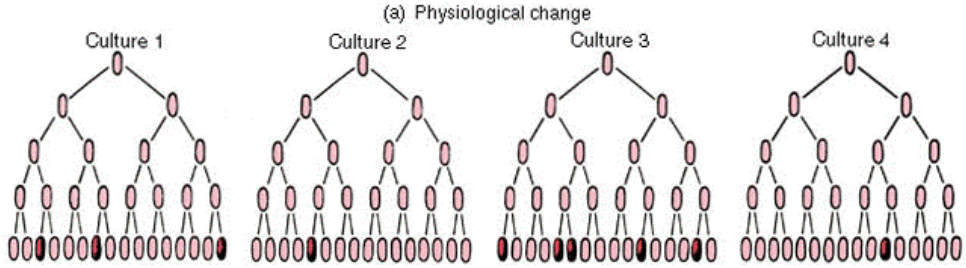
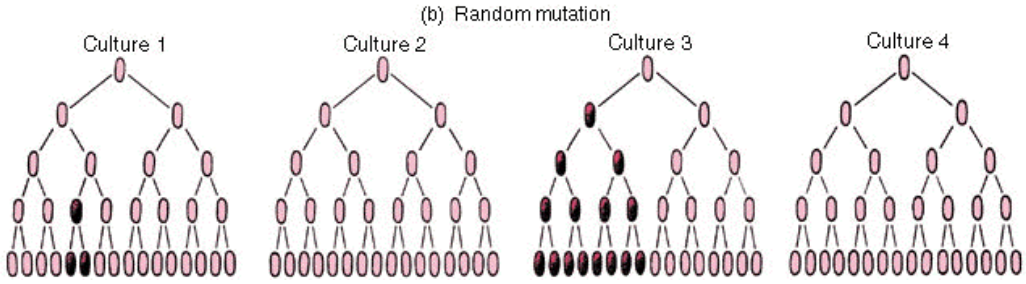


Versus



Luria and Delbruck tested the above hypothesis by starting 20 very small cultures (0.2 ml) and 1 large culture (10 ml) with low numbers of bacteria. The cultures were allowed to grow for numerous generations and them 0.2 ml aliquots were plated on phage plates (all of the small cultures and aliquots of the large culture) and the # of resistant bacteria were counted. If the physiological hypothesis was correct, then one would expect that each plate would contain the same number of phage resistant bacteria. However, if the random mutation hypothesis was correct, some of the small cultures may have had the random phage resistance mutation occur early and thus almost the entire population is phage resistant while other small cultures may have had the random phage resistance mutation occur very late early and thus almost the entire population is not phage resistant. They found a large fluctuation in the number of phage resistant bacteria on plates from the small 0.2 ml cultures (Table 7-3), and thus random mutation was the correct hypothesis. In the larger culture, the fluctuations are averaged out and so you do not see the large variation in #s.

4. Microbial screening systems

a) Screening for forward mutations from wild type to auxotrophy: Plate bacteria on complete media to form colonies. Replica plate many, many colonies to plates with and without the nutrient you are testing for auxotrophy. Compare the plus and minus nutrient X plates to look for a colony that appears on the plus nutrient plate but not the minus nutrient plate. If you expect the mutation frequency to be 1 in 10,000, then you will need to replica plate at least 10,000 colonies to get the 1 mutant.

b) Enrichments are important in screening because they reduce the number of organisms that you have to screen. Enrichment for auxotrophs works by selectively removing or killing growing microorganisms while they are in medium that allows only the prototrophs to grow.

(1) Filtration enrichment (Figure 7-18)

(2) Penicillin enrichment

IV. Mutations and cancer

A. Cancer is a group of diseases characterized by rapid, uncontrolled proliferation of cells within a tissue resulting in the formation of a tumor. Cancer has many causes and phenotypes but the fundamental mechanism underlying all cancers is genetic.

B. There are two types of genes that are involved in cancer formation.

1. Tumor suppressor genes are genes that encode a product that normally stops cell division. Mutations in these genes result in uncontrolled activation of cell division and therefore tumor formation. Mutations are generally recessive and thus you need mutations in both alleles to have cancer. A mutation in one allele predisposes the carrier to cancer.

a) Rb gene - retinoblastoma (retinal cancer)

b) BRCA1 - hereditary breast cancer gene

c) p53 gene mutations are found in a variety of cancers including breast, lung, bladder, and colon cancers. Over 1/2 of all cancers are associated with p53.

2. Proto-oncogenes are genes that encode a product that normally controls cell division (kind of like an on/off switch). Mutations in these genes make the gene product permanently in the on position which results in uncontrolled activation of cell division and therefore tumor formation.

a) N-ras – neuroblastoma (tumor formed of embryonic ganglion cells), leukemia

b) N-myc – neuroblastoma

c) man – mammary carcinoma

V. Mutagens in genetic dissection

A. Mutagens are agents that cause mutations at a rate higher than the spontaneous rate.

B. Mutagens can be chemical (i.e. cigarette smoke, mustard gas) or radiation (i.e. UV, X-rays,)