IV SEMESTER

COURSE-9:- MOLECULAR BIOLOGY & MICROBIAL GENETICS

UNIT-1

DNA/RNA as genetic material, Replication of DNA.

1. Experimental evidences that established DNA & RNA as genetic material. Genome organization in prokaryotes and eukaryotes.
2. Replication of DNA in prokaryotes: Bidirectional and unidirectional replication, Semiconservative replication, proof of semiconservative replication (Messelson - stahl experiment). Mechanism of DNA Replication in prokaryotes: step by step process, Enzymes and factors involved in replication - Primase, Helicase, Gyrase, DNA polymerases, DNA ligase, SSB proteins.
3. Extra chromosomal genetic elements: General characters, types and applications of plasmids and transposons.

Unit-2: Concept of gene, Transcription:-

1. Classical concept of gene: Muton, Recon and cistron; one gene-one enzyme and one gene-one polypeptide and onegene-One product hypotheses.
2. Modern concept of gene: Definition of gene; Open reading frame; structural, constitutive and regulatory genes: Uniinterrupted genes, Split genes - concept of introns and exons.

3.- Protein synthesis in Prokaryotes: Transcription - Definition, difference from replication, promoter, RNA polymerase, mechanism of transcription. RNA Splicing in eukaryotes.

Unit: 3:-

Translation and regulation of gene expression:

Protein Synthesis in Prokaryotes.

3:1. Genetic code: salient features, Wobble hypothesis.

3:2. Translating - charging of tRNA, aminoacyl tRNA synthetase, Mechanisms of initiation, elongation and termination of polypeptides. Inhibitors of protein synthesis.

3:3. Regulation of gene expression in bacteria - lac operon.

Unit-4: Mutations and DNA repair:-

4.1 :- Mutations: Definition and types of Mutations (spontaneous & induced, Somatic and germline): Physical and chemical mutagenes;

4:2:- Molecular basis of mutations (base pair changes, frame shifts, deletions, inversions, tandem duplications, insertions); functional mutants (loss and gain of function mutants); uses of mutations.

4:3:- outlines of DNA repair mechanisms: Direct repair, Mismatch Repair, Recombination Repair, SOS Repair.

Unit- 5 :-

Genetic recombination in bacteria :

1. Conjugation - discovery, F - factor, Ft & Hfr, mechanism of conjugation, applications of conjugation.
2. Transformation - Discovery, mechanism of transformation, competence factors affecting transformation and application of transformation.
3. Transduction :- Discovery, Mechanism and types of transduction.

→ Re write plasmids & Transposons ←

07/01/25

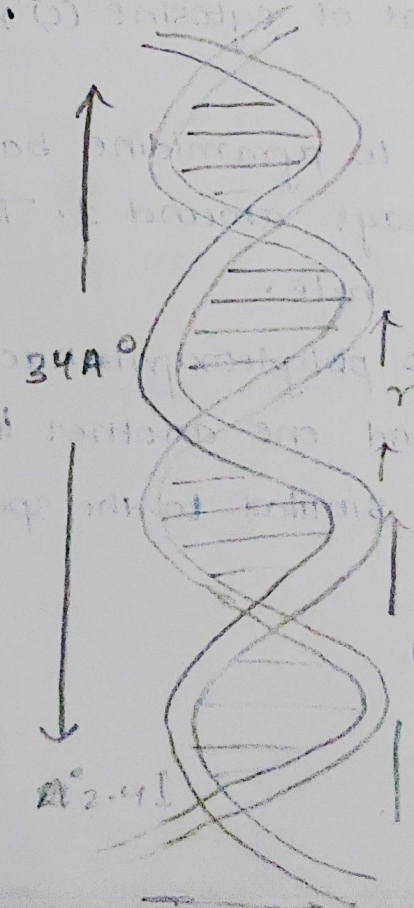
UNIT - 1

1. Nucleic acids are also bipolymers of high molecular weight with mononucleotides as their repeating units, just as aminoacids are the repeating units of proteins. The nucleic acid contain carbon, hydrogen, oxygen, nitrogen and phosphorous.
2. There are two kinds of Nucleic acids.
   1. RiboNucleic acid (RNA) &
   2. Deoxy RiboNucleic acid (DNA).
3. DNA constituents the genetic materials where as ribonucleic acids (RNA) function as working copies of DNA and participate in protein synthesis.

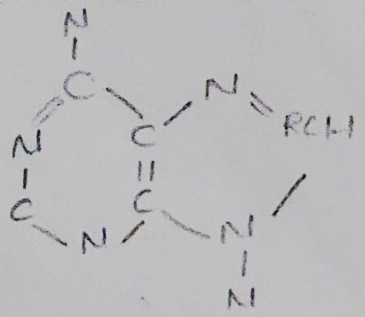
1. Deoxy Ribo Nucleic Acid :- (DNA).

J. Watson and F. Crick proposed a model for explaning the structure of DNA molecule.

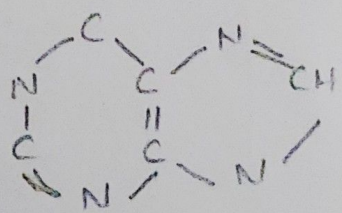
Structure of DNA :-



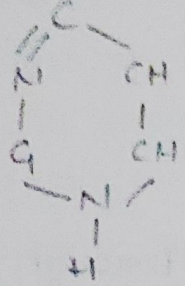
1. DNA is the chemical basis of heredity.
2. DNA is composed of four deoxyribonucleotides, deoxydenylate, deoxyguanylate, deoxycytidylate & deoxythymidylate.
3. These units are combined through 3' ⟶ 5' phosphodiester bonds to polymerase into a long chain.
4. The nucleotide is formed by a combination of base + sugar + phosphoric acid. The 3'-hydroxyl of one sugar is continued to the 5'-hydroxyl of another sugar through a phosphate group.
5. The polymer possess a polarity, one end has a 5'-hydroxyl or phosphate terminus while the other has 3'-phosphate or hydroxyl moiety.
6. In DNA molecules the concentration of Adenosine (A) nucleotides equal that of thymine (T) nucleotides (A = T). The concentration of Guanosine(G) nucleotides equal that of cytosine (C) nucleotides (G≡C).
7. The ratio of purine to pyramidine bases in the DNA molecule is always around 1. This is known as chargaff's rule.
8. DNA consists of two polydeoxyribonucleotide chain twisted around one another in a right handed double helix similar to the spiral staircase.



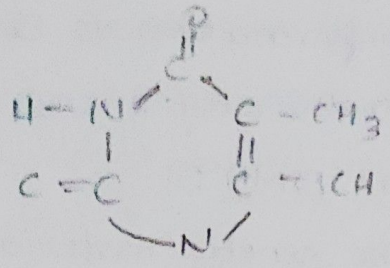
Adenine



Guanine.



Cytosine



Thymine.

9. The base pairing rule is maintained. The two stands are complementary to eachother. In other words, the Adenine of one strand will pair with cytosine. The base pairing (A with T and G with C) is through hydrogen bond.

10. The DNA strands are held together by hydrogen bonds between the purine and pyrimidine bases. These are two hydrogen bonds between A and T while there are three hydrogen bonds between G and C. The GC bond is G≡C . therefore stronger than the AT bond A=T. So regions rich in the GC bond pair are more resistant to denaturation.

11. The two strands in a DNA molecule run antiparallel which means that one strand runs in the 5' to 3'. direction. while the other is the 3' to 5' direction.

12. In the DNA molecule, the genetic information resides on one strand called the template stand or sense strand. The opposite strand is called antisence strand.

13. DNA can exists in several conformation depending upon the base composition and under different physical conditions. In all these conformations the same base paring rules apply, changes do not affect the information content of the DNA. The most

common conformation is D-DNA, other types are A-DNA, C-DNA, and Z-DNA.

Functions of DNA:-

1. DNA is the genetic material.
2. DNA controls every function of cell through protein synthesis.
3. DNA has two important functions heterocatalytic and autocatalytic. In heterocatalytic function, DNA directs the synthesis of chemical molecules eg:- synthesis of RNA, proteins etc.

In autocatalytic function DNA directs the synthesis of DNA itself.

Ribonucleic Acid (RNA):-

RNA is polymer of ribonucleotides of adenine, uracil, guanine and cytosine joined together by 3'-5' phosphodiester bonds. Thymine is absent in RNA. RNA found in the nucleus.

Granules, ribosomes, mitochondria and cytoplasm. The pentose sugar of the nucleotide is D-ribose.

Structure of RNA:-

RNA is single strand and having ribose sugar, Nitrogenous bases and phosphoric acid. There are three forms of RNA in the cell.

There- (i) M-RNA (ii) t-RNA (iii) r-RNA

each of them having different role in protein synthesis.

Primary structure of RNA:-

1. The primary structure of RNA defined as the number

and sequence of ribonucleotides in the chain.

2. Each line as strand is held together by the nucleotides bound to each other by 3', 5' phosphodiester bonds joining 5'-OH of the next.

Secondary structure of RNA :-

It involves the various coil formation of the polyribonucleotide chain.

Tertiary structure of RNA :-

1. It involves the folding of the molecule into three dimensional structure.
2. The cross-linkage also occurs as various sites stabilized by hydrophobic and hydrogen bonds producing a compactly coiled globular structure.

Functions of RNA :-

The RNA are synthesized from DNA and play important role in the process of protein synthesis.

(a). mRNA transfers genetic information from genes (DNA) to ribosomes for protein synthesis.

b. t-RNA tranyer aminoacids to mRNA for protein synthesis.

c. r-RNA provide structural frame work of ribosomes.

Experimental evidences that established DNA & RNA as genetic material :-

Exp :- 1 : Frederick Griffith Bacterial transformation :-

In 1928, British bacteriologist federick Griffith conducted a series of experiments using streptococcus pneumonia bacteria and mice Griffith was not trying to identify the genetic material, but rather trying to develop a vaccine against pheromonia.

They are used two related strains of bacteria.

R-strain :-

When grown in a petridish the R-bacteria formed colonies or clumps of related bacteria that had well-defined edges of the rough appear on the R-bacteria that had were non-virulent meaning that did not cause sickness when injected into a mouse.

S-strain :-

S-bacteria formed colonies that were rounded and smooth the smooth appearance was due to a polysaccharide or sugar based coat produced by the bacteria. This coat protected the S bacteria from the mouse capable of causing disease.

In this experiment :-

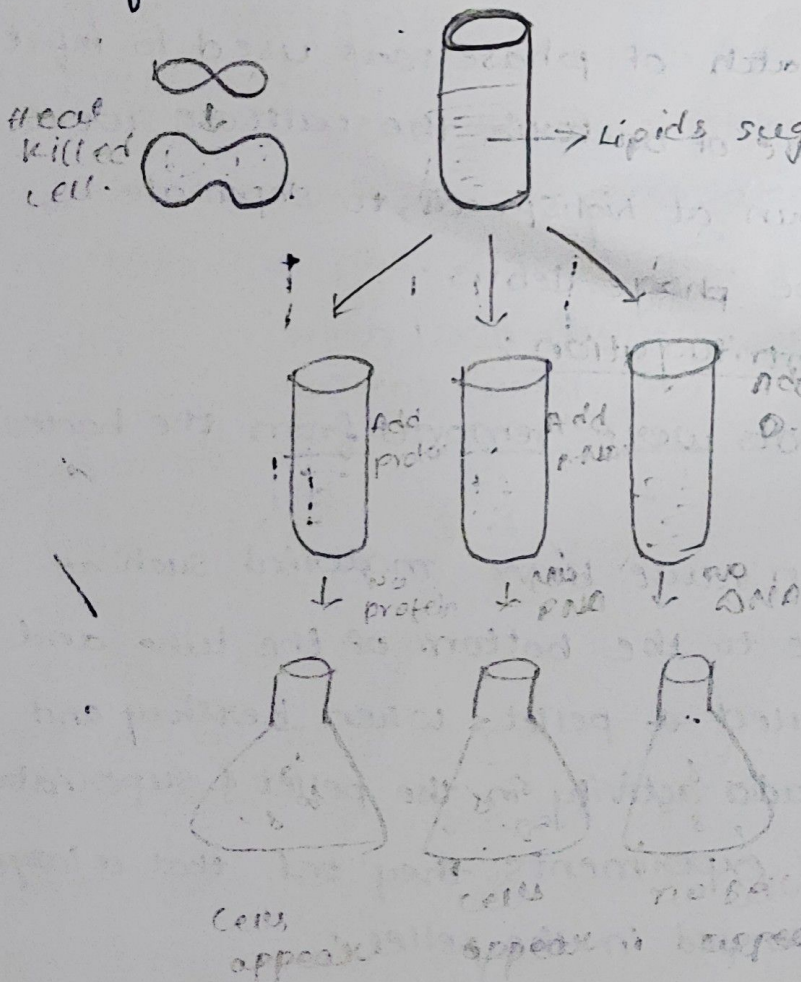
1. When griffith injected mice with the non-virulent bacteria (strain R) the mice survived.
2. The griffith injected mice with virulent bacteria (strains) the mice died.
3. When Griffith injected mice with heat killed virulent bacteria (strains) the mice survived as the bacteria had been killed.
4. When Griffith injected mice with a mix R. The mice were found to have died.
5. From the Griffith concluded that the transformed into virulent S cell.
6. This indicated that three was same form of transferable genetic material present with in the cells.

Exp:2:- Avery, mc carty and nacleoid :-

Identifying the transforming Principle:-

Later Oswald Avery, Colin macleod and macylm mccarty in 1944, repeated Griffith experiment in an vitro system in order to identify the nature of the transforming substance responsible for converting a non-virulent strain into virulent strain.

They observed that DNA, RNA and protein isolated from the heat killed S-strain when added to R-strain changed their surface character from rough to smooth and also made them pathogenetic but when the extract was treated with DNase the transforming ability was lost R-Nase and proteases didnot affect the transformation. This phenomenon of R strain change in into S-strain is refferred to a transformation.



Exp:3:-

The Hershey and chase Experiment:-

The scientists believed that the genetic material was protein in 1952 hersey & chase were the one to conclusively prove that DNA as the genetic material. Hershey & chase use bacteriophage to experiment used bacteriophage to experiment.

Labelling:-

Some virus were grown on a medium containing radioactive phosphorous.

viruses:-

Grown on radioactive phosphorous have radioactive DNA but not protein since DNA contains phosphorous but protein doesnot.

Infection:-

Each batch of phase was used to infect a different culture of bacteria the culture were centrifuged or spun at highspeeds, to separate bacteria from the phage debris.

Blending and Centrifugation:-

The coats were removed from the bacteria by blending.

* The centrifugation cause bave material such as bacteria to move to the bottom of the tube and from a lump called a pellet. When hershey and chase measured radio activity in the pellet & supernatant from both of their experiments they tal that a large amount of P^{BR} appeared in the pellet.

Indirect Evidence:-

(a) Localization: The genetic substance should have a fixed location with in the cell. If it has no

fixed location the genes are not able to function properly. The specific function of DNA located at the feulgen reaction with the most specific one for DNA feulgen staining strains chromosome magneta colour against the clear cytoplasmic background.

(b) stability :-

Various macromolecules present within the cell are continously being anobolised and catabolised.

(c) Sensitivity to mutagens :-

Mutagens is an important characteristic feature of the genetic material the agents capable of including mutations are called mutagens. Different types of radiation UV-ray, x-ray, γ-ray and a variety chemical compounds acts as mutagens.

(d). DNA content :-

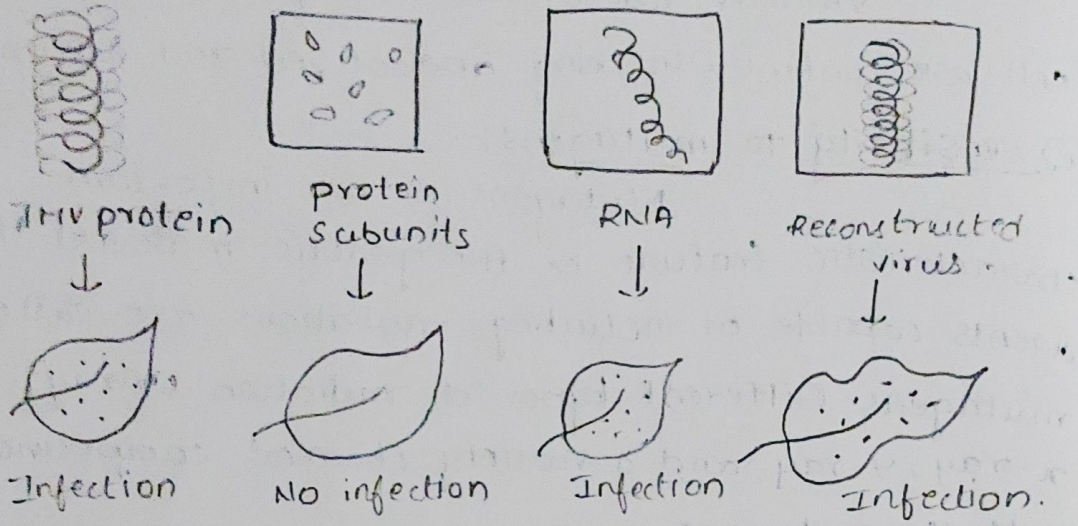
One of the striking features of the genetic material. is the correlation between DNA content and the number of chromosome sets.

Cells	Mean DNA feulgen content (picograms)	pressured chromosome set (plody).
Spermatids	1·68	Haploid (n)
Liver	3·16	Diploid (2n)
liver	6·30	Tetraploid (4n)
Liver	12·30	Octoploid (8n)

RNA as Genetic material :-

The genome of viruses may be DNA & RNA most of the plant viruses have RNA as then hereditary material 'traenkent cobrat 1957 conducted experiments on tobacco mosaic virus (TMV) to demonstrate that in some viruses RNA acts genetic material. TMV is a small virus composed

of a single molecule of spring like RNA encapsulated in a cylindrical protein coat. Different strains of TMV can be identified on the basis of differences in the chemical composition of their protein coats and difference in symptoms tobacco leaves.



In the other exp: two strains of TMV i.e. type A and type B showing symptoms the protein RNA parts were separated and chemera (hybrid) viruses were synthesized using RNA of type A & protein of type B and vice versa.

Structure and organization of Prokaryotic DNA:-

The DNA of a cell packaged as a double stranded DNA molecule is called its genome. In prokaryotes, the genome consists of a single double stranded, DNA molecules in the form of loop or circle. Some prokaryotes also have smaller DNA loops called plasmids that are not necessary for normal growth. Antibiotic resistance is a trait that often spreads through the exchange of plasmids in a bacterial colony supper coiled DNA is more tightly wound than is normally the case in a cell

(more than 10 nucleotides) per turn of the helix. DNA carries all the information is carried on a different section of the DNA these sections are called genes.

If is very living thing DNA is found in structure of the cell chromosomes DNA was discovered in 1869 by swiss found in structures, researcher fedrick mischer, who first identified DNA is often mistakely given to some watson and Grick.

Watson and Grick model of DNA :-

Importance of phosphodiester bond ~~the~~ DNA is a blue print of human that stores data and transfer it to the next generation.

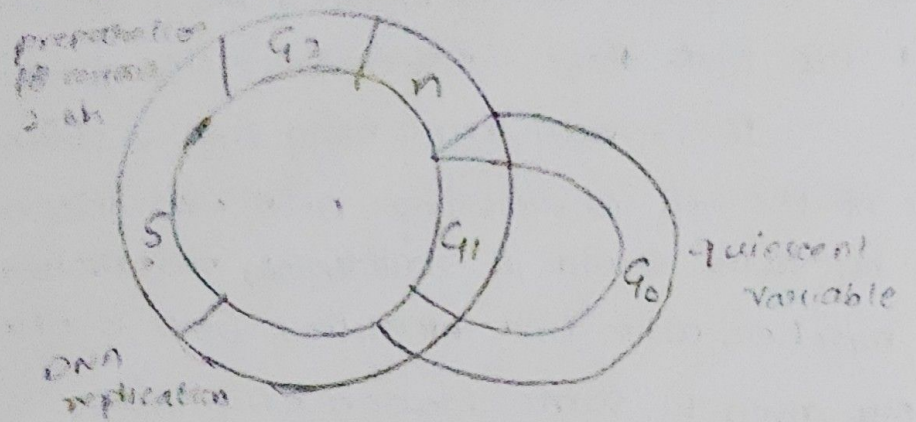
properties of major forms of DNA :-

particulars	A DNA	B DNA	Z DNA
Helix	Right Handed	Right Handed	left handed
Base pairs per turn	~11	~10.5	-12
Helical diameter (in nm)	2.6	2.0	1.8
Helical length(nm)	2.6	3.4	3.7
shape	Broadest	Intermediate	Narrowest.
Major Grove	wide, deep	Narrow, deep	Flat
minor Groove	Narrow, Shallow	Broad, Shallow	Narrow, deep.

Replication of DNA :-

DNA replication is the basis of inheritance it is the fundament process occuring in all cells for transfer of genetic information to daughter cells. Each cell must replicate its

DNA before division.



Commitment to DNA replication (10h)

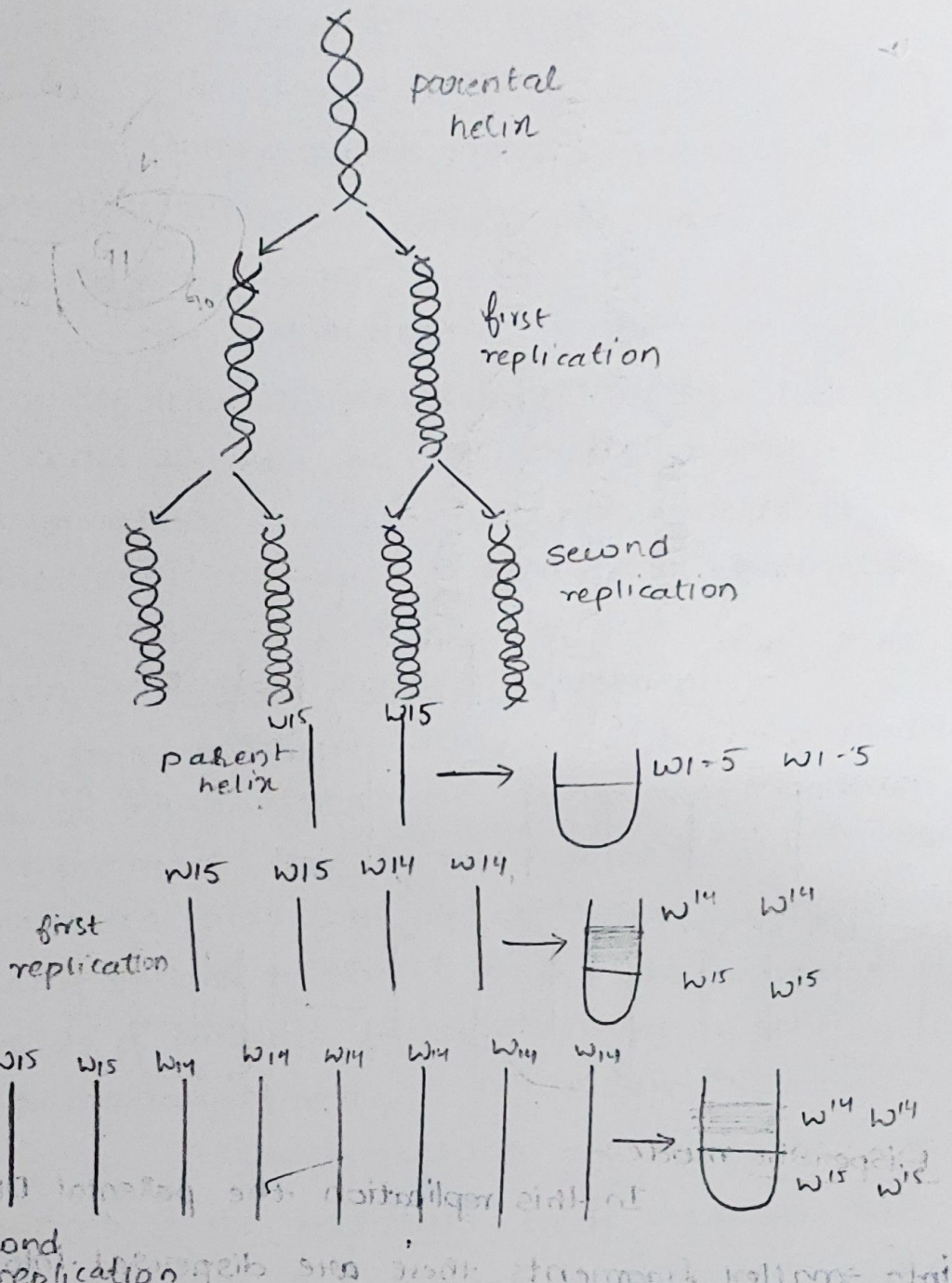
Genetic material is transmitted from parent the offspring and from cell to cell for this The genetic material must be copied. This process of copying genetic material is called as replication of DNA or the process on which DNA makes exact copies of itself.

* In this process DNA uses templates for the synthesis of new strands.
* These are three different modes for DNA replication.

1. Conservative mode
2. Semi conservative mode or half conservative mode
3. Dispersive mode.

1. Conservative mode :—

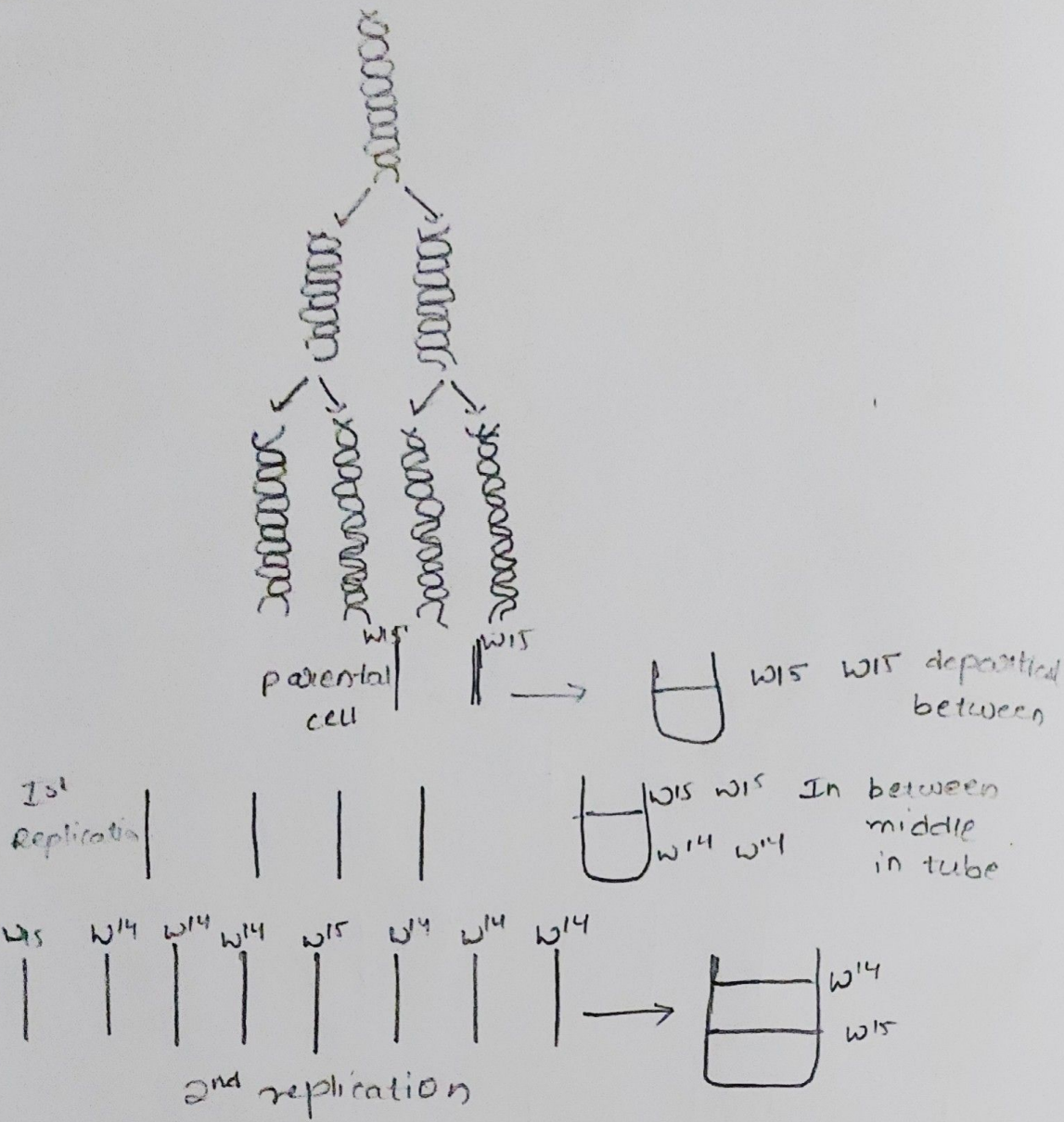
The newly synthesized strands come together to form their a helix and the parental strand. This alleles to conserve the reassociates the parental helix.



2. Semi Conservative mode:—

1. This model was proposed by John cairns.

2. It invdves strands separation of parental DNA after replication in the two DNA duplex one new strand and one old strand associates.



3. Dispersive mode :-

In this replication the parental DNA is into smaller fragments these are dispersed into the new double helical following replication (mix to form). Hence each strand of despersive contain of both old & new fragments

Bidirectional replication :-

It is a process that involves replicating changes made to copy of a table to another copy and then replicating changes made to the second copy back to the first.

Unidirectional replication :-

The process of replicating data from a source to target or the process of replicating DNA in one direction.

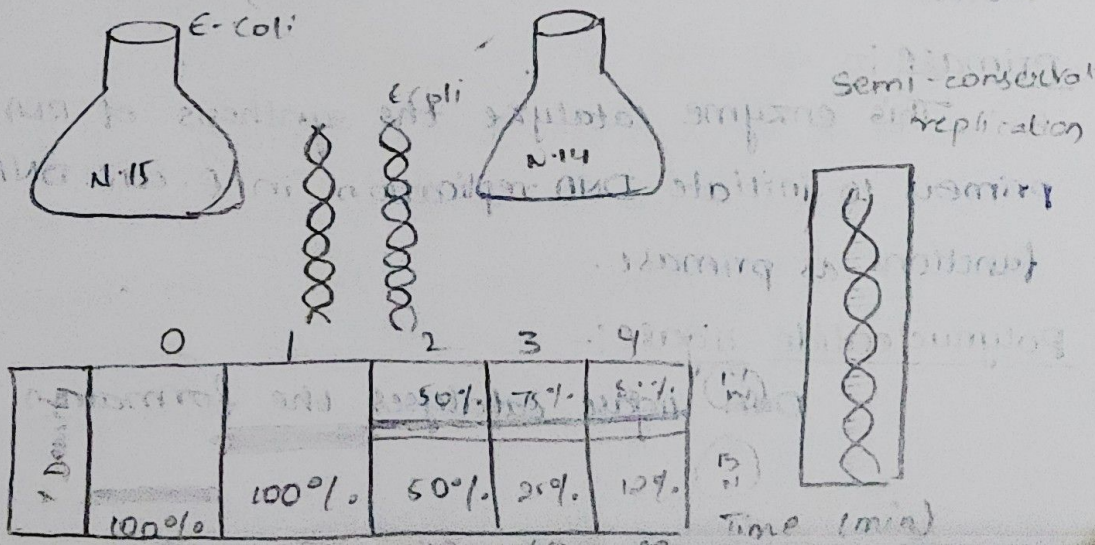
Central Dogma :-

The central dogma of molecular biology explains the flow of genetic information within a cell DNA codes for RNA via - the process of transcription the information flow was always considered to be unidirectional always considered to be unidirectional DNA → RNA → protein.

Meselson and Stahl cracked experiments :-

DNA replication using E-Coli bacteria model system. In the year 1957 gave experimental evidence. The experiment done by meselon and stahl demonstrated that DNA replicated semi conservatively meaning that each strand in a DNA molecule serve as a template for synthesis at a new complementary strand.

Although meselon and stahl their experiment in the bacterium E.coli we know that semi-conservative DNA replication is a universal mechanism shared by all organisms.



Enzymes Involved in DNA replication:-

DNA polymerase:-

This is the main enzyme required for DNA replication activity for discovered by cornberg in 1956 co. has DNA polymerases.

A DNA Polymerase - I :-

Filling if gap after removal RNA primer DNA repair, removal of RNA primers.

→ The exonuclease activity $5' \longrightarrow 3'$ and $3' \longrightarrow 5'$

B. DNA polymerase II :-

The polymerization is slower than the Pol I it also consists of a single polypeptide chain pol - II acts as backup enzyme and DNA repair.

→ It has $5' - 3'$ polymerase & $3' - 5'$ exonucleases activities.

C. DNA polymerase III :-

1. DNA polymerase III is a large multiplication complex that acts as the main work nourse of replication.
2. In synthesis nucleotide strands by adding new nucleotides to the 3' end of a growing DNA molecule. molecule $3' - 5'$ exonuclease activity.

primase :-

This enzyme catalyze the synthesis of RNA primers to initiate DNA replication in E. coli. DNA functions as primase.

Polynucleotide ligase:-

DNA ligase catalyses the formation of

phophodiester linkage between two immediate neighbour nucleotides of a DNA strand thus it seals the nicks remaining in a DNA strand either following replications on DNA repair. This enzyme cannot fill gaps in DNA strands

Helicase :-

Helicase effects strand separation at the fork and uses one ATP molecule for each base that is separation in E-coli DNA functions as helicase. This protein isohexomer and its moves with replication fork.

Single strand binding protein (SSB) :-

Bint to single stranded DNA and prevents it form forming duplex DNA or secondary structures. SSB binds as a monomer but it binds cooperatively in that binding of one SSB molecule facilitates binding of more SSB monomers same DNA strand.

DNA Gyrase :-

Topoisomerase as also known as gyrase it helps to reliere that stress on DNA when unwinding by causing breaks and then revealing the DNA.

Ligase :-

Seals the gaps between the okazaki fragments to create one continous DNA strand.

Semi Conservative mechanism of DNA replication :-

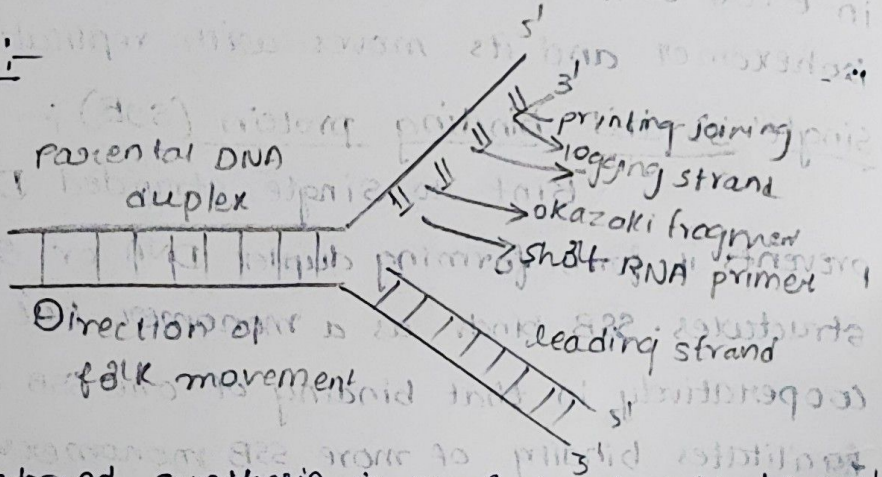
John (cairns 1963) through his autoradiography experiments provide the first evidence contains the E-coli chromosome.

1. Initiation.
2. Elongation &
3. Termination.

Initiation :-

DNA replication begins from origin in E-coli replication origin is called oric which consists of 245 base pair and contains DNA sequences that are highly conserved among bacterial replication origin the types of conserved sequence are found at oric three repeats of 13bp (GATRCTNTTNTTTT) and four five repeated of a bp (TTA TCC A CA) called 13mer and 9mer respectively.

2. Elongation :-



(iii) Leading strand synthesis is more a straight forward proces which begins with the synthesis of RNA primer by primase replication origin.

(i) Logging strand synthesized in shott fragments called okazaki fragments. At first RNA primer is synthesized by primase and as in leading straining DNA polymerase bind to RNA.

3. Termination :-

Eventually the two replication fork of circular E.coli chromosome meet at termination recognizing sequences (ter). The ter sequences of 23bp are arranged on the chromosome. Ter-Tus complex can arrest the replication fork from only one direction.

* When either of the fork encounter Ter-Tus complex replication.
* Find few hundred bases of DNA between these large protein complexes are replicated by two interlinked (catenated) chromosome.
* In E.coli DNA topisome rase IV (Type II) cut the two strand of one circular DNA and segregates each of the circular DNA and finally join the strand. Transfers to two daughter cells.

03/02/24:

Plasmids:-

Plasmids are small circular DNA fragments, double stranded, self-replicating, extra chromosomal DNA molecule.

2. The term plasmid was coined by Joshua Lederberg

3. The physical nature of plasmids is quite simple. They are small double stranded DNA molecules Ex:- Streptomyces spp and Borrelia spp).

4. The plasmid DNA isolated from the bacterial cells exists in the supercoil configuration.

5. Plasmids are present in only 1-3 copies, whereas others may be present over 100 copies. Copy number is controlled by genes on the plasmid and by interactions between the host and the plasmid

6. Borrelia burgdorferi that causes Lyme disease, for convenience, possess 17 different circular and linear plasmids.

7. Plasmid is not accommodated and is lost during subsequent replication.

Structure of plasmids:-

1. Origin of Replication:-

In plasmids, this region is A=T rich region as it is easier to separate the strands during replication.

2. Selectable marker site:-

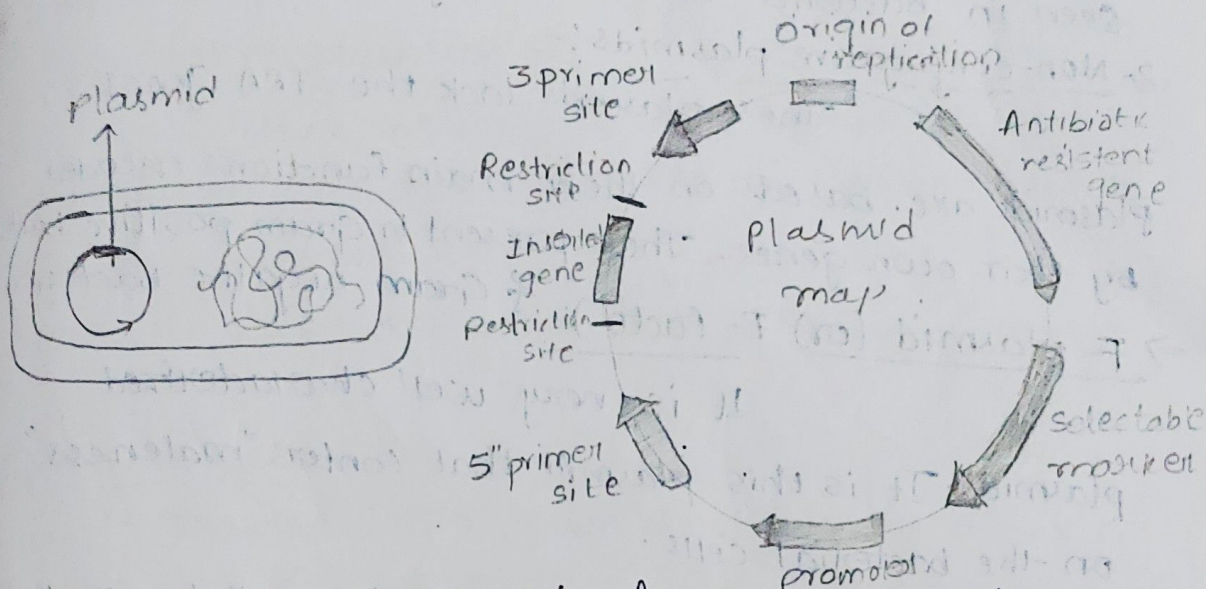
This region consists of Antibiotic resistance genes which are useful in identification.

3. promoter region:-

The region where the transcriptional machinery is loaded.

4. primer binding site :-
This is short sequence of single strand DNA useful in DNA amplification & sequencing.

5. Multiple cloning sites :-
Where the restriction enzymes can bind and cleave the double stranded structure.



1. The size of plasmid varies from 2kb to 200kb
2. It is not required for growth and development of the cell.
3. Most plasmids contain the TRA gene, which is essential in transferring the plasmids from one cell to another.

Transfer of plasmid :-
Plasmid DNA can be transmitted among bacteria by Transformation
Transduction (or) conjugation.

It is not required for inheritance of traits.
* Many plasmids are incapable of self-transmission and transferred at high frequency from cells that carry a self-transmissible plasmid
* The process of conjugation involves two cells :- a donar cell and a recipient cell.
* The donar cells form a conjugation bridge now as pilus and attaches to the recipient cell.

* One copy of the plasmid is transferred from donar to recipient cell

Types of plasmids :-

1, Conjugative plasmids :-

Plasmid contain TRA gene are seen in bacteria. It includes synthesis of sex pilli

2. Non-conjugative plasmids :-

These plasmids lack the TRA genes.

plasmids are based on their main functions encoded by their own genes. These present in Gram positive bacteria & Gram negative bacteria.

⇒ F plasmid (or) F- factor)

It is very well characterized plasmid. It is this plasmid that confers "maleness" on the bacterial cells.

* The term sex factor is also used to refer to F-plasmid
F-plasmid. (IS3, Tn1000, IS3 and IS2 genes)
* It is circular ds DNA molecule of 99,159 base pairs.

ii, R- plasmids :-

These are called resistant plasmids.

Example is 94·3 kilobase - pair plasmid.

iii, Virulence plasmids :-

It confers the pathogenicity on the host bacterium. Ex :- Ti- plasmids of Agrobacterium tumefaciens include crown gall disease of angiospermic plants.

* Which includes extensive secretion of water ~~with~~ and salts into the bowel.

iv, Col plasmids :-

These plasmids confers the ability to host bacterium to kill other bacteria by secreting bacteriocins. col E1 plasmid of E-coli

v. Metabolic plasmids :-

Also called degradative plasmids, possess genes to code enzymes such as pesticides (2,4-dichloro-phenoxyacetic acid, and sugars.

* TOL - (pWW0) plasmid of pseudomonas putida is an example.

vi. Degradative plasmids :-

From the dead plants & animals, it helps in degradation and digestion of the dead organic matter.

vii. Virulence plasmids :-

It carries the genes which are responsible for causing disease

Determining the Host Range :-

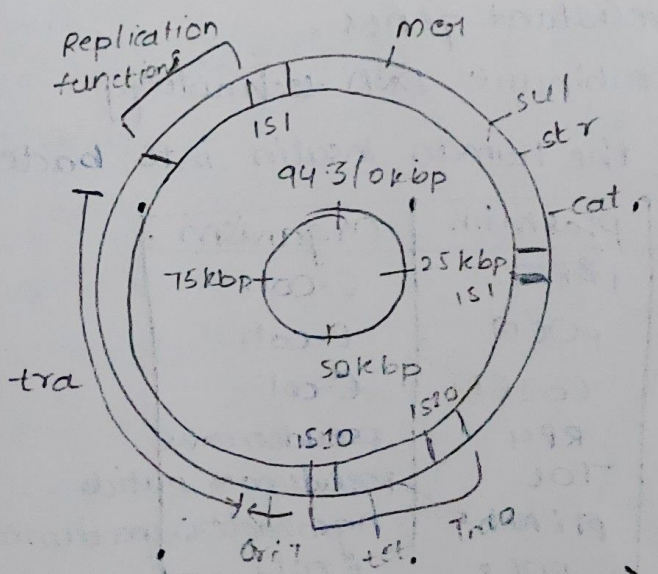
Plasmid will replicate on the other host known as transformation

* A bacterium might possess resistance to any antibiotic due to the presence of a particular gene.
* Such resistance property can be transferred to other bacteria too when they will uptake those resistant genes.

Ex :- kanamycin resistant gene.

Replication of plasmids :-

They have their own replication origins.



These large plasmids carry genes that code for enzymes that are specific for plasmid replication.

* 1/10 or less of the total time of cell division cycle.
* It is the bidirection replication around the DNA.
* plasmids of gram-negative bacteria replicate by unidirectional method.
* phage ϕX 174

Plasmid Curing :-

It can be eliminated from bacterial cells, and it called curing.

* Curing treatment agents are UV rays, ionizing radiation.

Use of plasmids as cloning vector :-

1. Small size, which makes the plasmid easy to isolate and manipulate
2. Independent origin of replication.
3. Amplification of the DNA becomes easy.
4. Selectable markers such as antibiotic resistance genes.
5. The plasmid vector is isolated from the bacterial cells at one site of restriction enzyme.
6. Gene cloning in bacteria is pBR 322

Applications :-

Antibiotic resistant genes.

* Used in Recombinant DNA technology
* insertion of the human insulin into bacteria.

S.No.	plasmids	Organism
1.	pBR322	E-Coli
2.	pUC19	E-coli
3.	ColE1	E-coli
4.	RP4	pseudomonas
5.	TOL	Pseudomonas putida.
6.	pTiAch5	Agrobacterium tumefaciens
7.	pUC8	E-coli

Transposons:-

Transponsable elements (TEs) also known as "Jumping genes" are DNA sequences that move from one location on the genome to another.

* These are first identified by geneticist Barbara McClintock.
* Both prokaryotes and eukaryotes which makes approximately 50% of the human genome and up to 90% of the maize genome.
* changing sites of genetic elements as transposition and those genetic elements were called by her as controlling elements.
* Controlling elements were later called transposable elements by Alexander Brink.
* A DNA sequence that is able to move or insert itself a new location in the genome.

Characteristics:-

1. Transposons cause mobilization, reshuffling & Rearrangements.
2. Transposons can cause disease due to mutations.
3. These carry an antimicrobial resistance gene.
4. The DNA sequence that code for enzyme which result in self-duplication and insertion into a new DNA site.
5. These transposons invalves in both recombination & Replication.
6. The target genes of these elements is invariably disrupted.
7. initiation of RNA synthesis. & these elements donot site for the origin of replication

Types of transposable elements:-

1, Insertion sequences (IS) or simple Transposons:-

These are shorter sequences (~168 bp to 5kb)

* These sequences carry the genetic information. Gene for enzyme transposase.
* It identified in bacteriophages in F factor plasmid of many bacteria.
* In E.coli's galactose operon having 768bp and 4-19 copies.
* The term ITR (Inverted Terminal Repeat) implies the sequence at 5' end of one strand is identical to the sequence at 5' end.
* The number of copies of several IS-elements like IS1, IS2, IS3, IS4 and IS5 are present.

```
5' - ACAGTTCAG.....CTGAACTGT - 3'
3' - TGTCAAGTC.....GACTTGACA - 5'
```

* These are not simple inversions, they are both inverted and complementary.

Direct repeats:-

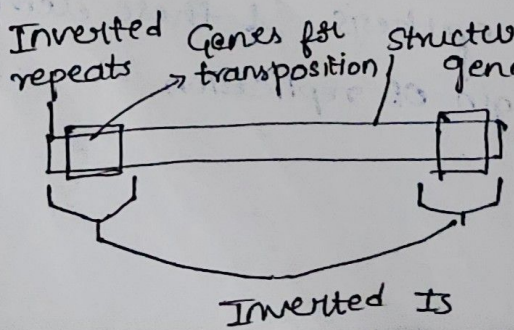
Short flanking direct repeats from 3 to 12 bp

* Replication of the single stranded DNA then creates the flaking direct repeats.

(2) Transposons (Tn) or Composite Transposons:-

Ex:- Antibiotic resistance or toxic genes

These are formed when two IS elements assosiate with a central segment containing one or more genes.

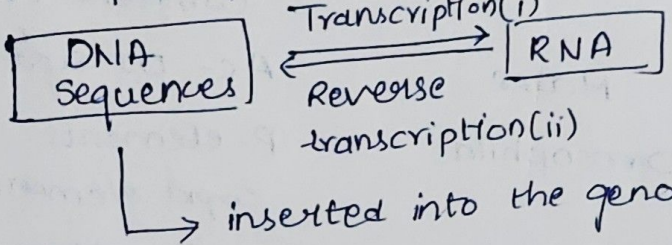


Retro transposons :-

The DNA element transcribed into RNA. The RNA is then reverse-transcribed into DNA.

* A lot of LTR at their end which can have more than 1000 bp in each of them.
* These are common in plants & serves as major component of the nuclear DNA.

Ex: The genome of maize is comprised of 49 to 78% retrotransposons.



Mechanism of Transposition :-

1. Conservative transposition :-

The element moves from the donar site into the target site. Also called cut and paste transposons.

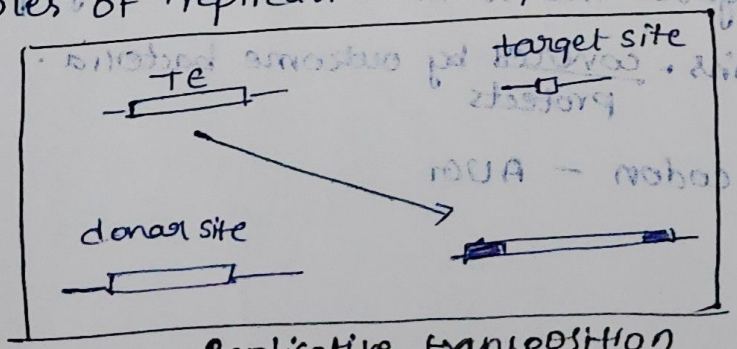
2. These are also called as non-replicative mechanism.

It is also known as cut & paste mechanism

3. Sleeping beauty transposon system is the best example.

2. Replicative transposition :-

The element moves a copy of itself to a new site via a DNA intermediate. Tn3, phage Mu are some examples of replicative transposons in bacteria.



Replicative transposition

The shift DNA sequence, considered as Junk DNA.

III. Retrotransposition :-

1. The transposon is synthesized by the normal process of transcription.
2. The conversion of RNA into DNA requires a special enzyme called reverse transcriptase.
3. The DNA copy of tranposon, integrates into the genome.

S.NO.	Organism	Type of transposons.
1.	Bacteria	IS element Composite tr-
2.	Maize	Ac- Ds system
3.	Drosophila	P-elements copia elements FB elements.
4.	humans	SINEs LINEs

Significance :-

These may change the structural & functional characteristics of genome.

2. These elements cause mutation by insertion/deletion etc
3. Useful as cloning vector also, in gene cloning.
4. Also used as a genetic markers.

Unit-2 Concept of Genes.

1. The term 'gene' was first coined by Johansson in 1907.
2. Hugo DeVries postulated one gene one character hypothesis which holds that a particular character of an individual is controlled by a specific chromomeres which appear as series of granules on the chromosome might be the genes.

Presently, the genes are regarded as specific DNA segments capable of producing a definite cell product, consisting of mutable sites which can exist in several forms and capable of self duplication.

3. The concept of gene was proposed by sutton in 1902, the term gene was first used by Johannsen in 1909, earlier, it was reffered to as Mendelian factor.

Genes are located in the chromosomes, where these are arranged in a single linear order. Genes are ordinarily transmitted from one generation to the next one. Each gene has a specific position in a specific chromosome. This position is called locus.

4. Mutation, a spontaneous change in the composition of a gene, may produce a mutant gene. The expression of mutant gene will be different from the original gene.
5. An allele is an alternative form of gene.

Classical Definition of Gene:- Gene is the unit of Function (one gene specifies one character),

recombination and mutation.

Modern concept of gene:-

Gene is the unit of genetic information i.e, the sequence of DNA that specifies one polypeptide. It includes coding as well as non-coding regulatory sequences.

Year	Scientist	Gene concept:
1866	G.J. Mendel	A unit factor that controls specific phenotypic trait.
1902	Sir A.E. Garrod	one-gene-one metabolic block theory.
1940	Beadle & Tatum	One-gene-one enzyme theory
1957	V.M. Ingram	one gene-one polypeptide theory.
1960	C. Yanofsky & coworkers	Gene is a unit of recombination.
Early 1970's	E.B. Lewis	Complementation test in Drosophila.

Units of a gene:-

A gene can be defined as a polynucleotide chain that consists of segments each controlling a particular trait.

Genes are considered as a unit of function (Cistron), a unit of mutation (mutant) and a unit of recombination (recon).

Cistron:-

One gene-one enzyme hypothesis of Beadle and Tatum was refined by several workers in coming years. A single mRNA is transcribed by a single gene.

A single mRNA is also transcribed by more than one gene and it is said to be polycistronic.

The concept has been given as one-gene-one protein hypothesis. The proteins are the polypeptide chain of aminoacids translated by mRNA.

⇒ Genes are present within the chromosome and their cis-trans effect govern the function.

⇒ S. Benzer termed the functional gene as cistron.

Recon:-

The smallest unit capable of undergoing recombination is known as recon.

Benzer (1955) found that the cultures of T_4 bacteriophage formed plaques on agar plates of E. coli. Normally T_4 found small plaques of smooth edges, whereas the mutant phage formed the larger plaques of rough edges.

Muton:-

The smallest unit of chromosome that undergoes mutational change. The small unit of DNA which may be changed is the nucleotide.

One gene-one enzyme hypothesis:-

The one gene-one enzyme hypothesis, proposed by Beadle and Tatum, states that each gene is responsible for producing a single enzyme. This concept was later refined to 'one gene-one polypeptide' as it was discovered that some proteins are composed of multiple polypeptide chains, each coded by a separate gene.

2) One gene - one polypeptide hypothesis :-

The one gene - one polypeptide hypothesis refines the earlier "one gene - one enzyme" concept. It states that each gene directs the synthesis of a single polypeptide chain, a component of a protein. This is more accurate as some proteins are made of multiple polypeptide chains, each from a different gene.

3) One gene - one product hypothesis :-

The hypothesis is a modern extension of the earlier "one gene - one enzyme / polypeptide" concepts. It states that one gene codes for one final functional product. This product can be a polypeptide chain (which may or may not be an enzyme), an RNA molecule (like tRNA or rRNA), or any other functional molecule directly transcribed from the gene. It acknowledges that not all genes code for proteins.

Open Reading Frame (ORF) :-

It is a continuous stretch of nucleotides in a DNA or RNA sequence that has the potential to be translated into a protein. It begins with a start codon, typically "AUG" in RNA (which codes for the amino acid methionine) and ends with a stop codon, such as "UAA", "UAG" or "UGA". These codons signal the beginning and termination of protein synthesis, respectively.

ORFs are important for gene expression because they indicate the regions of the genome that can be translated into functional proteins. The sequence blw the start and stop codon is considered the coding sequence, which determines the aminoacid sequence of the resulting protein. The length and composition of the ORF are key to understanding the gene's function, as they dictate the structure and properties of the protein.

In genomic analysis, identifying ORFs is crucial for annotating genes and understanding how the genetic code is used to produce proteins.

Structural genes:-

Structural genes are those that code for proteins or RNA molecules that have a direct role in the organism's structure or function.

2. They provide the blueprint for the synthesis of enzymes, structural proteins, and other molecules necessary for the cell's structure and functioning.

3. Example:- Genes coding for enzymes, cytoskeletal proteins etc.

Constitutive Genes:-

These are the genes that are always expressed at a constant level, regardless of external signals or environmental conditions.

2. These genes are essential for basic cellular functions and are constantly active.

Example:- Genes encoding for housekeeping proteins like ribosomal RNA (rRNA) or enzymes involved in basic metabolic processes (eg: glycolysis).

3. Regulatory Genes:-

Regulatory genes are involved in controlling the expression of other genes. They produce regulatory proteins (eg: transcription factors) that can either promote or inhibit the transcription of specific target genes.

2. They regulate the timing, location and amount of gene expression, thus controlling various cellular processes like differentiation, development, and responses to stimuli

Example:- Genes that produce transcription factors, repressors, or activators that regulate the expression of structural genes.

(4) Uninterrupted Genes:-

These genes are genes that consist of a continuous sequence of coding regions (exons) without interruptions like introns.

2. The entire gene is translated into protein without the need for splicing, making the gene simpler in terms of its expression.

3. Example:- Found in prokaryotes (eg:- bacterial genes), where genes typically do not contain introns.

(5) Split Genes:-

These genes that have both coding (exons) and non-coding (introns) regions. The

exons are separated by introns, and the introns are removed during RNA processing before the gene can be translated into proteins.

2. This structure allows for alternative splicing, where different combinations of exons can be joined to produce multiple protein variants from the same gene.

Example:- Most genes in eukaryotes, such as those in humans, are split genes, and they undergo splicing during mRNA processing.

Concept of Introns and Exons:-

It is crucial in understanding how genes are structured and gene expression is regulated, particularly in eukaryotic organisms. These terms refer to the different parts of a gene and its mRNA transcript.

1. Exons:-

Exons are the coding regions of a gene that contains the actual information needed to synthesize proteins. They are the sequences that remain in the mRNA after the splicing process.

2. Exons are the parts of the gene that are transcribed into mRNA and translated into proteins.

Example:-

In the gene for a specific enzyme, the exons will contain the instruction (codons) that determine the sequence of aminoacids in the enzyme's structure.

Introns :-

Introns are the non-coding regions of a gene that are transcribed into mRNA but are removed (spliced) during RNA processing before translating.

2. Although introns do not directly code for proteins, they play roles in gene regulation, alternative splicing, and may have functions in the control of gene expression. Their presence in genes allow for the possibility of alternative splicing, where different combinations of exons can be joined together to produce different protein isoforms.

- Example :-

In a gene that codes for a protein, the introns are transcribed into pre-mRNA, but during processing, they are removed, and the exons are joined to form the mature mRNA that will guide protein synthesis.

RNA processing (splicing) :-

After the initial transcription of a gene into pre-mRNA, the introns are removed through a process called splicing. The remaining exons are then connected together to form the mature mRNA that can be translated into a protein. This process is essential because it ensures that only the exonic sequences, which carry the protein-coding information, are used in the final mRNA.

Alternative splicing :-

presence of introns is alternative splicing. This process allows a single gene to produce multiple different proteins by selectively including or excluding different exons in the final mRNA transcript. This greatly increases the diversity of

proteins an organism can produce without needing additional genes.

Gene structure:-

* A gene might consist of a series of exons and introns. example: Gene A might be structured like this:
  - Exon 1 - Intron 1 - Exon 2 - Intron 2 - Exon 3

* After transcription, the pre-mRNA would like
  - Exon 1 - Intron 1 - Exon 2 - Intron 2 - Exon 3.

* After splicing, the introns would be removed, and the final mature mRNA would consist only of the exons:
  - Exon 1 - Exon 2 - Exon 3

Importance of Introns and Exons:-

Exons:- They carry the actual coding information for protein synthesis.

Introns:- Although they don't directly code for proteins, they are important for regulating gene expression and increasing the potential for producing different proteins from a single gene via alternative splicing.

This concept allows the regulation of gene expression and the creation of different proteins, providing flexibility and complexity to eukaryotic organisms.

Protein synthesis in Prokaryotes : Transcription :-

Transcription is the process in which an RNA molecule is synthesized from a DNA template. In prokaryotes, it is the first step in protein synthesis, where the genetic information encoded in a gene is transcribed into messanger RNA (mRNA), which is then translated into protein.

Both transcription & replication involve copying genetic information.

Transcription :- To produce a single-stranded RNA copy (mRNA) of a gene to carry genetic information for protein synthesis

Replication :- It is to create an exact copy of the entire genome (DNA) to ensure genetic material is passed to the next generation.

⇒ Template :-

Transcription :- Uses one strand of DNA to synthesize RNA.

Replication :- Both strands of the DNA are used to create two identical double-stranded DNA molecules

⇒ Enzymes Involved :-

Transcription :- RNA polymerase is the enzyme responsible for synthesizing RNA.

Replication :- DNA polymerase is responsible for synthesizing the new DNA strands.

⇒ product :-

- Transcription :- Results in RNA (mRNA, tRNA, & rRNA).
- Replication :- Results in a new DNA molecule.

① promoter :-

It is a specific sequence of DNA located upstream of a gene that signals the start of transcription. It is recognized by RNA polymerase and its associated sigma factor.

2. The promoter serves as a binding site for RNA polymerase and directs the enzyme to the correct starting point for transcription.

② RNA polymerase :-

It is an enzyme responsible for synthesizing RNA from a DNA template during transcription.

In prokaryotes, RNA polymerase consists of a core enzyme and a sigma factor, which is responsible for recognizing the promoter region.

It reads the DNA template strand and synthesizes a complementary RNA strand in the 5' to 3' direction.

Mechanism of Transcription :-

1. Initiation
2. Elongation
3. Termination.

Mechanism of Transcription

Initiation :-

The RNA polymerase binds to the promoter region of the DNA

2. The enzyme covers about 60 base pairs of the promoter including the -10 and -35 boxes.
3. The σ-subunit then dissociates from the open promoter complex having the core enzyme

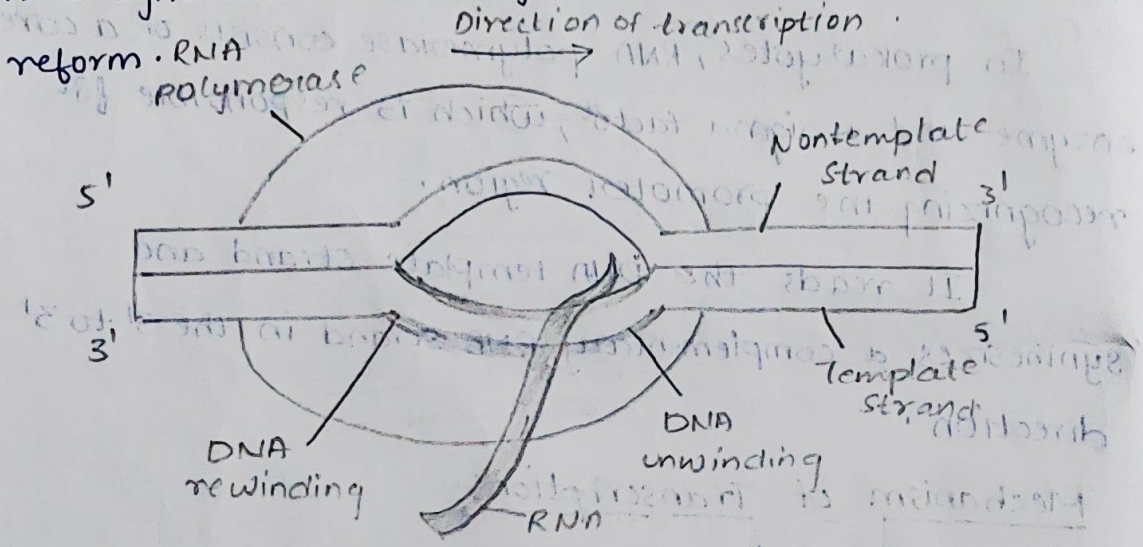
Elongation :-

RNA polymerase moves along the DNA molecule.

The enzyme adds ribonucleotides to the 3' end of the growing RNA molecule.

During transcription, only a small portion of the double helix is unwound at any point of time. It extends over 12-17 base. This imposes strain on the DNA molecule.

The RNA is released from the template DNA as it is synthesized allowing the DNA double helix to reform.



elongation of transcription.

It takes place by addition of activated ribonucleoside triphosphates (ATP, UTP, GTP and CTP) to one strand of the DNA template.

For each nucleotide added to the growing RNA chain, pyrophosphate (PPi) is given off.

Termination :-

Transcription terminates when RNA polymerase reaches a terminator sequence on the DNA, signalling the end of the gene.

* The RNA polymerase dissociates from the DNA, and the newly synthesized mRNA is released.

Termination can be of two types.

(i) Rho independent termination and

(ii) Rho dependent termination.

(1) Rho independent termination:-

In Ecoli, termination occurs at sequence known as palindromes.

The first half of the sequence is followed by its exact complement in the second half.

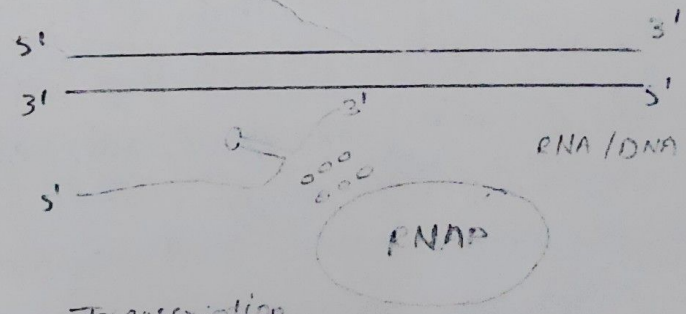
In single-stranded RNA molecules, this allow the feature of first half of the sequence to base pair with second half to form, it known as stem-loop structure.

The stem-loop structure sequence is followed by a run of 5-10. As the DNA which form weak A-U base pairs.

RNA polymerase pauses just after the stem-loop and that the weak A-U bp break causing the transcript to detach from the template.

(2) Rho-dependent termination:-

On binding of a protein called Rho(p). Rho protein will have helicase function. It disrupts bp blw the template and the transcript when the polymerase pauses after the stem loop. The termination of transcription involves the release of transcript and the core enzyme.



Transcription termination

Rho-dependent termination

Transcription in Eukaryotes :-

It is carried out in the nucleus of the cell by one of three RNA polymerases.

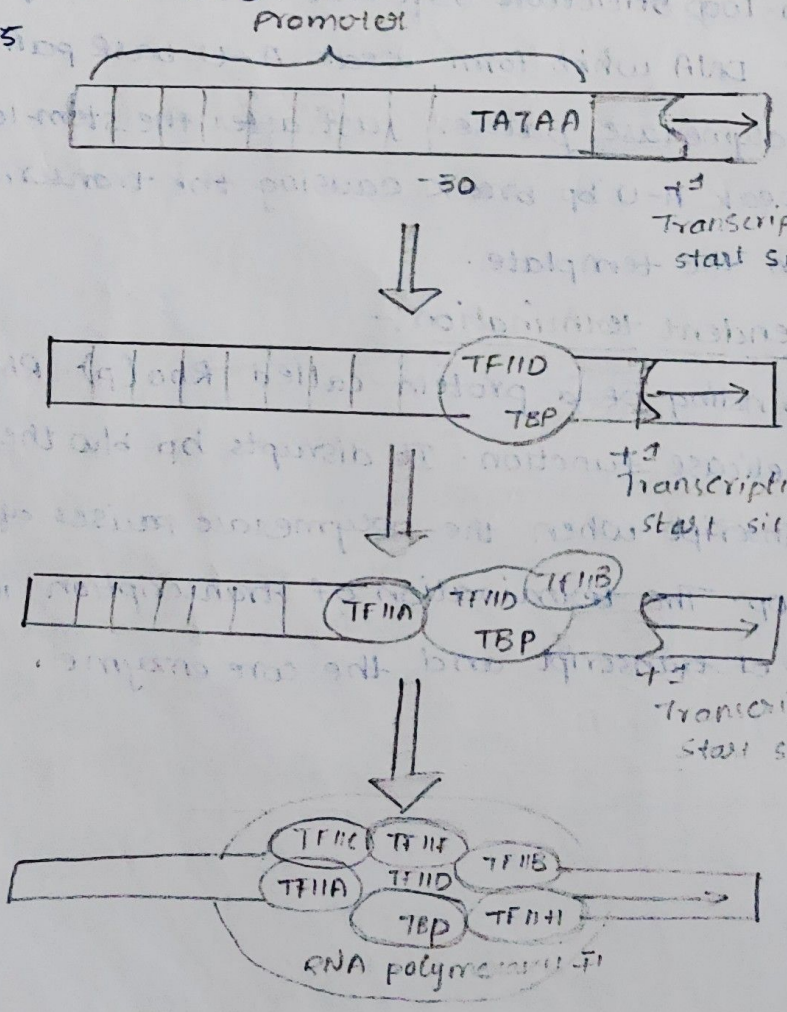
1. Initiation.
2. Elongation
3. Termination.

Initiation :-

RNA polymerase bind to the promoter, short DNA sequence known as a TATA box. TATA boxes is well characterized.

TATA box :-

It is a consensus sequence of 8 bases (TATAAAAA), having A-T bp, and occupies a fixed position at -25



Elongation phase:-

RNA polymerase II now starts moving along the DNA template. RNA synthesis occurs in the 5' → 3' direction.

1. It use ATP to unwind the DNA helix.
2. It phosphorylates RNA polymerase II which cause this enzyme to change its conformation and dissociate from other proteins in the initiation complex.

Termination phase:-

Elongation by RNA polymerase II in eukaryotes takes place by 1,000-2,000 nucleotides beyond the end of the gene being transcribed.

RNA splicing in Eukaryotes:-

It is the process by which introns are removed from the pre-mRNA, and the exons are joined together to form the mature mRNA.

Splicing ensures that only the coding regions are present in the final mRNA, which is then used to synthesize proteins.

Formation of the Spliceosome:-

The splicing process involves the spliceosome, a complex of small nuclear RNA and proteins. The spliceosome recognizes the specific splice sites at the intron-exon boundaries.

Removal of Introns:-

The spliceosome cuts the RNA at the 5' end of the intron and the 3' end. The intron is then removed as a lariat

Exon joining:- The exons on either side of the introns are joined together, and the mature mRNA is produced

→ The mature mRNA, which now contains only exons, is ready to be exported from the nucleus to the cytoplasm.

Genetic code :-

Introduction :-

Genetic code is the sequence of nucleotide in a gene coded in triplets. It determines the sequence of aminoacids in protein synthesis.

The genetic code is proposed by F.H.C. Crick. It holds the existence of a triplet code i.e, the sequence of three nitrogenous bases, codes for one amino acid.

Characteristics of Genetic code :-

1. Genetic code is a triplet
2. The code is degenerate i.e, more than one code for an amino acid.
3. The code is non-overlapping i.e, the same letter is not used for 2 different codons.
4. The code is non-ambigious i.e - a particular codon will always code for the same amino acid
5. The code is commaless - No punctuations are needed between any 2 codons i.e, after one Aminoacid is coded, the 2nd aminoacid will be automatically coded by the 3 letters.
6. The code is universal - In all living organisms the same genetic code is used.

   Eg: UUU codes for phenylalamine in Bacteria, it will codes for the same aminoacid in man or higher plants.
7. Codons are carried by messanger RNA to the ribosomes.
8. Out of 64 codons, 61 of which code for amino acids and three for chain termination.

9. The anticodons are the complementary to the bases in a transfer RNA that are complementary to the 3 bases of a specific code in a messanger RNA.

10. Genetic code was cracked by Khorana & Nirenberg.

START CODON:- (AUG)

The codon AUG (adenine-uracil-guanine) serves as the start codon, signaling the beginning of protein synthesis. It codes for the aminoacid methionine in eukaryotes (formylmethionine in prokaryotes).

STOP CODON:-

There are three stop codons - UAA, UAG & UGA - which signal the termination of protein synthesis. They do not code for any aminoacid but instead cause the ribosome to release the completed polypeptide chain.

Triplet code:-

A codon is made up of three nucleotides, each derived from the four nucleotides in RNA (adenine, cytosine, guanine, uracil). This triplet is the basic unit that encodes genetic information.

Initiation codon:-

The codon AUG codes for aminoacid methionine. This is positioned first in a cistron & initiates the synthesis of a polypeptide chain initiation codon.

Non-sense codons:-

The codons UAA, UAG and UGA donot code for any aminoacid hence there are known as non-sense codons. These are positioned at the

of cistron and are utilized to stop synthesis, hence those are also known as termination codons.

Degenerate codes:-

These are 64 possible codons in a triplet code of which 61 have shown to code for amino acids, since only 20 aminoacid take part in protein synthesis. It is obvious that there are many more codons than aminoacid types. Except for tryptophan and methionine, which have a single code each.

Wooble Hypothesis:-

There are more than one codon for one amino-acid. This is called degeneracy of genetic code. To explain the possible cause of degeneracy of codons, in 1966 Francis crick proposed "the wobble hypothesis".

According to the wooble hypothesis, only the first two bases of the codon have a precise pairing with the bases of the anticodon of tRNA, while the pairing between the third bases of codon and anticodon may wobble.

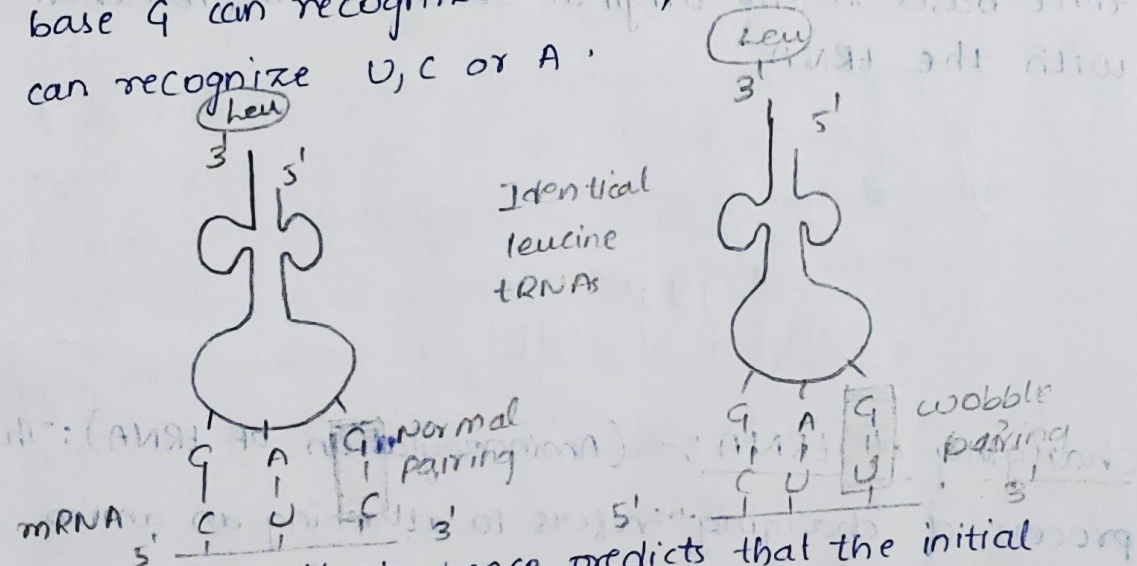
The phenomenon permits a single tRNA to recognize more than one codon. ∴, although there are 61 codons for amino acids, the number of tRNA is far less which is due to wobbling.

Wobble Hypothesis Statement:-

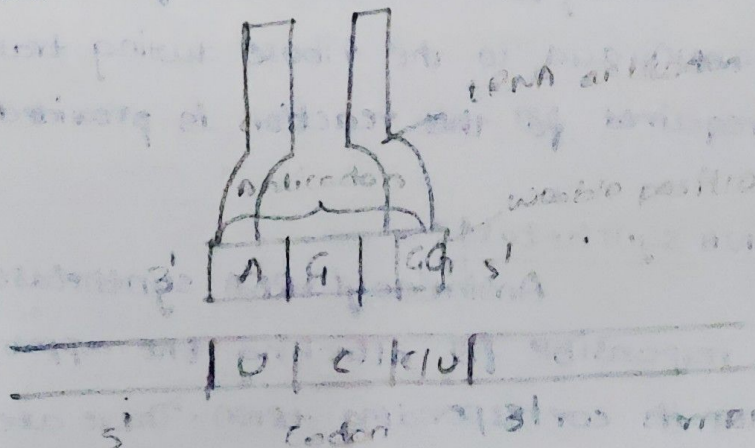
The wobble hypothesis states that the base at 5'end of the anticodon is not spatially confined as the other two bases allowing it to form hydrogen bonds with any of several bases located at

the 3' end of a codon.

1. The first two bases of the codon make normal (canonical) H-bond pairs with the 2nd and 3rd bases of the anticodon.
2. At the remaining position, less stringent rules apply and non-canonical pairing may occur. The wooble hypothesis thus proposes a more flexible set of base-pairing rules at the third position of the codon.
3. The relaxed base-pairing requirement, or "wobble", allows the anticodon of a single form to tRNA to pair with more than one triplet in mRNA.
4. The rules: first base U can recognize A or G, first base G can recognize U or C, and first base I can recognize U, C or A.



Crick hypothesis hence predicts that the initial two ribonucleotides of triplet codes are often more critical than the third member in attracting the correct tRNA.

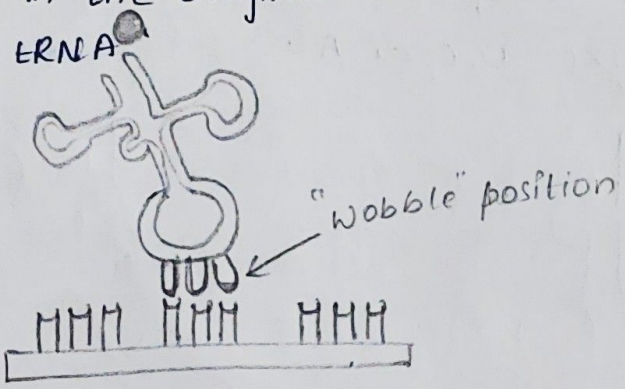


1. A wobble base pair is a pairing between two nucleotides in RNA molecules that does not follow Watson-Crick base pair rules.

2. The four main wobble base pairs are guanine-uracil (G-U), hypoxanthine-uracil (I-U), hypoxanthine-adenine (I-A), and hypoxanthine-cytosine (I-C)

3. In order to maintain consistency of nucleic acid nomenclature, "I" is used for hypoxanthine because hypoxanthine is the nucleobase of ionsine

4. Inosine displays the true qualities of wobble, in that is the first nucleotide in the anticodon then any of three bases in the original codon can be matched with the tRNA



→ **Charging of tRNA**:- (Aminoacylation of tRNA): The process of "charging" refers to attaching an amino acid to its corresponding transfer RNA (tRNA) molecule. This is carried out by an enzyme called aminoacyl tRNA synthetase. The aminoacid is covalently bonded to the tRNA molecule, which is essential for the tRNA to carry the aminoacid to the ribose during translation. The energy required for this reaction is provided by ATP.

Aminoacyl tRNA synthetase:-

Aminoacyl tRNA synthetase is an enzyme responsible for attaching the appropriate aminoacid to its corresponding tRNA. There are 20

different aminoacyl tRNA synthetases, one for each aminoacid. The enzyme is highly specific, ensuring that only the correct aminoacid is linked to its matching tRNA.

Mechanism of Initiation of polypeptide Synthesis :-

The small ribosomal subunit binds to the mRNA, facilitated by initiation factors.

2. The initiator tRNA, carrying methionine (in eukaryotes) or formylmethionine (in prokaryotes), binds to the start codon (AUG) of the mRNA.

3. The large ribosomal subunit then joins the small subunit, forming the complete ribosome.

4. This results in the initiation complex, where the tRNA is positioned in the P site of the ribosome

Mechanism of elongation of polypeptide chain :-

The elongation of the polypeptide chain involves three main steps.

- Codon recognition :- The aminoacyl tRNA, carrying the correct aminoacid, binds to the A site of the ribosome, matching its anticodon with the mRNA codon.
- peptide Bond formation :- The enzyme peptidyl transferase in the ribosome catalyzes the formation of a peptide bond between the aminoacid in the P site and the one in the A site.
- Translocation :- The ribosome moves along the mRNA, shifting the tRNA from the A site to the P site, and the empty tRNA moves to the E site, where it is released.

Mechanism of Termination of polypeptide Synthesis:-

Termination occurs when the ribosome reaches a stop codon (UAA, UAG & UGA) on the mRNA:

- Release factors bind to the stop codon, prompting the release of the polypeptide chain from the tRNA in the P site
- The ribosome dissociates, releasing the newly synthesized polypeptide and the mRNA.

Inhibitors of protein synthesis :-

Several substances can inhibit protein synthesis at various stages. These include

- **Chloramphenicol:-** Inhibits peptidyl transferase activity in prokaryotic and eukaryotic ribosomes.
- **Tetracycline:-** Blocks the attachment of aminoacyl tRNA to the ribosome's A site, inhibiting elongation.
- **Erythromycin:-** Prevents translocation by binding to the ribosome.
- **Streptomycin:-** Causes misreading of the mRNA in prokaryotes, leading to incorrect aminoacid incorporation.
- **Rifampicin:-** Inhibits bacterial RNA polymerase, preventing transcription and subsequent translation.

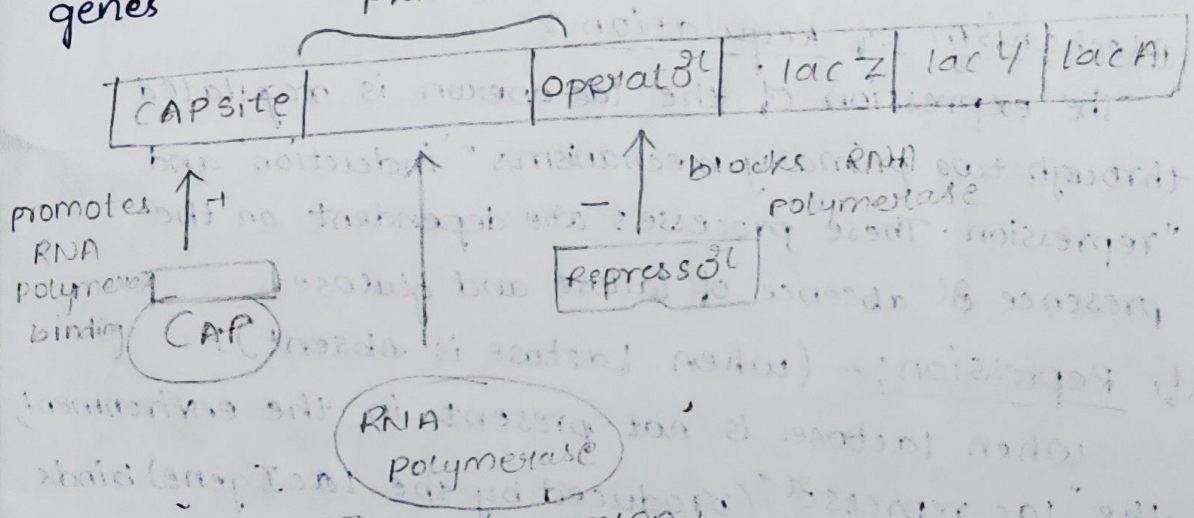
Regulation of Gene Expression in Bacteria-

Lac operon :-

The regulation of gene expression in bacteria is an essential process that allow cells to adapt to their environment by switching genes on or off in response to external stimuli. The "lac operon" found in e-coli and some other bacteria and some other bacteria. The lac operon is involved in the metabolism of lactose, a sugar;

Structure of lac operon :-

The "lac operon" consists of the three structural genes involved in lactose metabolism, along with regulatory elements that control the expression of these genes



The lac operon

1. "Structural Genes":

"Lac Z": Encodes "β-galactosidase", an enzyme that breaks down lactose into glucose and galactose.

"Lac Y":- Encodes "lactose permease", a protein that transports lactose into the cell.

"Lac A":- Encodes "thiogalactoside transacetylase", which is involved in detoxifying certain byproducts of lactose metabolism.

② Regulatory Elements :-

• Promoter :- (P)

A region where RNA polymerase binds to initiate transcription of the lac operon

• Operator (O) :-

A regulatory DNA sequence located b/w the promoter and the LacZgene. The repressor protein binds here to block transcription.

• lacI gene :-

Located upstream of the lac operon, it encodes the "lac repressor" protein that regulates the operon. The lac repressor binds to the operator region to prevent transcription when lactose is not present.

Mechanism of Regulation :-

The expression of the lac operon is regulated through two primary mechanisms "induction" and "repression. These processes are dependent on the presence & absence of lactose and glucose.

(1) Repression :- (when Lactose is Absent) :-

When lactose is not present in the environment, the "lac repressor" (produced by the LacIgene) binds to the "operator (O)" region of the lac operon.

This binds physically blocks "RNA polymerase" from transcribing the operon's genes, preventing the production of enzymes like "β-galactosidase" and "lactose permease".

Thus the operon is off, and the cell does not waste energy producing enzymes that are not needed.

2, Induction:- "(When lactose is present)"

When lactose is available, it enters the bacterial cell through lactose permease.

Some of the lactose is converted into "allolactose" which acts as an 'inducer'.

Allolactose binds to the "lac repressor", causing it to undergo a conformational change.

The altered repressor can no longer bind to the operator region, so the RNA polymerase is free to transcribe the lac operon genes.

This allows the production of "B-galactosidase" (to breakdown lactose) and 'lactose permease" (to bring more lactose into the cell).

③ Catabolic Repression:- (Effect of Glucose):-

1, when glucose is present in the environment, bacteria preferentially use glucose as an energy source because it is more efficient than lactose.

2, The presence of glucose cause the concentration of CAMP (cyclic AMP) to drop inside the cell.

3, CAMP is needed to activate "CAP (catabolite activator protein)". When glucose is low and CAMP levels are high, CAMP binds to CAP, and the CAMP-CAP complex binds to the "CAP binding site" near the promoter region of the lac operon.

4, The binding of the CAMP-CAP complex helps RNA polymerase bind more effectively to the promoter, thus promoting transcription of the lac operon genes.

5, When glucose levels are high, CAMP levels fall, reducing the efficiency of transcription and essentially turning the operon off or at a low level, even in the presence of Lactose.

24/01/25 UNIT - 4

~~TRANSCRIPTION~~ &

MUTATION OF DNA REPAIR:

Mutations :-

Mutation refer to sudden heritable change in the phenotype of an individual.

2. In molecular terms, mutations is defined as the permanent and relatively rare change in the number or sequence of nucleotides.

3. Mutation was first discovered by Wright in 1791. Later mutations was reported / coined by Hugo de Vries in 1900, in Drosophila.

Importance :-

1. Mutations are responsible for sustenance of life

2. It is the source of all genetic variation, thus the raw material of evolution,

3. Mutations have detrimental effects, and mutation is the source of many human diseases and disorders.

Classification of mutations :-

1. In multicellular organisms, mutations categorizes into 1. Somatic mutations
2. Germline mutations.

Somatic mutations :-

Mutations that are in the somatic tissues of the body. Mutations are not transmitted to progeny. The extent of the phenotypic effect depends upon whether the mutation is dominant or recessive.

Germ-line mutation:-

These mutations arise in cells that ultimately produce gametes. A germline mutations can be passed to future generations, producing individual organisms that carry the mutation in all their somatic and germline cells.

Spontaneous mutations:-

The spontaneous mutations occur suddenly in the nature and their origin is unknown. They are also called "background mutation" and have been reported in many organisms such as maize, bread molds, micro-organisms (bacteria and viruses), Drosophilia, mice, man, etc.

Induced mutations:-

The mutations can be induced artificially in the living organisms by exposing them to abnormal environment such as radiation, certain physical conditions (i.e, temperature) and chemicals.

Physical mutagens:-

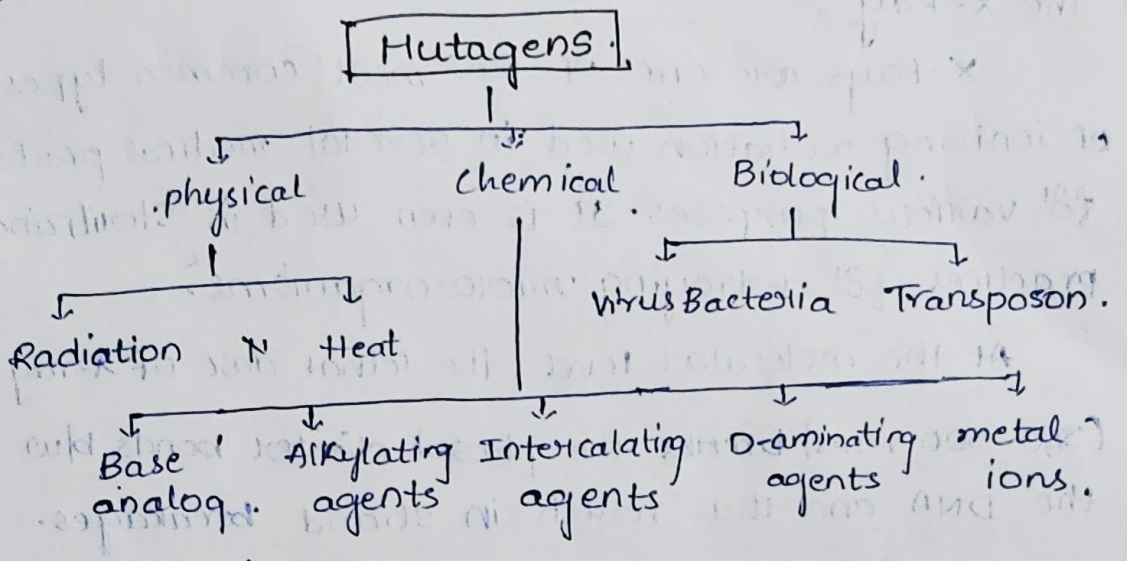
These include ionizing radiation, such as X-rays, gamma rays and alpha particles. Ultra-violet radiation can also behave as potential mutagens.

Chemical mutagens:-

Elements such as arsenic, nickel, and chromium are considered to be mutagens. Some organic compounds like benzene are also considered to be mutagenic in nature.

Biological mutagens :-

It involves transposons and viruses. Furthermore, certain bacteria such as Helicobacter pylori can increase the risk of developing stomach cancer.



physical mutagens :-

1. Radiations as Mutagens :-

Radiation is the most important among the physical mutagens. Radiations damaging the DNA molecules fall in the wavelength range below 340nm and photon energy above 1 electro-volt (eV) The destructive radiation consists of ultraviolet (UV) rays, X rays, Y-rays, alpha(α) rays, beta(β) rays, neutrons, etc.

2. Ultraviolet (UV-Rays) Radiation :-

The UV-light is a non-ionizing type of radiation having less energy in it, used in the sterilization and decontamination process during the cell culture and microbiological experiments. The DNA and protein absorb UV light of 260 and 280nm, respectively.

1) UV-A :- Nearly visible range (320nm) causes pyrimidine dimers.

2. UV-B :- (290-320nm) emitted by the sunlight. These

UV rays are highly lethal to our DNA.

* UV-C :- (180-290nm) is one of the most energy-consuming forms of UV which is extremely lethal.

(5) The X-Rays :-

X-Rays are one of the most common types of ionizing radiation used in several medical practices for various purposes. It is even used in sterilizing practices for destroying micro-organisms.

At the molecular level, the lethal dose of x-ray (350-500 rems) breaks the phosphodiester bonds b/w the DNA and thus results in strand breakages.

Mutagen	Source	Characteristics
X-rays	x-ray machine	Electromagnetic radiation, penetrates tissues from a few millimeters to many centimeters.
Gamma rays	Radioisotopes & nuclear reaction	Electromagnetic radiation produced by radioisotopes and nuclear reactors.
Neutrons	Nuclear reactors & accelerators	There are different types produced in nuclear reactors, uncharged particles, penetrate tissues to many centimeters, source is ^{235}U.
Beta Particles	Radioactive isotopes & accelerators.	Produced in particle accelerators & from radioisotopes, are electrons, ionize, shallowly penetrating.
Alpha Particles	Radioisotopes	Derived from radioisotopes
protons	Nuclear reactors & accelerators	derived from hydrogen nucleus.
Ion beam	particle accelerators	produced positively charged ions are accelerated at a high speed

Group of mutagen	Name of chemical	Mode of action.
Alkylating agent	Ethyl methane sulphonate	AT to GC transistions
	Methyl methane sulphonate	GC to AT transitions
	Ethyl Ethane sulphonate	
	Ethylene imines	
Base analogues	5-Bromo Uracil	AT to GC transistions
	2-Amino purine	AT to GC transistions
Acridine Dyes	Acriflavin, Proflavin	Deletion, addition & Frameshift.
Chemicals changing the specificity of Hydrogen bonding	Nitrous acid Hydroxylamine Sodium azide	AT to GC transitions GC to AT transitions

Spontaneous mutations :-

Spontaneous mutations arise without exposure to external agents. This class of mutations may result from errors in DNA replication or from the action of mobile genetic elements such as transposons. Replication errors can occur when the nitrogenous base of a template nucleotide takes on a rare tautomeric form.

Tautomerism :-

It is the relationship between two structural isomers that are in chemical equilibrium and readily change into one another. Bases typically exist in the keto form. However they can at times take on either an imino or enol form. These tautomeric shifts change the hydrogen bonding characteristics of the bases, allowing purine for purine & pyramidine for pyrimidine substitution that can eventually lead to a stable

alteration of the nucleotide sequence.

2. Induced mutations :-

Virtually any agent that directly damages DNA, alters its chemistry, or in some way interferes with its functioning will induce mutations. Mutagens can be conveniently classified according to their mode of action.

The common types of chemical mutagens are base analogs, DNA-modifying agents, and intercalating agents. A number of physical agents (eg: radiation) damage DNA and also are mutagens.

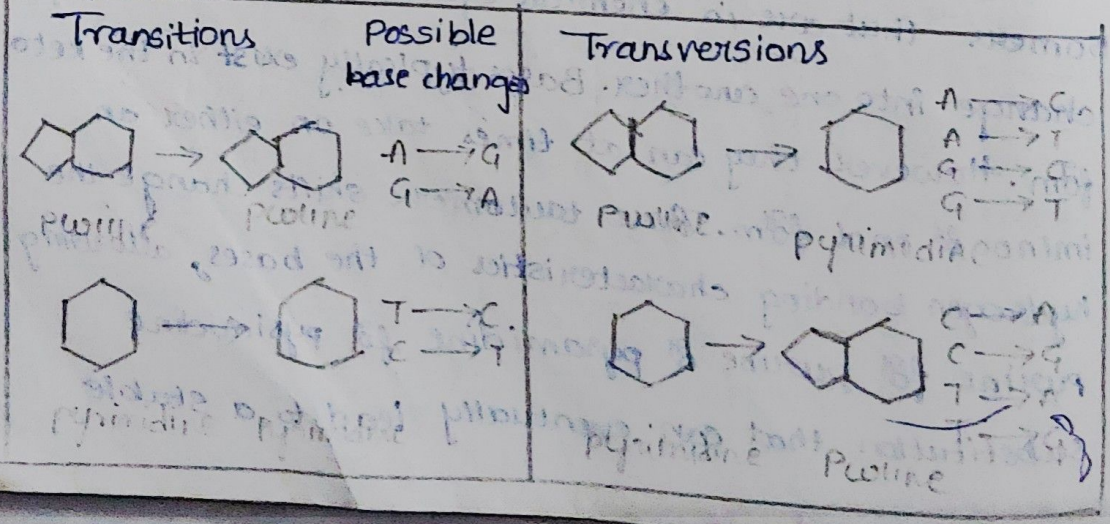
3. Base pair changes :-

The simplest type of gene mutation is a base substitution, the alteration of a single nucleotide in the DNA. Because of the complementary nature of the two DNA strands, the base of the corresponding nucleotide on the opposite strand also will be altered in the next round of replication. A base substitution therefore usually leads to base-pair substitution.

⇒ It is of two types.

1. Transition :- Here a purine is replaced by a different purine or a pyrimidine is replaced by a different pyrimidine.

2. Transversion :- Here a purine is replaced by a pyrimidine and vice versa.



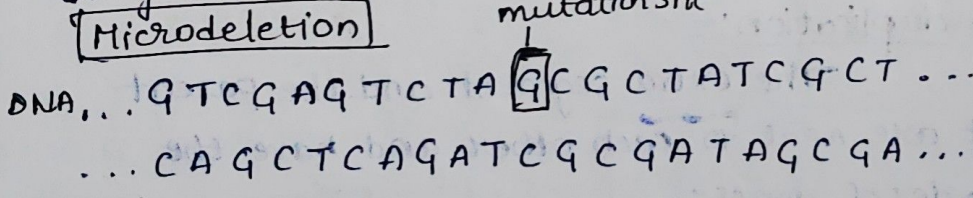
4. Frame shift mutations :-

A frame shift mutation in a gene refers to the insertion or deletion of nucleotide bases in numbers that are not multiples of three. This is important because a cell reads a gene's code in groups of three bases when making a protein. Each of these "triplet codons" corresponds to one of 20 different aminoacids used to build a protein. If a mutation disrupts this normal reading frame, then the entire gene sequence following the mutation will be incorrectly read.

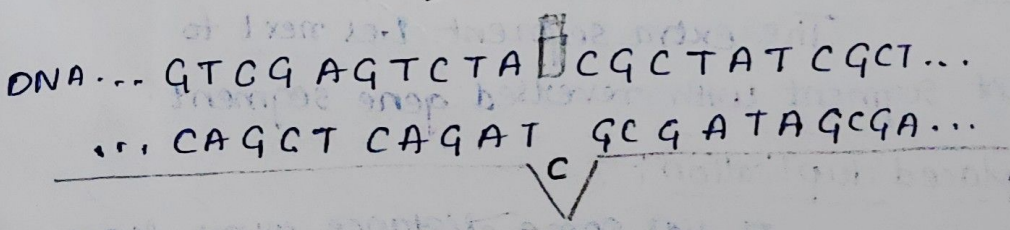
Deletion :-

A deletion is a type of mutation that involves the loss of one or more nucleotides from a segment of DNA. A deletion can involve the loss of any number of nucleotides, from a single nucleotide to an entire piece of an chromosome. It causes a large number of genetic diseases.

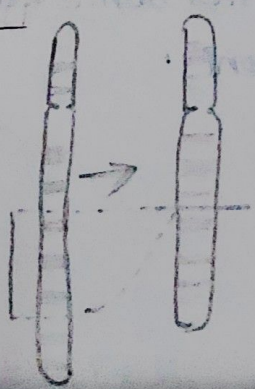
Microdeletion



Deletion



Macrodeletion :-



Inversion :-

Inversion mutations occur when a section of DNA breaks away from a chromosome during the reproductive process and then reattaches to the chromosome in reversed order. This changes the genetic code and can make it more difficult to read.

An inversion can disrupt a gene, either by truncating the coding region of the gene or by placing its regulatory regions far from the gene itself. This latter case can in turn lead to differences in gene expression.

7. Duplication :-

It is the doubling or repetition of chromosome segment during chromosome duplication. As a result of it, a set of genes gets doubled or repeated. The extra set of genes is generally called as "repeat". There are 3 types of duplication.

i. Tandem duplication :-

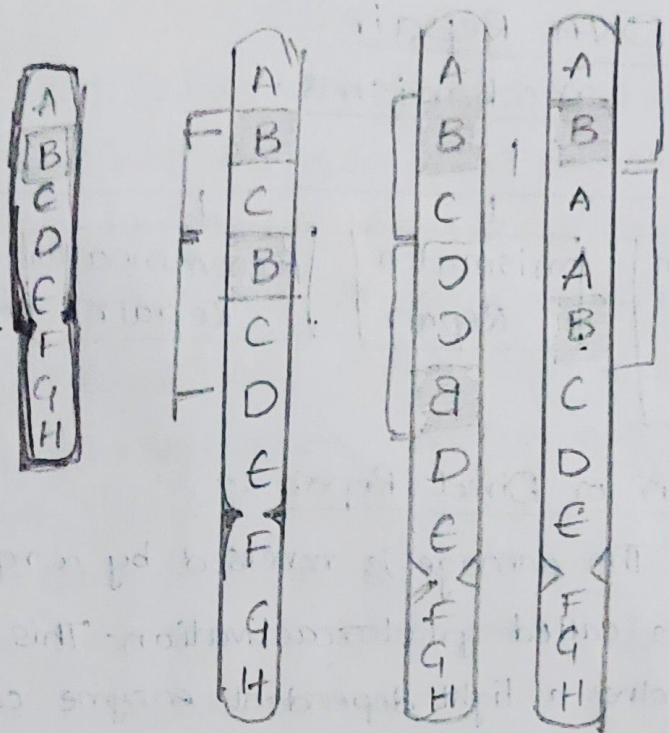
The extra segment and the parent segment are next to each other and have the same order of genes.

ii. Reverse tandem duplication :-

The extra segment lies next to parent segment with reversed gene segment.

iii. Displaced duplication :-

It lies some distance away from the parent segment.



Insertion :-

An insertion mutation involves the addition of one or more nucleotides into a segment of DNA. An insertion can involve the addition of any number of nucleotides, from a single nucleotide to a entire piece of a chromosome.

This insertion mutation involves three base pairs then there will be no frameshift, and thus it can be potentially less harmful than having the insertion of just one base pair.

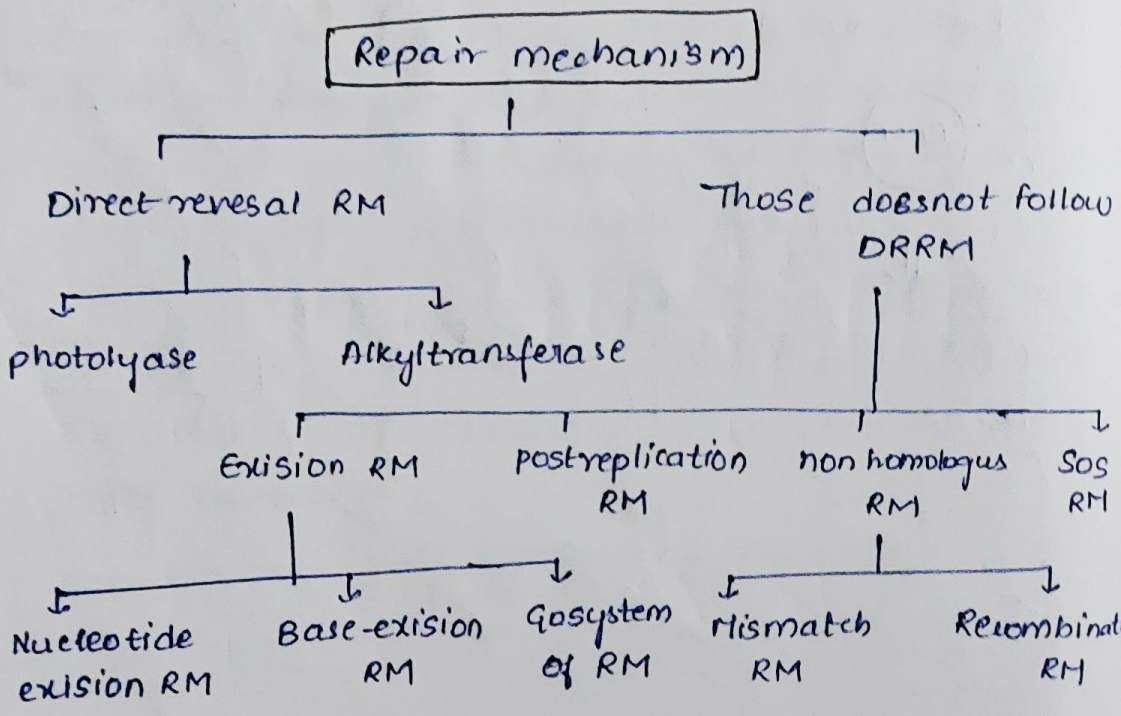
Insertion can involve huge pieces of DNA also. i.e, at chromosomal level.

Based on size of insertion, it can be a microinsertion and macroinsertion.

Repair Mechanism.

When DNA is damaged or when mistakes occur in its synthesis, it is important that it is repaired rapidly. Some damage can be directly reversed, where as other types of damage requires that the faulty stretch of DNA is removed and replaced. In DNA replication intervens between damage and repair the consequences are often more extreme.

There are different types of repair mechanisms. These are therapatic agents possible when the mutations are occur



Direct Reversal Repair mechanism (DRRM):-

RM is done in the same pathway, in reversal direction to the mutation occurs.

photolyase:- When bacteria damaged by UV light. They are exposed to an intense source of visible light (320-370nm). A large propotion of the damaged cells recover. This process is known

as photoreactivation, and it is due to reactivation by visible light of an enzyme which cleaves the pyramidine dimers and restores the two bases to their original form. The photoreacting enzyme - photolyase has been obtained in pure form. (A similar enzyme occurs in eukaryotes and may be deficient in people who suffered from the xeroderma pigmentosum characterized by extreme sensitivity light dependent.

Alkyl transferases :-

Enzyme covalently attached to alkyl groups of N_2 bases are effected. So the enzyme confirmation is changed and its activity is altered. So this type of category is said to be as a sucidial enzyme

Those does not follow DRRM

Exision RM :-

This kind of DNA Repair involves a sequence of enzyme catalysed steps in which the thiamine dimers are removed from DNA molecules and a new DNA segment of DNA synthesized. This is not light dependent.

a) Nucleotide exision Repair meehanism :-

If the dimer distortion is more sevrere the nucleotide repair meehanism come into actions

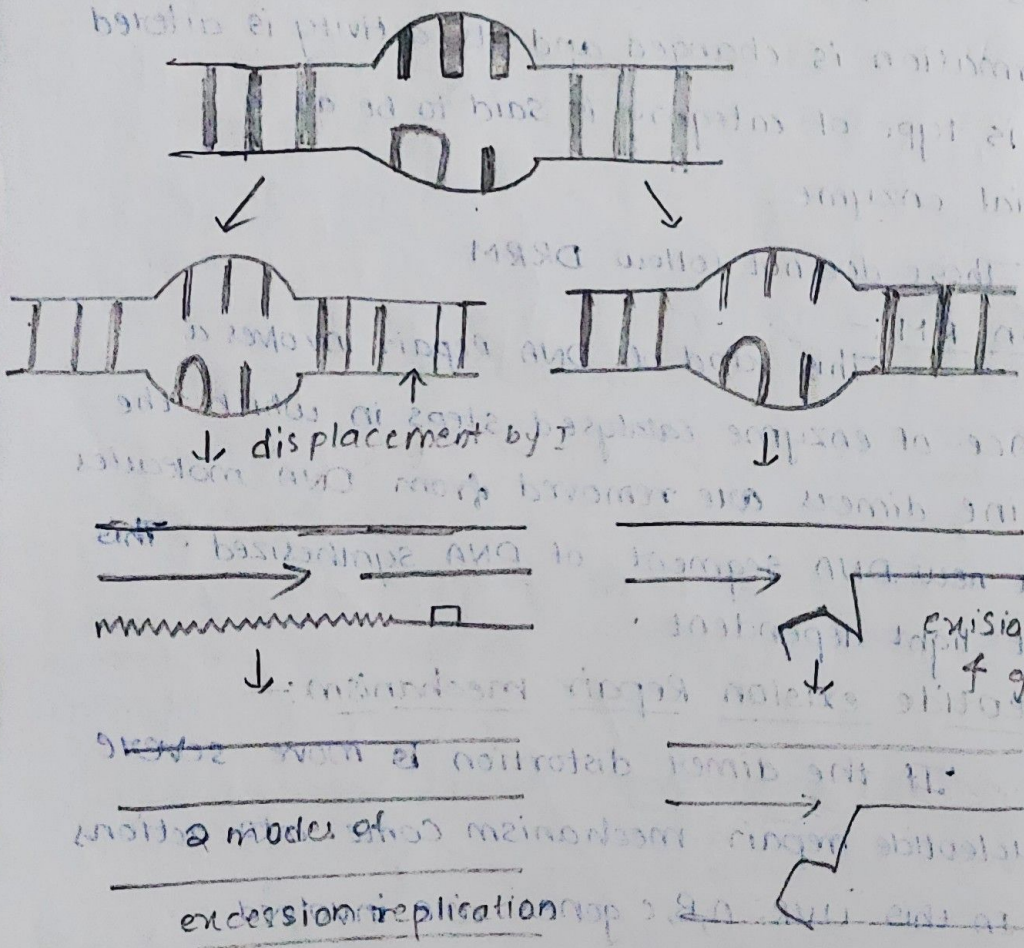
In this UVR-A,B,C genes are involved

* UVR-A product recognise the distortion site and makes UVR-B protein to bind to the distorted area. and then UVR-C protein act as endonucleases and make a cut in the distorted area (either side of the dimer) so it is called "exoisomerases".

The UVR-B cut 4 bases on one side of the dimer and UVR-C makes cut of 7 8 bases on other side. so the stretch of DNA lost by UVR-B and UVR-C is around 12-14 bases: (8+4 dimer loss)

* A DNA polymerase, probably Pol-I then fills the gap by resynthesising the missing segment using the complementary strand as template.
* DNA ligase close the gap and joins the resynthesizing segment to the polynucleotide chain.

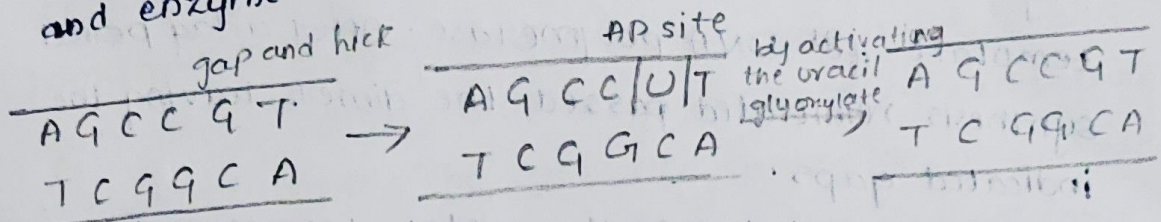
b) Base exision



(b) Base Exision Repair mechanism:-

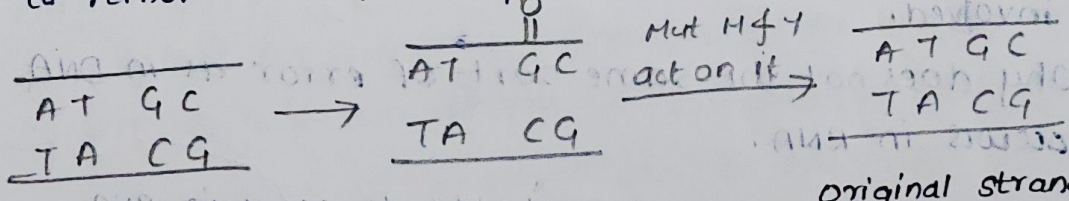
1. The bases are repaired.
2. The undesired bases in the DNA are removed, with the help of the enzyme uracyl or DNA glycosylate

This make a cut in the N-Glycocitide bond & form a Ap site (a purine (δ) apurimidine). The removed U as to be replaced by C. This capacity has + only to one enzyme AP endonuclease. This recognise the site, cut and the portion of DNA is lost. Then pol-I and ligase fill the gap and nick. Then Pol I and ligases and enzyme.



(3) Go system:-

Guanine on oxidation give 8-oxy 7-hydro guanidine (or) Guanidine oxide (G-O) Two proteins Mut-H & Mut-y both cooperatively with one another to eliminate this G-O, Mut-M a recognise the G-O and cut it to recognise the G-O, it is not possible to remove the G-O completely.



Post transcription repair mechanism:-

Another type of dark repair of U.V damage that occurs in the E-coli involves both replication and recombination and hence called post replication recombination. Each details are unknown but the process clearly involves the replication of the damaged chromosomes followed by recombination. This follows:-

a) Segment of a DNA molecule containing adjacent thiamine bases.

b) Formation of thyamine bases during UV radiation.

c) Replication of dimer containing DNA gaps are present in the nascent strands because of the irability of the distorted dimer containing region of the parental strands to function as template for DNA polymerase. After a lag period synthesis reinitiated passed the dimers, leaving the indicated gaps.

d) Recombination between the sister chromosomes producing one undamaged DNA molecule and one doubly damaged DNA molecule the net effect is the production of one undamaged chromosome from two damaged ones or quite possibly, one viable product from two inviable ones. Nucleases, DNA polymerases and DNA ligases and undoubtedly involved.

- Why does not the same sort of error as in DNA occurs in RNA.

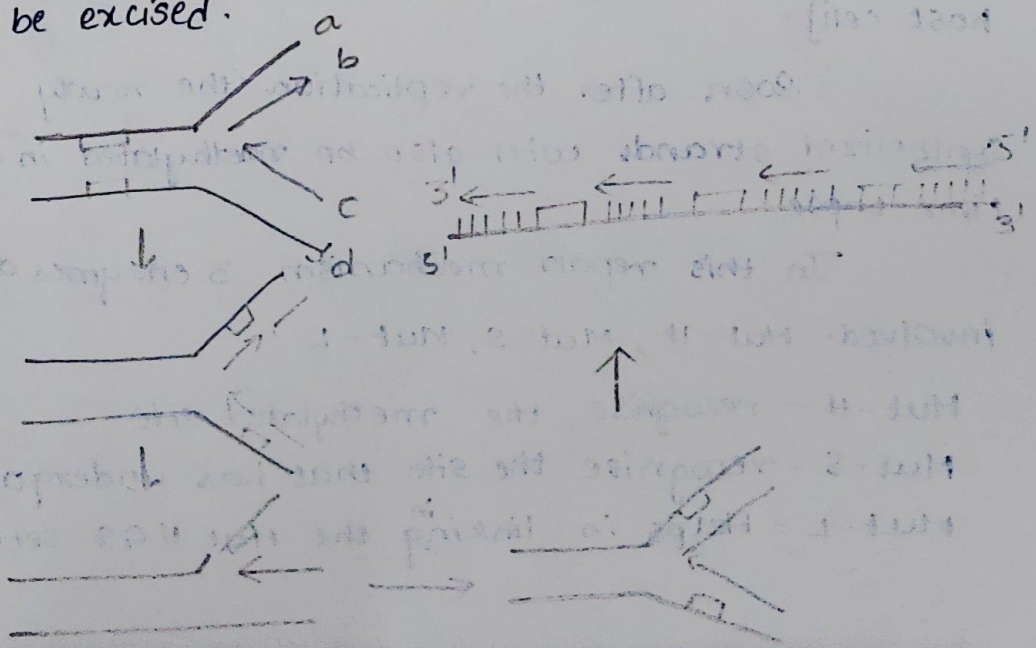
—These errors probably do occur in RNA also but the life time of the RNA is relatively short and there are many copies form of each kind of molecule with which to perform their assigned function.

Non-homologous RM:—

Recombinant RM:— This occurs mainly in the homologus chromosomes due to the presence of the thymine dimers in the template strand that particular portion is not accessible to the replication. So the gapped

DNA daughter strands are produced during replication. With the help of the recombination mechanism called sister-strand exchange, proper double-strand molecules can be made.

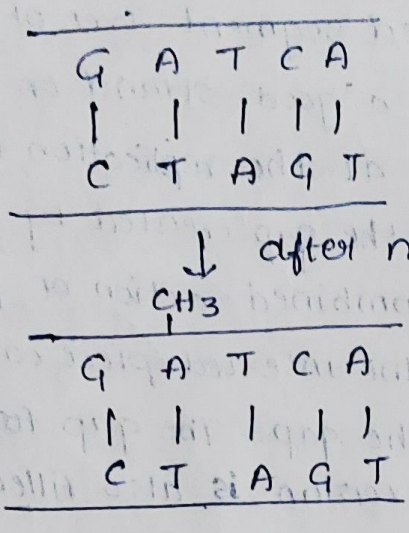
This essential idea in sisters strand exchange is that a single stranded segment free of any defects is excised from a "good" strand on the homologue DNA segment at the replication fork and some how inserted into the gap created by excision of a thyamine dimer. The combined action of polymerase I and DNA ligase joins the inserted piece to adjacent regions thus filling in the gap. The gap formed in the donar molecule by excision is also filled incompletely by polymerase-I and ligase. If this exchange and gap filling are done for each thyamine dimer, two complete daughter single strands can be formed and each can serve in the next round of replication as a templatt for synthesis of normal DNA molecule. The system fails it two dimers in opposite strands are very nearer to one another because then no undamaged sister strand segments are unavailable to be excised.



Signature :

Mismatch RM :-

This system inorder to protect it from the nucleases, the adenine are methylate. It is a post replication Repair mechanism.



[Nucleases have the capacity to break the DNA strands these are produced when the foreign DNA particles enters the host cell. The host nucleases can't detect the foreign DNA and serves the DNA. So inorder to protect it from the self nucleases A of the one DNA molecules undergo methylation. (The methylate DNA can't be cleared by nucleases). The unmethylated foreign particles will be acted upon by the nucleases are cause damage and so protect the host cell].

Soon after the replication the newly synthesized strands will also be methylated in a time Lapse.

In this repair mechanism 3 enzymes are involved. Mut-H, Mut-S, Mut-L.

Mut-H – recognise the methylated site
Mut-S - recognise the site that has undergo.
Mut-L - Helps in linking the Mut H & S sets in

SOS Repair :- [Save oursoul]

* It includes bypass system.
* A complex, indivisible repair process i.e, used to repair, DNA when extensive damage has occured. Maximum SOS repair is the major cause of UV. induced mutagenesis. In this repair Rec-A gene are involved.

[U.V radiation doesnot occur in Rec-A bacterium]. When damage occur Rec-A binds to DNA [where damage occur].

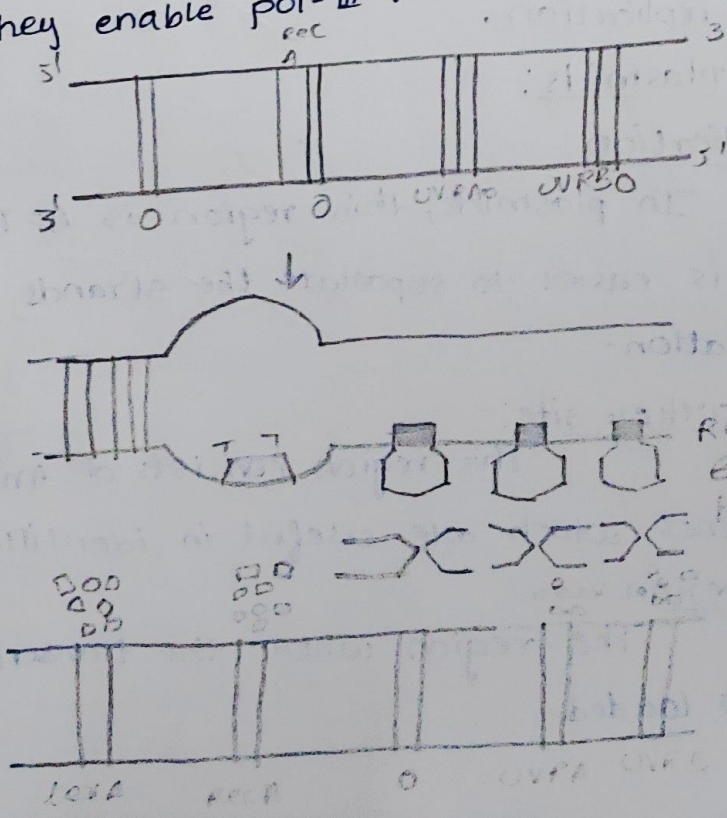
* In this repair pol-3 are involved. pol-III utilises the residual base pairing ability to the thymine dimer. and put two Adenines on daughter strand.
* In this SOS repair UHVC' and UMV 'D' are also involved the role of gene product unknown

But three hypothesis are there.

a) They facilitate tight binding of Rec-A at the small distortion.

b) They facilitate binding of pol-III to the distorted region.

c) They enable pol-III from Rec-A or Rec-A DNA.



Signature :

1. Introduction to Transformation:-

Transformation is a process by which bacteria take up foreign genetic material (DNA) from their environment. This is one of the three main types of horizontal gene transfer, along with transduction and conjugation. Transformation plays a crucial role in genetic engineering, bacterial evolution, and biotechnology.

This process was first demonstrated by Frederick Grifith in 1928 while studying Streptococcus pneumonia; he observed the non-virulent strains of bacteria could become virulent when exposed to heat killed virulent strains, leading to the discovery of the transforming principle" later identified as DNA by Avery, Macleod, and Mc Carty in 1944.

Mechanism of Tranformation:-

The transformation process involved several key steps:-

1. Uptake of DNA:-

For transformation to occur, bacteria must be in a state of competence, meaning they can take up foreign DNA.

Competence can be:-

- "Natural Competence": Some bacteria (Bacillus subtills "Streptococcus pneumonia") naturally develop comptence during specific growth pheases.
- Artificial Competence":- Other bacteria (eg:- E-coli can be induced to take up DNA through chemical treatments (eg: Calcium chloride and heat shock) or electroporation.

Once competent, bacteria can recognize and bind to extracellular DNA through surface receptors. This DNA is usually from lysed bacterial cells and can be single-stranded or double-stranded.

2, DNA Binding and Transport.

After Binding, the DNA is transported across the bacterial cell membrane through specialized transport proteins. In gram-positive bacteria, this process occurs through DNA translocases. In Gram negative bacteria, additional porins and channel proteins help the DNA pass through the outer membrane.

Typically, one strand of the double-stranded DNA is degarded by nucleases, while the other strand is transported into the cytoplasm.

3, Integration into the Bacterial Genome:—

Once inside, the single-stranded DNA aligns with homologus regions in the bacterial chromosome. The RecA protein facilitates homologus recombination, allowing the foreign DNA to replace or integrate into the bacterial genome. If the incoming DNA doesnot integrate, it is usually degraded.

In some cases, if the DNA remains as a plasmid, it can replicate independently and confer new traits to the bacterium.

4. Expression of new traits:—

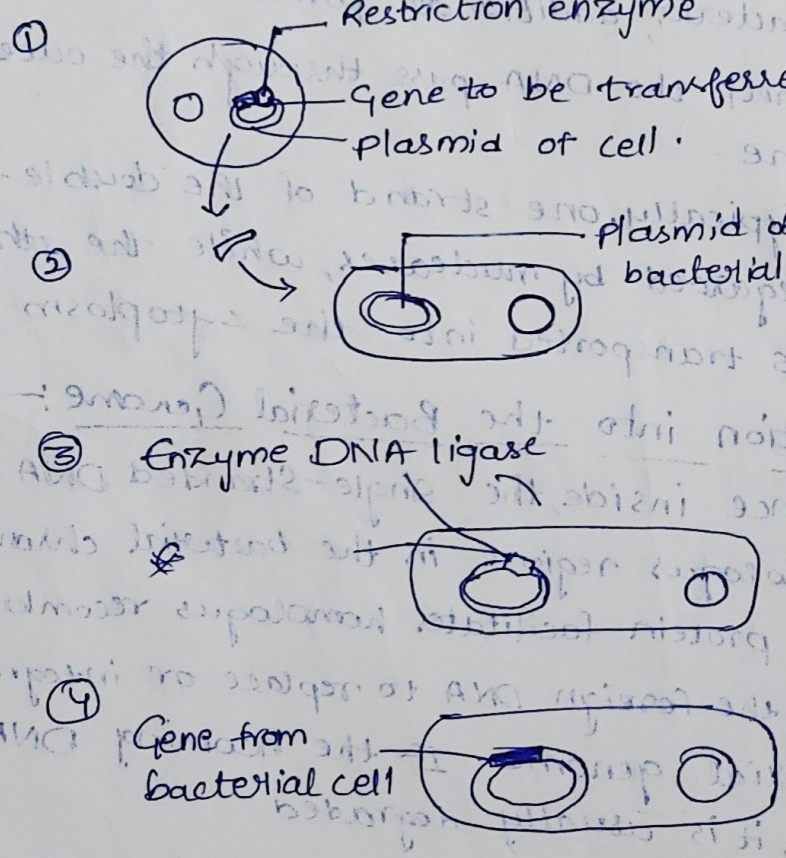
If the incorporated DNA contains functional genes, the bacterium can express new traits. These may include.

- Antibiotic resistance
- Virulence factors.

—Metabolic capabilities

Conclusion:-

Transformation is a fundamental mechanism of horizontal gene transfer that allows bacteria to acquire new genetic traits. Onderstanding this process has led to advancements in genetics medicine & biotechnology, making it a powerful tool for scientific research & industrial applications.



Conjugation:-

If is a process of horizontal gene transfer in bacteria that allows the direct transfer of genetic material from one bacterium to another via cell-to cell contact. Unlike transformation (involves uptake of free DNA) & transduction (involves DNA transfer through bacteriophages), a conjugation requires a physical connection blwd donar & a receipient cell.

This process plays a crucial role in bacterial evolution, antibiotic resistance, and genetic diversity, it was first discoverd by Joshua Lederberg & Edward Tatum in 1946 in E-coli, demonstrating that bacteria can exchange genetic information.

Mechanism of conjugation :-

It occurs in several steps, beginning with cell contact & ending with the replication & expression of the transferred DNA in the recipient cell.

1. Formation of cell-to-cell contact :-

- Conjugation is initiated when a donor cell (F^+) containing a conjugative plasmid, such as the F (fertility) plasmid, extends a pilus (sex pilus) toward a recipient cell (F^-) that lacks the plasmid.
- The pilus is a proteinaceous appendage encoded by genes in the F plasmid. It acts as a bridge, allowing the donor to attach to the recipient.
- once contact is made, the pilus retracts, bringing the two cells close together. A specialized conjugation pore or bridge forms between them, facilitating DNA transfer.

2. Activation of the DNA transfer process"

Within the donor cell, a protein complex called the relaxosome" assembles at a specific site on the F-plasmid known as the "origin of transfer (oriT).

- The enzyme 'Relaxase' part of the (relaxosome) makes a "single-stranded cut" at the oriT site.
- Relaxes remaines attached to the cut DNA strand and interacts with the coupling proteins that guide it toward the conjugation channel.

3. Transfer of Single stranded DNA through the Conjugation Bridge.

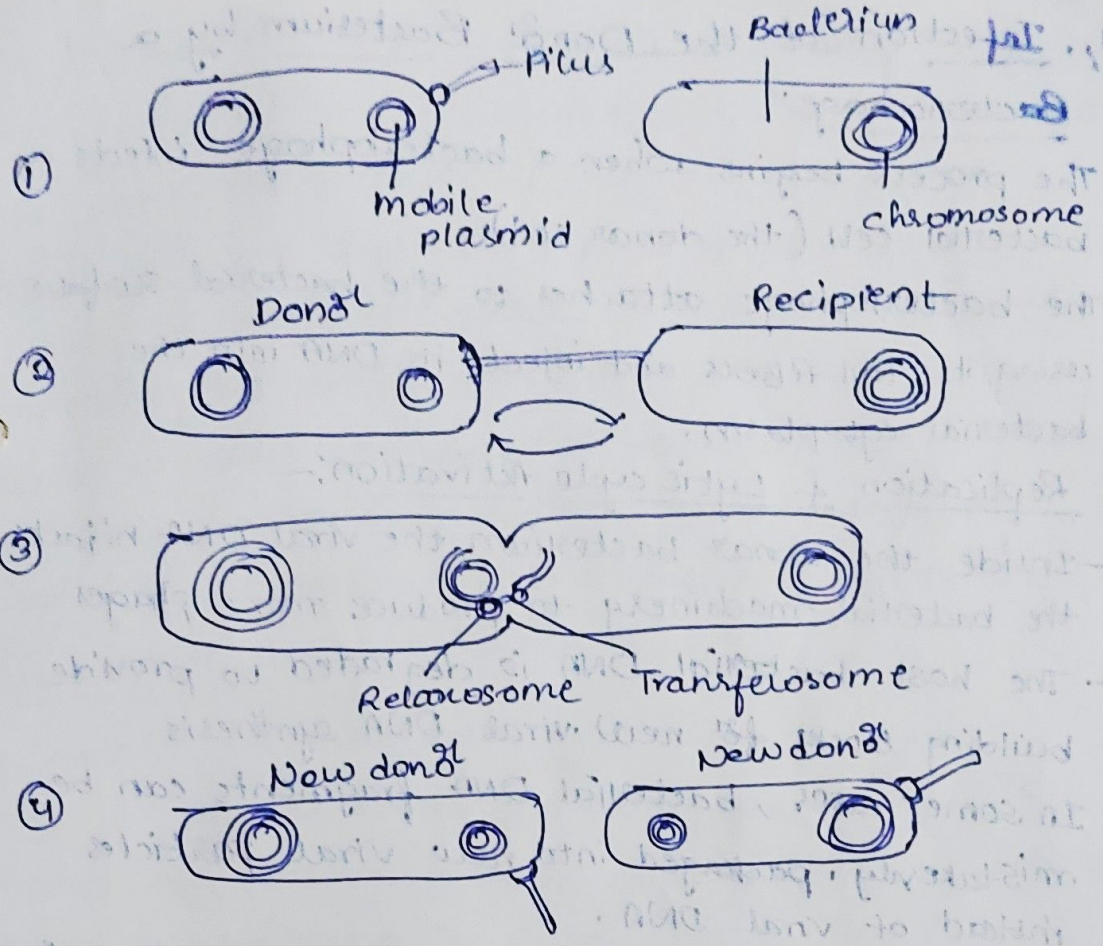
- The "T4 secretion system" a type of bacterial secretion system, transports the single-stranded DNA from the donor to the recipient through the conjugation bridge.
- As the single strand moves into the recepient cell, a process called "rolling circle replication" begins in the donor.
- This ensures that while one strand is being transferred a complementary strand is synthesized in both the donor and the recipient cells, restoring the plasmid to its double-stranded from in each.

4. Completion of DNA transfer & separation :-

- Once the entire single strand of the F plasmid has entered the recipient, the conjugation bridge disassembles, & the cells separate.
- DNA polymerase in the recipient completes the synthesis of the complementary strand, converting the single-stranded DNA into a functional double-stranded plasmid.
- The recipient cell, originally F^-, is now, an F^+ cell & can initiate conjugation with other F^- cells, spreading the plasmid further.

Conclusion:-

Bacterial Conjugation is a crucial mechanism of genetic exchange that facilitates adaptation, antibiotic resistance, & evolution in microbial populations. By understanding its mechanisms, scientists can develop strategies to combat antibiotic resistance, utilize bacteria in (bacteriologist) biotechnology, & explore new avenues in microbial research.



(3) Transduction :-

It is a process of horizontal gene transfer in bacteria where genetic material is transferred from one bacterium to another via bacteriophages (virus that infect bacteria). This process was first discovered by Joshua Lederberg & Norton Zinder in 1952 while studying 'Salmonella' bacteria.

Unlike conjugation, & transformation, transduction relies on viral infection to introduce new genetic material into bacteria. It plays a crucial role in bacterial evolution, genetic diversity, and the spread of antibiotic resistance.

Mechanism of Transduction :-

The process of transduction involves the following steps :-

1. Infection of the Donor Bacterium by a Bacteriophage"

- The process begins when a bacteriophage infects a bacterial cell (the donor cell)
- The bacteriophage attaches to the bacterial surface using its tail fibers and injects its DNA into the bacterial cytoplasm.

(2) Replication & Lytic cycle Activation:—

- Inside the donar bacterium, the viral DNA hijacks the bacterial machinery to produce more phages.
- The host bacterial DNA is degraded to provide building blocks for new viral DNA synthesis.
- In some cases, bacterial DNA fragments can be mistakenly packaged into new viral particles instead of viral DNA.

(3) "Lysis of the Donar cell and Release of phages"

- Inside the donar bacterium, the viral DNA hijacks the bacterial machinery to produce more phages.
- The host bacterial DNA is degraded to provide building blocks for new viral DNA synthesis.
- In once new viral particles are assembled, the bacterial cell undergoes "lysis", releasing phages into the environment.
- Some of these phages contain bacterial DNA instead of viral DNA.

(4) "Infection of the Recipient Bacterium"

- These defective phages, carrying bacterial DNA, infect a new bacterial cell (the recipient cell).
- Instead of causing a lytic infection, the bacterial DNA carried by the phage may integrate into the recepient's genome through homologous recombination.

...onor Bacterium by a

...n a bacteriophage infects a
... cell)
...hes to the bacterial surface
...injects its DNA into the

...e Activation:—
...rium, the viral DNA hijacks
...to produce more phages.
... is degraded to provide
...iral DNA synthesis.
... DNA fragments can be
..., new viral particles

...nd Release of phages"
...ium, the viral DNA hijacks
...to produce more phages.
... is degraded to provide
...ral DNA synthesis.
...les are assembled, the
...lysis, releasing phages into

...ntain bacterial DNA

...ent Bacterium"
...carrying bacterial DNA, infect
...the recipient cell).
...ytic infection, the bacterial
...ge may integrate into
... through homologous

5. Integration and Expression of Transferred Genes"

- If recombination occurs, the recipient cell incorporates the donor bacterial genes into its chromosome.
- The recipient bacterium may acquire new traits, such as antibiotic resistance or toxin production, depending on the transferred genes.
- Since transduction doesnot require direct contact between bacterias, it enables gene transfer even between distantly related bacterial species.

Types of Transduction:—

① Generalized Transduction:—

Occurs when random bacterial DNA fragments are mistakenly packaged into a bacteriophage during the lytic cycle.

- Any gene from the donor bacterium can be transferred to the recipient.
- The transferred DNA doesnot replicate independently but may integrate into the recipient's genome through homologous recombination.

ex:- Salmonella & E-coli

② Specialized Transduction:—

Occurs when a specific segment of bacterial DNA is transferred by a bacteriophage during the lysogenic cycle.

- Only genes adjacent to the prophage insertion site in the bacterial chromosome are mistakenly excised along the viral genome.
- This type of transduction is carried out by temperate phages that integrate into the bacterial chromosome as a prophage.

Ex:- The Lambda (λ) phage infecting E-coli is a well-known

Conclusion :-

It is a critical mechanism of horizontal gene transfer in bacteria, facilitating the exchange of genetic material through bacteriophages. Generalized transduction allows the random transfer of bacterial genes, which s