

Biomolecules are the molecules that occur naturally in living organisms. Biomolecules include macromolecules like proteins, carbohydrates, lipids and nucleic acids. DNA / RNA -

It also includes small molecules like primary and secondary metabolites and natural products.

Biomolecules consists mainly of carbon and hydrogen with nitrogen, oxygen, sulphur, and phosphorus.

Biomolecules are very large molecules of many atoms, which are covalently bound together.

Classes of Biomolecules

There are four major classes of biomolecules:

- Carbohydrates ✓
- Proteins ✓
- Nucleic acids (DNA & RNA) ✓
- Lipids ✓

A) STRUCTURE AND FUNCTION OF BIOMOLECULES

1. CARBOHYDRATES :

- These are poly hydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis. Carbohydrates have the empirical formula $(CH_2O)_n$.
- They provide the body with source of fuel and energy, it aids in proper functioning of our Brain, Heart and Nervous, Digestive and Immune system. Deficiency of carbohydrates in the diet causes fatigue, poor mental function.
- Carbohydrates are the most abundant biomolecules on earth.
- Plants make carbohydrates from CO_2 by photosynthesis.
- Oxidation of carbohydrates is the central energy yielding pathway in most non-photosynthetic cells.
- Carbohydrate is an organic compound, it comprises of only oxygen, carbon and hydrogen. The oxygen: hydrogen ratio is usually is 2:1.
- Carbohydrates are hydrates of carbon; technically they are polyhydroxy aldehydes and ketones.
- Carbohydrates are also known as saccharides, the word saccharide comes from Greek word **sakkron** which means sugar.

There are three major classes of carbohydrates:

a. Monosaccharides :

Monosaccharides, or simple sugars, consist of a single polyhydroxy aldehyde or ketone unit. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose.

b. **Oligosaccharides :**

Oligosaccharides consist of short chains of monosaccharide units, or residues, joined by characteristic linkages called glycosidic bonds. The most abundant are the disaccharides, with two monosaccharide units. **Example:** sucrose (canesugar)

c. **Polysaccharides**

The polysaccharides are sugar polymers containing more than 20 or more monosaccharide units, and some have hundreds or thousands of units. Example: starch. Polysaccharides are of two types based on their function and composition. Based on function, polysaccharides are of two types: storage and structural.

Storage polysaccharide- starch.

Structural polysaccharide - cellulose.

Monosaccharides

- The word "Monosaccharides" derived from the Greek word "Mono" means Single and "saccharide" means sugar.
- Monosaccharides are polyhydroxy aldehydes or ketones which cannot be further hydrolysed to simple sugar.
- Monosaccharides are simple sugars. They are sweet in taste. They are soluble in water. They are crystalline in nature.
- They contain 3 to 10 carbon atoms, 2 or more hydroxyl (OH) groups and one aldehyde (CHO) or one ketone (CO) group.

Monosaccharides are again classified on the basis of

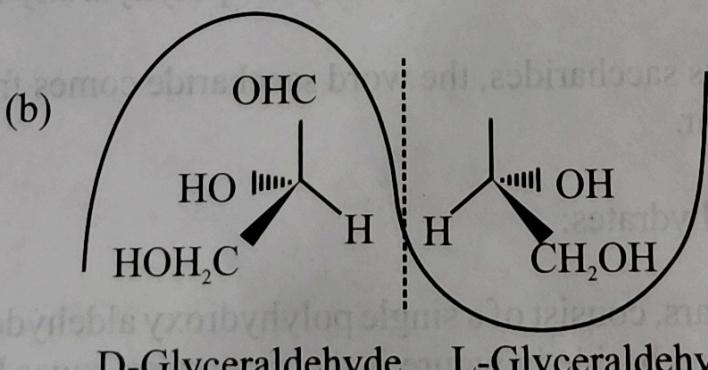
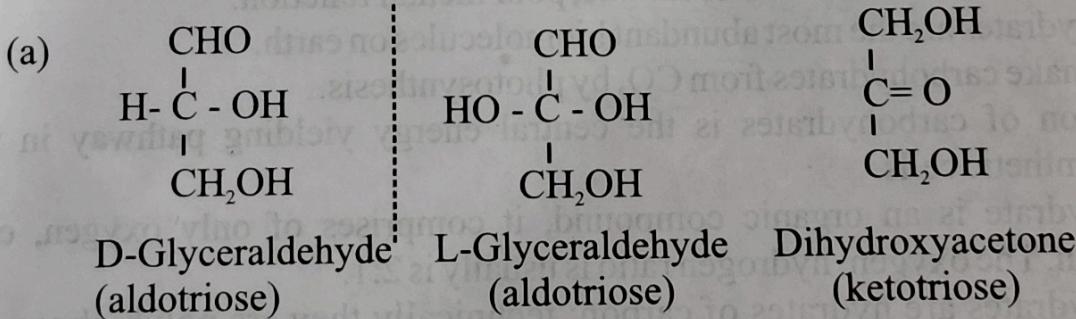
i. **Nature of Carbonyl Group :**

- **Aldoses** : Monosaccharides possesses aldehyde group (-CHO). **Example :** Glucose
- **Ketoses** : Monosaccharides possesses ketone group (=C=O). **Example :** Fructose

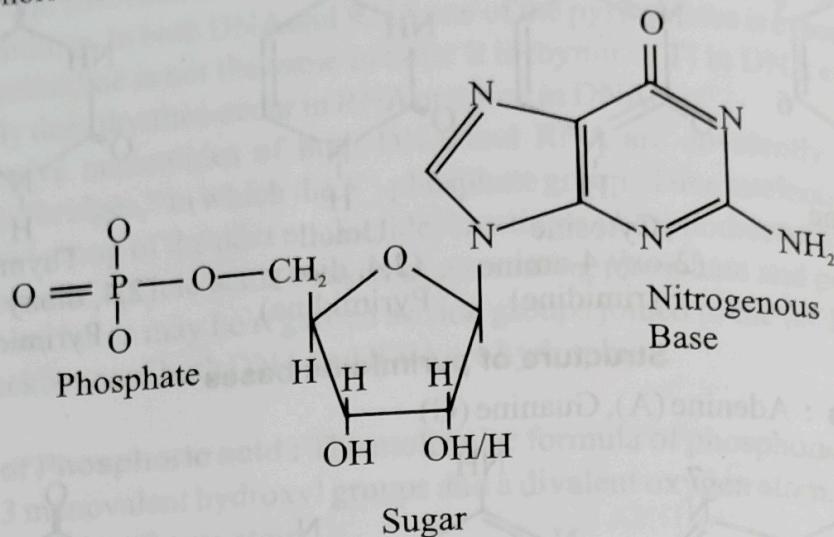
ii. **Nature of carbon atoms :**

- **Trioses** : Trioses are "Monosaccharides" containing 3 carbon atoms. The molecular formula of triose is C₃H₆O₃,

Example: Glyceraldehyde and Dehydroxy acetone



- i. Nitrogenous base(Purine and Pyrimidine)
- ii. Sugar(Ribose and deoxyribose)
- iii. Phosphoric acid (H_3PO_4)

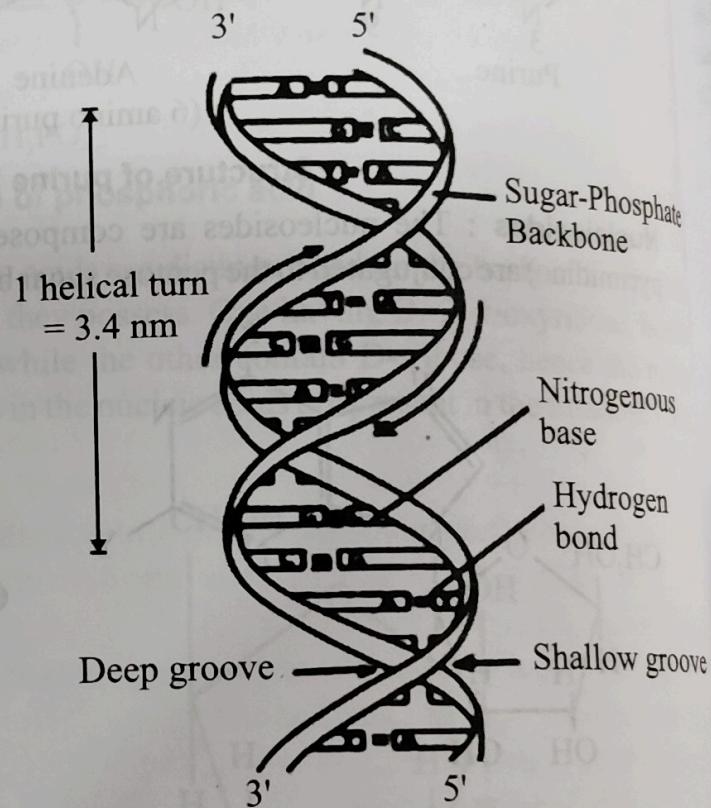


Structure of DNA:

The structure of DNA was a mystery before the 1950s. It was at the beginning of 1950s, James Watson (American biologist) and Francis Crick (British physicist) after combining the available physical and chemical data and based on their research introduced the double-helix model for DNA. The work of Rosalind Franklin and Maurice Wilkins helped Watson and Crick in their discovery of the double helix structure of DNA. This model for the DNA structure was widely accepted and is known as Watson and Crick's model of DNA.

DNA is a double-stranded molecule. It is made of two helical chains or strands that are spirally coiled around a common axis to form a right-handed double helix like a twisted ladder.

Each strand has two ends; 5' end with a phosphate group and 3' end with a hydroxyl group. The DNA strands run in opposite directions which mean the 5' end of one chain and 3' end of another chain are on the same side, so they are antiparallel to each other. One strand runs from 5' to 3' direction and is known as sense strand and the opposite strand runs from 3' to 5' direction, which is known as antisense strand.



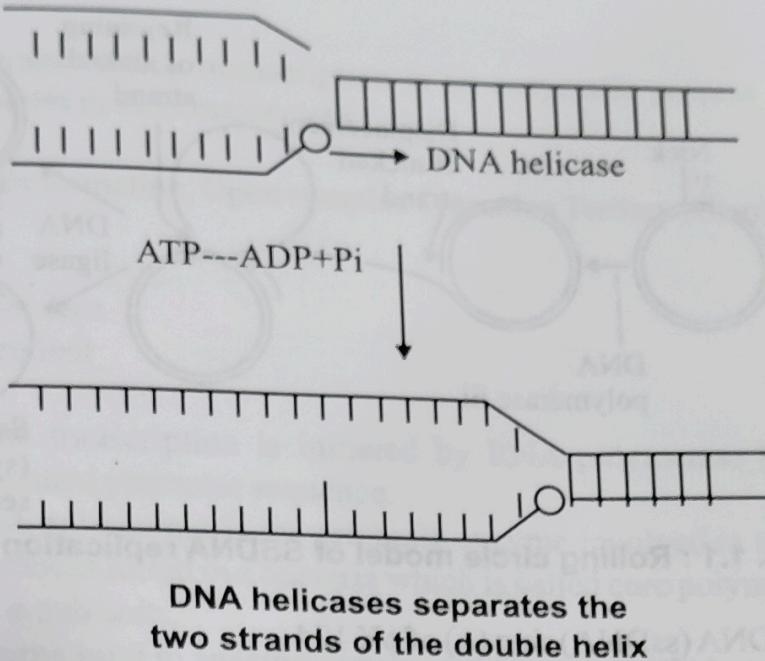
Watson and Crick Model of DNA Molecule

- The diameter of DNA is uniform and is around 2 nm.
- The distance between each turn or the length of each spiral turn is 3.6 nm (earlier 3.4 nm).
- The distance between base pairs or two successive rings is 0.34 nm.
- There are 10.5 nucleotides per turn or in one complete spiral turn or one complete rotation of 360 degrees (earlier 10 nucleotides).
- The spiral arrangement of chains creates major and minor grooves between the two chains or strands. The major groove is large, whereas, the minor groove is small. Major groove occurs when backbones are apart and minor groove occurs when they are close.
- The alternating deoxyribose sugar and phosphate groups are located on the outside of the double helix. So, it makes the backbone of the helix.
- Each DNA strand consists of a long sequence of four bases that include Adenine (A), Cytosine (C), Guanosine (G) and Thymine (T). The bases on one strand are bonded or paired with the complementary bases on the opposite strand.
- The pyrimidine (Thymine and Cytosine) and purine (Adenine and Guanine) bases are located inside the double helix.
- A specific purine base is bonded or make pair with a specific pyrimidine base through hydrogen bonds. For example, Adenine (A) pairs with Thymine (T) and Guanine (G) pairs with Cytosine (C).
- Adenine and Thymine are joined through two hydrogen bonds ($A=T$), whereas, Guanine and Cytosine are joined through three Hydrogen bonds ($G\equiv C$).
- The double helix structure is stabilized by hydrogen bonds that are formed between purine and pyrimidine bases.
- These base pairs are known as complementary base pairs and form the steps of the DNA ladder. So, the two DNA strands are complementary to each other. The number of A bases is equal to the number of T bases and, similarly, the number of G bases is equal to C bases. So, the number of purine bases is always equal to the number of pyrimidine bases. This is also suggested by a rule called Chargaff's rule.
- The sides of the helix or strands are made up of alternating sugar and phosphate molecules of nucleotides that are linked by 3'-5' phosphodiester bond. Whereas, the steps of the ladder are made of nitrogen bases that are connected to sugars by glycosidic bonds. If we imagine the double helix structure as a ladder, the sugar and phosphate group can be considered as sides and the bases would be the step.

The structure of DNA helped understand the various functions of DNA such as how it is replicated and how the information is carried by it is used to make proteins by the cells.

Structure of RNA

With the discovery of molecular structure of the DNA double helix in 1953, researchers turned to the structure of ribonucleic acid (RNA). It is a type of molecule that consists of a long chain of nucleotide units. Each nucleotide consist of nitrogenous base, a ribose sugar, and a phosphate group. RNA is very similar to DNA, but differ in few important structural details. In the cell, RNA is usually single stranded, while DNA is usually double stranded. RNA nucleotide contain ribose while DNA contains deoxyribose and RNA has the uracil rather than thymine that is present in DNA.



5. **Topoisomerases** : As the strands of DNA are unwound by helicase activity, the double stranded DNA in front of the fork becomes increasingly supercoiled. These supercoils are removed by topoisomerases. This enzyme removes.

ii) **Rolling Circle Model (in circular DNA)**

- This model was proposed by Gilbert and Dressler in 1968. This is the current model for explaining viral DNA replication in single stranded DNA viruses for example ϕ X 174 and transfer of *E. coli* sex factor (plasmid).
- ϕ X 174 chromosomes consist of a single stranded DNA ring (+ve strand). The chromosome first becomes double stranded by the synthesis of a negative strand. Synthesis is presumed to begin at a specific initiation point on the template ring.
- One strand of the parental duplex ring is now cut at a specific point by an endonuclease. As a result of cut (nick) linear strand with 3' and 5' ends is created.
- 3' end serves as a primer for the synthesis of a new DNA strand under the catalytic action of DNA polymerase. The unbroken strand is used as the template for this purpose and complimentary strand is synthesized. Thus the parental DNA molecule itself is used as a primer for initiating replication.
- The 5' end of broken strand becomes attached to the plasma membrane of the host bacterium.
- The unbroken strand rolls and unwinds as synthesis proceeds, leaving a "tail" which is attached to the membrane. New DNA is synthesized on 3' ends and the tail. In the tail region, new DNA is synthesized in discontinuous segments in the 5' → 3' direction. This synthesis is presumed to start by the synthesis of an RNA primer under the catalytic action of RNA polymerase.
- The tail is cut off by a specific endonuclease into a unit length rod of daughter cells. The rod may undergo circularization to form a new circular molecule.
- During this process the gap is closed by DNA ligase. The newly formed circular molecule can in turn become new rolling circle.

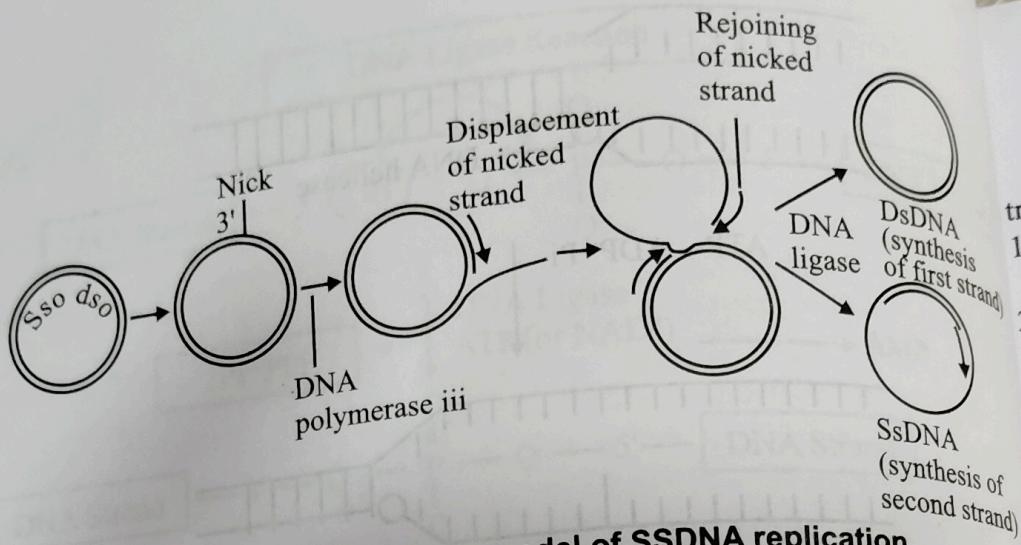


Fig. 1.1 : Rolling circle model of SSDNA replication

- a. Single stranded DNA (ssDNA) ring (+) of ϕ X 174.
- b. Synthesis of negative strand (-) and formation of double stranded (dsDNA) replicative form.
- c. Nicking of one parental strand by endonuclease.
- d. Parental strand rolls and unwinds. 5' end attaches to the host membrane. New DNA synthesized on the 3' end and at the 'tail'.
- e. Further unwinding and synthesis. Note that the tail region synthesizes discontinuous segments.
- f. Tail is cut by specific endonuclease into unit length progeny rod.
- g. Circularization of rods to form new circular molecule, which can become new rolling circles. During circularization the gap is closed by a ligase.

b) TRANSCRIPTION (RNA Synthesis)

The process of synthesis of RNA by copying the template strand of DNA is called transcription. During replication entire genome is copied but in transcription only the selected portion of genome is copied. The enzyme involved in transcription is RNA polymerase. Unlike DNA polymerase it can initiate transcription by itself, it does not require primase. More exactly it is a DNA dependent RNA polymerase.

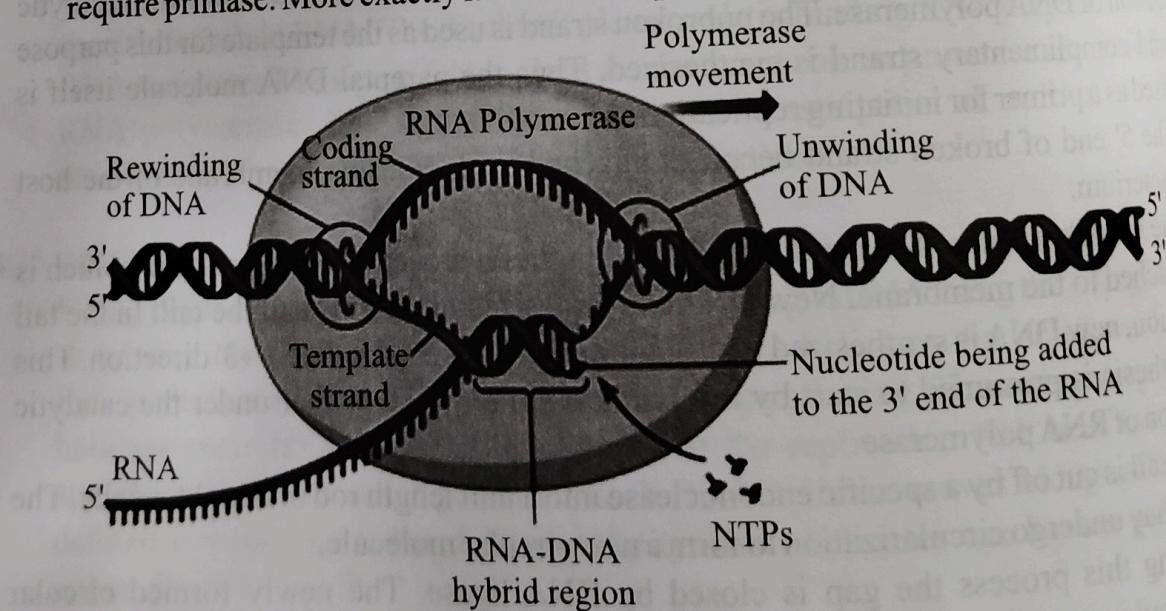


Fig. 1.2 : Rolling circle model of SSDNA replication

The step of transcription com
1. Initiation:
closed com
Elongation
Terminatio
a) Rho-
b) Rho-

1. Initiation

specific p
Bacteria
RNA pc
of α , β ,
The co
The bi
factor.
The c
holoe
In ca
elem
from
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In s
ma
Th
aC
dc
i)

The step of transcription -transcription is an enzymatic process the mechanism of transcription completes in three major steps

Initiation:

Closed complex formation, Open complex formation Tertiary complex formation

Elongation

Termination :

- a) Rho-dependent
- b) Rho-independent

Initiation: The transcription is initiated by RNA polymerase holoenzyme from a specific point called promotor sequence.

Bacterial RNA polymerase is the principle enzyme involved in transcription. Single RNA polymerase is found in a bacteria which is called core polymerase and it consists of α , β , β' and ω sub units.

The core enzyme bind to specific sequence on template DNA strand called promotor. The binding of core polymerase to promotor is facilitates and specified by sigma (σ) factor. ($\sigma 70$ in case of E. coli).

The core polymerase along with σ -factor is called Holo-enzyme ie. RNA polymerase holoenzyme.

In case of e. coli, promotor consists of two conserved sequences 5'-TTGACA-3' at -35 element and 5'-TATAAT-3' at -10 element. These sequence are upstream to the site from which transcription begins. Binding of holoenzyme to two conserve sequence of promotor form close complex.

In some bacteria, the altered promotor may exist which contain UP-element and some may contain extended -10 element rather than -35 element.

The UP-element is recognized by a carboxyl terminal domain of α -sub unit called α CTD (carboxyl terminal domain) which is connected to α NTD (Amino terminal domain) by flexible linker.

i) **Closed complex:** Binding of RNA polymerase holoenzyme to the promotor sequence form closed comolex

ii) **Open complex:** after formation of closed complex, the RNA polymerase holoenzyme separates 10-14 bases extending from -11 to +3 called melting. So that open complex is formed. This changing from closed complex to open complex is called isomerization.

iii) **Tertiary complex:**

a) RNA polymerase starts synthesizing nucleotide. It does not require the help of primase. If the enzyme synthesize short RNA molecules of less than 10 bp, it does not further elongates which is called abortive initiation. This is because σ 3.2 acting as mimic of RNA and it lies at middle of RNA exit channel in open complex.

b) When the RNA polymerase manage to synthesize RNA more than 10 bp long, it eject the σ 3.2 region and RNA further elongates and exit from RNA exit channel. This is the formation of tertiary complex.

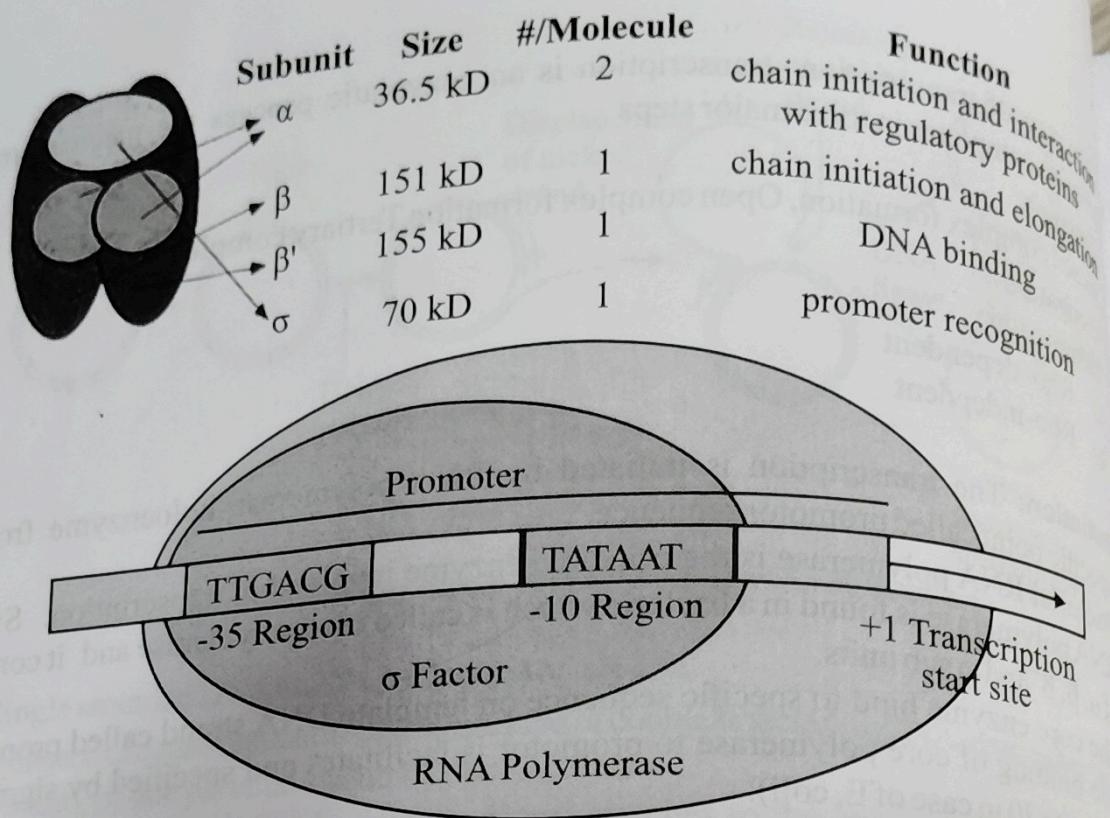


Fig. 1.3 : Prokaryotic RNA Polymerase:Holoenzyme Enzyme

2. **Elongation :** After synthesis of RNA more than 10 bp long, the σ-factor is ejected, the enzyme move along 5'-3' direction continuously synthesizing RNA. The synthesized RNA is proofread. Hydrolytic editing. For this the polymerase back track by one or more nucleotide and cleave the RNA removing the error and synthesize the correct one. The Gre factor enhance this proof reading process. Pyrophospholytic editing another mechanism removing altered nucleotide.

3. **Termination :** There are two mechanism of termination.
 - a) **Rho independent :** In this mechanism, transcription is terminated due specific sequence in terminator DNA. The terminator DNA contains inverted repeat which cause complimentary pairing as transcript RNA form hair pin structure. This invert repeat is followed by larger number of TTTTTTTT (~8 bp) on template DNA. The uracil appear in RNA. The load of hair pin structure is tolerated by A=U base pair so the RNA get separated from RNA-DNA heteroduplex.
 - b) **Rho dependent :** In this mechanism, transcription is terminated by rho protein. It is ring shaped single strand binding ATPase protein. The rho protein bind the single stranded RNA as it exit from polymerase enzyme complex and hydrolyse the RNA from enzyme complex. The rho protein does not bind those RNA whose protein is being translated. Rather it binds to RNA after translation. In bacteria transcription and translation occur simultaneously so rho protein bind the RNA after translation has completed but transcription is still on.

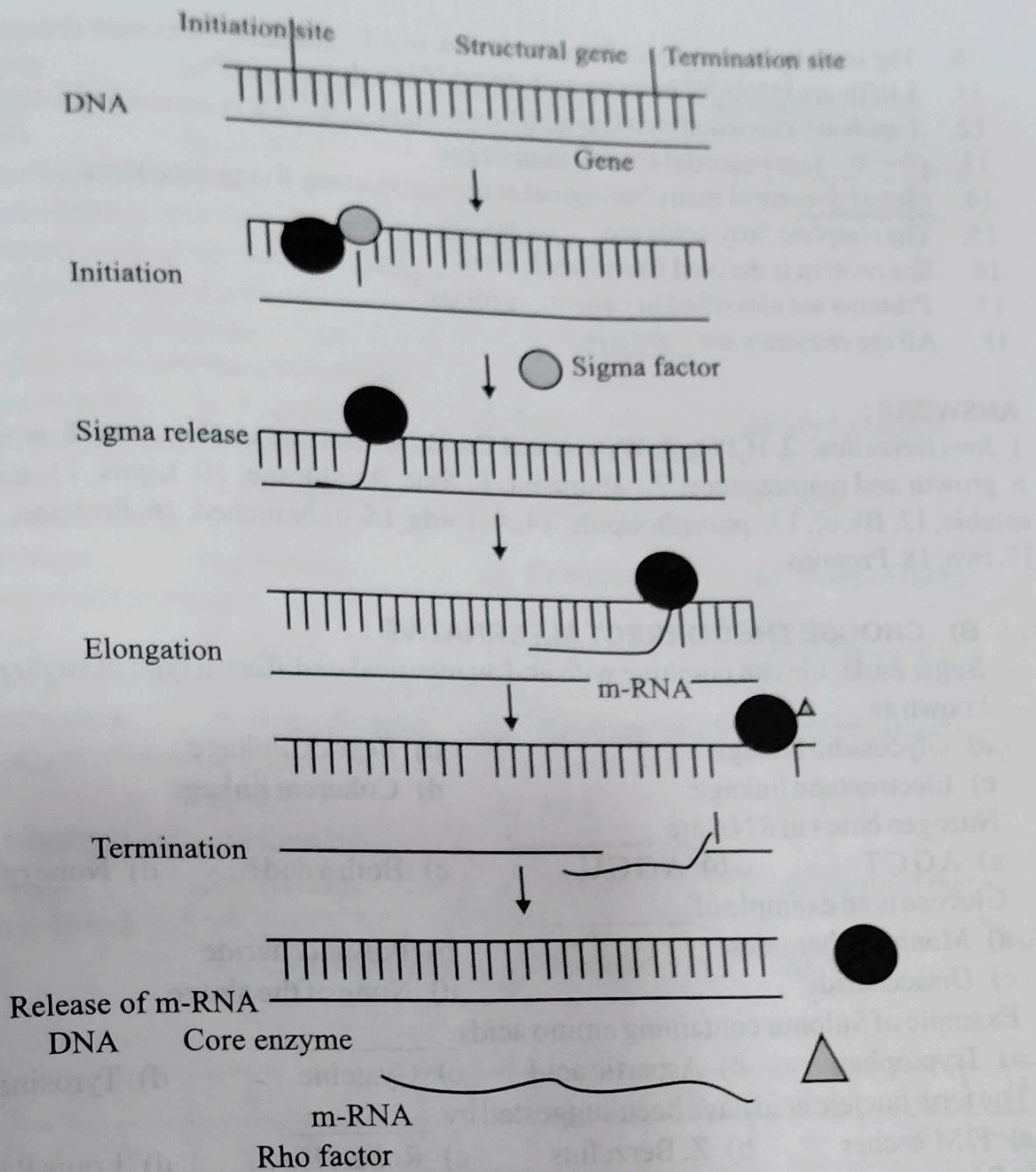


Fig. 1.4 : Steps involved in RNA synthesis (Transcription)

EXERCISE

A) FILL IN THE BLANKS

1. The name of protein was first suggested by Jons Bezerlius.
2. The molecular formula of phosphoric acid is H_3PO_4 .
3. The structure of DNA was proposed by watson and Crick.
4. Sucrose is made up of Glucose and Fuctose Molecule.
5. All protein polymers of amino acids.
6. Biomolecules are required to living organisms for their growth and maintenance.
7. Carbohydrates are the abundant substances in nature.
8. Carbohydrates classified in to four types.
9. Carbohydrates acts as a storage molecules.

10. The term lipids originate from the Greek word Lipos.
11. Lipids are insoluble in water and soluble in solvent
12. Lipids are classified by a German biochemist Bloor.
13. phospho are essential for cell membrane. Lipids.
14. Steroids control many biological activities in many living organisms.
15. The simplest fatty acids are unbranched.
16. The protein is derived from Greek word peotēios
17. Proteins are classified in two groups.
18. All the enzymes are proteins.

ANSWERS:

1. Jons Berzelius, 2. H_3PO_4 , 3. Watson and Creek, 4. Glucose and Fructose, 5. amino acids,
6. growth and maintenance, 7. abundant, 8. four, 9. storage, 10. Lipos, 11. insoluble and soluble, 12. Bloor, 13. phospholipids, 14. steroids, 15. unbranched, 16. Proteios, 17. two, 18. Proteins.

1. B) CHOOSE THE CORRECT ALTERNATIVE :

1. Sugar molecule can combine with and an identical or different type of sugar molecule known as _____.
 ✓ a) Glycosidic linkage b) Peptide linkage
 c) Electrostatic linkage d) Coherent linkage
2. Nitrogen bases in RNA are _____.
 a) AGCT ✓ b) AGCU c) Both a and b d) None of the above
3. Glucose is an example of _____.
 ✓ a) Monosaccharides b) Polysaccharide
 c) Disaccharide d) None of the above.
4. Example of Sulphur containing amino acids _____.
 a) Tryptophan b) Aspartic acid c) Cysteine d) Tyrosine
5. The term nucleic acid have been suggested by _____.
 ✓ a) F. Miescher b) Z. Berzelius c) Robert Koch d) Louis Pasteur
6. In RNA, thymine base is replaced by _____.
 ✓ a) Uracil b) Adenine c) Guanine d) Cytosine
7. Which of the following enzymes are used to join bits of DNA?
 a) DNA ligase ✓ b) DNA polymerase
 c) Primase d) Endonucleases
8. Semi-conservative replication of DNA was first demonstrated in _____.
 ✓ a) Escherichia coli b) Streptococcus pneumoniae
 c) Salmonella typhi d) Drosophilla melanogaster.
9. During the replication of DNA, the synthesis of the DNA on lagging strand takes place in segments, these segments are called _____.
 a) Satellite segments b) Double helix segments
 c) Kornbeg segments ✓ d) Okazaki segments.
10. Unwinding of DNA is done by _____.
 a) Helicase b) Ligase c) Hexonucleases d) Topoisomerase

11. In prokaryotes transcription occurs _____.
 a) Nucleus b) Cytoplasm c) Cytosol
 d) Matrix
12. Anticodons of amino acids are present on _____.
 a) r-RNA b) m-RNA c) t-RNA
 d) DNA
13. Function of topoisomerase in DNA replication is _____.
 a) Polymerization of deoxyribonucleotides.
 b) Polymerization of ribonucleotides.
 c) Unwinds the DNA double helix.
 d) Removal of supercoils in DNA
14. Choose different word from following
 a) Carbohydrates b) Proteins c) Fats
 d) Hydrocarbon
15. carbohydrate molecule contain
 a) Sodium b) Phosphorus c) Oxygen
 d) Zinc
16. Fructose is known as
 a) Fruit sugar b) Sucrose c) Cane sugar
 d) None of these
17. Fat is triglyceride which is
 a) Liquid b) Solid c) Gas
 d) Both a and b
18. Vanaspati ghee is manufactured by the process
 a) Chlorination b) Solidification c) Hydrogenation
 d) Chelation
19. 1gm lipids provides kcal heat
 a) 9.3 b) 9.1 c) 10.5 d) 10.1
20. Hairs, wool and nails are form by
 a) Collagen b) Keratin c) Elastin
 d) None of these
21. Insulin is an example of _____.
 a) Fat b) Carbohydrates c) Protein
 d) Lipid

ANSWERS :

1	2	3	4	5	6	7	8	9	10	11
a	b	a	c	a	a	b	a	d	d	b
12	13	14	15	16	17	18	19	20	21	
c	d	d	c	a	d	c	a	b	c	

1. C) ANSWER IN ONE SENTENCE

- Define nucleotide.
- What are heteropolysaccharide?
- Define glycolipid.
- Define nucleosides.
- What are triglycerides?
- Define protein.
- Who discovered DNA structure?
- Define Wax.
- What is Phospholipid?
- Write two examples of polyunsaturated fatty acids

Other Related Definitions :

- **Genetics** : Genetics is the branch of biology concerned with the study of inheritance, including the interplay of genes, DNA variation and their interactions with environmental factors.
- **Genome** : Total set of hereditary elements in a cell or organism.
- **Genotype** : Refer to genetic constitution of cell for example TT, Tt or tt for height of plant.
- **Phenotype** : Expression of genotype in observable manner is known as phenotype.
- **Mutation** : Sudden inheritable change in genetic material is known as mutation.
- **Allele** : An allele is one of two or more versions of DNA sequence (a single base or a segment of bases) at a given genomic location. An individual inherits two alleles, one from each parent, for any given genomic location where such variation exists. If the two alleles are the same, the individual is homozygous for that allele. If the alleles are different, the individual is heterozygous.

Definition of Cistron, Muton and Recon:

The gene has been variously considered as a unit of recombination (recon), a unit of mutation (muton) and a unit of function (cistron). These three units are not identical. Cistron is the largest unit in size followed by recon and muton. A gene consists of several cistron, a cistron contains many recons, and a recon contains a number of mutons. However, if the size of a recon is equal to muton, there would be no possibility in recon for consisting of several mutons.

- a) **Cistron** : S., Benzer called the unit of function as a cistron. In other words, Benzer called the functional gene as a cistron. Cistron represents a segment of the DNA molecule and consists of a linear sequence of nucleotides, which controls some cellular function. In *E. coli* cistron may contain about 1500 base pairs. Some cistrons may contain as many as 30,000 base pairs. The cistron begin with initiation codon and ends with a terminating codon. Each cistron is responsible for coding one m-RNA molecule which in turn controls the formation of one polypeptide chain. Each cistron consists of hundreds of mutons and recons.
- b) **Recon** : It is a unit of recombination. It is the smallest unit capable of recombining genetically. Recombination studies on microbes indicate that structurally the recon consists of one or two pairs of nucleotides, possibly only one pair.
- c) **Muton** : It is a unit of mutation. The shortest chromosomal unit capable of undergoing mutation has been called the muton. The muton consists of one or many pairs of nucleotides within the DNA molecule.

Gene within gene:

In 1940s, Beadle and Tatum proposed one-gene - one protein hypothesis which explains that one gene code for one protein/ polypeptide. However, if one gene consists of 1,500 base pairs, a protein of 500 amino acids in length would be synthesized. In addition, if the same sequence read in two different ways, two different proteins would be synthesized by the same sequence of base pairs. It means the same DNA sequence can synthesize more than one protein

9. **Chain Termination Codons** : The 3 triplets UAA, UAG, UGA do not code for any amino acid. When any one of them occurs immediately before the triplet AUG or GUG, it causes the release of the polypeptide chain from the ribosome. They are also called as stop codons. They are also called chain termination codons because these codons are used by the cell to signal the natural end of translation of a particular peptidyl chain. However, their inclusion in any mRNA results in the abrupt termination of the message at the point of their location even though the polypeptide chain has not been completed.
10. **Sense Codons** : 61 codons, which code for particular amino acids are termed as sense codons.

C) OUTLINE OF TRANSLATION :

Protein synthesis is the creation of proteins by cells that uses DNA, RNA, and various enzymes. In biological systems, it is carried out inside the cell. In prokaryotes, it occurs in the cytoplasm. In eukaryotes, it initially occurs in the nucleus to create a transcript (mRNA) of the coding region of the DNA. The transcript leaves the nucleus and reaches the ribosomes for translation into a protein molecule with a specific sequence of amino acids. It generally includes transcription, translation, and post-translational events. The basic mechanism of protein synthesis is that DNA makes RNA, which in turn makes protein. The central idea or dogma of protein synthesis is as follow:

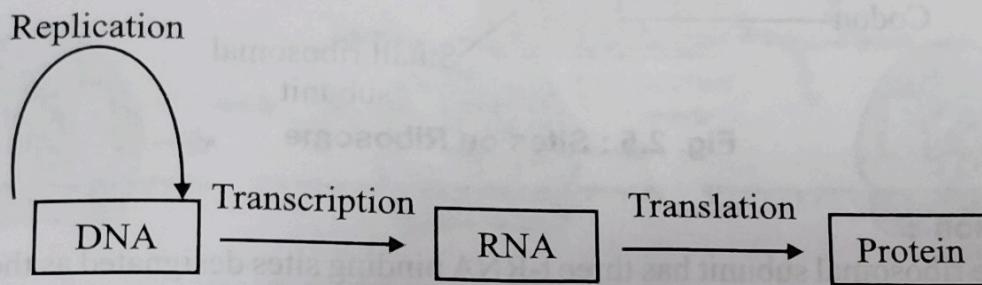


Fig. 2.4 : Central dogma of protein synthesis

Translation (Protein Synthesis) :

- **Translation** : The translation process occurs in association with ribosomes and involves the three types of RNA common to all types of cells, i.e., m-RNA, r-RNA and t-RNA. Since m-RNA molecules carry the genetic message, they must interact directly with the ribosomes. Translation can be broken into a series of stages as follows:

1) Initiation :

Initially, the small ribosomal subunit (30S in prokaryotes, 40S in eukaryotes) binds to m-RNA at a site "upstream" of the initiator codon. Some bacterial m-RNA molecules carry the nucleotide sequence "5'-AGGAGG" (the Shine-Dalgarno sequence) which binds to the complimentary sequence "3'-UCCUCCA" (the anti-Shine-Dalgarno sequence) on the 16S ribosomal-RNA portion of the ribosome. In bacteria lacking a Shine-Dalgarno sequence, the binding of m-RNA to the small ribosomal subunit involves interaction between ribosomal proteins and purine rich regions of RNA upstream of the initiator. In eukaryotes a sequence beginning with "A" or "G" followed by "CCACC" (the Kozak consensus sequence) serves as the ribosome binding site. Once the binding of m-RNA to the small ribosomal subunit has occurred, a number of translation initiation factors (IF) bind to the ribosome along with a t-

RNA carrying the amino acid methionine. In prokaryotes, this is N-formylmethionine (fmet) rather than the methionine commonly incorporated into proteins. Following the binding of this aminoacyl-t-RNA molecule, the second ribosomal subunit (50S in prokaryotes or 60S in eukaryotes) binds to the m-RNA.

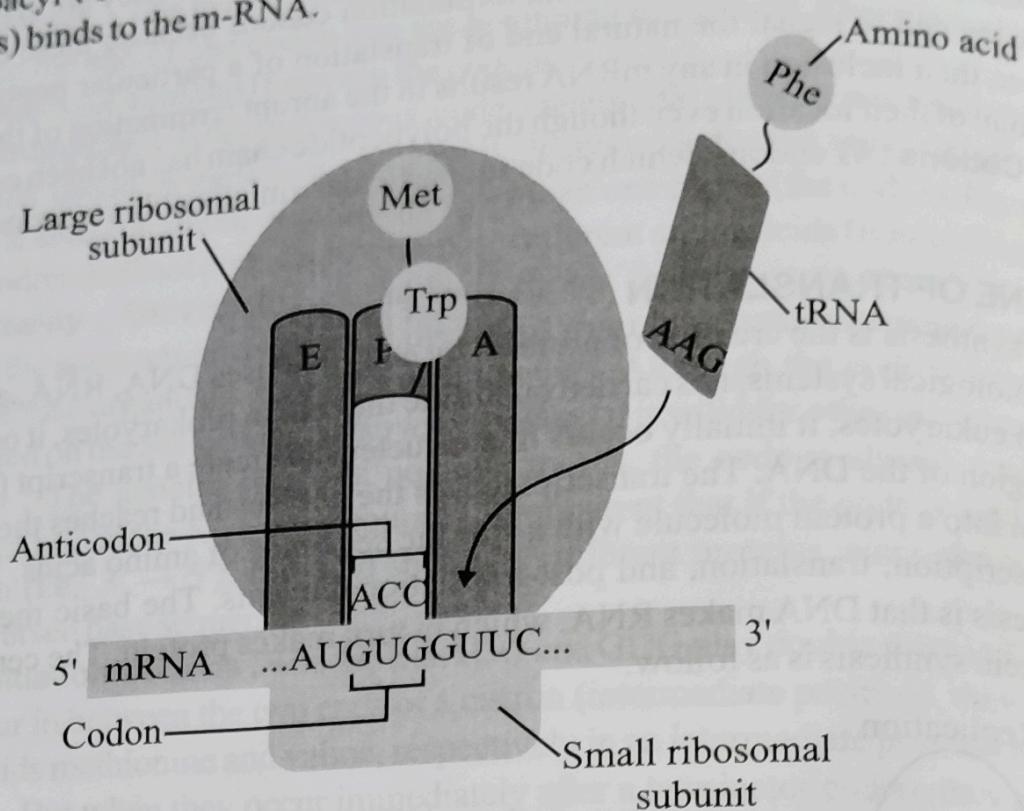


Fig. 2.5 : Sites on Ribosome

2) Elongation :

The large ribosomal subunit has three t-RNA binding sites designated as the A-site, the P-site and the E-site. The A-site is the amino acid binding site and binds with incoming t-RNA molecules (aminoacyl-t-RNA), i.e., those carrying individual amino acids to the ribosome. The P-site is the polypeptide binding site, and once translation has begun, will bind with the peptidyl-t-RNA molecule, i.e., the one holding the growing polypeptide chain. The E-site binds with the exiting-t-RNA, i.e., the free tRNA molecule remaining after the amino acid it was carrying has been added to the growing peptide chain.

The binding of aminoacyl-t-RNA molecules involves interaction between t-RNA anticodons and m-RNA codons. Hydrogen bonds forming between the complementary base sequences insure that each amino acid will be incorporated in the correct location. A ribosomal enzyme called peptidyl transerase catalyses chemical reactions resulting in the formation of peptide bonds between adjacent amino acids. Without this enzyme activity, proteins cannot be made. Peptidyl transferase activity apparently involves RNA molecules, not ribosomal proteins. Translation requires that the ribosomes travel along m-RNA molecules from the 5' end toward the 3' ends. As they do, the codons associated with the A-site change and new aminoacyl-t-RNA molecules can bind. As peptide bonds are formed between adjacent amino acids, the growing peptide chain is transferred from the t-RNA in the P-site to the one occupying the A-site (explaining the name peptidyl transferase). The t-RNA released by the transfer of the peptide chain, occupies the E-site until the ribosome moves, and is then released.

released. These "exiting" t-RNA molecules can again interact with aminoacyl-t-RNA synthetase enzymes, pick up new amino acids, and return to the ribosome, i.e., they can be used over and over again.

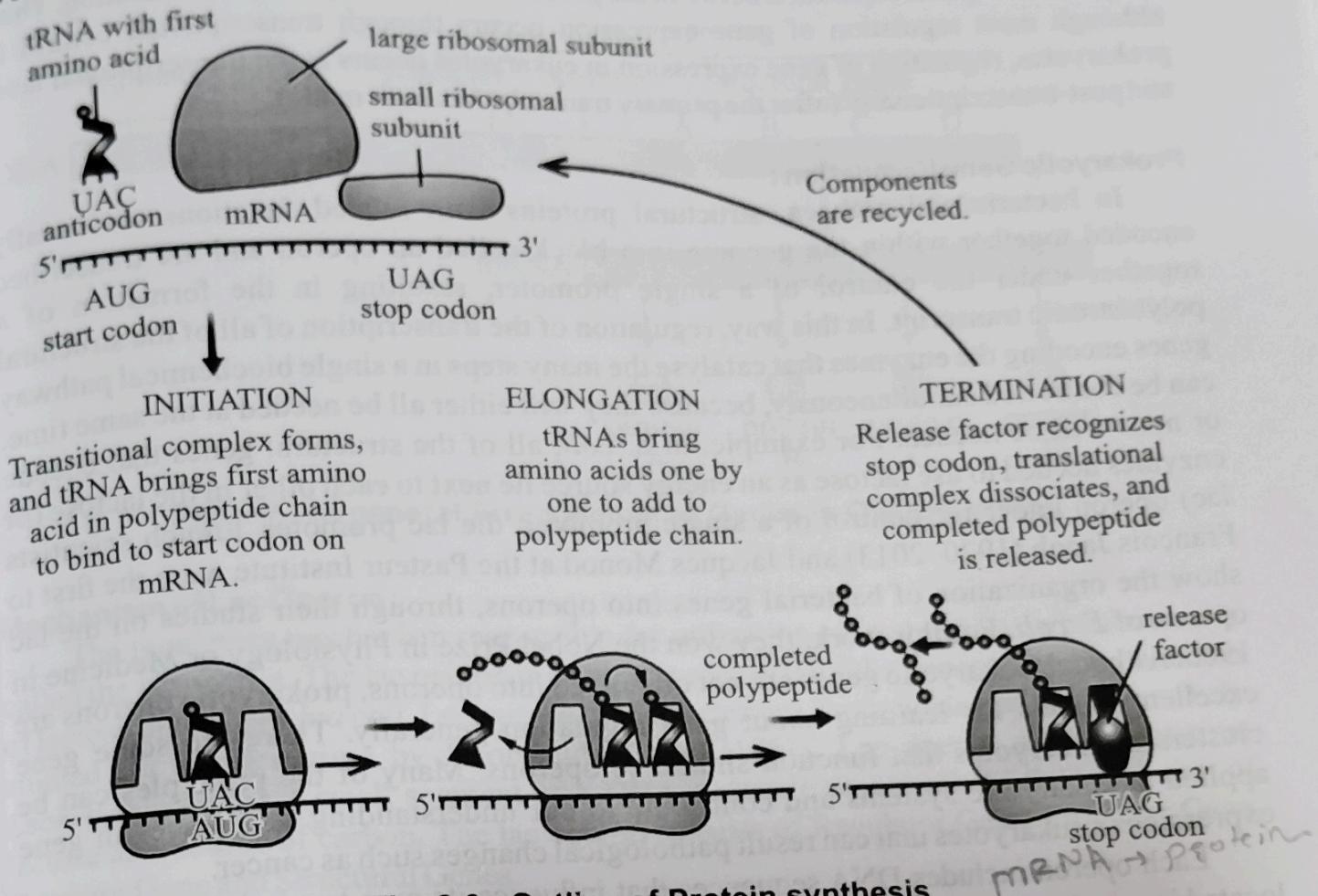


Fig. 2.6 : Outline of Protein synthesis

3) Termination:

Translation is terminated when the ribosome reaches a stop or terminator codon. In both prokaryotic and eukaryotic cells, the process involves the interaction of two or more proteins called chain release factors. When a stop codon is recognized, the finished polypeptide chain is released from the last t-RNA, i.e., the covalent bond attaching the last amino acid added to its corresponding t-RNA molecule is hydrolysed. Following this reaction, the t-RNA is released from the ribosome, and the two ribosomal subunits separate, releasing the m-RNA.

C) GENE REGULATION:

Genomic DNA contains both structural genes, which encode products that serve a cellular structures or enzymes, and regulatory genes, which encode products that regulate gene expression. The expression of a gene is a highly regulated process. Whereas regulating gene expression in multicellular organisms allows for cellular differentiation, in single-celled organisms like prokaryotes, it primarily ensures that a cell's resources are not wasted making proteins (especially enzymes- which use up energy as they are synthesized and they are speeding up chemical reactions) that the cell does not need at that time.

including DNA replication, repair, and expression, as well as enzymes involved in core metabolism. In contrast, there are other prokaryotic operons that are expressed only when needed and are regulated by repressors, activators, and inducers.

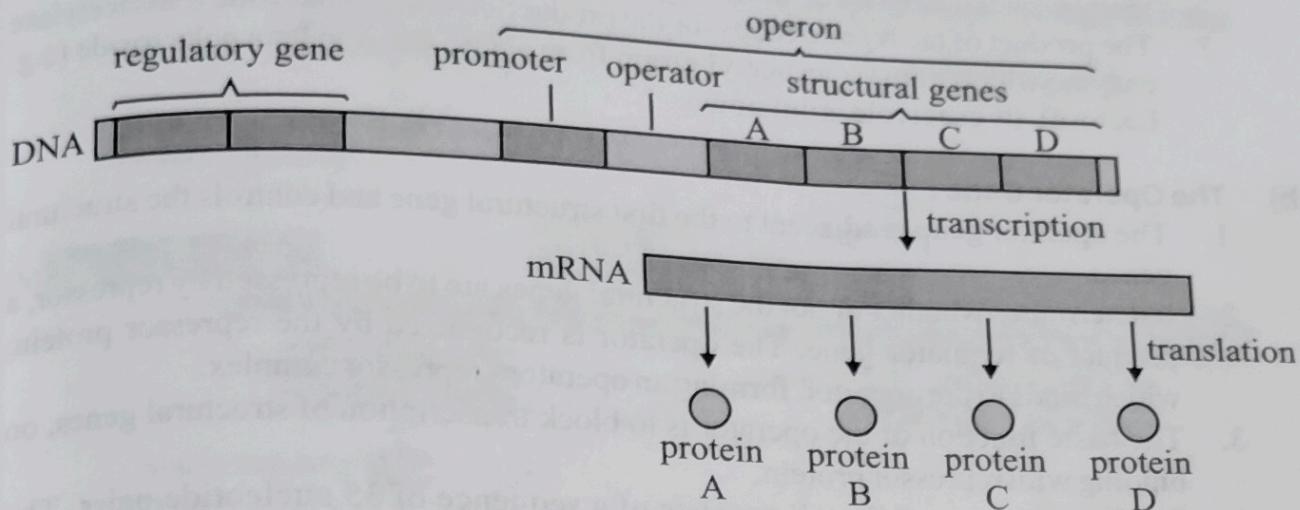


Fig. 2.7 : In general Arrangement of Genes in Operon Model

Mechanism of Lac Operon :

The regulatory mechanism responsible for utilization of lactose as a carbon source is called the lac operon. The lac operon is an operon required for the transport and metabolism of lactose in *Escherichia coli*. Lac operon of the bacterium *Escherichia coli* was extensively studied for the first time by Jacob and Monod (1961). Lac operon of the bacterium *Escherichia coli* is a small segment of a DNA molecule that regulates the utilization of lactose as a source of carbon. The lac operon consists of Regulator Gene, Promoter Gene, Operator Gene and Structural Genes.

a) The Structural Genes:

1. The structural gene directs synthesis of cellular proteins through messenger RNA (mRNA) and determines the sequence of amino acids in the protein synthesized.
2. Each structural gene may be controlled independently and transcribe separate m-RNA molecule (monocistronic), or all the structural genes of an operon may be controlled collectively and may form one long polycistronic mRNA molecule.
3. Lac operon of the bacterium *Escherichia coli* has three structural genes Lac Z, Lac Y and Lac A.
4. These three structural genes together transcribe single long polycistronic mRNA molecule, which controls the synthesis of three different enzymes.
5. The Lac Z gene codes for the production of β -galactosidase enzyme which breaks (β -1,4 glycosidic bond) lactose into glucose and galactose to be utilized in the cell. When lactose is absent in the medium only one or two molecules of β -galactosidases are present in the cell. But when lactose is added to the medium the structural genes are turned on and production of β -galactosidase starts and within two to three minutes about 3000 molecules are synthesized by the Z gene.

6. The Lac Y gene codes for β -galactoside permease (Lactose permease) enzyme which facilitates the entry of lactose into the cell. This enzyme spans the membrane and brings lactose into the cell from the outside environment. The membrane is otherwise essentially impermeable to lactose.
7. The product of lac A gene codes for the production of β -galactosidase enzyme which transfer an acetyl group from acetyl Co-A to beta galactoside (Lactose). Its exact role is unknown.

b) The Operator Gene :

1. The operator gene is adjacent to the first structural gene and controls the structural genes.
2. It determines whether or not the structural genes are to be repressed by repressor product of regulator gene. The operator is recognized by the repressor protein which binds to the operator, forming an operator-repressor complex.
3. The basic function of the operator is to block transcription of structural genes by binding with repressor protein.
4. The lac operator of *E. coli* consists of a sequence of 35 nucleotide pairs. 11 base pairs in the lac operator show a two-fold symmetry (palindromic sequences).
5. The lac operator gene binds to active lac repressor protein (which is a tetramer four subunits) and forms an operator-repressor complex, which in turn blocks transcription of Z, Y and A genes by blocking the path of RNA polymerase.

c) The Promoter Gene:

1. The promoter gene lies between the operator gene and regulator gene, and adjacent to the operator gene.
2. Like operators the promoter region consists of palindromic sequence (sequence, two fold symmetry) of nucleotides on its CRP site.
3. During transcription of structural genes, RNA polymerase (a transcribing enzyme) binds to the promoter region of lac operon.
4. When the operator gene is functional, the RNA polymerase moves over it reaches the structural genes to perform transcription.
5. The promoter region becomes much more attractive to RNA polymerase presence of c-AMP & CRP Protein (cyclic AMP receptor protein).
6. c-AMP and CRP protein form a complex which binds to the lac promoter which stimulates transcription and protein synthesis. This is known as positive control.

d) The Regulator Gene :

1. It is also called as Repressor gene.
2. In lac-operon, it is called i-gene because it produces an inhibitor or repressor.
3. The regulator gene synthesizes the repressor protein which binds to operator gene and controls the transcription of structural genes.
4. Repressor is meant for blocking the operator gene so that the structural genes unable to form mRNAs.

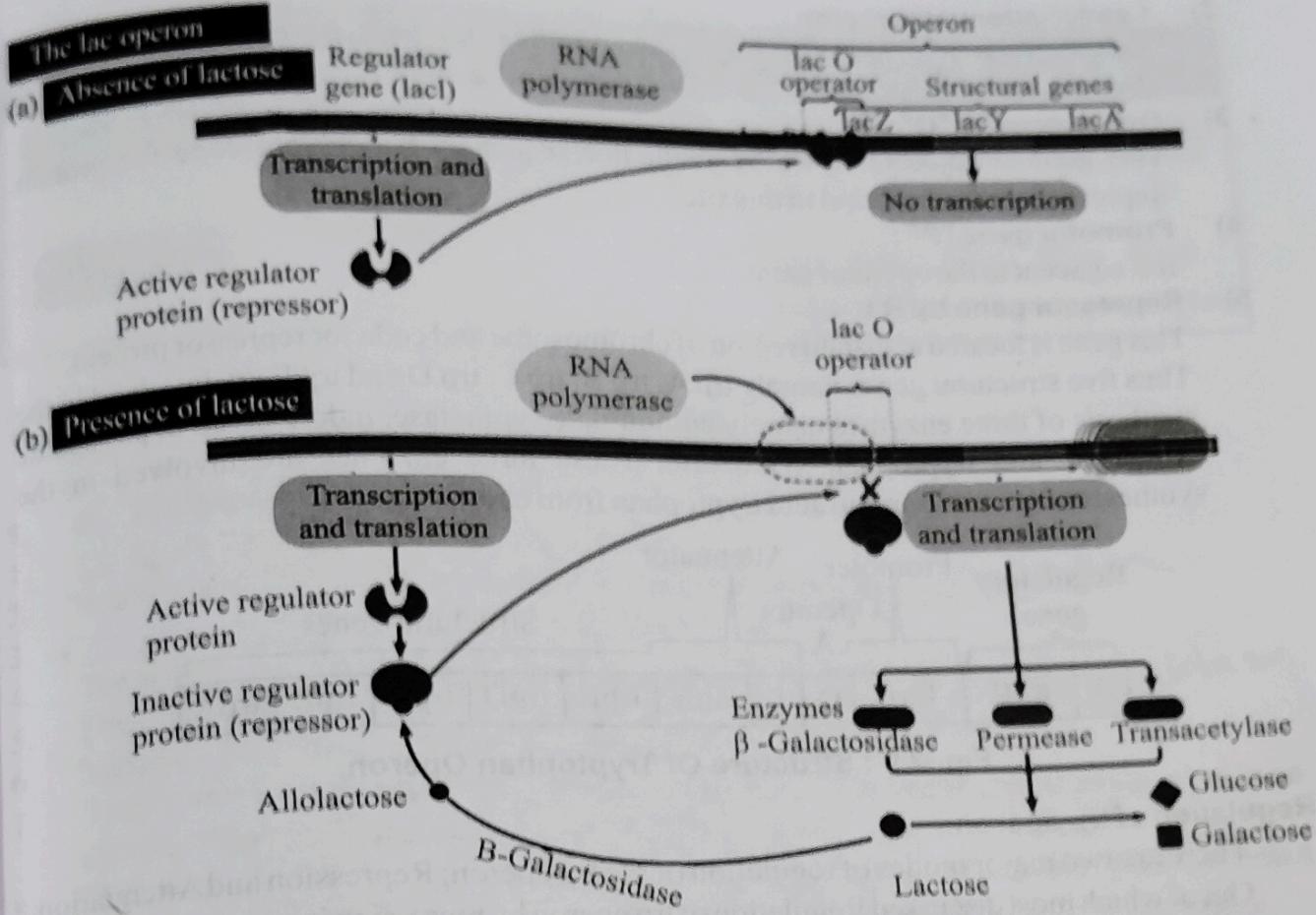


Fig. 2.8 : The lac operon

Trp (tryptophan) operon :

The trp operon of *E. coli* controls the biosynthesis of tryptophan in the cell from the initial precursor chorismic acid. It is a negatively regulated biosynthetic operon. Trp operon promotes the production of tryptophan when tryptophan is not present in the environment. This operon was discovered in 1953 by Jacques Monod and colleagues. The trp operon in *E. coli* was the first repressible operon to be discovered. This operon works in the opposite way of the inducible lactose operon. lac operon is induced by lactose, while trp operon is inhibited by a tryptophan.

Structure of tryptophan Operon : trp operon of *E. coli* contains following genes.

1. Structural genes:

There are five structural genes in trp operon. These are

- trp A :** This gene codes for enzyme tryptophan synthetase (enzyme A).
- trp B :** This gene also codes for enzyme tryptophan synthetase (enzyme B).
- trp C :** This gene codes for enzyme indole glycerol phosphate synthetase (enzyme C).
- trp D :** This gene codes for enzyme anthranilate synthetase (enzyme D).
- trp E :** This gene also codes for enzyme anthranilate synthetase (enzyme E).

They are used to kill cancer cells without having to resort to difficult surgery, is used in sterilizing medical equipment (as an alternative to autoclaves or chemical means), removing decay-causing bacteria from many foods or preventing fruit and vegetables from sprouting to maintain freshness and flavor.

Genetic Recombinations :

The term genetic recombination refers to the 'changes in the sequence of genetic nucleotides brought about by transfer of genes between the organisms. Genetic recombination is the process of forming new genetic combination in offspring by exchanges between genetic materials (as exchange of DNA sequences between DNA molecules). Genetic recombination in bacteria is caused by exchange of genetic material (DNA) between chromosome (large circular DNA) of one cell and segment of chromosome (fragment of DNA) of another cell. The term recombination is usually applied to more or less permanent changes in the organism. The site of recombination is localized between two adjacent nucleotides.

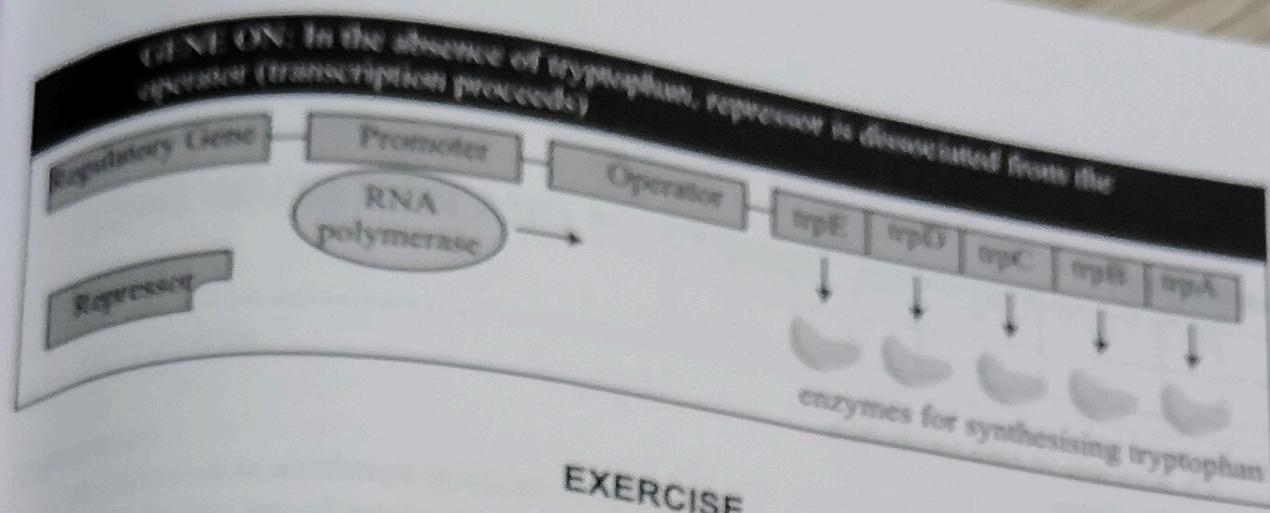
In eukaryotes, which are the diploid organisms, gene recombination is brought about during fertilization. During fertilization, haploid male gametes and female gametes fuse to form the diploid zygote. Bacteria (prokaryotic organism) are the haploid organism, and were thought to multiply only by binary fission. However, three different mechanisms were later discovered for transferring genes from one bacterial cell to another. Gene transfer in bacterial cells produce not completely diploid zygote but partially diploid zygote called merozygote. The original genome of the recipient cell is called the endogenote. The fragment of DNA introduced from a donor cell into a recipient cell is called the exogenote.

Mechanism of Recombination:

The mechanism of recombination has been explained by three general groups of theories.

1. Breakage and Reunion
2. Breakage and Copying
3. Complete Copy Choice.

1. **Breakage and reunion :** The breakage and reunion concept has been long used to explain genetic recombination. For example 'a' and 'b' are the two genetic loci and a^+ and b^+ their alleles. During synapsis the homologous chromosomes break between 'a' and 'b' and a^+ and b^+ . The piece then rejoin cross-wise, producing recombinants in which one chromosome contains 'a' and b^+ and the other a^+ and 'b' loci. The recombinant chromosome contains two pieces of parental chromosome information. Breakage and reunion type of recombination does not depend upon the synthesis of new DNA molecules. There is merely mutual transfer of DNA segments from one parental chromosome to the other.
2. **Breakage and copying :** The recombinant chromosome is formed by the breakage of one chromosome followed by copying of the other. Thus the two parental chromosomes 'ab' and a^+b^+ produce a recombinant which contains ab^+ and a^+b . The 'a' section of one parent is attached to the b^+ section copied from the other chromosome.



EXERCISE

A) Fill in the Blanks

- G. J. M. is known as father of Genetics.
- Sudden inheritable change in genetic material is known as Mutation.
- Jacob & Monod first time explain Operon model.
- The genetic code consists of 64 different codons.
- The Lac Z gene codes for the production of β -galactosidase enzyme.
- Initiation codes for N- amino acid, formylmethionine.
- Two or more independent pairs of genes which affect the same characteristics but in an additive fashion is known as cumulative gene.
- The gene which suppresses or inhibits the expression of another gene is called Inhibitory gene.
- If the two alleles are the same, the individual is homozygous.
- The shortest chromosomal unit capable of undergoing mutation has been called the Muton.

ANSWERS:

1. Gregor Johann Mendel, 2. Mutation, 3. Jacob & Monod, 4. 64, 5. β - galactosidase, 6. N-formylmethionine, 7. Cumulative gene, 8. Inhibitory gene, 9. Homozygous, 10. Muton

B) Choose Correct Alternative

1. The Lac Z gene codes for the production of which of the following enzyme?
 - a) β galactosidase
 - b) α galactosidase
 - c) Transacetalase
 - d) Permease
2. Genetic constitution of cell is known as _____.
 - a) Phenotype
 - b) Mutation
 - c) Genotype
 - d) Recon
3. Classical concept of gene was introduced by _____.
 - a) Sutton
 - b) Antonty Van Leewenhook
 - c) Mendel
 - d) Summner
4. _____ proposed one-gene - one protein hypothesis.
 - a) Sutton
 - b) Antonty Van Leewenhook
 - c) Mendel
 - d) Beadle and Tatum

Sickle cell anemia has an autosomal recessive inheritance pattern and leads to abnormal shaped red blood cells. The molecular change in sickle cell disease (SCD) is base substitution of valine for glutamate in the sixth position of beta globulin gene leading to production of abnormal hemoglobin S. In sickle cell anemia, once the hemoglobin S form is deoxygenated it has a tendency to polymerize that causes changes in red cell membrane structure and function leading to sickling of red cells and decrease in red cell deformability.

B) MOLECULAR BASIS OF MUTATION

- Gene mutation at molecular level involves substitution of one base by another, or addition or deletion of one or more bases.
- Mutations may occur in an organism by two mechanisms.
 - i) Spontaneous mutation
 - ii) Induced mutation.

SPONTANEOUS MUTATIONS

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

Tautomerism :

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

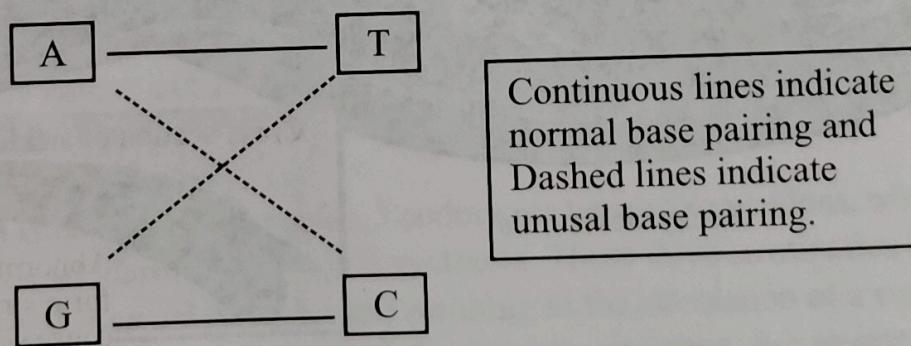
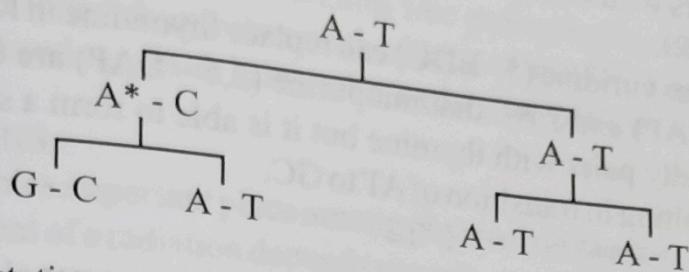


Fig. 3.6 : Tautomerism

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



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

INDUCED MUTATIONS

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

CHEMICAL MUTAGENS

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

1. Base Analogues :

- Base analogues are a chemical compound similar to one of the four bases of DNA.
- A base analogue may be incorporated into newly synthesized DNA instead of a normal base.
- These compounds have different base pairing properties.
- They replace bases and cause stable mutations.
- A very common and widely used base analogue is 5 - Bromouracil (5-BU) that is structurally very similar to thymine.

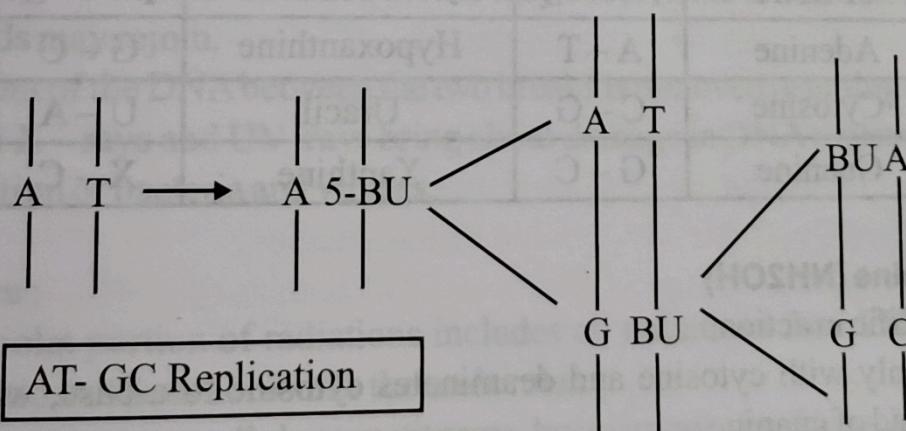


Fig. 3.7 : Mutation by Base Analogues

3. **Complete copy choice:** According to the copy choice theory, 'a' part of one parental strand serves as a template for the synthesis of a copy. The copying process is then suddenly switched over to the other parental strands. The recombinant carries some genetic factors of one parental strand and some of the other. Thus, the 'a' section is copied from one parent and the b+ from the other. The complementary recombinant type is produced by an entirely independent recombination event.

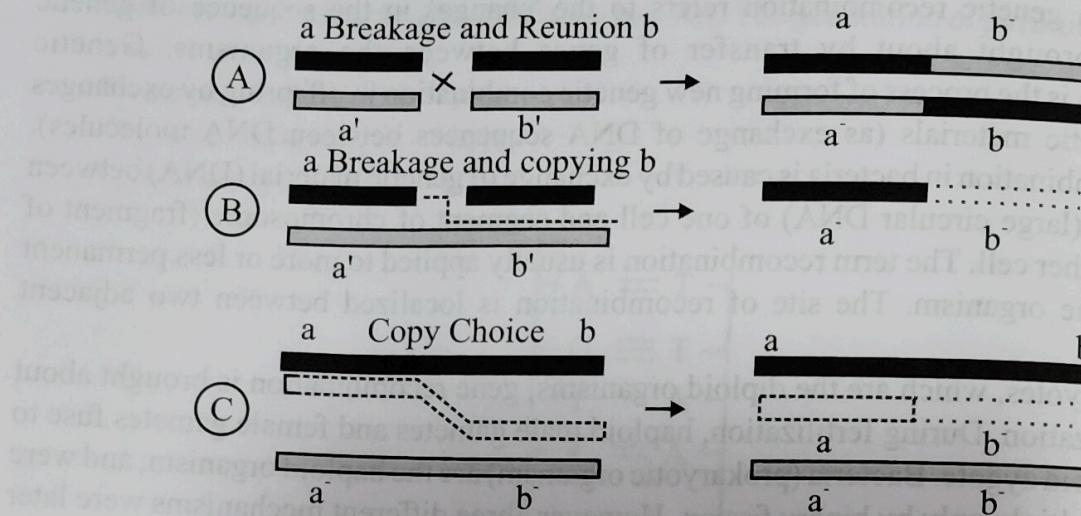


Fig. 3.9 : Mechanism of Recombination

Transfer of Genetic Material in Prokaryotes: Mechanism of Gene Transfer:

There exists three different mechanisms of gene transfer in prokaryotes. These mechanisms in the order of their discovery are as follows.

1. **Transformation :** In this mechanism, DNA is transferred as 'naked' DNA from one cell to another cell.
2. **Conjugation :** In this mechanism DNA is transferred from one cell (donor cell) to another (recipient cell) after coming in direct contact.
3. **Transduction :** In this mechanism DNA is transferred from one cell (donor cell) to another cell (recipient cell) by bacterial virus (bacteriophage)

a) BACTERIAL TRANSFORMATION :

This is one of the mechanisms of gene recombination.

Definition:

Transformation is the process of genetic recombination in which fragment of DNA from one enters and integrates in another cell. It is the process of genetic recombination where cell free or naked DNA containing a limited amount of genetic information is transferred from one bacterial cell to another. This is the asexual method of gene transfer.

Discovery of Transformation:

The process of bacterial transformation was discovered by an English health officer Frederich Griffith in 1928. This transformation was first discovered in bacterium *Streptococcus pneumoniae* or *Diplococcus pneumoniae*. He used two strains of *Diplococcus pneumoniae*. These are:

1. **'S' Strain (Capsulated *Diplococcus pneumoniae*)** : It is a wild strain of *Diplococcus pneumoniae* surrounded by polysaccharide capsule and forms colonies with smooth surface on solid nutrient medium. Hence, capsulated *Diplococcus pneumoniae* also called as smooth cells or S cells. This strain is virulent (pathogenic), and if injected into mice produces pneumonia and cause death.
 2. **'R' Strain (Non-capsulated *Diplococcus pneumoniae*)** : It is a mutant strain of *Streptococcus pneumoniae* and do not have a capsule and produces rough colonies on solid nutrient medium. Hence, non-capsulated *Diplococcus pneumoniae* also called as rough cells or R cells. It is avirulent (non-pathogenic) and if injected into mice, do not cause any harm. The mutant, non-capsulated strain had a defective gene so that it could not produce the capsule.

Griffith's Experiment:

To discover transformation process, Griffith performed following experiment:
He injected living, virulent, capsulated cells of *D. pneumoniae* to mice.

1. He injected living, virulent, capsulated cells of *Diplococcus pneumoniae* (S cells) into mice. He found that mouse suffers from severe septicemia and dies of pneumonia.
 2. Griffith injected avirulent, non-capsulated *Diplococcus pneumoniae* (R cells) into mice and it was found that it resulted in no development of pneumonia & mice remains healthy.
 3. Thirdly, Griffith injected a group of mice with heat killed capsulated *Diplococcus pneumoniae* (heat killed 'S' cells) into mice and again mice were found to be unaffected and it resulted into no development of pneumonia keeping mice healthy.
 4. Later on, he injected a group of mice with a mixture of heat killed capsulated 'S' cells and live, non-capsulated (R cells) cells. He was very much surprised to see that mice were infected and died within few days.

He isolated live, virulent, capsulated S cells from the blood of such died mice. From the above experiment Griffith concluded that some substance have been transformed from heat killed 'S' cell to living 'R' cell and thus converting 'R' cell to 'S' cell. Griffith called this change of avirulent, non-capsulated 'R' cell into virulent, capsulated 'S' cell as Transformation or Griffith effect.

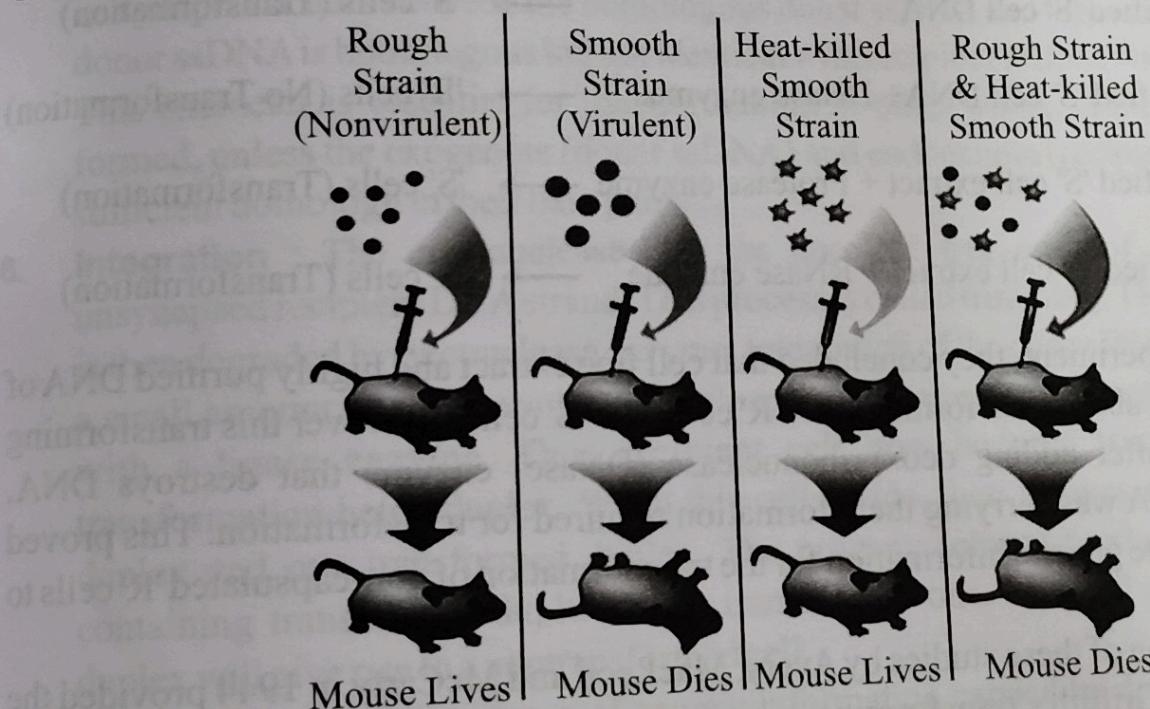


Fig. 3.10 : Griffith's Experiment

proved for first time that DNA (gene) is a carrier of genetic information. Later on genetic transformation has been demonstrated in several other bacteria including *Haemophilus influenza*, *Bacillus subtilis*, *Neisseria meningitidis*, certain strains of *Streptococci*, *Staphylococci*, *Rhizobium* etc. Transformation process also reported in higher organisms including insect *Drosophila*, *Bombyx*, mice & in vitro cultured cells of human being.

Mechanism of Transformation :

The various events of bacterial transformation are represented below.

1. **Lysis of donor cell :** There is a release of chromosome upon the lysis of donor cell.
2. **Fragmentation :** The bacterial chromosome (DNA) is randomly fragmented into about 100 pieces, each piece consisting of about 20 genes. Therefore, all 20 genes can be transferred simultaneously.
3. **Binding :** Donor DNA fragment comes in contact with recipient cell by random collision. Donor DNA fragment binds to the surface of competent recipient cell. Binding generally take place at various receptor sites which are associated with mesosome, dsDNA transformation takes place more effectively while ssDNA transformation takes place less effectively (only 0.5%).
4. **Penetration :** Binded donor DNA penetrates into the recipient cell. In most cases, double stranded (ds) molecule of DNA is converted to single stranded DNA (ssDNA). In *Bacillus subtilis* both strands of DNA enter into the cell and then one strand is degraded. According to one hypothesis, one end of ds DNA enters the cell where an endonuclease digest one of the strand, while the other strand is pulled into the cell. After penetration the donor DNA migrates from periphery of the cell to the recipient DNA. Penetrating DNA must have minimum length of 750 base pairs.
5. **Synapsis :** Penetration is followed by synapsis. The recipient DNA unwinds and initiates pairing with the single stranded donor DNA, called as synapsis. Base pairing i.e synapsis occurs between the homologous donor ssDNA and the recipient DNA. The donor ssDNA is homologous but not identical with recipient ssDNA with which it pairs. This character is essential for transformation. In other words, recombinants are not formed, unless the exogenote (donor ssDNA) and endogenote (recipient ssDNA) have sufficient homology in their base pairs.
6. **Integration :** The endonuclease cuts the unpaired free ends of donor DNA and unsynapsed recipient DNA strand. This process is called trimming. This DNA fragment is then degraded by exonuclease enzyme. Integration of the donor DNA strand involves a small amount of repair synthesis to close the gap present, followed by final sealing with a ligase enzyme. Thus recipient cell now become transformed cell or transformation heteroduplex. When this cell divide, then it gives rise to one normal duplex and one transformed duplex. The progeny (clone) produced from the cell containing transformed duplex is the transformed one. The cell containing normal duplex will give rise to a nontransformed cell.
7. Lastly, there is the expression of genetic information carried by donor DNA fragments through the recipient cell and its progeny.

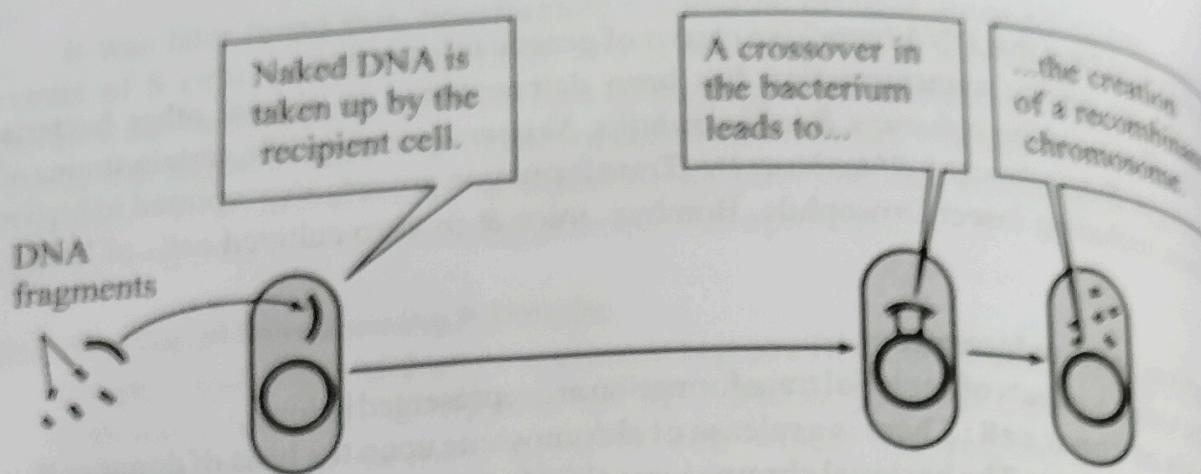


Fig. 3.11 : Mechanism of Transformation

Competence:

The recipient bacteria must be competent for accepting donor DNA fragments undergoing transformation process. The cell wall of recipient bacteria becomes permeable to the donor DNA during the competence phase. Freifelder (1987) has defined competence as a physiological state that permits a cell to take up transforming DNA.

The competence of bacteria is not a permanent feature but occurs only at certain times in the life cycle. The duration and the time of occurrence of the competence stage varies with bacterial species and condition of growth. Competence is commonly observed towards the end of the logarithmic growth phase, just before the establishment of stationary phase. There are several factors that affect competence such as growth medium temperature, degree of aeration, etc. influences the development of competence in cells.

On the basis of development of competent state the organisms undergoing transformation can be divided into two groups: organisms always present in competent stage (e.g. *Neisseria*) and the organisms transiently competent in late exponential phase of growth (e.g. *S. pneumoniae*).

b) TRANSDUCTION:

Definition:

Transduction is the process of gene recombination in which there is a transfer of a fragment of DNA from one bacterial cell (donor) to another (recipient cell) by bacteriophages (viruses), this is the asexual method of gene transfer.

Discovery of Transduction:

The phenomenon of transduction was discovered by Joshua Lederberg and Norton Zinder in 1952, while searching for sexual conjugation in *Salmonella* species. Usually, transduction occurs most readily between the closely related species of same genus of bacterium i.e intragenic. In addition, intergenic transduction has been shown between the closely related enteric bacteria such as between *E. coli* and *Salmonella* or *Shigella* species.

Experiment of Zinder and Lederberg :

Transduction was first discovered in *Salmonella typhimurium*. They used following auxotrophic mutants of *Salmonella typhimurium*.

Discovery of Conjugation:

Conjugation was first discovered by Joshua Lederberg and Edward Tatum (1946) in bacterium *Escherichia coli*.

Experiment of Lederberg and Tatum

E. coli does not require any growth factor for their cultivation. Lederberg and Tatum produced two auxotrophic (defective) strains of *E. coli* by mutation.

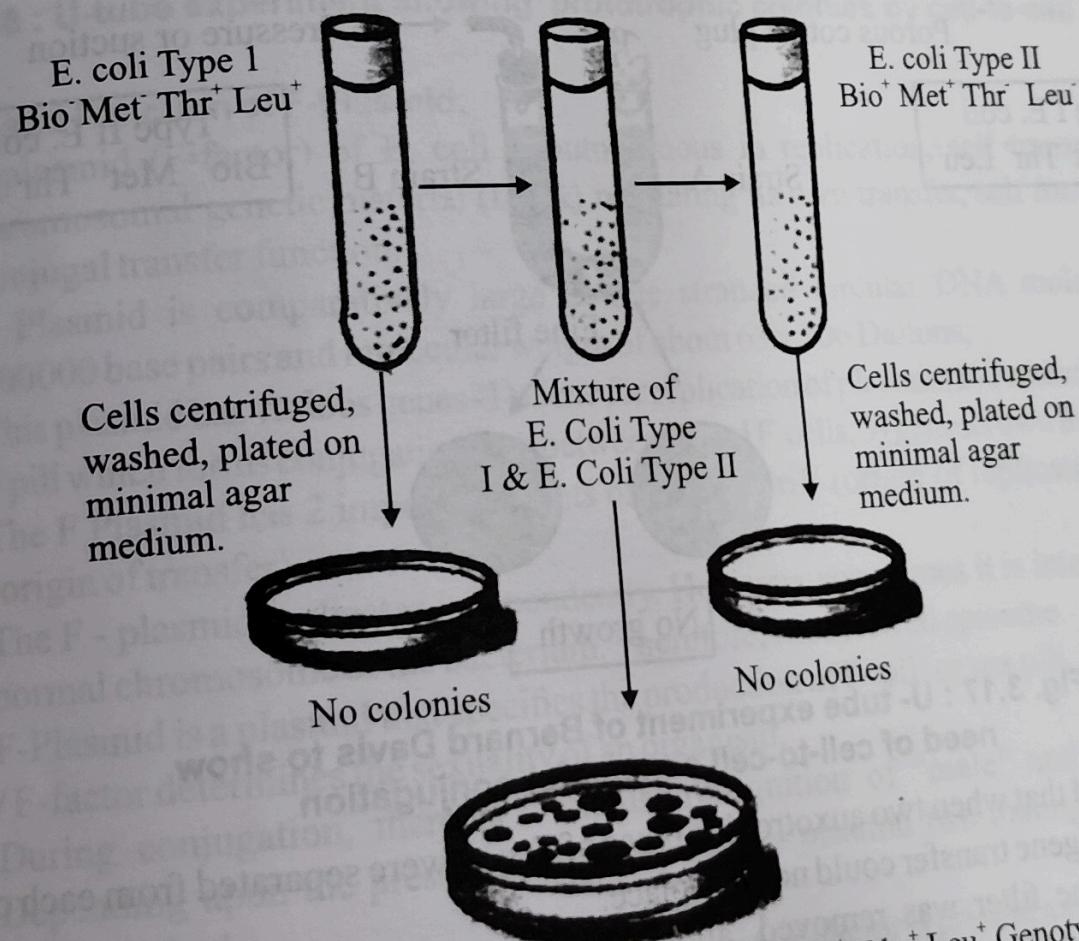
- Type I : cannot produce Biotin and Methionine but produce Threonine and Leucine.
- Hence genotype of this parental type is designated as

Bio⁻ Met⁻ Thr⁺ Leu⁺

- Type II : Cannot produce Threonine and Leucine but produce Biotin and Methionine.
- Hence genotype of this parental type is designated as

Bio⁺ Met⁺ Thr⁻ Leu⁻

- Type I *E. coli* (Bio⁻ Met⁻ Thr⁺ Leu⁺) was plated on a minimal agar medium containing none of the growth factor. It was found that Type I *E. coli* cannot grow on the medium.
- Type II *E. coli* (Bio⁺ Met⁺ Thr⁻ Leu⁻) was plated on a minimal agar medium containing none of the growth factor. Again there was found to be no growth.



PROTOTROPHIC COLONIES with Bio⁺ Met⁺ Thr⁺ Leu⁺ Genotype
Conclusion: Gene transfer (genetic recombination) between two different strains taken place

Fig. 3.16 : Lederberg – Tatum Experiment

- When Hfr mates with F⁻, transfer of entire chromosome takes ~100 minutes (in E. coli) but very rare.
- Usually, the conjugation terminates before the entire chromosome moves across.
- Only a part of the F factor strand is transferred to the recipient cell along with the chromosome. So recipient F⁻ remains F⁻.
- In a cross between Hfr cell and F⁻ cell, F⁻ cells always remains F⁻ because separation of cells occurs before the final transfer of F factor.

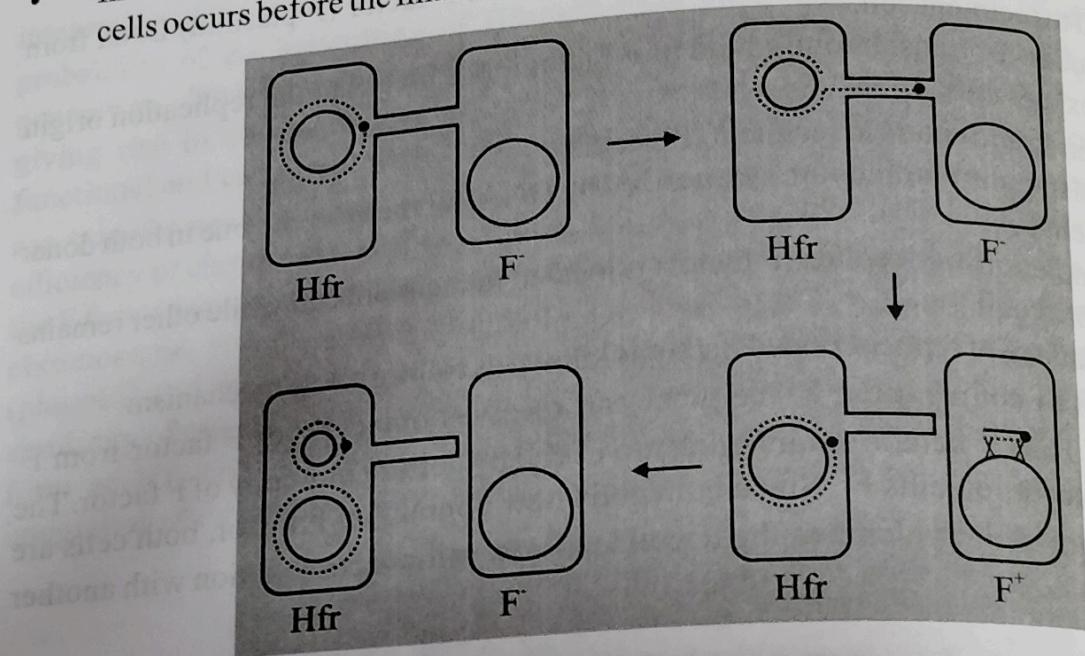


Fig. 3.21 : Conjugation Between Hfr and F- cells.

EXERCISE

1. A) FILL IN THE BLANK

- Segment of DNA capable of undergoing mutation is known as Muton.
- Mutation occurring due to mutagenic agents are called as Induced mutation.
- The formation of thymine dimer is caused by Ultra violet light.
- Mutation due to addition or deletion of nucleotide is called as Frameshift mutation.
- In nonsense mutation, codon in mRNA mutated to Stop codon.
- Transfer of DNA from one bacterial cell to other cell by bacteriophage is called as Transduction.
- In conjugation, the fragment of DNA is transferred from donor cell to recipient cell by direct cell to cell contact.

ANSWERS :

1. Muton, 2. Induced, 3. Ultra-violet, 4. Frameshift mutation, 5. Stop codon, 6. Transduction
7. Conjugation

- B) CH
1. 1. Insertion
2. a) Bas
c) Nit
Mutat
3. a) Sp
c) Si
Griffi
4. Lay Tran
5. a) S
c) E
In
6. Lay
- ANSWER
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B) CHOOSE THE CORRECT ALTERNATIVE:

1. Mutation in which purine is replaced by purine is known as _____ mutation.
a) Transition b) Transversion c) Inversion d) Frame shift
2. Insertion and deletion mutation is caused by _____.
a) Base analogues b) Acridine dye c) Nitrous oxide d) Hydroxylamine
3. Mutation occurring due to acridine dye are _____.
a) Spontaneous mutation b) Frame shift mutation
c) Silent mutation d) Non sense mutation
4. Griffiths experiment proved:
a) Transformation b) Transduction
Transformation was first discovered in:
a) *Streptococcus pneumoniae* b) *Salmonella typhimurium*
c) *E. coli* d) None of the above
5. In _____ naked fragment of DNA is transformed from one cell to other.
a) Transformation b) Transduction
c) Conjugation d) All of the above
6. In _____ naked fragment of DNA is transformed from one cell to other.
a) Transformation b) Transduction
c) Conjugation d) All of the above

ANSWERS:

1	2	3	4	5	6
a	b	b	a	a	a

1. C) ANSWER IN ONE SENTENCE.

1. What is mutation?
2. What are induced mutations?
3. What are transition mutations?
4. What are transversion mutations?
5. What is conjugation?
6. What is transduction?

2. SHORT ANSWER QUESTIONS

1. Describe in brief nonsense mutation.
2. Describe in brief spontaneous mutation.
3. Describe ultraviolet rays as mutagenic agent.
4. Describe in brief mutation by nitrous oxide.
5. Describe in brief physical mutagens.
6. Describe in brief base pair substitution mutation.
7. Describe in brief induced mutation.
8. Explain molecular basis of spontaneous mutation.
9. Describe in brief missense and nonsense mutation.
10. Explain mutagenic effect of 5- Bromo uracil.
11. Explain in brief Frame- shift mutation.
12. Describe in brief Griffiths experiment.
13. Explain formation of Hfr cell.

A) INTRODUCTION TO BASIC TECHNIQUE OF GENETIC ENGINEERING

Genetic engineering is a technique, where genes are transferred from one cell to another to produce recombinant DNA. The recombinant DNA is used for the synthesis of valuable products such as insulin, drug etc. Genetic engineering implies "changing genes" these changes may occur as the result of -

- The transfer of a gene from the normal location to a cell which does not normally contain it & Altering its sequence in some way, so that it's a different gene.
- transfer of altered gene to new cell.
- Integration of gene with a carrier molecule plasmid and transfer to a new cell.

Concepts of Genetic Engineering :

The manipulation of genetic make-up of living cells by inserting desired genes through a DNA vector, is the genetic engineering. In France, this technology is called biomolecular engineering.

In 1973, Stanley Cohen and Herbert Boyer designed a methodology for transferring certain genes from one organism to another. Now it is a popular technique in almost all branches of applied biology. Gene is a small piece of DNA that encodes for a specific protein. A desired gene is inserted into a vector DNA so that a new combination of vector DNA is formed. The DNA formed by joining DNA segments of two different organisms is called recombinant DNA (rDNA) or chimeric DNA. The gene is introduced into a cell in the form of recombinant DNA; hence the gene manipulation is known as recombinant DNA technology. The organism whose genetic make-up is manipulated using recombinant DNA technique is called as recombinant or genetically manipulated organism (GMO). Genetic engineering has many applications in Agriculture, Animal sciences, Industry and Medicine.

Introduction to Basic Technique of Genetic Engineering:

The basic technique of genetic engineering is simple. Genetic engineering is the construction of a recombinant DNA molecule, which contains DNA fragments (genes) from different and unrelated organisms. Genetic engineering or rDNA technology involves several steps as:

- Isolation of the desired gene of DNA
- Selection & Isolation of Vector
- Insertion of the desired gene into a cloning vector to create rDNA or chimeric DNA.
- Introduction of recombinant vectors into host cells (eg. Bacteria)
- Multiplication and selection of clones containing the recombinant molecules
- Expression of the gene to produce desired product.

Isolation of the desired gene:

Specific DNA fragment or desired gene is to be identified, isolated and purified. DNA of interest is known as passenger DNA, target DNA or donor DNA.

Selection & Isolation of Vector:

If a desired gene is to be introduced into a host cell, a carrier molecule that can transport the gene into the host cell is required. Such a molecule is called a cloning vehicle, carrier molecule, or a vector. A vector is a self-replicating molecule of DNA to which the donor molecule can be linked. The vector should also be able to propagate in the host organism or cell. Commonly plasmids and the DNA of viruses are used as vectors.

Construction of Recombinant Vector:

The donor DNA is inserted into the vector to produce a donor-vector hybrid DNA molecule. This hybrid is also called a recombinant DNA, a chimera or a recombinant vector as it contains DNA from unrelated sources.

Introduction of the Recombinant Vector into the Host Cell:

The recombinant vector is introduced into a suitable host cell. Such host cell is also called target cells. Inside the host cell the recombinant DNA is either linked to the host chromosome or remains free in the cytoplasm. Inside the target cell the recombinant vector DNA may multiply. Thus numerous copies of recombinant DNA are formed. This means, the desired gene, it carries also multiplied.

Selection & Multiplication of host cells carrying Recombinant DNA:

The cells, which have successfully taken up the recombinant DNA molecule, are selected. These cells are allowed to divide. After a large number of cell divisions, a colony of identical host cell is produced. At the cellular level it is a clone of cell. Each cell in a clone carries one or more recombinant DNA molecules. Thus the desired gene carried by the recombinant DNA is cloned.

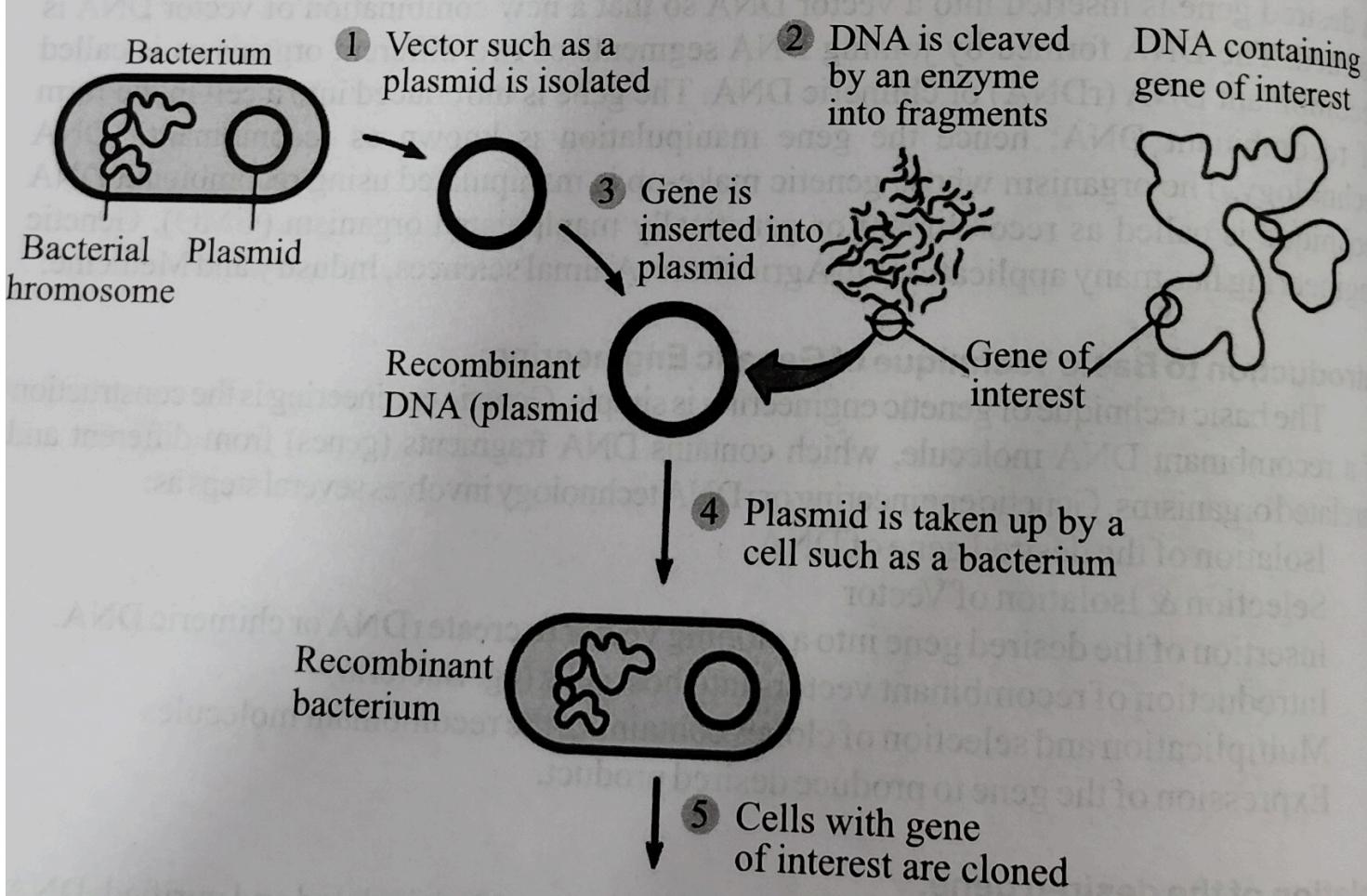


Fig. 4.1 : Genetic engineering

E. coli DNA ligase and T 4 DNA ligase are extensively used for covalently joining DNA fragments.

E. coli DNA ligase joins the cohesive or sticky ends of DNA fragments, whereas T 4 DNA ligase has the ability to join the blunt ends of DNA fragments.

3. **Polymerases**: Polymerases carry out the process of polymerization of nucleotides resulting in the formation of polynucleotide chain. Polymerase synthesize The polymerase that polymerizes the deoxyribonucleotides are called DNA Polymerases and the polymerase that polymerizes the ribonucleotides are called RNA Polymerases.

In 1957, for the first time A. Kornberg discovered an enzyme in *E. coli* which polymerized the deoxyribonucleotide triphosphate on a DNA template and produced new complementary strand of DNA. This enzyme was called DNA polymerase. In prokaryotes, following three types of DNA polymerases are found.

- DNA polymerase I (Pol I)
- DNA polymerase II (Pol II)
- DNA polymerase III (Pol III)

Pol III is the main and efficient polymerization enzyme and synthesizes new DNA strand on DNA template by polymerizing nucleotides in 5' to 3' direction. DNA polymerase enzyme also shows Exonuclease activity.

4. **DNA Modifying Enzymes:**

DNA modifying enzymes modify DNA molecules by removing or adding specific chemical groups. Some important DNA modifying enzymes are as like;

- i) **Alkaline phosphatase** : Alkaline phosphatase catalyses the removal of 5'-phosphate group from RNA, DNA and ribo-and deoxyribonucleotide triphosphates. This enzyme is a dimeric glycoprotein with a molecular weight 14,000. It is obtained from *E. coli* or calf intestinal tissue. It is a zinc containing enzyme with four atoms of zinc per molecule. Alkaline phosphatase is used for the dephosphorylation of 5'- phosphorylated ends of DNA and RNA. This enzyme is also used for mapping and finger printing studies.
- ii) **Polynucleotide kinase** : Polynucleotide kinase has the reverse effect and catalyses the transfer of the terminal phosphate group of ATP to a free 5' end of a DNA or RNA molecule. It is obtained from *E. coli* infected with T 4 bacteriophage. Polynucleotide kinase is used to label 5' terminal of DNA and RNA with [r2P] ATP. This 5' terminal labeling is used in mapping of restriction sites, DNA or RNA fingerprinting, hybridization studies and sequence analysis of DNA.
- iii) **Terminal deoxynucleotidyl transferase (Terminal transferase)** : deoxynucleotidyl transferase catalyses the repetitive addition of mononucleotide units from a deoxynucleoside triphosphate to the terminal 3'-hydroxyl group of a DNA molecule. The nucleotides can be attached to ssDNA as well as dsDNA. It is obtained from calf thymus tissue. This enzyme has a molecular weight of 32000 and consists of two subunits. Terminal transferase is used to add homopolymer tail to DNA fragments.

C) RANGE OF DNA MANIPULATING ENZYMES:

- Recombinant DNA technology/gene manipulation involves cutting, joining, and transporting of DNA molecules. Each process is carried out by specific enzymes. There are several enzymes, which are used to manipulate the structure of DNA and RNA in a defined way. All these enzymes are together called manipulating enzymes. There are broad classes of enzymes that are used in DNA manipulation techniques. These enzymes are
1. Nucleases
 2. Ligases
 3. Polymerases
 4. DNA Modifying enzymes
 - a. Topoisomerases
 - b. Reverse transcriptase

These enzymes are described below.

1. **Nucleases:** Nucleases are enzymes that hydrolyse nucleic acids by breaking down polynucleotide chain into its component nucleotides. They can cut, shorten or degrade nucleic acid molecules. The nucleases enzyme that attacks and breaks DNA is called deoxyribonucleases (DNase). So also enzyme that attacks and breaks RNA is called ribonucleases (RNase). They attack either at 3'-OH end or 5'-phosphate end of the chain. Some important examples of nucleases are: Nuclease SI, DNase 1, Exonuclease 1. Nuclease Bal 31 etc. Nucleases are of two types -

- a) **Exonucleases:** Some nucleases attacks on outer free ends of the polynucleotide chain and removes terminal nucleotides one at a time. These are called exonucleases. It breaks phosphodiester bond either in 5' to 3' direction or in 3' to 5' direction. The enzyme moves stepwise along the chain and removes nucleotides one by one. Thus, the whole polynucleotide chain is digested broken down.

- b) **Endonucleases:** Some nucleases attack within the inner portion of one or double strands and break the phosphodiester bond (sugar-phosphate backbone) non-terminal sites. These are called endonucleases. If the polynucleotide chain is single stranded (eg. in DNA viruses), the attack of endonuclease will break chain into two pieces. Endonuclease makes the 'nick' on double stranded DNA. In double stranded DNA the nick contains two free ends that in turn act as template for DNA replication. The most important group of nuclease enzymes used in genetic engineering are restriction endonucleases.

2. **Ligases:** DNA ligase is a group of enzymes that brings about joining individual DNA fragments. These enzymes play active role in DNA mechanism, DNA replication and genetic recombination's. DNA ligases are used in genetic engineering. Since ligases join DNA fragments, they are molecular sutures. A DNA ligase seals a nick in the polynucleotide chain by 3'-OH and 5' monophosphate group forming phosphor-di ester bonds. This can join a 5'- phosphate group only when both are terminals of adjacent nucleotides. The enzyme cannot seal a gap when one or more nucleotides are absent.

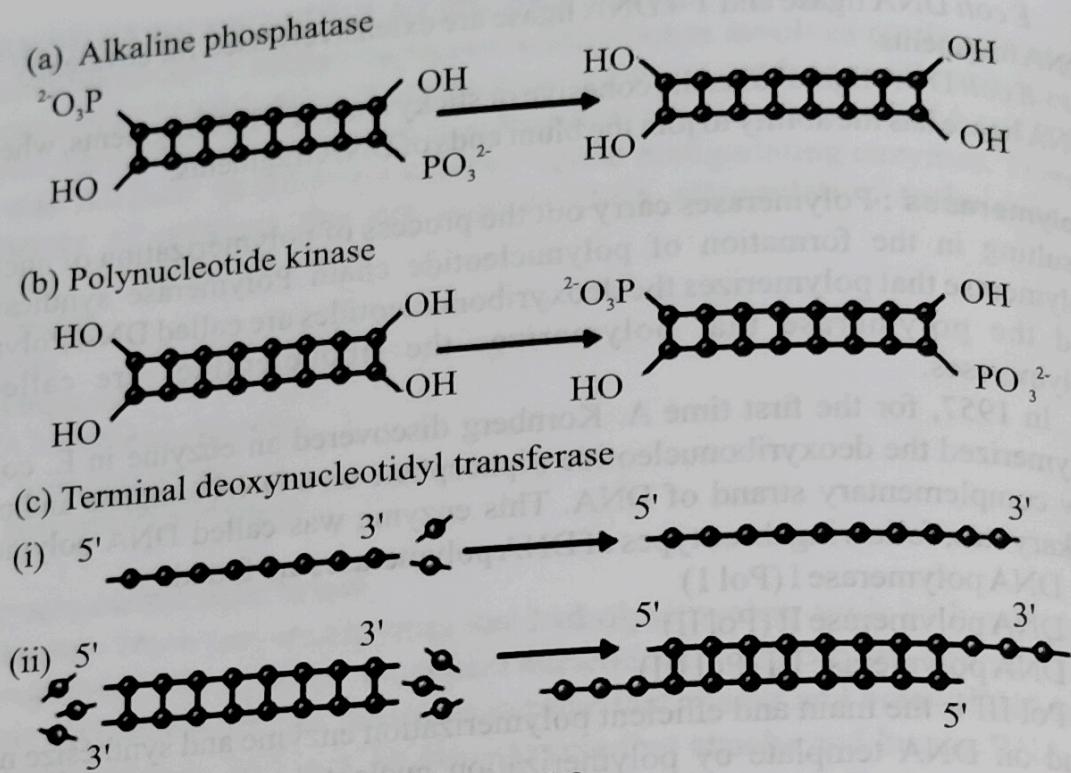


Fig. 4.3 :

Topoisomerase:

Topoisomerases are group of enzyme that controls the supercoiling in DNA. It removes the supercoils from covalently closed, circular DNA molecules. These are the superhelix relaxing proteins. In supercoiling, the DNA molecule coils up like a telephone cord, which shortens the molecule. The topoisomerases act by catalyzing a three step process.

1. The cleavage of one or both strands of the DNA
2. Passing of a segment of DNA through this break
3. The resealing of the DNA break

This enzyme has both nicking (cutting) and closing (joining) enzymatic activity.

There are two main classes of topoisomerases; i.e. Topoisomerase I - Topoisomerase I which binds to DNA and cuts a single strand of DNA duplex, passes the uncut strand through the break, then reseals the break. Thus removes the accumulated supercoils. These topoisomerases do not require any expenses of ATP. (ii) Topoisomerase II - Topoisomerase II binds to DNA and creates double stranded break, passes the uncut DNA through the gap, then reseals the break. These topoisomerases requires ATP consumption.

The removal of DNA supercoiling is essential for DNA transcription and replication. Topoisomerases serve to maintain both the transcription and replication of DNA. During DNA replication, Type II Topoisomerases or topo II, plays an important role in the replication fork progression by continuous removal of the excessive positive supercoils that starts from the unwinding of the DNA strand.

Reverse transcriptase:

Reverse transcriptase is an enzyme isolated from Avian Myeloblastosis Virus (AMV). It is an RNA-dependant DNA polymerase. This enzyme requires RNA template and a primer with

3'-OH group
transcriptas
has severa
dependant
enzyme is
Gene
receives d
host cell.
has to int
chromos
chromos
termed
Cloning

D) VI

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- 4.
- 5.

Type

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- 5.
- 6.
- 7.

Characteristic Features of an Ideal Plasmid Vector :

All the plasmids present in the bacterial cells are not useful for the genetic engineering works. Because some plasmids lack the property of carrying the foreign DNA fragment along with their own sequences. Some plasmids replicate only in the cells from which they were isolated, but fail to replicate in the host cell. A plasmid vector must have the following characteristic features for the successful gene cloning as given below;

1. The plasmid DNA must be smaller in size.
2. The plasmid must have the capacity to transfer the foreign DNA fragment into the host cell.
3. It must be a shuttle vector which replicates both in the donor cell and in the recipient cell.
4. It must integrate the foreign DNA fragment into the genome of the host cell.
5. The plasmid must have one or more genetic markers to detect the presence of cloned genes in the organism.
6. The plasmid should not have any pathogenic property.
7. The plasmid must have its own regulatory genes for the replication.

Properties of Plasmids :

Plasmids have the following characteristics:

1. Plasmids are circular double stranded molecule.
2. They are genetically dispensable elements in the sense that they are usually non essential for the growth of cells.
3. They replicate independently or autonomously.
4. They have an origin of replication site naturally in them.
5. They are passed on to the daughter cells during cell division.
6. They may carry very important genes for antibiotic resistant toxin production, for antibody production, for degradation of a large number of unusual substrates such as, herbicides or industrial effluents and genes for nitrogen fixation. These confer the phenotypic traits of plasmids.
7. They propagate the DNA fragments linked to them by in vitro ligation.
8. They may be present in large number of copies (more than 2 to hundreds).
9. They can acquire chromosomal genes by several mechanisms.
10. They rely on the DNA replication enzymes of the host cell for their replication ;however ,the initiation of replication is controlled by plasmid genes.
11. Certain plasmids do not show any phenotypic traits. Such plasmid called cryptic plasmids.
12. They have high transformation efficiency.
13. They have convenient selectable markers such as antibiotic resistance, k production, etc. for transformants and recombinants.
14. They have the ability to clone reasonably large pieces of DNA, say about 5 kb pairs
15. They are of low molecular weight.
16. They have single sites of a large number of restriction enzymes, prefer in genes with a readily scorable phenotypes.
17. They are easily isolated and purified.

Plasmid Types:

Bacterial Plasmids as Vector:

Bacteria are the common source of natural plasmids. For the purpose of cloning experiments, new artificial plasmids with specific properties are constructed in naturally occurring plasmids. Such artificial plasmids are superior and efficient.

Most of the plasmids used as vector have been obtained from pSC101 or Ca (*E. coli*) plasmid that codes for production of colicin antibiotics) plasmids. One of such specifically created plasmid for use in cloning is pBR322.

Importance of PBR 322 Cloning Vector:

Most popular plasmid vector is pBR322. pBR322 is an artificial pl derived from three different naturally occurring plasmids. It is regarded as the "house" of a gene-cloning laboratory. The plasmid pBR322 is an ideal cloning vector, the most common plasmid used in gene cloning experiments.

P Denotes that it is a plasmid;

BR Indicates the laboratory in which the plasmid was originally constructed. BR stands for Bolivar and Rodriguez who constructed this plasmid.

322- It is a number given to distinguish this plasmid from others developed in the same laboratory. For ex. pBR 325, pBR 327, PBR 328, PBR 345 etc 322-

Structure of pBR322:

pBR 322 plasmid is 4363 bp long and is 4.4 kb in size. PBR 322 has restriction sites for over 20 restriction endonucleases. It also has an origin of replication and restriction sites for cleavage by variety of restriction enzymes such as Eco RI, Bam HI, Pvul and Sal I.

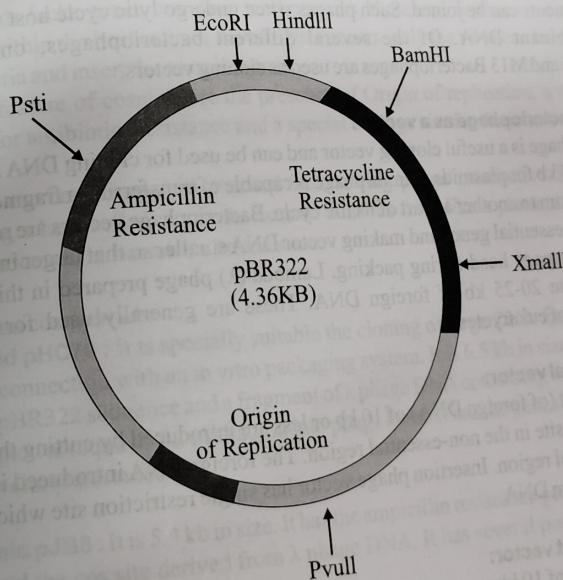


Fig. 4.4 : *E. coli* pBR322 plasmid showing restriction sites and resistance genes
INTRODUCTION AND TOOLS OF GENETIC ENGINEERING

- region of lambda DNA using restriction endonucleases λ . phage replacement vector are constructed as follows a) The vector DNA ie. λ . phage DNA is cleaved with Bam HI to generate the long (19kb) and short (9 kb) ends. These are purified. The target fragments are also prepared by cleaving DNA with Rau HI and thioglycollate treated with alkaline phosphatase to prevent them from ligating or joining each other.
- b) The λ arms and the target fragments are now ligated together at relatively high concentration to form long linear products. The recombinant phage DNA is now packed in vitro into a preparation of head and tail proteins. The viruses thus constructed are allowed to infect E. coli where they multiply.

Cosmids:

- Cosmids are the novel and effective derived cloning vectors
- Cosmid is an artificial plasmid containing 'cos' site of a phage DNA.
- It is formed by introducing the fragment of the λ phage DNA with the cos site into a plasmid.
- Cosmids can be defined as the vector derived from plasmids which contains cos site of lambda phage.
- Cosmids were first developed in 1978 by Barbara Hohn and John Collins.
- Cosmid possess properties of both plasmid and λ phage.
- Cosmid contain a cos site of λ phage, which is essential for packing of nucleic acid into protein coat and essential genes of plasmid such as origin of replication a marker gene for antibiotic resistance.
- A Cosmids lacks genes encoding viral proteins, therefore, neither viral particles are formed within the host cell nor the cell lysis occurs, only the virus can be able to infect the bacteria and insert the cosmid into it.
- Special feature of cosmids are the presence of Origin of replication, a marker gene coding for antibiotics resistance and a special cleavage site for the insertion of foreign DNA
- Cosmid can be packaged in vitro and the resulting 'phage' is infective. However, after infecting a host cell (E. coli) the cosmid behaves fully like a plasmid and replicates independent of the bacterial chromosome.

Two important cosmids are;

- **Cosmid pHC79** : It is specially suitable for cloning of large DNA fragments (upto 40 kb) in connection with an in vitro packaging system. It is 6.5 kb in size. It contains part of the pBR322 sequence and a fragment of λ phage DNA containing the cos region. The cos region is required for packaging into phage head. It has the genes for ampicillin and tetracycline resistance.
- **Cosmid pJB8** : It is 5.4 kb in size. It has the ampicillin resistance genes of plasmid PBR 322 and the cos site derived from λ phage DNA. It has several possible cloning sites, e.g. Bam HI, Hind III, and Sal I.

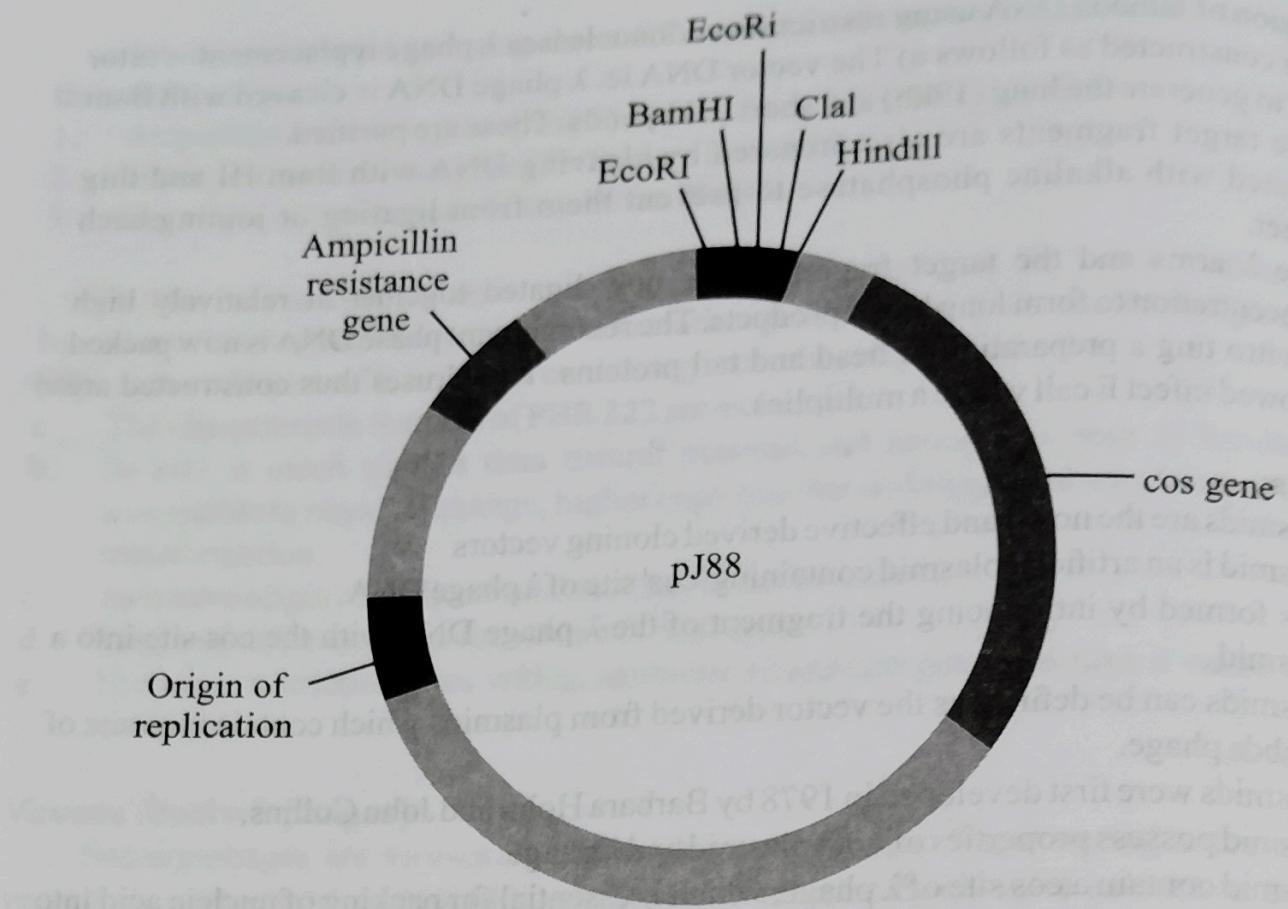


Fig. 4.5 :

EXERCISE

phosphate

1. A) FILL IN THE BLANKS:

1. Alkaline Catalyses the removal of 5' phosphate group from DNA.
2. Reverse transcriptase is RNA dependent DNA polymerase.
3. Plasmid vector should have Low molecular weight.
4. Bacteriophages are viruses that infects bacteria.
5. Cosmid is an Artificial plasmid containing cos site & λ phase.

ANSWERS :

1. Alkaline phosphatase, 2. RNA, 3. Low, 4. Viruses , 5. Artificial

1. B) MULTIPLE CHOICE QUESTIONS:

1. Which of these are most widely used in genetic engineering?
 - a) Plastid
 - b) **Plasmid**
 - c) Mitochondrion
 - d) Ribosome
2. What is the source of EcoRI?
 - a) Escherichia coli R 1
 - b) Escherichia coli RI 13
 - c) **Escherichia coli R Y 13**
 - d) Escherichia coli R X 13

3. Which of the following is a cloning vector?
- DNA of *Salmonella typhimurium*
 - Ti plasmid
 - Amp' and Tet' loci
 - Times minus PBR322
- The source of Bam HI restriction enzymes is
- Bacillus subtilis*
 - Bacillus amyloliquefaciens*
 - Thermophilus aquaticus*
 - All of the above
- The source of Taq DNA polymerase
- Thermophilus aquaticus*
 - Tryponema pallidum*
 - Thermus aquaticus*
 - All the above three bacteria

ANSWERS:

1	2	3	4	5
b	c	b	b	c

- C) ANSWER IN ONE SENTENCE:
- What is the role of DNase enzymes ?
 - What is pBR 322 ?
 - What is polynucleotide kinase ?
 - Define cosmid
 - What is alkaline phosphatase ?
 - What is the function of enzyme DNA ligase ?

2. SHORT ANSWER QUESTION (4 MARK EACH)

- What are restriction endonucleases? Explain two types of cuts introduced by endonuclease in DNA.
- What are vectors used in recombinant DNA technology? Discuss their ideal properties.
- Describe any two nucleases used for DNA manipulations.
- Explain in short plasmid vectors with examples.
- Explain the action of any two DNA modifying enzymes.
- What is genetic engineering? Give the outline of different steps involved in genetic engineering.
- Explain in brief pBR 322.
- What are cosmids? Explain in brief.
- Explain in brief DNA modifying enzymes.
- Describe the importance of Nuclease enzyme in genetic engineering.
- Describe plasmid as vectors and give its ideal characters.
- Briefly discuss basic technique of genetic engineering.

B) METABOLISM

Glycolysis:

Glucose occupies a central position in the metabolism of plants, animals, and many microorganisms. It is relatively rich in potential energy, and thus a good fuel; the complete oxidation of glucose to carbon dioxide and water. Glucose is not only an excellent fuel; it is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions. A bacterium such as *Escherichia coli* can obtain from glucose the carbon skeletons for every amino acid, nucleotide, coenzyme, fatty acid, or other metabolic intermediate it needs for growth.

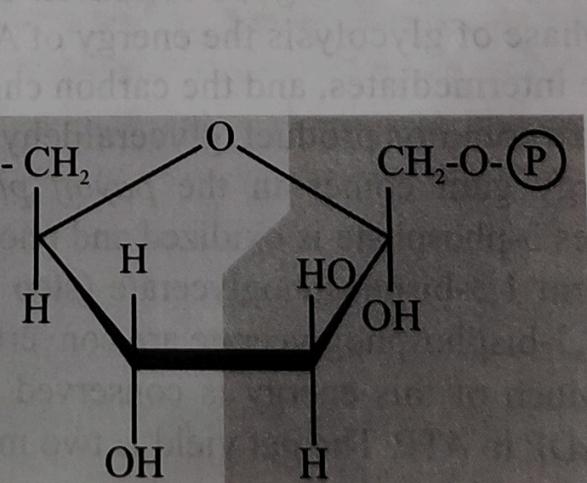
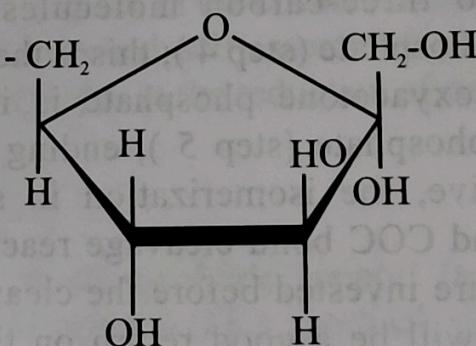
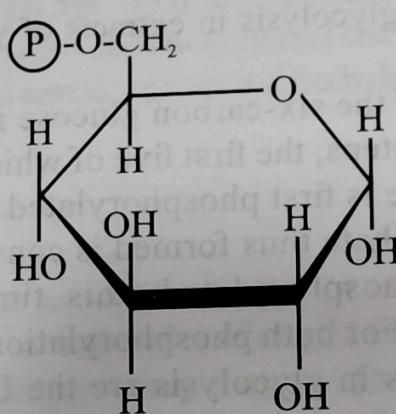
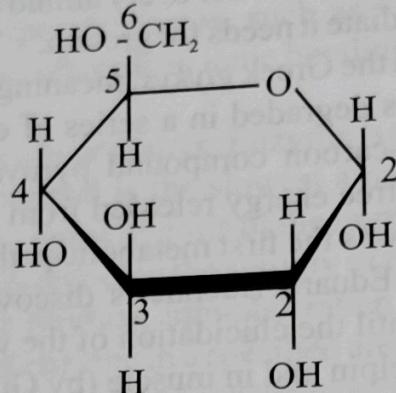
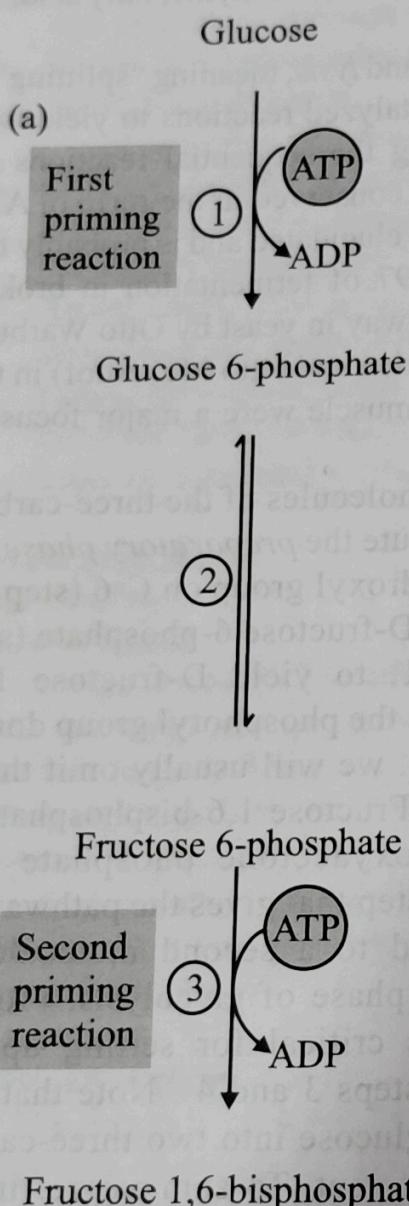
In **glycolysis** (from the Greek *glykys*, meaning "sweet," and *lysis*, meaning "splitting"), a molecule of glucose is degraded in a series of enzyme-catalyzed reactions to yield two molecules of the three-carbon compound pyruvate. During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH. Glycolysis was the first metabolic pathway to be elucidated and is probably the best understood. From Eduard Buchner's discovery in 1897 of fermentation in broken extracts of yeast cells until the elucidation of the whole pathway in yeast by Otto Warburg and Hans von Euler-Chelpin and in muscle (by Gustav Embden and Otto Meyerhof) in the 1930s, the reactions of glycolysis in extracts of yeast and muscle were a major focus of biochemical research.

The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in ten steps, the first five of which constitute the *preparatory phase*. In these reactions, glucose is first phosphorylated at the hydroxyl group on C-6 (step 1). The D-glucose 6-phosphate thus formed is converted to D-fructose 6-phosphate (step 2), which is again phosphorylated, this time at C-1, to yield D-fructose 1,6-bisphosphate (step 3). For both phosphorylations, ATP is the phosphoryl group donor. As all sugar derivatives in glycolysis are the D isomers, we will usually omit the D designation except when emphasizing stereochemistry. Fructose 1,6-bisphosphate is split to yield two three-carbon molecules, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (step 4); this is the "lysis" step that gives the pathway its name. The dihydroxyacetone phosphate is isomerized to a second molecule of glyceraldehyde 3-phosphate (step 5), ending the first phase of glycolysis. From a chemical perspective, the isomerization in step 2 is critical for setting up the phosphorylation and COC bond cleavage reactions in steps 3 and 4. Note that two molecules of ATP are invested before the cleavage of glucose into two three-carbon pieces; later there will be a good return on this investment. To summarize: in the preparatory phase of glycolysis the energy of ATP is invested, raising the free-energy content of the intermediates, and the carbon chains of all the metabolized hexoses are converted into a common product, glyceraldehyde 3-phosphate.

The energy gain comes in the *payoff phase* of glycolysis. Each molecule of glyceraldehydes 3-phosphate is oxidized and phosphorylated by inorganic phosphate (not by ATP) to form 1,3-bisphosphoglycerate (step 6). Energy is then released as the two molecules of 1,3-bisphosphoglycerate are converted to two molecules of pyruvate (steps 7 through 10). Much of this energy is conserved by the coupled phosphorylation of four molecules of ADP to ATP. The net yield is two molecules of ATP per molecule of gluco-

used, because two molecules of ATP were invested in the preparatory phase. Energy is also conserved in the payoff phase in the formation of two molecules of NADH per molecule of glucose.

In the sequential reactions of glycolysis, three types of chemical transformations are particularly noteworthy: (1) degradation of the carbon skeleton of glucose to yield pyruvate, (2) phosphorylation of ADP to ATP by high-energy phosphate compounds formed during glycolysis, and (3) transfer of a hydride ion to NAD forming NADH.



Preparatory phase

Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate

- 1) Hexokinase
- 2) phosphohexabe isomerase
- 3) phosphofructokinase-1
- 4) Aldolase
- 5) Triose phosphate isomerase

Cleavage of g-carbon Sugar Phosphate to two 3-carbon Sugar phosphates

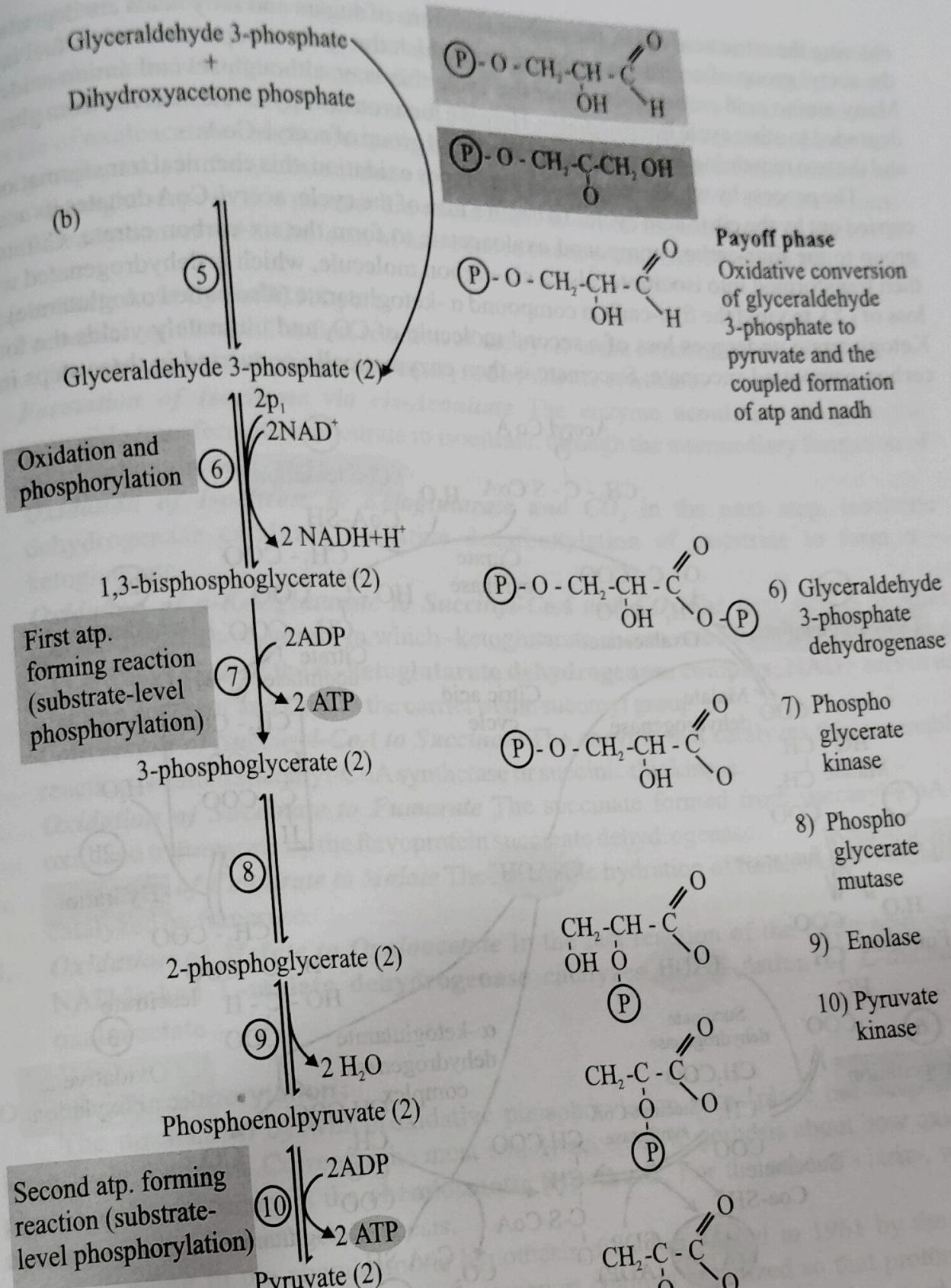


Fig. 5.4 : Glycolysis Pathway

TCA Cycle: Conversion of Pyruvate to Acetyl CoA

In aerobic organisms, glucose and other sugars, fatty acids, and most amino acids are ultimately oxidized to CO_2 and H_2O via the citric acid cycle and the respiratory chain.

force, ATP is synthesized in a reversal of the ATP hydrolysis reaction. A similar process takes place in prokaryotes, with electron flow causing the protons to move outward across the plasma membrane. ATP synthesis occurs when these protons diffuse back into the cell. The proton motive force also may drive the transport of molecules across membranes and the rotation of bacterial flagella and thus plays a central role in prokaryotic physiology. The chemiosmotic hypothesis is accepted by most microbiologists. There is considerable evidence for the generation of proton and charge gradients across membranes. However, the evidence for proton gradients as the direct driving force for oxidative phosphorylation is not yet conclusive. In some halophilic marine bacteria, sodium ions may be used to drive ATP synthesis.

Whatever the precise mechanism, ATP synthesis takes place at the F₁F₀ ATPase or ATP synthase. The mitochondrial F₁ component appears as a spherical structure attached to the inner membrane surface by a stalk and the F₀ component, which is embedded in the membrane. The F₁F₀ ATPase is on the inner surface of the plasma membrane in bacteria. F₀ participates in proton movement across the membrane, and this movement through a channel in F₀ is believed to drive oxidative phosphorylation. F₁ is a large complex in which three α subunits alternate with three β subunits. The γ subunit extends downward from the $\alpha_3\beta_3$ complex; it composes part of the stalk and interacts with F₀. The δ subunit also is located in the stalk. Much of the γ subunit is positioned in the center of F₁, surrounded by the α and β subunits.

The γ subunit rotates rapidly in a counterclockwise direction within the $\alpha_3\beta_3$ complex much like a car's crankshaft and causes conformational changes that drive ATP synthesis at the active sites on the β subunits. Thus the ATP synthase is the smallest rotary motor known, much smaller than the bacterial flagellum.

Many chemicals inhibit the aerobic synthesis of ATP and can even kill cells at sufficiently high concentrations. These inhibitors generally fall into two categories. Some directly block the transport of electrons. The antibiotic piericidin competes with coenzyme Q; the antibiotic antimycin A blocks electron transport between cytochromes b and c; and both cyanide and azide stop the transfer of electrons between cytochrome a and O₂ because they are structural analogs of O₂. Another group of inhibitors known as uncouplers stops ATP synthesis without inhibiting electron transport itself. Indeed, they may even enhance the rate of electron flow. Normally electron transport is tightly coupled with oxidative phosphorylation so that the rate of ATP synthesis controls the rate of electron transport. The more rapidly ATP is synthesized during oxidative phosphorylation, the faster the electron transport chain operates to supply the required energy. Uncouplers disconnect oxidative phosphorylation from electron transport; therefore the energy released by the chain is given off as heat rather than as ATP. Many uncouplers like dinitrophenol and valinomycin may allow hydrogen ions, potassium ions, and other ions to cross the membrane without activating the F₁F₀ ATPase. In this way they destroy the pH and ion gradients. Valinomycin also may bind directly to the F₁F₀ ATPase and inhibit its activity.

Electron Transport Chain :

The mitochondrial **electron transport chain** is composed of a series of electron carriers that operate together to transfer electrons from donors, like NADH and FADH₂,

acceptors, such as O₂. The electrons flow from carriers with more negative reduction potentials to those with more positive potentials and eventually combine with O₂ and H⁺ to form water. The electrons move down this potential gradient much like water flowing in a series of rapids. The difference in reduction potentials between O₂ and NADH is large, about 1.14 volts, and makes possible the release of a great deal of energy. The potential changes at several points in the chain are large enough to provide sufficient energy for ATP production, much like the energy from waterfalls can be harnessed by turbines and used to generate electricity. The electron transport chain breaks up the large overall energy release into small steps. Some of the liberated energy is trapped in the form of ATP.

Electron transport at these points may generate proton and electrical gradients. These gradients can then drive ATP synthesis. The electron transport chain carriers reside within the inner membrane of the mitochondrion or in the bacterial plasma membrane. The mitochondrial system is arranged into four complexes of carriers, each capable of transporting electrons part of the way to O₂. Coenzyme Q and cytochrome c connect the complexes with each other. The process by which energy from electron transport is used to make ATP is called **oxidative phosphorylation**. Thus as many as three ATP molecules may be synthesized from ADP and Pi when a pair of electrons passes from NADH to an atom of O₂.

The earlier discussion has focused on the eucaryotic mitochondrial electron transport chain. Although some bacterial chains resemble the mitochondrial chain, they are frequently very different. They vary in their electron carriers (e.g., in their cytochromes) and may be extensively branched. Electrons often can enter at several points and leave through several terminal oxidases. Bacterial chains also may be shorter and have lower P/O ratios than mitochondrial transport chains. Thus prokaryotic and eucaryotic electron transport chains differ in details of construction although they operate using the same fundamental principles.

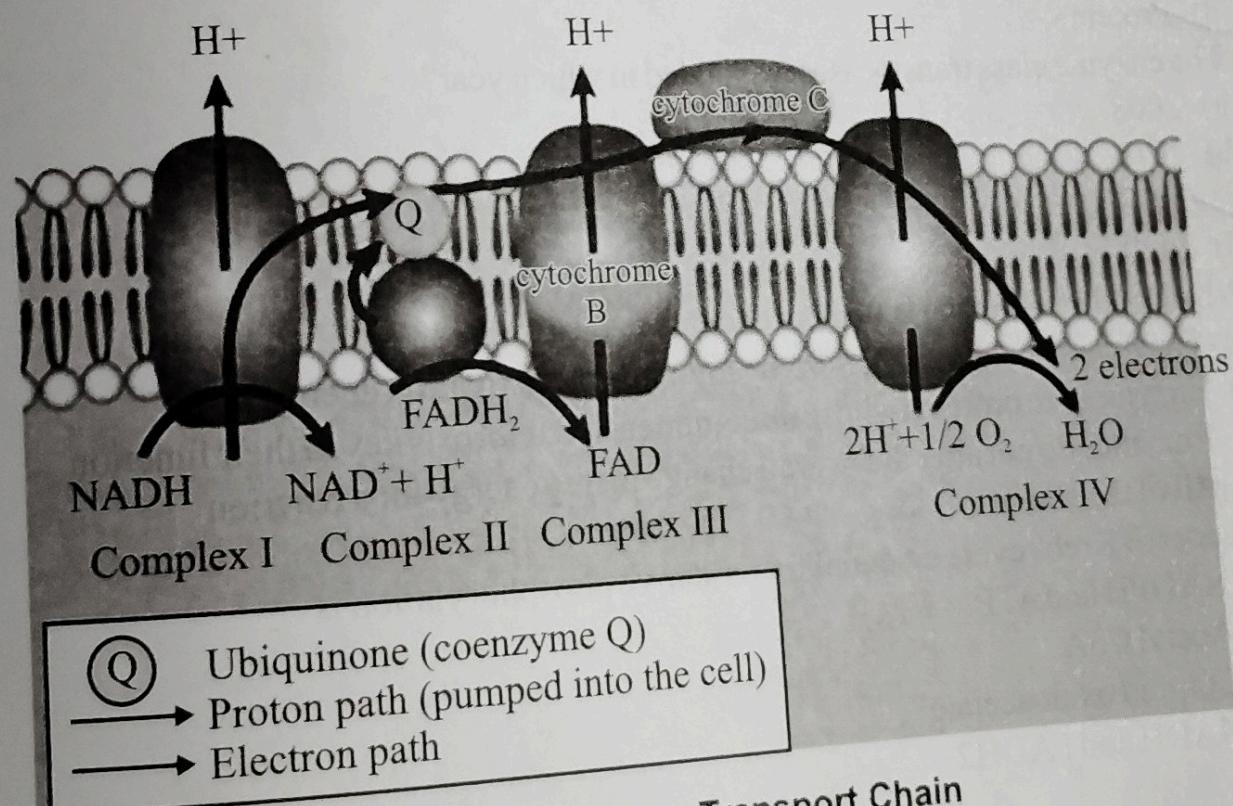
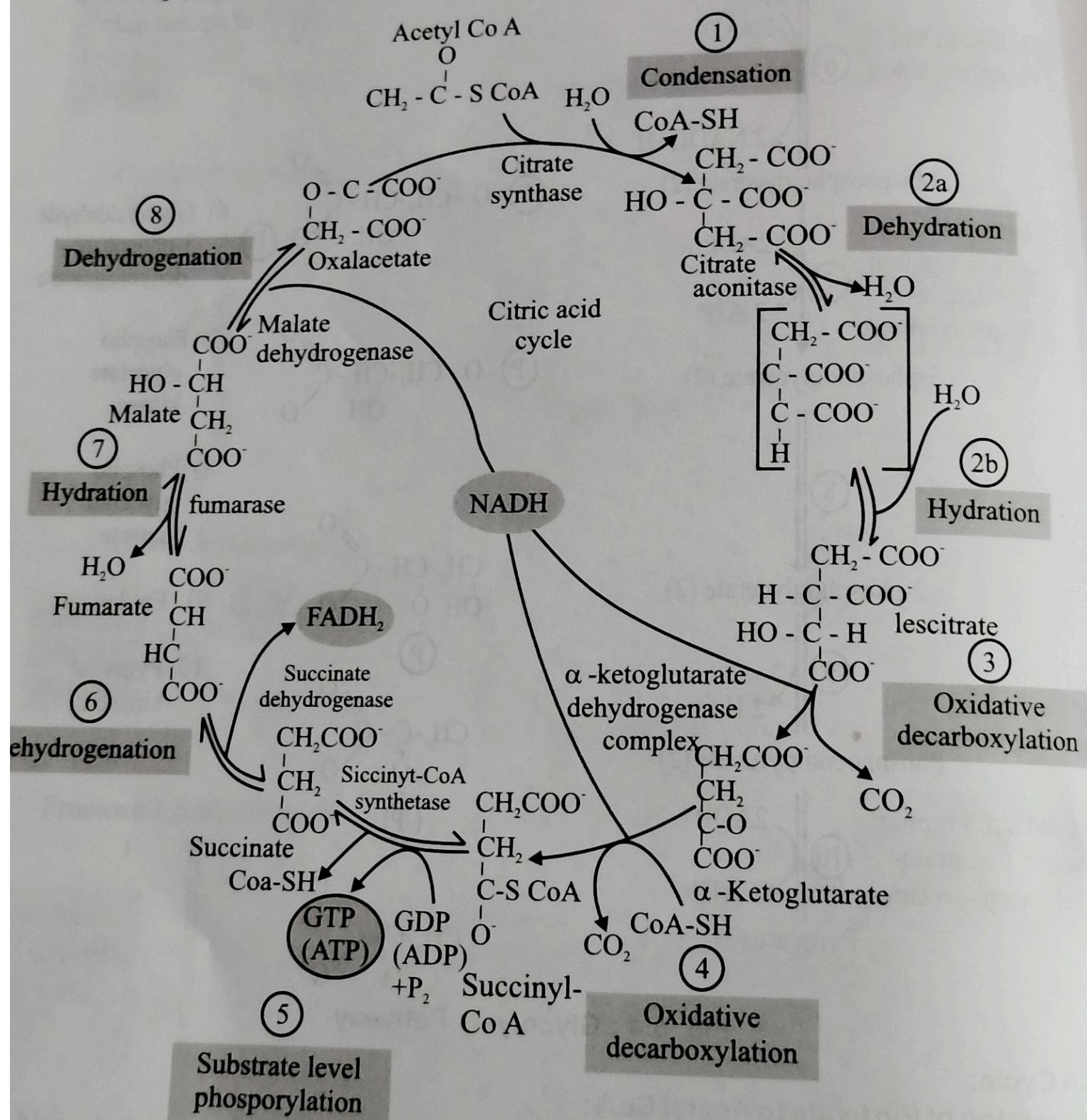


Fig. 5.5 : Electron Transport Chain

entering the citric acid cycle, the carbon skeletons of sugars and fatty acids are degraded to the acetyl group of acetyl-CoA, the form in which the cycle accepts most of its fuel input. Many amino acid carbons also enter the cycle this way, although several amino acids are degraded to other cycle intermediates. Here we focus on how pyruvate, derived from glucose and the two remaining carbons become the acetyl group of acetyl-CoA.

The process by which acetyl-CoA undergoes oxidation, this chemical transformation is carried out by the citric acid cycle. To begin a turn of the cycle, acetyl-CoA donates its acetyl group to the four-carbon compound oxaloacetate to form the six-carbon citrate. Citrate is then transformed into isocitrate, also a six-carbon molecule, which is dehydrogenated with loss of CO₂ to yield the five-carbon compound α -ketoglutarate (also called oxoglutarate). α -Ketoglutarate undergoes loss of a second molecule of CO₂ and ultimately yields the four-carbon compound succinate. Succinate is then enzymatically converted in three steps into



EXERCISE

1. A) FILL IN THE BLANKS :

1. Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by Ferments.
2. Alanine racemase is an example of Isomerase.
3. Group transfer reactions are catalyzed by Transferases.
4. The active site of an enzyme refers to the specific region of an enzyme where a substrate binds.
5. trypsin is obtained by rubbing pancreatic tissue with glycerine.
6. Isozymes were first described by Hunter in 1957.
7. Enzymes which have an additional site along with active sites are allosteric enzymes.
8. In glycolysis Fructose 1,6- is split to yield two three-carbon molecules, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate.
9. The enzyme Aconitase catalyzes the reversible transformation of citrate to isocitrate.
10. The process by which energy from electron transport is used to make ATP is called oxidative phosphorylation.

ANSWERS :

1. Ferments, 2. Isomerases, 3. Transferases, 4. Specific region, 5. glycerine, 6. Hunter and Markert , 7. Allosteric enzymes, 8. Fructose 1,6-bisphosphate , 9. Aconitase, 10. oxidative phosphorylation.

1. B) MULTIPLE CHOICE QUESTIONS :

1. The nature of an enzyme is
 - a) Lipid
 - b) Vitamin
 - c) Carbohydrate
 - d) Protein
2. The enzyme class translocases was added in which year?
 - a) 2008
 - b) 2012
 - c) 2018
 - d) 2020
3. Which of the following statement about enzymes is true?
 - a) Enzymes accelerate reactions by lowering the activation energy
 - b) Enzymes are proteins whose three-dimensional form is key to their function
 - c) Enzymes do not alter the overall change in free energy for a reaction
4. Product of Krebs cycle essential for oxidative phosphorylation is
 - a) NADPH and ATP
 - b) Acetyl CoA
 - c) CO₂ and oxaloacetate
 - d) NADH and FADH₂

5. A single molecule of glucose generates how many molecules of acetyl CoA? Which enters the Krebs cycle.
- 4
 - 2
 - 1
 - 3
6. Krebs cycle occurs in aerobic respiration due to
- Oxygen is a reactant
 - Oxygen has a catalytic function
 - Electron transport chain requires aerobic conditions to operate
 - All of the above
7. Glycolysis is the conversion of
- Fructose into phosphoenolpyruvate
 - Glucose into pyruvate
 - Fructose into pyruvate
 - Glucose into phosphoenolpyruvate
8. In EMP pathway, the process by which ATP is formed from ADP is
- Substrate-level phosphorylation
 - Oxidative phosphorylation
 - Reduction
 - Photo phosphorylation
9. Which of the following terminal cytochromes is responsible for donating electrons to oxygen?
- Cyt b
 - Cyt c
 - Cyt a3
 - Cyt a1
10. EC number is made up of how many components which are separated by full stops.
- 8
 - 6
 - 2
 - 4

ANSWERS:

1	2	3	4	5	6	7	8	9	10
d	c	d	d	b	c	b	a	c	d

1. C) ANSWER IN ONE SENTENCE

- In TCA cycle the reversible hydration of fumarate to L-malate is catalyzed by what enzyme?
- What is the name of an enzyme which catalyzes Oxidative decarboxylation of isocitrate to form α -ketoglutarate?
- An enzyme with restricted mobility, attached to an inert, insoluble material is known as