Molecular Basis of Inheritance

QUICK RECAP

- Genetic material is the substance which not only controls the formation and expression of traits in an organism but can also replicate and pass on from a cell to its daughter cell or from one generation to next.
- Nucleic acids are of two types - DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). In all organisms DNA serves as the carrier of genetic information which expresses itself through RNA but in viruses either DNA or RNA can serve as genetic material.

DNA AS GENETIC MATERIAL

- The most conclusive evidences in support of DNA as the genetic material came from the following three avenues of approach on microorganisms – transformation of bacteria, mode of infection of bacteriophages and conjugation of bacteria.
- **Frederick Griffith,** conducted a series of experiments with bacteria *Streptococcus pneumoniae*. He experimented with smooth (S) or virulent and rough (R) or nonvirulent strains of *Streptococcus pneumoniae*. He conducted a series of experiments as summarised in the given table.

Table : Summary of Griffith's experiments

	Bacteria injected	Effect on mice
(i)	Live virulent (S-type)	Died
(ii)	Live non-virulent (R-type)	Survived
(iii)	Heat killed virulent or S-type	Survived
(iv)	Live non-virulent or R-type + Heat killed virulent or S-type	Some Died

- In the last experiment, Griffith did not inject living S-type into mice but living S-type appeared in the blood and caused the death of mice. Griffith concluded that there was some factor in heat killed S-type that transformed live R-type into live S-type. This must be due to the transfer of the genetic material. However, the biochemical nature of genetic material was not defined from his experiments.
- In 1944, **Avery, McCarty** and **MacLeod** fractionated the killed S-type bacteria into three components-DNA, carbohydrate and protein.
- DNA fraction was further divided into two parts : one with deoxyribonuclease or DNase and the other without it. The four components were then added to separate culture tubes containing R-type bacteria as shown below:

R-type +Protein S-type	R-type
R-type +Carbohydrate S-type	R-type
R-type +(DNA of S-type + DNase)	R-type
R-type +DNA of S-type	R-type +S-type

- They also discovered that protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNases) did not affect transformation, so the transforming substance was not a protein or RNA. Digestion with DNase did inhibit transformation, suggesting that the DNA caused the transformation. They concluded that DNA is the hereditary material.
- 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. Hershey and Chase worked to discover whether it was protein or DNA from the viruses that entered the bacteria.
- They incorporated radioactive isotope of phosphorus (^{32}P) into phage DNA and that of sulphur (^{35}S) into proteins of a separate phage culture. These phage types were used independently to infect the bacterium *Escherichia coli*. After sometime, this mixture was agitated on a blender to separate the empty phage capsids from the surface of bacterial. When ^{35}S was used, all radioactive material was limited to empty viral protein coats.
- Bacteria which were infected with viruses that had radioactive DNA were radioactive indicating that DNA was the material that passed from the virus to the bacteria.
- These results indicated that the DNA of the bacteriophage and not the protein enters the host, where viral replication takes place. Therefore, DNA is the genetic material of bacteriophage.

DNA

- DNA is the largest macromolecule which is composed of small monomeric units called nucleotides. The length of DNA is defined as number of nucleotides present in it which is the characteristic of an organism.

- Each nucleotide is made up of a pentose sugar (deoxyribose type), a phosphate group and a nitrogenous base.
- A subunit composed of only sugar and the nitrogen base is known as **nucleoside.** Nitrogenous base is linked to pentose sugar through N-glycosidic linkage to form a nucleoside.
- The four nucleosides differ from each other in the type of the base, which could be adenine (A), guanine (G), thymine (T), or cytosine (C). The adenine and guanine are the purines and thymine and cytosine are the pyrimidines. (In RNA, uracil is present instead of thymine).
- In a nucleotide, N-base is attached to deoxyribose sugar at C-1 and phosphoric acid is attached at C-5 of sugar.
- Adjacent nucleotides are joined together by phosphodiester bonds between C–3 and C–5 of different deoxyribose sugars of two adjacent nucleotides (3′-5′ phosphodiester linkage) to form a polynucleotide chain.
- The polynucleotide chains show polarity as one end of the chain has a sugar residue with C-3 not linked to another nucleotide having free 3′-OH group and the other end has sugar residue with C-5 linked to a phosphate group (not linked to another nucleotide). These are named as 3' and 5' ends (three and five prime ends) of polynucleotide chain, respectively.
- The two polynucleotide chains are antiparallel to each other and are held together by hydrogen bonding between specific pairs of purines and pyrimidines.
- The pairing is always between A and T, and G and C. There are two hydrogen bonds between A and T and three between G and C.
- The stacking of bases creates two types of grooves called **major** and **minor grooves.**
- Both polynucleotide strands in a double helix remain separated by a distance of 20Å. The coiling of double helix is **right handed** and one complete turn occurs every 34Å so the pitch of one turn is 34Å.
- Distance between base pairs is 3.4Å and since the pitch of one turn is 34Å, so there are 10 base pairs in each turn.
- In 1950, **Erwin Chargaff** formulated important generalisations about DNA structure, these generalisations are called **Chargaff's rules** in his honour. They are summarised as follows :
  - The purines and pyrimidines are always in equal amounts, *i.e.*, **A + G = T + C**.
  - The amount of adenine is always equal to that of thymine, and the amount of guanine is always equal to that of cytosine, *i.e.*, **A = T** and **G = C**.
  - The base ratio **A + T / G + C** may vary from one species to another, but is constant for a given species.
- DNA packing in prokaryotes is carried out with the help of RNAs and non-histone basic proteins like polyamines.
- DNA packing in eukaryotes is carried out with the help of lysine and arginine rich basic proteins called **histones.** The unit of compaction is **nucleosome.** Histones are of five types-H_1, H_2A, H_2B, H_3 and H_4. Four of them (H_2A, H_2B, H_3 and H_4) are organised to form a unit of eight molecules called as histone octamer. The negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called nucleosome.
- A typical nucleosome contains 200 bp of DNA helix. Nucleosomes constitute the repeating unit of a structure in nucleus called chromatin, (thread-like stained bodies seen in nucleus). The nucleosomes in chromatin are seen as 'beads-on-string' structure when viewed under electron microscope.
- In a typical nucleus, some regions of chromatin are loosely packed (and stain light) and are referred to as **euchromatin,** which is transcriptionally active chromatin. The chromatin that is more densely packed and stains dark is called as **heterochromatin**, which is inactive.

RNA

- Ribonucleic acid (RNA) is a single-stranded structure consisting of an unbranched polynucleotide chain, but it is often folded back on itself forming helices. The four nitrogenous bases found in RNA are adenine, cytosine, guanine and uracil. Uracil is present in RNA at place of thymine. In RNA, every nucleotide residue has an –OH group present at 2′ position in the ribose.

Types of RNA

- The RNA may be mainly of two types – genetic RNA and non-genetic RNA. **Genetic RNA** is seen in most of the plant viruses and some animal viruses, *e.g.,* in TMV, polio virus, influenza virus, etc., RNA acts as genetic material.

Table : Different RNA viruses and the nature of genetic RNA associated with them

	Virus	Types of RNA
1.	**Plant viruses**	
	TMV	Single stranded
	Wound tumor	Double stranded
2.	**Animal viruses**	
	Influenza,	Single stranded
	Rous sarcoma,	
	Poliomyelitis	Double stranded
	Reovirus	
3.	**Bacteriophages**	
	MS_2, F_2, R_{17}	Single stranded

- On the basis of molecular size and function, three main forms of non genetic RNA are – ***m*RNA, *t*RNA** and ***r*RNA**.
- *m*RNA constitutes about 2-5% of cellular RNA; *t*RNA is about 15% and *r*RNA about 70-80%.
- **Messenger ribonucleic acid** (*m*RNA) or informational RNA is a molecule of RNA that is transcribed from a gene and then translated by ribosomes in order to manufacture protein.
- ***t*RNA** or transfer RNA is also known as **soluble RNA or adaptor RNA**. *t*RNAs are smallest, bearing 70-80 nucleotides and sedimentation coefficient of 4S.
- **Ribosomal RNA** is a component of the ribosomes, the protein synthetic factories in the cell. It is formed in nucleolus. ***r*RNA is the most stable type of RNA**.

DNA *v/s* RNA

- DNA is a better genetic material than RNA because of the following reasons:
  - DNA is chemically less reactive and structurally more stable as its nucleotides are not exposed except when they have to express their effect.
  - Presence of thymine in DNA instead of uracil, provides stability to DNA.
  - Hydrogen bonding between purines and pyrimidines and their stacking make DNA more stable for storage of genetic information.
  - DNA is capable of undergoing slow mutations required of genetic material.
  - It possesses the power of repairing.
- Since, DNA is more stable while RNA is more reactive, both the types of nucleic acids have been retained in genetic expression.

DNA Replication

- DNA replication is the unique process of making an identical copy of a double stranded DNA, using existing DNA as a template for the synthesis of new DNA strands prior to cell division (in S phase of cell cycle).
- **Meselson and Stahl (1958)** proved that DNA replicates by **semi-conservative** method by experimenting on *E.coli.* They grew *E.coli* for many generations in medium containing the heavy isotopes of nitrogen, *i.e.,* ^{15}N. They grew bacterial cells having DNA labelled with ^{15}N in ^{14}N medium and found that F_1 generation has DNA density intermediate between the two. This implied that, the newly synthesised DNA possess one strand contributed by parent DNA and other newly synthesised.
- Replication of DNA is energetically **highly expensive**. It requires a set of enzymes, mainly **DNA polymerases**. These enzymes catalyse **polymerisation** of a large number of nucleotides very accurately in a very short time. Replication requires abundant energy that comes from breakdown of triphosphates

of deoxyribonucleotides. **Enzyme helicase** acts over the Ori site and unwinds the two strands of DNA. The separated strands are stabilised by means of **single stranded binding proteins (SSBPs)**. Unwinding creates tension in the uncoiled part by forming more supercoils. Tension is released by enzymes topoisomerases. They cause nicking of one strand of DNA (for removing coils) and resealing the same. Whole of DNA does not open in one stretch due to very high energy requirement but the point of separation proceeds slowly towards both the directions. It gives the appearance of **Y-shaped** structure called **replication fork**.

- A small strand of RNA, called **RNA primer**, is synthesised at the 5′ end of new DNA strand. It is essential for initiation of new DNA chains as it is required by DNA polymerases to add nucleotides. DNA polymerases catalyse polymerisation only in one direction, that is 5′ → 3′. Consequently, on one strand called leading strand (the template with polarity 3′ → 5′), the replication is continuous, while on the other strand called lagging strand (the template with polarity 5′ → 3′), it is discontinuous. Discontinuous replication occurs because only a small stretch of template exposes at a time. The discontinuously synthesised fragments are later joined by the enzyme **DNA ligase.**

Central Dogma

- **Crick (1958)** proposed the central dogma of molecular biology. Central dogma is the unidirectional flow of information from DNA to RNA and from RNA to polypeptide.
- **H. Temin and Baltimore (1970)** introduced the concept of reverse central dogma, *i.e.*, formation of DNA from RNA. It is also called **teminism** and occurs in retroviruses.

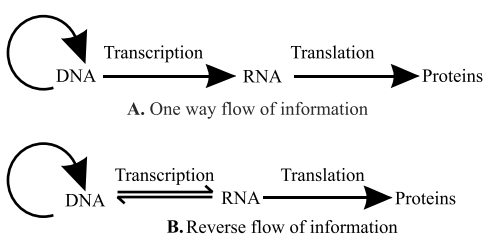


Fig.: Diagrammatic representation of flow of transcriptional information from DNA.

Transcription

- The process of copying genetic information from one strand of the DNA into RNA is termed as transcription.
- The segment of DNA that takes part in transcription is called **transcription unit**. It has three components a promoter, the structural genes and a terminator. Besides a promoter, eukaryotes also require an enhancer. Promoter is located upstream of structural gene. Terminator region is present downstream (3′ end of coding strand) of structural gene. The promoter has an AT rich region, called **TATA box** also called **Pribnow box.**
- In structural gene transcription can occur only in 5′→ 3′ direction. The strand of DNA taking part in transcription is called template strand.
- In prokaryotes RNA polymerase is only of one type and can transcribe all types of RNAs. It has σ subunit attached with core enzyme having 5 subunits - β′, β, α_1, α_2 and ω.
- In eukaryotes, three major classes of RNA polymerases are found in the nucleus. RNA polymerase I synthesises precursors for the large ribosomal RNAs. RNA polymerase II synthesises the precursors for *m*RNAs and small nuclear RNAs. RNA polymerase III participates in the formation of *t*RNAs and 5S *r*RNAs.
- The DNA segment which codes for polypeptide chain is called **cistron**. The structural gene in a transcription unit could be monocistronic (mostly in eukaryotes) which codes for only one protein or polycistronic (mostly in bacteria or prokaryotes) which codes for many proteins.
- Prior to transcription the nucleotides are activated through phosphorylation. Enzyme **phosphorylase** is required along with energy for this.
- Transcription takes place in three steps - **initiation, elongation and termination.**
- The first step in transcription is binding of RNA polymerase to DNA. The specific

region on the DNA where the enzyme binds to form **closed promoter complex** is known as **promoter region.** It is located on the 5′ end of the gene to be transcribed which signals where to start RNA synthesis. It also determines which DNA strand is to be transcribed. Thus a promoter region has RNA polymerase recognition site and RNA polymerase binding site.

- When the closed promoter complex forms, DNA remains double helical. It then isomerises and causes unwinding and separation of DNA strands to form open promoter complex, a highly stable structure.
- RNA polymerase contains two nucleotide binding sites: initiation site and elongation site.
  - **Initiation site** binds only with purine triphosphates (ATP and GTP). The initiating nucleoside triphosphate binds to the enzyme in open promoter complex and forms hydrogen bond with complementary DNA base (base pairing).
  - **Elongation site** is then occupied by a nucleoside triphosphate that is selected strictly on the basis of its ability to form hydrogen bond with the next base in DNA strand. The two nucleotides are then joined together and the first base pair is released from initiation site.
- In prokaryotes, the transcription product directly function as *m*RNA but in eukaryotes the result of transcription is hnRNA.
- In eukaryotes, hnRNA contains both the exons (expressing sequences) and the introns (interrupting sequences) that are non-functional. Hence, it is subjected to a process called **splicing** where the introns are removed and exons are joined in a defined order.
- hnRNA undergoes additional processing called as **capping** and **tailing.** In capping, methyl guanosine triphosphate is added to the 5′ – end of hnRNA. In tailing, adenylate residues are added at 3′ – end in a template independent manner.

Genetic Code

- Sequence of nitrogenous bases or nucleotides in a polynucleotide chain (DNA molecule) which determines sequence of amino acids in a polypeptide chain is called **genetic code.** The 64 distinct triplets determine the 20 amino acids. The DNA sequence of a gene is divided into a series of units of three bases called **codon.**
- The salient features of genetic code are as follows:
  - The codon is **triplet.** 61 codons code for amino acids and 3 codons (UAA, UAG and UGA) do not code for any amino acids, hence they function as stop codons.
  - One codon codes for only one amino acid, hence, it is **unambiguous** and **specific.**
  - Some amino acids are coded by more than one codon, hence the code is **degenerate.**
  - The codon is read in *m*RNA in a **continuous** fashion. There are no punctuations.
  - The code is nearly **universal** : *i.e.,* found in all living organisms.
  - AUG has dual functions. It codes for methionine (met), and it also act as initiation codon.
- **Wobble hypothesis** was given by **F.H.C. Crick** (1966). According to this, third nitrogenous base of a codon is not much significant and codon is specified by first two bases. Hence the same *t*RNA can recognise more than one codons differing only at third position.

TRANSLATION

- Translation or protein synthesis is a process during which the genetic information stored in the sequence of nucleotides in an *m*RNA molecule is translated, following the dictation of the genetic code, into the sequence of amino acids in the polypeptide which requires the functions of a large number of macromolecules.

- Protein synthesis occurs over the **ribosomes (protein factories).** Each ribosome has two unequal parts, small and large. The **larger subunit** of ribosomes has a groove for pushing out the newly formed polypeptide and protecting the same from cellular enzymes. The **smaller subunit** fits over the larger one like a cap but leaves a tunnel for *m*RNA. The two subunits come together only at the time of protein formation. The phenomenon is called **association.** $\mathbf{Mg^{2+}}$ is essential for it. Ribosomes usually form groups during active protein synthesis, known as **polyribosomes** or **polysomes.**
- There are three reactive sites in a ribosome – **P-site** (peptidyl transfer or donor site.) **A-site** (amino-acyl or acceptor site) and **E** or **exit site.** P-site is jointly contributed by both ribosomal subunits. A-site and E-site are largely confined to the larger subunit of ribosome.
- The synthesis of polypeptide can be considered in terms of **initiation, elongation** and **termination stages.** These fundamental processes have additional stages : **activation of amino acids** before their incorporation into polypeptide and the **post-translational processing** of the completed polypeptide. Both these processes play important roles in ensuring the proper function of the protein product. Amino acids are activated in the presence of ATP and linked to their cognate *t*RNA-a process commonly called as charging of tRNA or **aminoacylation of *t*RNA** to be more specific. The translational of *m*RNA begins with the formation of **initiation complex.** Initiation factors are designated as **IFs in prokaryotes** and **eIFs in eukaryotes.** Elongation requires the initiation complex, aminoacyl-*t*RNAs, **elongation factors** and GTP.
- The sequence of the second triplet in *m*RNA dictates which charged *t*RNA molecule will become positioned at the A site. Once it is present, **peptidyl transferase** catalyses the formation of the peptide bond that links the two amino acids together. At the same time, the covalent bond between the amino acid and the *t*RNA occupying the P site is hydrolysed (broken). The product of this reaction is a dipeptide, which is attached to the 3′ end of *t*RNA still residing in the A site. Soon after the establishment of first peptide linkage and slipping of the freed *t*RNA of P-site, the ribosome or *m*RNA rotates slightly. The process is called translocation. It requires a factor called tranlocase. As a result of translocation the A-site codon alongwith peptidyl-*t*RNA complex reaches the P-site. A new codon is exposed at the A-site. It attracts a new aminoacyl *t*RNA complex. The process of bond formation and translocation is repeated. One by one all the codons of *m*RNA are exposed at the A-site and get decoded through incorporation of amino acids in the peptide chain. The peptide chain elongates.
- **Termination** is signalled by the presence of one of there termination codons in the *m*RNA **(UAA, UAG, UGA),** immediately following the final coded amino acid. Three release factors **RF-1**, **RF-2** and **RF-3** recognise termination codons and contribute in release of the free polypeptide and last *t*RNA, now uncharged, from the ribosome. In eukaryotes, a single release factor **eRF** recognises all the 3 termination codons.

REGULATION OF GENE EXPRESSION

- The control over the functioning of genes is called regulation of gene expression.
- There are two types of gene regulations; positive and negative. In negative gene regulation, the genes continue to express their effect till their activity is suppressed. This type of gene regulation is also called **repressible regulation.**
- Positive gene regulation is the one in which the genes remain non-expressed unless they are induced to do it. It is, therefore, **inducible regulation.** As the genes express their effect through enzymes, their enzymes are also called inducible enzymes and repressible enzymes.
- **Jacob** and **Monod** (1961) from their study in bacterial genetics proposed that genetic material has a number of functional units called **operons.** An operon consists of minimum four types of genes-regulator, operator, promoter and structural.

- Regulator gene forms a biochemical for suppressing the activity of operator gene. Operator gene receives the product of regulator gene. It allows the functioning of the operon when it is not covered by the biochemical produced by regulator gene. The functioning of operon is stopped when operator gene is covered. Promoter gene provides point of attachment to RNA polymerase required for transcription of structural genes. Structural genes transcribe *m*RNA for polypeptide synthesis.
- *Lac* operon of *Escherichia coli* is an inducible operon system which was discovered by Jacob and Monod (1961).
- It has three structural genes, *z*, *y*, and *a*. In the induced operon the structural genes transcribe a polycistronic *m*RNA which produces three enzymes. These are β-galactosidase, galactoside permease and galactoside transacetylase respectively.
- β-galactosidase brings about hydrolysis of lactose to form glucose and galactose. Galactoside permease is required for entry of lactose into the bacterium. Galactoside transacetylase can transfer acetyl group from acetyl CoA to β-galactoside.
- The substance whose addition induces the synthesis of enzyme is called **inducer.** It is a chemical which attaches to repressor and changes its shape so that repressor cannot bind to operator.
- The repressor of *lac* operon is a tetrameric protein. It is made up of 4 subunits each having molecular weight of 40,000. If lactose is added, the repressor is rendered inactive so that it cannot attach to operator gene and synthesis of *m*RNA takes place.

Human Genome Project

- With the establishment of genetic engineering techniques where it was possible to isolate and clone any piece of DNA and availability of simple and fast techniques for determining DNA sequences, a very ambitious project of sequencing human genome was launched in the year 1990 called as Human Genome Project.
- Some of the **important goals of HGP** were as follows :
  - Identify all the genes present in human genome;
  - Determine the sequences and numbers of all the base pairs that make up human genome;
  - Store this information in databases;
  - Identify various genes that cause genetic disorders.
  - Improve tools for data analysis;
  - Transfer related technologies to other sectors, such as industries;
  - Address the ethical, legal, and social issues (ELSI) that may arise from the project.
- The human genome project was coordinated by the U.S. Department of Energy and the National Institute of Health. Additional contributions came from Japan, France, Germany, China and others.
- The basic strategies of human genome project are mapping, sequencing and functional analysis.
- Mapping is to prepare genetic and physical maps of human genome.
- Sequencing the entire genomic DNA of humans is limited because of limitations of the squencing techniques.
- Functional anaysis is to relate each gene with its function.

DNA Fingerprinting

- DNA fingerprinting involves identifying differences in some specific regions in DNA sequence called as repetitive DNA, because in these sequences, a small stretch of DNA is repeated many times.
- These repetitive DNA are separated from bulk genomic DNA as different peaks during density gradient centrifugation. The bulk DNA forms a major peak and the other small peaks are referred to as satellite DNA. Depending on base composition (A : T rich or G:C rich), length of segment, and number of repetitive units, the satellite DNA is classified into many categories, such as micro-satellites, mini-satellites etc. These sequences normally do not code for any proteins and show high degree of polymorphism hence form the basis of DNA fingerprinting.

- **Minisatellites or Variable Number Tandem Repeat (VNTRs)** are very specific in each individual and vary in number from person to person but are inherited. Each individual inherits these repeats from his/her parents which are used as genetic markers in a personal identity test.
- The major steps in DNA fingerprinting are as follows :
  - DNA is extracted from the cells. DNA can be amplified by PCR or polymerase chain reaction.
  - DNA is cut into fragments with restriction enzymes.
  - Chopped DNA fragments are passed through electrophoresis. The separated fragments can be visualised by staining them with a dye.
  - Double-stranded DNA is then split into single-stranded DNA using alkaline chemicals.
  - Separated DNA sequences are transferred from gel onto a nitrocellulose or nylon membrane.
  - The nylon sheet is then immersed in a bath, where probes or markers radioactive synthetic DNA segments of known sequences are added. The probe hybridises VNTR (Southern blotting).
  - X-ray film is exposed to the nylon sheet gives dark bands at the probe sites. Thus hybridised fragments are detected by autoradiography.
  - DNA fingerprinting helps in solving parentage dispute as well as identifying criminals.

Previous Years' CBSE Board Questions

6.1 The DNA

VSA/MCQs (1 mark)

1. Name the transcriptionally active region of chromatin in a nucleus. *(Delhi 2015)*

2. Name the negatively charged and positively charged components of a nucleosome. *(Delhi 2015C)*

3. Name the specific components and the linkages between them that form deoxyguanosine. *(AI 2013C)*

4. Name the two basic amino acids that provide positive charges to histone proteins. *(Delhi 2012C)*

5. If the base adenine constitutes 31 percent of an isolated DNA fragment, then what is the expected percentage of the base cytosine in it? *(Delhi 2011C)*

SA I (2 marks)

6. Although a prokaryotic cell has no defined nucleus, yet DNA is not scattered throughout the cell. Explain. *(2018)*

7. Describe the structure of a nucleosome. **(***Delhi 2017***)**

8. What is central dogma ? Who proposed it? *(2/5, AI 2015C)*

9. Draw a schematic diagram of a part of double stranded dinucleotide DNA chain having all the four nitrogenous bases and showing the correct polarity. *(Delhi 2012)*

10. (a) Draw a neat labelled diagram of a nucleosome.
(b) Mention what enables histones to acquire a positive charge. *(AI 2012)*

11. How do histones acquire positive charge? *(Delhi 2011)*

SA II (3 marks)

12. (a) A DNA segment has a total of 1000 nucleotides, out of which 240 are adenine containing nucleotides. How many pyrimidine bases this DNA segment possesses?
(b) Draw a diagrammatic sketch of a portion of DNA segment to support your answer. *(Delhi 2015)*

13. (a) A DNA segment has a total of 1,500 nucleotides, out of which 410 are guanine containing nucleotides. How many pyrimidine bases this segment possesses?
(b) Draw a diagrammatic sketch of a portion of DNA segment to support your answer. *(Delhi 2015)*

14. (a) A DNA segment has a total of 2,000 nucleotides, out of which 520 are adenine containing nucleotides. How many purine bases this DNA segment possesses?
(b) Draw a diagrammatic sketch of a portion of DNA segment to support your answer. *(Delhi 2015)*

15. Draw a labelled diagram of a nucleosome. Where is it found in a cell? *(Foreign 2014)*

16. The base sequence in one of the strands of DNA is TAGCATGAT.
(a) Give the base sequence of its complementary strand.
(b) How are these base pairs held together in a DNA molecule?
(c) Explain the base complementarity rules. Name the scientist who framed this rule. *(Delhi 2011)*

LA (5 marks)

17. (a) How are the following formed and involved in DNA packaging in a nucleus of a cell?
(i) Histone octamer
(ii) Nucleosome
(iii) Chromatin
(b) Differentiate between euchromatin and heterochromatin. *(Delhi 2016)*

18. (a) Explain the chemical structure of a single stranded polynucleotide chain.
(b) Describe the salient features of the double-helix structure of DNA molecule. *(AI 2012C)*

19. (a) Mention the contributions of the following scientists:
(i) Maurice Wilkins and Rosalind Franklin
(ii) Erwin Chargaff
(b) Draw a double-stranded dinucleotide chain with all the four nitrogen bases. Label the polarity and the components of the dinucleotide. *(AI 2011C)*

6.2 The Search for Genetic Material

VSA/MCQs (1 mark)

20. Why is RNA more reactive in comparison to DNA? *(Delhi 2015C)*

SA I (2 marks)

21. Why is DNA considered a better genetic material? *(AI 2013C)*

SA II (3 marks)

22. Explain the discovery made by Hershey and Chase using radioactive sulphur and phosphorus in their experiment. *(2020)*

23. Why is DNA a better genetic material when compared to RNA? *(Delhi 2015C)*

24. Frederich Griffith claimed that R-strain *Streptococcus pneumoniae* had been transformed by heat-killed S-strain bacteria. Explain the findings. *(AI 2012C)*

LA (5 marks)

25. (a) State the 'central dogma' as proposed by Francis Crick. Are there any exceptions to it? Support your answer with a reason and an example.
(b) Explain how the biochemical characterisation (nature) of 'Transforming Principle' was determined, which was not defined from Griffith's experiments. *(2018)*

26. List the criteria a molecule that can act as genetic material must fulfill. Which one of the criteria are best fulfilled by DNA or by RNA thus making one of them a better genetic material than the other? Explain. *(Delhi 2016)*

27. Answer the following questions based on Hershey and Chase experiments :
(a) Name the kind of virus they worked with and why?
(b) Why did they use two types of culture media to grow viruses in? Explain.
(c) What was the need for using a blender and later a centrifuge during their experiments?
(d) State the conclusion drawn by them after the experiments. *(Delhi 2016)*

28. (a) Describe the series of experiments of F. Griffith. Comment on the significance of the results obtained.
(b) State the contribution of MacLeod, McCarty and Avery. *(AI 2016)*

29. How did Hershey and Chase established that DNA is transferred from virus to bacteria? *(Delhi 2015)*

30. (a) Describe the experiment which demonstrated the existence of "transforming principle".
(b) How was the biochemical nature of this "transforming principle" determined by Avery, MacLeod and McCarty? *(Foreign 2015)*

31. How did Alfred Hershey and Martha Chase conclusively establish that DNA is the genetic material ? Explain. *(Foreign 2015)*

32. How did Griffith prove transforming principle in genetics? Explain the procedure. *(Delhi 2015C)*

33. Describe the Hershey and Chase experiment. Write the conclusion drawn by the scientist after their experiment. *(AI 2014)*

34. (a) Describe the various steps of Griffith's experiments that led to the conclusion of the transforming principle.
(b) How did the chemical nature of the transforming principle get established? *(AI 2014, 2013C)*

35. (a) Write the conclusion drawn by Griffith at the end of his experiment with *Streptococcus pneumoniae*.
(b) How did O. Avery, C. MacLeod and M. McCarty prove that DNA was the genetic material? Explain. *(AI 2013)*

36. Name the scientists who proved experimentally that DNA is the genetic material. Describe their experiment. *(Delhi 2012)*

37. Describe Frederick Griffith's experiment on *Streptococcus pneumoniae*. Discuss the conclusion he arrived at. *(Delhi 2012)*

38. Describe with help of labelled diagrammatic sketches the experiments conducted by Hershey and Chase. Write the inference drawn by them. *(Delhi 2012C)*

6.3 RNA World

SA II (3 marks)

39. Describe the structure of a RNA polynucleotide chain having four different types of nucleotides. *(Delhi 2013)*

40. It is established that RNA is the first genetic material. Explain giving three reasons. *(Delhi 2012)*

6.4 Replication

VSA/MCQs (1 mark)

41. Write the dual purpose served by deoxyribonucleoside triphosphates in polymerisation. *(2018)*

42. Name the source of energy for the replication of DNA. *(1/3, Delhi 2015C)*

43. Why is it not possible for an alien DNA to become part of chromosome anywhere along its length and replicate normally? *(AI 2014)*

44. What will happen if DNA replication is not followed by cell division in a eukaryotic cell? *(AI 2014C)*

45. Name the enzyme and state its property that is responsible for continuous and discontinuous replication of the two strands of a DNA molecule. *(Delhi 2013)*

46. Name the enzyme that joins the small fragment of DNA of a lagging strand during DNA replication. *(Delhi 2013C)*

SA I (2 marks)

47. Discuss the role, the enzyme DNA ligase plays during DNA replication. *(Delhi 2016)*

48. Show DNA replication with the help of a diagram only. *(Delhi 2014C)*

49. Draw a neat labelled sketch of a replicating fork of DNA. *(Delhi 2012)*

50. State the dual role of deoxyribonucleoside triphosphates during DNA replication. *(Delhi 2011)*

SA II (3 marks)

51. Describe the experiment where Mathew Meselson and Franklin Stahl used heavy isotope of nitrogen. *(2020)*

52. Explain the mechanism of DNA replication with the help of a replication fork. What role does the enzyme DNA ligase play in a DNA replication fork? *(Delhi 2019)*

53. Answer the following questions based on Meselson and Stahl's experiment on *E.coli*:
(a) Write the name of the chemical substance used as the only source of nitrogen in the experiment.
(b) Why did they allow the synthesis of the light and the heavy DNA molecules in the organism?
(c) How did they distinguish the heavy DNA molecules from the light DNA molecules? Explain.
(d) Write the conclusion the scientists arrived at, at the end of the experiment. *(AI 2019)*

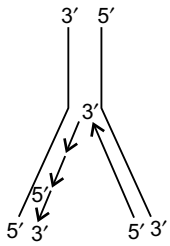
54. Describe the experiment that helped demonstrate the semi-conservative mode of DNA replication. *(Delhi 2016)*

55. How was a heavy isotope of nitrogen used to provide experimental evidence to semi-conservative mode of DNA-replication? *(Foreign 2015)*

56. Describe Meselson and Stahl's experiment to prove that the DNA replication is semi-conservative. *(3/5, AI 2015C)*

57. (a) Why did Meselson and Stahl use ^{14}N and ^{15}N isotopes in the sources of nitrogen present in the culture medium in their experiment? Explain.
(b) Write conclusion drawn by the them from the experiment. *(Delhi 2012C)*

58. Why do you see two different types of replicating strands in the given DNA replication fork? Explain. Name these strands.



(Delhi 2011)

59. Answer the following questions based on Meselson and Stahl's experiment:

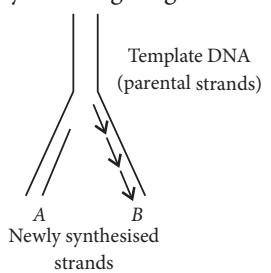
(a) Write the name of the chemical substance used as a source of nitrogen in the experiment by them.

(b) Why did the scientists synthesise the light and the heavy DNA molecules in the organism used in the experiment?

(c) How did the scientists make it possible to distinguish the heavy DNA molecule from the light DNA molecule? Explain.

(d) Write the conclusion the scientists arrived at after completing the experiment. *(AI 2011)*

60. (a) Identify the polarity at *A* and *B* respectively in the figure given below.



(b) Explain the mechanism the figure represents. *(AI 2011C)*

LA (5 marks)

61. (a) Name the stage in cell cycle where DNA replication occur.

(b) Explain the mechanism of DNA replication. Highlight the role of enzymes in the process.

(c) Why is DNA replication said to be semi-conservative? *(AI 2016)*

62. Describe Meselson and Stahl's experiment that was carried in 1958 on *E. coli*. Write the conclusion they arrived at after the experiment. *(AI 2016)*

63. (a) Draw a labelled diagram of a "replicating fork" showing the polarity. Why does DNA replication occur within such 'forks'?

(b) Name two enzymes involved in the process of DNA replication, along with their properties. *(AI 2015)*

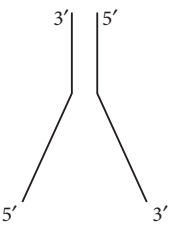
64. Explain the process of DNA replication with the help of a replicating fork. *(Delhi 2015C)*

65. (a) Explain the process of DNA replication with the help of a schematic diagram.

(b) In which phase of the cell cycle does replication occur in eukaryotes? What would happen if cell division is not followed after DNA replication. *(Delhi 2014)*

66. Describe Meselson and Stahl's experiment and write the conclusion they arrived at. *(Foreign 2014)*

67.



(a) Identify the structure shown above.

(b) Redraw the structure as a replicating fork and label the parts.

(c) Write the source of energy for this replication and list the enzymes involved in this process.

(d) Mention the difference in the synthesis based on the polarity of the two template strands. *(AI 2013C)*

68. How did Meselson and Stahl experimentally prove that DNA replication is semi-conservative? Explain. *(AI 2013C)*

69. State the aim and describe Meselson and Stahl's experiment. *(Delhi 2012)*

6.5 Transcription

VSA/MCQs (1 mark)

70. In a bacterium when RNA polymerase binds to the promoter on a transcription unit during transcription, it

(a) terminates the process

(b) helps remove introns

(c) initiates the process
(d) inactivates the exons. *(2020)*

71. What is a cistron? *(AI 2015)*

72. Write the function of RNA polymerase II. *(Foreign 2015)*

73. Name the enzyme that transcribes hnRNA in eukaryotes. *(Delhi 2015C)*

74. Differentiate between exons and introns. *(1/3, AI 2015C)*

75. Which one out of *Rho* factor and Sigma factor act as initiation factor during transcription in a prokaryote? *(Delhi 2013C)*

76. Which one of an intron and an exon is the reminiscent of antiquity? *(AI 2013C)*

SA I (2 marks)

77. Differentiate between a template strand and coding strand of DNA. *(2/3, Delhi 2015C)*

78. State the difference between the structural genes in a transcription unit of prokaryotes and eukaryotes. *(AI 2014)*

79. A template strand is given below. Write down the corresponding coding strand and the *m*RNA strand that can be formed, along with their polarity.
3′ ATGCATGCATGCATGCATGCATGC 5′ *(Foreign 2014)*

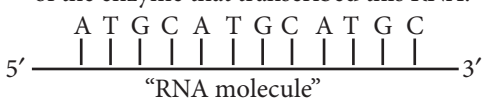
80. State the functions of the following in a prokaryote:
(a) *t*RNA
(b) *r*RNA *(2/3, AI 2012)*

81. Differentiate between a cistron and an exon. *(AI 2012C)*

82. Differentiate between exons and introns. *(AI 2012C)*

SA II (3 marks)

83. Construct and label a transcription unit from which the RNA segment given below has been transcribed. Write the complete name of the enzyme that transcribed this RNA.



(Delhi 2019)

84. Write the help of a schematic diagram, explain the location and the role of the following in a transcription unit:
Promoter, Structural gene, Terminator. *(Delhi 2014C)*

85. (a) What are the transcriptional products of RNA polymerase III?
(b) Differentiate between 'Capping' and 'Tailing'.
(c) Expand hnRNA. *(AI 2014C)*

86. (a) Construct a complete transcription unit with promotor and terminator on the basis of the hypothetical template strand given below:

(b) Write the RNA strand transcribed from the above transcription unit along with its polarity. *(Delhi 2012)*

87. Describe with the help of a schematic representation the structure of a transcription unit. *(3/5, AI 2012C)*

88. (a) Name the enzyme that catalyses the transcription of hnRNA.
(b) Why does the hnRNA need to undergo changes? List the changes hnRNA undergoes and where in the cell such changes take place. *(AI 2011)*

89. Monocistronic structural genes in eukaryotes have interrupted coding sequences. Explain. How are they different in prokaryotes? *(Delhi 2011C)*

90. Differentiate between the following:
(a) Promoter and terminator in a transcription unit.
(b) Exon and intron in an unprocessed eukaryotic *m*RNA. *(AI 2011C)*

LA (5 marks)

91. Name the type of cells and the process by which hnRNA is formed. Describe the processing mechanism it undergoes before it becomes functional. *(2020)*

92. (a) Describe the process of transcription in bacteria.
(b) Explain the processing the hnRNA needs to undergo before becoming functional *m*RNA of eukaryotes. *(AI 2016)*

93. Explain the process of transcription in prokaryotes. How is the process different in eukaryotes? *(AI 2015)*

94. Explain the process of transcription in eukaryotes. *(Foreign 2015)*

95. Describe the process of transcription in a bacterium. *(AI 2014C)*

96. (a) Describe this process of synthesis of fully functional *m*RNA in a eukaryotic cell.

(b) How is the process of *m*RNA synthesis different from that in prokaryotes? *(AI 2012)*

97. (a) Describe the process of transcription in prokaryotes.

(b) Mention how is the process different in eukaryotes. *(AI 2012C)*

6.6 Genetic Code

VSA/MCQs (1 mark)

98. Give an example of a codon having dual function. *(Delhi 2016)*

99. How does a degenerate code differ from an unambiguous one? *(Foreign 2015)*

100. Write the specific features of the genetic code AUG. *(1/5, AI 2013)*

101. Mention the role of the codons AUG and UGA during protein synthesis. *(Delhi 2011)*

SA I (2 marks)

102. State a functional difference between the following codons.

(a) AUG and UAA

(b) Specific and Degenerate *(2020)*

103. Differentiate between the genetic codes given below :

(a) Unambiguous and Universal

(b) Degenerate and Initiator (*AI 2017*)

104. Following are the features of genetic codes. What does each one indicate?

Stop codon; Unambiguous codon; Degenerate codon; Universal codon.

(AI 2016)

105. (a) Name the scientist who suggested that the genetic code should be made of a combination of three nucleotides.

(b) Explain the basis on which he arrived at this conclusion. *(Delhi 2014)*

106. One of the salient features of the genetic code is that it is nearly universal from bacteria to humans. Mention two exceptions to this rule. Why are some codes said to be degenerate? *(Foreign 2014)*

107. Genetic codes can be universal and degenerate. Write about them, giving one example of each. *(2/5, AI 2013)*

108. Explain the structure of a *t*RNA and state why it is known as an adapter molecule. *(2/5, AI 2012C)*

SA II (3 marks)

109. (a) Name the scientist who postulated the presence of an adapter molecule that can assist in protein synthesis.

(b) Describe its structure with the help of a diagram. Mention its role in protein synthesis. *(Foreign 2014)*

110. (a) Name the enzyme responsible for the transcription of *t*RNA and the amino acid the initiator *t*RNA gets linked with.

(b) Explain the role of initiator *t*RNA in initiation of protein synthesis. *(Delhi 2012)*

111. Unambiguous, universal and degenerate are some of the terms used for the genetic code. Explain the salient features of each one of them. *(AI 2011)*

112. (a) Name the scientist who called *t*RNA as an adapter molecule.

(b) Draw a clover leaf structure of *t*RNA showing the following:

(i) Tyrosine attached to its amino acid site.

(ii) Anticodon for this amino acid in its correct site (codon for tyrosine is UAC).

(c) What does the actual structure of *t*RNA look like? *(AI 2011)*

LA (5 marks)

113. (a) Describe the structure and function of a *t*RNA molecule. Why is it referred to as an adapter molecule?

(b) Explain the process of splicing of hnRNA in a eukaryotic cell. (*AI 2017*)

114. (a) What is a genetic code?
(b) Explain the following :
Degenerate code; Unambiguous code; Initiator code. *(Delhi 2014C)*

6.7 Translation

VSA/MCQs (1 mark)

115. Write the two specific codons that a translational unit of *m*RNA is flanked by one on either sides. *(AI 2015C)*

116. Which one of the two sub-units of ribosome encounters an *m*RNA? *(Delhi 2013C)*

SA I (2 marks)

117. What is aminoacylation? State its significance. *(AI 2016)*

118. State the functions of ribozyme and release factor in protein synthesis respectively. *(AI 2015C)*

119. Where does peptide bond formation occur in a bacterial ribosome and how? *(Foreign 2014)*

120. Explain aminoacylation of *t*RNA. *(Delhi 2014C, 2/5, AI 2013)*

LA (5 marks)

121. Explain the relationship of ribosomes, tRNA and mRNA during the process of translation in prokaryotes. *(2020)*

122. (a) Write the contributions of the following scientists in deciphering the genetic code.
George Gamow; Hargobind Khorana; Marshall Nirenberg; Severo Ochoa
(b) State the importance of a genetic code in protein biosynthesis. *(Delhi 2019)*

123. How do *m*RNA, *t*RNA and ribosomes help in the process of translation? *(AI 2015)*

124. Name the major types of RNAs and explain their role in the process of protein synthesis in a prokaryote. *(Foreign 2014)*

125. Explain the process of translation. *(Delhi 2014C)*

6.8 Regulation of Gene Expression

VSA/MCQs (1 mark)

126. Differentiate between the following:
Inducer and repressor in *lac* operon. *(1/3, Delhi 2011C)*

SA I (2 marks)

127. How would *lac* operon operate in *E. coli* growing in a culture medium where lactose is present as source of sugar? *(AI 2014C)*

128.

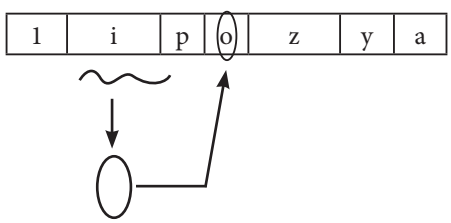
i	p	o	x	y	a

Given above is a schematic representation of the *lac* operon in *E.coli*. What is the significant role of '*i*' gene in switching on or off the operon? *(AI 2013C)*

SA II (3 marks)

129. How are the structural genes inactivated in *lac* operon in *E.coli* ? Explain. *(Delhi 2012)*

130. Given below is a schematic representation of *lac* operon:



(a) Identify i and p.
(b) Name the 'inducer' for this operon and explain its role. *(Foreign 2011)*

LA (5 marks)

131. Explain the expression of *lac* operon genes in *E. coli* growing in lactose containing cultural medium. *(2020)*

132. Explain the role of different genes in a *lac* operon when in a switched on state. *(2020)*

133. Study the schematic representation of the genes involved in the *lac* operon given below and answer the questions that follow:

p	*i*	*p*	*o*	*z*	*y*	*a*

(a) Identify and name the regulatory gene in this operon. Explain its role in 'switching off' the operon.

(b) Why is the *lac* operon's regulation referred to as negative regulation?

(c) Name the inducer molecule and the products of the genes '*z*' and '*y*' of the operon. Write the functions of these gene products. *(AI 2019)*

134. Write the different components of a *lac*-operon in *E. coli*. Explain its expression while in an 'open' state. (*AI 2017*)

135. Explain the role of lactose as an inducer in a *lac* operon. *(Delhi 2016)*

136. Sketch a schematic diagram of *lac* operon in switched on position. How is the operon switched off? Explain. *(AI 2015C)*

137. Describe how the *lac* operon operates, both in the presence and absence of an inducer in *E. coli*. *(AI 2014)*

138.

i	p	o	z	y	a

Given above is the schematic representation of *lac* operon of *E. coli*. Explain the functioning of this operon when lactose is provided in the growth medium of the bacteria. *(Delhi 2013C)*

139. (a) State the arrangement of different genes that in bacteria is referred to as 'operon'.

(b) Draw a schematic labelled illustration of *lac* operon in a 'switched on' state.

(c) Describe the role of lactose in *lac* operon. *(AI 2011)*

6.9 Human Genome Project

VSA/MCQs (1 mark)

140. Choose the chromosome in a human that possesses least number of genes.

(a) 21st chromosome

(b) Autosome

(c) X chromosome

(d) Y chromosome *(2020)*

141. Write the scientific importance of single nucleotide polymorphism identified in human genome. *(1/5, Foreign 2014)*

142. Mention the contribution of genetic maps in human genome project. *(AI 2011)*

143. Mention any two ways in which Single Nucleotide Polymorphism (SNPs) identified in human genome can bring revolutionary change in biological and medical sciences. *(AI 2011C)*

SA I (2 marks)

144. Which human chromosome has (a) maximum number of genes, and which one has (b) fewest genes? *(2/5, Foreign 2014)*

145. Expand 'BAC' and 'YAC'. Explain how they are used in sequencing human genome. *(Delhi 2011C)*

SA II (3 marks)

146. What are 'SNPs'? Where are they located in a human cell? State any two ways the discovery of SNPs can be of importance to humans. *(2020)*

147. (a) List the two methodologies which were involved in human genome project. Mention how they were used.

(b) Expand 'YAC' and mention what was it used for? (*AI 2017*)

148. (a) What do 'Y' and 'B' stand for in 'YAC' and 'BAC' used in Human Genome Project (HGP). Mention their role in the project.

(b) Write the percentage of the total human genome that codes for proteins and the percentage of discovered genes whose functions are known as observed during HGP.

(c) Expand 'SNPs' identified by scientists in HGP. *(AI 2016)*

LA (5 marks)

149. (a) List any four major goals of human genome project.

(b) Write any four ways the knowledge from HGP is of significance for humans.

(c) Expand BAC and mention its importance. *(2020)*

6.10 DNA Fingerprinting

VSA/MCQs (1 mark)

150. How is repetitive/satellite DNA separated from bulk genomic DNA for various genetic experiments? *(Delhi 2014)*

151. How does DNA polymorphism arise in a population? *(Delhi 2014)*

SA I (2 marks)

152. Write the full form of VNTR. How is VNTR different from 'Probe'? *(AI 2011)*

SA II (3 marks)

153. "A very small sample of tissue or even a drop of blood can help determine paternity". Provide a scientific explanation to substantiate how it is possible. *(AI 2019)*

154. (a) Expand VNTR and describe its role in DNA fingerprinting.
(b) List any two applications of DNA fingerprinting technique. *(2018)*

155. A number of passengers were severely burnt beyond recognition during a train accident. Name and describe a modern technique that can help in handing over the dead to their relatives. (*Delhi 2017*)

156. Following the collision of two trains a large number of passengers are killed. A majority of them are beyond recognition. Authorities want to hand over the dead to their relatives. Name a modern scientific method and write the procedure that would help in the identification of kinship. *(Delhi 2015)*

157. "A very small sample of tissue or even a drop of blood can help determine paternity". Provide a scientific explanation to substantiate the statement. *(AI 2015)*

158. Explain the significance of satellite DNA in DNA fingerprinting technique. *(AI 2015)*

159. A burglar in a huff forgot to wipe off his blood-stains from the place of crime where he was involved in a theft and fight. Name the technique which can help in identifying the burglar from the blood-stains. Describe the technique. *(AI 2013)*

160. In a maternity clinic, for some reason, the authorities are not able to hand over the two new-born babies to their respective parents. Name and describe the technique you would suggest to sort out the matter. *(AI 2013)*

161. (a) Explain DNA polymorphism on the basis of genetic mapping of human genome.
(b) State the role of VNTR in DNA fingerprinting. *(AI 2013)*

LA (5 marks)

162. (a) Name the type of DNA that forms the basics of DNA fingerprinting and mention two features of this DNA.
(b) Write the steps carried out in the process of DNA fingerprinting technique and mention its application. *(2020)*

163. (a) Differentiate between repetitive and satellite DNA.
(b) How can satellite DNA be isolated? Explain.
(c) List two forensic applications of DNA fingerprinting. *(Delhi 2012C)*

Detailed Solutions

1. Euchromatin is the transcriptionally active region of chromatin in a nucleus.

2. The negatively charged and positively charged components of a nucleosome are DNA and histones respectively.

3. The specific components that form deoxyguanosine are guanine (nitrogenous base) and deoxyribose (pentose sugar) linked together by glycosidic bond.

4. Lysine and arginine are the two basic amino acids that provide positive charge to histone proteins.

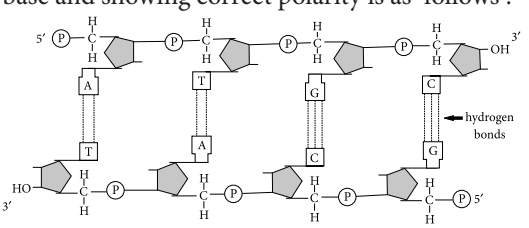
5. According to Chargaff's rule,
[A] + [G] = [C] + [T] = 50%
Therefore, if [A] = 31%, then [T] = 31%
[C] + [T] = 50%
Therefore, [C] = 50% – 31% = 19%

6. In prokaryotes, DNA lies in the cytoplasm which is supercoiled (coiled and recoiled) with the help of RNAs and non-histone basic proteins like polyamines. DNA being negatively charged is held in place with the help of these proteins that have positive charges in a region termed as nucleoid. The DNA in nucleoid is organised in large loops held by proteins.

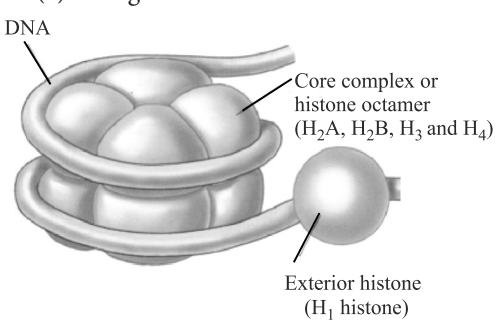
7. DNA packing in eukaryotes is carried out with help of lysine and arginine rich basic proteins called histones. The unit of compaction is called nucleosome. There are five types of histone proteins - H_1, H_2A, H_2B, H_3 and H_4. Four of them (H_2A, H_2B, H_3 and H_4) occur in pairs to produce histone octamer, called nu body or core of nucleosome. Their positively charged ends are towards the outside. They attract negatively charged strands of DNA. DNA over nu body forms 1¾ turns to form nucleosome core. A typical nucleosome contains 200 bp of DNA helix. DNA connecting two adjacent nucleosomes is called interbead or linker DNA. It bears H_1 histone protein.Nucleosome chain gives a beads-on-string appearance.

8. Concept of central dogma was proposed by Crick in 1958. It refers to the flow of information from DNA to *m*RNA (transcription) and then decoding the information present in *m*RNA in the formation of polypeptide chain or protein (translation).

9. Diagrammatic representation of double stranded DNA chain having all four nitrogenous base and showing correct polarity is as follows :



10. (a) Diagram of nucleosome is as follows:



(b) Histones are rich in basic amino acids lysine and arginine, hence they are positively charged.

11. *Refer to answer 10(b).*

12. (a) According to Chargaff's rule,
[A] + [G] = [C] + [T]
Also [A] = [T] and [C] = [G]
As [A] = [T], therefore [T] = 240
[A] + [T] = 240 + 240 = 480
As total number of nucleotides = 1000,
therefore [G] + [C] = 1000 – 480 = 520
[G] = [C]

$$\text{therefore, } [G] = [C] = \frac{520}{2} = 260$$

Thus, total number of pyrimidines.
i.e. [C] + [T] = 260 + 240 = 500
(b) *Refer to answer 9.*

13. (a) Cytosine and thymine are pyrimidines. According to Chargaff's Rule, purines and pyrimidine base pairs are in equal amount, therefore
Total nucleotides = 1500
[A + G + C + T] = 1500
[A] = [T] and [G] = [C]
Guanine = 410
Therefore, A + 410 + 410 + T = 1500
A + T + 820 = 1500
A + T = 1500 – 820
A + T = 680

$$\therefore \quad T = \frac{680}{2} = 340$$

Therefore, total pyrimidine,
C + T = [410 + 340] = 750
(b) *Refer to answer 9.*

14. (a) According to Chargaff's rule,
[A] + [G] = [C] + [T]
Also [A] = [T] and [G] = [C]
As [A] = [T], therefore [A] = [T] = 520
[A] + [T] = 520 + 520 = 1040
As total number of nucleotides = 2000
therefore, [G] + [C] = 2000 – 1040 = 960

$$[G] = [C] = \frac{960}{2} = 480$$

Thus, total number of purines *i.e.*
[A] + [G] = 520 + 480 = 1000
(b) *Refer to answer 9.*

15. *Refer to answer 10 (a).*
A section of negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called nucleosome. Nucleosome

constitute the repeating unit of a structure in nucleus called chromatin, thread like stained bodies seen in nucleus. The nucleosomes in chromatin are seen as 'beads on string' structure when viewed under electron microscope.

16. (a) The base sequence of the complementary strand is ATCGTACTA.

(b) The base pairs in a DNA molecule are held together by hydrogen bonds.

(c) Base complementarity rules or Chargaff's rules are the important generalisations made by Chargaff (1950) on the bases and other components of DNA. These rules are as follows:

(i) Purine and pyrimidine base pairs are in equal amount, that is,

adenine + guanine = thymine + cytosine.

$[A + G] = [T + C]$, *i.e.*, $\frac{[A+G]}{[T+C]} = 1$

(ii) Molar amount of adenine is always equal to the molar amount of thymine. Similarly, molar concentration of guanine is equalled by molar concentration of cytosine.

$[A] = [T]$, *i.e.*, $\frac{[A]}{[T]} = 1$; $[G] = [C]$, *i.e.*, $\frac{[G]}{[C]} = 1$

(iii) Sugar deoxyribose and phosphate occur in equimolar proportions.

(iv) A–T base pairs are rarely equal to C–G base pairs.

(v) The ratio of $\frac{[A+T]}{[G+C]}$ is variable but constant for a species. It can be used to identify the source of DNA. The ratio is low in primitive organisms and higher in advanced ones.

17. (a) (i) Histone octamer : Histones are positively charged proteins, rich in basic amino acid residues lysines and arginines. These amino acids carry positive charges on their side chains. There are five types of histone proteins : H_1, H_2A, H_2B, H_3 and H_4. Four of them (H_2A, H_2B, H_3 and H_4) are organised in pairs to from a unit of eight molecules called histone octamer, nu body or core of nucleosome. Negatively charged DNA wraps around this octamer to form nucleosome.

(ii) Nucleosome : It is the compaction unit. The positively charged ends of histone octamer attract the negatively charged strands of DNA. The DNA is thus wrapped around the positively charged histone octamer to form a structure called nucleosome. Around 200 bp of DNA is wrapped around the nu body or histone octamer for $1\frac{3}{4}$ turns. DNA connecting two adjacent nucleosomes is called linker DNA which bears H1 histone protein. Nucleosome and linker DNA together constitute chromatosome. Nucleosome chain gives a bead on string appearance under electron microscope.

(iii) Chromatin : The nucleosomal organisation has approximately 10 nm thickness, which further gets condensed and coiled to produce a solenoid (having 6 nucleosomes per turn) of 30 nm diameter. This solenoid structure further undergoes coiling to produce a chromatin fibre of 30-80 nm thickness. These chromatin fibres are further coiled and condensed to form chromatid which further forms chromosome at metaphase stage of cell division.

The packaging can be summarised as follows :

DNA → Nucleosome → Solenoid → Chromatin fibre

(2nm diameter) (10 nm diameter) (30 nm diameter) (30-80 nm diameter)

↓

Chromatid (700 nm diameter)

↓

Chromosome (1400 nm diameter)

(b) The differences between euchromatin and heterochromatin are as follows :

	Euchromatin	**Heterochromatin**
(i)	These are the regions where chromatin is loosely packed.	These are the regions where chromatin is densely packed.
(ii)	It stains lighter.	It stains darker.
(iii)	This is transcriptionally active.	It is transcriptionally inactive or late replicating.

18. (a) A polynucleotide chain is formed by the end to end polymerisation of a large number of nucleotides. A nucleotide is a condensation product of three chemicals – a pentose sugar, phosphoric acid and a nitrogenous base.

The nitrogen base combines with the sugar molecule at its carbon atom 1′ in a glycosidic bond

(C–N–C) by one of its nitrogen atoms (usually 1 in pyrimidines and 9 in purines).

The phosphate group is connected to carbon 5′ of the sugar residue of its own nucleotide and carbon 3′ of the sugar residue of the next nucleotide by phosphodiester bonds. –H of phosphate and –OH of sugar are eliminated as H_2O during each ester formation.

At the end of the polynucleotide chain, last sugar has its 5–C free while at the other end 3–C of first sugar is free. They are respectively called 5′ and 3′ ends.

(b) Watson and Crick proposed the double helix model for structure of DNA in 1953. Its salient features are as follows:

- In a DNA double helix, two polynucleotide chains are coiled to form a helix. Sugar-phosphate forms backbone of this helix while bases project inwards towards each other.
- Complementary bases, pair with each other through hydrogen bonding. Purines (adenine, guanine) always pair with their corresponding pyrimidines (thymine, cytosine). Adenine pairs with thymine through two hydrogen bonds while guanine pairs with cytosine through three hydrogen bonds.
- The helix is right-handed.
- The plane of one base pair stacks over the other in a double helix. This provides stability to the helix along with hydrogen bonding.
- The two chains of DNA have antiparallel polarity, 5′ → 3′ in one and 3′ → 5′ in other.
- The pitch of helix is 3.4 nm (34 Å) with roughly 10 base pairs in each turn. The average distance between two adjacent base pairs comes to about 0.34 nm (0.34×10^{-9} m or 3.4 Å).
- DNA is acidic. For its compaction, it requires basic (histone) proteins. The histone proteins are positively charged and occupy the major grooves of DNA at an angle of 30° to helix axis.

19. (a) (i) Maurice Wilkins and Rosalind Franklin (1953) carried out X-ray diffraction studies to study the structure of DNA molecule. The fine X-ray photographs of DNA taken by them showed that DNA was a helix with a width of 2.0 nm. One turn of the helix was 3.4 nm with 10 layers of bases stacked in it. Watson and Crick (1953) worked out the first correct double helix model from the X-ray photographs of Wilkins and Franklin.

(ii) Erwin Chargaff (1950) proposed generalisations called Chargaff's rules (or base complimentarity rules) about DNA. These generalisations are as follows:

- Purine and pyrimidine base pairs are in equal amount, that is,
  adenine + guanine = thymine + cytosine.
  $[A + G] = [T + C]$, *i.e.*, $\frac{[A+G]}{[T+C]} = 1$
- Molar amount of adenine is always equal to the molar amount of thymine. Similarly, molar concentration of guanine is equal to molar concentration of cytosine.
  $[A] = [T]$, *i.e.*, $\frac{[A]}{[T]} = 1; [G] = [C]$, *i.e.*, $\frac{[G]}{[C]} = 1$
- Sugar deoxyribose and phosphate occur in equimolar proportions.
- A – T base pairs are rarely equal to C – G base pairs.
- The ratio of $\frac{[A+T]}{[G+C]}$ is variable but constant for a species.

(b) *Refer to answer 9.*

20. RNA is more reactive in comparison to DNA because:

- 2′ –OH group present in ribose sugar of every nucleotide of RNA is a reactive group. It makes RNA highly reactive, labile and easily degradable.
- RNA functions as an enzyme, therefore is reactive and unstable.

21. (i) DNA is chemically less reactive and structurally more stable as its nucleotides are not exposed except when they are to express their effect.

(ii) Presence of thymine in DNA instead of uracil in RNA, provides stability to DNA.

(iii) Hydrogen bonding between purines and pyrimidines and their stacking make DNA more stable for storage of genetic information.

(iv) DNA is capable of undergoing slow mutations.

(v) It has power of repairing.

Thus, DNA which is stable enough not to change with different stages of life cycle, age or with change in metabolism of the organism, is a better material for the storage of genetic information.

22. Hershey and Chase used following two types of culture media :

(i) Medium that contained radioactive phosphorus (P^{32}).

(ii) Medium that contained radioactive sulphur (S^{35}).

They used two different kinds of culture media to detect whether the genetic material is DNA or protein.

Viruses grown in the medium with radioactive phosphorus contained radioactive DNA but not radioactive protein as DNA contains phosphorus but protein does not. Similarly, viruses grown on radioactive sulphur medium contained radioactive protein but not radioactive DNA because DNA does not contain sulphur.

Hence, their experiment proved that DNA is the genetic material.

23. The criteria which makes DNA a better genetic material than RNA are as follows :

(i) DNA is chemically less reactive and structurally more stable than RNA as its nucleotides are not exposed except when they are to express their effect whereas 2′ –OH group in ribose sugar of every nucleotide of RNA makes it more reactive. RNA also functions as an enzyme and is therefore more reactive and unstable.

(ii) Presence of thymine in DNA instead of uracil in RNA provides stability to DNA.

(iii) Hydrogen bonding between purines and pyrimidines and their stacking make DNA more stable for storage of genetic information than RNA.

(iv) DNA has power of repairing and there is no such repairing mechanism in RNA.

24. Transformation is the phenomenon by which the DNA isolated from one type of cell, when introduced (artificially or naturally) into another type, is able to bestow some of the properties of the former to the latter.

Griffith observed transformation in *Streptococcus pneumoniae* (bacterium responsible for causing pneumonia). He grew bacteria on a culture plate, some produced smooth shiny colonies (S) while others produced rough colonies (R). Mice infected with the S strain (virulent) die from pneumonia infection but mice infected with the R strain do not develop pneumonia.

S strain → Injected into mice → Mice die

R strain → Injected into mice → Mice live

Griffith observed that heat-killed S strain bacteria, when injected into the mice, did not kill them.

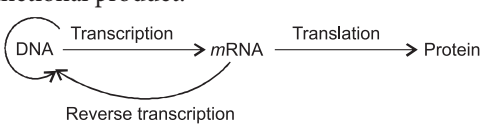
S strain (heat-killed) → Inject into mice → Mice live

When he injected a mixture of heat-killed S strain and live R bacteria, the mice died. Moreover, he recovered living S strain bacteria from the dead mice.

S strain (heat - killed) + R strain (live)
→ Injected into mice → Mice die

From the experiment Griffith concluded that the R strain bacteria had been transformed by the heat-killed S strain bacteria as some 'transforming principle' transferred from heat killed S strain enabled the R strain to become virulent.

25. (a) Francis Crick (1958) proposed 'Central dogma 'in molecular biology which states that the flow of information first occurs from DNA to *m*RNA by the process of transcription and then the information present in *m*RNA is decoded for the formation of polypeptide chain by the process of translation. The central dogma suggests that DNA contains the information needed to make all of our proteins, and that RNA is a messenger that carries this information to ribosomes. The ribosomes serve as factories in the cell where the information is translated from a code into the functional product.



Yes, central dogma differs in retroviruses, *e.g.*, HIV, etc. where it is called central dogma reverse (inverse flow of information) *i.e.*, from RNA to DNA. RNA of these viruses first synthesises DNA through reverse transcription and DNA then transfers information to RNA which takes part in translation of coded information to form a polypeptide.

(b) In 1928, Frederick Griffith performed the transformation experiment using *Streptococcus pneumoniae*. When he injected heat killed, virulent S strain along with non-virulent, live R strain in

mice, then the mice died. It showed that something from dead S strain transformed the non-virulent R strain into virulent one. During the course of his experiment, a living organism (bacteria) had changed in physical form. This phenomenon was called transformation by him. He concluded that the R strain bacteria had somehow been transformed by the heat-killed S strain bacteria. Some 'transforming principle', transferred from the heat-killed S strain, had enabled the R strain to synthesise a smooth polysaccharide coat and become virulent. This must be due to the transfer of the genetic material. However, the biochemical nature of genetic material was not defined from his experiments. Now, Avery, MacLeod and McCarty worked to determine the biochemical nature of transforming principle in Griffith's experiment. They later purified biochemicals (proteins, DNA, RNA, etc.) from the heat-killed S cells to see which ones could transform live R cells into S cells. They discovered that DNA alone from S bacteria caused R bacteria to become transformed. They also discovered that protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNases) did not affect transformation. Digestion with DNase did inhibit transformation, suggesting that the DNA caused the transformation and they concluded that DNA is the hereditary material.

26. A molecule that can act as genetic material must fulfill the following criteria :

(a) It should be able to generate its replica.

(b) It should chemically and structurally be stable.

(c) It should provide the scope for slow changes that are required for evolution.

(d) It should be able to express itself in the form of 'Mendelian characters'.

Also refer to answer 23.

27. (a) Hershey and Chase worked with virus T_2 bacteriophage. T_2 bacteriophage is a bacterial virus which has ability to infect *Escherichia coli* and it possess linear double stranded DNA (deoxyribose nucleic acid) as genetic material, therefore they used this bacteriophage or virus for their work.

(b) Hershey and Chase used following two types of culture media :

(i) Medium that contained radioactive phosphorus (P^{32}).

(ii) Medium that contained radioactive sulphur (S^{35}).

They used two different kinds of culture media to detect whether the genetic material is DNA or protein.

Viruses grown in the presence of radioactive phosphorus contained radioactive DNA but not radioactive protein because DNA contains phosphorus but protein does not. Similarly, viruses grown on radioactive sulphur contained radioactive protein but not radioactive DNA because DNA does not contain sulphur.

(c) Blender was used in experiment to remove the empty phage capsids (or ghosts) sticking to the surface of bacteria.

Centrifuge was used to separate virus particle from the bacteria. The bacterial cultures were centrifuged. Both the supernatant and the pellets were checked for radioactivity. In culture with radioactive S^{35} it was found that phage with labelled protein did not make bacteria labelled. Instead, radioactivity was restricted to supernatant which was found to contain only capsid. On the other hand, in the second culture with P^{32} it was found that supernatant containing capsid was not radioactive instead bacteria become labelled proving that only DNA of the phage entered the bacteria.

(d) Hershey and Chase from this experiment concluded that genetic material is DNA and not the protein.

28. (a) *Refer to answer 24.*

(b) Avery, MacLeod and McCarty (1944) performed the biochemical characterisation of the 'transforming principle' of Griffith's experiment. They separated the extract of smooth, virulent bacteria into protein, DNA and carbohydrate fractions. Each fraction was separately added to a culture medium containing live rough bacteria. Only the culture that received the DNA fraction of the extract from virulent bacteria produced smooth bacteria. This proved that DNA was the transforming agent. When DNA fraction was treated with deoxyribonuclease (an enzyme that digests DNA), it became inactive and incapable of transforming the rough strain into the smooth strain. This confirmed that DNA is the genetic material.

The following table represent the result of Avery's experiment.

Table : Results of Avery's Experiment

Mixture	Result
R-type bacteria + carbohydrates of S-type bacteria	R-type bacteria
R-type bacteria + protein of S-type bacteria	R-type bacteria
R-type bacteria + DNA of S-type bacteria	S-type bacteria
R-type bacteria + DNA of S-type bacteria + deoxy-ribonuclease	R-type bacteria

29. Alfred D. Hershey and Martha Chase, choose T_2 bacteriophage as their experimental material. They decided to see which of the bacteriophage components-protein or DNA-entered bacterial cells and directed reproduction of the virus.

Hershey and Chase experiment is based on the fact that DNA but not the protein contains phosphorus, and similarly sulphur is present in proteins (cysteine and methionine) but not in DNA. They incorporated radioactive isotope of phosphorus (^{32}P) into phage DNA and that of sulphur (^{35}S) into proteins of separate phage cultures. These phage types were used independently to infect the bacterium *Escherichia coli*. After sometime, the cultures were agitated in a blender to separate the empty phage capsids from the surface of bacterial cells and the two were separated by centrifugation. Hershey and Chase showed that in bacterial cells, infected with virus containing radioactive phosphorus (^{32}P), radioactivity was associated with bacterial cells and also, appeared in the progeny phage. However, in bacterial culture where radioactive sulphur (^{35}S) was used, all radioactive material was limited to phage 'ghosts' (empty viral protein coats).

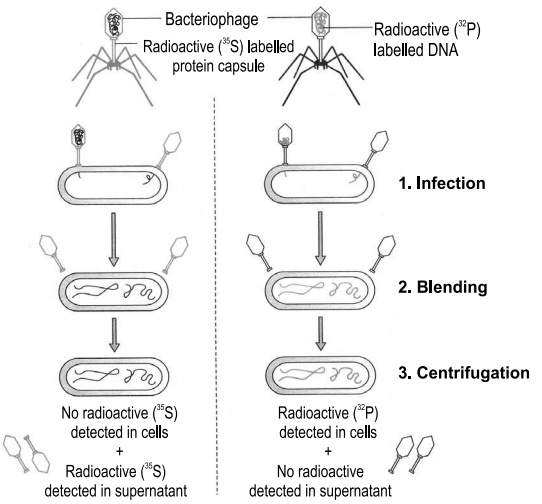
These results indicated that the DNA of the bacteriophage and not the protein enters the host, where viral replication takes place. Therefore, DNA is the genetic material of T_2 bacteriophage. It directs protein coat synthesis and allows replication to occur.

30. (a) *Refer to answer 24.*

(b) *Refer to answer 28 (b).*

31. *Refer to answer 29.*

Diagrammatic representation of Hershey and Chase experiment is as follows:



32. *Refer to answer 24.*

33. *Refer to answer 29.*

34. (a) *Refer to answer 24.*

(b) *Refer to answer 28 (b).*

35. (a) From his experiment Griffith concluded that R strain bacteria has been transformed by heat killed S strain bacteria as some 'transforming principle' from it enabled R strain bacteria to become virulent.

(b) *Refer to answer 28 (b).*

36. The unequivocal proof that DNA is the genetic material came from the experiments of Alfred Hershey and Martha Chase (1952).

They decided to see which of the bacteriophage components (protein or DNA) entered bacterial cells and directed reproduction of the virus.

Their experiment is based on the fact that DNA but not the protein contains phosphorus, and similarly sulphur is present in proteins but not in DNA. They incorporated ^{32}P (radioactive isotope of phosphorus) into phage DNA and ^{35}S (radioactive isotope of sulphur) into proteins of a separate phage culture. These phage types were independently used to infect the bacterium *Escherichia coli*.

After sometime, this mixture was agitated in a blender. And the two were separated by

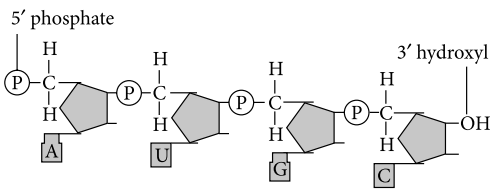
centrifugation. Hershey and Chase observed that when ^{32}P was used, all radioactivity was associated with bacterial cells and if followed, appeared in the progeny phage. However, when ^{35}S was used, all radioactive material was limited to the protein coats.

These results confirmed that the DNA is the genetic material.

37. *Refer to answer 24.*

38. *Refer to answers 31 and 36.*

39. RNA is single stranded or chain (ds RNA is reported in wound tumour virus, rice dwarf virus) which is formed by end to end polymerisation of a number of ribonucleotides or ribotides. Four types of ribonucleotides occur in RNA. They are adenosine monophosphate, guanosine monophosphate, uridine monophosphate and cytidine monophosphate. A ribonucleotide is formed of ribose sugar, phosphoric acid and a nitrogen base. The four nitrogen bases present in RNA are adenine, guanine (purines), cytosine and uracil (pyrimidines). The union of nitrogen base is with carbon 1′ of ribose sugar by glycosidic bond through 3 N or 9 N region. Phosphate combines with carbon 5′ of its sugar and carbon 3′ of next sugar by phosphodiester bond. Figure representing polynucleotide chain of RNA is as follows:



40. The first genetic material was RNA. It can be explained as :

(a) Metabolism, splicing and translation evolved around RNA.

(b) The first biocatalysts were RNAs. Even now some enzymes are made of RNAs, *e.g.,* ribozyme.

(c) RNAs worked well in early unstable environmental conditions. As the environment become stable, RNAs were replaced in two of its functions (i) By small chemical modifications RNA gave rise to DNA as genetic material. (ii) For biocatalysis, RNA was replaced by protein enzymes. The latter were more stable, more efficient and more varied.

41. Deoxyribonucleoside triphosphates such as dATP, dCTP, dGTP and dTTP serve dual purpose during DNA replication. They act as substrates *i.e.,* nucleotides for the replication process as well as provide energy for the polymerisation of nucleotides by cleavage of high energy terminal phosphate bonds.

42. The sources of energy for the replication of DNA are phosphorylated nucleotides or deoxyribonucleoside triphosphates *i.e.*, deATP, deCTP, deGTP and deTTP.

43. It is not possible for an alien DNA to become a part of the chromosome anywhere along the length and replicate normally because of absence of origin of replication (*ori*). It is the sequence where DNA replication starts. This site is also necessary for binding of DNA polymerase to start DNA replication. As this site may not present in all alien DNA molecules hence they cannot replicate normally.

44. DNA replication doubles the amount of DNA in a cell and cell division again halves the amount of DNA *i.e.*, maintains the normal amount of DNA in the daughter nuclei. Thus, if DNA replication is not followed by cell division in a eukaryotic cell, then amount of DNA will increase than normal, resulting in abnormal conditions such as polyploidy.

45. The enzyme DNA-dependent DNA polymerase catalyse the polymerisation of deoxynucleotides.

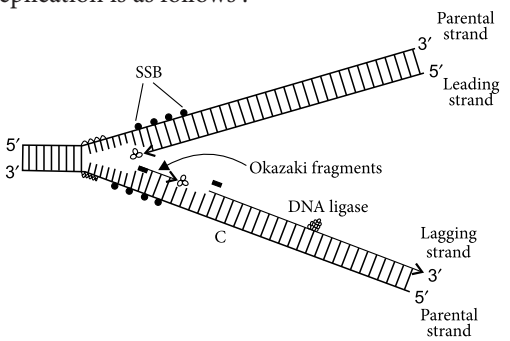
It catalyses polymerisation only in 5′ → 3′ direction thereby resulting in continuous replication on DNA strand with 3′ → 5′ polarity and discontinuous replication on strand with 5′ → 3′ polarity.

46. The enzyme DNA ligase joins the small fragments of DNA of a lagging strand during DNA replication.

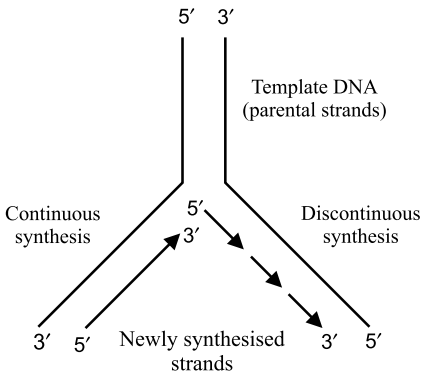
47. DNA ligase is an enzyme that catalyses the repair of a single strand break by formation of covalent phosphodiester bond between adjacent 3′ hydroxyl and 5′ phosphoryl groups in double stranded DNA. Hence, the enzyme helps in sealing gaps in DNA fragments and acts as molecular glue.

During replication of DNA, short replicated fragments/segments of DNA or Okazaki fragments are present on the lagging strand. These fragments are joined to form a continuous strand with the help of enzyme DNA ligase.

48. Diagrammatic representation of DNA replication is as follows :



49. Diagrammatic representation of replicating fork is as follows:



50. Deoxyribonucleoside triphosphates (or phosphorylated nucleotides) *i.e.,* deATP, deCTP, deGTP and deTTP serve dual purpose during DNA replication. They act as substrates for the replication process as well as provide energy for the polymerisation of nucleotides.

51. The work of Mathew Meselson and Franklin Stahl (1958) on *E. coli* proved semi-conservative replication of DNA.

They first grew bacteria *Escherichia coli* in a medium containing heavy isotope ^{15}N for several generations. This led to the incorporation of heavy isotope in all nitrogen-containing compounds including bases. They were able to extract the bacterial DNA and centrifuge it in caesium chloride solution. Depending on the mass of the molecule, the DNA would settle out at a particular point in the tube.

The ^{15}N bacteria were then transferred to a growth medium containing the normal, lighter isotope of nitrogen, ^{14}N, where they reproduced by cell division. Meselson and Stahl found that DNA of the first generation was hybrid or intermediate (^{15}N and ^{14}N). It settled in caesium chloride solution at a level higher than the fully labelled DNA of parent bacteria ($^{15}N^{15}N$). The second generation of bacteria after 40 minutes, contained two types of DNA, 50% light ($^{14}N^{14}N$) and 50% intermediate ($^{15}N^{14}N$). At succeeding generation times, the DNA extracts were found to have a lower proportion of ^{15}N as more ^{14}N was incorporated into the bacterial DNA. This observation is possible only if both strands separate during replication and one strand act as template for synthesis of new strand of DNA having ^{14}N. This was conclusive evidence for the semi-conservative method of DNA replication.

52. *Refer to answer 49.*

The figure represents the mechanism of DNA replication, during which leading strand and lagging strand are formed on the two different template strands. DNA-polymerase can polymerise nucleotides only in 5′ → 3′ direction. Since the two strands of DNA run in antiparallel directions, the two templates provide different ends for replication. Replication, over the two templates, thus proceeds in opposite directions. On strand with polarity 3′→ 5′ replication is continuous because 3′ end of the latter is always open for elongation. It is called leading strand. Replication is discontinuous on the other template with polarity 5′ → 3′ because only a short segment of DNA strand can be built in 5′ → 3′ direction due to exposure of a small stretch of template at one time. Short segments of replicated DNA are called Okazaki fragments. Okazaki fragments are later joined together by means of enzyme, DNA ligase to form lagging strand. *Also refer to answer 47.*

53. (a) NH_4Cl (Ammonium chloride) was used as a source of nitrogen by Meselson and Stahl in their experiment.

(b) They synthesised light and heavy DNA molecules in the organism (*E. coli* bacterium) so as to separate different generations on the basis of density gradient centrifugation.

(c) The DNA molecules from each generation, were tested through density gradient centrifugation using cesium chloride. The heavy

DNA molecules settled at the bottom whereas successively light DNA molecules settled at the surface. In this way, the DNA molecules were distinguished.

(d) From the experiment scientists concluded that DNA replication is semi-conservative in nature.

54. *Refer to answer 51.*

55. *Refer to answer 51.*

56. *Refer to answer 51.*

57. (a) Meselson and Stahl used ^{14}N and ^{15}N isotopes for their experiment because ^{15}N is a heavy isotope of nitrogen and can be separated from ^{14}N by density gradient centrifugation using caesium chloride.

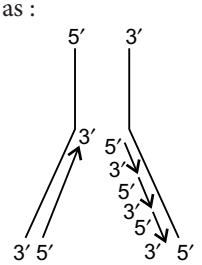
Using two different isotopes of nitrogen helped them to isolate the different generations of *E. coli* bacteria from each other, *e.g.*, they found that DNA of first generation was hybrid (^{15}N and ^{14}N), it settled in density gradient centrifugation at a level higher than the fully labelled DNA of parent bacteria (^{15}N ^{15}N). Succeeding generations were also found to settle toward the surface.

(b) From the experiment they concluded that DNA replication is semi-conservative.

58. The DNA-dependent DNA polymerase catalyse polymerisation in one direction only *i.e.*, 5′ → 3′. Therefore, in the strand with polarity 3′ → 5′ replication is continuous whereas on the other strand with polarity 5′ → 3′ replication is discontinuous. Therefore, two different types of replicating strands are seen on the DNA replicating fork. The two strands are named leading strand and lagging strand respectively.

59. *Refer to answer 53.*

60. (a) The polarity of template strands and newly synthesised strands in the given figure can be represented as :



(b) *Refer to answer 52.*

61. (a) DNA replication occurs during S-phase of the cell cycle.

(b) DNA replication is a multistep complex process, which requires a dozen enzymes and protein factors. It begins at a particular spot called origin of replication or *ori*.

Separating the two strands of DNA is accomplished by the helicase enzymes that travel along the helix, opening the double helix as they move. Unwinding also creates a coiling tension in front of the moving replication fork, a structure that will be formed when DNA replication begins. This tension is reduced by topoisomerases.

The very important DNA synthesising enzyme is DNA polymerase III. It along with other DNA polymerases (I and II) has the ability to elongate an existing DNA strand but cannot initiate the synthesis.

All the three DNA polymerases function in 5′ → 3′ direction only for DNA polymerisation and have 3′ → 5′ exonuclease activity.

To initiate DNA synthesis, a small segment of RNA (10 to 60 nucleotides) called an RNA primer complementary to the template DNA is synthesised by a unique RNA polymerase known as primase.

While on the one strand the DNA synthesis is continuous in 5′→ 3′ direction, on the other strand, DNA is synthesised in small stretches resulting in discontinuous DNA synthesis. This happens in the opposite direction to the first strand but maintains the overall 5′ → 3′ direction as required. Such a process is also referred to as semi-discontinuous replication. The short stretches of DNA, each primed by RNA are called Okazaki fragments.

RNA primer are then removed, and the gap is filled by DNA synthesis by DNA polymerase I. The enzyme ligase then seals these fragments.

The strand which supports the continuous DNA synthesis is the leading strand and the one, which is replicated in short stretches is called the lagging strand.

(c) DNA replication is said to be semi-conservative since daughter DNA duplex comprise of one parental and one newly synthesised strand.

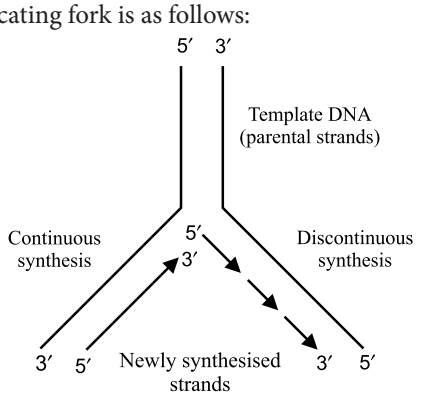
62. The work of Mathew Meselson and Franklin Stahl (1958) on *E.coli* proved semi-conservative replication of DNA.

They first grew *Escherichia coli* bacteria in a medium containing heavy isotope of nitrogen (^{15}N) for several generations. This led to the incorporation of heavy isotope in all nitrogen-containing compounds including bases. They were able to extract the bacterial DNA and centrifuge it in caesium chloride solution. Depending on the mass of the molecule, the DNA would settle out at a particular point in the tube (heavy DNA molecule can be distinguished from normal DNA by centrifugation in cesium chloride density gradient).

The ^{15}N bacteria were then transferred to a growth medium containing the normal, lighter isotope of nitrogen, ^{14}N, where they reproduced by cell division. Extracts of DNA from the first generation offspring were shown to have a lower density, since half the DNA was made up of the original strand containing ^{15}N and the other half was made up of the new strand containing ^{14}N. At succeeding generation times, the DNA extracts were found to have a lower proportion of ^{15}N as more ^{14}N was incorporated into the bacterial DNA. This was conclusive evidence for the semi-conservative method of DNA replication.

The conclusion they arrived at after the experiment is that the DNA replication is semi-conservative. Semi-conservative means that when the double stranded DNA helix was replicated, each of the two double stranded DNA helices of newly synthesised strands consisted of one strand coming from the original helix and one newly synthesised. So, in this way at each replication, one strand of parent DNA is conserved in the daughter while the second is freshly synthesised.

63. (a) Diagrammatic representation of replicating fork is as follows:



Due to high energy requirement whole of DNA does not open in one stretch. The point of separation proceed slowly towards both directions. It gives the appearance of Y-shaped structure called replication fork.

(b) Two enzymes involved in the process of DNA replication are:

(i) Helicase - causes the unwinding of DNA strand.

(ii) Topoisomerase - releases the tension of DNA strand.

64. DNA replication occurs during S-phase of cell cycle. It is a multistep complex process which requires over a dozen enzymes and protein factors. It begins at a particular spot called origin of replication or *ori*.

Replication of DNA is energetically highly expensive. The main enzyme of DNA replication is DNA dependent DNA polymerase.

Deoxyribonucleoside monophosphates occur freely inside the nucleoplasm. They are first phosphorylated and changed to active forms. The phosphorylated nucleotides are deATP (deoxyadenosine triphosphate), deGTP (deoxyguanosine triphosphate), deCTP (deoxycytidine triphosphate) and deTTP (deoxythymidine triphosphate).

Enzyme helicase (unwindase) acts over the *Ori* site and unzips (unwinds) the two strands of DNA by destroying hydrogen bonds. Unwinding creates tension in the uncoiled part by forming more supercoils. Tension is released by enzymes topoisomerases. With the help of various enzymes both the strands of DNA become open for replication. However, whole of DNA does not open in one stretch due to very high energy requirement. The point of separation proceeds slowly towards both the directions. In each direction, it gives the appearance of Y-shaped structure called replication fork.

RNA primer is a small strand of RNA which is synthesised at the 5′ end of new DNA strand with the help of DNA specific RNA polymerase enzyme called primase.

Prokaryotes have three major types of DNA synthesising enzymes called DNA polymerases III, II and I. In eukaryotes five types of DNA polymerases are found - α, β, γ, δ and ε, but the major three being α, δ and ε. The two separated

DNA strands in the replication fork function as templates.

As replication proceeds, new areas of parent DNA duplex unwind and separate so that replication proceeds rapidly from the place of origin towards the other end. RNA primer is removed and the gap filled with complementary nucleotides by means of DNA polymerase I. DNA polymerase can polymerise nucleotides only in $5' \rightarrow 3'$ direction on $3' \rightarrow 5'$ strand because it adds them at the $3'$ end. Since the two strands of DNA run in antiparallel directions, the two templates provide different ends for replication. Replication over the two templates thus proceeds in opposite directions. One strand with polarity $3' \rightarrow 5'$ forms its complementary strand continuously because $3'$ end of the latter is always open for elongation. It is called leading strand. Replication is dicontinuous on the other template with polarity $5' \rightarrow 3'$ because only a short segment of DNA strand can be built in $5' \rightarrow 3'$ direction due to exposure of a small stretch of template at one time. Short segments of replicated DNA are called Okazaki fragments.

Okazaki fragments are joined together by means of enzyme, DNA ligase. DNA strand built up of Okazaki fragments is called lagging strand. *Also refer to answer 49.*

65. (a) *Refer to answers 64 and 49.*

(b) In eukaryotes, the replication of DNA takes place during S-phase of the cell cycle. The replication of DNA and cell division cycle should be highly coordinated. A failure in cell division after DNA replication results into polyploidy (a chromosomal anomaly).

66. *Refer to answer 62.*

67. (a) Given structure represents replicating fork. The two parental strands of DNA unwind at the start of replication.

(b) *Refer to answer 63.*

(c) *Refer to answer 42.*

Various enzymes required during DNA replication are helicase, phosphorylase, topoisomerase, DNA polymerase I, II and III, primase and DNA ligase.

Helicase : It acts over the *ori* site and unzips the two strands of DNA.

Phosphorylase : It is required for phosphorylation of deoxyribonucleotides.

Topoisomerase : It helps in nicking of one strand of DNA to release tension.

DNA polymerase I : It is a major repair enzyme and it has $5' \rightarrow 3'$ exonuclease activity.

DNA polymerase II : It is a minor repair enzyme.

DNA polymerase III : It does addition and polymerisation of new bases.

Primase : It helps in formation of RNA primer.

DNA ligase : Okazaki fragments are joined by DNA ligase.

(d) DNA polymerase can polymerise nucleotides only in $5' \rightarrow 3'$ direction on $3' \rightarrow 5'$ strand because it adds them at the $3'$ end. Since the two strands of DNA run in antiparallel directions, the two templates provide different ends for replication. Replication over the two templates thus proceeds in opposite directions. One strand with polarity $3' \rightarrow 5'$ forms its complementary strand continuously because $3'$ end of the latter is always open for elongation. It is called leading strand. Replication is dicontinuous on the other template with polarity $5' \rightarrow 3'$ because only a short segment of DNA strand can be built in $5' \rightarrow 3'$ direction due to exposure of a small stretch of template at one time.

68. *Refer to answer 62.*

69. The aim of the experiment of Meselson and Stahl was to prove semi-conservative replication of DNA. *Also refer to answer 62.*

70. (c)

71. Cistron is a segment of DNA consisting of a stretch of base sequences that codes for one polypeptide chain, one transfer RNA (*t*RNA), ribosomal RNA (*r*RNA) molecule or performs any other specific function in connection with transcription, including controlling the functioning of other cistrons.

72. In eukaryotes, RNA polymerase II transcribes precursor of *m*RNA, the heterogeneous nuclear RNA (hnRNA).

73. *Refer to answer 72.*

74. Exons are the segments in genes which contain coding nucleotide sequences. These sequences are ultimately translated into polypeptide. Thus, exons carry genetic information. Introns are the segments in genes

which contain non-coding nucleotide sequences. These do not form part of *m*RNA and are removed during the processing of hnRNA.

75. Sigma (σ) factor acts as an initiation factor during transcription in a prokaryote.

76. Intron is considered to be as the reminiscent of antiquity.

77. Differences between template strand and coding strand are as follows :

	Template strand	**Coding strand**
(i)	Strand of DNA having 3′ → 5′ polarity.	Strand of DNA having 5′→3′ polarity.
(ii)	Participates in transcription.	Do not take part in transcription.

78. Differences between structural gene in prokaryotes and structural gene in eukaryotes are as follows :

	Structural gene in prokaryotes	**Structural gene in eukaryotes**
(i)	Consists of functional coding sequences.	Consists of both exons and introns.
(ii)	Information is continuous as only exons are present.	Information is split due to presence of introns in between exons.
(iii)	Splicing does not take place.	Splicing occur to make functional *m*RNA.

79. The corresponding coding strand is :
5′ TACGTACGTACGTACGTACGTACG 3′
The corresponding *m*RNA strand is
5′ UACGUACGUACGUACGUACGUACG 3′

80. (a) *t*RNA helps in transferring amino acids to ribosome for synthesis of polypeptide chain. *t*RNA reads the genetic codes, carries amino acids to the site of protein synthesis and acts as an adapter molecule.
(b) *r*RNA is the most abundant RNA. Prokaryotic ribosomes are of three types 23S, 16S and 5S. 23S and 5S occur in large subunit of ribosome while 16S is found in smaller subunit. It plays structural and catalytic role during translation.

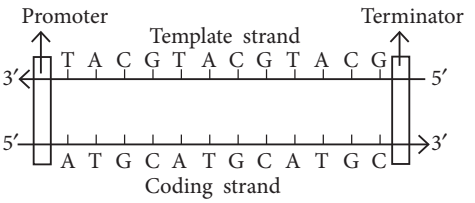
81. Cistron is segment of DNA consisting of a stretch of base sequences that codes for one polypeptide chain, one transfer RNA (*t*RNA), ribosomal RNA (*r*RNA) molecule or performs any other specific function in connection with transcription, including controlling the functioning of other cistrons.
Exons are the regions of a gene, which become part of *m*RNA and code for the different proteins.

82. Differences between introns and exons are:

	Introns	**Exons**
(i)	Regions of a gene which do not form part of *m*RNA.	Regions of a gene which become part of *m*RNA.
(ii)	Removed during the processing of *m*RNA.	Code for the different proteins.

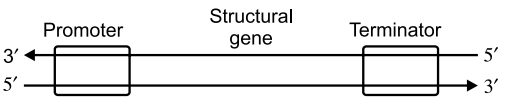
83. RNA segment that has been transcribed from a transcription unit which has the polarity (5′ → 3′) have uracil at the place of thymine.
Given RNA strand :

For the given RNA, the transcription unit is given as:



DNA dependent RNA polymerase is an enzyme that transcribed this RNA segment.

84. Schematic representation of transcription unit is as follows:

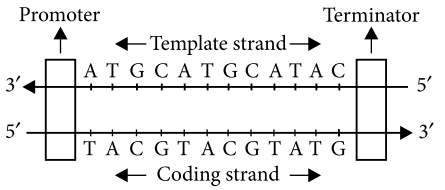


(i) Promoter - The promoter is located towards 5′-end of the coding strand. It is a DNA sequence that provides binding site for RNA polymerase, and it is the presence of a promoter in a transcription unit that defines the template and coding strands.
(ii) Structural gene - The structural genes code for the enzymes and proteins. It transcribe the *m*RNA for the same.

(iii) Terminator - The terminator is located towards 3′-end of the coding strand and it defines the end of the process of transcription.

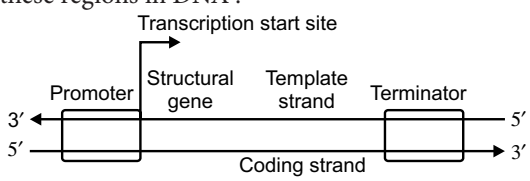
85. (a) The transcriptional products of RNA polymerase III are *t*RNA, 5S*r*RNA and snRNA.
(b) In capping, additional nucleotides (methyl guanosine triphosphate) are added to the 5′-end of hnRNA. In tailing, adenylate residues (200 - 300) are added at the 3′-end in a template independent manner.
(c) Heterogeneous nuclear RNA.

86. (a)



(b) 5′ U A C G U A C G U A U G 3′

87. A transcription unit in DNA is defined by these regions in DNA :



88. (a) RNA polymerase II transcribes hnRNA.
(b) Post transcription processing of hnRNA is required to convert primary transcript of all types of RNA into functional RNAs. The eukaryotic transcription involves certain complexity, one of the complexity is that the primary transcript contain both the exons and the introns (which are non-functional). Hence, it is subjected to a process called splicing where the introns are removed and exons are joined in a defined order. The transcribed eukaryotic RNA or heterogeneous nuclear RNA (hnRNA), undergoes additional processing called as capping and tailing.
In capping, an unusual nucleotide (methyl guanosine triphosphate) is added to the 5' - end of hnRNA. In tailing, adenylate residues (200 - 300) are added at the 3′-end in a template independent manner. It is the fully processed hnRNA, now called *m*RNA. This process takes place in the nucleus of a cell and then *m*RNA is transported out of the nucleus for translation.

89. In eukaryotes, monocistronic structural genes could be said as interrupted, as both introns and exons are present. *Also refer to answers 88 and 78.*

90. (a) Differences between promoter and terminator are as follows:

	Promoter	**Terminator**
(i)	Located upstream of structural gene.	Located downstream of structural gene.
(ii)	Has RNA polymerase binding and recognition site.	Rho factor required for termination.
(iii)	In many cases, promoter has AT rich region.	It has stop signal and also possess 4-8 A-nucleotides.

(b) *Refer to answer 82.*

91. In eukaryotic cells, the primary transcript is often larger than the functional RNA and is known as heterogeneous RNA or hnRNA. It undergoes modification termed as post-transcriptional processing to become functional RNA. It has four steps.
(i) Cleavage : Larger RNA precursors are cleaved to form smaller RNAs.
(ii) Splicing : Eukaryotic transcripts possess extra segments called introns or intervening sequences or non-coding sequences. They do not appear in mature or processed RNA. The functional coding sequences are called exons. Splicing is removal of introns and fusion of exons to form functional RNAs.
(iii) Terminal additions (capping and tailing) : Additional nucleotides are added to the ends of RNAs for specific functions, *e.g.*, CCA segment in *t*RNA, cap nucleotides at 5′ end of *m*RNA or poly-A segments (200-300 residues) at 3′ end of *m*RNA. Cap is formed by modification of GTP into 7-methyl guanosine or 7 mG.
(iv) Nucleotide modifications :They are most common in *t*RNA-methylation (*e.g.*, methyl cytosine, methyl guanosine), deamination (*e.g.*, inosine from adenine), dihydrouracil, pseudouracil, etc.

92. (a) Transcription is the process of copying genetic information from one strands of DNA into RNA. A transcription unit of a DNA has three regions a promoter, a structural gene and a terminator.

Bacterial structural gene in a transcription unit is polycistronic. Transcription requires a DNA dependent RNA polymerase. Prokaryotes have only one DNA dependent RNA polymerase which synthesises all types of RNA.

Three major types of RNAs in a bacteria are *m*RNA (messenger RNA), *t*RNA (transfer RNA), and *r*RNA (ribosomal RNA). All three RNAs are needed to synthesise a protein in a cell. The *m*RNA provides the template, *t*RNA brings amino acids and reads the genetic code, and *r*RNAs play structural and catalytic role during translation. In bacteria/prokaryotes, transcription occurs in contact with cytoplasm as their DNA lies in the cytoplasm.

RNA polymerase binds to promoter and initiates transcription (Initiation). It uses nucleoside triphosphates as substrate and polymerises the *m*RNA strand in a template depended fashion following the rule of complementarity. It also facilitates opening of the helix and continues elongation. Only a short stretch of RNA remains bound to the enzyme. Once the polymerases reaches the terminator region, the nascent RNA falls off, so also the RNA polymerase. This results in termination of transcription.

(b) *Refer to answer 91.*

93. Mechanism of transcription in prokaryotes :

In bacteria/prokaryotes, transcription occurs in contact with cytoplasm as their DNA lies in the cytoplasm.

(a) Activation of ribonucleotides – The four types of ribonucleotides are adenosine monophosphate (AMP), guanosine monophosphate (GMP), uridine monophosphate (UMP) and cytidine monophosphate (CMP). They occur freely in the nucleoplasm. Prior to transcription the nucleotides are activated through phosphorylation. Enzyme phosphorylase is required alongwith energy. The activated or phosphorylated ribonucleotides are adenosine triphosphate (ATP), guanosine triphosphate (GTP), uridine triphosphate (UTP) and cytidine triphosphate (CTP).

(b) Binding of RNA polymerase to DNA duplex – On a signal from the cytoplasm, DNA segment become ready to transcribe. The RNA polymerase enzyme binds to a specific site, called promoter, in the DNA double helix. Prokaryotes have only one RNA polymerase that synthesise all types of RNA. The promoter also determines which DNA strand is to be transcribed. Thus, a promotor region has RNA polymerase recognition site and RNA polymerase binding site.

(c) Base pairing – Ribonucleoside triphosphates present in the surrounding medium come to lie opposite the nitrogen bases of the DNA template (anti-sense strand). They form complementary pairs; U opposite A, A opposite T, C opposite G and G opposite C. A pyrophosphate is released from each ribonucleoside triphosphate to produce ribonucleotide.

(d) Formation of RNA chain – With the help of RNA polymerase the adjacent ribonucleotides held over DNA template join to form RNA chain. A single RNA polymerase recognise promoter and initiation region in prokaryotes. As the RNA chain formation initiates, the sigma (σ) factor of the RNA polymerase separates. RNA polymerase (core enzyme) moves along the DNA template causing elongation of RNA chain at the rate of some 30 nucleotides per second. RNA synthesis stops as soon as polymerase reaches the terminator region. Rho factor (ρ) has ATP-ase activity and also possesses 4-8 adenine ribonucleotides.

(e) Separation of RNA chain – With the help of rho factor, the fully formed RNA chain is now released. One gene forms several molecules of RNA, which are released from the DNA template one after the other on completion. The released RNA is called primary transcript.

(f) Duplex formation – As the RNA chain is released, the transcribed region of the DNA molecule gets hydrogen bonded to the sense strand and the two are spirally coiled to assume the original double helical form. The protective protein coat is added again to the DNA duplex. Gyrases, helicases and helix stabilizing proteins are released.

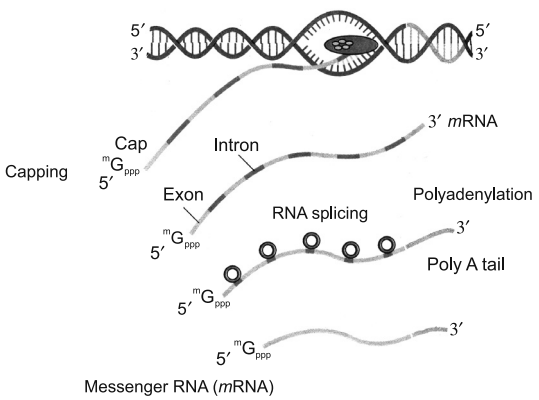
Differences between prokaryotic and eukaryotic transcription are :

	Prokaryotic transcription	**Eukaryotic transcription**
(i)	It occurs in contact with cytoplasm.	It occurs inside the nucleus.
(ii)	There is no specific period for its occurrence.	Major part of transcription occurs in G_1 and G_2 phases.

(iii)	It is coupled to translation.	Transcription and translation are spacially separated.
(iv)	Products of transcription become effective *in situ*.	Products of transcription come out of the nucleus for functioning in cytoplasm.
(v)	There is only one RNA polymerase.	There are three types of RNA polymerases.
(vi)	RNA polymerase does not have separate transcription factors.	Transcription factors are involved in recognition of promotor site.
(vii)	*m*RNA is generally polycistronic.	*m*RNA is generally monocistronic.
(viii)	Splicing is generally not required.	In most of the cases splicing is required for removing intervening sequences.

94. In eukaryotes, transcription occurs throughout I-phase in differentiated cells but more so in G_1 and G_2 phases of cell cycle inside the nucleus. Depending upon the requirement, a structural gene may transcribe one to numerous RNA molecules. The transcription products move out into cytoplasm for translation. Transcription requires a DNA dependent RNA polymerase. Eukaryotes have three RNA polymerase, Pol I (Pol A) (for ribosomal or *r*RNAs except 5S *r*RNA). Pol II (for *m*RNA, snRNAs) and Pol III (for transfer or *t*RNA, 5S *r*RNA., and some snRNAs). Eukaryotic RNA polymerases also require transcription factors for initiation.

Prior to transcription, the nucleotides are activated through phosphorylation. Enzyme phosphorylase is required alongwith energy. Each DNA transcription segment has a promoter region, initiation site, coding region and a terminator region. RNA polymerase (common in prokaryotes and specific in eukaryotes) binds itself to the promoter region. The two strands of DNA uncoil progressively from the site of polymerase binding. One of the two strands of DNA ($3' \rightarrow 5'$) functions as a template for transcription of RNA. Transcript formation occurs in $5' \rightarrow 3'$ direction. Ribonucleoside triphosphate present in the surrounding medium form complementary pairs. With the help of RNA polymerase the adjacent ribonucleotides held over DNA template join to form RNA chain. In eukaryotes, there are separate transcription factor and RNA polymerase for activation of transcription. RNA polymerase (core enzyme) moves along the DNA template causing elongation of RNA chain at the rate of some 30 nucleotides per second. RNA synthesis stops as soon as polymerase reaches the terminator region. In eukaryotes, the transcription unit yields a monocistronic *m*RNA. Diagrammatic representation of transcription in eukaryotes is as follows:



95. *Refer to answer 93.*

96. (a) *Refer to answers 94 and 91.*

(b) *Refer to answer 93.*

97. (a) *Refer to answer 93.*

(b) *Refer to answer 93.*

98. AUG codon has dual functions. It codes for methionine (met) and also acts as an initiation codon for polypeptide synthesis.

99. The difference between unambiguous and degenerate codons is :

Unambiguous codons	Degenerate codons
They specify only one amino acid.	More than one codons that code for a single amino acids.

100. *Refer to answer 98.*

101. AUG has dual functions. It functions as initiation codon during protein synthesis and also codes for methionine. UGA does not specify any amino acid hence it functions as terminator codon.

102. (a) AUG or methionine is an initiation codon as polypeptide synthesis is signalled by it whereas UAA (ochre) is termination codon as polypeptide chain termination is signalled by it.
(b) Genetic code is non-ambiguous i.e. one codon specifies only one amino acid which implies that codons are specific. Degeneracy of code implies that some amino acids can be influenced by more than one codon. *e.g.*, Phenylalanine is specified by two codons UUU and UUC.

103. (a) Differences between unambiguous and universal genetic codes are:

	Unambiguous code	**Universal code**
(i)	A codon specifies only one amino acid.	The codons specifies the same amino acids from a virus to a tree or human being.
(ii)	*E.g.,* AUG codes for methionine.	*E.g.,* from bacteria to human UUU would code for phenylalanine.

(b) Difference between the degenerate and initiator code is:

Degenerate code	**Initiator code**
More than one codon code for a single amino acid.	Signal polypeptide synthesis. It has dual functions.

104. (i) Stop codon : Codons that do not code for any amino acids and signal polypeptide chain termination. *E.g.*, UAA, UAG, UGA.
(ii) Unambiguous codon : Codons that specify only one amino acid and not any other. *E.g.*, AUG codes for methionine.
(iii) Degenerate codon : More than one codons codes for a single amino acid. In degenerate codons, generally the first two nitrogen bases are similar while the third one is different. *E.g.*, UUU and UUC codes for phenylalanine.
(iv) Universal codon : A codon that is applicable universally *i.e.,* specifies the same amino acid from a virus to a tree or human being.

105. (a) George Gamow suggested that the genetic code should be made up of three nucleotides.
(b) He proposed that since there are only 4 bases and if they have to code for 20 amino acids, the code should constitute a combination of bases made up of three nucleotides. Combination of 4^3 ($4 \times 4 \times 4$) would generate 64 codons; generating many more codons than required.

106. Exceptions to the universality of genetic code are:
(i) UAA and UGA are termination codons and do not code for any amino acid. But in *Paramecium* and some other ciliates, these codons code for glutamine.
(ii) Genetic code is non-overlapping in most organisms. But, $\phi \times 174$ has 5375 nucleotides that code for 10 proteins which require more than 6000 bases. Three of its genes E, B and K overlap other genes. Nucleotide sequence at the beginning of E gene is contained within gene D. Likewise gene K overlaps with genes A and C. A similar condition is found in SV-40.
More than one codons code for a single amino acid, thus are called degenerate codons. In degenerate codes, the first two nitrogen bases are similar while the third one is different, *e.g.*, UUU and UUC are the degenerate codes that code for amino acid phenylalanine.

107. Genetic code is universal *i.e.*, a codon specifies the same amino acid from a virus to a tree or human being. Example : *m*RNA from chick oviduct introduced in *Escherichia coli* produces ovalbumen in the bacterium exactly similar to one formed in chick.
Genetic code is degenerative *i.e.*, all other amino acids, except tryptophan and methionine, are specified by two (*e.g.* phenylalanine – UUU, UUC) to six (*e.g.*, arginine–CGU, CGC, CGA, CGG, AGA, AGG) codons. They are therefore called degenerate or redundant codon. In degenerate codons, generally the first two nitrogen bases are similar while the third one is different.

108. Structure of *t*RNA can be explained by means of L-form model (Given by Klug 1974) and by means of clover leaf model (given by Holley 1965).

In *t*RNA molecule, about half of the nucleotides are base paired to produce paired stems. Five regions are unpaired or single stranded - AA-binding site, T ψ C loop, DHU loop, extra arm and anticodon loop.

(i) Anticodon Loop. It has 7 bases out of which three bases form anticodon (nodoc) for recognising and attaching to the codon of *m*RNA.

(ii) AA-Binding Site. It is amino acid binding site. The site lies at the 3′ end opposite the anticodon and has CCA – OH group. The 5′ end bears G. Amino acid or AA binding site and anticodon are the two recognition sites of *t*RNA.

(iii) T ψ C Loop. It has 7 bases out of which ψ (pseudouridine) and rT (ribothymidine) are unusual bases. The loop is the site for attaching to ribosome.

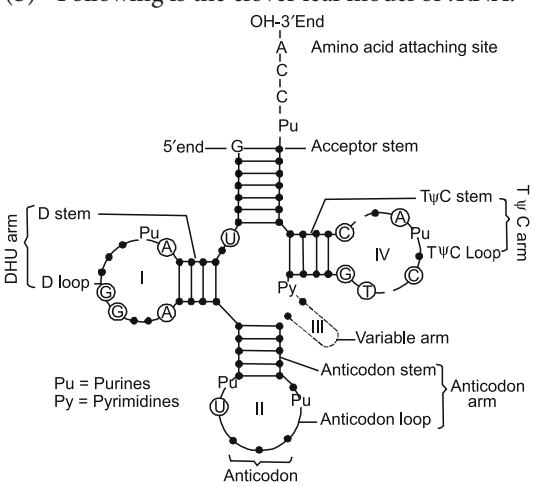
(iv) DHU Loop. The loop contain 8–12 bases. It is largest loop and has dihydrouridine. It is binding site for aminoacyl synthetase enzyme.

(v) Extra Arm. It is a variable side arm or loop which lies between T ψ C loop and anticodon. It is not present in all *t*RNAs.

*t*RNA is known as an adapter molecule because it transfers amino acids to ribosomes during protein synthesis for synthesis of polypeptides.

109. (a) Crick postulated the presence of *t*RNA as an adapter molecule.

(b) Following is the clover leaf model of *t*RNA.



Role of *t*RNA in protein synthesis:

(i) *t*RNA is an adapter molecule meant for transferring amino acids during protein synthesis. *t*RNA binds to a particular amino acid at 3′ end. The charged *t*RNA take the same amino acid to *m*RNA over particular codons corresponding to their anticodons.

(ii) *t*RNA holds peptidyl chains over the *m*RNAs.

(iii) The initiator *t*RNA has the dual function of initiation of protein synthesis as well as bringing in of the first amino acid.

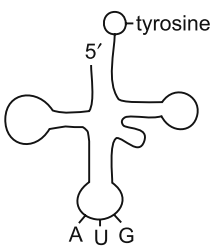
110. (a) Enzyme RNA polymerase III is responsible for the transcription of *t*RNA. Methionine is the amino acid with which the initiator *t*RNA gets linked.

(b) Anticodon of initiator *t*RNA *i.e.*, *t*RNA linked with methionine carries this amino acid to *m*RNA, establishes temporary hydrogen bonds with the initiation codon (AUG) of *m*RNA to start the process of protein synthesis.

111. *Refer to answer 104.*

112. (a) Francis Crick

(b)



(c) Actual structure of *t*RNA is compact and looks like an inverted L.

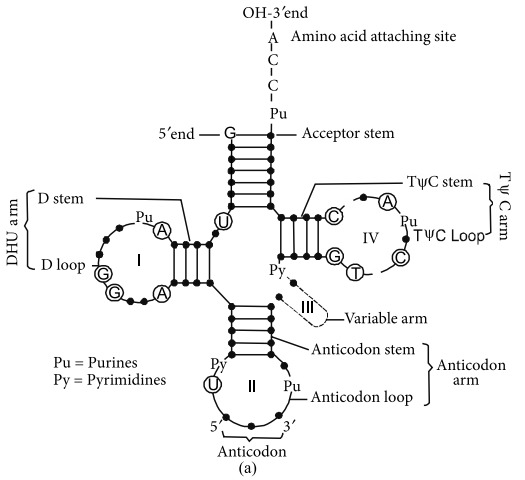
113. (a) Structure of *t*RNA can be explained by means of L-form model (Given by Klug 1974) and by means of clover leaf model (given by Holley 1965).

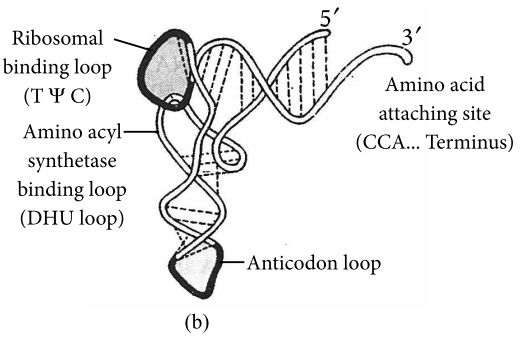
In tRNA molecule, about half of the nucleotides are base paired to produce paired stems. Five regions are unpaired or single stranded - AA-binding site, T ψ C loop, DHU loop, extra arm and anticodon loop.

(i) Anticodon Loop - It has 7 bases out of which three bases form anticodon (nodoc) for recognising and attaching to the codon of *m*RNA.

(ii) AA-Binding Site - It is amino acid binding site. The site lies at the 3′ end opposite the anticodon and has CCA – OH group. The 5′ end bears G. Amino acid or AA binding site and anticodon are the two recognition sites of *t*RNA.

(iii) T ψ C Loop - It has 7 bases out of which ψ (pseudouridine) and rT (ribothymidine) are unusual bases. The loop is the site for attaching to ribosome.
(iv) DHU Loop - The loop contains 8–12 bases. It is largest loop and has dihydrouridine. It is binding site for aminoacyl synthetase enzyme.
(v) Extra Arm - It is a variable side arm or loop which lies between T ψ C loop and anticodon. It is not present in all *t*RNAs.
Following are the (a) clover leaf model and (b) L-form model of *t*RNA.





Functions of *t*RNA are:
(i) *t*RNA is meant for transferring amino acids during protein synthesis. *t*RNA binds to a particular amino acid at 3′ end. The charged *t*RNA take the same amino acid to *m*RNA over particular codons corresponding to their anticodons.
(ii) *t*RNA holds peptidyl chains over the *m*RNAs.
(iii) The initiator *t*RNA has the dual function of initiation of protein synthesis as well as bringing in of the first amino acid.

*t*RNA is known as an adapter molecule because it transfers amino acids to ribosomes during protein synthesis for synthesis of polypeptides.
(b) Eukaryotic transcripts possess extra segments called introns or intervening sequences or non-coding sequences. RNA contains both the exons and introns. The functional coding sequences are called exons. Splicing is removal of introns and fusion of exons to form functional hnRNAs.
Each intron starts with dinucleotide GU and ends with dinucleotide AG (GU-AG rule). They are recognised by components of splicing apparatus of Sn-RNPs or small nuclear ribonucleoproteins (*viz*, U1, U2, U4, U5, U6). A complex called spliceosome is formed between 5′ end (GU) and 3′ end (AG) of intron. Energy is obtained from ATP. It removes the intron. The adjacent exons are brought together. The ends are then sealed by RNA ligase.

114. (a) The relationship between the sequence of amino acids in a polypeptide and nucleotide sequence of DNA or *m*RNA is called genetic code.
(b) *Refer to answer 104.*
Initiator codons are AUG and rarely GUG, which code respectively for methionine and valine. They initiate the process of translation.

115. The two specific codons are initiation codon on one side (AUG or GUG) and termination codon (UAA, UAG or UGA) on the other side of *m*RNA.

116. Smaller sub-unit of ribosome encounters *m*RNA during initiation of protein synthesis.

117. Aminoacylation or charging of the *t*RNA is the process during which the amino-acyl-adenylate-enzyme complex reacts with *t*RNA specific for the amino acid to form aminoacyl-*t*RNA complex. Enzyme and AMP are released. *t*RNA complexed with amino acid is sometimes called charged *t*RNA. The amino acid is linked to 3-OH-end of *t*RNA through its -COOH group,

$$AA \sim AMP — E + tRNA \rightarrow AA\text{-}tRNA + AMP + E$$

Aminoacyl adenylate enzyme

The aminoacyl-*t*RNA complex specific for the initiation codon reaches the P-site to initiate the process of protein synthesis.

118. Ribozyme (catalytic RNA) is present in ribosome and joins the amino acids together by peptide bond formation to form protein chains. Release factor (RF) is GTP dependent. It binds to the stop codon, terminates translation and release the complete polypeptide from the ribosome.

119. Peptide bond formation occurs in the peptidyl transferase centre present in the larger sub-unit of ribosome. Peptide bond formation occurs between the nascent polypeptide chain and the new amino acid resulting in the elongation of polypeptide chain. An aminoacyl *t*RNA complex reaches the A-site and attaches to *m*RNA codon next to initiation codon with the help of its anticodon. The step requires GTP and an elongation factor. A peptide bond (—CO —NH—) is established between the carboxyl group (—COOH) of amino acid attached to *t*RNA at P-site and amino group (—NH_2) of amino acid attached to *t*RNA at A-site. The reaction is catalysed by enzyme peptidyl transferase which is an RNA-enzyme.

A lot of energy is consumed in protein synthesis. For every single amino acid incorporated in the peptide chain one ATP and two GTP molecules are used.

120. *Refer to answer 117.*

121. (i) *m*RNA - Messenger RNA bring coded information from DNA and takes part in its translation by bringing amino acids in a particular sequence during the synthesis of polypeptide. However, the codons of *m*RNA are not recognised by amino acids but by anticodons of their adapter molecules (*t*RNAs → aa-*t*RNAs). Translation occurs over the ribosomes. The same *m*RNA may be reused time and again. In the form of polysome, it can help synthesise a number of copies simultaneously.

(ii) *t*RNAs -They are transfer or soluble RNAs which pick up particular amino acids (at CCA or 3′ end) in the process called charging. The charged *t*RNAs take the same to *m*RNA over particular codons corresponding to their anticodons. A *t*RNA can pickup only a specific amino acid though an amino acid can be specified by 2-6 *t*RNAs. Each *t*RNA has an area for coming in contact with ribosome (T ψ C) and the enzyme amino acyl *t*RNA synthetase (DHU).

(iii) Ribosomes - Protein synthesis occurs over the ribosomes, Ribosomes are, therefore, also called protein factories. Each ribosome has two unequal parts, small and large. The larger subunit of ribosome has a groove for pushing out newly formed polypeptide and protecting the same from cellular enzymes. The smaller subunit fits over the larger one like a cap but leaves a tunnel for *m*RNA. The two subunits come together only at the time of protein formation. Mg^{2+} is essential for it. Soon after the completion of protein synthesis, the subunits separate.

122. (a) George Gamow suggest triplet code *i.e.*, the genetic code should be made up of three nucleotides.

He proposed that since there are only 4 bases and if they have to code for 20 amino acids, the code should constitute a combination of bases made up of three nucleotides. A combination of 4^3 $(4 \times 4 \times 4)$ would generate 64 codons; generating many more codons than required.

Hargobind Khorana developed the technique of synthesising RNA molecules with well defined combination of bases (homopolymers and copolymers). He observed that these bases stimulated the formation of polypeptides having alternately similar amino acids such as cysteine-valine-cysteine.

Marshall Nirenberg found out the method of protein synthesis in cell free systems.

Severo Ochoa discovered polynucleotide phosphorylase which could polymerise ribonucleotides to produce RNA without any template.

(b) The genetic code consists of 64 triplets or codons. With three exceptions (UAA, UAG and UGA), each codon encodes for one of the 20 amino acids used in the biosynthesis of proteins. The genetic code determines the relationship between the B nucleotide codon on the *m*RNA and the insertion of the correct amino acid into a protein during protein biosynthesis.

123. *Refer to answer 121.*

124. The three type of RNA are ribosomal RNA, messenger RNA and transfer RNA. *Also refer to answer 121.*

125. The process of decoding of the message from *m*RNA to protein with the help of *t*RNA, ribosome and enzyme is called translation (protein synthesis). Protein synthesis occurs over ribosomes.

The 4 main steps in protein synthesis (translation) are : activation, initiation, elongation and termination of polypeptide chain.

The newly synthesised *m*RNA joins the smaller subunit of ribosome at 5′ end. *m*RNAs carry the codon and *t*RNAs carry the anticodon for the same codon. Activation of amino acid is catalysed by the enzyme aminoacyl *t*RNA synthetase in the presence of ATP. In presence of ATP an amino acid combines with its specific amino acyl-*t*RNA synthetase to produce aminoacyl adenylate enzyme complex. This reacts with *t*RNA to form aminoacyl-*t*RNA complex. Activated *t*RNA is taken to ribosome *m*RNA complex for initiation of protein synthesis.

Initiation of protein synthesis is accomplished with the help of initiation factor which are 3 (IF3, IF2, IF1) in prokaryotes and 9 in eukaryotes (eIF2, eIF3, eIF1, eIF4A, eIF4B, eIF4C, eIF4D, eIF5, eIF6). The ribosome binds to the *m*RNA at the start codon (AUG) that is recognised only by the initiator *t*RNA. A polypeptide chain forms as *t*RNAs deliver amino acids to the ribosome. Large ribosomal subunit binds the initiation complex forming two (A and P) binding site for *t*RNA molecules. The first site is P site or peptidyl site which is occupied by $tRNA^{met}$. The second site is A or amino acyl site and is positioned over the second codon. The ribosome proceeds to the elongation phase of protein synthesis. During this stage, complexes composed of an amino acid linked to *t*RNA, sequentially bind to the appropriate codon in *m*RNA by forming complementary base pairs with the *t*RNA anticodon. The ribosome moves from codon to codon along the *m*RNA. Amino acids are added one by one, translated into polypeptide sequences dictated by DNA and represented by *m*RNA. The enzyme peptidyl synthetase catalyses the formation of peptide bond between the carboxylic group of amino acid at P site and amino group of amino acid at A site. Enzyme translocase brings about the movement of *m*RNA by one codon.

The termination of protein synthesis occur when a non-sense codon reaches at A site of ribosome. The chain detaches from the ribosome. A release factor binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome. Two subunits of ribosomes dissociate with the help of dissociation factor.

126. Inducer is a chemical (substrate, hormone or some other metabolite) which after coming in contact with the repressor, changes the latter into non-DNA binding state so as to free the operator gene. The inducer for *lac*-operon of *Escherichia coli* is lactose (actually allolactose, or metabolite of lactose.)

Repressor is a regulator protein meant for blocking the operator gene so that the structural genes are unable to form *m*RNAs.

127. When lactose is present in the culture medium, then the *lac* operon in *E. coli* is switched on. It is because the inducer (lactose) binds to the repressor protein thereby inactivating it. It prevents binding of repressor to the operator. Consequently, RNA polymerase gets access to the promoter and transcription of structural genes proceeds.

128. '*i*' gene is regulator gene. It produces a repressor, which binds to the operator gene and stops its working. This '*i*' gene exerts a negative control over the working of structural genes.

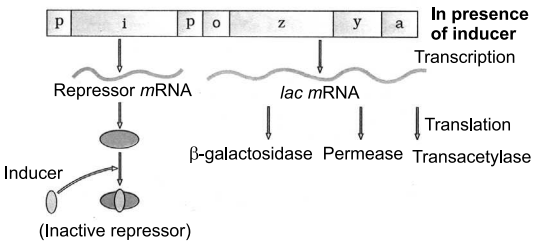
129. *Lac* operon in *E.coli* consists of structural genes, an operator gene, a promoter gene, a regulator gene, a repressor and an inducer. The structural genes are inactivated in the absence of an inducer (*i.e.* lactose). It is because in the absence of an inducer, the repressor binds to the operator gene making it non-functional. RNA polymerase enzyme cannot move over it to reach the structural genes. Thus, structural genes are inactivated and transcription cannot take place.

130. (a) *i* = Regulator gene

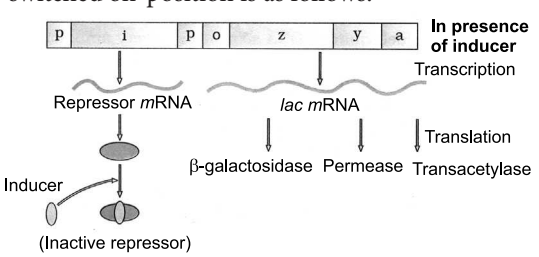
p = Promoter gene

(b) 'Inducer' for the given operon is 'lactose'. Its role is to bind with repressor, change the latter into non-DNA binding state so as to free the operator gene and switch on the *lac* operon.

131. *Lac* operon is the operon of *E. coli* associated with lactose metabolism. It is an inducible operon that consists of a regulator, a promoter, an operator and three structural genes z, y and a. The structural genes are activated in presence of lactose that acts as an inducer. The structural genes are normally inactivated, as a repressor molecule binds to an operator gene preventing transcription. These genes get activated when lactose binds to the repressor disabling it from binding with operator. Now operator gene becomes free, permitting transcription thus expression of structural genes.
Its expression can be explained with the schematic diagram of *lac* operon in open state as follows:



132. In *lac* operon, the regulatory gene is called i gene because it produces an inhibitor or repressor. Inducer for the *lac* operon is lactose. Its role is to bind with repressor, change the latter into non-DNA binding state so as to free the operator gene and switch on *lac* operon.
The products of gene *z* and *y* of the *lac* operon are β-galactosidase and premease respectively. β-galactosidase brings about hydrolysis of lactose to form glucose and galactose. Galactoside permease is required for entry of lactose into the bacterium.
Schematic diagram of lac operon in 'switched on' position is as follows:

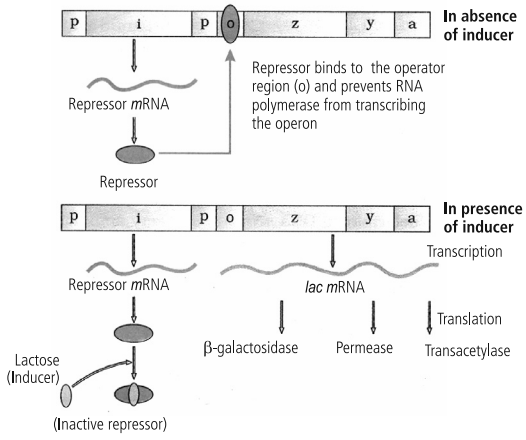


The operon gets switched 'off' in the absence of lactose (inducer). The repressor molecule binds with the operator region of the operon and prevents RNA polymerase from transcribing the operon.

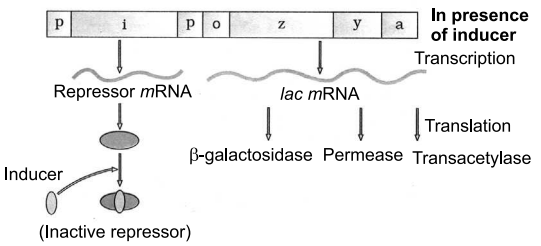
133. (a) The given schematic representation is of *lac* operon. In *lac* operon, the regulatory gene is called *i*-gene because it produces an inhibitor or repressor. The repressor binds to operator gene and stops the operator from working.
In the absence of an inducer (*i.e.,* lactose), the repressor binds to the operator gene making it non-functional. RNA polymerase enzyme cannot move over it to reach the structural genes. Thus, structural genes are inactivated and transcription cannot take place.
(b) As regulatory gene exerts a negative control over the working of structural genes, therefore regulation of *lac* operon is called negative regulation.
(c) 'Inducer' for the given operon is 'lactose'. Its role is to bind with repressor, change the latter into non-DNA binding state so as to free the operator gene and switch on the *lac* operon.
The products of the genes 'z' and 'y' of the *lac* operon are β-galactosidase and permease respectively. β-galactosidase brings about hydrolysis of lactose to form glucose and galactose. Galactoside permease is required for entry of lactose into the bacterium.

134. *Refer to answer 131.*

135. An operon is a part of genetic material (or DNA) which acts as a single regulated unit having one or more structural genes, an operator gene, a promoter gene, a regulator gene, a repressor and an inducer or corepressor (from outside).
Lactose acts as an inducer in *lac* operon. The repressor molecule coded by *i* gene is inactivated by interaction with the lactose. This allows RNA polymerase to access the promoter and transcription proceeds. The operon gets switched 'off' in the absence of lactose as the repressor molecule binds with the operator region of the operon and prevents RNA polymerase from transcribing the operon.
Diagrammatic representation of *lac* operon is as follows:



136. Schematic diagram of lac operon in 'switched on' position is as follows:



The operon gets switched 'off' in the absence of lactose (inducer). The repressor molecule binds with the operator region of the operon and prevents RNA polymerase from transcribing the operon.

137. *Refer to answer 135.*

138. *Refer to answer 131.*

139. (a) The arrangement of different genes in the operon of bacteria is as following:
Regulator gene, Promoter gene, Operator gene and Structural genes.
(b) *Refer to answer 136.*
(c) Inducer is a chemical (substrate, hormone or some other metabolite) which after coming in contact with the repressor, changes the latter into non-DNA binding state so as to free the operator gene.
The inducer for *lac* operon of *Escherichia coli* is lactose (actually allolactose, or metabolite of lactose). In the presence of inducer (lactose), the repressor gets inactivated due to its interaction with it. This allows RNA polymerase to access the promoter and tranciption proceeds. Hence, the *lac* operon is switched on.

When lactose is digested, glucose and galactose are formed. Then, the *lac* operon will stop due to the accumulation of glucose and galactose in the cell as they cannot be used as an inducer for *lac* operon.

140. (d) : Y-chromosome

141. Single nucleotide polymorphism (SNPs or snips) help in finding chromosomal locations for disease associated sequences and tracing human history.

142. Genetic maps have helped in gene sequencing, DNA fingerprinting, tracing human history, etc.

143. (i) In finding chromosomal locations for disease associated sequences
(ii) In tracing human history

144. (a) Chromosome 1 has most genes (2968).
(b) Chromosome Y has fewest (231).

145. BAC = Bacterial Artificial Chromosome.
YAC = Yeast Artificial Chromosome.
BAC and YAC are the vectors into which DNA fragments are inserted to form rDNA (recombinant DNA) using recombinant DNA technology and are then multiplied in suitable host.

146. 'SNP's stands for single nucleotide polymorphism. About 1.4 million single base DNA differences or SNPs have been identified in humans. SNPs occur normally throughout a person's DNA almost once in every 1000 nucleotides on average. Their number may be more than 10 million. They are helpful in finding chromosomal locations with disease associated sequences and tracing human history.

147. (a) The two methodologies involved in human genome project (HGP) are :
(i) Expressed sequence tags or ESTs, *i.e.*, identification of all the genes that are expressed as RNA and sequencing the same.
(ii) Sequence annotation, *i.e.*, sequencing whole set of genome and then assigning the functions to different regions.
HGP followed the second methodology which involves the following steps:
(i) The whole DNA of the cell is isolated and broken randomly into fragments.

(ii) They are inserted into specialised vectors like BAC (bacterial artificial chromosomes) and YAC (yeast artificial chromosome).
(iii) The fragments are cloned in suitable hosts like bacteria and yeast. PCR (polymerase chain reaction) can also be used for cloning or making copies of DNA fragments.
(iv) The fragments are sequenced as annotated DNA sequences.
(v) The sequences were then arranged on the basis of some overlapping regions. It necessitated the generation of overlapping fragments for sequencing.
(vi) Computer based programmes were used to align the sequences.
(vii) The sequences were then annotated and assigned to different chromosomes. All the human chromosomes have been sequenced, 22 autosomes, X and Y.
(viii) With the help of polymorphism in microsatellites and restriction endonuclease recognition sites, the genetic and physical maps of the genome have also been prepared.
(b) YAC is a vector and is expanded as yeast artificial chromosome. This vector is used to clone DNA fragments of more than 1 Mb in size, therefore, they have been exploited extensively in mapping the large genome *i.e.*, human genome project. They also contain restriction enzyme sites and genes which act as selectable markers in yeast.

148. (a) In human genome project, 'Y' stands for yeast in YAC (yeast artificial chromosomes) and 'B' stands for bacterial in BAC (bacterial artificial chromosomes). These are specialised vectors used during sequencing in human genome project.
(b) In human genome, less than 2 percent of the genome codes for proteins and functions of only 50% of discovered genes are known.
(c) Human genome has SNPs at 1.4 million locations. Expanded form of SNPs is Single Nucleotide Polymorphism.

149. (a) Four major goals of human genome project are :
(i) Identification of all the approximately 20,000-25,000 genes in human DNA.
(ii) To determine the sequences of the 3 billion chemical base pairs that make up human DNA.
(iii) To store this information in databases.
(iv) To improve tools for data analysis.
(b) Four ways in which knowledge of HGP is of significant for humans are:
(i) SNPs will be helpful in finding chromosomal locations with disease associated sequences and tracing human history.
(ii) Satellite DNA *viz.*, minisatellite sequences called VNTRs are used in DNA fingerprinting.
(iii) Repetitive sequences called junked DNA provide information about chromosome structure, dynamics and evolution.
(iv) It will be possible to study how various genes and proteins work together.
(c) *Refer to answer 145.*

150. Repetitive/satellite DNA can be separated from bulk genomic DNA by using density gradient centrifugation.

151. DNA polymorphism in a population arise due to mutations.

152. VNTR stands for Variable Number of Tandem Repeats.
VNTRs are short nucleotide repeats in DNA that are specific to each individual and vary in number from person to person. DNA probes, are radioactive, have repeated base sequence complementary to VNTRs .

153. DNA fingerprinting helps in determining the paternity from a small sample of tissue or a drop of blood. DNA fingerprinting is a technique for identifying individuals, generally using repeated sequences in the human genome that produce a pattern of bands which is unique for every individual. Important for DNA fingerprinting are short nucleotide repeats that vary in number from person to person, but are inherited. These are the Variable Number of Tandem Repeats or VNTRs. The VNTRs of two persons may be of the same length and sequence at certain sites, but vary at others.
DNA fingerprints can be prepared from extremely minute amounts of blood, semen, hair bulb or any other cells of the body.

154. (a) VNTR stands for Variable Number of Tandem Repeats that are short nucleotide repeats in DNA which are specific to each individual and vary in number from person to person but

are inherited. DNA probes, are radioactive, have repeated base sequence complementary to VNTRs. Satellite DNA show high degree of polymorphism. The term polymorphism is used when a variant at a locus is present with a frequency of more than 0.01 population. VNTRs are important for DNA fingerprinting technique and are also referred as "minisatellites". Each individual inherits these repeats from his/her parents which are used as genetic markers in a personal identity test. For example, a child might inherit a chromosome with six tandem repeats from the mother and the same tandem repeated four times in the homologous chromosome inherited from the father. One half of VNTR alleles of the child resemble that of the mother and other half with that of the father. As a result the size of VNTR varies from 0.1 to 20 kb. Consequently after hybridisation with VNTR probe, the autoradiogram gives many bands of differing sizes. These bands give characteristic pattern for an individual DNA which is used to identify individuals.

(b) Applications of DNA fingerprinting are as follows:

(i) Paternity or maternity disputes can be solved by DNA fingerprinting as it can identify the real genetic mother, father and the offspring.

(ii) This technique is being used to identify genes connected with hereditary diseases.

(iii) It is useful in detection of crime and legal pursuits.

(iv) It can identify racial groups, their origin, historical migrations and invasions.

155. DNA fingerprinting technology can help to identify the dead individuals. It is a technique of determining nucleotide sequences of certain portion of DNA which are unique to each individual. DNA fingerprints can be prepared from extremely minute amounts of blood, semen, hair bulb or certain other cells of the body. The major steps are as follows :

(i) DNA is extracted from the cells. It is cut into fragments with restriction enzymes. The fragments of DNA also contain VNTRs (Variable Number Tandem Repeats) which vary in number from person to person.

(ii) DNA fragments are passed through gel electrophoresis. Fragments of particular size having VNTRs are multiplied using PCR. Alkaline chemical is used to split dsDNA to ssDNA.

(iii) Separated DNA sequences are transferred from gel onto a nitrocellulose or nylon membrane.

(iv) Radioactive DNA probes complementary to VNTRs are poured over the nylon membrane. Some of them bind with VNTRs (Southern Blotting).

(v) X-ray film is exposed to the nylon sheet which gives dark bands at the probe sites. Thus, hybridised fragments are detected by autoradiography. The dark bands on X-ray film represent the DNA fingerprints (DNA profiles).

156. By using DNA fingerprinting technique, the kinships can be identified. *Also refer to answer 155.*

157. *Refer to answer 153.*

158. Satellite DNA are very specific in each individual, vary in number from person to person and are inherited. These sequences show high degree of polymorphism. Each individual inherits the satellite DNA from, his/her parents which are used as genetic markers in DNA fingerprinting.

159. DNA fingerprinting is a technique of determining nucleotide sequences of certain areas of DNA which are unique to each individual. DNA fingerprints can be prepared from extremely minute amounts of DNA sample from blood, semen, hair bulb or any other cells of the body. *Also refer to answer 155.*

160. *Refer to answer 153.*

161. (a) DNA polymorphism is variation at genetic level which arises due to mutations. The polymorphism in DNA sequences is the basis of genetic mapping of human genome as well as DNA fingerprinting. If an inheritable mutation is observed in a population at high frequency, it is referred to as DNA polymorphism.

(b) Short nucleotide repeats in the DNA are very specific in each individual and vary in number from person to person but are inherited. These are the 'Variable Number Tandem Repeats' (VNTRs). These are also called "minisatellites".

Each individual inherits these repeats from his/her parents which are used as genetic markers in a personal identity test. For example, a child might inherit a chromosome with six tandem repeats from the mother and the same tandem repeated four time in the homologous chromosome inherited from the father. One half of VNTR alleles of the child resemble that of the mother and other half with that of the father.

162. (a) *Refer to answer 161 (a) and (b).*
(b) *Refer to answer 154 (b).*

163. (a) Difference between repetitive and satellite DNA are as follows :

Repetitive DNA	Satellite DNA
Repetitive DNA consist of short identical sequences which are repeated several hundred or thousand times. It is of three types, terminal repeats, tandem repeats and interspersed repeats. The disposition of repetitive element consist either in arrays of tandemly repeated sequences or in repeats dispersed throughout the genome.	The proportion of the DNA of a eukaryotic cell that consists of very large numbers of copies of a tandem repeatedly short nucleotide sequence. It occurs mainly around the centromeres and telomeres of the chromosomes. The highly repetitive nature of this DNA fraction gives it a distinctive base composition, and consequently when samples of DNA are centrifuged, it forms so-called 'satellite bands' quite separate from the band representing the bulk of the cell's DNA.

(b) Satellite DNA can be isolated from bulk genomic DNA by density gradient centrifugation.
(c) DNA fingerprinting is very useful in detection of crime and legal pursuit.