UNIT 3 GENETIC MUTATIONS

In most organisms genes are segments of DNA (in some viruses genetic material is RNA). DNA is a highly stable molecule that replicates with an amazing accuracy. Despite this, changes in DNA structure and errors of replication do occur.

- In the broad sense the term mutation refers to all heritable changes in the genome, excluding those resulting from incorporation of genetic material from other organism.
- A mutation is an abrupt qualitative or quantitative change in the genetic material of an organism.
- In biology, a **mutation** is a permanent change of the nucleotide sequence of the genome of an organism, virus, or extrachromosomal DNA or other genetic elements.
- Mutations result from damage to DNA or to RNA genomes, errors in the process of replication, or from the insertion or deletion of segments of DNA by mobile genetic elements. Damage to DNA / RNA may occure due to physical or chemical mutagenic agents.
- Mutation can result in several different types of change in sequences. Mutations in genes can have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. Mutations can also occur in nongenic regions.
- Organisms have mechanisms such as DNA repair to prevent or correct (revert the mutated sequence back to its original state) mutations.
- Mutations can be both beneficial and detrimental. Beneficial means, they are the source of all genetic variation, the raw material of evolution. Without mutations and the variation they generate, organisms could not adapt to a changing environment and risk extinction. On the other hand, most mutations have detrimental effects (prevent the gene from functioning properly or completely), and mutation is the source of many human diseases and disorders.
- Mutation may be intragenic or intergenic. Intragenic mutations or point mutations include alternations in the structure of the DNA molecule within a gene. There is a change in the normal bas sequence of the DNA molecule. This change results in the modification of the structural characteristics or enzymatic capacities of the organism. The unit of a gene

mutation is the *muton*. The muton may consist of one more nucleotide pairs.

- Intergenic mutations, of which chromosomal changes in structure are examples, involve long regions of DNA, i.e. many genes. These include deletion or addition of segments of chromosomes, resulting in the deficiency & duplication, respectively.
- It is usually stated that, 'mutations occur in random manner, the frequency of spontaneous mutation is usually low, ranging from 10^{-7} to 10^{-12} per organism.'' These spontaneous mutations are easily detected.
- **Hot spots-** mutations occur much more frequently in certain regions of the genes than in others. These favoured regions of mutations are called as 'Hot spots'.

- **Types of gene mutations**

Gene mutations are of two main types,

1. Base pair substitutions or switches (Transition, Transversion)
2. Frameshift mutations (Deletion, Insertion)

1. Base pair substitutions or switches (Transition, Transversion)

Base pair substitutions are the most common mutations. They result in the incorporation of wrong bases during replication or repair of DNA. Examples of base pair switches are from A- T to G-C, C-G or T-A. In base pair switches one base of a triplet codon is substituted by another, resulting in a changed codon.

i. Original (wild type) message of reading frame.

CAT GAT CAT GAT CAT GAT CAT.....

ii. Substitution or replacement. A replaced by G

CAT GAT CGT GAT CAT GAT CAT.....

If the mutated codon specifies another amino acid it will result in amino acid substitution in the polypeptide chain during translation.

Substitutions usually result in a simple change of one amino acid in the polypeptide chain synthesized. Such changes are called missense mutations.

If a single base substitution modifies a codon to a termination codon, the chain may terminate at the point of mutation (e.g. CAG to UAG). Such mutations are called nonsense mutations. They result in incomplete polypeptide chains which may not be biologically active.

Base pair substitutions are of two main types, transitions and transversions.

i) Transitions

If a purine base is replaced by another purine base (A by G or G by A) or a pyrimidine by another pyrimidine (T by C or C by T) the substitution is called a transition. Transitions are by far the most common types of mutations.

ii) Transversions

If a purine base is substituted by a pyrimidine, or vice versa, the substitution is called a transversion. It will be seen that each base pair can undergo one kind of transition and two kinds of transversions. In general, transition mutations code for chemically similar amino acids while transversions show a greater possibility of inserting amino acids with different charges.

Although transitions and transversions can cause nonsense mutations, the chances of missense mutations are greater.

Mutations leading to base pair substitutions presumably take place in two steps. Let us consider a mutation in a DNA double strand in which the purine base A is substituted by another purine base G (transition). When the DNA replicates it will give rise to two chains, one normal like the parent chain and the other mutant. Since the mutated base G pairs with C, the mutant DNA will have G-C at the point of mutation. Thus both chains have altered bases at the point of mutation.

Inversion

If a segment of DNA is removed and reinserted in a reverse direction it results in an inversion. As in substitution, the message is out of phase only in the triplets involved in the inversion.

CAT GAT TAC GAT CAT GAT CAT.....

Massage out of frame

2. Frameshift Mutations – (Deletion, Insertion)

A mutation in which there is deletion or insertion of one or a few nucleotides is called a frameshift mutation. The name is derived from the fact that there is a shift in the reading frame backward or forward by one or two nucleotides.

Deletion or Addition or of one or two bases results in a new sequence of codons which may code for entirely different amino acids. This result in a drastic change in the protein synthesized.

The protein is usually nonfunctional. It should be noted that if the reading frame shifts by three nucleotides, the resulting protein is normal, except that it may lack one amino acid or may contain an extra amino acid.

The site of the mutation has an important bearing on whether the protein formed will be slightly or drastically altered.

Since translation, takes place in the 5'-->3' direction, a frameshift mutation near the 3' end of the gene results in only the terminal part of-the polypeptide chain being altered. This may result in a functional protein. The several variants of haemoglobin are believed to have arisen in this manner.

Deletion

Removal of one or a few bases from a nucleotide chain is called a deletion. It will be seen that the removal of even one base will throw the genetic message out of frame beyond the point of deletion. A new sequence will be established. This will happen on deletion of any number of bases not divisible by three.

Original (wild type) message or reading frame

CAT GAT CAT GAT CAT GAT CAT

Deletion.

- C

CAT GAT ATG ATC ATG ATC AT

Message out of frame

Addition or Insertion.

The genetic message will be similarly disturbed if one or a few bases are added (insertion), provided that the number of such bases is not divisible by three.

+G

CAT GAT GCA TGA TCA TGA TCA T

Message out of frame

If there is simultaneous deletion and addition of a base, then the message will be out of frame only in the triplets between the deletion and addition.

Deletion and insertion

-C + C

CAT GAT ATG ATC ATC GAT CAT

Message out of frame

Explanation of mutation

Mutations can be explained by the following analogy. Suppose that the genetic message is contained in the sentence:

THE MAN WHO HAS ONE EYE CAN SEE YOU.

In this sentence each word consisting of three letters represents a codon. If the letter W of WHO is removed (deletion) the sentence becomes

THE MAN HOH ASO NEE YEC ANS EEY OU-.

The sentence is meaningless after the word MAN.

If the letter A is added after MAN (insertion), then also the sentence becomes meaningless after MAN.

THE MAN AWH OHA SON EEY ECA NSE EYO U-.

If the letter H of WHO is replaced by U (substitution) the sentence becomes:

THE MAN WUO HAS ONE EYE CAN SEE YOU. In this case only one word, WUO, becomes meaningless.

If the letters of WHO are reversed (inversion) the sentence becomes:

THE MAN OHW HAS ONE EYE CAN SFE YOU.

In this case also only one word is meaningless (OHW).

If the letter W of WHO is removed and the letter Z is added after ONE (deletion and insertion) the sentence becomes:

THE MAN HOB ASO NEZ EYE CAN SEE YOU.

Only the words involving the deletion and the insertion are meaningless.

MISSENSE MUTATION

A missense mutation is one, which results in the replacement of one amino acid in a polypeptide chain by another. As results of mutation one base of

a codon may another base. The changed codon may then code for another amino acid. A missense mutation can be caused by substitution, deletion or insertion.

Missense mutations arising by substitution results in protein which differ from their normal counterparts only a single amino acid. Such proteins therefore frequently have normal biological activity. One of the codons for *phenylalanine* is UUU. A single base substation (U-G) changes it to UGU, the codon for cytosine. Thus the protein formed after mutation is identical to the normal protein except that phenylalanine is substituted by cytosine. About half the known human haemoglobins have amino acid substitutions involving base transversions.

Amino acid in normal haemoglobin	***Base substitution***	***Amino acid in mutant haemoglobin***
Lysine (AAA)	A ⟶ G	Glutamic acid (GAA)
Glycine (GGU)	G ⟶ A	Aspartic acid (GAU)
Asparagines (AAU)	U ⟶ A	Lysine (AAA)
Glutamic acid (GAA)	A ⟶ U	Valine (GUA)

NONSENSE MUTATION

Of the 64 codons 61 code for amino acids, while three are *termination codons* which do not specify any amino acid. The three termination codons are UAA, UAG and UGA. Any mutation resulting in the alteration of a codon specifying an amino acid to a termination codon is called as *nonsense mutation.* Thus if the codon UAC (for tyrosine) undergoes a one-base substitution (C to G) it becomes UAG, a termination codon.

A nonsense mutation brings about termination of polypeptide synthesis at the point (unless there is *genetic suppression.* As a result the polypeptide chain synthesized is incomplete. Such chains are likely to be biologically inactive. Since a nonsense mutation brings about a relatively drastic change in the enzyme synthesized it is more likely to have a deleterious effect on the phenotype than missense mutation.

Polypeptide chain synthesis takes place in the 5' to 3' direction. Therefore a nonsense mutation near the 5' end results in very short chain with

probably very little or no biological activity. Conversely, a nonsense mutation near the 3' end results in a chain which is nearly complete, and which may be some or normal biological activity.

MUTATION IN TERMINATION CODONS

Mutations, which are the reverse of nonsense mutations, also occur. Thus mutations can convert a *termination codon* specifying some amino acid. The α chain of human haemoglobin is normally 141 amino acid residues long. A mutation (U to C) converts the *termination codon* UAA to CAA, the codon for *glutamine.* Chain synthesis therefore proceeds beyond the normal termination point, producing a polypeptide chain containing 172 amino acids.

SILENT MUTATION

Any gene mutation, which does not result in phenotypic expression, is called a *silent mutation.*

1. The genetic code is degenerate, i.e. more than one codon may specify an amino acid. For example both AAG and AAA specify lysine. If the codon AAG undergoes a mutation to AAA the latter codon will still specify lysine. When a mutated triplet codes for the same amino acid as the original there is no change in the amino acid. This mutation is of the silent type; because of DNA there is no alteration in the amino acid sequence of the protein synthesized.
2. The codon change may be result in an amino acid substitution but this is not sufficient to modify the function of the protein appreciably.
3. The three mutations may occur in a gene that is no longer functional or whose protein is not essential at the particular stage of testing.
4. Simultaneous presence of suppressor mutations may cause a mutation to become silent. In genetic suppression a second mutation at a different site neutralize the effects of the first mutation (see ‘genetic suppression).

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❖ **Types of mutations on the basis of molecular mechanism**

There are mainly two types of mutations on the basis of molecular mechanism

A) SPONTANEOUS
B) INDUCED

A) SPONTANEOUS MUTATIONS: -

1. The mutations that occur suddenly in **nature** under **natural conditions** are called Spontaneous mutations.
2. Their origin **is unknown**.
3. They are also called as **background mutations.**
4. They arise by the action of **mutagenic agents** present in the **environment.**
5. These mutagenic agents include cosmic rays, radioactive compounds, heat and naturally occurring base analogues such as caffeine.
6. Spontaneous mutations have been reported in maize, bread molds, bacteria, viruses, mice, man, *Drosophila* etc.
7. *Spontaneous mutations* on the molecular level can be caused by

- **Tautomerism** — A base is changed by the repositioning of a hydrogen atom, altering the hydrogen bonding pattern of that base, resulting in incorrect base pairing during replication.
- **Depurination** — Loss of a purine base (A or G) to form apurinic site (AP site).
- **Deamination** — Hydrolysis changes a normal base to an atypical base containing a keto group in place of the original amine group. Examples include A → HX (hypoxanthine) and C → U, which can be corrected by DNA repair mechanisms; and 5MeC (5-methylcytosine) → T, which is less likely to be detected as a mutation because thymine is a normal DNA base.
- **Slipped strand mispairing** — Denaturation of the new strand from the template during replication, followed by renaturation in a different spot ("slipping"). This can lead to insertions or deletions.

A) SPONTANEOUS MUTATIONS:

Replica plating

Delbruck in 1952 first time used replica-plating technique to prove the spontaneous mutation phenomenon. The process involves making of identical copy of master plate by using velvet cloth, which acts as several inoculating needles at a time. The given culture of *E. coli* is grown on nutrient agar plate to form well-isolated colonies. This is called as master plate. It does not contain streptomycin.

This plate is replicated on nutrient agar plate with streptomycin with the help of sterile velvet stamp. Both the plates are incubated at 37^0 C for 24 hours. The position of streptomycin resistant mutant on master plate is determined by corresponding position of colony on replica plate, which is containing streptomycin. As master plate without streptomycin was not exposed to any mutagenic agent, the type of mutation is spontaneous mutation.

Requirements: -

1. *E. coli* culture
2. Nutrient agar plate without streptomycin
3. Nutrient agar plate with streptomycin
4. Velvet stamp

Procedure: -

1. A round piece of velvet cloth is wrapped on the bottom of stamp having a diameter of petriplate. It is sterilized.
2. From the given culture of *E. coli*, 0.1 ml is spread on the nutrient agar plate and incubated at 37^0 C for 24 hours.
3. The plate showing isolated colonies is selected. This plate serves as master plate. This plate is labelled as A and B at two ends of diameter. Similar labelling is made with streptomycin containing nutrient agar plate. Velvet stamp is also labelled as A and B at two ends of diameter.
4. The stamp is gently pressed on the surface of master plate and then on streptomycin containing nutrient agar plate by coinciding the A and B marks both the plates.
5. The plates are incubated at 37^0 C for 24 hours.

6. Streptomycin resistant mutant *E. coli* only can grow on the replica plate, where as sensitive *E. coli* cells are killed. The appearance of mutants were already appeared on master plate, which was never exposed, to any mutagenic agent. The position of mutant colony on master plate corresponds to the position of these colonies on streptomycin containing nutrient agar plate. Thus we can prove spontaneous mutation, which occur in absence of mutagenic agents.

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Molecular mechanism of spontaneous mutation

Truly spontaneous mutations that will be dealt with here are those arising from *Tautomerism*. Spontaneous mutations can also arise as a result of ambiguity of base pairing.

Tautomerism: - the ability of a molecule to exist in more than one chemical forms is called *Tautomerism* (see figure A). All the four common bases of DNA (adenine, guanine, cytosine and thiamine) have unusual tautomeric forms, which are, however, rare. The normal bases of DNA are usually present in the *keto* form. As a result of tautomeric rearrangement they can be momentarily transformed into the rare *enol* form in which the distribution of electrons is slightly different.

Normal base pairing DNA is A-T & G-C. The tautomeric forms are, however, capable of unusual (forbidden) base pairing (figure B & C.) like T-G, G-T, and C-A & A-C.

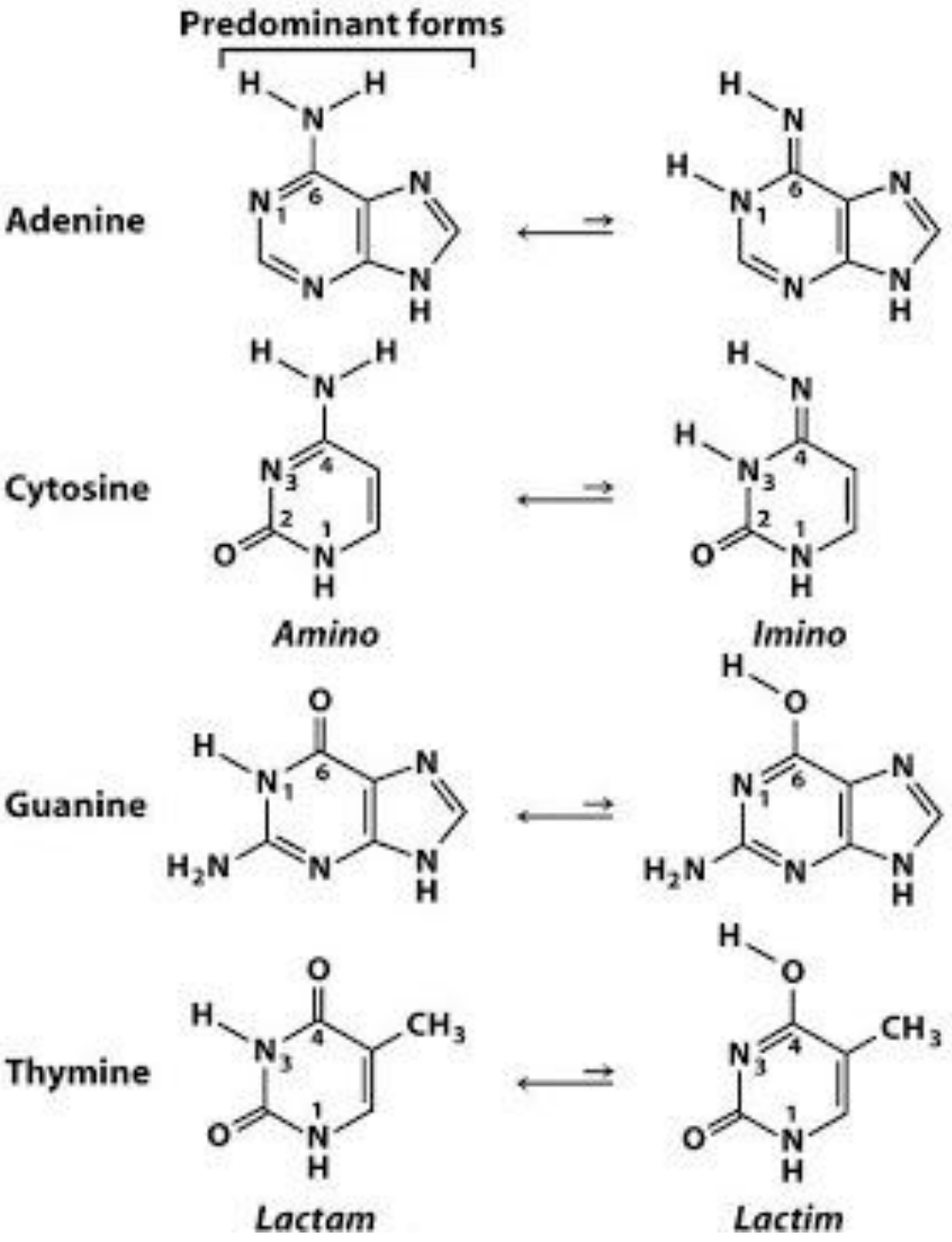

Predominant forms
Adenine
Cytosine
Amino
Imino
Guanine
Thymine
Lactam
Lactim

Fig. A

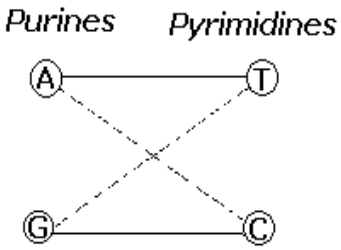


Fig. B Normal (Contnuous) lines and forbidden (dashed lines) base pairing

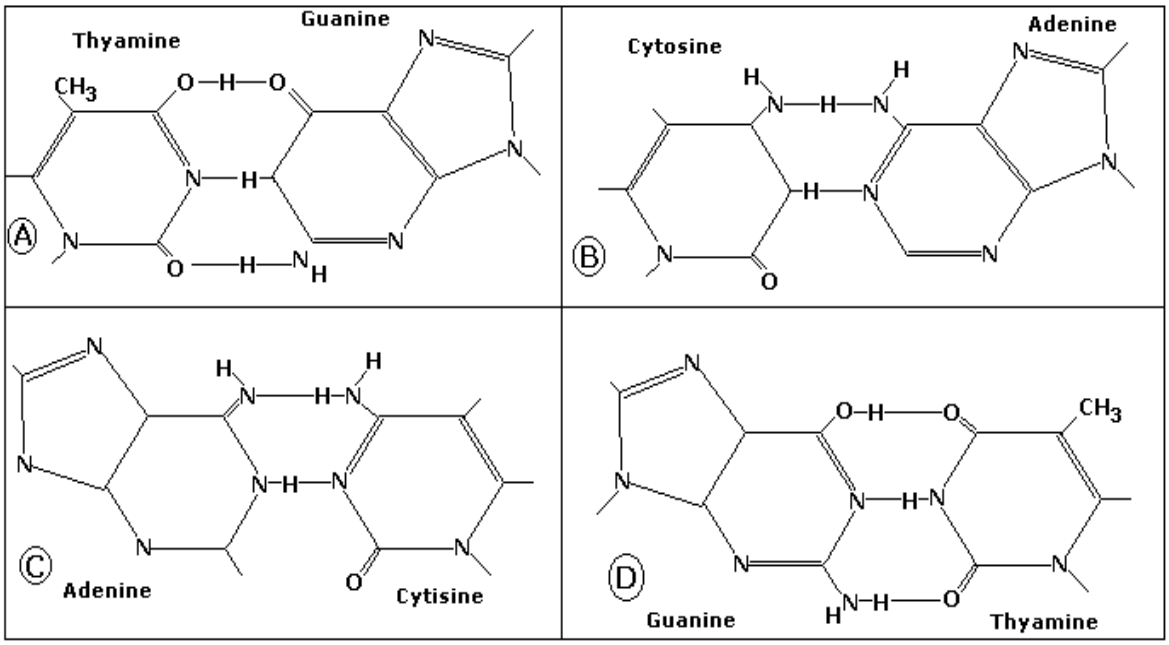
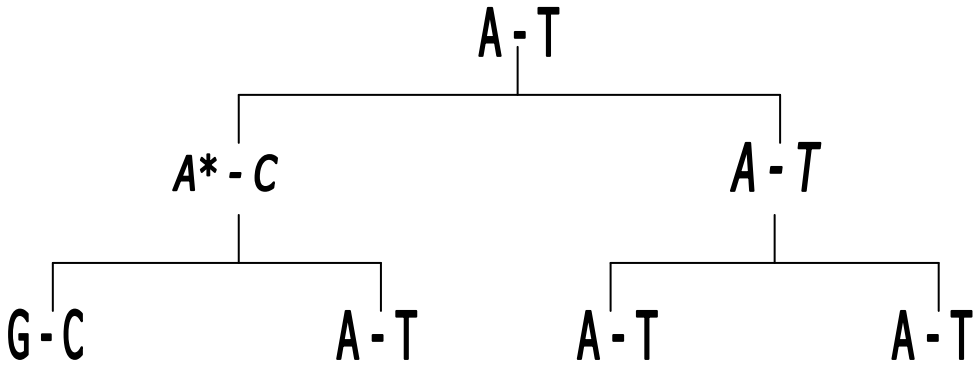


Fig. Abnormal or forbidden base pairing resulting from tautomerism

Fig. C (A) Thyamine-guanine. (B) Cytisine-adenine.
(C) Adenine-cytosine (D) Guanine-thymine

This unusual base pairing results in misreplication of the DNA strand giving rise to mutants in some of the progeny. Thus A* a rare tautomer of adenine (A) pairs with cytosine. This leads to G-C pairing in the next generation.



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B) INDUCED MUTATIONS: -

1. The mutations that can be artificially induced in the living organisms by exposing them to abnormal environment such as radiations, physical conditions (e.g. Temperature) and chemicals are called Induced Mutations.
2. The agents which induce artificial mutations are called mutagens or mutagenic agents.
3. Induced mutations are caused in organisms by mutagenic agents which are of two types:

   Physical agents: X-rays, UV-rays, α, β and γ-rays.

   Chemical agents: Nitrous oxide, Hydroxylamine, Alkylating agents. Base analogues, Proflavine, Acridine orange, etc.

4. It helps **evolution** and development of more **genetic variations.**

- **Mutagenesis by Physical and Chemical agents (Molecular mechanism of induced mutation)**

A variety of agents increase the frequency of mutation. Such agents are called *mutagens*. They include

I) Physical mutagenic agents (Radiations, UV light, X rays)
II) Chemical mutagens

I) Physical mutagenic agents (Radiations, UV light, X rays).

Radiations:

Among the physical mutagens, radiation is the most important. The energy content of a radiation depends upon its wavelength. In general, the shorter the wavelength the greater the energy value of the radiation. High-energy radiations can change the atomic structure of a substance by causing the *loss of an electron* and the formation of an *ion.* Sometimes an electron pair may be moved from an inner to an outer orbital shell. This brings about *excitation* of

the atom. In this excited state the atom is highly reactive and is called a *free radical.* Radiation which brings about such a state is called *ionizing radiation.*

The most common sources include **cobalt-60 and cesium-137.** Radiation is the energy emitted by naturally occuring radioactive elements (like **radon and uranium**) or man-made sources like those found (and created) in nuclear reactors. High energy radiation from a radioactive material or from X-rays is absorbed by the atoms in water molecules surrounding the DNA. This energy is transferred to the electrons which then fly away from the atom. Left behind is a **free radical**, which is a highly dangerous and highly reactive molecule that attacks the DNA molecule and alters it in many ways. Ionizing radiations such as X-rays, gamma rays and alpha particles may cause-

- Ionizing radiation has greater penetration power than UV-radiation and produces free radicals which tend to labilize molecules.
- Alterations in nucleic acids caused by radiation are of great genetic importance. High-energy ionizing radiation and ultraviolet (UV) light are important mutagenic agents.
- This type of radiation causes single-strand breaks in DNA and produce deletions.
- Radiation can cause double strand breaks in the DNA molecule.
- Radiation can directly damage DNA or can cause the formation of chemicals (i.e. reactive oxygen species or ROS) that can then damage DNA or other cell components.
- Radiation of this type comes in different types and can cause different types of damage to cells and tissues.

Exposure to radiation from radioactive materials has been well documented. Analyses of survivors of the atomic bombs dropped on **Japan** during World War II showed large increases in leukemias shortly after the exposure and then increases in other **cancer** types over the following decades.

Dangerous amounts of radioactive materials have also been accidentally released from nuclear power plants. Radiation exposures due to the accidental release of radioactive materials from the **Chernobyl** nuclear reactor have been associated with increases in thyroid **cancer** and other malignant cancers.

UV light:

Both DNA and RNA preferentially absorb UV-light, causing their nitrogen-containing bases to become highly reactive free radicals. The resulting instability causes the conversion of one base to another (a purine to another purine or a pyrimidine to another pyrimidine). If this change occurs in mRNA, only a few inactive proteins will be formed because mRNA is soon broken down. Substitutions in DNA, however, may have a lasting effect. All the proteins coded by the DNA may be defective. Moreover, if the mutation happens to take place in germ cells the mutated DNA strands could be passed on to succeeding generations.

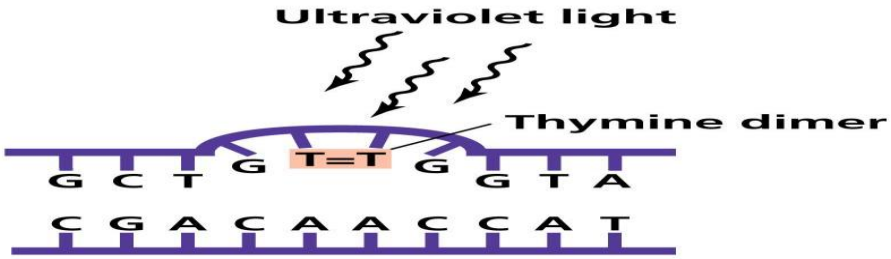
The primary mutagenic effect of UV-light appears to be due to the production of *thymine dimers* (Fig.). The 5, 6 unsaturated bonds of adjacent pyrimidines become covalently linked to form a cyclobutane ring. Irradiation of a bacterial culture and subsequent extraction of DNA yields three possible types of pyrimidine dimers in DNA:

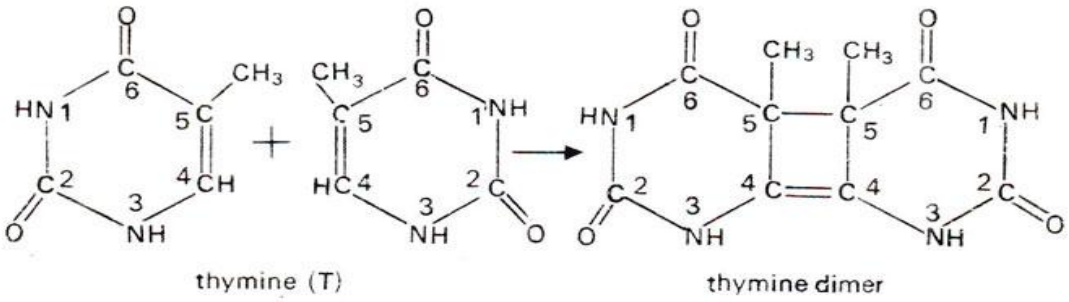
Thymine-thymine	—	50%
Thymine-cytosine	—	40%
Cytosine-cytosine	—	10%

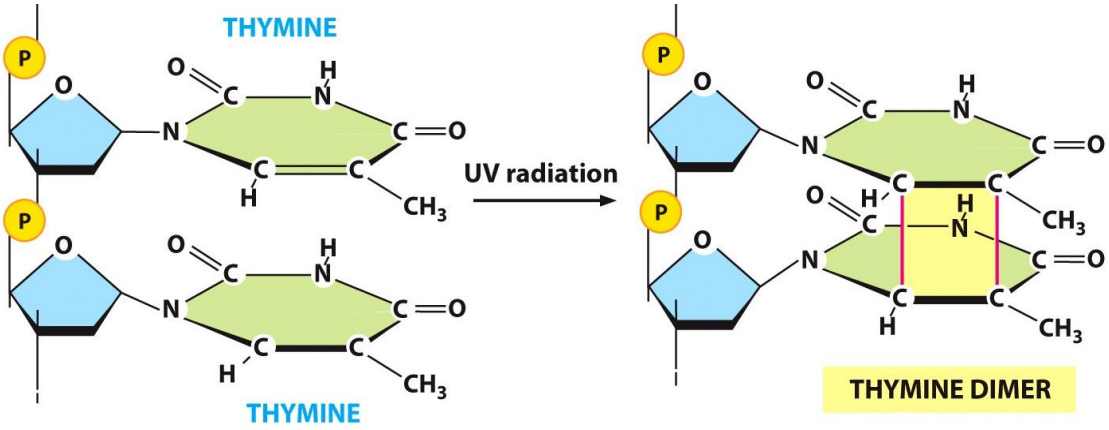
Pyrimidine dimers can also be formed between adjacent strands. In RNA pyrimidine dimers are formed between adjacent *uracil* and *cytosine* rings. Pyrimidine dimers cannot fit into the DNA double helix and cause distortion of the molecule. If the damage is not repaired, replication is blocked, leading to lethal effects.

Distortions in DNA caused by thymine dimers can be corrected by a repair mechanism. An *exonuclease* recognizes the distorted region and excises it. A second enzyme, *DNA polymerase* inserts the correct bases in the gap. A third enzyme *ligase,* joins the inserted bases. The DNA is thus restored to its original condition.

UV-radiation also causes addition of water molecules to pyrimidines in both DNA and RNA resulting in the formation of *photohydrates*. The water molecule is added across the C5-C6 double bond.







X Rays

X-rays bring about mutations by breaking the phosphate ester linkages in DNA. The breakage may take place at one or more points. As a result a large number of bases are lost *(deletion)* or *rearranged.* In double-stranded DNA breaks may occur in one or both strands. Only the latter types are lethal. Sometimes two double-stranded breaks may occur in the same molecule and the two broken ends may rejoin. The part of the DNA between the two breaks is eliminated, resulting in a deletion. The damage caused to nucleic acids by UV light and X rays is utilized to sterilize bacteria and viruses.

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II) CHEMICAL MUTAGENS: -

Chemical mutations can classify according to the way in which they bring about mutations:

(1) *Base analogues*, which are incorporated into DNA instead of normal bases
(2) *Agents modifying purines* and *pyrimydines*
(3) Agents producing *distortions in DNA.*

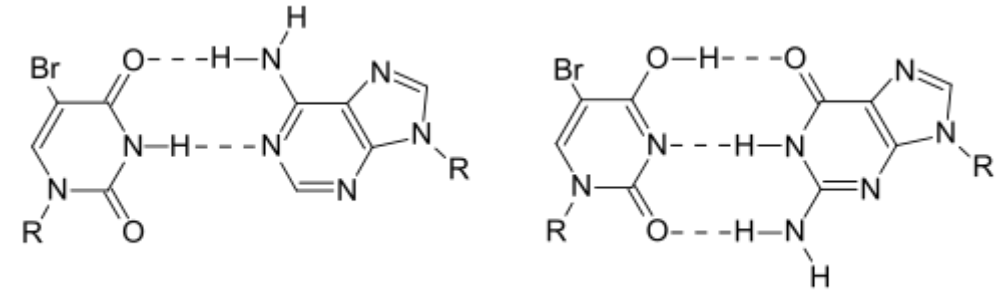
The agents in categories (1) and (3) require replication for their action, while agents in category (2) can modify even non-replicating DNA.

(1) Base analogues: A chemical substance resembling a base is called a *Base analogue*. A base analogue may be incorporated into newly synthesized DNA instead of normal base.

The pyrimidine analogue *5-bromouracil* (5-BU) is structurally very much similar to *thymine*. If bacteriophages are grown in the presence of 5-BU they incorporate in the substance as if it were thymine. 5-BU does not have a lethal action because it is incorporated in place of T and functions almost normally. 5-BU can, however undergo internal rearrangements (*tautomerism)* from the usual *keto* state to the rare *enol* state. 5-BU now pairs with *Guanine* instead of *adenine*, the natural part of thymine (see the fig. D). Thus there is 5-BU- G pairing instead of T-A pairing. Because of this property 5-BU is used in the chemotherapy of virus infection and cancer. By pairing with guanine it disturbs the normal replication mechanism of microorganism.

5-bromodeoxyuridine (5-BDU) can replace Thymidine in DNA.

2-Aminopurine (2-AP) and 2, 6 Diaminopurine (2, 6 DAP) are purine analogues.



2 aminopurine can be read as either *adenine or guanine*. It normally pairs with *thymine* but can also form a hydrogen bond with *cytosine*. It can therefore produce A-T→ G-C transitions. 2-AP and 2, 6-DAP is less effective as mutagens than 2-BU and 5-BDU.

DNA from any sources contain *methylated base*. Methylation of the bases takes place after the synthesis of the polynucleotide. Thus *cytosine* on Methylation becomes 5-*methylcytosin* (see the fig. **E**) in many organism DNA contains both cytosine and 5-methyl cytosine. The amount of guanine is equal to the sum of these two bases. Methylation appears to protect DNA from enzymes formed under the direction of invading viruses. *5-hydroximethyl cytosine* formed when there is a hydroxymethyl (-CH_2OH) group at the fifth position of cytosine. The bacteriophage T2 contains 5-hydroxymethyl cytosine instead of cytosine. Similarly in a bacteriophage of *bacillus* there is *hydroxymethyl uracil* instead of *uracil* and 5-*dihydroxypentyl uracil* instead of *thiamine*. It should be noted that the methylated bases mentioned above are normal constituents of DNA and are not mutagens.

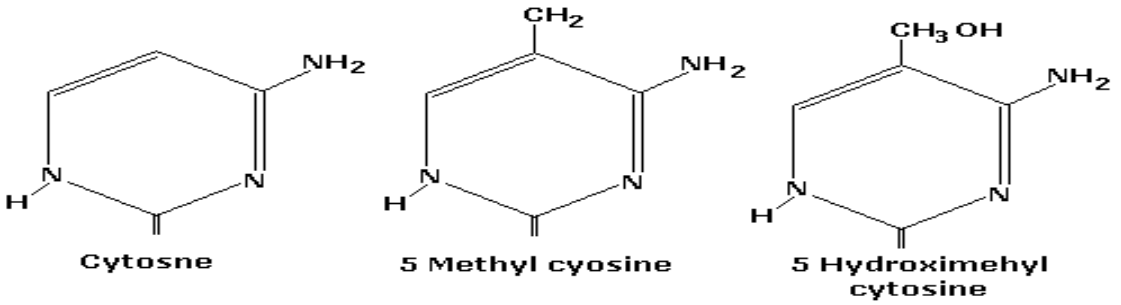


Fig. E Methylation of cytosine resulting in formation of 5-methyl cytosine and 5-hydroxymethyl cytosine.

(2) Agents modifying purines and pyrimidines or agents which labilize the bases include

i) *Nitrous oxide,*
ii) Hydroxylamine and
iii) A*lkylating agents.*

i) Nitrous oxide (HNO_2) reacts with bases containing amino groups. It can change the structure of such bases by deamination (removal of the amino group). When purines or pyrimidines containing the amino group are treated with nitrous oxide, *the **amino group*** (-NH_2) is replaced by the *hydroxyl group* (-OH). The order of frequency of deamination is adenine, cytosine and guanine.

Deamination of *adenine* results in the formation of *hypoxanthine* (**Fig. F**). The pairing behaviour of hypoxanthine is like that of *guanine.* Therefore hypoxanthine pairs with *cytosine* rather than with *thymine*. Thus A-T pairing is replaced by C-G pairing. The deamination of *cytosine* (at the (6) sigma position) results in the formation of *uracil*. The hydrogen bonding properties of uracil are similar to those of *thymine*. Therefore, instead of C-G pairing there is U-A pairing.

Guanine is deaminated to *xanthene*. There is no change in pairing behaviour in this case, because xanthine behaves like guanine and pairs with cytosine. Instead of C-G pairing there is X-C pairing. Thus deamination of guanine is not mutagenic.

NORMAL BASES OF DNA	**NORMAL PAIRING**	**BASE FORMED BY DEAMINATION**	**NEW PAIRING**
Adenine	A-T	Hypoxanthine	G-C
Cytosine	C-G	Uracil	U-A
Guanine	G-C	Xanthine	X-C

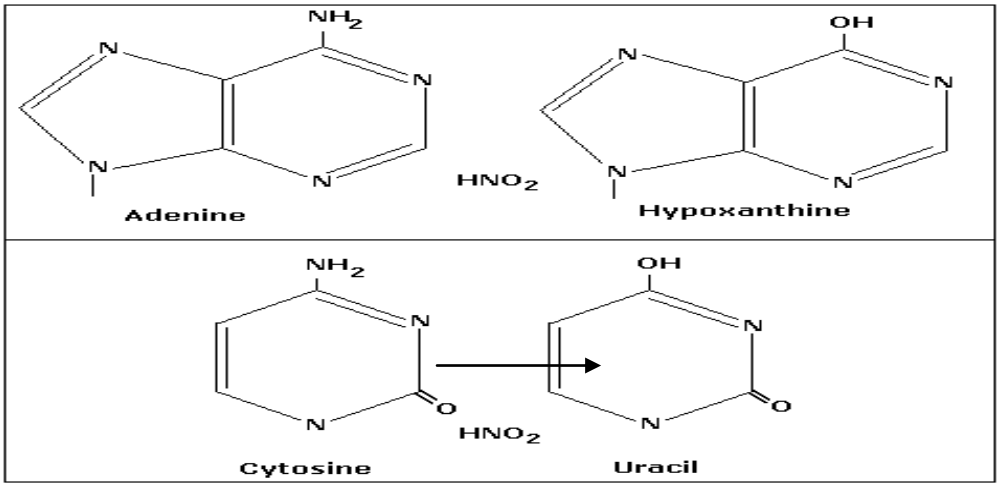


Fig. F Deamination by nitrous acid

The bases formed after deamination of adenine and cytosine has a different pairing behaviour. As a result changes in DNA take place in 50 % of the progeny. Deamination of guanine, however, does not result in a heritable mutation, since there is no change in pairing behaviour of the deaminated base (xanthine).

ii) Hydroxylamine (NH_2OH) is very specific in its action. It reacts mainly with cytosine and guanine residues and brings about transition and mispairing. It

deaminates cytosine to a base, which pairs with adenine instead of guanine. Thus C-G pairing is changed to A-T pairing.

iii) Alkylating agents are the most widely used mutagenic reagents. They include:

Dimethyl sulphonate (DMS)	$-CH_3SO_3CH_3$
Ethyl methane sulphonate (EMS)	$-CH_3CH_2SO_3CH_3$
Ethyl ethane sulphonate (EES)	$-CH_3CH_2SO_3CH_2CH_3$

The main chemical reaction of these agents is alkylation at the N-7 position of *guanine* residues or at the N-3 position of *adenine* residues. Alkylation increases the probability of ionisation and introduces pairing errors. The base sugar-linkage undergoes hydrolysis and releases the base from the DNA molecule. This creates the gap in one chain.

EMS specifically removes *guanine* from the chain. During replication the chain without gaps will gives rise to normal DNA. In the chain with gaps, however any base (A, T, G or C) may be inserted across the gap. This may be a correct base or an incorrect one. In the next replication the gap is filled by a base, which is complementary to the inserted base. Where the correct base is inserted the DNA is normal. Insertion of an incorrect base may result in a *transversion* (purine replaced by a pyrimidine and *vice-versa*) or a *transition* (purine replaced by a purine and pyrimidine by a pyrimidine).

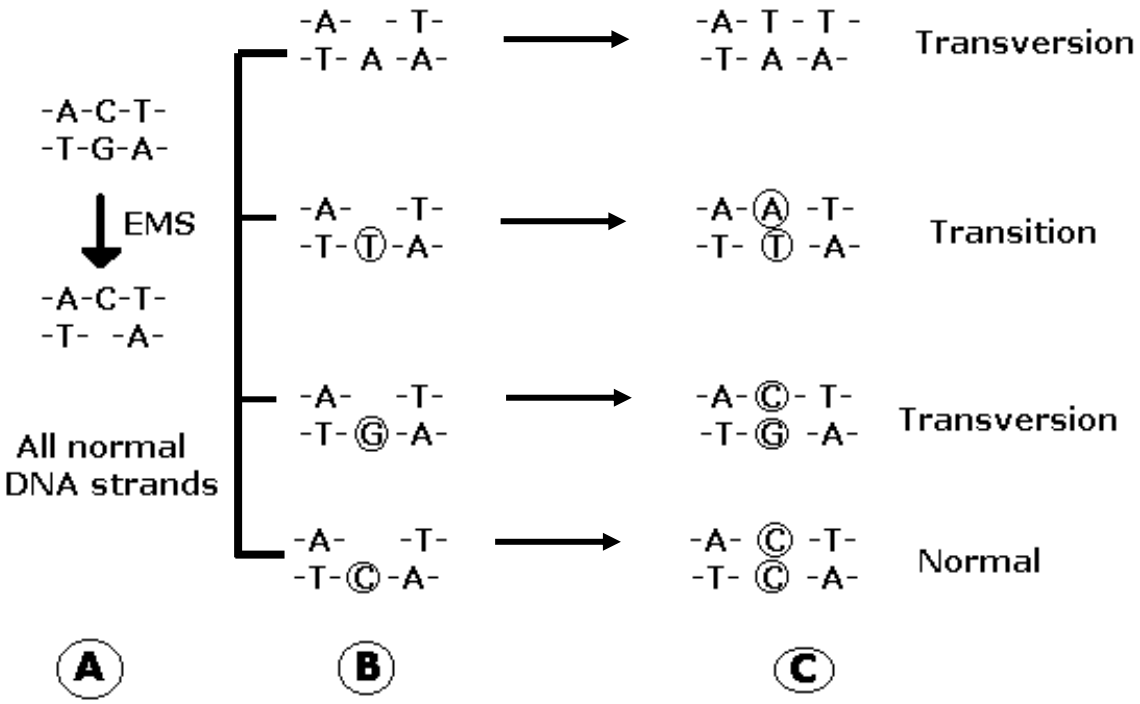


Fig. Effect of the alkylating agent ethyl methane sulphonate (EMS) on DNA
(A) EMS removes G and creates a gap in DNA.
(B) 1st replication. A/T/G/C unserted across the gap.
(C) 2nd replication. complementory base folls the gap.

3) Agents producing distortions in DNA. Certain acridine dyes such as *proflavine* and *acridine orange* (see fig. G) cause mutations by *insertion or deletions of bases.* Crick's work on acridine mutants has provided strong evidence for the genetic code. The acridines are planer (flat) molecules, like the purine bases, and can be intercalated between the bases of the DNA helix (Fig. H). This distorts the structure of DNA and can result in deletion of insertion of bases during recombination. (Fig. J).

i) Intercalation resulting in insertion of base. Intercalation of the acridine molecule between two bases of the template strand results in the lengthing of the DNA molecule. During replication a base (X') is inserted at random opposite the acridine molecule in the new chain. In the next replication a complementary base (X) will pair with the newly inserted base. Thus the new DNA has an additional base pair.

ii) Intercalation resulting in deletion of base. The acridine molecule may be inserted in the new chain during synthesis. This blocks the base in the template strand and does not permit any base, and in the next replication produces DNA with a deficient base pair.

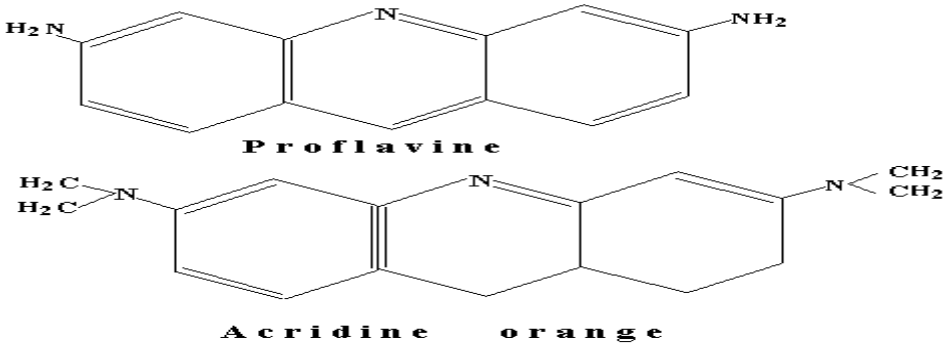


Fig. G The acridine dyes proflavine and acridine orange.

Fig. H Inserted of acridine dye molecule (black) between bases of DNA

Acridine mutagen

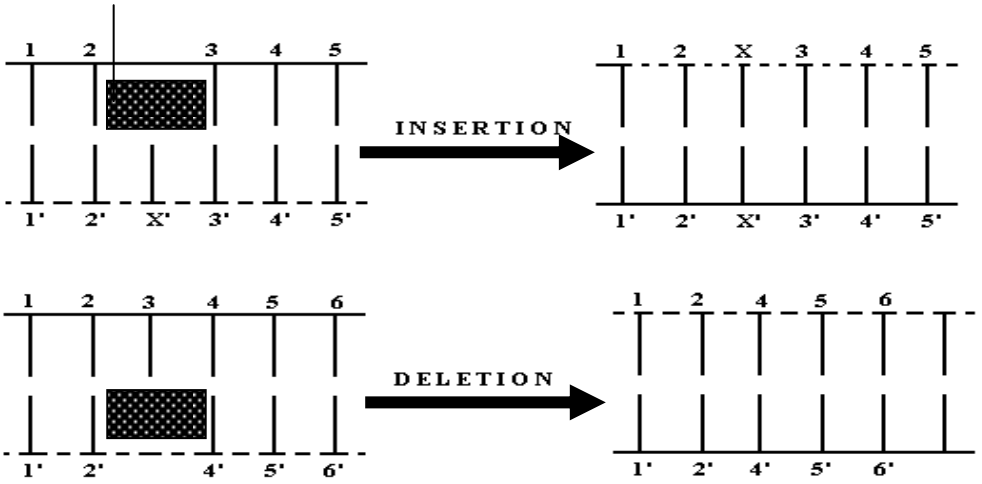


Fig. J Digram of mutagenicof acridine dyes.

FRST REPLICATION **SECOND REPLICATION**

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- **GENETIC SUPPRESSION**

The effect of a mutation on the phenotype can be reversed, so that the original wild type phenotype is brought back. This reversal may be due to true reversion suppression.

In a true reversion there is a reversal of the original genetic change. A C to A mutation would change the codon GCU (alanine) to GAU (aspartate). This may result in the enzyme formed becoming inactive. In a true reversion a reverse mutation from A to C would restore the codon for alanine (GAU GCU). Such a mutation is called back mutation.

In suppression a change at a different site brings about phenotype correction of the mutation. True reversions can be distinguished from suppressions: only suppressed mutants yield recombinats in which the mutant phenotype is again produced.

Suppression mutations are of two types,

A) Intragenic suppression and

B) Extragenic suppression.

A) Intragenic suppression: - In intragenic suppression a mutation in a gene is suppressed by another mutation in the same gene. The effects of a previous mutation in a cistron are removed or reduced by another mutation in the same cistron. Intragenic suppression may be divided onto several types.

1. Intracodon suppression: - A codon that has undergoes change as result of mutation may undergo another mutation to a codon that is less harmful to enzyme function. Thus mutation of GCU (alanine) to GAU (aspartate) may result in an inactive enzyme. A second mutation A to C would give the codon GUU for valane and may restore enzyme activity partially or fully.

GCU $\xrightarrow{\text{mutation}}$ GAU $\xrightarrow{2^{nd}\text{ mutation}}$ GUU

Alanine	aspartate	valine
Active enzyme	inactive enzyme	active enzyme

Since the deleterious effect of the first mutation is suppressed by another mutation within the codon, the suppression is called as intracodon suppression.

2. Reading frame mutation: - A second mutation at a different site in the gene may neutralize the first mutation. This results in an altered enzyme, which differs from the wildtypebe only a few amino acids. Thus the addition of a base a few steps away from earlier deletion can suppress the effects of the deletion. These changes brought about by a shift in the reading frame opposite in direction to that caused by the first mutation. The effects of a deletion and an addition are shown in the following hypothetical sequences:

mRNA GUU CGU UUU CCU CGA ACU GAC GCA AUC GGU A
polypeptide Val- Leu- Phe- Pro- Arg- Thr- Asp- Ala- Ileu- Gly

Normal mRNA and polypeptide

-U
mRNA GUU CUG UUC CUC GAA CUG ACG CAA UGG GUA A

Polypeptide Val– Leu – Phe - Leu –Glu - Leu - Thr – Gln - Ger - Val-

Deletion of U from the third codon causes a shift in reading frame (phe is not affected because of degeneracy in the code). This results in changed amino acids (*italic*) and the protein becomes inactive.

MRNA GUU CUG UUC CUC GAA CUG ACU GCA AUC GGU
Polypeptide- Val – Leu –Phe – Leu –Glu – Leu – Thr –Ala –Ileu - Gly-

Addition of U restores the original reading frame beyond the point of addition. The amino acid sequence is normal, except for the few residues between the two mutations. The polypeptide may be partially or fully active.

3. Suppression can also take place by an amino acid substitution some distance away from the site of the primary mutation. In the *tryptophan synthatase A gene of E coli a* primary mutation (glycine to Glutamic acid) resulted in a non-functional enzyme. The effect of this mutation was corrected by a second mutation (*tyrosine to cytosine*) taking place 36 amino acid residues away in the same gene. This mutation restored the activity of the enzyme. Neither mutation by itself permits the synthesis of a functional enzyme.

B) Extragenic or intergenic suppression. If the deleterious effects of a mutation in a gene are overcome by a mutation in another gene, the process is called as *extragenic or intergenic* suppression. In the strict sense the term *suppressor mutation* refers to intergenic events only, and not intragenic events. The essential feature of intergenic suppression is that the interacting mutational events take place in two separate genes. These two genes nay even be located on different chromosomes.

The termination of polypeptide chain synthesis is brought about by the *termination codons* (UAA, UAG, or UGA). A mutation which coverts a codon specifying an amino acid into a termination codon chain such chains are usually inactive. The effect of a nonsense mutation can be suppressed by mutations in other genes (*intergenic suppression*). Such suppressor mutations results in viable proteins.

Altering the anticodon of tRNA molecules is one method of suppressing the effects of nonsense codons. One of the codons for glutamine (Gln) is CAG. This codon is recognized by the anticodon GUC of Gln-tRNA. The glutamine codon CAG may undergo mutation become UAG (CAG $\longrightarrow$UAG), which is a *termination codon.* This codon does not specify any amino acid and results in polypeptide chain termination. The incomplete chain formed is usually inactive.

The effect of the nonsense codon can be suppressed by mutations in other genes. The normal anticodon for *tyrosine tRNA codon* 5'CAG3' is 3' AUG 5'. A suppressor mutation can convert this anticodon to 3' AUG 5' through a G to C substitution. This mutated tyrosine tRNA anticodon can recognize the nonsense codon UAG as a codon for tyrosine. Thus tyrosine is added to the chain instead of glutamine. The mutant protein is active. In this case, the suppressor gene functions by producing a tRNA that reads a termination codon. (Normally no tRNA has an anticodon that can be read by a termination codon).

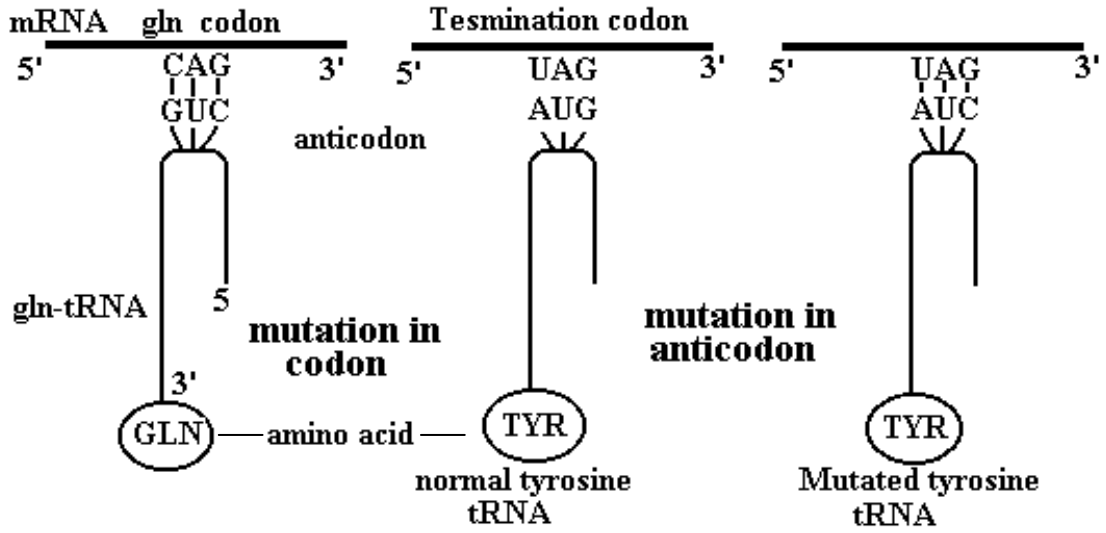
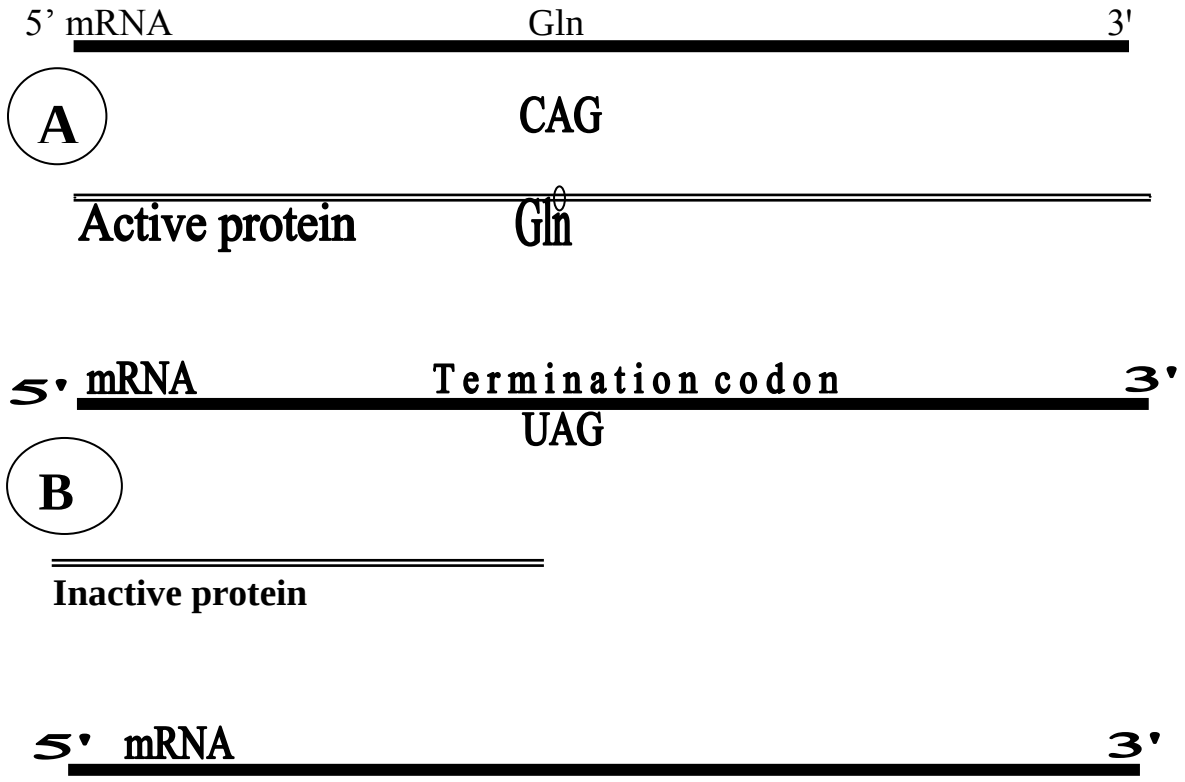


Fig. Intergenic suppression. Deletion effect of mutation of glutamine (Gln) codon (CAG) to termination codon (UAG) is neutralized by mutation of normal tyrosine tRNA anticodon (AUG) to AUC which reads the termination codon as for tyrosine.



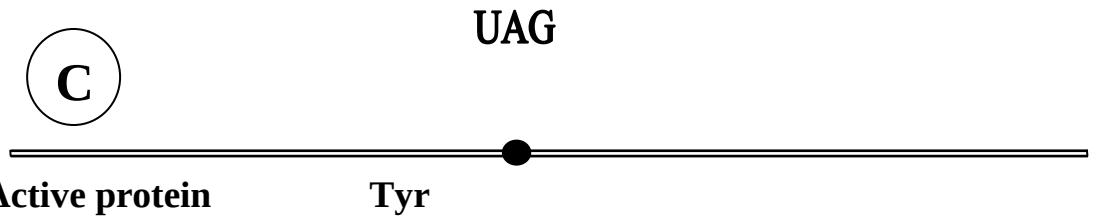


Fig. Intergenic suppression (refer above figure)

1. mRNA with CAG codon for glutamic (Gln) codes normal active protein.
2. CAG undergoes mutation to UAG, a termination codon. The protein is terminated at UAG and is incomplete and inactive.
3. A second mutation modifies tyrosine tRNA anticodon from AUG to AUC. The mutated tRNA reads the termination codon UAG as a codon for tyrosine and inserts tyrosine (Tyr).

The type of suppression mutation mentioned above can only be selected if the tRNA, which has undergone mutation, is not essential for synthesis. *E. Coli* has two different tyrosine tRNA which recognize both tyrosine codons UAC and UAC. One tRNA species continues to termination codon as a codon for tyrosine.

Suppressor gene. It has been seen that in intergenic suppression the effects of a harmful mutation are neutralized by a second mutation in another gene. Genes whose activity results in suppression of mutations in the other genes are called *suppressor genes*. There are suppressor genes for each of the three termination codons, UAG (*amber*), UAA (*ochre*) and UGA (*opal*). The *amber* mutants of the bacteriophage T4 cannot grow on most strains of *E. coli*. They can however, grow on certain strains called *permissive strains*. These genes restore the function of the mutated gene in T4 DNA, and thus enable the bacteriophage to grow in the host cell. There are three *amber suppressor* genes, which suppress the amber termination codon UAG. These are called as su^1, su^2 and su^3 (or sup D, sup E, sup F). They act by producing changes in the anticodon can of certain tRNA species. The tRNAs with modified anticodons can now read the termination codon UAG. One suppressor gene inserts *serine* in the termination position. Another inserts *glutamine*, while the third inserts *tyrosine*. The tyrosine-inserting suppressor gene acts by changing the tyrosine tRNA anticodon from 3' AUG 5' to 3' AUC 5'. The latter anticodon reads the termination codon UAA. There are two *ochre suppressor* genes, *sup B* and *sup C*.

Opal suppressor genes, which suppress the opal termination codon, UGA have also been found. Here suppression takes by the insertion of *tryptophan* in the termination position (UGA). Normal tryptophan tRNA reads only the UGG codon. The suppressor mutation causes it to read the *termination codon* UGA, at the same time retaining its ability to read the *tryptophan codon* UGG.

Suppression of UAG and UGA is about 50% UAA suppression is less efficient and is about 1-5%.

Frameshift suppression. Certain frameshifst mutations resulting from the insertion of nucleotides can also be masked by suppressor genes. In *salmonella typhimurium* glycine tRNA contains the *nucleotide quadruplet* CCCC inserted of the *triplet* CCC in the anticodon position. The extra nucleotide permits the reading of four nucleotides in mRNA at a time, and thus restores the reading frame to the original position.

In another example the anticodon for phenylalanine tRNA reads the quadruplet UUUC instead of the triplet UUU as the codon for phenylalanine.

It should be noted that more insertion of an amino acid in the termination codon positions it not sufficient to bring about suppression. The inserted amino acid must be able to produce a functional protein. If a wrong amino acid is inserted a mutant may be produced which does not give rise to functional protein. The mutated tRNA gene producing such a change cannot be a suppressor gene.

Another factor, which must be considered, is the effect of suppressors on normal chain termination. When the termination codon is suppressed, polypeptide chain synthesis will continue beyond the normal termination point. This will result in the production of abnormal longs chains, causing cell growth to stop. Suppressor strains protect themselves against this possibility by having *double termination codons,* e.g. UAA and UAG in MS2 coat protein mRNA.

5' UCC GGC AUC UAC **UAA UAG ACG CCG….**

This acts as a safely device if one of the termination codon is suppressed. Since the suppression of UAA is relative poor (1-5% as compared to about 50% for UAG and UGA) it is possible that normal codon for chain termination, and genes terminate in UAA alone.

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Site directed mutagenesis

Site-directed mutagenesis, also called **site-specific mutagenesis** or **oligonucleotide-directed mutagenesis**, is a molecular biology technique in which a mutation is created at a defined site in a DNA molecule. In general, this form of mutagenesis requires that the wild type gene sequence be known.

Basic mechanism

The basic procedure requires the synthesis of a short DNA primer containing the desired base change. This synthetic primer has to hybridize with a single-stranded DNA containing the gene of interest. The single stranded fragment is then extended using a DNA polymerase, which copies the rest of the gene. The double stranded molecule thus obtained is then introduced into a host cell and cloned. Finally, mutants are selected.

In 1987 Kunkel *et al.* introduced an improvement to this technique that eliminated the need for selection of the mutants. The plasmid to be mutated is transformed into an *E. coli* strain deficient in two enzymes, UTPase and uracil deglycosidase. The UTPase deficiency prevents the breakdown of UTP, a nucleotide that normally replaces dTTP in RNA, resulting in an abundance of UTP; the uracil deglycosidase deficiency prevents the removal of UTP from newly-synthesized DNA. As the double-mutant *E. coli* replicates the transformed plasmid, its enzymatic machinery incorporates UTP, resulting in a distinguishable copy. This copy is extracted, and then incubated with the Klenow fragment, dNTPs, DNA ligase, and an oligonucleotide containing the desired mutation, which attaches by base pairing to the complementary wild type gene sequence. The ensuing reaction replicates the UTP-containing plasmid using the oligonucleotide as primer, thus incorporating the desired mutation. This forms a chimeric plasmid, with one strand unmutated and containing UTP, and the other strand mutated and containing dTTP. When this plasmid is transformed into an *E. coli* strain with normal UTPase and uracil deglycosidase, the UTP-containing strand is broken down, whereas the mutation-containing strand is replicated, forming a plasmid lacking UTP but containing the desired mutation on both strands.

Cassette mutagenesis

Cassette mutagenesis involves the cleavage by a restriction enzyme at a site in the plasmid and subsequent ligation of an oligonucleotide containing the mutation in the gene of interest to the plasmid. Usually the restriction enzyme that cuts at the plasmid and the oligonucleotide is the same, permitting sticky ends of the plasmid and insert to ligate to one another.

PCR site-directed mutagenesis

The same result can be accomplished using polymerase chain reaction with oligonucleotide "primers" that contain the desired mutation. As the primers are the ends of newly-synthesized strands, by engineering a mis-match during the first cycle in binding the template DNA strand, a mutation can be introduced. Because PCR employs exponential growth, after a sufficient number of cycles the mutated fragment will be amplified sufficiently to separate from the original, unmutated plasmid by a technique such as gel electrophoresis, and reinstalled in the original context using standard recombinant molecular biology techniques.

For plasmid manipulations, this technique has largely been supplanted by a PCR-like technique where a pair of complementary mutagenic primers is used to amplify the entire plasmid. This generates a nicked, circular DNA which can undergo repair by endogenous bacterial machinery. However, this process does not amplify the DNA exponentially, but linearly. Yields are complicated by the fact that the product DNA must undergo the nick repair and are not supercoiled, resulting in reduced efficiency of bacterial transformation. Finally, the product DNA is of the same size as the plasmid. Therefore, the template DNA must be eliminated by enzymatic digestion with a restriction enzyme specific for methylated DNA. The template, which for this technique should be biosynthesized, will be digested, but the mutated plasmid is preserved because it was generated *in vitro* and is therefore unmethylated.