B. Sc. PART II

3 S MICROBIOLOGY

MOLECULAR BIOLOGY AND GENETIC ENGINEERING

UNIT - II

GENE: REGULATION AND MUTATION

- **Gene regulation Mechanisms –**
  - ***lac* operon ,**
  - ***trp* operon.**

b) Mutation- Definition & types of mutations –
Base pair substitution, frame-shift, point, missense, nonsense & silent mutations, Random Vs. Directed mutation, Rate of mutation, Effect of Mutation on Phenotype,

c) Genetic suppressions:- Intragenic (Intracodon suppression, reading frame Suppression) and extragenic suppression (Non sense and Missense Suppression).

d) Molecular basis of spontaneous and induced mutations –
Spontaneous mutation (Tautomerism),
Induced Mutation (Chemical Mutagens) e.g. Base analogues, Nitrous Oxide, Hydroxylamine, Acridine dyes,
Physical mutagens e.g. X-rays, Gamma rays, U.V. light.

Unit II : Gene : Regulation and Mutation

b) MUTATION

A) Gene Regulation

- The DNA present in a cell may contain from a few to thousands of genes.
- Not all genes are active at one time.
- As development proceeds certain genes becomes active while others becomes inactive, i.e. the genes are “switched on” and “switched off ” at different times. This process is called *differential gene action*.
- When genes are active they direct the formation of enzyme, which affect certain traits.
- Thus enzyme synthesis is induced and repressed at different times.
- Although a cell has the genes to produce hundreds of enzymes, only the enzymes required at a particular time are produced.
- Cells are not flooded with unnecessary enzymes.
- Thus there is a mechanism that regulates the gene action i.e. induction and repression of enzyme synthesis.

Operon Model

- Francois Jacob and Jacques Monod (1961) of Pasteur Institute in Paris proposed the model to explain the regulation of gene action.
- For this and some other major contribution in biochemistry Jacob and Monod were awarded the Nobel Prize in medicine in 1965.
- The model proposed by these workers is called the ***operon model***.

Mechanism of *Lac Operon*

- The regulatory mechanism responsible for utilization of lactose as a carbon source is called the *lac* operon.
- The ***lac* operon** is an operon required for the transport and metabolism of lactose in *Escherichia coli*.
- *lac* operon of the bacterium *Escherichia coli* was extensively studied for the first time

by Jacob and Monod (1961).

- *lac operon* of the bacterium *Escherichia coli* is a small segment of a DNA molecule that regulates the utilization of lactose as a source of carbon .
- The *lac operon* consist of the following components:

Regulator Gene, Promoter Gene, Operator Gene, Structural Genes.

1) <u>The Structural Genes</u> –

- The structural gene directs synthesis of cellular proteins through messenger RNA (m – RNA) and determines the sequence of amino acids in the protein synthesized.
- Each structural gene may be controlled independently and transcribe separate m-RNA molecule (monocistronic), or all the structural genes of an operon may be controlled collectively and may form one long polycistronic m – RNA molecule.
- *Lac operon* of the bacterium *Escherichia coli* has three structural genes Z, Y and A.
- These three structural genes together transcribe single long polycistronic m – RNA molecule, which controls the synthesis of three different enzymes.
- The product of ***Lac* Z** gene is the **- galactosidase** enzyme that cleaves the - 1,4 glycosidic bond of lactose to form glucose and galactose. When lactose is absent in the medium only one or two molecules of **- galactosidase** are present in the cell. But **w**hen lactose is added to the medium the structural genes are turned on and production of **- galactosidase** starts and within two to three minutes about 3000 molecules are synthesized by the Z gene.
- The product ***Lac* Y** gene is the **-galactoside permease (Lactose permease)** enzyme which facilitates the entry of lactose into the cell. This enzyme spans the cell membrane and brings lactose into the cell from the outside environment. The membrane is otherwise essentially impermeable to lactose.
- The product of ***lac*A** gene is the enzyme **-galactoside transacetylase** whose role is unknown.

Lac A

Structural genes

Lac Y

Lac Z

Operator Gene
Promotor Gene
Regulator Gene

RNA polymerase

Lactose operon

lac m- RNA

m-RNA
+
Ribosomes

lac repressor

-Galactosidase **Galactoside permease** **Acetylase**

Fig. *lac* operon showing its genes and their products

2) <u>The Operator Gene</u> –

- The operator gene is adjacent to the first structural gene and controls the structural genes.
- It determines whether or not the structural genes are to be repressed by repressor, a product of regulator gene.
- The operator is recognized by the ***repressor*** protein, which binds to the ***operator***, forming an ***operator - repressor*** complex.
- The basic function of the operator is to block transcription of structural genes, on

binding with repressor protein.

- The ***lac* operator** of *E. coli* consists of a sequence of 35 nucleotide pairs. The base pairs in the *lac* operator show a two-fold symmetry *(palindromic sequences).*
- The *lac* operator gene binds to active *lac* repressor protein (which is a tetramer of four subunits) and forms an **operator-repressor complex**, which in turn blocks the transcription of Z, Y and A genes by blocking the path of RNA polymerase.

3) <u>The Promoter Gene</u> –

- The promoter gene lies between the operator gene and regulator gene, and is continuous with the operator gene.
- Like operators the promoter region consists of palindromic sequence (sequence of two fold symmetry) of nucleotides on its CRP site.
- During transcription of structural genes, RNA polymerase (a transcribing enzyme) binds to the promoter region of *lac* operon.
- The promoter region becomes much more attractive to RNA polymerase in presence of c-AMP & CRP Protein (cyclic AMP receptor protein).
- c-AMP and CRP protein form a complex which binds to the lac promoter which stimulates transcription.
- CRP-c-AMP complex binds to the promoter and enhances the attachment of RNA polymerase to the promoter and thus increases transcription and protein synthesis. This is known as positive control.

4) <u>The Regulator (Repressor) Gene</u> -

- Repressor gene determines the transcription of structural genes.
- The regulator gene directs the synthesis of a **repressor protein**, which may be an active repressor or an inactive repressor.
- Repressor (protein) is a product of regulator gene that determines the transcription of structural genes.
- When lactose is present, it acts as an inducer of the operon. It enters the cell and binds to the Lac repressor protein (inducer-repressor complex) and induces a conformational

change in repressor protein. Ultimately repressor protein becomes inactive and can not bind to the operator. Now the RNA polymerase move along the structural genes and transcribes m-RNA which later synthesizes enzymes required for lactose utilization. Lactose can now be metabolized.

- In absence of lactose, the lac repressor protein returns to its original conformation and binds to the operator gene. This blocks the path of RNA polymerase enzyme so that it can not move along the structural genes. Thus structural genes are unable to transcribe m-RNA, & consequently, no enzymes (protein) are made.

FIG. LACTOSE PRESENT

Lac **A**

Lac **Y**

Lac *Z*

RNA Polymerase

RNA Polymerase moves along structural gene & synthesize m-RNA & consequently proteins.

Structural genes

Operator Gene

Regulator Gene

Promotor Gene

Start codon

m-RNA + Ribosomes

can not bind to operator

lac m- RNA

Conformational Change

Inactive repressor

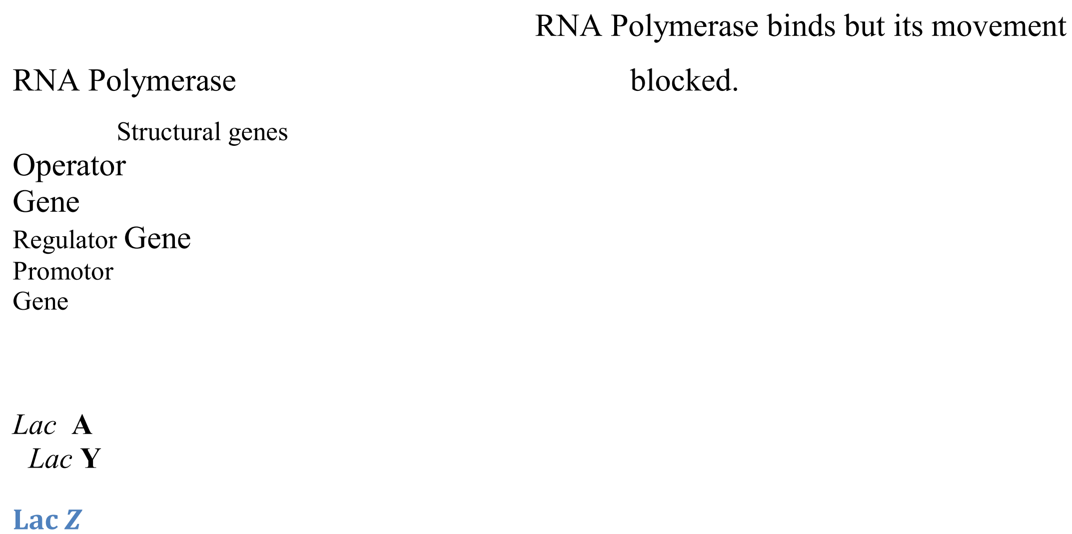
lac repressor

-Galactosidase **Galactoside permease** **Acetylase**

Lactose (inducer)

RNA Polymerase binds but its movement blocked.

RNA Polymerase



Structural genes

Operator Gene

Regulator Gene

Promotor Gene

Lac **A**

Lac **Y**

Lac *Z*

m-RNA
+
Ribosomes

Binds to operator

No m- RNA & No Proteins

FIG . LACTOSE ABSENT

lac repressor (active)

<u>trp (tryptophan) operon</u>

- The *trp* operon of *E. coli* controls the biosynthesis of tryptophan in the cell from the initial precursor chorismic acid.
- It is a negatively regulated biosynthetic operon.
- Trp operon promotes the production of tryptophan when tryptophan is not present in

the environment.

- This operon was discovered in 1953 by Jacques Monod and colleagues,
- The trp operon in *E. coli* was the first repressible operon to be discovered.
- This operon works in the opposite way of the inducible lactose operon.
- lac operon is induced by lactose, while trp operon is inhibited by a tryptophan.

Structure of *trp* Operon

trp P

O *trpL* *trp E* *trp D* *trp C* *trp B* *trp A* *trp R*

Control region

- This operon contains five structural genes: trp E, trp D, trp C, trp B, and trp A.
- Products of these structural genes are-

***trp* Operon Gene**	**Gene Function**
trp E	Gene for anthranilate synthetase enzyme
trp D	Gene for anthranilate synthetase enzyme
trp C	Gene for glycerolphosphate synthetase enzyme
trp B	Gene for tryptophan synthetase enzyme
trp A	Gene for tryptophan synthetase enzyme

- It contains an operator which binds to the repressor protein and blocks the transcription.
- It also contains a promoter which binds to RNA polymerase.
- Promoter; operator sequence is found in the promoter.
- It also contains *trpR* gene, which is not a part of the operon, synthesize the repressor protein that regulates the operon by binding to the operator.
- *trp* Operon also contains *trp L* (Leader sequence) gene that codes for leader peptide. Leader sequence and attenuator (A) sequence is found in the leader. which allows for graded regulation. It allows for graded regulation.
- In this trp operon, unlike the *lac* operon, the gene for the repressor is not adjacent to

the promoter, but rather is located in another part of the *E. coli* genome. Another difference is that the operator resides entirely within the promoter.

Working of *trp* operon (Repression)

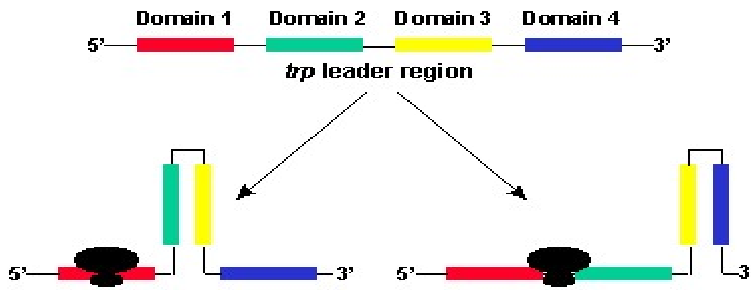
- When tryptophan is present, it binds to the tryptophan repressor, and causes a change in conformation, which allows the repressor to bind the operator. The operator is blocked by the repressor protein, which prevents RNA polymerase from binding or transcribing the operon, so tryptophan is not produced.

- When tryptophan is not present, the repressor cannot bind the operator (repressor protein is liberated from operator in tryptophan's absence), so transcription can occur and produce a group of enzymes required for biosynthesis of tryptophan. This is therefore a negative feedback mechanism.
- This regulatory action is complimented by the process of attenuation.
- Leader sequence (L) controls the expression of the operon through a process called attentuation.

Attenuation of the *trp* Operon

- Leader sequence has four domains. Domain 3 of the mRNA can base pair with either domain 2 or domain 4. If domain 3 pairs with domain 4, a stem and loop structure forms on the mRNA and transcription stops. This structure forms when the level of tryptophan is high in the cell. If domain 3 pairs with domain 2, then the stem and loop structure does not form and transcription continues through the operon, and all of the enzymes required for tryptophan biosynthesis are produce. These events occur when tryptophan is low in the cell.

- If domain 4 is deleted, the stem and loop structure can not form and transcription of the operon will occur even in the presence of tryptophan. Domain 4 is called the **attenuator** because its presence is required to reduce (attenuate) mRNA transcription in the presence of high levels of tryptophan.



<u>Low Tryptophan Level</u>
- Slow translation of Domain I peptide
- Domain 2 -3 pairing occurs
- Normal full gene transcription

<u>High Tryptophan Level</u>
- Fast translation of Domain I peptide
– Domain 2 blocked by ribosomes
– Domain 3 – 4 pairing occurs
- Attenuation of transcription occurs
- Only 10% of normal mRNA is made

<u>Products of *trp* Operon</u>

trp P

O *trpL* *trp E* *trp D* *trp C* *trp B* *trp A* *trp R*

m-RNA

Leader peptide

Anthranilate synthetase Tryptophan synthetase

Glycerolphosphate synthetase

Repressor

- **<u>IN THE ABSENCE OF TRYPTOPHAN</u>**

trp P

O *trpL* *trp E* *trp D* *trp C* *trp B* *trp A* *trp R*

m-RNA

ON

Leader peptide

Anthranilate synthetase Tryptophan synthetase

Glycerolphosphate synthetase

Repressor

- **<u>IN THE PRESENCE OF TRYPTOPHAN</u>**

trp P

O *trpL* *trp E* *trp D* *trp C* *trp B* *trp A* *trp R*

NO m-RNA & NO TRANSCRIPTION

OFF

Repressor

B) Mutation

Definition –

In the broad sense the term mutation refers to all the heritable changes in the genome, excluding those resulting from incorporation of genetic materials from other organisms. A mutation is an abrupt qualitative or quantitative change in the genetic material of an organism.

Mutation may be **intragenic** or **intergenic**.

Intragenic mutations (point mutation) – involves changes (alterations) in the normal base sequence of the DNA molecule within a gene. These changes modify structural characteristics or enzymatic capabilities of an organism.

Intergenic mutations – involves changes in long region of DNA i,e many genes. This includes addition or deletion of segment of chromosome.

DIFFERENT TYPES OF MUTATIONS

1) Base Pair Substitutions –

Substitutions of base pairs are the commonest mutations. In base pair substitution, one base of triplet codon is substituted by another, resulting in a changed codon. They result in the incorporation of wrong base pairs during replication or repair of DNA.

- Original message of reading frame

  C A T G A T C **A** T G A T C A T G A T -------

2) Substitution or Replacement

A replaced by G

C A T G A T C G T G A T C A T GAT --------

Message out of frame

Fig. Mutation by substitution

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

Base pair substitutions are of two main types.

- Transition mutation B) Transversion mutation

A) Transition mutation – These are the most common type of mutations. If a purine base 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.

Fig. Transition

T – A

A – T G – C

C – G

B) Transversion mutation – These are the most rare type of mutations. If a purine base is substituted by a pyrimidine, or vice versa, the substitution is called a transversion. Eight types of changes are possible in transversion such as AT---TA, AT ---- GC, GC ---- CG, GC --- TA, TA ----GC, CG ----- GC, CG ----- AT.

T –A

G – C

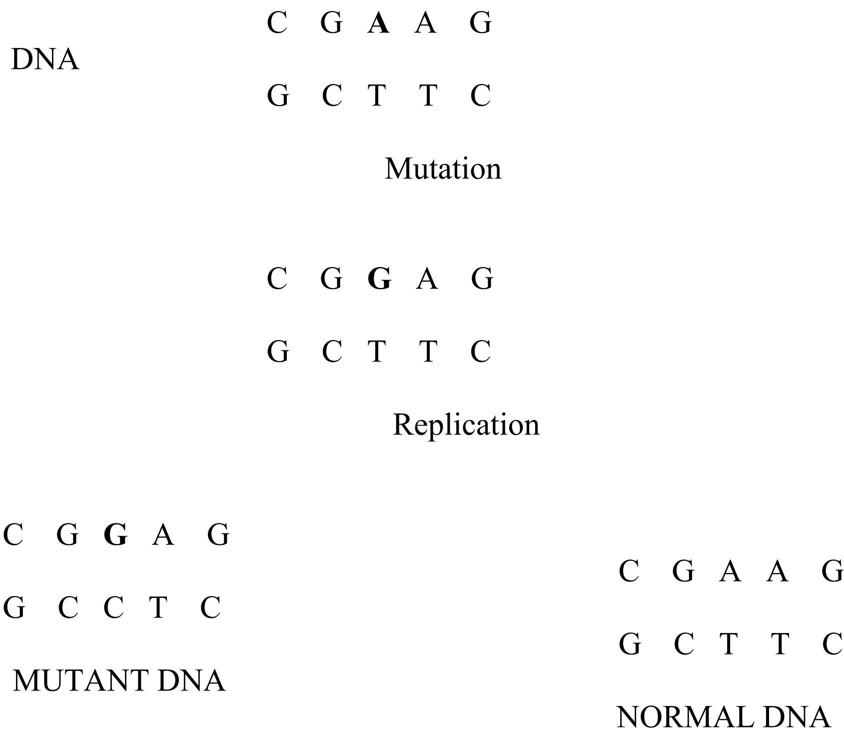
A – T

C – G

Fig. Transversion

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 with another purine base G (transition).



When DNA replicates, it will give rise to two duplexes, one normal like the parent duplex and the other mutant. Since the mutated base G, pairs with C, the mutant DNA will have G C pair at the point of mutation. Thus both chains of mutant DNA have altered bases at the point of mutation.

<u>Inversion</u> – 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 frame only in the triplet involved in the inversion.

C A T G A T **T A C** G A T C A T G A T

2) <u>Frameshift Mutations</u> -

A mutation in which there is a 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.

Addition or deletion 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 resulting protein is usually nonfunctional.

If 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. A change in only one amino acid can have a drastic effect on the phenotype.

The site of mutation also determines whether protein formed will be slightly or drastically changed. 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 functional protein.

Deletion – Removal of one or a few bases from a nucleotide chain is called a deletion. Removal of even one base throws 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 3.

Original message or reading frame

C A T G A T **C** A T G A T C A T G A T -------

Deletion - C

C A T G A T A T G A T C A T G A T -------

Message out of frame

Insertion – The genetic message is similarly disturbed if one or few bases are added (insertion), provided that the number such bases are not divisible by 3.

Original message or reading frame

C A T G A T C A T G A T C A T G A T -------

Insertion + G

C A T G A T G C A T G A T C A T G A T C A

Message out of frame

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

Deletion and Insertion

- C   + C

C A T G A T C A T G A T C A T G A T C A T -------

C A T G A T **A T G A T C A T C** G A T C A T -------

Message out of frame

A mutation 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.

Deletion- 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.

Insertion- If letter A is inserted after MAN, then also the sentence becomes meaningless after MAN.

THE MAN AWH OHA SON EEY ECA NSE EYO U

3) Missense Mutations

Missense mutation is a type of point mutation. When one amino acid in a polypeptide chain is replaced by the other amino acid, this type of mutation is known as missense mutation. In this mutation one base of a codon may be substituted by another base. The changed codon may then code for another amino acid.

Missense mutation occurs by insertion, deletion or substitution of a single base into a codon. For example one base of a codon for phenylalanine is UUU. A single base substitution (U ---- G) changes it to UGU, which codes for cysteine. Thus the protein formed after mutation is identical to normal protein except that phenylalanine is substituted by cysteine. In spite of substitution of single amino acid, many proteins are still functional. This depends on type and location of amino acid.

For example, if a nonpolar amino acid in the polypeptide chain is replaced by a polar amino acid, it will drastically change the three dimensional structure of protein and also

change the function. But if the polar amino acid is replaced by the another polar amino acid, there will be little or no effect on protein. A missense mutation, substituting single amino acid of normal hemoglobin by valine, causes sickled blood cells and sickle cell anemia.

4) Non-sense Mutations

Out of 64 codons for amino acids, 3 codons are termination codons, which do not specify any amino acids and terminates protein synthesis. These three termination codons are UAA, UAG AND UGA. Any mutation (change) resulting in the alternation of a codon specifying an amino acid to a termination codon is called a nonsense mutation. For example the codon UAC (for tyrosine) undergoes a one base substitution (C ---- G), it becomes UAG, a termination codon.

A nonsense mutation brings about termination of polypeptide chain synthesis at that point. As a result the polypeptide chain synthesis is incomplete. Such chains are likely to be biologically inactive. The nonsense mutations bring about drastic change in the expression of phenotypic characters because in this mutation the structure and the function of enzyme are changed.

Polypeptide synthesis takes place in 5 ---- 3 direction. Therefore nonsense mutation near 5 end results in a very short chain with little or no biological activity. While nonsense mutation near 3 end results in a chain, which is nearly complete and may have some biological activity.

5) Silent Mutations

Any gene mutation, which does not affect the phenotypic expression, is called a silent mutation. Silent mutations does not result in phenotypic expression because the code is degenerate i,e more than one codon 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, even after change in the base sequence of DNA. This mutation is of silent type because even after change in base sequence of DNA, there is no change in the amino acid sequence and expression of phenotypic characters.

Random Vs. Directed Mutations

It is usually stated that mutations occurs in a random manner means they are not directed according to the requirement of an organism. Thus mutations take place in the Darwinian sense and not in the Lamarckian sense.

According to Lamarckian theory, changes in the organism are brought as a result of sensible want of the organism in response to environmental conditions. As per this theory mutation would have to be directed towards some objective.

According to Neo-Darwinism, mutations are random and are not directed towards some objective.

Rate of Mutation

The rate of mutation is defined as the probability of occurrence of a particular mutation per cell division. The frequency of spontaneous mutations is usually low, ranging from 10^{-7} to 10^{-12} per organism. The rate of detectable mutations in average gene is 1 in $10^{6.}$ However, most methods used for estimating the rate of mutation, underestimates their rate/ frequency due to many reasons. Firstly, lethal leaves no progeny for estimating the rate of mutation. Secondly a mutation, which brings about slight change in the phenotype, may remain undetected.

Mutations occur much more frequently in certain regions of the gene than in others. The favored regions are called 'hot spots'. Mutations involving single nucleotide can revert to normal gene structure and are reversible. In many cases the rate of reverse mutation is similar to the rate of forward mutation. In rare cases the rate of forward mutation is much greater than the rate of backward mutations.

Effect of Mutations on the Phenotype

According to the effect of mutation on the phenotype, mutations may be classified as lethals, subvitals and supervitals. Lethal mutations results in the death of the cell or organism in which they occurs. subvital mutations reduce the chances of survival of the organism in which they are found. Supervital mutations on the other hand may results in the improvement of the biological fitness under certain conditions. There may also be mutations, which are neither harmful nor beneficial to the organism in which they occur.

C) <u>GENETIC SUPPRESSIONS</u>

- The effect of mutation on the phenotype can be reversed, so that the original wildtype phenotype is brought back. This reversal may be due to true reversion or suppression.
- In a true reversion there is a reversal of the original genetic change.
- In suppression a change at a different site brings about phenotypic correction of the mutation.
- True reversion can be distinguished from suppression: only suppressed mutants yield recombinants in which the mutant phenotype is again produced.

Suppression mutations are of two types.

- Intragenic suppression and
- Extragenic suppression

1. Intragenic Suppression –

- Certain mutation in a gene is suppressed by another mutation in the same gene. Such types of genetic suppressions are known as intragenic suppression.
- The effect of a previous in a cistron are removed or reduced by another mutation in the same cistron.
- Intragenic suppression may be divided into several types. Intracodon Suppression and Reading Frame Suppression are discussed below.

1A) Intracodon Suppression –

- In these, deleterious effect of the first mutation is suppressed by another mutation

within the codon, the suppression is called intracodon suppression.

- A codon that has undergone a change as result of mutation may undergo another mutation to a codon that is less harmful to enzyme function.
- For example – mutation of GCU (alanine) to GAU (aspartate) may results in an inactive enzyme. A second mutation A U would give a codon GUU for valine and may restore enzyme activity partially or fully.

	Mutation		2nd Mutation	
GCU		GAU		GUU
Alanine		Aspartate		Valine
Active Enzyme		Inactive Enzyme		Active Enzyme

1B) Reading Frame Suppression

- In these, deleterious effect of the first mutation is suppressed/ neutralized by second mutation at a different site in the gene. This type of suppression is called_reading frame suppression.
- The addition of a base a few codons away from an earlier deletion can suppress the effects of the deletion.
- The amino acid sequence in the polypeptide chain formed after second mutation becomes normal, except for the few amino acids between the two mutations. Thus an altered enzyme differs from the wild type (normal) by only a few amino acids.
- The effects of a deletion and an addition are shown in the following hypothetical sequence.

m-RNA - GUU CUG UUU CCU CGA ACU GAC GCA AUC GGU A

Polypeptide- Val - Leu -Phe - Pro - Arg - Thr - Asp - Ala - Ileu - Gly -

Normal m – RNA and polypeptide chain

- U

m-RNA - GUU CUG UUC CUC GAA CUG ACG CAA UCG GUA

Polypeptide - Val - Leu -Phe - ***Leu - Glu - Leu - Thr - Gln - Ser - Val***

Deletion of U from the Third codon (first Mutation)

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

+U

m-RNA - GUU CUG UUC CUC GAA CUG ACU GCA AUC GGU

Polypeptide - Val - Leu -Phe - ***Leu - Glu - Leu - Thr -*** Ala **-** Ileu - Gly

Addition of U in the Seventh codon (Second Mutation)

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

2) Extragenic Suppressions

- These kinds of suppressions are also known as intergenic suppressions.
- If the harmful effects of a mutation in one gene are overcome by a mutation in another gene, the process is called extragenic or intergenic suppressions.
- The essential feature of intergenic suppressions is that the interacting mutational events take place in two separate genes.
- These two genes may even be located on different chromosomes.

2A) **Nonsense Suppressions**

- The termination of polypeptide chain synthesis is brought about by the termination codons (UAA, UAG or UGA).

- A mutation that converts a codon specifying an amino acid into a termination codon (nonsense mutation) results in the formation of an incomplete polypeptide chain. Such chains are usually inactive.
- The effect of such nonsense mutation can be suppressed by mutations in other genes. Such types of intergenic suppressions are known as nonsense suppressions.
- Such suppressor mutations result in viable proteins.
- One of the methods of suppressing the effects of nonsense mutation is altering the anticodon of t – RNA.
- Example – For glutamine (Gln) amino acid one of the codon is **CAG**. This codon is recognized by anticodon **GUC** of Gln-tRNA. The glutamine codon CAG mutated to UAG (CAG UAG), which is a termination codon. This codon does not recognize any amino acid but terminates polypeptide chain synthesis. As a result inactive and incomplete polypeptide chains are formed.

  The effect of this nonsense codon UAG can be suppressed by mutation in other genes. The normal anticodon for tyrosine t-RNA is AUG. A suppressor mutation can convert this anticodon to AUC through a G ------ C substitution. This mutated tyrosine t-RNA anticodon can recognize the nonsense codon UAG as a codon for tyrosine and thus tyrosine is added to the chain instead of glutamine amino acid. As a result mutant protein becomes active.

Normal Mutated

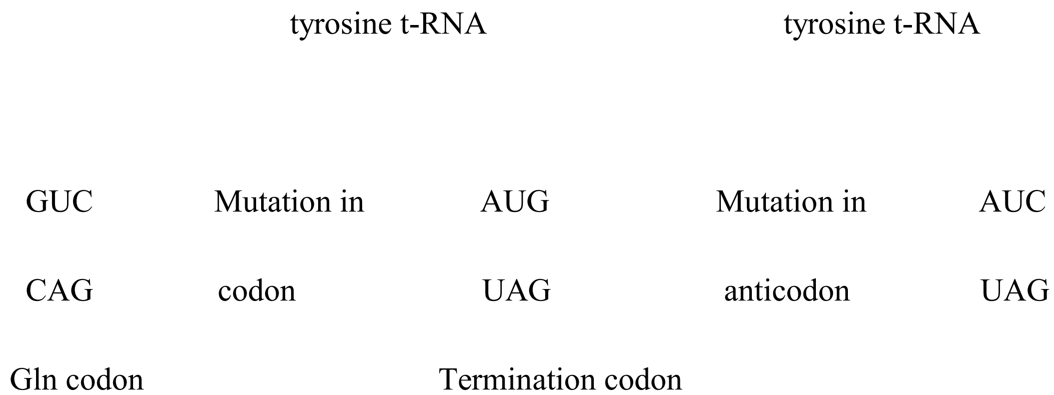


Fig. **Intergenic suppression** : Deleterious effect of mutation of glutamine codon (CAG) to termination codon (UAG) is neutralized by mutation of tyrosine t-RNA anticodon (AUG) to AUC which reads the termination codon as a codon for tyrosine.

m-RNA

Active protein

A. m- RNA with CAG codon for Glutamine (Gln) codes normal active protein.

Inactive protein

B. CAG undergoes mutation to UAG, a termination codon. The protein is terminated at UAG and is incomplete and inactive.

Active protein

C. A second mutation modifies Tyrosine t- RNA anticidon from AUG to AUC. The mutated t-RNA reads the termination codon UAG as a codon for tyrosine And insert Tyrosine (Tyr).

- **MOLECULAR BASIS OF MUTATION**

- Gene mutation at molecular level involves substitution of one base by another, or addition or deletion of one or more bases.
- Mutations may occur in an organism by two mechanisms,
  - Spontaneously called spontaneous mutation or

  ii) Through physical or chemical agents called induced mutation.

SPONTANEOUS MUTATIONS

- Mutations, which occur under natural conditions, are called spontaneous mutations. In other words, the mutation that occurs naturally without any effort is called spontaneous mutation.
- Spontaneous mutations are under the control of nature and are very rare (ranging from 10^{-6} to 10^{-8} per generation) that they occur once in every million individuals.
- Some spontaneous mutations arise by the action of mutagens present in the environment. These mutagens include high-energy radiations, radioactive compounds, temperature fluctuations and naturally occurring base analogues like caffeine.
- Most commonly spontaneous mutations are brought about by different structural rearrangement or tautomerism.

Tautomerism

- The ability of a molecule to exist in two structural isomeric forms, which are mutually interconvertible, is called tautomerism.
- All the four common bases of DNA (adenine, guanine, thymine and cytosine) have unusual tautomeric forms, which are, however rare.
- The normal bases of DNA are usually present in the keto form. As a result of tautomerism they can be transformed into a rare enol form in which the distribution of electrons is slightly different.
- In DNA, normal base pairing is A – T and G – C. However, the tautomeric forms are capable of unusual (forbidden) base pairing like A – C, C – A, G – T and T – G.

Continuous lines indicate normal base pairing and

Dashed lines indicate unusual base pairing.

Purines **Pyrimidines**

A
T

G
C

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

A – T

A* - C A – T

G – C A – T A – T A – T

- Spontaneous mutations can also arise as a result of ambiguity of base pairing during replication.

Tautomerism in Adenine

Common Keto state Rare Enol state

Tautomerism in Thymine

Common Keto state Rare Enol state

Tautomerism in Guanine

Common Keto state Rare Enol state

Tautomerism in Cytosine

Common Keto state Rare Enol state

INDUCED MUTATIONS

- Some agents induce mutations artificially. These agents, which induce mutations artificially, are called mutagens and such mutations induced by mutagens are called induced mutations.
- The frequency of such mutations is higher than spontaneous mutations.
- Various mutagens are classified into two major groups.

  A) Chemical mutagens

  B) Physical mutagens

CHEMICAL MUTAGENS

- Different chemical mutagens, which increase the frequency of mutation, are base anologues, nitrous oxide, hydroxylamine, acrydine dyes etc.

Base Analogues-

- Base analogues are a chemical compound similar to one of the four bases of DNA.

- A base analogues may be incorporated into newly synthesized DNA instead of a normal base.
- These compounds have different base pairing properties.
- They replace bases and cause stable mutations.
- A very common and widely used base anologue is 5 – BromoUracil (5–BU) that is structurally very similar to thymine.

Thymine **5 – Bromouracil**

- 5-Bu functions like thymine and pairs with adenine (A). 5-Bu undergoes internal rearrangement (tautomerism) from the usual keto state to the rare enol state. 5-Bu now pairs with guanine, which is a first mutagenic step of replication. In the next round of replication G pairs with C. Thus there is a generation of GC base pair from the AT base pair (AT ------- GC).

AT ----- GC Replication

G BU **G C**

Mutant DNA

G BU

G C **G BU**

G BU

A BU

GC ------- AT Replication

A T

Mutant

- The 5 – Bromodeoxyuridine (5 –BDU) can replace thymidine in DNA molecule. The 2- aminopurine (2 –AP) and 2,6 – diaminopurine (2,6 – DAP) are the purine analogues. The 2 –AP normally pairs with thymine but it is able to form a single hydrogen bond with cytosine resulting in transition of AT to GC.

2) Nitrous Oxide

- Nitrous oxide reacts with bases containing amino group (-NH_2 group).
- It changes the structure of such bases by deamination (removal of the -NH_2 group).
- When purine or pyrimidine containing the -NH_2 groups are treated with nitrous oxide,

the -NH_2 group is replaced by hydroxyl group (-OH group) and changes it's specificity of hydrogen bonding.

- Deamination of adenine results in the formation of Hypoxanthine. The pairing behavior of which is like a Guanine. Hence it pairs with Cytosine instead of Thymine. Thus A – T pairing is replaced by G – C pairing.
- Deamination of cytosine (at the 6 – position) results in the formation of uracil by replacing -NH_2 group with -OH group. The hydrogen bonding property of uracil is similar to those of thymine. Therefore C – G pairing is replaced by U – A pairing.
- Guanine is deaminated to Xanthine. There is no change in pairing behaviour in this case, because Xanthine behaves like Guanine and pairs with Cytosine. Instead of G – C pairing, there is X – C pairing. Thus deamination of Guanine is not mutagenic.
- The base formed after deaminations of adenine and cytosine have different pairing behaviour.as a result changes in DNA takes place in 50% of the progeny. Deamination of guanine, however, does not result in a heritable mutation, since there is no change in the pairing behaviour of the deaminated base (xanthine).

Normal bases of DNA	Normal pairing	Bases formed by deamination	New pairing
Adenine	A – T	Hypoxanthine	G – C
Cytosine	C – G	Uracil	U – A
Guanine	G – C	Xanthine	X – C

3) Hydroxylamine (NH_2OH)

- It is very specific in action.
- It reacts mainly with cytosine and deaminates cytosine to a base, which pairs with adenine instead of guanine.
- Thus C – G pairing is replaced to A – T.

4) Acrydine Dyes

- Certain fluorescent acrydine dyes such as proflavin and acrydine orange causes mutations by addition or deletion of bases.
- They produce distortion in DNA.
- The acydines are planner (Flat) molecules like purine bases, and in aqueous solution these dyes insert themselves between the bases of the DNA helix. This distorts the structure of DNA and can result in insertion or deletion of bases during recombination.

- **Intercalation Resulting in Insertion of Bases** –

- Intercalation of acrydine molecules between two bases of the template strand results in lengthening of the DNA molecule.
- During replication a base (X)is inserted at random opposite the acrydine 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.

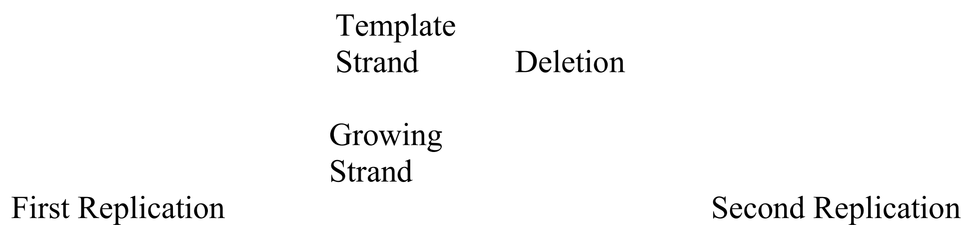
Acrydine molecule

Insertion

DNA Helix First Replication Second Replication

- **Intercalation Resulting in Deletion of Base**

- Acrydine molecule may be inserted in the new chain during synthesis.
- This blocks the base in template strand and does not permit any base to pair with it.
- The chain produced is thus deficient in one base, and in the next replication produces DNA with a deficient base pair.



PHYSICAL MUTAGENS

- Radiations occupies important place among physical mutagens.
- The energy content of a radiation depends upon its wavelength.
- Radiations with long wavelength contain less energy. Low energy radiations include visible light and radio waves.
- Radiations having shorter wavelength contains high energy. High-energy radiations include X – rays, Gamma () rays, Cosmic rays and Ultra Violet rays.
- High-energy radiations can penetrate all the forms of matter and can change the atomic structure of a substance.
- High-energy radiations are of two kinds – a) Ionizing Radiations & b) Non Ionizing Radiations.

a) Ionizing Radiations - These radiations have wavelengths 0.0008 A^0 – 150 A^0 and includes alpha, beta, X – rays, Gamma () rays and Cosmic rays. These radiations have sufficient energy and bring about ionization of molecules by pulling electrons away from molecule and ionize them and are therefore called ionizing radiations.

b) Non Ionizing Radiations – these radiations do not ionize the molecules and includes ultra violet portion of electromagnetic radiations.

X – Rays

- X – rays brings about mutations by breaking the phosphate ester linkages in DNA.
- The breakage may take place at one or more points and as a result, a large number of bases are lost or rearranged.
- X – rays may break the DNA either in one or both strands. If break occurs in both strands, it becomes lethal.
- Sometimes, two double- stranded breaks may occur in the same molecule and thee two broken ends may rejoin.
- The segment of the DNA between the two breaks is removed resulting in a deletion.
- Since both X – rays and UV- rays bring about damage in DNA molecule; they are used in sterilization of bacteria and viruses.

Ultraviolet Rays

- The ultraviolet portion of radiations includes all radiations from150 to 3900 A^0. It is less energetic and does not ionize the molecules.
- It is absorbed by many cellular constituents, but primarily by the nucleic acids.
- UV- rays causes damage in the DNA duplex of the bacteria and phages.
- The DNA molecule is the target molecule for UV- rays but not the proteins.
- The absorption of UV- rays by DNA brings nitrogen containing bases to highly excited state. The excited DNA leads to cross-linking, single strand breaks and base damage as minor effects.
- The primary mutagenic effect of the UV- light appears to be due to the production of thymine dimmer.
- The 5, 6 unsaturated bonds of adjacent pyrimidines become covalently bonded to form cyclobutane ring.
- Irradiation of a bacterial culture and subsequent extraction of DNA yields three possible types of pyrimidine dimmers in DNA. The generation of pyrimidine

dimmer is a major mutagenic effect.

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

A T

G C

T A

T A

C G

Distortion of DNA by
Thymine dimmer

- Pyrimidine dimmer can also be formed between adjacent strands.
- Pyrimidine dimmer cannot fit into the DNA helix and causes distortion of DNA helix. If damage is not repaired, replication is blocked, leading to lethal effects.
- UV- radiations also cause addition of water molecule to pyrimidine in both DNA and RNA, resulting in the formation of photohydrate. The water molecule is added across the C5 – C6 double bond.

Gamma Ray

- Gamma () rays are shorter wavelength radiations with higher energy level and strong penetration power.
- Gamma rays have energies above 10 electron volt and wavelength less than 10 picometers,.
- Gamma rays are ionizing radiation and are thus biologically hazardous.
- Gamma radiation is often used to kill living organisms, in a process called irradiation. They are used to kill cancer cells without having to resort to difficult surgery.

- Gamma radiation is used in sterilizing medical equipment (as an alternative to autoclaves or chemical means), removing decay-causing bacteria from many foods or preventing fruit and vegetables from sprouting to maintain freshness and flavor.
- Gamma-rays are energetic enough that they produce reactive ions (charged atoms or molecules) when they react with biological molecules; thus they are referred to as ionizing radiation.
- Ionizing radiation produces a range of damage to cells and organisms primarily due to the production of free radicals of water (the hydroxyl or OH radical).
- Free radicals possess unpaired electrons and are chemically very reactive and will interact with DNA, proteins, lipids in cell membranes, etc
- Gamma rays produces a range of effects on DNA both through free radical effects and direct action.
- They cause breaks in one or both strands of DNA which can lead to rearrangements, deletions of bases, damage to bases, and crosslinking of DNA to itself.