04 Molecular Basis of Inheritance

Syllabus

4.1 The Discovery of DNA

INTEXT QUESTION

Q.1 Can you recall?

i. What is nucleic acid?

Ans : Nucleic acids are long polymer chains made from monomers known as nucleotides. Nucleic acids are included in the class of macromolecules.

ii. What are the types of nucleic acid?

Ans : Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) are the two types of nucleic acid.

iii. What are the functions of nucleic acid?

Ans : Functions of nucleic acids are as follows:

i. By the process of transcripton, DNA gives rise to RNA, which in turn helps in protein synthesis.

ii. DNA is the genetic material which carries hereditary information from one generation to next except for certain viruses.

iii. RNA acts as the genetic material in certain classes of viruses.

iv. Cellular metabolism along with differentiation and development of an organism is controlled by DNA.

v. Mutations occuring in nucleic acids help organisms evolve.

iv. What is difference between DNA in prokaryotes and eukaryotes?

Ans :

	DNA in Prokaryotes	DNA in Eukaryotes
i.	DNA is freely suspended in the cytoplasm.	DNA is present in membrane bound nucleus.
ii.	DNA is compactly arranged in the form of chromosomes without the aid of histone proteins.	DNA is compactly arranged in the form of chromosomes with the aid of histone proteins.
iii.	Introns are absent.	Introns are present.

4.2 The Genetic Material is DNA

Q.2 Explain Griffith's experiment in detail.

Ans :

i. In 1928, a British medical officer Frederick Griffith performed an experiment on bacterium Streptococcus pneumoniae that causes pneumonia in humans and other mammals. Griffith used two strains or two genetic varieties of Streptococcus to find a cure for pneumonia, which was a common cause of death at that time.

ii. The two strains used were :
   a. Virulent, smooth, pathogenic and encapsulated S type.
   b. Non-virulent, rough, non-pathogenic and non-capsulated R type.

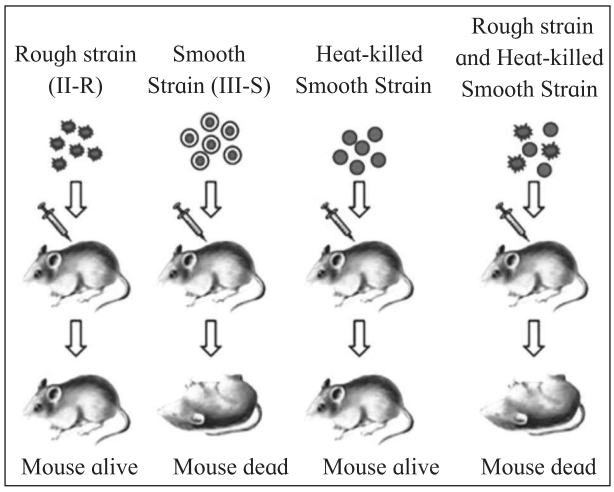
iii. Griffith conducted four experiments on these bacteria.
   a. First, when he injected bacteria of strain R to mice, the mice survived because it did not develop pneumonia.
   b. Second, when he injected bacteria of strain S to mice, the mice developed pneumonia and died.

c. In the third experiment, he injected heat-killed strain S bacteria to mice, once again the mice survived.

d. In fourth experiment, he mixed heat-killed S bacteria with live bacteria of strain R and injected to mice. The mice died and Griffith recovered large numbers of live strain S bacteria from the blood of the dead mice.

iv. In these four experiments, something had caused harmless strain R bacterium to change into deadly S strain bacterium.

v. Griffith concluded that the R-strain bacterium must have taken up, to what he called a "transforming principle" from the heatkilled S bacterium, which allowed R strain to get transformed into smooth-coated bacterium and become virulent.



Q.3 Explain the Avery, McCarty and MacLeod's experiment.

Ans :

i. In 1944, U.S. microbiologists Oswald T. Avery, Colin M. MacLeod and Maclyn McCarty first evidenced to prove the DNA is a genetic material (transforming principle), through the experiments.

ii. They purified DNA, RNA, Proteins (enzymes) and other materials from cell free extract of S cells/ strain and mixed with heat killed S strain and R cells seperately to confirm which one could transform living R cells into S cells.

iii. Only DNA was able to transform harmless strain R into deadly strain S.

iv. They also discovered that protein–digesting enzymes (proteases), RNA-digesting enzyme (RNAases) did not affect transformation, so the transforming substance was neither a protein nor RNA.

v. DNA digested with DNAse did inhibit the transformation, suggesting that DNA caused the transformation.

Q.4 Explain the experiment performed by Hershey and Chase with a suitable diagram.

Ans :

i. Hershey and Chase worked with viruses that infect bacteria i.e. bacteriophages, which are composed of DNA and protein.

ii. They used radioactive phosphorous 32P in the medium for some viruses and radioactive sulphur 35S for some others.

iii. They grew some viruses on a medium that contained radioactive phosphorus and some others on medium that contained radioactive sulphur.

iv. Viruses grown in the presence of radioactive phosphorus contained radioactive DNA, but not radioactive proteins because DNA contains phosphorus but proteins do not.

v. Similarly, viruses grown on radioactive sulphur contained radioactive protein but not radioactive DNA because DNA does not contain sulphur.

vi. Radioactive phages were allowed to infect *E.coli* bacteria grown on the medium containing normal 'P' and 'S'.

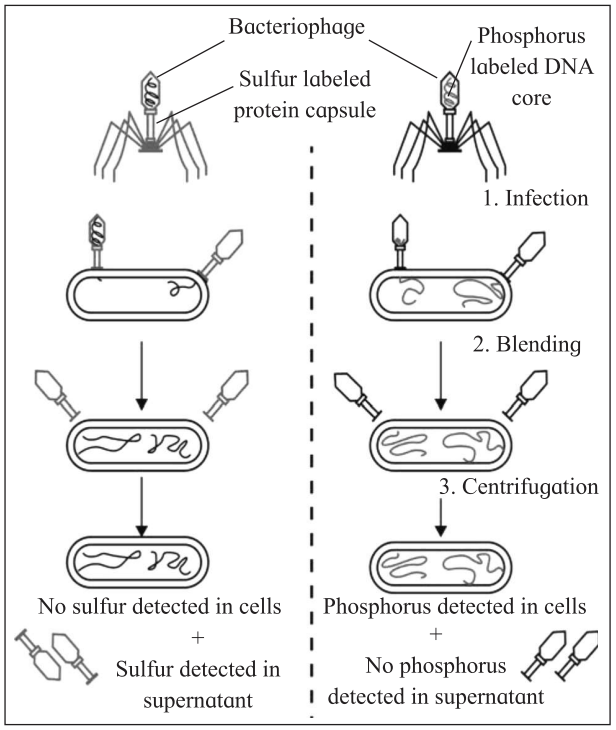
vii. Then, as the infection proceeded, the viral coats were removed with the help of centrifuge.

viii. Bacteria which were infected by viruses with radioactive DNA, were radioactive, indicating that DNA was the material that passed from the viruses to the bacteria.

ix. Bacteria which were infected by viruses having radioactive sulphur (protein) were not radioactive.

x. This indicates that proteins from the viruses, did not enter the bacteria.

xi. DNA is, therefore, the genetic material that is passed from virus to bacteria



Q.5 Do you know ?

i. Multiple forms of DNA and their differences.

Ans :

Properties	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~ 26 Å	~ 20 Å	~ 18 Å
Base pairs per helical turn	11	10	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å

ii. Significance of different forms of DNA.

Ans : DNA structures important for interactions with proteins in recombination, gens expression and replication. They may also play different roles in the formation of nucleosomes and other complex structures involving DNA.

Q.6 Can you recall?

i. What are the chemical components of DNA?

Ans :

i. DNA is a nucleic acid formed from several deoxyribo-nucleotides.

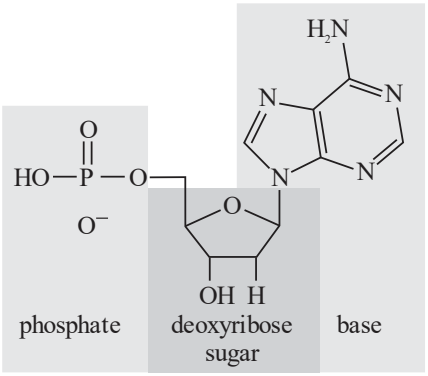
ii. Each deoxyribo-nucleotides has three components - Deoxyribose sugar, Phosphate group and Nitrogen base.

iii. Deoxyribose sugar is a pentose sugar having a pentagonal structure.

iv. The phosphate group is phosphoric acid (H_3PO_4) it helps in linkage of deoxyribo-nucleotides.

v. Nitrogen bases can be classified majorly two types i.e. Purines and Pyrimidines.

vi. Purines are double ring compounds and include Adenine (A) and Guanine (G), while Pyrimidines are single ring compounds which include Cytosine (C) and Thymine (T).



ii. What is a chromosome?

Ans : The filamentous bodies composed of DNA molecule present in the eukaryotes nucleus is known as chromosome.

MULTIPLE CHOICE QUESTIONS

Entrance Set 1

1. Identify the correct order of organisationof genetic material from largest to smallest.
   (a) Genome, chromosome, gene, nucleotide
   (b) Chromosome, genome, nucleotide, gene
   (c) Chromosome, gene, genome, nucleotide
   (d) Genome, chromosome, nucleotide, gene.

Solution:(a)

In genome all the genes are contain in a single set of chromosomes. The instructions in our genome are present in the form of DNA. DNA has a complicated structure in the form of a double helix. Single strands of DNA are coilded up into structures called chromosomes. Within the chromosomes,

segments of DNA are "read" together to form genes. Thus, a gene is a segment of DNA or chromosome situated at a specific locus (gene locus) which carries coded information associated with a specific function and can undergo crossing over as well as mutation. A nucleotide is the basic unit of DNA made up of a pentose sugar, phosphoric acid and a nitrogenous base.

2. Transformation was discovered by
   (a) Meselson and Stahl
   (b) Hershey and Chase
   (c) Griffith
   (d) Watson and Crick.

Solution:(c)

Transformation was first studied by S.F. Griffith in 1928 while studying Streptococcus pneumoniae. He found that R-type non virulent bacteria pick up virulence from heat killed virulent S-type bacteria and transform into virulent forms. It was this experiment which indicated presence of a 'transforming principle' which was later found out to be DNA, by Avery et al.

3. The unequivocal proof of DNA as the genetic material came from the studies on a
   (a) bacterium (b) fungus
   (c) viroid (d) bacterial virus

Solution:(d)

The unequivocal proof that DNA is the genetic material came from the experiments of Alfred Hershey and Martha Chase (1952). They worked with viruses that infect bacteria called bacteriophages.

4. Similarity in DNA and RNA is that
   (a) both are polymer of nucleotides
   (b) both have similar pyrimidine
   (c) both have similar sugar
   (d) both are genetic material.

Solution:(a)

Deoxyribonucleic acid and ribonucleic acid as the name suggests are made up of several nucleotide monomers.Each nucleotide consists of pentose sugar, phosphate group and nitrogenous bases. DNA has deoxyribose sugar whereas RNA has ribose sugar. The bases in DNA molecule are A, T, G and C whereas in RNA, thymine is absent and instead uracil is found.

5. DNA is mainly found in
   (a) nucleolus (b) nucleus only
   (c) cytoplasm only (d) none of these

Solution:(b)

DNA is mainly foudn in nucleus. It is associated with RNA and proteins to form compact chromosomes. But some amount of DNA is also found in chloroplasts and mitochondria. This is called extra-chromosomal DNA.

6. In prokaryotes, the genetic material is
   (a) linear DNA without histones
   (b) circular DNA without histones
   (c) linear DNA with histones
   (d) circular DNA with histones.

Solution:(b)

The genetic material of prokaryotes is circular and single stranded DNA. It has no association of histones. The eukaryotic genetic material is linear and double stranded DNA. It is associated with histone proteins to form nucleosome unit.

7. The transforming principle of Pneumococcus as found out by Avery, MacLeod and McCarty was
   (a) mRNA (b) DNA
   (c) protein (d) polysaccharide.

Sol. (b) : The transforming principle of *Pneumococcus* as found out by Avery, MaclLeod and McCarty repcated Griffith's experiment successfully. They separated the proteins, carbohydrates and DNA of S III strains and separately mixed them in the pure cultures of R II. Only DNA could bring about transformation of R II type into S III and not the proteins or the carbohydrates.

8. Who proved that DNA is basic genetic material?
   (a) Griffith
   (b) Watson
   (c) Boveri and Sutton
   (d) Hershey and Chase

Sol. (d): Hershey and Chase proved that DNA is a

basic genetic material. Hershey and Chase, 1952, by using P^{35} and S^{35} with a T-2 type phage concluded that DNA is the genetic material.

Try yourself

9. A molecule that can act as a genetic material must fulfil the traits given, EXCEPT
(a) It should provide the scope for slow changes that required for evolution
(b) it should be able to express itself in the form of 'Mendelian characters'
(c) it should be able to generate its replica
(d) it should be unstable structurally and chemically.

10. In Griffith's experiment, the conversion of R-type to S-type of *Streptococcus pneumoniae* when mixed with heat killed S-type is called
(a) mutation
(b) transduction
(c) transfection
(d) transformation

11. Transformation was discovered by
(a) Meselson and Stahl
(b) Hershey and Chase
(c) Griffith
(d) Watson and Crick

12. **Assertion (A):** Mice infected with the S strain (virulent) die from pneumonia infection but mice infected with R strain do not develop pneumonia.
Reason (R): This is because the S strain bacteria have a mucous (polysaccharide) coat, while R strain does not.
Which of the following is true?
(a) Both (A) and (R) are true and (R) is the correct explanation of (A).
(b) Both (A) and (R) are true, but (R) is not the correct explanation of (A).
(c) (A) is true, but (R) is false
(d) (A) is false, but (R) is true.

13. The result of the following reaction/ experiment carried out by Avery et al on *Streptococcus pneumaniae* has proved conclusively that DNA is the genetic material;
(a) Live 'R' strain + DNA from 'S' strain + RNase
(b) Live 'R' strain + DNA from 'S' strain + DNase
(c) Live 'R' strain + Denatured DNA of 'S' strain + protease
(d) Heat killed 'R' strain + DNA from 'S' strain + DNase

14. The final proof for DNA as the genetic material came from the experiments of
(a) Griffith
(b) Hershey and Chase
(c) Avery Macleod and McCarty
(d) Har Gobind Khorana

15. Which scientist experimentally proved that DNA is the sole genetic material in bacteriophage?
(a) Jacob and Monod
(b) Beadle and Tatum
(c) Meselson and Stahl
(d) Hershey and Chase

4.3 DNA Packaging

Q.7 Explain the packaging of DNA in Prokaryotes with the help of a suitable diagram.

Ans :

i. In prokaryotes like *E. coli*, cell size is almost 2-3μ long.

ii. They do not have well organized nucleus. It is without nuclear membrane and nucleolus.

iii. The nucleoid is small, circular, highly folded, naked ring of DNA which is 1100μ long in perimeter, containing about 4.6 million base pairs.

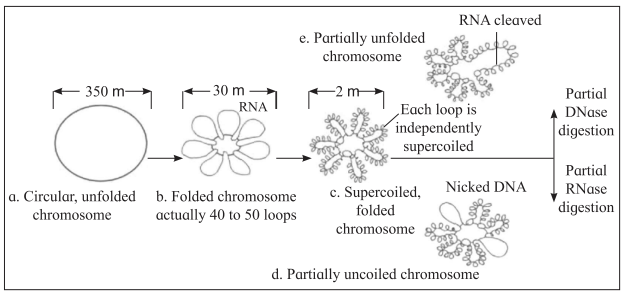
iv. The 1100μ long nucleoid is to be fitted or packaged into a cell which is hardly 2-3μ long.

v. Hence the negatively charged DNA becomes circular, reducing the size to 350μm in diameter. This is further reduced to 30μm in diameter because of folding/looping. 40-50 domains (loops) are formed.

vi. Formation of loops is assisted by RNA connectors.

vii. Each domain is further coiled and supercoiled, thereby reducing the size down to 2μ in diameter.

viii. This coiling (packaging) is assisted by positively charged HU (Histone like DNA binding proteins) proteins and enzymes like DNA gyrase and DNA topoisomerase I, for maintaining super coiled state.



TEXTUAL QUESTION

Q.8 Write short note on DNA packaging in eukaryotic cell.

Ans :

i. The organization of DNA is much more complex in eukaryotes.

ii. Histones are the proteins that are rich in lysine and arginine residues. Both these amino acid residues are basic amino acids and carry positive charges with them.

iii. So, histones are a set of positively charged, basic proteins (histones + protamine). These histones organize themselves to make a unit of 8 molecules known as **histone octamer**.

iv. The negatively charged helical DNA is wrapped around the positively charged histone octamer, forming a structure known as **nucleosome**.

v. The nucleosome core is made up of two molecules of each of four types of histone proteins viz. H_2A, H_2B, H_3 and H_4. H_1 protein binds the DNA thread where it enters (arrives) and leaves the nucleosome.

vi. One nucleosome approximately contains 200 base pair long DNA helix wound around it.

vii. About 146 base pair long segment of DNA remains present in each nucleosome.

viii. Nucleosomes are the repeating units of chromatin, which are thread-like, stained (coloured) bodies present in nucleus. These look like **'beads-on-string'**, when observed under an electron microscope.

ix. DNA helix of 200 bps wraps around the histone octamer by 1¾ turns.

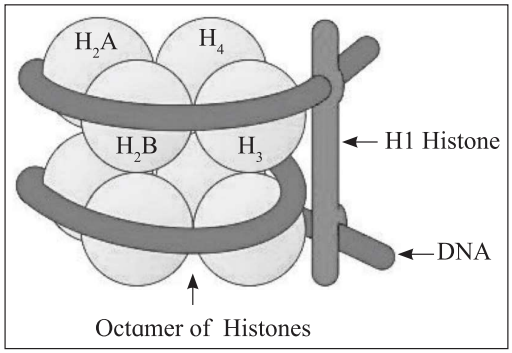
x. Six such nucleosomes get coiled and then form solenoid that looks like coiled telephone wire.

xi. The chromatin is packed to form a **solenoid structure** of 30 nm diameter ($300A^0$) and further supercoiling tends to form a looped structure called **chromatin fiber**, which further coils and condense at metaphase stage to form the **chromosomes**.

xii. The packaging of chromatin at higher levels, need additional set of proteins that are called Non-Histone Chromosomal proteins (NHC).

Q.9 What are nucleosomes? Draw a neat, labelled diagram of nucleosome.

Ans : The negatively charged helical DNA is wrapped around the positively charged histone octamer, forming a structure known as **nucleosome**.



TEXTUAL QUESTION

★Q.10 Which are the nucleosomal 'core' histones?

Ans : The nucleosome core is made up of two molecules of each of four types of histone proteins viz. H_2A, H_2B, H_3 and H_4. H_1 protein binds the DNA thread where it enters (arrives) and leaves the nucleosome.

Q.11 Write a detailed note on Non-Histone Chromosomal Proteins (NHC)

Ans :

i. Non-Histone Chromosomal Proteins are additional sets of proteins that contribute to the packaging of chromatin at a higher level.

ii. **Heterochromatin:**

a. In eukaryotic cells, some segments of chromonema / chromosome during

interphase and early prophase remain in a condensed state. These region constitute heterochromatin.

b. This term was proposed by Heitz.

c. These regions are localized near centromere, telomeres and are also intercalated.

d. It is genetically mostly inactive.

e. It stains strongly and appears dark.

f. Heterochromatin is 2 to 3 times more rich in DNA than in the euchromatin.

iii. **Euchromatin:**

a. The regions of chromonema which are in non-condensed state, constitute euchromatin.

b. Euchromatic regions stain light.

c. Euchromatin is genetically very much active and fast replicating.

iv. Euchromatin is transcriptionally active, while heterochromatin is transcriptionally almost inactive.

MULTIPLE CHOICE QUESTIONS

Set 2

1. The association of histone H_1 with a nucleosome indicates that

(a) DNA replication is occuring

(b) the DNA is considensed into a chromatin fibre

(c) the DNA double helix is exposed

(d) transcription is occuring.

Solution:(b)

Histones help in packaging of DNA. In eukaryotes, DNA packaging is carried out with the help of positively charged basic proteins called histones. Histones are of five types- H_1, H_2A, H_2B, H_3 and H_4. H_1 is attached over the linker DNA. Histone contains a large proportion of the positively charged (basic) amino acids, lysine and arginine in their structure. DNA is negatively charged due to the phosphate groups on its backbone. The result of these opposite charges is strong attraction and therefore, high binding affinity between histones and DNA.

2. What are the structures called that give an appearance as 'beads-on-string' in the chromosomes when viewed under electron microscope?

(a) Genes (b) Nuceleotides

(c) Nucleosomes (d) Base pairs

Solution:(c)

Nucleosomes appear as 'beads-on-string' in the chromosome when viewed under electron microscope. The beads in 'beads-on-string' arrangement are complexes of histones and DNA. The bead plus the connecting DNA leads to the next bead from the nucleosome. Nucelosome is the fundamental unit of organization on which the higher-order packaging of chromatin is built. The bead of each nucleosome contains eight histone molecules in which two copies each of H_2A, H_2B, H_3 and H_4 are found.

3. One turn of the helix in a B-form DNA is approximately

(a) 2 nm (b) 20 nm

(c) 0.34 nm (d) 3.4 nm.

Solution:(d)

DNA or deoxyribose nucleic acid is the largest macromolecule made of the helically twisted two antiparallel polydeoxyribonucleotide strands held together by hydrogen bonds. The two strands of DNA are together called DNA duplex. It has a diameter of 20A. One turn spiral has a distance of 34A or 3.4 nm.

4. The eukaryotic genome differs from the prokaryotic genome because

(a) the DNA is complexed with histone in prokaryotes

(b) the DNA is circular and single stranded in prokaryotes

(c) repetitive sequences are present in eukaryotes

(d) genes in the former case are organized into operons.

Sol. (b): Genome refers to the total sets of chromosomes carried by each cell of the organism. In prokaryotes the genetic material is circular and single stranded DNA. It has no

association of histones. The eukaryotic genetic material is linear and double stranded DNA. It is associated with histone proteins to form nucleosome unit.

5. Nucleosome core is made of
(a) H_0, H_2A, H_2B and H_3
(b) H_1, H_2A, H_2B, H_4
(c) H_1, H_2A, H_2B, H_3 and H_4
(d) H_2A, H_2B, H_3 and H_4

Sol. (d) : Nucleosome core is made up of H_2A, H_2B, H_3 and H_4. It is about 7-10 nm in diameter, consisting of histones around which a DNA strand, about 120 base pair long is wrapped in chromosomes.

6. Initiation codon of protein synthesis (in eukaryotes) is
(a) GUA (b) GCA
(c) CCA (d) AUG.

Sol. (d) : AUG codes for methionine and is initiation or starts the synthesis of polypeptide. UAA (ochre), UAG (amber) and UGA (opal) do not specify any amino acid so they are called termination codons. CUU, CUC, CUA and CUG codes for leucine whereas GCU, GCC, GCA and GCG codes for alanine.

7. A DNA with unequal nitrogen bases would most probably be
(a) single stranded
(b) double stranded
(c) triple stranded
(d) four stranded

Sol. (a) : A DNA with unequal nitrogen bases would most probably be single stranded. Nitrogenous bases are unequal in number in single stranded DNA, because they do not possess complementary base pairs.

Try yourself

8. A typical nucleosome "bead" is made up of
(a) 8 histone molecules and 146 base pairs of DNA
(b) 8 histone molecules and 200 base pairs of DNA
(c) 4 histone molecules and 146 base pairs of DNA
(d) 4 histone molecules and 200 base pairs of DNA

9. What is the approximate size of nucleus in a typical mammalian cell?
(a) 2.2 meter (b) 1.2 meter
(c) 10^{-4} meter (d) 10^{-6} meter

10. In one nucleosome, which one of the following histone molecule is NOT double?
(a) H4 (b) H3
(c) H2 (d) H1

11. Nucleosome contains
(a) only histone protein
(b) both DNA and histone protein
(c) only DNA
(d) both DNA and RNA.

12. A nucleosome along with linker DNA consists of
(a) eight molecules of histones and 146 base pairs
(b) eight molecules of histones and 200 base pairs
(c) nine molecules of histones and 146 base pairs
(d) nine molecules of histones and 200 base pairs

13. The association of histone H_1 with a nucleosome indicates
(a) transcription is occurring
(b) DNA replication is occurring
(c) the DNA is condensed into a chromatin fibre
(d) the DNA double helix is exposed

4.4 DNA Replication

Q.12 Can you recall?

i. What is the backbone of the DNA structure?

Ans : The backbone of the DNA structure formed of sugar-phosphate.

ii. Name the nitrogen bases of DNA.

Ans : The nitrogen bases found in DNA are Purine:adenine and guanine; Pyrimidines : cytosine and thymine.

iii. What are Nucleoside and Nucleotide?

Ans :

i. The nitrogen base combined with pentose sugar is called nucleoside, where nitrogen

base is attached by glycosidic bond.

ii. Nucleoside is basic in nature and the nitrogen base may be purine or pyrimidine.

iii. DNA is a nucleic acid formed from several deoxyribo-nucleotides.

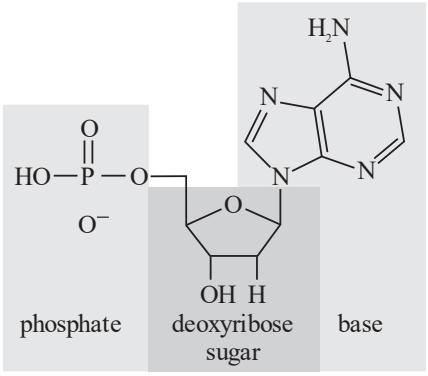
iv. Each deoxyribo-nucleotides has three components - Deoxyribose sugar, Phosphate group and Nitrogen base.

v. Deoxyribose sugar is a pentose sugar having a pentagonal structure.

vi. The phosphate group is phosphoric acid (H_3PO_4) it helps in linkage of deoxyribo-nucleotides.

vii. Nitrogen bases can be classified majorly two types i.e. Purines and Pyrimidines.

viii. Purines are double ring compounds and include Adenine (A) and Guanine (G), while Pyrimidines are single ring compounds which include Cytosine (C) and Thymine (T).



iv. Is the double helix right or left handed?

Ans : DNA helix mab lie either right handed i.e. in A or B form or left handed as in Z form.

Q.13 Find out

What is the key difference between DNA in prokaryotic and eukaryotic cells?

Ans : Cellular metabolism along with differentiation and development of an organism is controlled by DNA.

Q.14 Write a note on functions performed by DNA to regulate and centrol activity of the cell.

Ans : As a carrier of genetic information, DNA has to perform two important functions:

i. **Heterocatalytic function:**

a. When DNA directs the synthesis of chemical molecules other than itself, then such functions of DNA are called heterocatalytic functions.

b. Eg. Synthesis of RNA (transcription), synthesis of protein (Translation), etc.

ii. Autocatalytic function:

a. When DNA directs the synthesis of DNA itself, then such function of DNA is called autocatalytic function.

b. Eg. Replication.

TEXTUAL

★Q.15 Explain the process of DNA replication.

Ans :

i. The process by which DNA duplicates itself is called replication. Through replication, it forms two copies that are identical to it.

ii. In eukaryotic organisms, replication of DNA takes place only once in the cell cycle. It occurs in the S-phase of interphase in the cell cycle.

iii. The model for Semiconservative replication was proposed by Watson and Crick, on the basis of antiparallel and complementary nature of DNA strands.

The process of semicoservative replication is as below:

iv. **Activation of Nucleotides:**

a. The four types of nucleotides of DNA i.e. dAMP, dGMP, dCMP and dTMP are present in the nucleoplasm.

b. They are activated by ATP in presence of an enzyme **phosphorylase**.

c. This results in the formation of deoxyribonucleotide triphosphates i.e. dATP, dGTP, dCTP and dTTP.

d. The process is known as Phosphorylation.

v. **Point of Origin or Initiation point:**

a. It begins at specific point 'O' - origin and terminates at point 'T'.

b. Origin is flanked by 'T' sites.

c. The unit of DNA in which replication occurs, is called replicon.

d. In prokaryotes, there is only one replicon however in eukaryotes, there are several replicons in tandem.

e. At the point 'O', enzyme endonuclease

nicks one of the strands of DNA, temporarily.

f. The nick occurs in the sugar-phosphate back bone or the phosphodiester bond.

vi. **Unwinding of DNA molecule:**

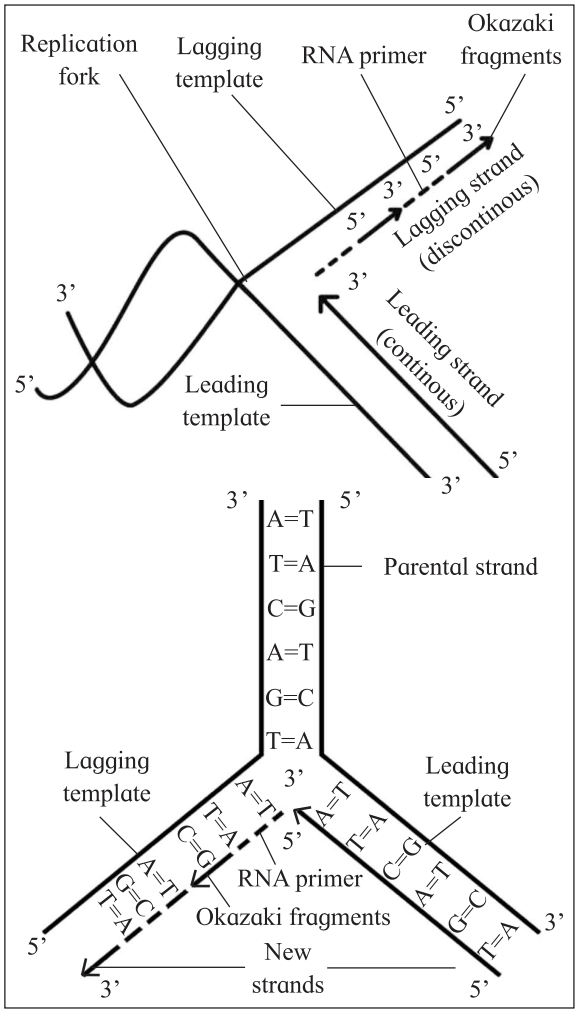
a. Now enzyme DNA helicase operates by breaking weak hydrogen bonds in the vicinity of 'O'.

b. The strands of DNA separate and unwind. This unwinding is bidirectional and continues as 'Y' shaped replication fork.

c. Each separated strand acts as template.

d. The two separated strands are prevented from recoiling (rejoining) by SSBP (Single strand binding proteins).

e. SSB proteins remain attached to both the separated strands so as to facilate synthesis of new polynucleotide strands.



vii. **Replicating fork:**

a. The point formed due to unwinding and separation of two strands appear like a Y-shaped fork, called replicating/replication fork.

b. The unwinding of strands imposes strain which is relieved by super-helix relaxing enzyme.

viii. **Synthesis of new strands:**

a. Each separated strand acts as mould or template for the synthesis of new complementary strand.

b. It begins with the help of a small RNA molecule, called RNA primer.

c. RNA primer get associated with the 3' end of template strand and attracts complementary nucleotides from surrounding nucleoplasm.

d. These nucleotides molecules bind to the complementary nucleotides on the template strand by forming hydrogen bonds (i.e. A=T or T=A; G ≡ C or C ≡ G).

e. The newly bound nucleotides get interconnected by phosphodiester bonds, forming a polynucleotide strand.

f. The synthesis of new complementary strand is catalyzed by enzyme DNA polymerase.

g. The new complementary strand is always formed in 5' → 3' direction.

ix. **Leading and Lagging strand:**

a. The template strand with free 3' end is called leading template and with free 5' end is called lagging template.

b. The process of replication always starts at C-3 end of template strand and proceeds towards C-5 end.

c. As both the strands of the parental DNA are antiparallel, new strands are always formed in 5' → 3' direction.

d. One of the newly synthesized strand develops continously towards replicating fork is called leading strand.

e. Another new strand develop discontinuously away from the replicating fork is called lagging strand.

f. **Maturation of Okazaki fragments:** DNA synthesis on lagging template takes place in the form of small fragments, called Okazaki fragments (named after scientist Okazaki).

g. Okazaki fragments are joined by enzyme DNA ligase.

h. RNA primers are removed by DNA polymerase and replaced by DNA sequence with the help of DNA polymerase-I in prokaryotes and DNA polymerase-á in eukaryotes.

i. Finally, DNA gyrase (topoisomerase) enzyme forms double helix to form daughter DNA molecules.

x. **Formation of daughter DNA molecules:**

a. At the end of the replication, two daughter DNA molecules are formed.

b. In each daughter DNA, one strand is parental and the other one is totally newly synthesized.

c. Thus, 50% is contributed by mother DNA. Hence, it is described as **semiconservative replication**.

Q.16 Explain the experimental confirmation of semi-conservative mode of DNA replication performed by Meselson and Stahl.

Ans :

i. In newly formed DNA molecule, one strand is old (i.e. conserved) and other strand is newly synthesized. Thus, it is called Semiconservative mode of replication.

ii. It is experimentally proved by Matthew Meselson and Franklin Stahl (1958) by using **equilibrium - density - gradient - centrifugation** technique.

iii. Meselson and Stahl in 1958 performed an experiment to prove semiconservative nature (mode) of replication.

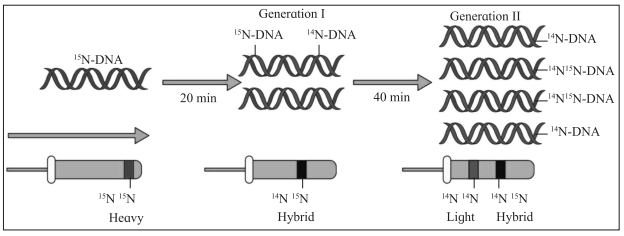
iv. They cultured bacteria *E.coli* in the medium containing ^{14}N (light nitrogen) and obtained equilibrium density gradient band by using 6M $CsCl_2$. The position of this band is recorded.

v. *E.coli* cells were then tranferred to 15N medium (heavy isotopic nitrogen) and allowed to replicate for several generations. At equilibrium point density gradient band was obtained, by using 6M $CsCl_2$. The position of this band is recorded.

vi. The heavy DNA (15N) molecule can be distinguished from normal DNA by centrifugation in a 6M Cesium chloride ($CsCl_2$) density gradient. The density gradient value of 6M $CsCl_2$ and ^{15}N DNA is almost same. Therefore, at the equilibrium point ^{15}N DNA will form a band. In this both the strands of DNA are labelled with ^{15}N.

vii. Such *E. coli* cells were they transferred to another medium containing ^{14}N i.e. normal (light) nitrogen. After first generation, the density gradient band for ^{14}N ^{15}N was obtained and its position was recorded. After second generation, two density gradient bands were obtained - one at ^{14}N ^{15}N position and other at ^{14}N position.

viii. The position of bands after two generations clearly proved that DNA replication is Semiconservative.



TEXTUAL

★Q.17 What is the function of RNA primer during DNA synthesis/replication?

Ans : The starting point for DNA polymerase to initiate a new DNA strand is provided by RNA primers.

★Q.18 Which enzyme does remove supercoils from replicating DNA?

Ans : Super helix relaxing enzyme removes supercoils from replicating DNA.

★Q.19 Why are Okazaki fragments formed on lagging strand only?

Ans :

i. The template strand with free 3' end is called

leading template and with free 5' end is called lagging template.

ii. The process of replication always starts at C-3 end of template strand and proceeds towards C-5 end.

iii. As both the strands of the parental DNA are antiparallel, new strands are always formed in 5' - 3' direction.

iv. One of the newly synthesized strand develops continously towards replicating fork is called leading strand.

v. Another new strand develop discontinuously away from the replicating fork is called lagging strand.

vi. Due to 5' → 3' polymerizing activity of DNA polymerase Okazaki fragments are formed only on lagging strand only.

Q.20 Use your brain?

i. List as many different enzyme activities required during DNA synthesis as you can.

Ans :

Enzyme	Function
Helicase (rep protein)	It is the DNA unwinding protein involved in the separation of the two DNA strands.
RNA primase	Controls synthesis of RNA primer.
DNA polymerase	Catalyses polymerisation in 5' → 3' direction.
R Nase	Removes RNA primer.
DNA ligase	Joins the Okazaki fragments
Gyrase	Relieves torsional strain generated by DNA unwinding.

ii. This type of replication is called semiconservative replication. Considering the meaning of these words, why DNA replication is called semiconservative replication?

Ans :

i. In each daughter DNA, one strand is parental and the other one is newly synthesized.

ii. Since 50% of parent DNA is conserved while 50% part is newly synthesized, DNA replication is called semiconservative replication.

MULTIPLE CHOICE QUESTIONS

Entrance Set 3

1. Match the following RNA polymerase with their transcribed products:
(1) RNA polymerase I (i) tRNA
(2) RNA polymerase II (ii) rRNA
(3) RNA polymerse III (iii) hnRNA
Select the correct option from the following:
(a) 1 - i, 2 - iii, 3 - ii
(b) 1 - i, 2 - ii, 3 - iii
(c) 1 - ii, 2 - iii, 3 - i
(d) 1 - iii, 2 - ii, 3 - i

Solution:(c)

2. The experimental proof for semi-conservative replication of DNA was first shown in a
(a) fungus (b) bacterium
(c) plant (d) virus

Solution:(b)

Semiconservative replication of DNA was proved by the work of Matthew Meselson and Franklin Stahl (1958) using bacterium Escherichia coli.

3. During DNA replication, Okazaki fragment are used to elongate
(a) the lagging strand towards replication fork
(b) the leading strand away from replication fork.
(c) the lagging strand away from the replication fork
(d) the leading strand towards replication fork.

Solution:(c)

Lagging strand is a replicated strand of DNA which is formed in short segments called Okazaki fragments. Its growth is discontinuous. The direction of growth fo the lagging strand in 3'- 5' through in each Okazaki fragment it in 5'-3'.

4. DNA-dependent RNA plymerase catalyses transcription on one strand of the DNA which is called the
(a) template strand (b) coding strand
(c) alpha strand (d) antistrand.

Solution:(a)

The strand of DNA on which RNA polymerase birds to catalyse transcription is called template strand. It is also known as master or antisense strand. It has the polarity of 3'- 5'.

5. Semi-conservative replication of DNA was first demonstrated in
(a) Escherichia coli
(b) Streptococcus pneumoniae
(c) Salmonella typhimurium
(d) Drosophila melanogaster.

Solution:(a)

Mathew Meselson and Franklin Stahl (1958) conducted various experiments using isotopically labelled DNA of Escherichia coli to provide evidence in favour of semi-converative mode of DNA replication.

6. In the DNA molecule,
(a) the proportion of adenine in relation to thymine varies with the organism
(b) there are two strands which run anti-parallel-one in 5' $\rightarrow$ 3' direction other in 3' $\rightarrow$ 5'
(c) the total amount of purine nucleotides and pyrimidine nucleotides is not always equal
(d) there are two strands which run parallel in the 5' $\rightarrow$ 3' direction.

Solution:(b)

A DNA molecule has two unbranched complementary strands which are spirally coiled. The two chains are antiparallel, i.e., they run parallel but in opposite direction. One chain has they polarity 5' $\rightarrow$ 3' whereas, other has 3' $\rightarrow$ 5'. Both are held together by hydrogen bonds between their bases, i.e., A = T and G = C and the amount of adenine is equal to thymine and guanine equals to cytosine. The base ratio A + T/G + C may vary from one species to another but is constant for a given species. The purine and pyrimidines are always in equal amount (A + G = T + C) but A + T is not necessarily equal to G + C.

7. Which one of the following pairs of nitrogenous bases of nuclei acids, is wrongly matched with the category mentioned against it?
(a) Guanine, Adenine - Purines
(b) Adenine,Thymine - Purines
(c) Thymine, Uracil - Pyrimdines
(d) Uracil, Cytosine - Pyrimidines

Solution:(b)

The two DNA chains are held together by hydrogen bonds between their nitrogenous bases. Adenine (A), a purine of one chain lies exactly opposite thymine (T), a pyrimidine of the other chain. Similarly, cytosine (C), a pyrimidine lies opposite guanine (G), a purine. Three hydrogen bonds occur between cytosine and guanine (C = G) at positions 1', 2', and 6' and two hydrogen bonds between adenine and thymine (A = T) at positions 1' and 6'.

8. Antiparallel strands of a DNA molecule means that
(a) one strand turns clockwise
(b) one strand turns anti-clockwise
(c) the phosphate groups of two DNA strands, at their ends, share the same position
(d) the phosphate groups at the start of two DNA strands are in opposite position (*pole*).

Solution:(d)

DNA is a type of nucleic acid that forms genetic material in many organisms. It consists of a long polymer of nucleotides which transcribes the coded information in the form of a triplet code of nucleotides in mRNA. It is a double helical molecule. The two strands of DNA run in opposite directions to one another with the hydrogen bonds between them. One strands of DNA has 5'-3' direction and the other strand has 3'-5' direction. So they are antiparallel. This direction is determined by the presence of a free phosphate or OH group at the end of the strand.

9. Which one of the following makes use of RNA template to synthesize DNA?
(a) DNA polymerase
(b) RNA polymerase
(c) Reverse transcriptase
(d) DNA dependent RNA polymerase
(2005)

Solution:(c)

$$DNA \xrightarrow{Transcription} mRNA \xrightarrow{Translation} Protein$$

This one-way flow of information from DNA to mRNA and then to protein is called the central dogma of molecular biology by F.H.C. Crick (1958). But later on two American workers H. Temin and D. Baltimore reported that DNA is also formed from RNA in retoviruses, e.g., HIV This is called reverse transcription or teminism, i.e.,

$$DNA \underset{\text{Reverse transcription}}{\overset{\text{Transcription}}{\rightleftharpoons}} mRNA \xrightarrow{Translation} Protein$$

This revenue transcription occurs under the influence of reverse transcriptase enzyme. So, HIV viruses does not follow central dogma.

10. Which one of the following hydrolyses internal phosphodiester bonds in a polynucleotide chain?
(a) Lipase (b) Protease
(c) Endonuclease
(d) Exonuclease

Solution:(c)

Endonucleases hydrolyse the internal phosphodiester bond. Exonucleases cleave the terminal nucleotides. Lipase digest fats and proteases break down proteins.

11. The following ratio is generally constant for a given species:
(a) A + G / C + T (b) T + C / G + A
(c) G + C / A + T (d) A + C / T + G

Solution:(c)

A DNA molecule has two unbranched complementary strands which are spirally coiled. The two chains are antiparallel, i.e., they run parallel but in opposite direction. One chain has they polarity 5' $\rightarrow$ 3' whereas, other has 3' $\rightarrow$ 5'. Both are held together by hydrogen bonds between their bases, i.e., A = T and G = C and the amount of adenine is equal to thymine and guanine equals to cytosine. The base ratio A + T/ G + C may vary from one species to another but is constant for a given species. The purine and pyrimidines are always in equal amount (A + G = T + C) but A + T is not necessarily equal to G + C.

12. mRNA is synthesised on DNA template in which direction.
(a) 5' $\rightarrow$ 3 (b) 3' $\rightarrow$ 5'
(c) both (a) and (b) (d) any.

Solution:(a)

mRNA is synthesized on DNA template in 5' $\rightarrow$ 3' direction. Synthesis of mRNA exhibits several features that are synonymous with DNA replication. RNA synthesis requires accurate and efficient initiation, elongation proceeds in the 5' $\rightarrow$ 3' direction (i.e., the polymerase moves along the template strand of DNA in the 5' $\rightarrow$ 3' direction) and RNA synthesis requires distinct and accurate termination. Transcription exhibits several features that are distinct from replication.

13. Method of DNA replication in which two strands of DNA separate and synthesize new strands?
(a) dispersive
(b) conservative
(c) semi-conservative
(d) non conservative.

Solution:(c)

The method of DNA replication is semi-conservative. According to the semi-conservative model proposed by Watson and Crick, each strand of the two double helices formed would have one old and one new strand. So, the parental identity is conserved upto half extent and hence DNA replication is sem-conservative.

14. In DNA, when AGCT occurs, their association is a per which of the following pair?
(a) AT-GC (b) AG-CT
(c) AC-GT (d) All of these.

Solution:(a)

DNA molecule has four bases - adenine, guanine, cytosine and thymine. Adenine always pairs with thymine and guanine pairs with cytosine. Their association is A-T and G-C.

15. If the sequence of bases in DNA is ATTCGATG, then the sequence of bases in its transcript will be
(a) GUAGCUUA (b) AUUCGAUG

(c) CAUCGAAU (d) UAAGCUAC

Sol. (d) : In transcription, mRNA is formed from DNA template and thymine of DNA is replaced by uracil of RNA. Uracil pairs with adenine.

DNA A T T C G A T G
| | | | | | | |
mRNA U A A G C U A C
(transcript)

16. If the DNA codons are ATG ATG ATG and a cytosine base is inserted at the beginning, then which of the following will result?
(a) CAT GAT GATG
(b) non-sense mutation
(c) C ATG ATG ATG
(d) CA TGA TGA TG

Sol. (a) : Nonsense mutationis a mutation which interconverts a nonsense to or from a sense-coding triplet, resulting in an abnomally foreshortened or elongated polypeptide chain. But in this example cytosine is added at the beginning so CAT GAT GAT G will result.

17. There are special proteins that help to open up DNA double helix in front of the replication fork. These proteins are
(a) DNA ligase
(b) DNA topoisomerase I
(c) DNA gyrase
(d) DNA polymerase I.

Sol. (b) : DNA is a double kelical molecule and it opens to form a replication fork for its replication. The two strands of DNA are joined with the help of H-bonds between the strands. Topoisonmerases are specialized to cause nicks or breaks in the double helix and helps separate the DNA stands. Helicase unwinds the DNA helix from that nick caused by the topoisomerase and this separates the two strands. DNA gyrase introduces negative supercoils in DNA strands of prokaryotes. DNA polymerase adds nucleotide units to the 3' end of a DNA chain. DNA ligase joins the ends of DNA.

18. During DNA replication, the strands separate by
(a) DNA polymerase (b) topoisomerase
(c) unwindase/helicase (d) gyrase

Sol. (c) : During DNA replication, the strands separate by unwindase/helicase. The molecule is unwound by DNA unwinding proteins called helicases. The helicases II and III get attached to lagging strand and protein to the leading strand. The formation of bands is avoided by single stranded DNA binding proteins (SSB).

19. Experimental material in the study of DNA replication has been
(a) Escherichia coli
(b) Neurospora crassa
(c) Pneumococcus
(d) Drosophila melanogaster.

Sol. (a) : Experimental material in the study of DNA replication has been *Escherichia coli. E.coli* fully labelled with ^{15}N is allowed to grow in ^{14}N medium. The two strands of DNA molecule of the first generation bacteria have different density and do not resemble parent DNA. Meselson and Stahl, 1958 by using ^{14}N and ^{15}N confirmed that the replication of DNA in *E.coli* is semi-conservative in nature.

20. Escherichia coli fully labelled with ^{15}N is allowed to grow in ^{14}N medium. The two strands of DNA molecule of the first generation bacteria have
(a) different density and do not resemble parent DNA
(b) different density but resemble parent DNA
(c) same density and resemble parent DNA
(d) same density but do not resemble parent DNA.

Sol. (a) : Experimental material in the study of DNA replication has been *Escherichia coli. E.coli* fully labelled with ^{15}N is allowed to grow in ^{14}N medium. The two strands of DNA molecule of the first generation bacteria have different density and do not resemble parent DNA. Meselson and Stahl, 1958 by using ^{14}N and ^{15}N confirmed that the replication of DNA in *E.coli* is semi-conservative in nature.

21. In the genetic dictionary, there are 64 codons as

(a) 64 amino acids are to be coded
(b) 64 types of tRNAs are present
(c) there are 44 nonsense codons and 20 sense codons
(d) genetic code is triplet.

Sol. (d)

22. DNA replication is
(a) conservative and discontinuous
(b) semi-conservative and semi-discontinuous
(c) semi-conservative and discontinuous
(d) conservative.

Sol. (b)

23. Genetic code consists of
(a) adenine and guanine
(b) cytosine and uracil
(c) cytosine and guanine
(d) all the above.

Sol. (d) : The genetic information is transfered from DNA to mRNA to protein. The proteins are made up of some 20 amino acids whose sequence is hidden in the sequence of nucleotides of mRNA. Hence, genetic code consists of all 20 amino acids. Thus genetic code is the relationship of amino acids sequence in a polypetide and nucleotide/base sequence in mRNA antisense strand and DNA.

Try yourself

24. The two strands of DNA are
(a) Similar and Parallel
(b) Complementary and parallel
(c) Complementary and antiparallel
(d) Similar and discontinuous.

25. Polymerization of DNA nucleotides during the synthesis of lagging strand occurs in
(a) 3' → 5' direction
(b) 5' → 3' direction
(c) any direction
(d) promoter to terminator direction

26. During DNA replication, the addition of nucleotides on the lagging strand occurs
(a) towards the replicating fork
(b) at a faster rate than leading strand
(c) continuously
(d) discontinuously

27. During DNA replication, Okazaki fragments are used to elongate
(a) the leading strand towards replication fork
(b) the lagging strand towards replication fork
(c) the leading strand away from replication fork
(d) the lagging strand away from the replication fork.

28. With respect to DNA synthesis, identify the correct combination of statements.
i. Always the direction of DNA polymerization 5' → 3' when referring to the polarity of strand being synthesized.
ii. DNA ligase forms hydrogen bonds between two newly synthesized DNA strands.
iii. DNA polymerases on their own cannot initiate the process of replication.
iv. DNA polymerase can catalyse polymerization in both 5'→3' and 3'→5' direction
(a) ii, iii and iv (b) i and ii
(c) i and iii (d) iii and iv

29. The experimental proof for semi-conservative replications of DNA was first shown in a
(a) Plant (b) Bacterium
(c) Fungus (d) Virus

30. *E. coli* bacteria grew in $^{15}NH_4Cl$ medium for several generations are allowed to grow in $^{14}NH_4Cl$ medium. After 2 generation, the bacteria are isolated from the medium and DNA of bacteria centrifuged in CsCl. The result of the density gradient of DNA is
(a) only hybrid DNA
(b) both hybrid and heavy DNA
(c) both heavy and light DNA
(d) both hybrid and light DNA

31. If *E. coli* is allowed to grow for 40 minutes in a medium containing N^{15}, then the number of N^{14} / N^{14} containing DNA would be
(a) zero (b) 20
(c) 10 (d) 2

32. The following is gradually used for creating density gradient during centrifugation
(a) NaCl (b) KCl
(c) CsCl (d) $MgCl_2$

33. *Escherichia coli*, in which both the strands of DNA are labeled with 15N is transferred to 14N medium and allowed to replicate for three generations. Find out the number of hybrid DNA molecules in the third generation.
(a) 2 (b) 8
(c) 10 (d) 12

34. **Assertion (A):** DNA replication is continuous on leading strand while on the lagging strand it is discontinuous.
Reason (R): This is because DNA polymerase cannot initialize polymerization without a primer.
The correct answer is:
(a) Both (A) and (R) are true, (R) is the correct explanation of (A).
(b) Both (A) and (R) are true but (R) is not the correct explanation of (A).
(c) (A) is true but (R) is false.
(d) (A) is false but (R) is true.

4.5 Protein Synthesis

Q.21 Define transcripton.

Ans : The process of copying of genetic information from one (template) strand of DNA into a single stranded RNA transcript, is termed as **transcription**.

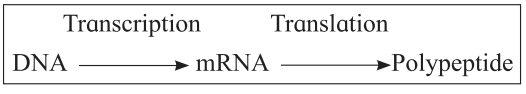
Q.22 Write a note on Central Dogma of molecular biology.

Ans :

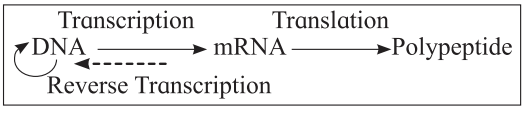
i. Double stranded DNA molecule gives rise to mRNA which acts as a messenger to programme the synthesis of a polypeptide chain (protein).

ii. This type of unidirectional flow of information from DNA to RNA to protein/ proteins is referred as central dogma of molecular biology.

iii.



The present concept of central dogma in retroviruses or riboviruses is given by Temin (1970) and Baltimore (1970):



Q.23 Can you recall?
i. What is transcription?

Ans : *Refer Q.21*

TEXTUAL

★Q.24 Describe the process of transcription in protein synthesis.

Ans :

i. In transcription, information of only one strand of DNA is copied into RNA. This strand of DNA acts as template.

ii. Enzyme RNA polymerase catalyses the formation of RNA transcript.

iii. Transcription occurs in the nucleus during G1 and G2 phases of cell cycle.

iv. DNA has promotor and terminator sites. Transcription starts at promotor site and stops at terminator site.

v. The process of transcription, in both Prokaryotes and Eukaryotes, involves three stages viz. Initiation, Elongation and Termination.

vi. After binding to promoter, RNA polymerase moves along the DNA and causes local unwinding of DNA duplex into two chains in the region of the gene.

vii. Exposed ATCG bases project into nucleoplasm.

viii. Only one strand functions as template (antisense strand) and the other strand is complementary which is actually a coding strand (sense strand).

ix. The ribonucleoside tri phosphates join to bases of DNA template chain.

x. As transcription proceeds, the hybrid DNA-RNA molecule dissociates and makes mRNA molecule free.

xi. When RNA polymerase reaches the terminator signal on the DNA, it leaves DNA and fully formed mRNA (primary transcript) is released.

Q.25 Write a note on structure of transcription unit.

Ans : Each transcribed segment of DNA is called transcription unit. It consists of **i. Promotor**,

ii. The structural gene, iii. **A terminator**. Two strands of DNA in the structural gene show following features :

i. **The promotor** is located towards 5' end of structural gene i.e. upstream. It is a DNA sequence that provides binding site for enzyme RNA polymerase. RNA polymerase binds to specific Promotor. In prokaryotes, the enzyme recognizes the promotor by its sigma factor sub unit.

ii. **Structureal genes** - two strands of DNA have opposite polarity. DNA dependent RNA polymerase catalyses polymerisation in 5 $\rightarrow$ 3' direction. So the DNA strand having 3' $\rightarrow$ 5' polarity acts as template strand. The other strand of DNA having 5' $\rightarrow$ 3' polarity is complementary to template strand. The sequence of bases in this strand, is same as in RNA (where Thymine is replaced by Uracil). It is the actual coding strand. The information on this strand of DNA is copied on mRNA. This is called sense strand.

iii. **The terminator** is located at 3' end of coding strand i.e. downstream. It defines the end of the transcription process.

Q.26 Write a note on different types of RNA polymerases present in prokayotes and eukaryotes?

Ans :

i. In bacteria, m-RNA does not require any processing because it has no introns. Prokaryotes posses only one type of RNA polymerase.

ii. In eukaryotes, there are three types RNA polymerases.

iii. RNA polymerase-I transcribes r-RNA.

iv. RNA polymerase-II transcribes m-RNA (primary transcript) and heterogeneous nuclear RNA (or hnRNA).

v. RNA polymerase-III is responsible for transcription of t-RNA and small nuclear RNA (snRNA).

Q.27 Explain transcription unit and gene.

Ans :

i. The DNA sequence coding for m-RNA/t-RNA or r- RNA is defined as a gene.

ii. Cistron is a segment of DNA coding for a polypeptide.

iii. A single structural gene in transcription unit is said to be **monocistronic** where as a long segment of DNA having set of various structural genes in one transcription unit is referred as **polycistronic**.

iv. Structral genes in eukaryotes have interrupted non-coding sequences (**introns**).

v. The coding sequences or expresssequences are defined are **exons**.

vi. Only exons appear in procesed mRNA in Eukaryotes.

Q.28 Write a note on processing of hnRNA.

Ans :

i. In eukaryotes, forms of RNA transcribed from DNA are called primary transcripts. Such transcripts undergo changes called processing or maturation before becoming functional.

ii. Primary transcript is non functional and contains both exons and introns.

iii. During processing only introns are removed by the process called splicing.

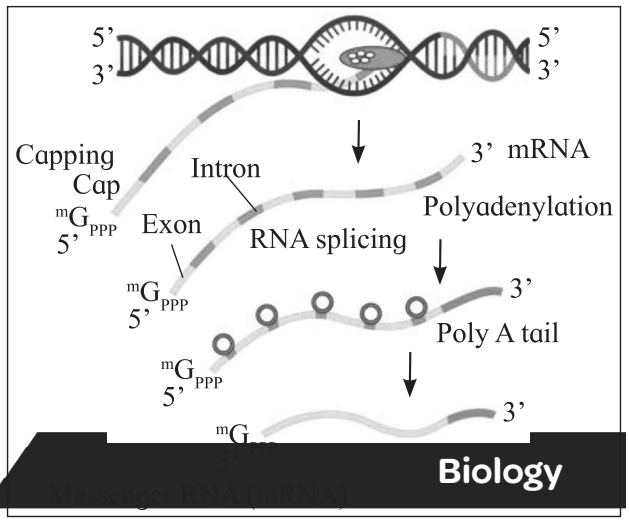
iv. Exons are joined in a definite sequence (order) by DNA ligase enzyme.

v. Heterogeneous nuclear RNA, undergoes the process of capping and tailing.

vi. In capping, methylated guanosine tri phosphate is added to 5' end of hnRNA.

vii. In tailing, polyadenylation take place at 3'end.

viii. It is the fully processed hnRNA, now called m-RNA. For translation m-RNA is transported out of the nucleus through nuclear pore.



Q.29 Can you recall?
How many nucleotides are present in codon?

Ans : There are three nucleotides in a codon.

Q.30 Why does each codon have 3 bases and not two?

Ans :

i. A single nitrogen base in a codon (singlet codon) will encode for only four different types of amino acids.

ii. A combination of two nitrogen bases (doublet codon) will specify only 16 different types of amino acids.

iii. A combination of three nitrogen bases (triplet codon) will specify 64 different types of amino acids.

iv. Hence G. Gamov (1954) suggested that in a codon, there must be combination of three consecutive nitrogen bases that will be sufficient to specify 20 different types of amino acids.

Q.31 Define terms:
i. Codon ii. Codogen

Ans :

i. **Codon:** A sequence of three adjacent nucleotides in mRNA that codes for amino acid is known as codon.

ii. **Codogen:** It is the smallest possible sequence (triplet) of nucleotides present on DNA stand which can specify one particular amino acid.

TEXTUAL

★Q.32 Enlist the characters of genetic code.

Ans : Genetic code of DNA has certain fundamental characteristics:-

i. **Genetic code is a triplet code:**
   a. Sequence of three consecutive bases constitute codon, which specifies one particular amino acid.
   b. Base sequence in a codon is always in 5'→ 3' direction.
   c. In every living organism genetic code is a triplet code.

ii. **Genetic code has distinct polarity:**
   a. Genetic code shows definite polarity i.e. direction. It, therefore, is always read in 5' → 3' direction and not in 3' → 5' direction.
   b. Otherwise message will change e.g. 5' AUG 3'.

iii. **Genetic code is non-overlapping:**
   a. Code is non overlapping i.e. each single base is a part of only one codon. Adjacent codons do not overlap.
   b. If non-overlapping, then with 6 consequtive bases only two amino acid molecules will be in the chain.
   c. Had it been overlapping type, with 6 bases, there would be 4 amino acid molecules in a chain. Experimental evidence is in favour of non-overlapping nature.

iv. **Genetic code is commaless:** There is no gap or punctuation mark between successive/ consecutive codons.

v. **Genetic code has degeneracy:**
   a. Usually single amino acid is encoded by single codon. However, some amino acids are encoded by more than one codons. e.g. Cysteine has two codons, while isoleucin has three codons.
   b. This is called degeneracy of the code. Degeneracy of the code is explained by Wobble hypothesis.
   c. Here, the first two bases in different codons are identical but the third one, varies.

vi. **Genetic code is universal:** By and large in all living organisms the specific codon specifies same amino acid. e.g. codon AUG always specifies amino acid methionine in all organisms from bacteria up to humans.

vii. **Genetic code is non-ambiguous:**
   a. Specific amino acid is encoded by a particular codon.
   b. Alternatively, two different amino acids will never be encoded by the same codon.

viii. **Initiation codon and termination codon:**
   a. AUG is always an initiation codon in any and every mRNA. AUG codes for amino

acid methionine.

b. Out of 64 codons, three codons viz. UAA, UAG and UGA are termination codons which terminate/stop the process of elongation of polypeptide chain, as they do not code for any amino acid.

ix. **Universal:** Usually in all organisms the specific codon specifies same amino acid.

x. **Codon and anticodon:**

a. Codon is a part of DNA e.g. AUG is codon. It is always represented as 5' AUG 3'.

b. Anticodon is a part of tRNA. It is always represented as 3' UAC 5'.

★Q.33 Why the genetic code is considered as commaless?

Ans : There is no gap or punctuation mark between successive/consecutive codons.

★Q.34 What is degeneracy og genetic code.

Ans :

i. Usually single amino acid is encoded by single codon. However, some amino acids are encoded by more than one codons. e.g. Cysteine has two codons, while isoleucin has three codons.

ii. This is called degeneracy of the code. Degeneracy of the code is explained by Wobble hypothesis.

iii. Here, the first two bases in different codons are identical but the third one, varies.

INTEXT

Q.35 Find out!

What is the amino acid sequence encoded by base sequence UCA UUU UCC GGG AGU of an mRNA segment?

Ans : The amino acid sequence encoded by the given base sequence is as follows: serine, phenylamine, serine, glycine, serine.

Q.36 Write a note on mutation.

Ans :

i. Mutation is a phenomenon in which sudden change in the DNA sequence takes place. It results in the change of genotype (i.e. character).

ii. Along with recombination, mutation is raw material for evolution as it also results in variations.

iii. During mutation, possibility of loss (deletion) or gain (insertion/duplication) of a segment of DNA results in alteration in the chromosome.

iv. Mutation can also occur due to change in a single base pair of DNA. This is known as point mutation. Eg. Sickle cell anaemia.

v. Deletion or insertion of base pairs of DNA causes frame – shift mutations or deletion mutation.

vi. Insertion or deletion of one or two bases changes the reading frame from the point of insertion or deletion.

vii. Insertion or deletion of three or multiples of three bases (insert or delete) results in insertion or deletion of amino acids and reading frame remains unaltered from that point onwards.

Q.37 Write a brief note on tRNA

Ans :

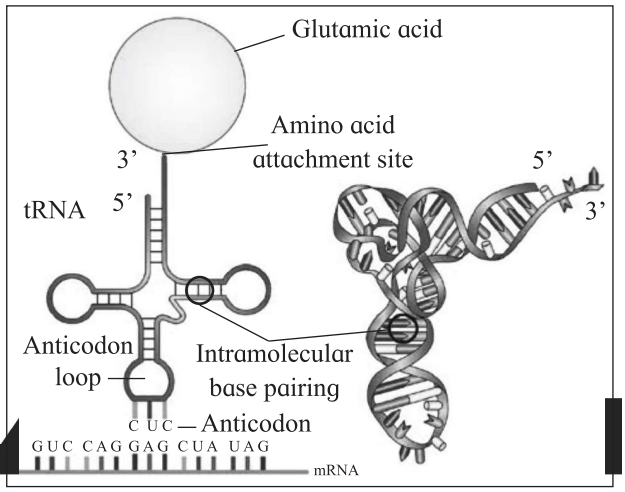
i. Cloverleaf structure (2 dimentional) of t-RNA possess an anticodon loop that has bases complementary to the codon. It is called **anticodon**.

ii. It shows amino acid acceptor end (3' end) having unpaired CCA bases (i.e. amino acid binding site) to which amino acid binds.

iii. For every amino acid, there is specific t- RNA.

iv. Initiator t-RNA is specific for methionine.

v. There are no t-RNA's for stop codons. In the actual structure, the t-RNA molecule looks like inverted L (3 dimentional structure).



INTEXT

Q.38 Can you recall?

i. What is mutation.

Ans : Mutation is a phenomenon in which sudden change in the DNA sequence takes place which results in the change of genotype and untimately variation in the phenotype.

ii. Name the molecule which carries anticodon?

Ans : tRNA carries on its anticodon loop.

iii. Why tRNA is called as adapter molecule.

Ans : Scientists considered that there has to be a mechanism in which t-RNA will read the codon and also simultaniously binds with the amino acid as amino acid does not have any special capacity to read the codon. So t-RNA is considered as an adapter molecule. This role of tRNA was understood much later.

iv. Name the different types of RNAs.

Ans : The three types of RNAs are ribosomal RNA (rRNA), messenger (mRNA) and transfer RNA (tRNA).

v. Name the site of protein synthesis.

Ans : Ribosomes are the site of protein synthesis.

vi. Which molecule carries information of protein synthesis from gene?

Ans : Information of protein synthesis from gene is carried by messenger RNA (mRNA).

vii. Which molecule carries amino acid from cytoplasm to ribosome?

Ans : Amino acid from cytoplasm to ribosome is carried by Transfer RNA (tRNA).

Q.39. Describe the process of translation in protein synthesis.

Ans :

i. Translation is the mechanism in which codons of mRNA are translated and specific amino acids in a sequence form a polypeptide on ribosomes.

ii. All types of proteins are synthesised by the cell, within itself (i.e. intracellularly).

iii. Process of translation requires amino acids, mRNA, tRNA, ribosomes, ATP, Mg^{++} ions, enzymes, elongation, translocation and release factors.

iv. Amino acids form raw material for protein synthesis. About 20 different types of amino acids are known to form proteins. These are available in the cytoplasm.

v. DNA controls synthesis of proteins having amino acids in specific sequence. This control is possible through transcription of m-RNA. Genetic code is specific for particular amino acid.

vi. RNAs serve as intermediate molecules between DNA and protein.

vii. Ribosomes serve as site for protein synthesis. Each ribosome consists of large and small subunits. These subunits occur separately in cytoplasm. Only during protein synthesis, these two subunits get associated together due to Mg^{++} ions.

viii. Mechanism of translation (i.e. synthesis of polypeptide chain). It involves three steps:

1. Initiation 2. Elongation
3. Termination

1. Initiation of Polypeptide chain:

a. Virulent, smooth,

a. Activation of amino acids is essential before translation initiates for which ATP is essential.

b. Small subunit of ribosome binds (attaches) to the m-RNA at 5' end.

c. Initiator codon, AUG is present on m-RNA which initiates the process of protein synthesis (translation).

d. Initiator t-RNA binds with initiation codon (AUG) by its anticodon (UAC) through hydrogen bonds.

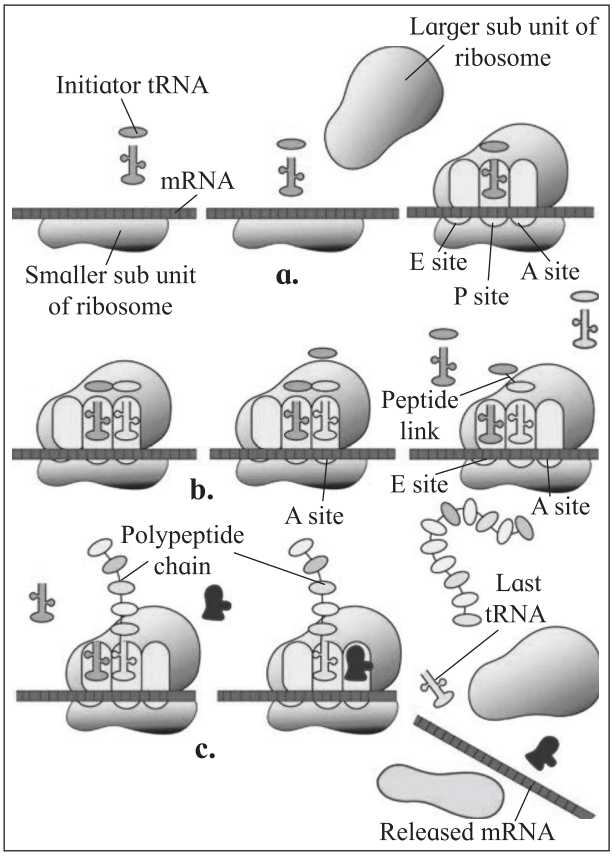
e. It carries activated amino acid methionine (in Eukaryotes) or formyl methionine (in prokaryotes).

f. Now the large subunit of ribosome joins with the smaller subunit, that requires Mg^{++} ions.

g. Initiator charged t-RNA (with activated amino acid methionine) occupies the P-site of ribosome and A-site is vacant.

2. **Elongations of polypeptide chain:**

a. During this process, activated amino acids are added one by one to first amino acid (methionine).
b. Amino acid is activated by utilising energy form ATP molecule.
c. This amino acid binds with amino acid binding site of t-RNA-This results in formation of t-RNA-amino acid complex.
d. Condon recognition- Amino acyl t-RNA molecule enters the ribosome at A-site. Anticodon binds with the codon by hydrogen bonds.
e. Amino acid on the first initiator t-RNA at P-site and amino acid on t-RNA at A-site join by peptide bond. Here enzyme Ribozyme acts as a catalyst. At this time first tRNA at 'P' site is kicked off.
f. Translocation-The t-RNA at A-site carrying a dipeptide at A-site moves to the P-site. This process is called **translocation**.
g. In translocation, both the subunits of ribosome move along in relation to tRNA and mRNA.
h. Hence, tRNA carrying dipeptide now gets positioned at 'P' site of ribosome, making 'A' site vacant. At this site, then next charged tRNA molecule carrying amino acid will be received.
i. During this process, first uncharged tRNA is discharged from E-site.
j. This process is repeated as amino acids are added to Polypeptide. It takes less than 0.1 second for formation of peptide bond.
k. As ribosome move over the m-RNA, all the codons on mRNA are exposed one by one for translation.



3. **Termination and release of polypeptide:**
a. At the 3' end of m-RNA, there is a stop codon (UAA/ UAG/ UGA). It is exposed at the A-site.
b. It is not read and joined by anticodon of any t-RNA.
c. The release factor binds to the stop codon, thereby terminating the translation process.
d. The Polypeptide is now released in the cytoplasm.
e. Two subunits of Ribosome dissociate and last tRNA is set free in the cytoplasm.
f. m-RNA also has some additional sequences that are not translated and are referred as untranslated regions (UTR).
g. The UTRs are present at both 5'-end (before start codon) and at 3'-end (after stop codon). They are required for efficient translation process.
h. Finally mRNA is also released in the cytoplasm. It gets denatured by nucleases immediately. Hence mRNA is short -lived.

Q.40 Can you tell?

i. Enlist different steps of protein synthesis.

Ans : Initiation, elongation and termination of polypeptide are steps of protein synthesis.

ii. Name the initiator codon of protein synthesis.

Ans : AUG is the initiator codon of protein synthesis.

iii. Explain in brief the process of initiation during protein synthesis.

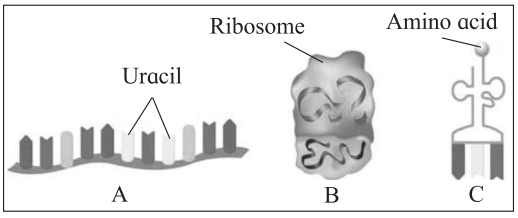
Ans. *Refer Q.39(i)*

iv. Name three binding sites of ribosome.

Ans : The three binding sites are:

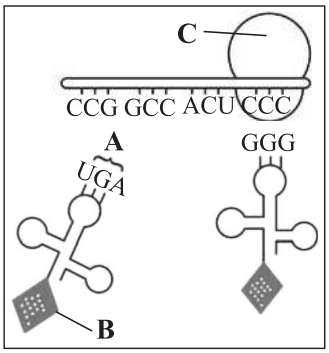
a. P site (peptidyl t-RNA site).
b. A site (aminoacyl - t-RNA site).
c. E site (Exit site)

Q.41 In the figure below A, B and C are three types of _____ .



Ans : In the given figure A (Messenger RNA), B (Ribosomal RNA) and C (Transfer RNA) are three types of Ribonucleic acids (RNA).

Q.42 Identify the labeled structures on the following diagram of translation.



Ans :
i. Part A is the **Anticodon**
ii. Part B is the **Amino Acid**
iii. Part C is the **Large subunit of ribosome**.

TEXTUAL

★Q.43 Justify the statement.
If the answer is false, change the underlined words to make the statement true.

i. The DNA molecule is double stranded and the RNA molecule is single stranded.

Ans :

i. DNA needs to be stable and resistant to enzymatic or oxidative alteration as the are responsible for storing genetic information.

ii. Phosphate groups keeps the Nitrogenous base pairs which stores the genetic information stacked inwards.

iii. DNA is further wrapped up around histone proteins into chromosomes which keep it condensed which wont be possible if it was single stranded.

iv. RNA acts as a template carrying information which is used in protein synthesis thus not meant to last long. Thus is single stranded.

ii. The process of translation occurs at the ribosome.

Ans : The process of translation occurs at the ribosomes as they are the site for protein synthesis Ribosome is responsible for holding mRNA in correct position.

iii. The job of mRNA is to pick up amino acids and transport them to ribosomes.

Ans : Its the job of tRNA to pick up amino acids and transport them to the ribosomes.

iv. Transcription must occur before translation may occur.

Ans : Transcription forms mRNA which acts as a template in the process of translation. Thus, transcription must occur before translation does.

MULTIPLE CHOICE QUESTIONS

Entrance Set 4

4.6 Regulation of Gene Expression.

Q.44 Regulation of gene expression occurs at which levels in eukoryotes?

Ans : In eukaryotes, the regulation can be at different levels like:

i. Transcriptional level (formation of primary transcript).

ii. Processing level (regulation of splicing).
iii. Transport of m-RNA from nucleus to the cytoplasm.
iv. Translational level.

Q.45 What is feedback repression?

Ans :

i. Repressible regulation of gene is seen when the end product of a biosynthetic pathway like amino acid, is provided in the medium.
ii. At this time, internal biosynthesis of amino acid stops.
iii. It is negative control so the metabolite (amino acid) turns off a set of genes involved in producing that metabolite.
iv. This is called feedback repression.

Q.46 Write a note on inducible enzymes and the phenomenon of induction.

Ans :

i. Certain bacteria like *E.coli* adapt to their chemical environment by synthesizing certain enzymes depending upon the substrate present.
ii. Such adaptive enzyme is called **inducible enzymes**.
iii. A set of genes will be switched on when there is necessity to metabolise a new substrate.
iv. This phenomenon is called **induction** and small molecule responsible for this, is known as **inducer**.
v. It is positive control.

4.7 Operon concept

Q.47 Describe the structure of 'Operon'.

Ans : Operon consists of following components :

i. Regulator gene (repressor gene)
ii. Promoter gene
iii. Operator gene
iv. Structural genes

i. **Regulator gene:**
   a. This gene controls the operator gene in cooperation with an inducer present in the cytoplasm.
   b. Regulator gene preceeds the promoter gene. It may not be present immidiately adjacent to operator gene.
   c. Regulator gene produces a protein called repressor protein.
   d. Repressor binds with operator gene and represses (stops) its action. It is called regulator protein.

ii. **Promoter gene:**
   a. This gene preceeds the operator gene. It is present adjacent to operator gene.
   b. The promoter gene marks the site at which the RNA Polymerase enzyme binds.
   c. When the operator gene is turned on, the enzyme moves over the operator gene and transcription of structural genes startS.
   d. Promoter gene base sequence determines which strand of DNA acts a template.

iii. **Operator gene:**
   a. It preceeds the structural genes. This controls the functioning of structural genes.
   b. It lies adjacent to the Structural genes.
   c. When operator gene is turned on by an inducer, the Structural genes produce m-RNA.
   d. Operator gene is turned off by a product of repressor gene.

iv. **Structural gene:**
   a. When lactose is added to the *E.coli* culture, the structural genes catalyse (produce) m-RNA which in turn produces polypeptides, on the ribosomes.
   b. The polypeptides formed, act as enzymes to caltalyse lactose in the cell.
   c. There are 3 structural genes in the sequence lac-Z, lac-Y and lac-A.
   d. Enzymes produced are β-galactosidase, β-galactoside permease and transacetylase respectively.

Q.48 Descibe the 'Lac Operon'.

Ans :

i. Lactose or lac operon of *E.coli* is inducible operon. The operon is switched on when a chemical inducer-lactose is present in the medium.
ii. Jacob and Monad proposed the classical model of Lac operon.
iii. The Lac operon consists of promoter site (P),

regulatory site (i) and operator site (O).

iv. It also has three structural genes, namely z, y and a each producing an enzyme.

v. The following three enzymes are required for the metabolism of lactose in the cell.

Name of gene	Enzyme produced	Function
lac z	β-galactosidase	Lactose $\xrightarrow{\beta\text{-galactosidase}}$ Glucose + Galactose
lac y	Permease	Entry of lactose in the cell
lac a	Transacetylase	Transfers acetyl group from Acetyl CoA to β-galactosidase

vi. If glucose is not available for cells, they will require another source of energy such as lactose.

vii. If lactose is not available, the repressor protein produced by repressor gene will attach to the operator and block RNS polymerase.

viii. Lactose acts as an inducer. If lactose is available, it will prevent the repressor from binding the operator, by forming inducer-repressor complex and allow RNA polymerase to transcribe mRNA.

ix. RNA polymerase will attach to promoter and will begin transcribing mRNA.

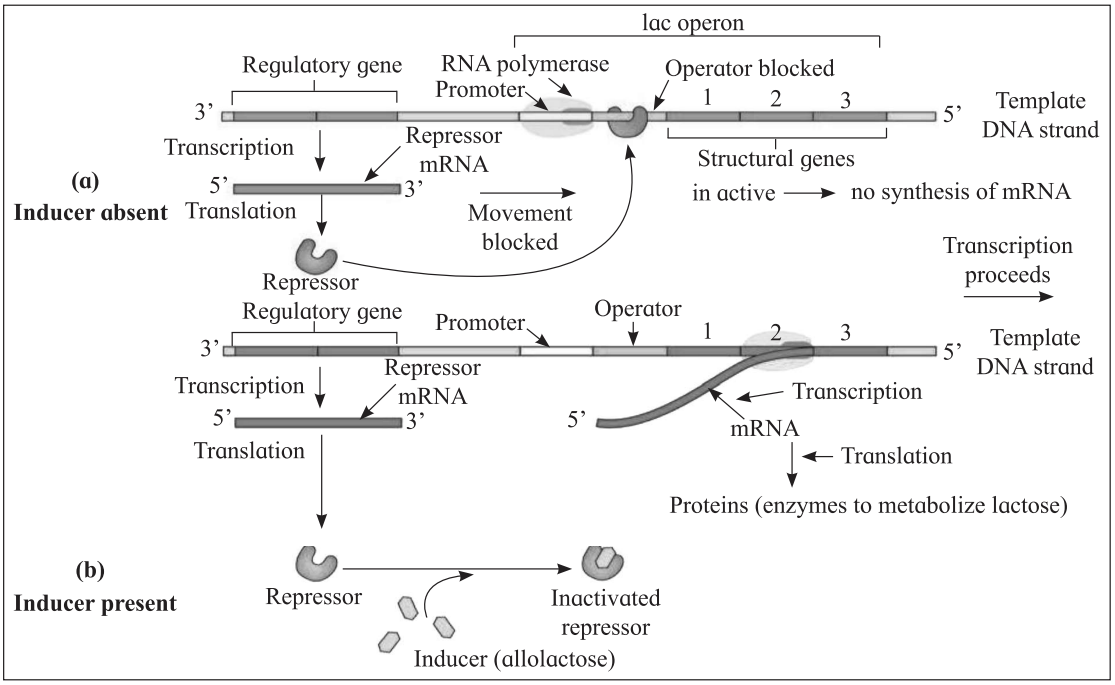
x. RNA polymerase first transcribes the lac z gene which is responsible for synthesizing β-galactosidase.

xi. RNA polymerase moves on to the next gene, lac y that synthesizes the enzymes permease.

xii. RNA polymerase finally moves to the lac a gene which is responsible for synthesizing transacetylase.

xiii. β-galactosidase, permease and transacetylase are enzymes in the metabolic pathway used to get energy from lactose.

xiv. After lactose is used up and levels decrease, the repressor will attach to the operator blocking the production of β-galactosidase, permease and transacetylase, so that lactose levels increase.



Q.49 Explain the role of Lactose in 'Lac Operon'.

Ans :

i. A few molecules of lactose enter into the cell by an enzyme permease.

ii. A small amount of this enzyme is present even when operon is switched off.

iii. A few molecules of lactose, act as inducer and bind to repressor.

iv. This repressor – inducer complex fails to join with the operator gene, which is then turned on.

v. Structural genes produce all enzymes. Thus, lactose acts as an inducer of its own break down.

vi. When the inducer level falls, the operator is blocked again by repressor. So structural genes are repressed/inactivated again. This is negative feedback.

Q.50 Use your brain power

If operator gene is deleted due to mutation, how will E.coli metabolise lactose?

Ans : If there was a mutation in the operator, the repressor protein will not bind to the operator (if there is no lactose in the environment). Mutations in the operator, prevent repressor binding, due to this the transcription will not be stopped and there will be continuous transcription. Mutation in the operator gene, would prevent the repressor from binding, thus yielding constitutive mutants or result in constitutive transcription (in which cellular production of a molecule occurs at a constant rate, which is not regulated by internal or external stimuli).

Q.51 Can you tell?

i. What is the role of a repressor gene?

Ans : *Refer Q.47-iii & iv.*

ii. Name the different structural genes in sequence of lac operon.

Ans : Lac-Z, Lac-Y and Lac-A are the different structural genes in sequence of Lac Operon.

iii. Which molecule does act as inducer molecule in lac operon?

Ans : Lactose molecule acts as a inducer molecule in Lac operon.

iv. In which condition, lac operon is switched off?

Ans : Lac operon is switched off when lactose is absent in the culture medium as there would be no requirement of lactose metabolizing enzymes.

4.8 Genomics

TEXTUAL

Q.52 What is genome?

Ans : The term **Genome** (introduced by H.Winkler in 1920) is the total genetic constitution of an organism. Alternatively, it is a complete copy of genetic information (DNA) or one complete set of chromosomes (monoploid or haploid) of an organism.

Q.53 What is Genomics?

Ans : The term **Genomics** (term coined by T.H. Roderick in 1986) is the study of genomes through analysis, sequencing and mapping of genes along with the study of their functions.

Q.54 Write a note on types of genomics and their significance.

Ans : Genomics study may be classified into two types:

i. **Structural genomics:** It involves mapping, sequencing and analysis of genome.

ii. **Functional genomics:** It deals with the study of functions of all gene sequences and their expression in organisms.

Q.55 Enlist the application of genomics.

Ans :

i. Structural and functional genomics is used for different purposes in the improvement of crop plant, human health and livestock.

ii. The knowledge and understanding acquired from genomics research can be applied in a number of different sectors, including medicine, biotechnology and social sciences.

ii. It helps in the treatment of genetic disorders through gene therapy.

iv. Genomics is used in agriculture to develop transgenic crops having more desirable characters.

v. Genetic markers developed in genomics, have applications in forensic analysis.

vi. Genomics can lead to introduce new gene in microbes to produce enzymes, therapeutic proteins and even biofuels.

4.9 Human Genome Project

Q.56 Write a note on Human Genome Project (HGP).

Ans :

i. The human genome project was initiated in 1990 under the International administration

of the Human Genome Organization (HUGO).

ii. This project was co-ordinated by the US department of Energy and National institute of health. Additional contributors included universities across the United States and international partners in the United Kingdom, France, Germany, Japan, India and China.

iii. The Human Genome Project formally began in 1990 and was completed in 2003.

iv. The main aims of project are:
   a. Mapping the entire human genome at the level of nucleotide sequences.
   b. To store the information collected from the project in databases.
   c. To develop tools and techniques for analysis of the data.
   d. Transfer of the related technologies to the private sectors, such as industries.
   e. Taking care of the legal, ethical and social issues which may arise from project.

Q.57 Use your brain power.

i. Why is HGP important.

Ans :

i. HGP (Human Genome Project) was closely associated with rapid development of a new area in biology, called **Bioinformatics**.

ii. The work of human genome project has allowed researchers to begin to understand the blueprint in building and constructing the human genome.

iii. As researchers learn more about the functions of genes and proteins, this knowledge will have a major impact in the fields like Medicine, Biotechnology and the Life sciences. Therefore HGP is very important.

ii. What have we learnt from Human Genome Project.

Ans :

i. Human Genome Project was to provide a complete and accurate sequence of the 3 billion DNA base pairs that make up the human genome and to find out the estimated number of human genes.

ii. Now about 33000 genes have been estimated to be present in humans.

Q.58 Genomes of which other organisms were aimed to study under HGP? State its significance.

Ans :

i. The project was also aimed to sequence the genomes of several other organisms such as bacteia e.g. *E.coli, Caenorhabditis elegans* (a free living non-pathogenic nematode), *Saccharomyces cerevisiae* (yeast), *Drosophila* (fruit fly), plants (rice and *Arabidopsis*), *Mus musculus* (mouse), etc.

ii. Complete genome sequences of these model organisms will be useful for comparative studies that will allow researchers to study gene functions in these organisms.

iii. It will lead to the understanding of gene structure and function in other species. Since we possess many of the genes same as these of flies, round worms and mice, such studies will lead to a greater understanding of human evolution.

Q.59 Can you tell?
Do different organisms have the same DNA.

Ans : No, even though nitrogenous bases are same in all organisms the sequence warrys and thus different organisms do not have the same DNA.

4.10 DNA Fingerprinting

Q.60 What is DNA fingerprinting?

Ans : The technique developed to identify a person with the help of DNA restriction analysis, is known as **DNA profiling** or **DNA fingerprinting**.

Q.61 What is Fingerprint.

Ans : Due to recombination of paternal and maternal genes, we differ from our parents. Differences also arise due to infrequent mutations that occur during gamete formation (cell division). Due to all these factors, every individual has its unique genetic make-up, which may be called its **Fingerprint**.

Q.62 Write a note on VNTRs?

Ans :

i. Some short sequences of nucleotides differ from person to person.

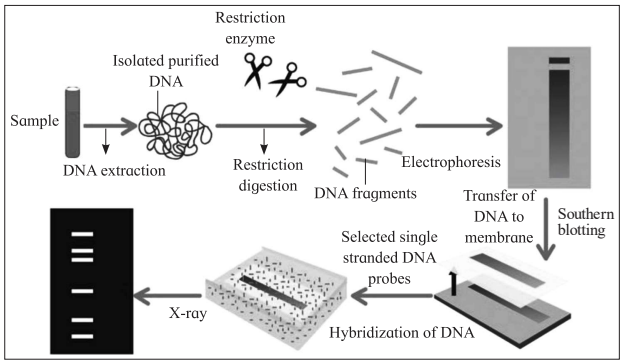
ii. In the population, every person shows unusual sequences of 20-100 base pairs, which are repeated several times.

iii. They are termed as Variable Number of Tandem Repeats (VNTRs).

Q.63 What is DNA fingerprinting and what are the steps of DNA fingerprinting?

Ans :

i. The technique developed to identify a person with the help of DNA restriction analysis, is known as **DNA profiling** or **DNA fingerprinting**.



ii. Steps involved in DNA finger printing are as follows:

a. **Isolation of DNA:** The DNA must be recovered from the cells or tissues of the body (host). Only small amount of tissue like blood, hair roots, skin, etc. is required.

b. **Restriction digestion:** The isolated DNA is treated with restriction enzymes. The restriction enzymes cut the DNA into small fragments having variable lengths. This phenomenon is called Restriction **Fragment Length Polymorphism** (RFLP).

c. **Gel electrophoresis:** The DNA samples are loaded for agarose gel electrophoresis under an electric influence. The DNA fragments, which are negatively charged move to the positive pole. The movement of these fragments depends on length of the fragments. This results in formation of bands. dsDNA splits into ssDNA by alkali treatment.

d. **Southern blotting:** The separated DNA fragments are transferred to a nylon membrane or a nitrocellulose filter paper by placing it over the gel and soaking them with filter paper overnight.

e. **Selection of DNA probe:** A known sequence of single-stranded DNA is prepared. It is called DNA Probe. DNA Probe is obtained from organisms or prepared by cDNA preparation method. The DNA probe is labelled with radioactive isotopes.

f. **Hybridization:** Probe DNA is added to the nitrocellulose filter paper containing host DNA. The single-stranded DNA probe pairs with the complementary base sequence of the host DNA strand. As a result DNA-DNA hybrids are formed on the nitrocellulose filter paper. Remaining single stranded DNA probe fragments are washed off.

g. **Photography:** The nitrocellulose filter paper is photographed on an X-ray film by autoradiography. The film is analysed to determine the presence of hybrid DNA.

Q.64 Enlist application of DNA fingerprinting.

Ans :

i. In forensic science, DNA finger printing is used to solve problems of rape and some complicated murder cases.

ii. DNA finger printing is used to find out the biological father or mother or both, of the child, in case of disputed parentage.

iii. DNA finger printing is used in pedigree analysis in cats, dogs, horses and humans.

❑❑❑