MOLECULARBIOLOGY AND MICROBIAL GENETICS

QUESTION BANK

Department of Microbiology
II B.Sc Microbiology Honours- IV SEMESTER
COURSE -9: MOLECULAR BIOLOGY AND MICROBIAL GENETICS
Model Question Paper

Time:2.30 Hrs **Max. marks:50**

Section-A **5X8=40**

(Answer all the questions. Draw the labelled diagrams when necessary.)

Unit	Q.N	Questions	Marks	BL	CO	PO
I	1	Interpret the experimental evidences that established DNA as a Genetic material.	8	3	1	4
		(OR)				
		Explain the mechanism of DNA replication in Prokaryotes	8	2	2	4
II	2.	Conclude and contrast the Classical concept of Gene	8	4	4	4
		(or)				
		Illustrate the Mechanism of Transcription	8	2	4	4
III	3	List the salient features of Genetic code	8	1	5	4
		(OR)				
		Evaluate the Gene expression in E. Coli by Lac operon concept	8	4	5	4
IV	4	Define Mutations. Describe different types of physical and chemical mutagens	1+7	1&3	6	4
		(OR)				
		Give outlines of different DNA repair mechanisms	8	2	6	4
V	5	Differentiate F factor and Hfr strains. Explain the mechanism of Conjugation in Bacteria	8	4&2	7	4

		(or)				
		Interpret the Lederberg and Zender experiment and explain mechanism of Transduction	4+4	3&2	7	4

Section -B

Answer the following questions 1X10=10

6. Give an example of a Microorganism having RNA as a Genetic material

7. Messelson – Stahl Experiment is to prove that the DNA replication is by Semiconservative method. (TRUE/FALSE)

8. One-gene one enzyme concept was given by----------

9. mRNA splicing takes place in only in Eukaryotes . (TRUE/FALSE)

10. What is wobble hypothesis?

11. Tetracycline is an inhibitor of protein synthesis. (TRUE/FALSE)

12. What is SOS repair?

13. UV radiation is a physical mutagen. (TRUE/FALSE)

14. What is transformation?

15. Integration of F plasmid in the recipient Bacteria is called Episome. (TRUE/FALSE)

Question bank

Section-A 5X8=40

(Answer all the questions. Draw the labelled diagrams when necessary.)

Unit	Q.N	Questions	Marks	BL	CO	PO
I	1.	Interpret the experimental evidence that established DNA as a Genetic material.	8	3	1	4
	2	Explain General characters of Plasmids and Transposons	8	2	3	4
	3	Explain the mechanism of DNA replication in Prokaryotes	8	2	2	4
II	4	Conclude and contrast the Classical concept of Gene	8	4	4	4
	5	Generalise the modern concept of gene in detail	8	3	4	4
	6	Illustrate the Mechanism of Transcription	8	2	4	4
III	7	List the salient features of Genetic code	8	1	5	4
	8	Outline the process of Translation in Prokaryotic organisms	8	2	5	4
	9	Evaluate the Gene expression in E. Coli by Lac operon concept	8	4	5	4
IV	10	Define Mutations. Describe different types of physical and chemical mutagens	1+7	1&3	6	4
	11	Demonstrate the molecular basis of mutations in detail	8	4	6	4
	12	Give outlines of different DNA repair mechanisms	8	2	6	4
	13	Differentiate F factor and Hfr strains. Explain the mechanism of Conjugation in Bacteria	8	4&2	7	4

V	14	Explain the mechanism of Translation and illustrate few applications of Transformation	4+4	2&3	7	4
	15	Interpret the Lederberg and Zender experiment and explain mechanism of Transduction	4+4	3&2	7	4

Section -B

Answer the following questions. Each question carries one mark (10x1=10)

1. Give an example of a Microorganism having RNA as a Genetic material
2. Messelson – Stahl Experiment is to prove that the DNA replication is by Semiconservative method. (TRUE/FALSE)
3. SSB protein function is to bind the Double stranded DNA during Replication (TRUE/FALSE)
4. Function of Enzyme Gyrase----------
5. Short length of DNA synthesized on Lagging strand are called----------
6. One-gene one enzyme concept was given by----------
7. Who gave the definition of Gene?
8. What is Recon?
9. Intron denote non coding regions and exons denotes coding regions (TRUE/FALSE)
10. ---------- region is the recognition site for RNA Polymerase during transcription.
11. mRNA splicing takes place in only in Eukaryotes (TRUE/FALSE)
12. Genetic code may be overlapping ((TRUE/FALSE)
13. What is wobble hypothesis?
14. What are the termination Codons?
15. Tetracycline is an inhibitor of protein synthesis. (TRUE/FALSE)
16. Define frame shift mutation--------
17. What is SOS repair?

18. Ethydium bromide is an alkylating agent (TRUE/FALSE)
19. UV radiation is a physical mutagen. (TRUE/FALSE)
20. What is transformation?
21. Grifith's experiment on Pneumococci leads to discovery of Transformation (TRUE/FALSE)
22. Bacterial conjugation was first described by---------
23. Integration of F plasmid in the recipient Bacteria is called Episome. (TRUE/FALSE)
24. What is abortive transduction?
25. ---------- ion enhance the competency during transformation.

Question Bank- Answers

1. DNA as a Genetic material

By the early 1900's, biochemists had isolated hundreds of different chemicals from living cells. Which of these was the genetic material? Proteins seemed like promising candidates, since they were abundant, diverse, and complex molecules. However, a few key experiments demonstrated that DNA, rather than protein, is the genetic material.

The important experimental evidences to state that the NDA is a Genetic material

1. Griffith Experiment:

In 1928, a young scientist Frederick Griffith discovered the transforming principle. In 1918, millions of people were killed by the terrible Spanish influenza epidemic, and pneumococcal infections were a common cause of death among influenza-infected patients. This triggered him to study the bacteria *Streptococcus pneumoniae* and work on designing a vaccine against it. It became evident that bacterial pneumonia was caused by multiple strains of *S. pneumoniae,* and patients developed antibodies against the particular strain with which they were infected. Hence, serum samples and bacterial isolates used in experiments helped to identify DNA as the hereditary material.

He used two related strains of *S. pneumoniae* and mice and conducted a series of experiments using them. When type II R-strain bacteria were grown on a culture plate, they produced rough

colonies. They were non-virulent as they lacked an outer polysaccharide coat. Thus, when RII strain bacteria were injected into a mouse, they did not cause any disease and survived.

When type I S-strain bacteria were grown on a culture plate, they produced smooth, glistening, and white colonies. The smooth appearance was apparent due to a polysaccharide coat around them that provided resistance to the host's immune system. It was virulent and thus, when injected into a mouse, resulted in pneumonia and death.

In 1929, Griffith experimented by injecting mice with heat-killed SI strain (i.e., SI strain bacteria exposed to high temperature ensuing their death). But, this failed to harm the mice, and they survived.

Surprisingly, when he mixed heat-treated SI cells with live RII cells and injected the mixture into the mice, the mice died because of pneumonia. Additionally, when he collected a blood sample from the dead mouse, he found that sample to contain live S-strain bacteria.

Conclusion of Griffith's Transformation Experiment

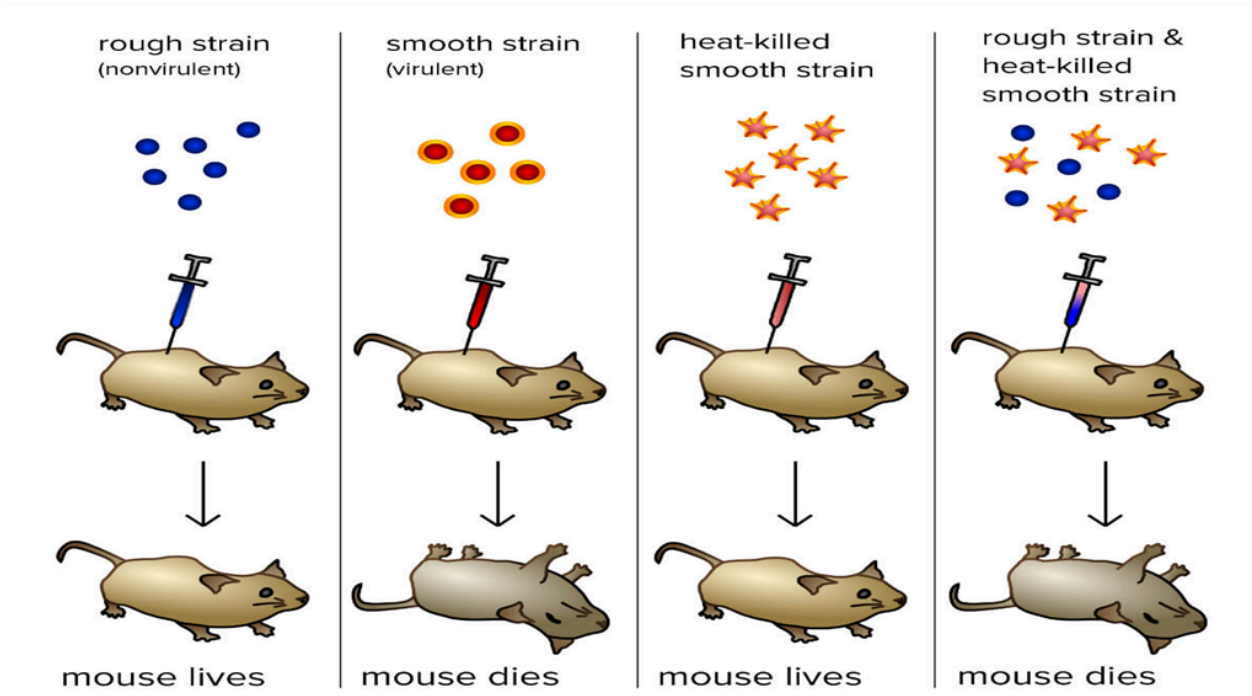


Figure: Griffith's Transformation Experiment

Based on the above results, he inferred that something must have been transferred from the heat-treated S strain into non-virulent R strain bacteria that transformed them into smooth coated and virulent bacteria. Thus, the material was referred to as the transforming principle.

2. Avery, McCarty, and MacLeod Experiment

During World War II, in 1943, Oswald Avery, Maclyn McCarty, and Colin MacLeod working at Rockefeller University in New York, dedicated themselves to continuing the work of Griffith in order to determine the biochemical nature of Griffith's transforming principle in an in vitro system. They used the phenotype of S. pneumoniae cells expressed on blood agar in order to figure out whether transformation had taken place or not, rather than working with mice. The transforming principle was partially purified from the cell extract (i.e., cell-free extract of heat-killed type III S cells) to determine which macromolecule of S cell transformed type II R-strain into the type III S-strain. They demonstrated DNA to be that particular transforming principle.

Initially, type III S cells were heat-killed, and lipids and carbohydrates were removed from the solution.

Secondly, they treated heat-killed S cells with digestive enzymes such as RNases and proteases to degrade RNA and proteins. Subsequently, they also treated it with DNases to digest DNA, each added separately in different tubes.

Eventually, they introduced living type IIR cells mixed with heat-killed IIIS cells onto the culture medium containing antibodies for IIR cells.

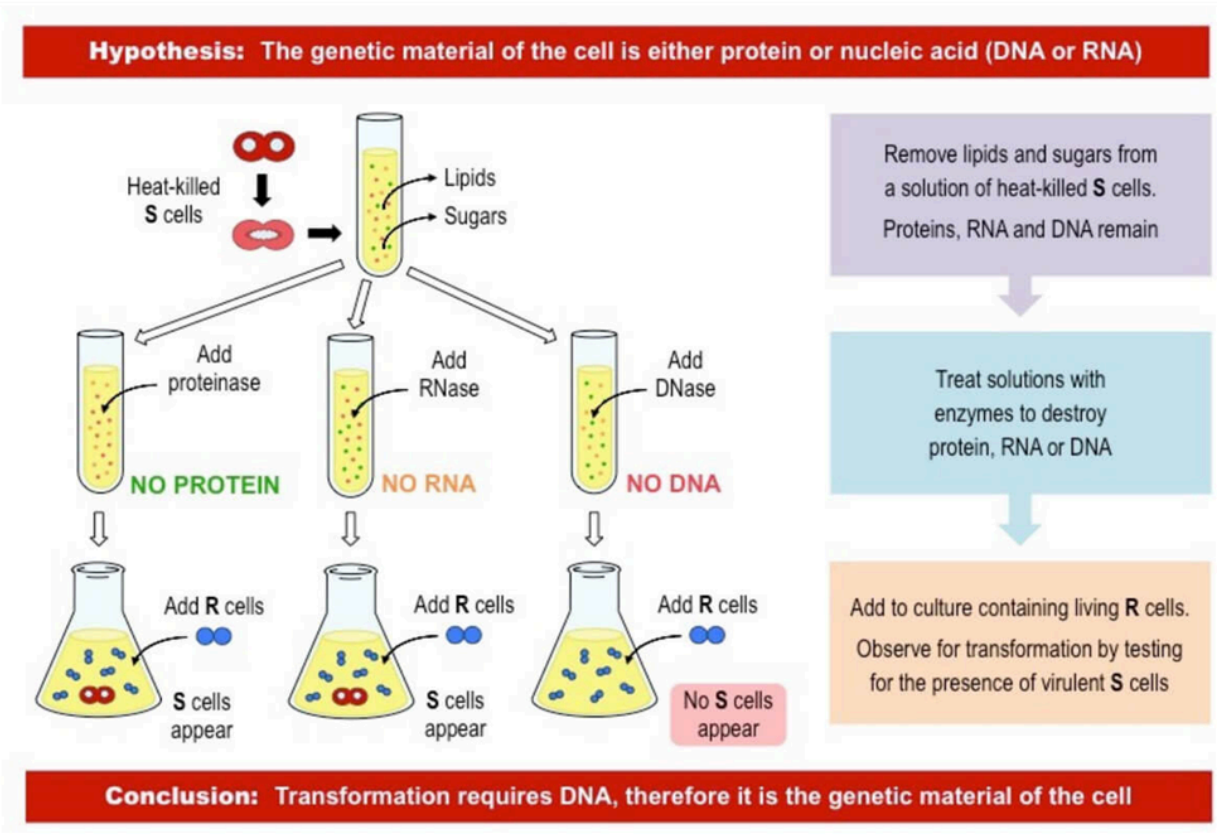


Figure: Avery, McCarty, and MacLeod Experiment

Observation of Avery, McCarty, and MacLeod Experiment

The culture treated with DNase did not yield transformed type III S strain bacteria which indicated that DNA was the hereditary material responsible for transformation.

Conclusion of Avery, McCarty, and MacLeod Experiment:-DNA was found to be the genetic material that was being transferred between cells, not proteins.

3. Hershey and Chase Experiment

Although Avery and his fellows found that DNA was the hereditary material, the scientists were reluctant to accept the finding. But,eight years after in 1952, Alfred Hershey and Martha Chase concluded that DNA is the genetic material. Their experimental tool was bacteriophages-viruses that attack bacteria which specifically involved the infection of *Escherichia coli* with T2 bacteriophage.

- Bacteriophage was allowed to grow on two of the medium: one containing a radioactive isotope of phosphorus(^{32}P) and the other containing a radioactive isotope of sulfur (^{35}S).
- Phages grown on radioactive phosphorus(^{32}P) contained radioactive P labeled DNA (not radioactive protein) as DNA contains phosphorus but not sulfur.
- Similarly, the viruses grown in the medium containing radioactive sulfur (^{35}S) contained radioactive ^{35}S labeled protein (but not radioactive DNA) because sulfur is found in many proteins but is absent from DNA.
- E. coli were introduced to be infected by the radioactive phages.
- After the progression of infection, the blender was used to remove the remains of phage and phage parts from the outside of the bacteria, followed by centrifugation in order to separate the bacteria from the phage debris.
- Centrifugation results in the settling down of heavier particles like bacteria in the form of pellet while those light particles such as medium, phage, and phage parts, etc., float near the top of the tube, called supernatant.

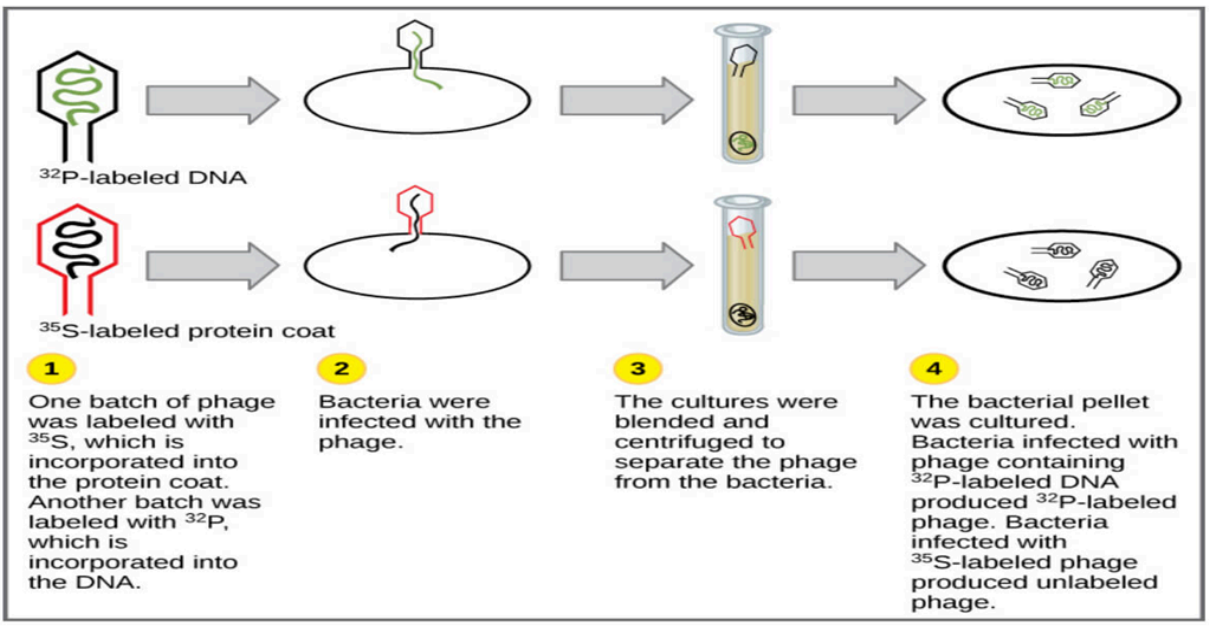


Figure: Hershey and Chase Experiment

Observation of Hershey and Chase Experiment

On measuring radioactivity in the pellet and supernatant in both media, ^{32}P was found in large amount in the pellet while ^{35}S in the supernatant that is pellet contained radioactively P labeled infected bacterial cells and supernatant was enriched with radioactively S labeled phage and phage parts.

Conclusion of Hershey and Chase Experiment: Hershey and Chase deduced that it was DNA, not protein which got injected into host cells, and thus, DNA is the hereditary material that is passed from virus to bacteria.

Q.2: Explain the general characters of Plasmids and transposons

Plasmids

Plasmids are small circular DNA fragments, double-stranded, self-replicating extra chromosomal structures found in many microorganisms.

The term Plasmid was coined by Joshua Lederberg in 1952.

Plasmids are important as genetic tools, which are used to introduce, manipulate or delete certain genes from the host cell.

Properties of plasmids:

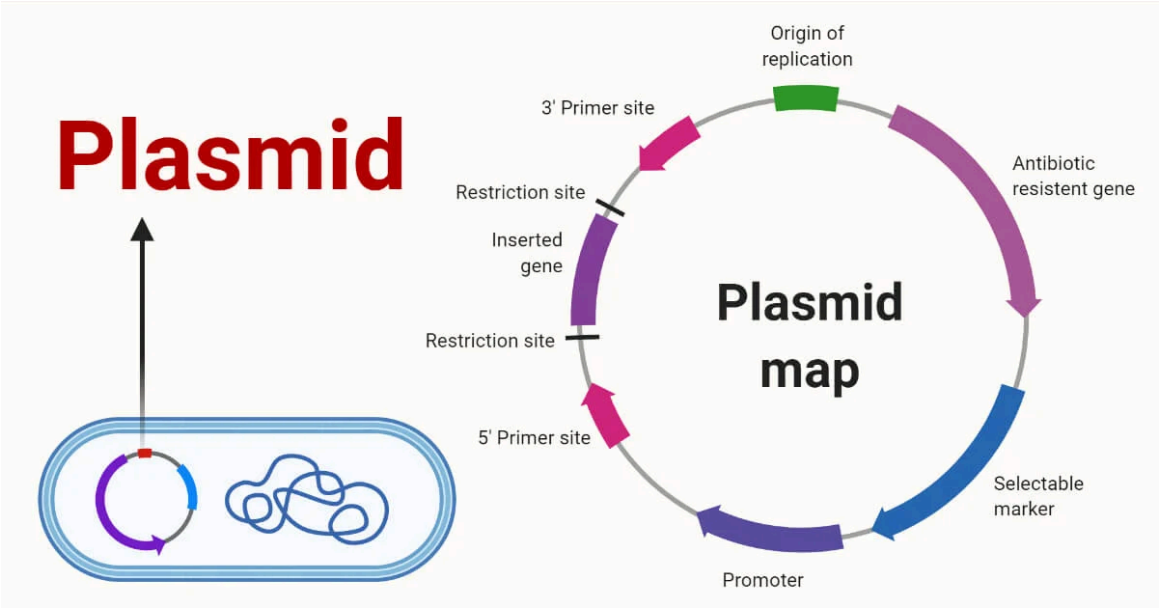
- They are extra chromosomal DNA fragments present in the cell.

- They are double stranded structures. Exceptions are the linear plasmids in bacteria *Streptomyces spp* and *Borrelia spp.*
- They can replicate independently.
- The absence of a plasmid in the cell does not affect cell functioning, but the presence of a plasmid in the cell is usually beneficial.
- Plasmids are also known as sex factors, conjugants, extra chromosomal replicons, or transfer factors.
- Copy number – the copy number refers to the number of copies of plasmid present in the bacterial cell. Usually, small plasmids are present in high numbers and large plasmids are present in few numbers.
- Compatibility of plasmids – this refers to the ability of two different plasmids to coexist in the same bacterial cell.

Structure of plasmids:

Every plasmid has certain essential elements. These are as follows –

- Origin of replication (OR) – This refers to a specific location in the strand where the replication process begins. In plasmids, this region is A=T rich region as it is easier to separate the strands during replication.
- Selectable marker site – This region consists of Antibiotic resistance genes which are useful in the identification and selection of bacteria that contain plasmids.
- Promoter region – this is the region where the transcriptional machinery is loaded.
- Primer binding site – this is the short sequence of single-strand DNA which is useful in DNA amplification and DNA sequencing.
- Multiple cloning sites – This site contains various sequences where the restriction enzymes can bind and cleave the double stranded structure.



Functions:

- The important use of plasmids is that they can be used as vectors to insert a specific gene into other organisms due to their capacity to incorporate a gene and replicate inside the cell.
- They are an important factor in bacteria as they carry antibiotic resistance genes.
- Degradative plasmids can be used to degrade industrial chemicals which are a threat to the environment.
- As plasmids are easy to manipulate, they are being used in gene therapy as well.
- Because plasmids are good vectors (a vehicle/factor which is used to transfer a gene from one organism to another) they are used in drug delivery and for hormone production in other cells.
- Plasmids are an important source of horizontal gene transfer.

Transposons General characters

Transposable elements (TE) or transposons can be defined as small, mobile DNA sequences that move around chromosomes with no regard for homology, and insertion of these elements may produce deletions, inversions, chromosomal fusions, and even more complicated rearrangements.

Transposable Elements

Some salient features of transposable elements are:

1. These are the DNA sequences that code for enzymes which result in self-duplication and insertion into a new DNA site.
2. Transposons are involved in transposition events which include both recombination and replication, which usually generates two copies of the original transposable elements. One of the copies remains at the parent site, whereas the other one reaches the target site on the host chromosome.
3. The integrity of the target genes of these elements is invariably disrupted by the presence of those elements.
4. Because transposons carry the genes for initiation of RNA synthesis, some previously dormant genes might be activated.
5. A transposable element doesn't have a site for the origin of replication. As a result, it cannot replicate without the host chromosome as plasmids or phages.
6. There is no homology between the transposon and its target site for insertion. These elements can insert at almost any position in the host chromosome or a plasmid. Some transposons might seem likely to enter at some specific positions (hot spots), they barely insert at base-specific target sites.

Applications:

- Transposable elements can be used as a genetic tool for the analysis of gene expression and protein functioning.
- These are used in genetic engineering to insert or remove specific genetic sequences, and also to cause frameshift mutation.
- The Tc1/mariner-class of TEs Sleeping Beauty transposon system is being studied for use in human gene therapy.

Q.3. DNA Replication

DNA replication is a fundamental genetic process that is essential for cell growth and division.

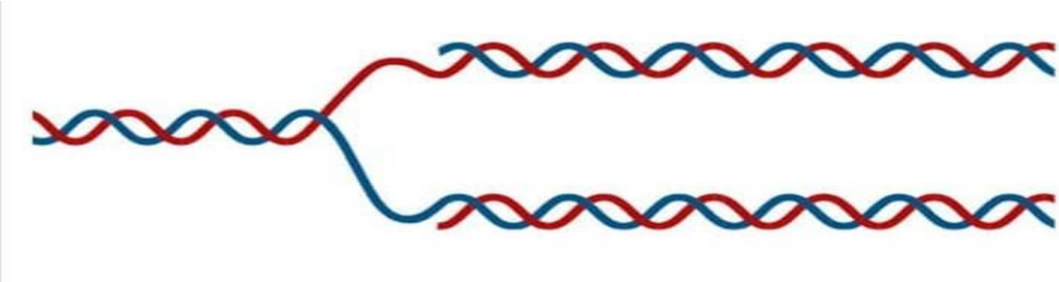
DNA replication involve the generation of a new molecule of nucleic acid, DNA, crucial for life. DNA replication is important for properly regulating the growth and division of cells. It conserves the entire genome for the next generation.

Enzymes and factors involved in replication

Steps in DNA Replication:

Step 1: Formation of Replication Fork

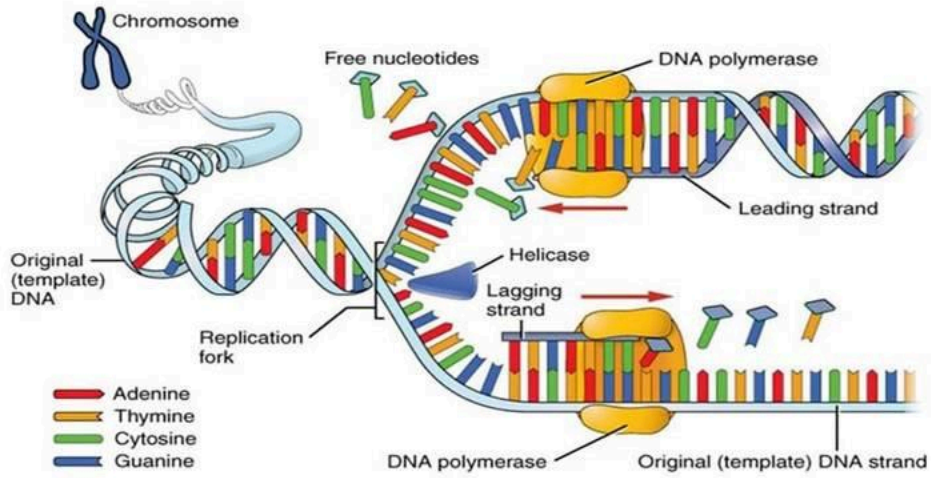
- Before DNA can replicate, this double-stranded molecule must unwind into two single strands to initiate the replication process
- DNA unwinds when the complementary base pairing between the double-stranded is broken, and the site to initiate this unwinding is denoted by specific regions (Adenine and Thymine rich).
- These specific coding regions are referred to as Origin of Replication (Ori) and thus the replication process begins.
- These origins are targeted by initiator proteins, which go on to recruit more proteins that can help the replication process by forming a replication fork around the Ori.
- Within this replication protein complex is an enzyme DNA helicase, which starts to unwind the DNA from its Ori and exposes two strands resembling a Y-like structure referred to as replication fork.
- The activity of helicase causes topological stress to the un-winded strand forming supercoiled DNA, this stress is relieved by Topoisomerase by negative supercoiling.
- The replication fork is bidirectional; one strand is oriented to 5′ to 3′ direction (leading strand) and the other strand is oriented to 3′ to 5′ direction (lagging strand) but the addition of nucleotide progress only in 5′ to 3′ direction.
- The formation of a replication fork exposing two single-stranded strands marks the beginning of Initiation.



Step 2: Initiation

- One strand runs from 5′ to 3′ direction towards the replication fork and is referred to as leading strand and the other strand runs from 3′ to 5′ away from the replication fork and is referred to as lagging strands.
- To this exposed single-stranded DNA, SSB proteins are adhered to prevent recoiling of DNA and to stabilize it.
- After which another enzyme DNA primase comes into action to synthesize a short stretch of RNA primer, which provides a free 3′ hydroxyl group for DNA polymerase can now add nucleotides and extend the new chain of nucleotides.

DNA Replication.



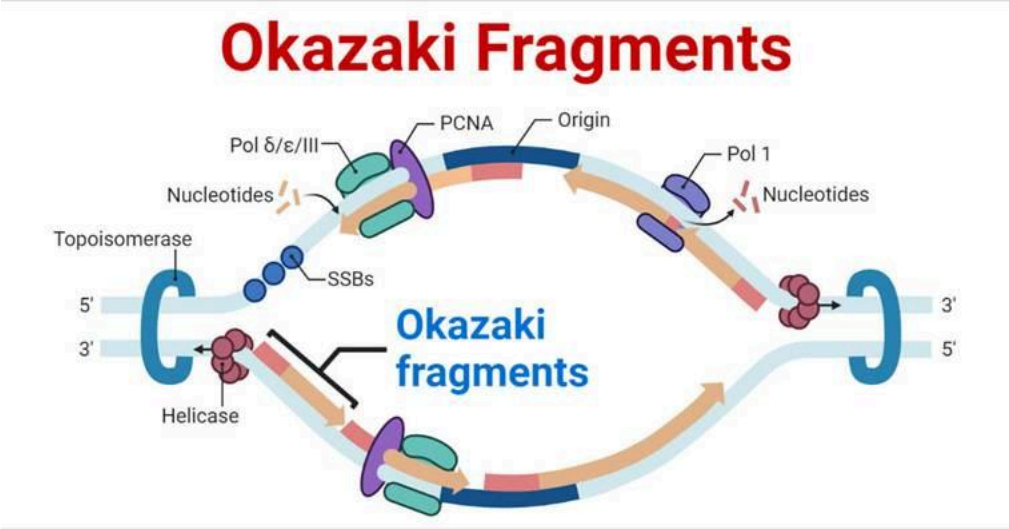
Step 3: Elongation

- Now that primer is added to unzipped two single-stranded DNA, these strands now act as a template for synthesizing new DNAs.
- The enzyme DNA polymerase synthesizes new nucleotide to match the template and add on to the free 3′ hydroxyl group provided by the primer in each single-stranded DNA
- The leading strand runs from 5′ to 3′ so the addition of nucleotides by DNA polymerase happens from 5′ to 3′ direction. As the replication fork progresses the addition of nucleotide is continuous thus only requiring the primer once.
- However, lagging strands is antiparallel and run from the 5′ to 3′ direction, the continuous addition of nucleotides is not possible as the replication fork progresses, DNA polymerase cannot add complementary nucleotides to the 5′ end. Therefore, multiple primers are required.
- Due to this phenomenon, the DNA nucleotides synthesis from lagging strands occurs in fragments. These fragments are termed Okazaki fragments.
- Hence, the leading strand using only one primer synthesizes nucleotides continuously, while the lagging strand uses multiple primers and thus synthesizes nucleotides discontinuously.

Step 4: Termination

- RNA primers of both leading and lagging strands are cleaved out or degraded by exonucleases activity of DNA polymerase, and the nicks or gaps so formed are filled with DNA and sealed by the enzyme DNA ligase.
- DNA polymerase also shows proofreading activity and check, remove and replace any errors.
- Interestingly, in eukaryotic organisms having linear DNA, when RNA primer at 5′ end of daughter strand is removed, there is not a preceding 3′ OH such that DNA polymerase can use it to replace with DNA.

- So, at the 5′ end of daughter strands, there is a gap (missing DNA). This missing DNA can cause a loss of information contained in that region. This gap must be filled before the next round of replication.
- For solving this end replication problem, researchers have found that linear ends of DNA called telomere are used which contain specific G: C rich repeats. These sequences are known as telomere sequences.
- These telomere sequences do not code anything but are essential to fill in the gap in the daughter strand and maintain the integrity of DNA.
- Eventually, the replication forks terminate at terminating recognizing sequences (ter).
- The ter sequences are of around 23 base pairs which facilitate as the binding sites for TUS protein.
- This ter- TUS complex arrest replication fork and terminate.



Q.4. classical concept of Gene

Classical Concept of Gene

Nature of Gene:

The characteristic of every organism is governed by factors of inheritance termed as genes. As centuries have passed the scientists have learned more and more about the nature of inheritance, and our concept of gene has undergone a remarkable evolution.

Definition 1860s-1900s: Gene as a Discrete Unit of Heredity:

The concept of the “gene” has evolved and become more complex since it was first proposed. There are various definitions of the term, although common initial descriptions include the ability to determine a particular characteristic of an organism and the heritability of this

characteristic. In particular, the word gene was first used by Wilhelm Johannsen in 1909, based on the concept developed by Gregor Mendel in 1866.

Definition 1910s: Gene as a Distinct Locus:

In the next major development, the American geneticist Thomas Hunt Morgan and his students were studying the segregation of mutations in Drosophila melanogaster. They were able to explain their data with a model that genes are arranged linearly, and their ability to crossover is proportional to the distance that separated them. The first genetic map was created in 1913 and Morgan and his students published The Mechanism of Mendelian Inheritance in 1915.

Definition 1940s: Gene as a Blueprint for a Protein:

Beadle and Tatum (1941), who studied Neurospora metabolism, discovered that mutations in genes could cause defects in steps in metabolic pathways. This was stated as the "one gene, one enzyme" view, which later became "one gene, one polypeptide."

1. One Gene-One Enzyme:

George Beadle and Edward Tatum conducted some experiments on Neurospora crassa. They saw that this pink bread mold can grow on medium containing only inorganic salts, a simple sugar, and one vitamin (biotin) and were called as minimal medium. This shows that these are capable of synthesizing all the other essential metabolites, such as purines, pyrimidine's, amino acids, and other vitamins, de novo required for the growth.

Furthermore, they found that the biosynthesis of these growth factors must be under genetic control. And mutations in genes whose products are involved in the biosynthesis of essential metabolites leads to produce mutant strains. These mutant strains require additional growth-factors.

Through this experiment they came to the conclusion that each mutation resulted in a requirement for one growth factor. By correlating their genetic analyses with biochemical studies of the mutant strains, they demonstrated in several cases that one mutation resulted in the loss of one enzyme activity.

This work, for which Beadle and Tatum received a Nobel Prize in 1958, was soon verified by similar studies of many other organisms in many laboratories. The one gene-one enzyme concept thus became a central part of molecular genetics.

2. One Gene-One Polypeptide:

Subsequent to the work of Beadle and Tatum, many enzymes and structural proteins were shown to be Heteromultimeric, that is, they contain two or more different polypeptide chains, with each

polypeptide encoded by a separate gene. For example, hemoglobin, which transport oxygen from our lungs to all other tissues of our bodies, are tetrameric proteins that contain two a-globin chains and two b-globin chains, as well as four oxygen-binding heme groups. Other enzymes, for example, E. coli DNA polymerase III and RNA polymerase II, contain many different polypeptide subunits, each encoded by a separate gene. Thus the one gene-one enzyme concept was modified to one gene-one polypeptide.

Definition 1950s: Gene as a Physical Molecule:

The fact that heredity has a physical, molecular basis was demonstrated by the observation that X rays could cause mutations. Griffith's (1928) demonstration that something in virulent but dead Pneumococcus strains could be taken up by live non-virulent Pneumococcus and transform them into virulent bacteria was further evidence in this direction. It was later shown that this substance could be destroyed by the enzyme Dnase (Avery et al. 1944).

In 1955, Hershey and Chase established that the substance actually transmitted by bacteriophage to their progeny is DNA and not protein. A practical view of the gene was that of the cistron, a region of DNA that in Trans could not genetically complement each other.

S. Benzer (1957) coined different terms for different nature of gene and genetic material

in relation to the chromosome on the basis of genetic phenomena to which they involve.

i) Genes as unit of transmission or cistron
ii) Genes as unit of recombination or recon
iii) Gene as unit of mutation or muton

• Benzer, in view of his work, coined the terms cistron (unit of function), recon (unit of recombination) and muton (unit of mutation).

CISTRON:

It is the largest element in a gene which encodes a polypeptide during protein synthesis. The term "cistron" refers to a test called cis-trans test, which is similar to a complementation test.

The term "cistron" is a unit of function. It is the unit of Function. It is the Gene in real sense capable of synthesizing a Polypeptide chain of an Enzyme.

RECON:

It is the smallest unit of DNA capable of undergoing Crossing Over and Recombination.

They are locations within a gene which participate in recombination. There is a minimum

distance between recons within a gene, and recombination cannot occur within a recon. The

term “recon” is a unit of recombination. The smallest segment of DNA capable of being separated and exchange with other chromosome is called recon. A recon consists of not more than two pairs of nucleotides.

MUTON:

It is the smallest unit of DNA which can undergo Mutation. They are elements within a gene that can undergo a mutation and lead to the production of mutant phenotype. The term “muton” is a unit of mutation. Each recon may have several mutons within them, and many mutons within a recon may stay linked because of recombination.Thus, a gene may have one more than one cistrons, a cistron may have several recons, and a recon may have several mutons.

EXONS AND INTRONS:

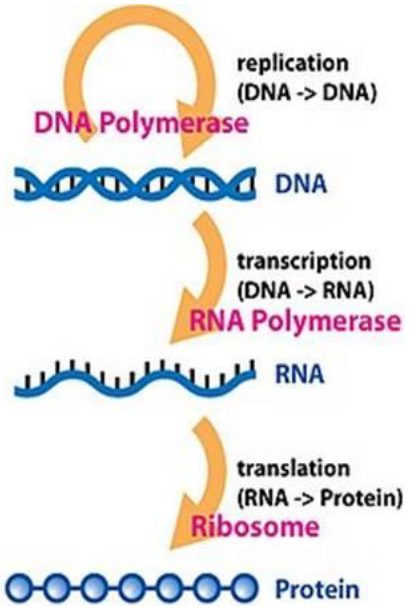
Exons are segments of a gene that encode mature mRNA for a specific polypeptide chain.

Introns are segments of a gene that do not encode mature mRNA. Introns are found in most genes in eukaryotes and in some gene of bacteriophage and archae .

Definition 1960s: Gene as Transcribed Code:

It was the solution of the three-dimensional structure of DNA by Watson and Crick in 1953 that explained how DNA could function as the molecule of heredity. Base pairing explained how genetic information could be copied, and the existence of two strands explained how occasional errors in replication could lead to a mutation in one of the daughter copies of the DNA molecule. From the 1960s on, molecular biology developed at a rapid pace.

The RNA transcript of the protein-coding sequences was translated using the genetic code (solved in 1965 by Nirenberg and co-workers into an amino acid sequence. Francis Crick (1958) summarized the flow of information in gene expression (“Central Dogma”).



Q. 5: Modern concept of gene

Modern Concept of Gene A gene can be described as a polynucleotide chain, which is a segment of DNA. It is a functional unit controlling a particular trait such as eye colour.

Beadle and Tatum concluded by various experiments that a gene is a segment of DNA that codes for one enzyme. They proposed one gene-one enzyme hypothesis. But as some genes code for proteins that are not enzymes, the definition of gene was changed to one gene-one protein hypothesis. Protein Hypothesis The concept of gene has undergone further changes as the new facts came to light. Since proteins are polypeptide chains of amino acids translated by mRNA, gene was defined as one gene-one polypeptide relationship. Some proteins have two or more different kinds of polypeptide chains, each with a different amino acid sequence. They are products of different genes. For example, hemoglobin has two kinds of chains a andβ chains, which differ in amino acid sequence and length. They are encoded by different genes. Thus, gene is defined as one gene one polypeptide relationship.

Structural and Regulatory Genes

Even the one gene-one polypeptide definition is not complete as it does not include gene which codes for rRNA and tRNA. Only mRNA is translated into proteins. Therefore genes which code for polypeptides and RNAs are called structural genes. In addition to structural genes, DNA also contains some sequences that have only regulatory function. These regulatory genes constitute signals, which “turn on” and “turn off” the transcription of structural genes and perform various other regulatory functions. In this way the definition of gene includes structural genes as well as regulatory genes.

Benzer coined terms for the gene; they are Cistron which is the unit of function, Recon which is the unit of recombination and Muton which is the unit of mutation.

Molecular Definition of a Gene

According to Lodish and others, gene is defined as the entire nucleic acid sequence that is necessary for the synthesis of a functional gene product, which may be polypeptide or any type of RNA.

In addition to structural genes (coding genes) it also includes all the control sequences and non-coding introns. Most prokaryotic genes transcribe polycistronic mRNA and most eukaryotic genes transcribe monocistronic mRNA.

Number of Genes on a Single Chromosome Total number of genes on a single chromosome is different in different organisms. Bacteriophage virus R17 consists of only three genes, SV40 consists of 5-10 genes. E. coli bacteria have more than 3000 genes on single 1 mm long chromosome. Size of a Gene In E. coli there are more than four million pairs of nucleotides (4638858 base pairs). It has been estimated that there are about 3000 genes in E. coli. The minimum size of a gene that encodes a protein can be directly estimated. Each amino acid of a polypeptide chain is encoded by a sequence of three consecutive nucleotides in a single strand of DNA. Therefore by measuring the size of the polypeptide chain, the size of a gene can be directly measured. The average polypeptide chain has about 450 amino acids, which are encoded by 1350 nucleotides. Therefore, in E. coli the number of genes will be around 3000 (4000000/1350=3000). Human genome contains about 30000 genes. A single copy of chromosome is composed of more than 3 billion base pairs. Coding regions of these genes take up only 3% of the genome.

Fine Structure of a Gene

A gene is present only in one strand of DNA, which is a double stranded helix. A gene consists of several different regions. The main region is the coding sequence which carries information regarding amino acid sequence of polypeptides.

The region on the left side of coding sequence (upstream or minus region) and on the right side (downstream or plus region) consists of fairly fixed regulatory sequences.

Regulatory sequences consist of promoters which are different in prokaryotes and eukaryotes. Types of Genes 1. Simple Genes: Simple genes have a coding sequence of bases in one DNA strand.

Upstream the coding region, the promoter is present. Downstream, the termination region is present.

2. Split Genes:

In most of eukaryotes, many non-coding sequences are present between coding sequences. The coding sequences of DNA of the genes are called exons. In between 2 exons are present non-coding sequences called introns. Exons alternate with introns.

Introns do not possess any genetic information and are not translated. Such genes are called split genes or interrupted genes.

Fig. Splicing

The mRNA transcribed from this DNA is called precursor mRNA (pre-mRNA) and contains exons as well as introns. The introns are removed by excision and discarded. This process is known as splicing. The remaining segments or exons are joined together to form the mature mRNA which takes part in translation. The mature mRNA is much smaller than the pre-mRNA for example α -globn has two introns, ovalbumin has seven introns and α-collagen has 52 introns.

3. Overlapping Genes:

Most genes consist of DNA sequences that code for one protein. But there are some sequences that code for more than one protein. Fredrick Sanger discovered this phenomenon in bacteriophage φ x 174. Overlapping genes are common in many viruses. Here the small length of viral DNA is exploited for synthesising different proteins. This is achieved in different ways. In some cases, one gene generates two proteins by having different starting points. Similarly, the same gene generates two proteins by terminating the expression at different points.

4. Jumping Genes or Transposons:

Earlier it was thought that genes are static and have definite and fixed locus. However, recently it has been discovered that segments of DNA can jump to new locations in the same or different chromosome. First of all it was discovered by Barbara Mc Clintock in Indian maize corn. These mobile genes are called transposable elements or transposons. They can jump within the genome, thus affecting the gene expression. Transposable elements are components of moderately repetitive class of DNA.

5. Variable Genes: Certain polypeptides are coded not by one gene but they are coded by more than one gene present on the same or different chromosomes.

Open Reading Frame

A gene is a segment of genome which is transcribed into RNA. If the RNA is a transcript of a protein coding gene then it is called messenger RNA or mRNA. The part of the protein coding gene which is translated into protein is called open reading frame. It has triplet nucleotide codons.

Open reading frame starts with an initiation codon and ends with a termination codon. The region of DNA before a gene is called an up-stream region denoted with a minus (-) sign while region after the gene is called downstream denoted with a plus (+) sign.

Q.6: Mechanism of Transcription

Transcription is the process by which the information in a strand of **DNA** is copied into a new molecule of messenger **RNA** (mRNA). In prokaryotic organisms transcription occurs in three phases known as initiation, elongation and termination.

Enzymes:

RNA is synthesized by a single RNA polymerase enzyme which contains multiple polypeptide subunits. In *E. coli*, the RNA polymerase has subunits: two α, one β, one β' and one ω and σ subunit (α2ββ'ωσ). This complete enzyme is called as the holoenzyme.

The σ subunit may dissociate from the other subunits to leave a form known as the core enzyme.

Initiation Phase:
During initiation, RNA polymerase recognizes a specific site on the DNA, upstream from the gene that will be transcribed, called a **promoter site** and then unwinds the DNA locally.

Promoter and Initiation:

The holoenzyme binds to a promoter region about 40–60 bp in size and then initiates transcription a short distance downstream (i.e. 3 to the promoter).

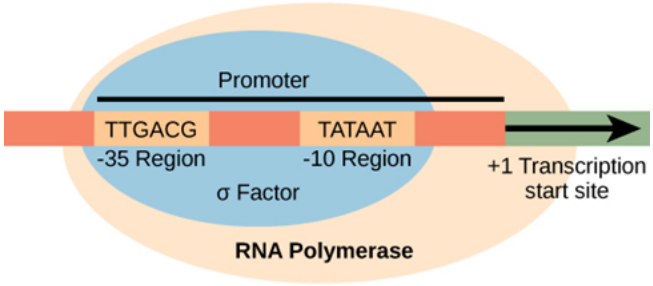
Within the promoter lie two 6 base pair sequences that are particularly important for promoter function.They are highly conserved between species.

Using the convention of calling the first nucleotide of a transcribed sequence as +1, these two promoter elements lie at positions –10 and –35, that is about 10 and 35 bp, respectively, upstream of where transcription will begin.

The –10 sequence has the consensus Because this element was discovered by Pribnow, it is also known as the Pribnow box. It is an important recognition site that interacts with the σ factor of RNA polymerase.

The –35 sequence has the consensus **TTGACA** and is important in DNA unwinding during transcriptional initiation.

RNA polymerase does not need a primer to begin transcription; having bound to the promoter site, the RNA polymerase begins transcription directly.



ELONGATION:

After transcription initiation, the σ factor is released from the transcriptional complex to leave the core enzyme (α2 ββω) which continues elongation of the RNA transcript.

The core enzyme contains the catalytic site for polymerization, within the β subunit.

The RNA polymerase then synthesizes RNA in the 5' →3' direction, using the four ribonucleoside 5-triphosphates (ATP, CTP, GTP, UTP) as precursors.

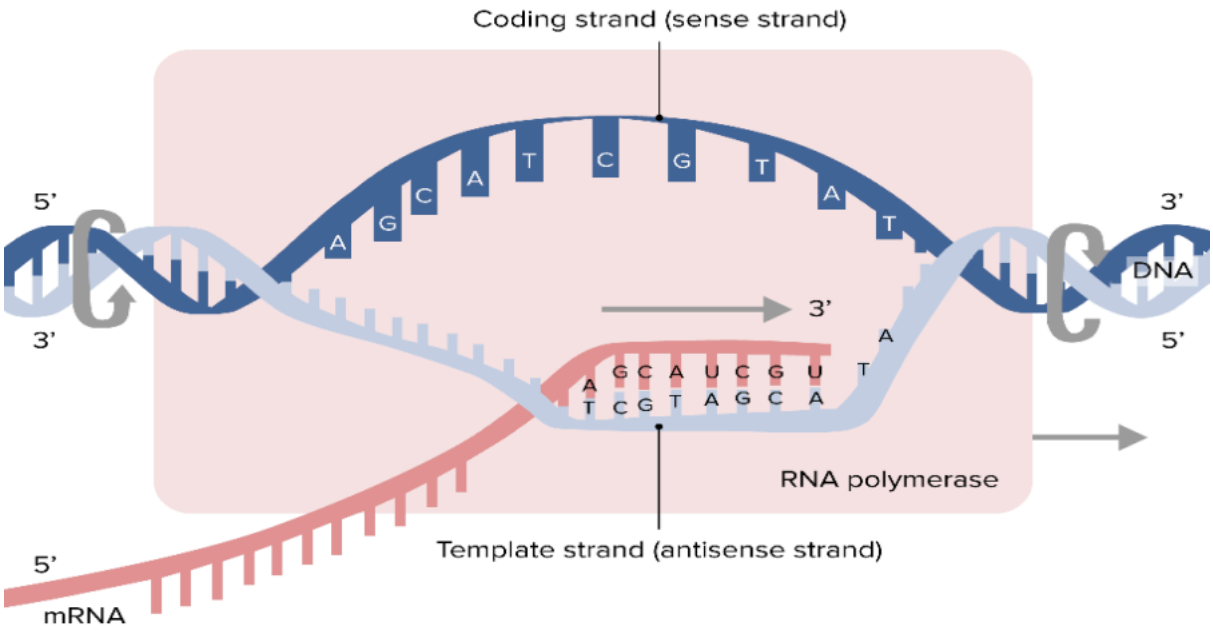
The 3-OH at the end of the growing RNA chain attacks the phosphate group of the incoming ribonucleoside 5-triphosphate to form a 3'5′ phosphodiester bond.

The DNA is unwound ahead of the transcription bubble and after the transcription complex has passed, the DNA rewinds.

Thus, during the elongation, the RNA polymerase uses the antisense (-) strand of DNA as template and synthesizes a complementary RNA molecule.

The RNA produced has the same sequence as the non-template strand, called the sense (+) strand (or coding strand) except that the RNA contains U instead of T.

The correct strand to be used as template is identified for the RNA polymerase by the presence of the promoter site.



TERMINATION:

There are two mechanism of termination.

1. Rho independent:

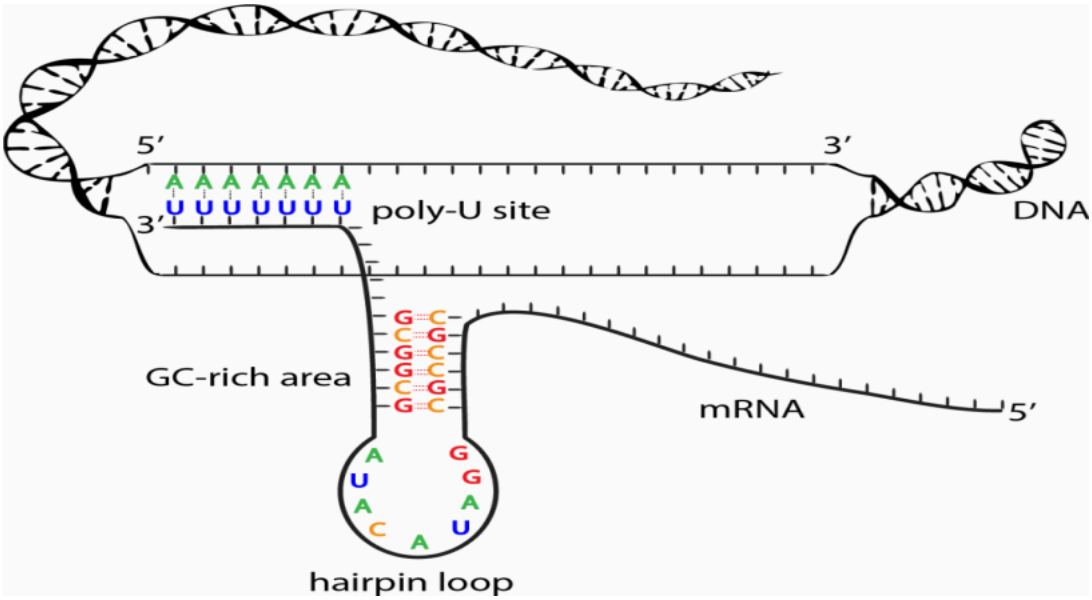
In this mechanism, transcription is terminated due to specific sequence in terminator DNA.

The terminator DNA contains GC rich sequence when RNA polymerase transcribe this sequence RNA get GC rich sequence which is complementary this cause RNA transcript to form hair pin like structure

The GC sequence is followed by larger number of AAAAAAA on template DNA.

When RNA polymerase transcribe this sequence the uracil appear in RNA.

The load of hair pin structure is not tolerated by A=U base pair so the RNA get separated from RNA-DNA hybrid

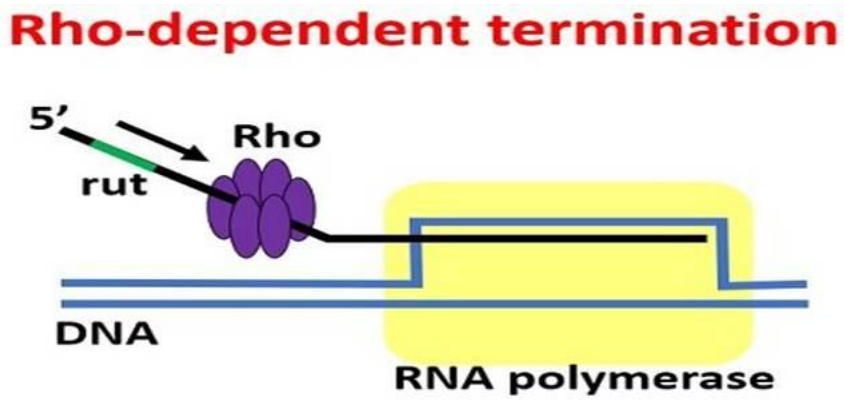


2. **Rho dependent:**

In this mechanism, transcription is terminated by rho (ρ) protein, It is ring shaped protein.

The rho protein bind the single stranded RNA and starts "climbing" up the transcript towards RNA polymerase.

When it reaches the transcription bubble, Rho pulls the RNA transcript and the template DNA strand apart, releasing the RNA molecule and ending transcription.



RNA processing:

In prokaryotes, RNA transcribed from protein-coding genes (messenger RNA, mRNA), requires little or no modification prior to translation. Many mRNA molecules begin to be translated even before RNA synthesis has finished.

Q.7: salient features of Genetic code

Genetic code

The **genetic code** consists of the sequence of nitrogen bases—A, C, G, U—in an mRNA chain. The four bases make up the "letters" of the genetic code. The letters are combined in groups of three to form code "words," called **codons**. Each codon stands for (encodes) one amino acid, unless it codes for a start or stop signal.

There are 20 common amino acids in proteins. There are 64 possible codons, more than enough to code for the 20 amino acids. The genetic code is shown in **Figure** below

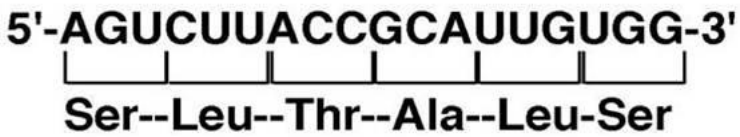
1st letter	Second Letter: U	Second Letter: C	Second Letter: A	Second Letter: G	3rd letter
U	UUU Phe UUC UUA Leu UUG	UCU UCC UCA UCG Ser	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G
C	CUU CUC CUA CUG Leu	CCU CCC CCA CCG Pro	CAU His CAC CAA Gln CAG	CGU CGC CGA CGG Arg	U C A G
A	AUU AUC AUA Ile AUG Met	ACU ACC ACA ACG Thr	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G
G	GUU GUC GUA GUG Val	GCU GCC GCA GCG Ala	GAU Asp GAC GAA Glu GAG	GGU GGC GGA GGG Gly	U C A G

Reading the Genetic Code

The codon AUG codes for the amino acid methionine. This codon is also the **start codon** that begins translation.

The **reading frame** is the way the letters are divided into codons. After the AUG start codon, the next three letters are read as the second codon. The next three letters after that are read as the third codon, and so on.

The mRNA molecule is read, codon by codon, until a **stop codon** is reached. UAG, UGA, and UAA are all stop codons. They do not code for any amino acids. Stop codons are also known as termination codons.



Reading frame: -

Properties of the Genetic Code:

(i) Triplet nature of the code. The codons for different amino acids, as well as those for chain termination, always consist of three successive nucleotides of DNA or m-RNA. The DNA and m-RNA codons are mutually complementary. For example, the DNA codon CGT is complementary to the m-RNA codon GCU (α-alanine).

(ii) Unambiguous nature of the code. One particular codon never codes for more than one amino acid.

(iii) Degeneracy of the genetic code. Most of the amino acids — except methionine and tryptophan — are coded by more than one codon. The number varies between 2 and 6.

(iv) The genetic code is non-overlapping and unpunctuated. This means that a sequence of nucleotides in m-RNA is read in groups of three without overlapping, and also without any gaps between successive triplets.

(v) The genetic code is non-overlapping and unpunctuated. This means that a sequence of nucleotides in m-RNA is read in groups of three without overlapping, and also without any gaps between successive triplets.

Wobble 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 Wobble Hypothesis, only the first two bases of the codon have a precise pairing with the bases of the anticodon of tRNA, while the pairing

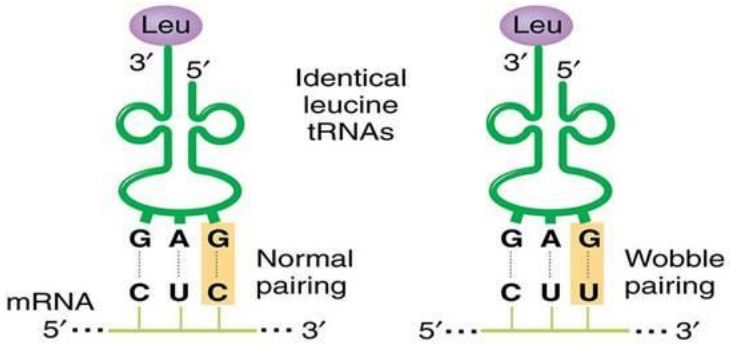
between the third bases of codon and anticodon may Wobble (wobble means move unsteadily). It states that

The first two bases of the codon make normal (canonical) H-bond pairs with the 2nd and 3rd bases of the anticodon.

At the remaining position, less stringent rules apply and non-canonical pairing may occur.

The relaxed base-pairing requirement, or "wobble," allows the anticodon of a single form of tRNA to pair with more than one triplet in mRNA.

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.



Q.8: TRANSLATION / PROTEIN SYNTHESIS

Protein Synthesis is a process of synthesizing proteins in a chain of amino acids known as polypeptides. It is the second part of the central dogma in genetics.

- It takes place in the ribosomes found in the cytosol or those attached to the rough endoplasmic reticulum.
- The functions of the ribosome are to read the sequence of the codons in mRNA and the tRNA molecules that transfer or transport or bring the amino acids to the ribosomes in the correct sequence.
- The translation process involves reading the genetic code in mRNA to make proteins.
- The entire translation process can be summarized into three phases: Initiation, elongation, and termination.

Important components of Translation:

Ribosomes

- Ribosomes are made of ribosomal RNA (rRNA) and proteins.The rRNA has the peptidyl transferase activity that bonds the amino acids.
- The ribosomes have two subunits of rRNA and proteins, a large subunit with three active sites (E, P, A) which are critical for the catalytic activity of ribosomes.

Transfer RNA (tRNA)

- Each tRNA has an anticodon for the amino acid codon it carries which are complementary to each other. For example; Lysine is coded by AAG, and therefore the anticodon that will be carried by tRNA will be UUC, therefore when the codon AAG appears, an anticodon UUC of tRNA will bind to it temporarily.

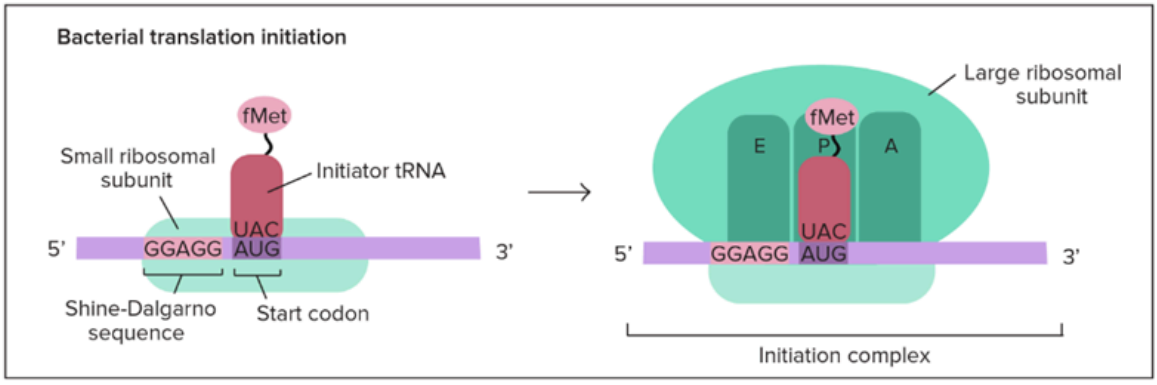
Enzymes:

- Peptidyl transferase is the main enzyme used in Translation. It is found in the ribosomes with an enzymatic activity that catalyzes the formation of a covalent peptide bond between the adjacent amino acids.
- In prokaryotes, the 23S subunit contains the peptidyl transferase between the A-site and the O-site of tRNA while in eukaryotes, it is found in the 28S subunit.

Overview of Protein synthesis:

Initiation

- Protein synthesis initiation is triggered by the presence of several initiation factors IF1, IF2, and IF3, including mRNA, ribosomes, tRNA.
- The small subunit binds to the upstream on the 5′ end at the start of mRNA. The ribosome scans the mRNA in the 5′ to 3′ direction until it encounters the start codon (AUG or GUG or UUG). When either of these start codons is present, it is recognized by the initiator fMet-tRNA (N-formylMet-tRNA). This initiator factor carries the methionine (Met) which binds to the P site on the ribosome.
- This synthesizes the first amino acid polypeptide known as N-formylmethionine. The initiator fMet-tRNA has a normal methionine anticodon therefore it inserts the N-formylmethionine. This means that methionine is the first amino acid that is added and appears in the chain.



Elongation

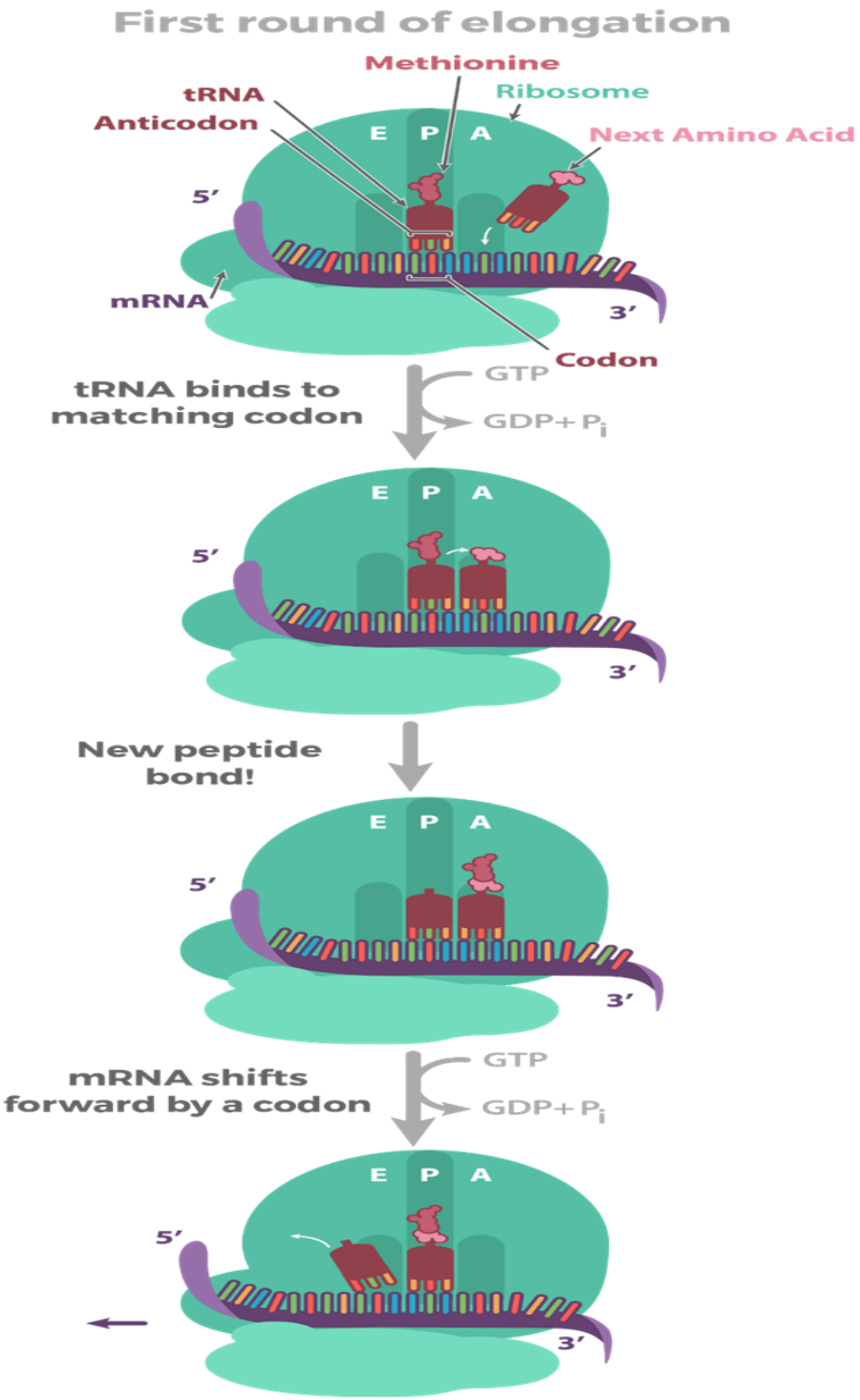
- The elongation of protein synthesis is aided by three protein factors i.e EF-Tu, EF-Ts, and EF-G.
- The ribosomal function is known to shift one codon at a time, catalyzing the processes that take place in its three sites.
- For every step, a charged tRNA enters the ribosomal complex and inserts the polypeptides that become one amino acid longer, while an uncharged tRNA departs.
- The bond created between each amino acid is derived from the Guanosine Triphosphate (GTP).
- The three sites (A, P, E) all participate in the translation process, and the ribosome itself interacts with all the RNA types involved in translation.

Termination

- Termination of the translation process is triggered by an encounter of any of the three stop codons (UAA, UAG, UGA). These triplet stop codons, however, are not recognized by the tRNA but by protein factors known as the release factors, (RF1 and RF2) found in the ribosomes.
- The RF1 recognizes the triplet UAA and UAG while RF2 recognizes UAA and UGA. A third factor also assists in catalyzing the termination process and it's known as Release factor 3 (RF3).
- When the peptidyl-tRNA from the elongation step arrives at the P site, the release factor of the stop codon binds to the A site. These releases the polypeptide from the

P site allowing the ribosomes to dissociate into two subunits by the energy derived from GTP, leaving the mRNA.

- After many ribosomes have completed the translation process, the mRNA is degraded allowing its nucleotides to be reused in other transcription reactions.



Q.9: GENE REGULATION/ LAC OPERON

Lac operon

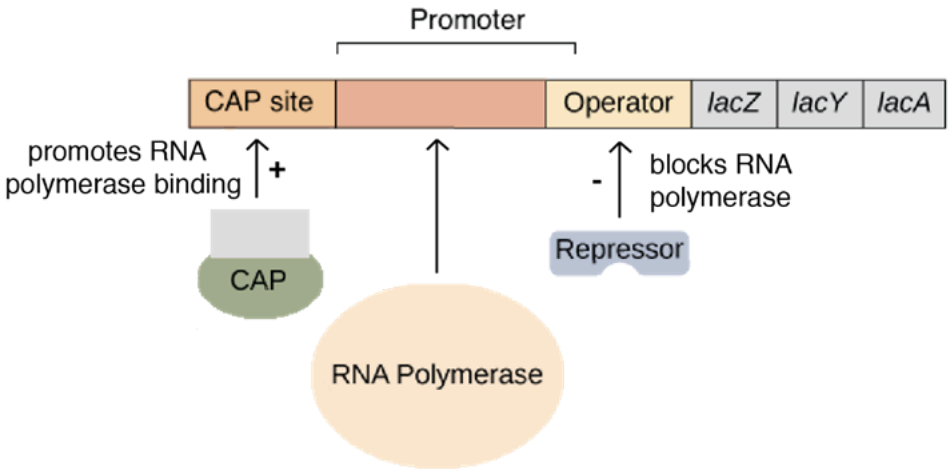
Lactose or lac operon of *Escherichia coli* is a cluster of three structural genes encoding proteins involved in lactose metabolism and the sites on the **DNA** involved in the regulation of the operon. It was Jacob and Monod in 1961 who proposed the operon model for the regulation of transcription.

The operon model proposes three elements:
A set of structural genes (i.e. genes encoding the proteins to be regulated);
An operator site, which is a DNA sequence that regulates transcription of the structural genes;
A regulator gene which encodes a protein that recognizes the operator sequence.

Lac operon codes for key enzymes involved in lactose metabolism:

1. **Lac-Z: Galactoside permease** (also known as lactose permease).Transports lactose into the cell across the cell membrane.
2. **Lac-Y: Galactosidase:** Hydrolyzes lactose to glucose and galactose.
3. **Thiogalactoside transacetylase- Lac A**

The *lac* operon:



Inducers and the Induction of Lac operon

Normally, E. coli cells make very little of any of these three proteins but when lactose is available it, causes a large and coordinated increase in the amount of each enzyme. Thus each enzyme is an inducible enzyme and the process is called induction.

The mechanism is that the few molecules of ß-galactosidase in the cell before induction convert the lactose to allolactose which then turns on the transcription of these three genes in the lac operon. Thus allolactose is an inducer.

Lac Operon in the absence of Inducer

In the absence of an inducer such as allolactose the lacI gene is transcribed and the resulting repressor protein binds to the operator site of the lac operon, (O), and prevents transcription of the lacZ, lacY and lacA genes.

Lac Operon in the presence of Inducer

During induction, the inducer binds to the repressor.

- This causes a change in the conformation of the repressor that greatly reduces its affinity for the lac operator site.
- The lac repressor now dissociates from the operator site and allows the RNA polymerase to begin transcribing the lacZ, lacY and lacA genes.
- They are transcribed to yield a single polycistronic mRNA that is then translated to produce all three enzymes in large amounts.
- The existence of a polycistronic mRNA ensures that the amounts of all three gene products are regulated coordinately.
- If the inducer is removed, the lac repressor rapidly binds to the lac operator site and transcription is inhibited almost immediately.

CRP/CAP regulation:

- High-level transcription of the lac operon requires the presence of a specific activator protein called catabolite activator protein (CAP), also called cAMP receptor protein (CRP).
- This protein, which is a dimer, cannot bind to DNA unless it is complexed with 3’5′ cyclic AMP (cAMP).
- The CRP–cAMP complex binds to the lac promoter just upstream from the binding site for RNA polymerase.
- It increases the binding of RNA polymerase and so stimulates transcription of the lac operon.
- Whether or not the CRP protein is able to bind to the lac promoter depends on the carbon source available to the bacterium.

Lac operon in the presence of Glucose:

When glucose is present, *E. coli* does not need to use lactose as a carbon source and so the lac operon does not need to be active. Thus the system has evolved to be responsive to glucose.

Glucose inhibits adenylate cyclase, the enzyme that synthesizes cAMP from ATP.

Thus, in the presence of glucose the intracellular level of cAMP falls, so CRP cannot bind to the lac promoter, and the lac operon is only weakly active (even in the presence of lactose).

Lac operon in the absence of Glucose:

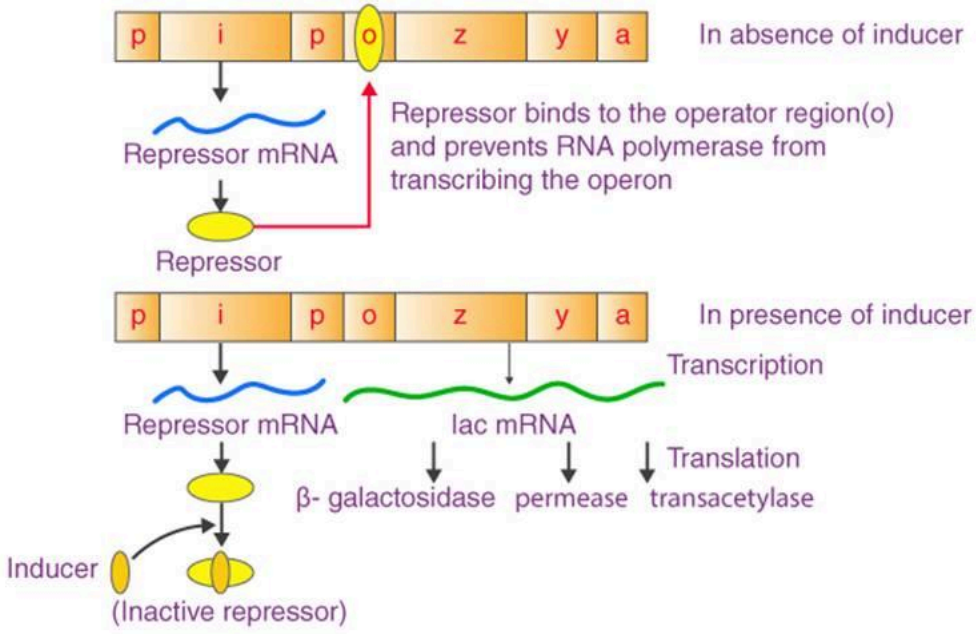
When glucose is absent, adenylate cyclase is not inhibited, the level of intracellular cAMP rises and binds to CRP.

Therefore, when glucose is absent but lactose is present, the CRP–cAMP complex stimulates transcription of the lac operon and allows the lactose to be used as an alternative carbon source.

In the absence of lactose, the lac repressor, of course, ensures that the lac operon remains inactive.

These combined controls ensure that the lacZ, lacY and lacA genes are transcribed strongly only if glucose is absent and lactose is present.

Regulation in Lac Operon:



Q.10; Define Mutations. Describe different types of physical and chemical mutagens

Physical and chemical mutagens

A mutagen is a physical or chemical agent that can cause mutations in DNA and raises their frequency above natural background levels.

They may directly damage DNA due to their actions, which most frequently leads to replication errors.

Mutations occur randomly, i.e., not directed according to the organism's requirements. Environmental factors cause most mutations, but they can also be created in the lab using chemicals, radiation, or physical stimuli.

Mutagen types: Mutagens can be classified into three major types based on their origin. They are:

1. Physical mutagen
2. Chemical mutagen
3. Biological mutagen

I.**PHYSICAL AGENTS**:

Physical Agents: **Ionizing radiations:** X-rays and gamma rays; alpha and beta rays; electrons, neutrons, protons, and other fast-moving particles. Ionizing radiation causes chromosomal changes such break, deletion, addition, inversion, and translocation by damaging the poly sugar-phosphate backbone of DNA.

Non-ionizing radiations

Ultraviolet (UV) light is a non-ionizing radiation that cause mutation. It cause the dimerization of pyrimidines by forming covalent bonds between adjacent pyrimidine bases. This particular form of pyrimidine dimerization alters the DNA structure, preventing the creation of the replication fork and potentially leading to mutations and cell death.

II. CHEMICAL MUTAGENS can be classified into different categories, such as:

1. Base analogues
2. Intercalating agents
3. Hydroxylating agents
4. Alkylating agents
5. Deaminating Agents

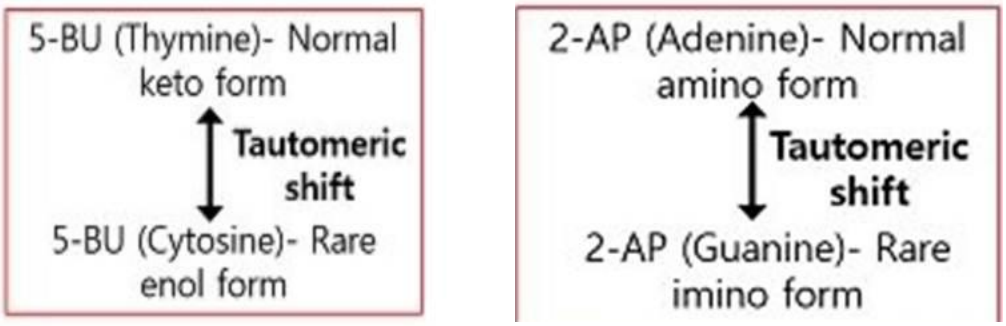
A. Base analogues

These substances have structural characteristics with bases like purines and pyrimidines.

5-Bromouracil and aminopurine are the two most prevalent base analogs that are considered to be chemical mutagens.

Base analogs are integrated into the DNA structure during replication because of the structural resemblances between these agents and DNA bases.

Like adenine, aminopurine can pair up with either C or T to form a base pair (though base pairing with C is rare).

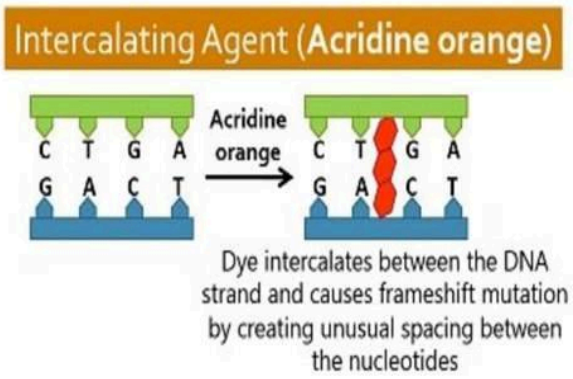


B. Intercalating agents

Intercalating molecules have a hydrophobic heterocyclic ring structure that resembles the base pair ring structure.

These agents embed themselves in the DNA helix, causing interference with transcription, replication, and mutation, most frequently a frameshift mutation.

Some of the common intercalating agents are: Ethidium bromide, Proflavine, Acridine orange, Actinomycin D , Daunorubicin.



C. Deaminating Agents

These occur in bases those possess **exocyclic amino group** in an order adenine, cytosine and guanine. A common deaminating agent, i.e. **nitrous acid** (HNO_2), replaces an $-NH_2$ or amino group with an –OH or ether group, thereby altering the **DNA bare pairing**.

It deaminates **adenine** into **hypoxanthine**, which forms complementary pairing with cytosine in place of thymine. It causes AT to GC mutations.

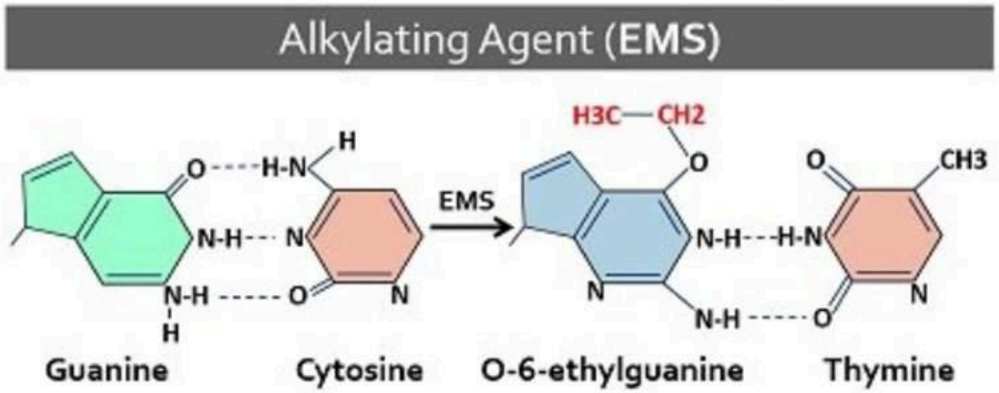
HNO2 converts **cytosine** into **uracil**, which forms complementary pairing with adenine instead of guanine. Therefore, it results in GC to UA mutations.

D. Alkylating agents

These chemicals cause DNA damage by inducing alkyl groups.

Alkyl group introduction boosts ionization, leading to base-pairing mistakes that eventually cause holes in the DNA strand and have a direct mutagenic effect on the DNA molecule.

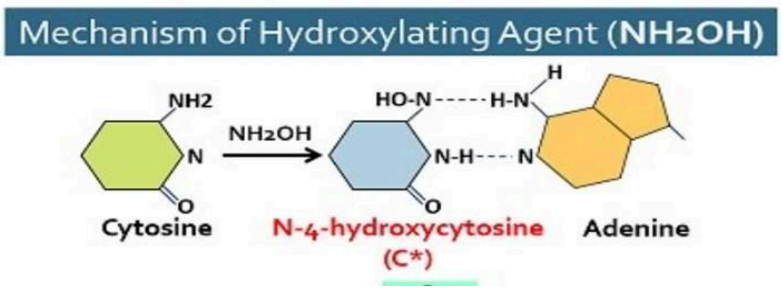
Some of the common alkylating agents are: Nitrous acid, Ethyl nitrosourea, Methylhydrazine, Vinyl chloride, Epoxides, Dimethyl and diethyl sulphonate, Methyl and ethyl methane sulphonate (MMS and EMS) ect.,



However, these substances can be eliminated from the DNA by the depurination process during the DNA repair process.

E. Hydroxylating agents:

They hydroxylate the C-4 nitrogen of cytosine. Hydroxylamine or NH2OH modifies **cytosine** into **hydroxycytosine** by adding OH. Then, hydroxycytosine pairs with adenine instead of guanine and causes GC→AT type of transition mutations.



Q. 11. TYPES OF MUTATIONS

Any sudden heritable change is called mutation. Gene mutations are the changes that occur in one or more genes that can lead to harmful diseases or illnesses.

Types of Gene Mutations:

Mutations or changes in the polynucleotide sequence of the gene, which can lead to structural alterations in the functioning of the gene, are known as gene mutation.

Gene mutations are of the following types:

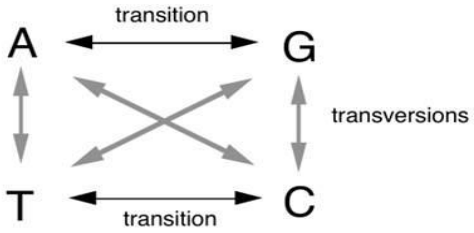
- Insertion: This type of gene mutation leads to the addition of a base in the gene sequence. This type is also more commonly known as addition mutation
- Inversion: This type of gene mutation is characterised by alteration in the gene sequence by the process of inversion when the gene sequence is pushed back into its original sequence
- Deletion: This type is self-explanatory; the base of a gene sequence gets deleted in these types of gene mutation
- Duplication: This gene mutation is characterised by duplicating the gene sequences
- Substitution: This type of gene mutation involves the replacement of certain bases of the gene sequence with other bases within the same gene
- Translocation: Movement of a gene may take place to a non-homologous chromosome and this is known as translocation.

Gene mutations at the molecular level: The two important types are

1. **Point mutation**
2. **Frame shift mutations**

Types of Point Mutations (Substitution) :

Nucleotide substitutions are one of two classes. In a transition, a purine nucleotide is replaced with a purine nucleotide, or a pyrimidine nucleotide is replaced with a pyrimidine nucleotide.In a transversion, a purine nucleotide is replaced with a pyrimidine nucleotide, or a pyrimidine nucleotide is replaced with a purine nucleotide.



A substitution mutation occurs when one base pair is substituted for another. For example, this would occur when one nucleotide containing cytosine is accidentally substituted for one containing guanine. There are three types of substitution mutations:

- Nonsense
- Missense
- Silent

A nonsense mutation occurs when one nucleotide is substituted and this leads to the formation of a stop codon instead of a codon that codes for an amino acid. A stop codon a certain sequence of bases (TAG, TAA, or TGA in DNA, and UAG, UAA, or UGA in RNA) that stops the production of the amino acid chain. It is always found at the end of the mRNA sequence, but if a substitution causes it to appear in another place, it will prematurely terminate the amino acid sequence and prevent the correct protein production.

Like a nonsense mutation, a missense mutation occurs when one nucleotide is substituted and a different codon is formed; but this time, the codon that forms is not a stop codon. Instead, the codon produces a different amino acid in the sequence of amino acids. For example, if a missense substitution changes a codon from AAG to AGG, the amino acid arginine will be produced instead of lysine.

In a silent mutation, a nucleotide is substituted but the same amino acid is produced anyway. This can occur because multiple codons can code for the same amino acid. For example, AAG and AAA both code for lysine, so if the G is changed to an A, the same amino acid will form and the protein will not be affected.

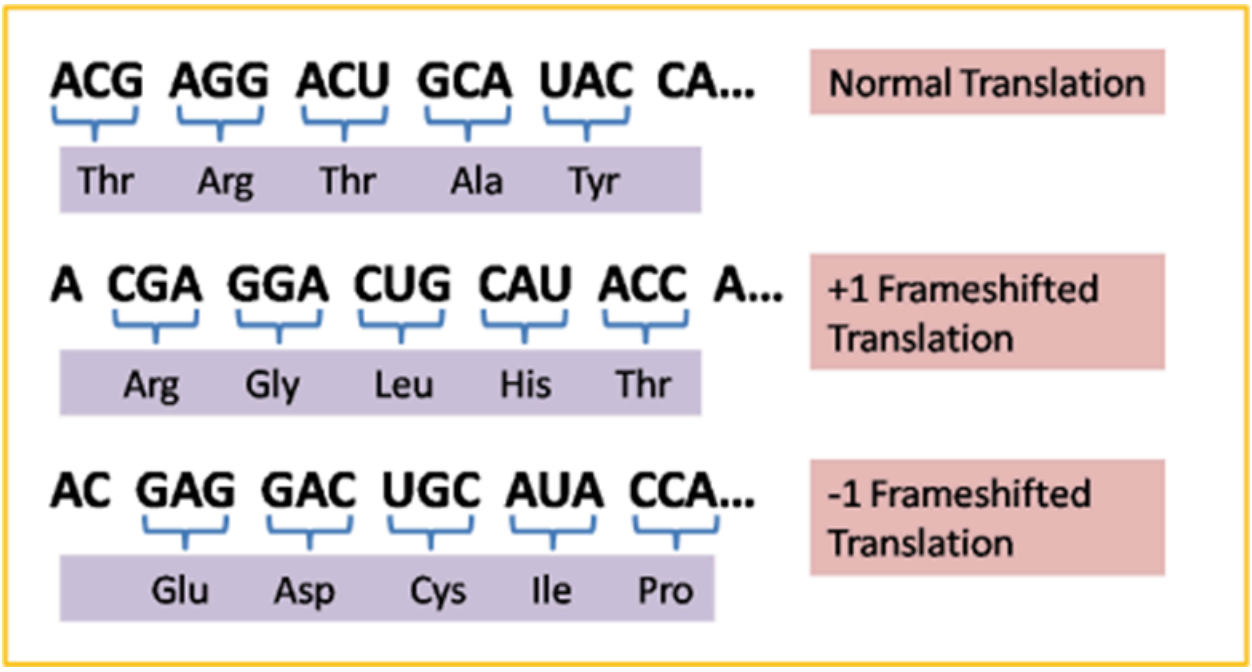
	No mutation	Point mutations			
		Silent	Nonsense	Missense	
				conservative	non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
protein level	Lys	Lys	STOP	Arg	Thr

Frameshift Mutations:

Insertion and Deletion

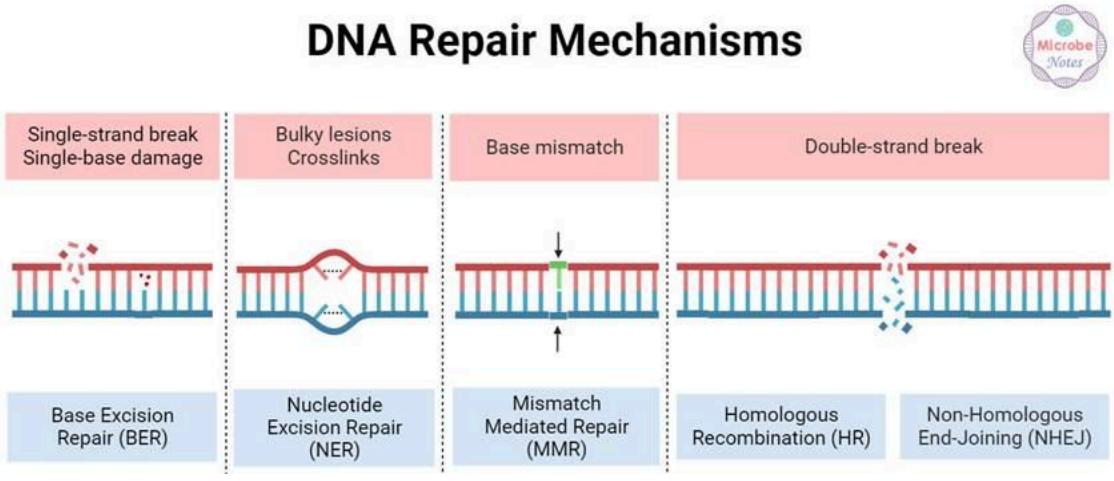
An insertion mutation occurs when an extra base pair is added to a sequence of bases. A deletion mutation is the opposite; it occurs when a base pair is deleted from a sequence. These two types of point mutations are grouped together because both of them can drastically affect the sequence

of amino acids produced. With one or two bases added or deleted, all of the three-base codons change. This is called a frameshift mutation.



Q.12:DNA Repair Mechanisms

DNA damage is a common event that can interfere with important cellular processes and lead to genomic defects and an increased risk of cancer. To ensure the integrity of their genomes, cells have evolved to develop mechanisms for DNA repair. These mechanisms help cells to cope with DNAdamage.



Various pathways exist for DNA repair. These include direct reversal, excision repair, mismatch repair, and repair of DNA breaks.

1. Direct reversal repair

Direct reversal repair is a DNA repair mechanism that directly fixes specific types of DNA damage without the need for excision or replacement.

Two examples of DNA damage that can be reversed are UV-induced lesions and alkylated bases. UV-induced lesions, caused by UV light, can be reversed through a process called photoreactivation, which uses visible light energy to break the damaged DNA structure, restoring the original pyrimidine bases.

2. Base excision repair (BER)

Base excision repair (BER) is a DNA repair mechanism that removes and replaces damaged bases. It involves the action of various DNA glycosylases. These enzymes recognize and remove damaged bases.

BER includes both short patch repair, where an abasic site is processed and filled by specific enzymes, and long patch repair, where gaps are tailored and DNA synthesis occurs followed by ligation.

3. Nucleotide excision repair

Nucleotide excision repair (NER) deals with large gaps and cross-linking gaps in DNA backbone caused by UV radiation or chemical exposure.
NER removes a fragment of nucleotides containing the damaged fragment and synthesizes a new DNA strand using the undamaged strand as a template.

NER consists of two pathways:

Global Genome NER (GG-NER) repairs bulky damages throughout the entire genome, including regions that are not actively transcribed.
Transcription-Coupled NER (TC-NER repairs damage that occurs on the transcribed DNA strand.
Mutations in NER pathway genes can lead to disorders such as xeroderma pigmentosum (XP) and certain other neurodegenerative conditions.

4. Mismatch repair

Mismatch repair (MMR) pathway repairs base mismatches and insertion-deletion loops that occur during replication. Most of these errors are fixed by the proofreading activity of DNA polymerase during replication, but some may be missed and need to be corrected later.

The MMR pathway involves three steps: recognition of mismatches, degradation of the error-containing strand, and synthesis of the correct DNA sequence.

Mutations in MMR genes can lead to Lynch syndrome, a hereditary condition associated with an increased risk of colon, ovarian, and other cancers.

5. Single-strand break repair (SSBR)

Single-stranded breaks (SSBs) in DNA can occur due to oxidative damage, abasic sites, or errors in the activity of the DNA topoisomerase enzyme.

These breaks can disrupt DNA replication, halt transcription, and activate cellular processes that can lead to cell death.

To protect the exposed single strand from breaking, PARP1 proteins coat the single strand and act as a shield.

SSBR can be accomplished through various pathways, including base excision repair, nucleotide excision repair, and mismatch repair.

6. Double-strand break repair

Double-strand breaks (DSBs) in DNA can be repaired through two pathways: homologous recombination (HR) and non-homologous end joining (NHEJ).

Homologous recombination (HR)

HR is a precise repair pathway that requires a matching DNA sequence as a template.

It primarily uses the sister chromatid, a copy of the damaged DNA, for repair.

HR is most active during the S, G2, and M phases of the cell cycle when sister chromatids are present.

Non-homologous end joining (NHEJ)

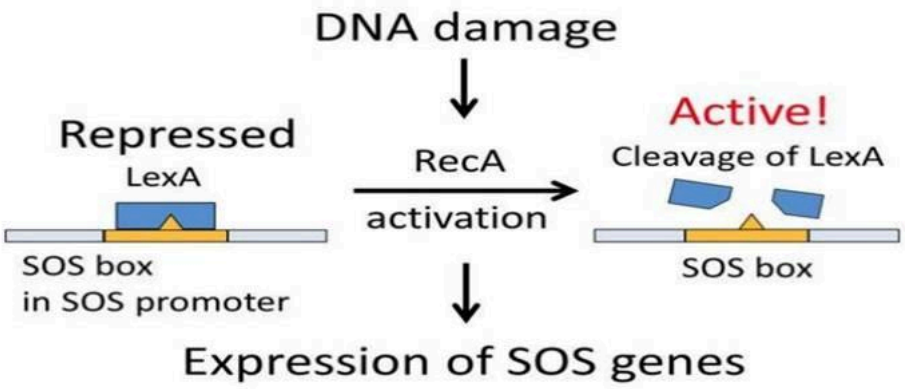
NHEJ is a simple and widely used mechanism that directly seals the broken ends of DNA without the need for a homologous DNA template. It can occur throughout the cell cycle.

SoS Repair:

The term “SOS repair” refers to a cellular response to UV damage. When bacterial cells suffer extensive damage to their DNA as a result of UV exposure, they turn on the coordinated expression of a large number of genes that are necessary for DNA repair.

These include the uvr genes needed for nucleotide excision repair and recA, which is involved in homologous recombination. In addition to these mechanisms, which can carry out error-free repair, the SOS response can also induce the expression of translesion polymerases encoded by the dinA, dinB and umuCD genes.

All of the genes induced in the SOS response are regulated by two components. The first is the presence of a short DNA sequence upstream of their coding region, called the SOS box. The second is a protein, the LexA repressor.



The genes controlled by the LexA repressor, as mentioned earlier, encode proteins that are necessary for accurate DNA repair as well as error-prone translesion synthesis. The various genes involved in DNA repair are induced in a specific order. In the initial stages, the repair genes that are derepressed are for nucleotide excision repair, followed by homologous recombination, both error-free mechanisms for repair. If the damage is too extensive to be repaired by these systems, error-prone repair mechanisms may be brought into play as a last resort.

Q.13. Differentiate F factor and Hfr strains. Explain the mechanism of Conjugation in Bacteria

Conjugation

Conjugation is the transfer of a plasmid or other self-transmissible DNA element and sometimes chromosomal DNA from a donor cell to a recipient cell via direct contact usually mediated by a conjugation pilus or sex pilus.

Recipients of the DNA transferred by conjugation are called transconjugants.

The process of conjugation can transfer DNA regions of hundreds to thousands of kilobases and has the broadest host range for DNA transfer among the methods for bacterial exchange.

Conjugation occurs in and between many species of bacteria, including Gram-negative as well as Gram-positive bacteria, and even occurs between bacteria and plants.

Broad-host-range conjugative plasmids have been used in molecular biology to introduce recombinant genes into bacterial species that are refractory to routine transformation or transduction methods.

The following process occurs during the transfer of F plasmid in *E. coli* by conjugation:

1. The F plasmid contains *tra* locus, which includes the *pilin* This gene, along with some regulatory proteins results in the formation of pilli on the F^+ cell surface.

2. The proteins present in the pilli attach themselves on the F^- cell surface. The pilli are responsible for making contact between the cells, but the transfer of plasmid doesn't occur through the pilli.

3. The *traD* enzyme, located at the base of the pilus, initiates membrane fusion.

4. Once the conjugation is initiated, enzyme relaxase creates a nick in the conjugative plasmid at the *oriT*

5. The nicked strand (called the T strand) then unwinds and is transferred to the recipient cell in the 5'-3' direction.

6. The complementary strand is synthesized in both cells; thus, both the donor and recipient are F^+.

7. In certain F^+ bacterial cells, the F element infrequently (about once in every 10,000 F^+ cells) becomes associated with the main bacterial chromosome in such a way that a copy of the chromosome instead is transferred through the conjugation tube from donor to recipient cell.

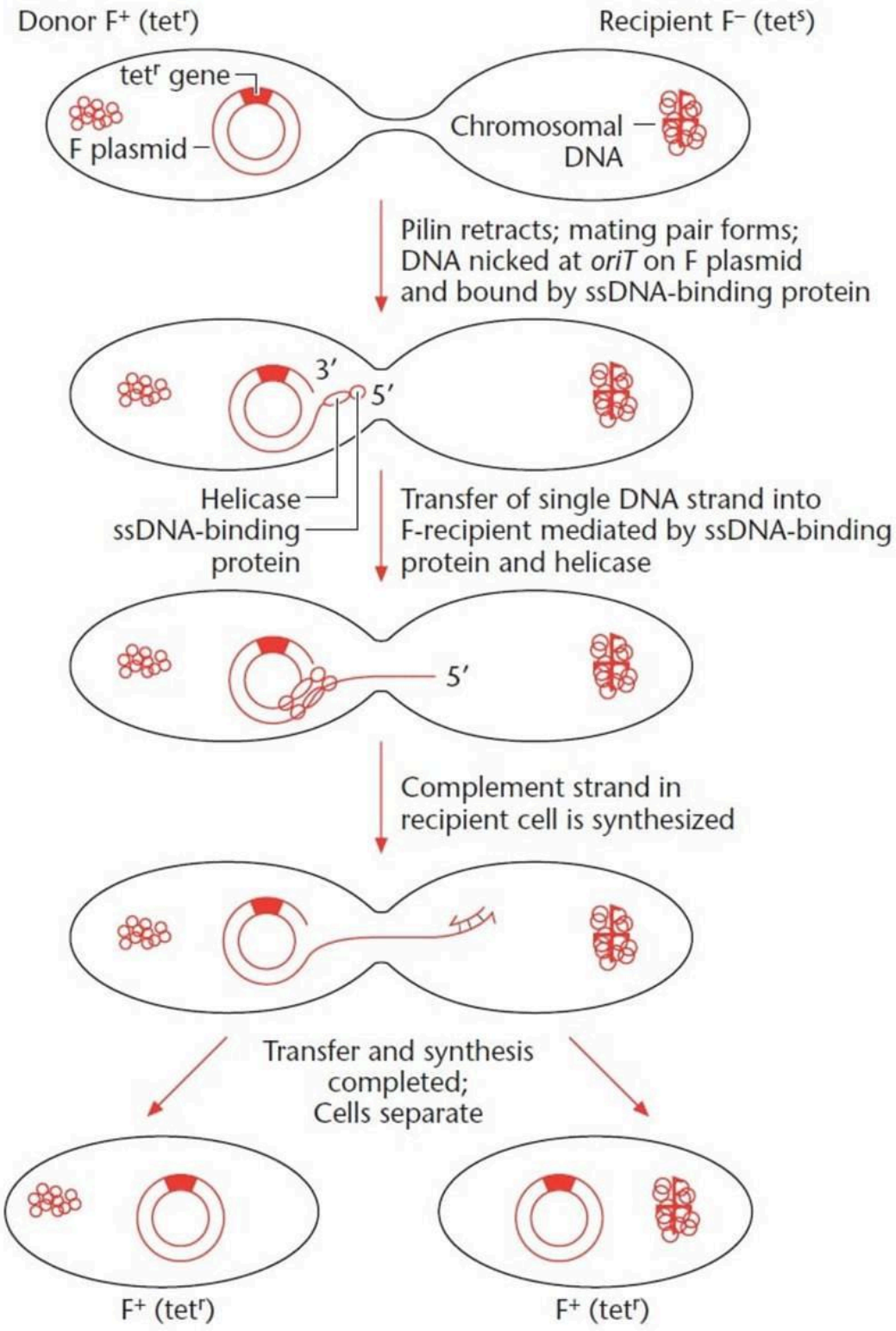
8. In the insertion process, the circular F element breaks at a particular point and becomes a linear segment of the bacterial chromosome.

9. An F^+ cell that carries such an integrated F element is known as an **Hfr cell** (Hfr stands for the *high frequency of recombination*).

10. The integrated F element of Hfr cells is ordinarily replicated passively along with the bacterial chromosome and in this way is transmitted from one Hfr generation to the next.

Applications;

Agrobacterium tumefaciens causes crown gall tumor in plants by transferring the T DNA element, a part of the *Ti* (tumor-inducing) plasmid present in this bacterium, into a plant cell where the T element becomes incorporated into the plant cell's genome.
Conjugative plasmids encoding antimicrobial resistance genes are called R plasmids which are transferred through *Shigella* spp that might result in a widespread outbreak of antibiotic-resistant *Shigella*-mediated dysentery.


Donor F+ (tetr)
Recipient F− (tets)
tetr gene
F plasmid
Chromosomal DNA
Pilin retracts; mating pair forms;
DNA nicked at oriT on F plasmid
and bound by ssDNA-binding protein
3′
5′
Helicase
ssDNA-binding protein
Transfer of single DNA strand into
F-recipient mediated by ssDNA-binding
protein and helicase
5′
Complement strand in
recipient cell is synthesized
Transfer and synthesis
completed;
Cells separate
F+ (tetr)
F+ (tetr)

Strains: Hfr

Hfr cell

1. When F-plasmid (sex factor) integrated with chromosomal DNA then such bacteria is known as **high frequency recombination (Hfr)** bacteria.

2. In the cross (conjugation) between Hfr cell and F- cell, frequency of recombination is very high but frequency of transfer of whole F-factor is very low.

3. Hfr cell acts as donor while F- cell acts as recipient.

4. At first F-factor makes sex pilus that joins donor and recipient cell then F- factor opens as replication origin then one strand is cut down. Now the 5' end of this strand enters into recipient cell through conjugation tube.

5. Since, replication origin lies somewhere in the middle of F- factor, portion of F-factor that lies at 5' end enters first into recipient cell but the portion situated at 3' end enters only when whole chromosomal DNA enters into the recipient cell.

6. To transfer whole chromosomal DNA, it takes 100 minutes in E. coli. In most of the cases, sex pilus (conjugation tube) breaks before transfer of whole chromosomal DNA takes place. So, frequency of transfer of whole F-factor is very low. After the cross between Hfr cell and F- cell, recipient cell remains recipient.

7. In this conjugation, chromosomal DNA is always almost transfer from donor to recipient cell together with portion of F- factor. So, frequency of recombination is high.

F –prime (F') cell:

Bacteria in which contains F-factor and a part of chromosomal DNA integrated in it is known as F-prime bacteria.

F' cells are formed from Hfr cell during induction of F- factor from chromosomal DNA in which F-factor carries a portion of chromosomal DNA along with it.

In the cross (conjugation) between F-prime (F') cell and F- cell, frequency of recombination is high as well as frequency of transfer of whole F-factor is also high.

Q.No.14: Explain the mechanism of Translation and illustrate few applications of Transformat

Bacterial transformation is the transfer of free DNA released from a donor bacterium into the extracellular environment that results in assimilation and usually an expression of the newly acquired trait in a recipient bacterium.

This process doesn't require a living donor cell and only requires free DNA in the environment.

The recipient that successfully propagates the new DNA is called the transformant.

During extreme environmental conditions, some bacterial genera spontaneously release DNA from the cells into the environment free to be taken up by the competent cells. The competent cells also respond to the changes in the environment and control the level of gene acquisition through a natural transformation process.

Transformation is adopted as the most common method of gene transfer as it is the best way for the transfer of artificially altered DNA into recipient cells.

The process of transformation can transfer DNA regions of one to tens of kilobases.

Bacterial transformation

Bacterial transformation is based on the natural ability of bacteria to release DNA which is then taken up by another competent bacterium.

The success of transformation depends on the competence of the host cell. Competence is the ability of a cell to incorporate naked DNA in the process of transformation

Organisms that are naturally transformable spontaneously release their DNA in the late stationary phase via autolysis.

Several bacteria, including *Escherichia coli*, can be artificially treated in the laboratory to increase their transformability by chemicals, such as calcium, or by applying a strong electric field (electroporation) or by using a heat shock.

Electroporation or heat shock increases the competence by increasing the permeability of the cell wall, which allows the entry of the donor DNA.

Similarly, transformants can be selected if the transformed DNA contains a selectable marker, such as antimicrobial resistance or utilization of a growth factor, such as an amino acid.

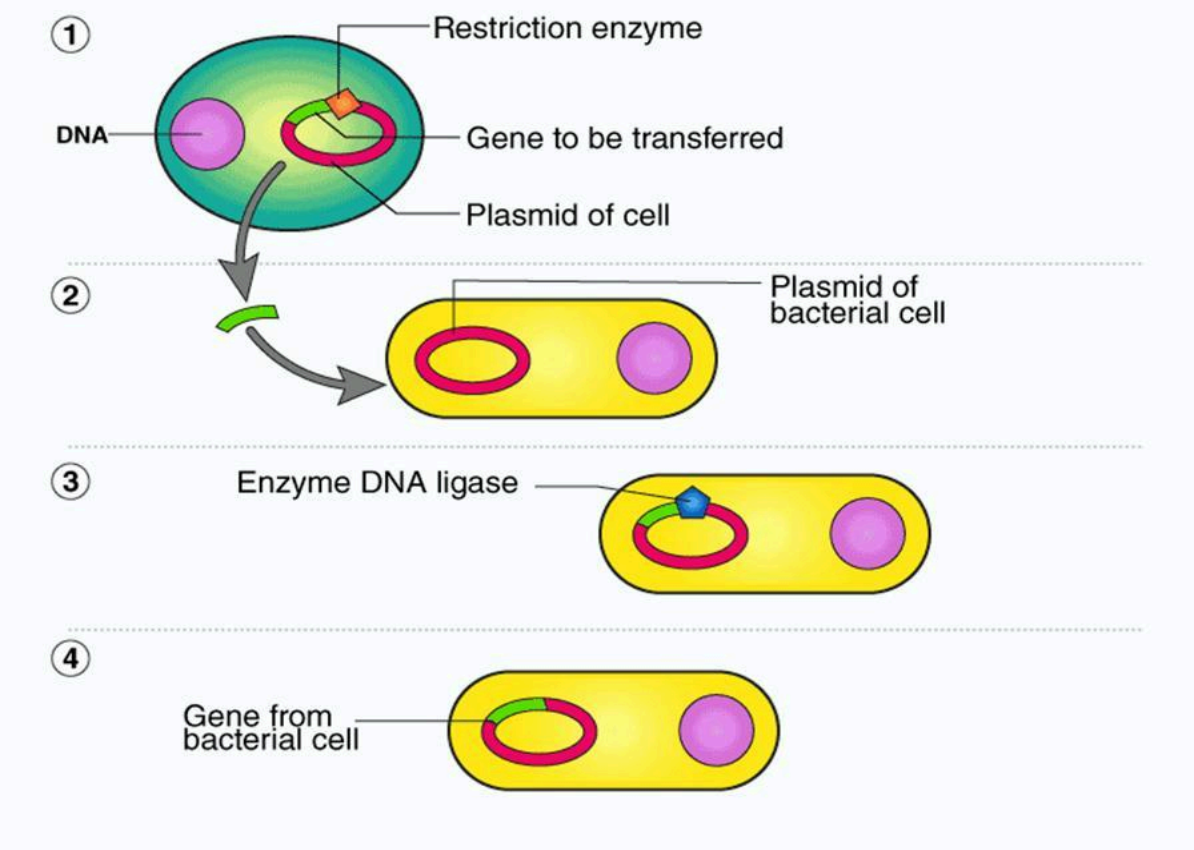
In most of the naturally competent bacteria, the free DNA binds to the bacteria, and the DNA is integrated into the chromosomal DNA.

Sometimes, the free DNA is inserted into a plasmid which is capable of replicating autonomously from the chromosome.

Plasmid encodes some enzymes and antibiotic-resistant markers which are later expressed in the transformed bacteria.

In this process of transformation, the donor DNA is first inserted into the plasmid. The plasmid containing the donor DNA is then inserted into the competent host bacteria.

After the transformation is completed, the bacteria containing the plasmid can be detected either by using a growth media supplemented with a particular antibiotic.



Types of Bacterial Transformation:

There are two forms of transformation:

Natural Transformation: In natural transformation, bacteria naturally have the ability to incorporate DNA from the environment directly.

Artificial Transformation: In the case of artificial transformation, the competence of the host cell has to be developed artificially through different techniques.

Examples :

The first and most prominent example of bacterial transformation is the transformation of DNA from smooth capsule-positive colonies of *Streptococcus pneumonia* to the rough capsule-negative colonies. This was the first mechanism of bacterial genetic exchange to be recognized.

Neisseria and *H. influenzae* take up DNA from their own species which occurs by species-specific recognition. Natural bacterial transformation is also observed in the case of *B. subtilis*.

Q.15: Interpret the Lederberg and Zender experiment and explain mechanism of Transduction

In the 1950s, Joshua Lederberg and Norton Zinder used a U-tube apparatus to demonstrate that genetic material could be transferred between bacteria by bacteriophages. This discovery, called transduction, was the first demonstration of gene transfer in an organism.

How the experiment worked

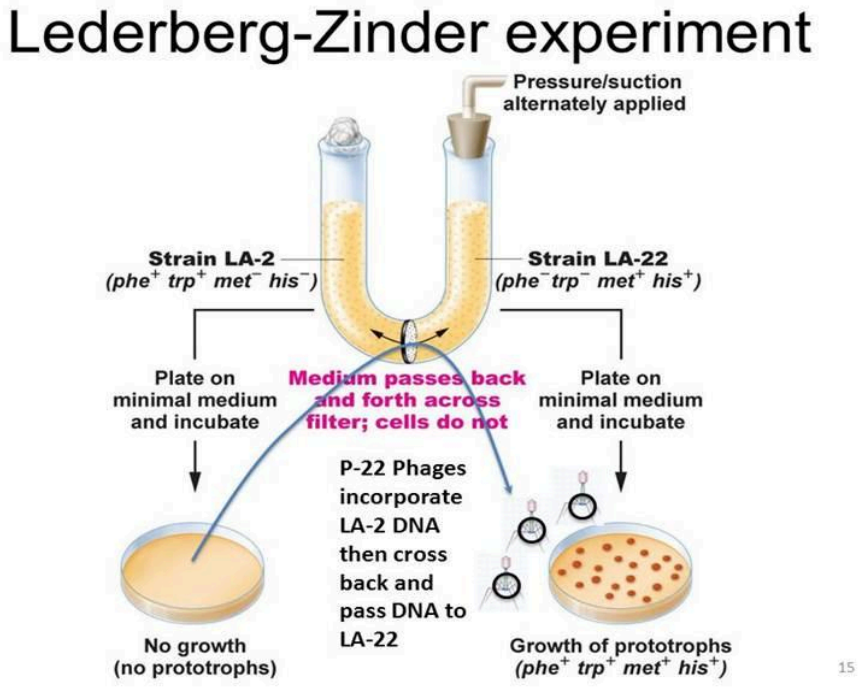
1. Each bacterial strain was placed in one arm of the U-tube.
2. A filter that prevented bacterial cells from passing through separated the two strains.
3. The experiment showed that genetic material could be transferred between the strains without cell-to-cell contact.

What they discovered

- The transfer of genetic material was caused by a filtrable agent (FA).
- FA had many of the same chemical, physical, and genetic properties as bacteriophage particles.
- Transduction could occur through either the lytic or lysogenic cycle of a bacteriophage.

Significance of the discovery

Transduction is a type of horizontal gene transfer that contributes to bacterial evolution. It also allows microbiologists to map and manipulate genes in bacteria and archaea.

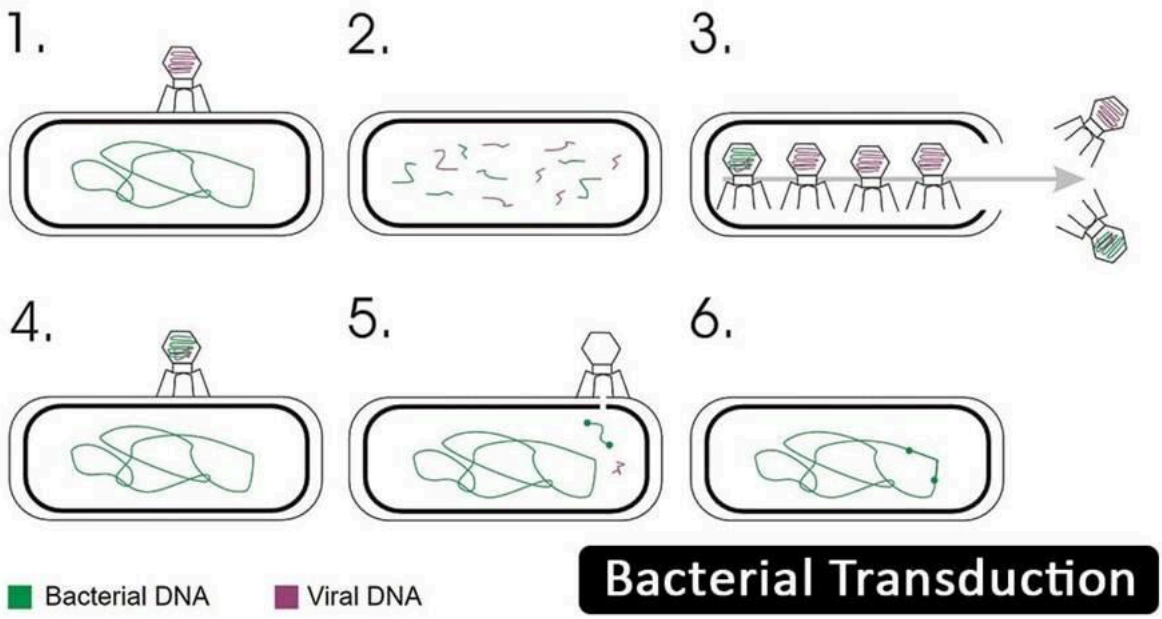


Bacterial Transduction- Principle

Transduction is the transfer of bacterial DNA from a donor to a recipient bacterium via a virus particle.

- The virus particle that infects bacteria is called a bacteriophage or phage, and the phages used for the transfer of DNA are called transfusing phages.
- Not all phages are transducing phages. The process of transduction can transfer DNA regions of tens to hundreds of kilobases.
- Due to the high specificity of phages for cell surface receptors, transduction has the narrowest host range of DNA transfer among the methods of bacterial genetic exchange.
- Transduction involves the carrying over of DNA (or gene transfer) from one organism to another by an intermediate agent, which is usually a bacteriophage.
- Transduction has an advantage over conjugation in that transduction doesn't require physical contact between the cell donating and the DNA and the cell receiving the DNA.
- Similarly, the process of transduction is resistant to the DNase enzyme while the transformation process is susceptible to DNase.

    · Transduction is a standard process employed by many molecular biologists to introduce a foreign gene into a host cell's genome.



Based on how the DNA is packaged within the viral particle, there are two types of transduction:

Generalized Transduction

· In generalized transduction, phage mistakenly packages bacterial DNA instead of their own phage DNA during phage assembly.

· This results in an infectious virus particle containing bacterial DNA, but one that can no longer replicate in the bacterium due to the loss of all of the phage DNA.

- The phage particle then attaches to a bacterial cell surface receptor and injects the packaged DNA into the cytoplasm of the bacterium.
- If the bacterial DNA in the phage is from the bacterial chromosome, the DNA recombines with the homologous DNA of the bacterial recipient to generate stable transductants. This process requires a host recombinase, recA.
- However, studies have indicated that the majority of transduced DNA is not stably integrated into the bacterial genome but rather remains extrachromosomal.
- Generalized transduction is used for mapping genes, mutagenesis, transferring plasmids and transposons, and determining whether different genera of bacteria have homologous genes

Specialized Transduction

- In specialized transduction, the phage undergoes lysogeny usually at specific locations in the bacterial genome called attachment sites.
- During this process, the phage genome usually integrates into the bacterial chromosome as virus replication is repressed during lysogeny.
- The phage genome then excises from the bacterial genome and, due to imprecise excision and recombination, adjacent bacterial genes are also excised.
- Unlike a generalized transducing phage, a specialized transducing phage contains both phage and bacterial genes.
- During the subsequent infection, the newly acquired gene is inserted into the bacterial genome along with phage DNA to form a new round of lysogeny.
- Specialized transduction is independent of host homologous recombination and recA but requires phage integrase.
- Specialized transduction is instrumental in the isolation of the genes in molecular biology, and in the discovery of insertion elements, which often serve as attachment sites for phage DNA integration.

Examples:

- A good example of a generalized transducing phage is P1, which can transduce *E. coli* DNA to numerous Gram-negative bacteria.
- *E. coli* phage lambda is a classic example of a specialized transducing phage that integrates its DNA precisely between operons encoding enzymes responsible for galactose (gal) and biotin (bio) utilization in the *E. coli* chromosome.