



### Can you recall?

1. What is nucleic acid?
2. What are the types of nucleic acid?
3. What are the functions of nucleic acid?
4. What is the difference between DNA in prokaryotes and eukaryotes?

### 4.1 The Discovery of DNA:

Modern understanding of DNA has evolved from the discovery of nucleic acid to the development of the double-helix model. In 1869, Friedrich Miescher began working with white blood cells which are the major component of pus from infections. He collected a lot of pus from bandages at the local hospital. He used a salt solution to wash the pus off the bandages. When he added a weak alkaline solution to the cells, the cells lysed and nuclei precipitated out of the solution. From the cell nuclei, he isolated a unique chemical substance to which he called **nuclein**. Chemically, nuclein has high phosphorus content. Moreover it showed acidic properties. Hence it was named as nucleic acid.

By the early 1900s, we knew that Miescher's nuclein was a mix (mixture) of proteins and nucleic acids. There are two kinds of nucleic acids. DNA (deoxyribonucleic acid) and RNA (ribonucleic acid).

### 4.2 The Genetic Material is a DNA:

By the early 1900s, geneticist knew that genes control the inheritance of traits, that genes are located on chromosome and that chemically chromosomes are mainly composed of DNA and proteins. Initially, most geneticists thought that protein are large, complex molecules and store information needed to govern cell metabolism. Hence it was assumed that proteins caused the variations observed within species.

On the other hand DNA thought to be small, simple molecule whose composition varied little among species. Over the time, these ideas about DNA were shown to be wrong. In fact DNA molecules are large and vary tremendously within and among species. Variations in the DNA molecules are different than the variation in shape, electrical charge and function shown by proteins so it is not surprising that most researchers initially favored proteins as the genetic material.

Over a period of roughly 25 years (1928-1952), geneticists became convinced that DNA and not protein, was the genetic material. Let us study three important contributions that helped cause this shift of opinion.

### Griffith's experiments :

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

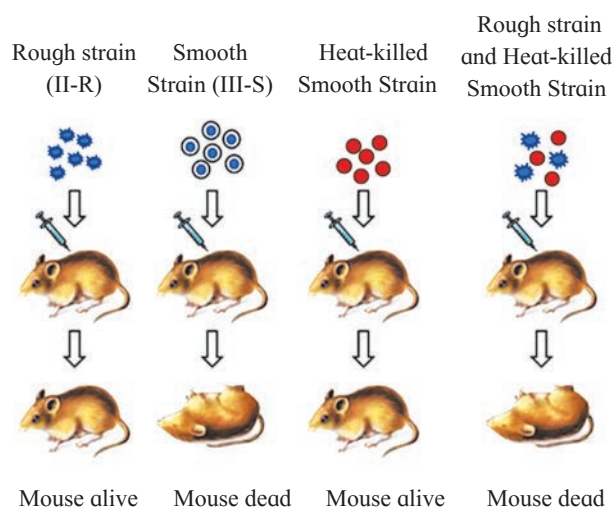
- i. Virulent, smooth, pathogenic and encapsulated S type.
- ii. Non-virulent, rough, non-pathogenic and non-capsulated R type.

Griffith conducted four experiments on these bacteria. First, when he injected bacteria of strain R to mice, the mice survived because it did not develop pneumonia. Second, when he injected bacteria of strain S to mice, the mice developed pneumonia and died. In the third experiment, he injected heat-killed strain S bacteria to mice, once again the mice survived. In fourth experiment, he mixed heat-killed S bacteria with live bacteria of strain R and injected to mice. The mice died and Griffith

recovered large numbers of live strain S bacteria from the blood of the dead mice.

In these four experiments, something had caused harmless strain R bacterium to change into deadly S strain bacterium. Griffith showed that the change was genetic. He suggested that genetic material from heat-killed strain S bacterium had somehow changed the living strain R bacterium into strain S bacterium.

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

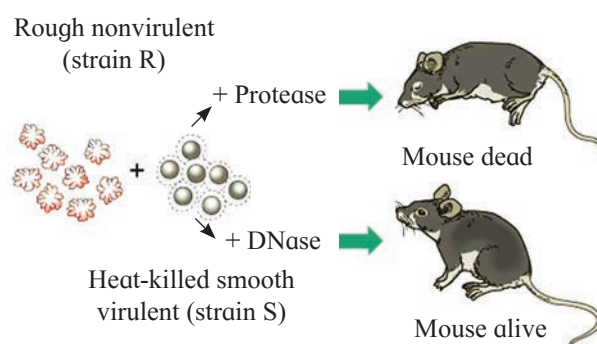


**Fig.4.1 : Griffith's experiment**

#### Avery, McCarty and MacLeod's experiment:

In 1944, after some 10 years of research and experimentation, U. S. microbiologists Oswald T. Avery, Colin M. MacLeod and Maclyn McCarty (all at Rockefeller University in New York) first evidenced to prove the DNA is a genetic material (transforming principle), through the experiments. They purified DNA, RNA, Proteins (enzymes) and other materials from cell free extract of S cells/ strain and mixed with heat killed S strain and R cells separately to confirm which one could transform living R cells into S cells. Only DNA was able to transform harmless strain R into deadly strain S.

They also discovered that protein-digesting enzymes (proteases), RNA-digesting enzyme (RNAases) did not affect transformation, so the transforming substance was neither a protein nor RNA. DNA digested with DNase did inhibit the transformation, suggesting that DNA caused the transformation. These experiments proved that the transforming principle is DNA but all biologists were not convinced.



**Fig. 4.2 : DNA transforms bacteria**

Finally, Alfred Hershey and Martha Chase (1952) proved that DNA is the genetic material and not proteins, by using bacteriophages.

#### Hershey - Chase Experiment:

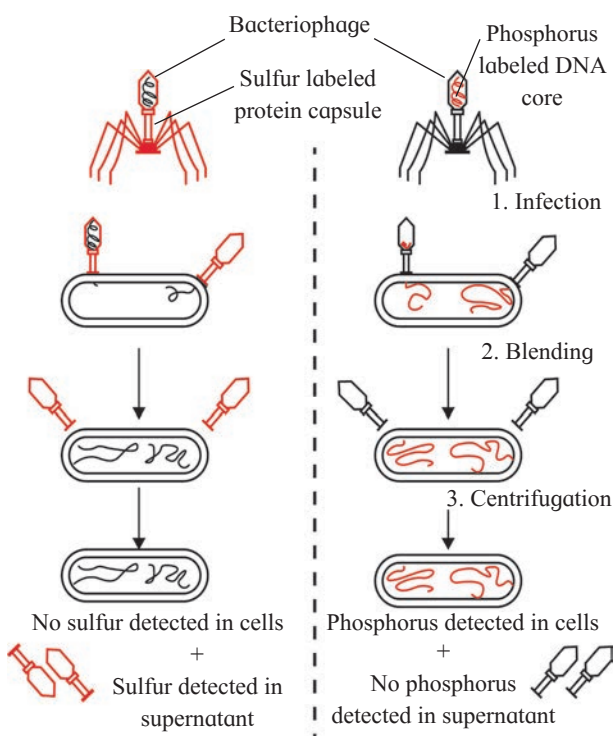
Hershey and Chase worked with viruses that infect bacteria i.e. bacteriophages, which are composed of DNA and protein. They used radioactive phosphorous  $^{32}\text{P}$  in the medium for some viruses and radioactive sulphur  $^{35}\text{S}$  for some others.

They grew some viruses on a medium that contained radioactive phosphorus and some others on medium that contained radioactive sulphur. Viruses grown in the presence of radioactive phosphorus contained radioactive DNA (labelled DNA), but not radioactive proteins because DNA contains phosphorus (labelled DNA) but proteins do not. Similarly, viruses grown on radioactive sulphur contained radioactive protein but not radioactive DNA because DNA does not contain sulphur.

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

the infection proceeded, the viral coats were removed with the help of centrifuge. Bacteria which were infected by viruses with radioactive DNA, were radioactive, indicating that DNA was the material that passed from the viruses to the bacteria. Bacteria which were infected by viruses having radioactive sulphur (protein) were not radioactive. This indicates that proteins from the viruses, did not enter the bacteria. DNA is, therefore, the genetic material that is passed from virus to bacteria (fig. 4.3).

In other words, sometime after infection, radioactivity for 'P' and 'S' was tested. Only radioactive 'P' was found inside the bacterial cell, indicating that DNA is the genetic material.



**Fig. 4.3 : Hershey - Chase Experiment**



### Do you know ?

1. Multiple forms of DNA and their differences.
2. Significance of different forms of DNA.



### Can you recall?

1. What are the chemical components of DNA?
2. What is a chromosome?

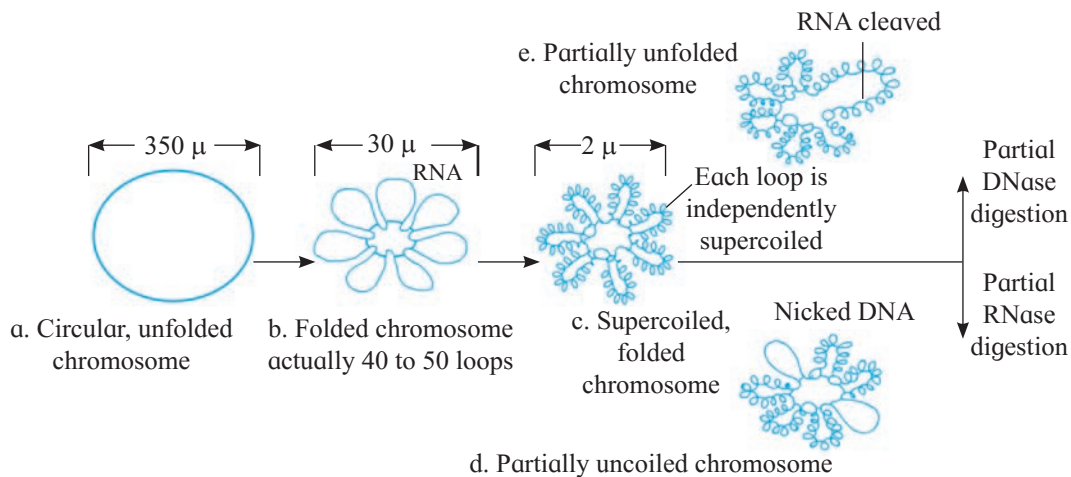
### 4.3 DNA packaging :

Length of DNA double helix molecule, in a typical mammalian cell is approximately 2.2 meters. (This can be worked out simply by multiplying the total number of base pairs with distance between the consecutive base pairs). Approximate size of a typical nucleus is  $10^{-6}$  m. How this long DNA molecule can be then accommodated in such a small nucleus? It, therefore, must be condensed, coiled and super coiled to fit inside such small nucleus.

#### Packaging in Prokaryotes:

In prokaryotes like *E. coli*, cell size is almost 2-3 $\mu$  long. They do not have well organized nucleus. It is without nuclear membrane and nucleolus. The nucleoid is small, circular, highly folded, naked ring of DNA which is 1100 $\mu$  long in perimeter, containing about 4.6 million base pairs.

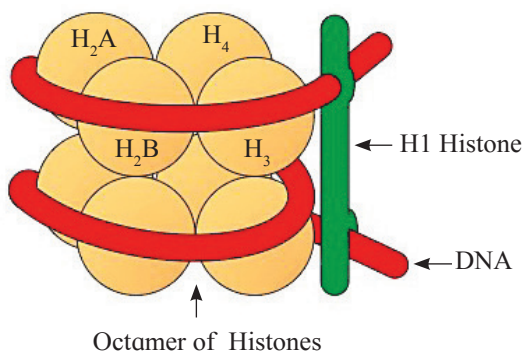
The 1100 $\mu$  long (approximately 1.1 mm, if cut and stretched out) nucleoid is to be fitted or packaged into a cell which is hardly 2-3 $\mu$  long. Hence the negatively charged DNA becomes circular, reducing the size to 350 $\mu$ m in diameter. This is further reduced to 30 $\mu$ m in diameter because of folding/ looping. 40-50 domains (loops) are formed. Formation of loops is assisted by RNA connectors. Each domain is further coiled and supercoiled, thereby reducing the size down to 2 $\mu$  in diameter. This coiling (packaging) is assisted by positively charged HU (Histone like DNA binding proteins) proteins and enzymes like DNA gyrase and DNA topoisomerase I, for maintaining super coiled state.



**Fig. 4.4 : DNA Packaging in Prokaryotes**

### Packaging in Eukaryotes:

Eukaryotes show well organized nucleus containing nuclear membrane, nucleolus and thread-like material in the form of chromosomes. In the chromosomes, DNA is associated with histone and non-histone proteins as was reported by R. Kornberg in 1974. The organization of DNA is much more complex in eukaryotes. Depending upon the abundance of amino acid residues with charged side chains, a protein acquires its charge. Histones are the proteins that are rich in lysine and arginine residues. Both these amino acid residues are basic amino acids and carry positive charges with them. So, histones are a set of positively charged, basic proteins (histones + protamine). These histones organize themselves to make a unit of 8 molecules known as **histone octamer**.

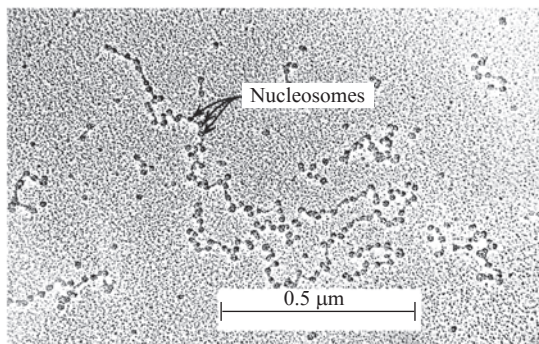


**Fig. 4.5 : Nucleosome**

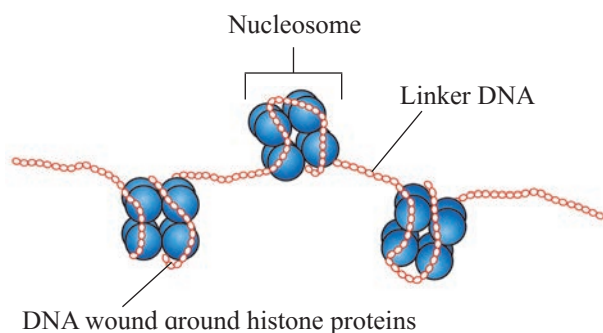
The negatively charged helical DNA is wrapped around the positively charged histone octamer, forming a structure known as **nucleosome**. The nucleosome core is made up of two molecules of each of four types of histone proteins viz.  $H_2A$ ,  $H_2B$ ,  $H_3$  and  $H_4$ .  $H_1$  protein binds the DNA thread where it enters (arrives) and leaves the nucleosome.

One nucleosome approximately contains 200 base pair long DNA helix wound around it (fig. 4.5). About 146 base pair long segment of DNA remains present in each nucleosome. Nucleosomes are the repeating units of chromatin, which are thread-like, stained (coloured) bodies present in nucleus. These look like '**beads-on-string**', when observed under an electron microscope. DNA helix of 200 bps wraps around the histone octamer by  $1\frac{3}{4}$  turns. Six such nucleosomes get coiled and then form solenoid that looks like coiled telephone wire. The chromatin is packed to form a **solenoid** structure of 30 nm diameter ( $300\text{\AA}$ ) and further supercoiling tends to form a looped structure called **chromatin fiber**, which further coils and condense at metaphase stage to form the **chromosomes**. The packaging of chromatin at higher levels, need additional set of proteins that are called Non-Histone Chromosomal proteins (NHC).

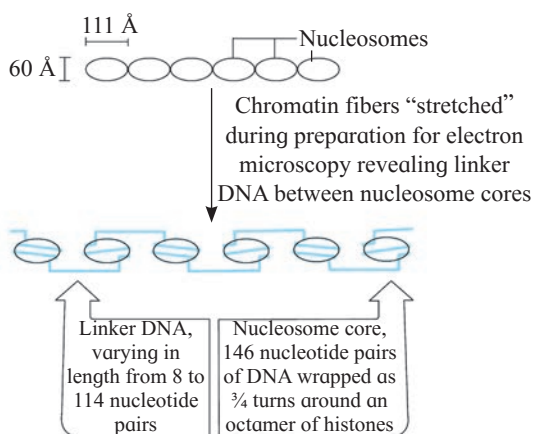




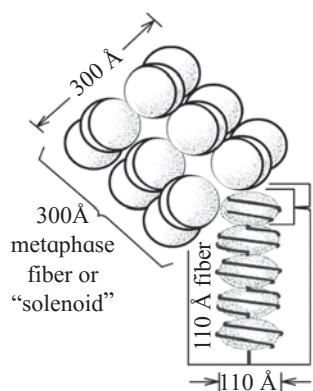
**Fig. 4.6 : Chromatin showing beads-on-string**



**A : Beads-on-string magnified**



**B : Chain of nucleosomes forming 10 to 11 nm thick fibre**



**C : Solenoid forming 30 nm thick fibre**

**Fig. 4.7 : DNA packaging**

**Non-Histone Chromosomal Proteins (NHC) :** These are additional sets of proteins that contribute to the packaging of chromatin at a higher level.

**Heterochromatin and Euchromatin :**

- 1. Heterochromatin:** In eukaryotic cells, some segments of chromonema/ chromosome during interphase and early prophase remain in a condensed state. These regions constitute heterochromatin. This term was proposed by Heitz. These regions are localized near centromere, telomeres and are also intercalated. It is genetically mostly inactive. It stains strongly and appears dark. Heterochromatin is 2 to 3 times more rich in DNA than in the euchromatin.
- 2. Euchromatin:** The regions of chromonema which are in non-condensed state, constitute euchromatin. Euchromatic regions stain light. Euchromatin is genetically very much active and fast replicating. Euchromatin is transcriptionally active, while heterochromatin is transcriptionally almost inactive.



#### Can you recall?

1. What is the backbone of the DNA structure?
2. Name the nitrogen bases of DNA.
3. What are Nucleoside and Nucleotide?
4. Is the double helix right or left handed?



#### Find out

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

### 4.4 DNA Replication :

The DNA molecule regulates and controls all the activities of the cell. Because of its unique structure, it is able to control the synthesis of other molecules of the cell. At the same time when the cell reproduces, the DNA also should duplicate itself to distribute

equally to the daughter cells. As a carrier of genetic information, DNA has to perform two important functions :

- a. **Heterocatalytic function** : When DNA directs the synthesis of chemical molecules other than itself, then such functions of DNA are called heterocatalytic functions. Eg. Synthesis of RNA (transcription), synthesis of protein (Translation), etc.
- b. **Autocatalytic function** : When DNA directs the synthesis of DNA itself, then such function of DNA is called autocatalytic function. Eg. Replication.

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

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

DNA replicates through Semiconservative mode of replication.

The model for Semiconservative replication was proposed by Watson and Crick, on the basis of antiparallel and complementary nature of DNA strands. The process of semiconservative replication is as below:

### 1. Activation of Nucleotides:

The four types of nucleotides of DNA i.e. dAMP, dGMP, dCMP and dTMP are present in the nucleoplasm. They are activated by ATP in presence of an enzyme **phosphorylase**. This results in the formation of deoxyribonucleotide triphosphates i.e. dATP, dGTP, dCTP and dTTP. The process is known as Phosphorylation.

### 2. Point of Origin or Initiation point:

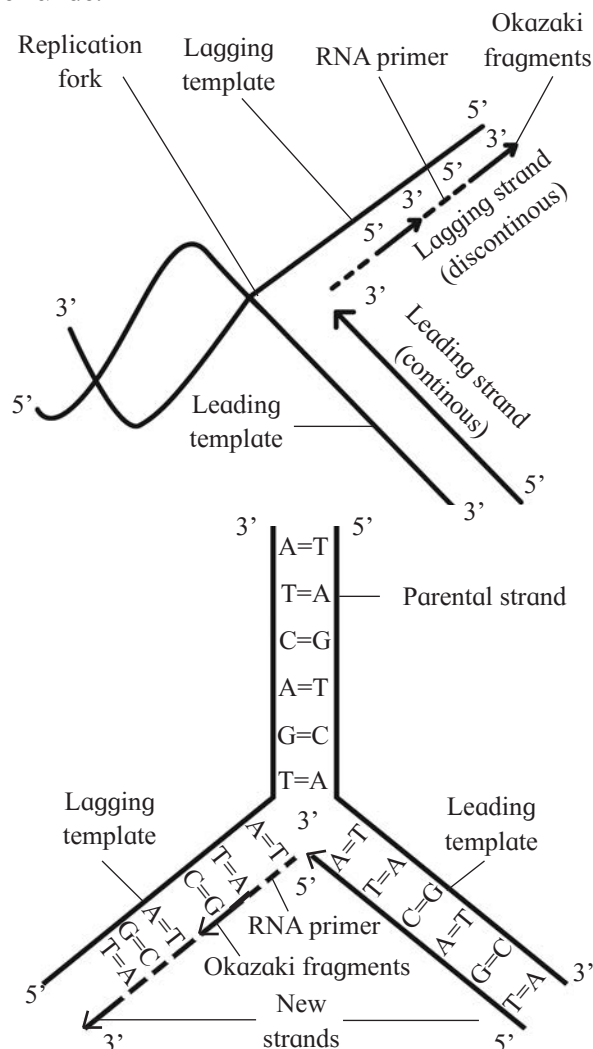
It begins at specific point 'O' -origin and terminates at point 'T'. Origin is flanked by 'T' sites. The unit of DNA in which replication occurs, is called replicon. In prokaryotes, there is only one replicon however in eukaryotes, there are several replicons in tandem.

At the point 'O', enzyme endonuclease nicks one of the strands of DNA, temporarily. The nick occurs in the sugar-phosphate backbone or the phosphodiester bond.

### 3. Unwinding of DNA molecule:

Now enzyme DNA helicase operates by breaking weak hydrogen bonds in the vicinity of 'O'. The strands of DNA separate and unwind. This unwinding is bidirectional and continues as 'Y' shaped replication fork. Each separated strand acts as template.

The two separated strands are prevented from recoiling (rejoining) by SSBP (Single strand binding proteins). SSB proteins remain attached to both the separated strands so as to facilitate synthesis of new polynucleotide strands.



**Fig. 4.8 : Semiconservative Replication of DNA**

#### 4. Replicating fork:

The point formed due to unwinding and separation of two strands appear like a Y-shaped fork, called replicating/ replication fork. The unwinding of strands imposes strain which is relieved by super-helix relaxing enzyme.

#### 5. Synthesis of new strands:

Each separated strand acts as mould or template for the synthesis of new complementary strand. It begins with the help of a small RNA molecule, called **RNA primer**. RNA primer get associated with the 3' end of template strand and attracts complementary nucleotides from surrounding nucleoplasm. These nucleotides bind to the complementary nucleotides on the template strand by forming hydrogen bonds (i.e. A=T or T=A; G  $\equiv$  C or C  $\equiv$  G). The newly bound nucleotides get interconnected by phosphodiester bonds, forming a polynucleotide strand. The synthesis of new complementary strand is catalyzed by enzyme DNA polymerase. The new complementary strand is always formed in 5'-3' direction.

#### 6. Leading and Lagging strand:

The template strand with free 3' end is called leading template and with free 5' end is called lagging template. The process of replication always starts at C-3 end of template strand and proceeds towards C-5 end. As both the strands of the parental DNA are antiparallel, new strands are always formed in 5'  $\rightarrow$  3' direction.

One of the newly synthesized strand develops continuously towards replicating fork is called leading strand. Another new strand develop discontinuously away from the replicating fork is called lagging strand.

#### Maturation of Okazaki fragments :

DNA synthesis on lagging template takes place in the form of small fragments, called Okazaki fragments (named after scientist

Okazaki). Okazaki fragments are joined by enzyme DNA ligase.

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

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

#### 7. Formation of daughter DNA molecules:

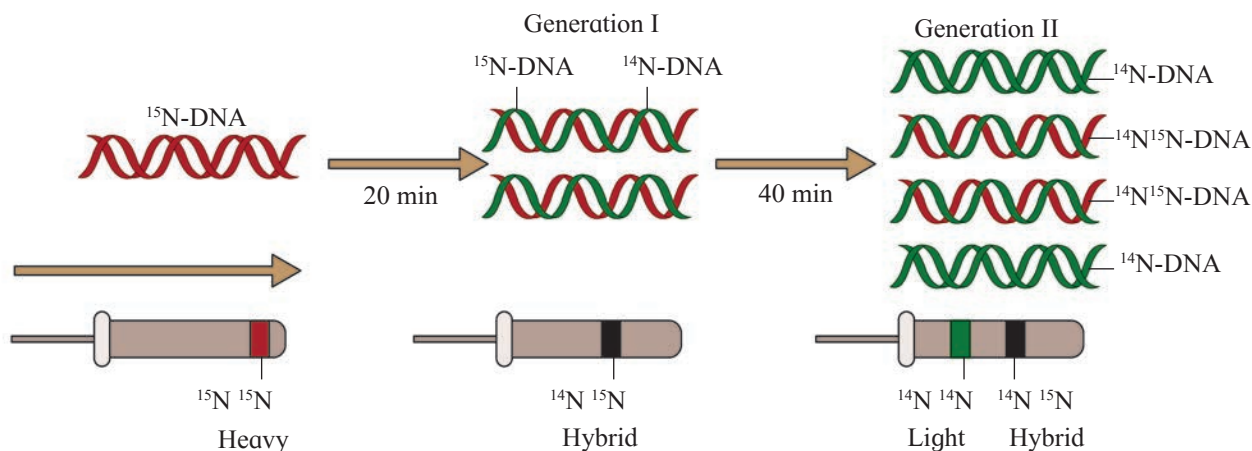
At the end of the replication, two daughter DNA molecules are formed. In each daughter DNA, one strand is parental and the other one is totally newly synthesized. Thus, 50% is contributed by mother DNA. Hence, it is described as **semiconservative replication**.

#### Experimental confirmation :

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

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

1. Meselson and Stahl in 1958 performed an experiment to prove semiconservative nature (mode) of replication.
2. They cultured bacteria *E.coli* in the medium containing  $^{14}\text{N}$  (light nitrogen) and obtained equilibrium density gradient band by using 6M  $\text{CsCl}_2$ . The position of this band is recorded.
3. *E. coli* cells were then transferred to  $^{15}\text{N}$  medium (heavy isotopic nitrogen) and allowed to replicate for several generations. At equilibrium point density gradient band was obtained, by using 6M  $\text{CsCl}_2$ . The position of this band is recorded.



**Fig. 4.9 : Interpretation of results of Meselson's experiment on the separation of DNA by equilibrium density gradient centrifugation**

- The heavy DNA ( $^{15}\text{N}$ ) molecule can be distinguished from normal DNA by centrifugation in a 6M Cesium chloride ( $\text{CsCl}_2$ ) density gradient. The density gradient value of 6M  $\text{CsCl}_2$  and  $^{15}\text{N}$  DNA is almost same. Therefore, at the equilibrium point  $^{15}\text{N}$  DNA will form a band. In this both the strands of DNA are labelled with  $^{15}\text{N}$ .
- Such *E. coli* cells were they transferred to another medium containing  $^{14}\text{N}$  i.e. normal (light) nitrogen. After first generation, the density gradient band for  $^{14}\text{N}^{15}\text{N}$  was obtained and its position was recorded. After second generation, two density gradient bands were obtained - one at  $^{14}\text{N}^{15}\text{N}$  position and other at  $^{14}\text{N}$  position.
- The position of bands after two generations clearly proved that DNA replication is Semiconservative.

#### 4.5 Protein synthesis :

Proteins are very important biomolecules. They serve as structural components, enzymes and hormones. The cell needs to synthesize new protein molecules. The process of protein synthesis includes **transcription** and **translation**. The process of copying of genetic information from one (template) strand of DNA into a single stranded RNA transcript, is termed as **transcription**. During this process, synthesis of complementary strand of RNA takes place (Except that the Adenine nitrogen base pairs with the Uracil base instead of Thymine).

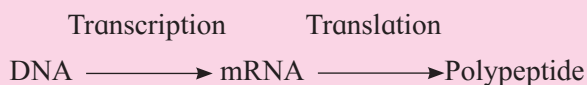
#### Central Dogma :

Double stranded DNA molecule gives rise to mRNA which acts as a messenger to programme the synthesis of a polypeptide chain (protein). This type of unidirectional flow of information from DNA to RNA to protein/ proteins is referred as **central dogma** of molecular biology. It was postulated by F.H.C. Crick in 1958.

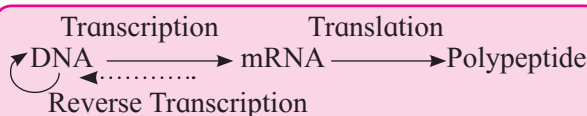


#### Use your brain power

- List as many different enzyme activities required during DNA synthesis as you can.
- This type of replication is called semi-conservative replication. Considering the meaning of these words, why DNA replication is called semiconservative replication?



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





Accordingly enzyme RNA dependent DNA polymerase, synthesizes DNA from RNA.



### Can you recall?

1. What is transcription?
2. How many nucleotides are present in a codon?
3. Name the molecule which carries anticodon?
4. What is mutation?

### A. Transcription:

During transcription, information of only one strand of DNA is copied into RNA. This strand of DNA acts as template. Enzyme RNA polymerase catalyses the formation of RNA transcript.

DNA is located in the nucleoid of Prokaryotes and in nucleus of Eukaryotes. DNA transcription takes place in nucleus in eukaryotes whereas translation occurs in cytoplasm. DNA transfers information to m-RNA which then moves to ribosomes. Transcription occurs in the nucleus during G1 and G2 phases of cell cycle. DNA has promotor and terminator sites. Transcription starts at promotor site and stops at terminator site. Actually the process of transcription, in both Prokaryotes and Eukaryotes, involves three stages viz. Initiation, Elongation and Termination.

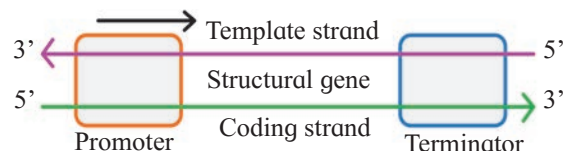
### Transcription Unit:

Each transcribed segment of DNA is called transcription unit. It consists of i. Promotor, ii. The structural gene, iii. A terminator. Two strands of DNA in the structural gene show following features :

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

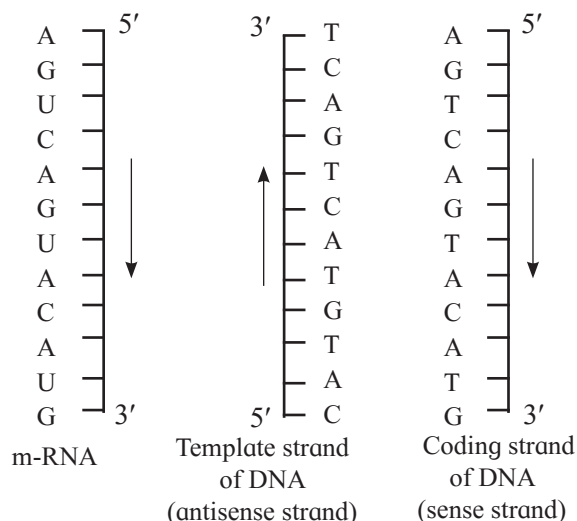
polymerase catalyses polymerisation in 5'→3' direction. So the DNA strand having 3'→5' polarity acts as template strand. The other strand of DNA having 5'→3' polarity is complementary to template strand. The sequence of bases in this strand, is same as in RNA (where Thymine is replaced by Uracil). It is the actual coding strand. The information on this strand of DNA is copied on mRNA. This is called sense strand.

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



**Fig. 4.10 : Transcription unit**

After binding to promotor, RNA polymerase moves along the DNA and causes local unwinding of DNA duplex into two chains in the region of the gene. Exposed ATCG bases project into nucleoplasm. Only one strand functions as template (antisense strand) and the other strand is complementary which is actually a coding strand (sense strand). The ribonucleoside tri phosphates join to bases of DNA template chain. As transcription proceeds, the hybrid DNA-RNA molecule dissociates and makes mRNA molecule free.



**Fig. 4.11 : Formation of Template and Coding strand of DNA**

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

As the mRNA grows, the transcribed region of DNA molecule becomes spirally coiled and attains (regains) double helical form.

In bacteria, m-RNA does not require any processing because it has no introns. Prokaryotes possess only one type of RNA polymerase. In eukaryotes, there are three types of RNA polymerases. RNA polymerase-I transcribes r-RNA. RNA polymerase-II transcribes m-RNA (primary transcript) and heterogeneous nuclear RNA (or hnRNA). RNA polymerase-III is responsible for transcription of t-RNA and small nuclear RNA (snRNA).



### Do you know ?

1. Many viruses contain RNA as genetic material and replicate by synthesizing first the DNA and then form RNA. This process is called reverse transcription. Such viruses are known as Retroviruses.
2. e.g. Human immunodeficiency virus (HIV) is responsible for causing AIDS.
3. In some cases like *E.coli*, a chain terminating protein, the rho factor stops the synthesis of mRNA.
4. The process of transcription as well as translation involves 3 stages - initiation, elongation and termination.



### Always Remember

#### Types of RNA and process of transcription:

In bacteria, there are three types of RNAs: m-RNA provides the encoded message, t-RNA brings specific amino acid, to the site of translation. r-RNA plays a role in providing binding site to m-RNA.

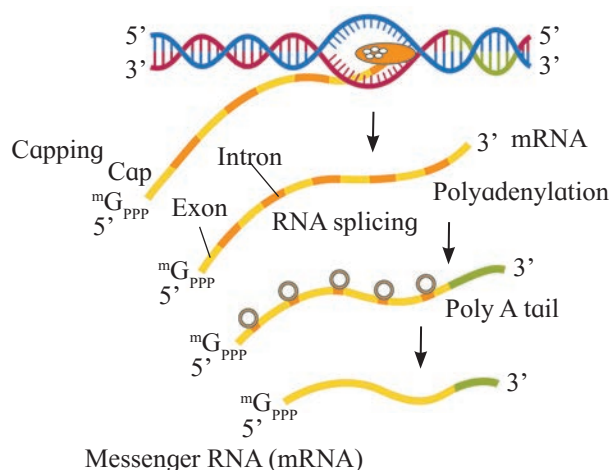
There is single DNA dependent RNA polymerase that catalyses transcription of all 3 types of RNA in bacteria. RNA polymerase binds to promoter and initiates transcription (initiation).

### Transcription unit and the gene:

The DNA sequence coding for m-RNA/ t-RNA or r-RNA is defined as a gene. Cistron is a segment of DNA coding for a polypeptide. A single structural gene in a transcription unit is said to be **monocistronic** whereas a long segment of DNA having a set of various structural genes in one transcription unit is referred to as **polycistronic**. Structural genes in eukaryotes have interrupted non-coding sequences (**introns**). The coding sequences or express-sequences are defined as **exons**. Only exons appear in processed mRNA in Eukaryotes.

### Processing of hnRNA:

In eukaryotes, forms of RNA transcribed from DNA are called **primary transcripts**. Such transcripts undergo changes called processing or maturation before becoming functional. Primary transcript is non-functional and contains both exons and introns. During processing, only introns are removed by the process called **splicing**.



**Fig. 4.12 : Transcription and Processing of hnRNA to mRNA in Eukaryotes**

Exons are joined in a definite sequence (order) by DNA ligase enzyme. Heterogeneous nuclear RNA, undergoes the process of capping and tailing. In **capping**, methylated guanosine tri phosphate is added to 5' end of hnRNA. In **tailing**, polyadenylation take place at 3' end. It is the fully processed hnRNA, now called m-RNA. For translation m-RNA is transported out of the nucleus through nuclear pore.

### Genetic Code:

It is already known that DNA is a master molecule of a cell that initiates, guides, regulates and controls the process of protein synthesis. To perform this complicated function, it must carry the requisite information for the synthesis of proteins. Obviously this information has to be verily located in the DNA itself. The site for storing this information lies in the sequence of nucleotides (i.e. nitrogen bases), as evidenced by Yanofski and Sarabhai (1964).

About, 20 different types of amino acids are involved in the process of synthesis of proteins. DNA molecule has 4 types of nitrogen bases to identify these 20 different types of amino acids. Question arises then, how is it possible that 20 types of amino acids are encoded by 4 types of nitrogen bases?

According to F.H.C. Crick, this information is stored in the form of coded language (cryptogram) called **genetic code**, that contains code words (**codons**) each one specifying (representing) specific amino acid. Genetic code, therefore, is a collection of base sequences that correspond to each amino acid.

A single nitrogen base in a codon (singlet codon) will encode for only four different types of amino acids. A combination of two nitrogen bases (doublet codon) will specify only 16 different types of amino acids. A combination of three nitrogen bases (triplet codon) will specify 64 different types of amino acids. Hence G. Gamov (1954) suggested that in a codon,

there must be combination of three consecutive nitrogen bases that will be sufficient to specify 20 different types of amino acids.

Thus, there would be 64 different codons (code words) in the dictionary of genetic code and that each code word has to be a triplet codon. Every three consecutive nucleotides in DNA will constitute a triplet codon. Genetic code is a triplet code, was evidenced first by Crick (1961) using "frame- shift mutation". However, M. Nirenberg and Matthaei were able to synthesize artificial m-RNA which contained only one type nitrogenous base i.e. Uracil (Homopolymer). This synthetic poly-U sequence was transferred to protein synthesizing enzymes. A small polypeptide molecule was produced/ formed by the linking of phenylalanine molecules. This explains that UUU codes for phenyl alanine. Later different homopolymer codons were deciphered. Codons formed by two or more bases were also tried.

**Dr. Har Gobind Khorana :** He devised a technique for artificially synthesizing m- RNA with repeated sequences of known nucleotides. By using synthetic DNA, Dr. Khorana prepared chains of polyribonucleotides with known repeated sequences of two or three nucleotides. eg. CUC UCU CUC UCU.

This resulted in formation of polypeptide chain having two different amino acids placed alternately (Leucine and Serine). Similarly polynucleotide chain with three- nitrogen base repeats gave polypeptide chain with only one amino acids. Eg. CUA CUA CUA CUA (leucine). Later, Severo Ochoa established that the enzyme (polynucleotide phosphorylase) was also helpful in polymerising RNA with defined sequences in a template- independent manner (i.e. enzymatic synthesis of RNA).

Finally Nirenberg, Matthaei and Ochoa deciphered all the 64 codons in the dictionary of genetic code.

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

**Fig. 4.13 : Dictionary of genetic code**

During replication and transcription, a nucleic acid is copied to form another nucleic acid. These two processes are based on complementarity principle. During translation, genetic information is transferred from a polymer of nucleotides to a polymer of amino acids. Here, complementarity principle does not exist.

It is evident that change in nucleic acid (genetic material) results in the change in amino acids of proteins. This clearly explains that genetic code directs the sequence of amino acids during synthesis of proteins.



#### Find out

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

#### Characteristic of Genetic code:

Genetic code of DNA has certain fundamental characteristics –

- Genetic code is a triplet code:** Sequence of three consecutive bases constitute codon, which specifies one particular amino acid. Base sequence in a codon is always in 5' → 3' direction. In every living organism genetic code is a triplet code.
- Genetic code has distinct polarity :** Genetic code shows definite polarity i.e. direction. It, therefore, is always read in 5' → 3' direction and not in 3' → 5' direction. Otherwise message will change e.g. 5' AUG 3'.
- Genetic code is non-overlapping :** Code is non overlapping i.e. each single base is a part of only one codon. Adjacent codons do not overlap. If non-overlapping, then with 6 consecutive bases only two amino acid molecules will be in the chain. Had it been overlapping type, with 6 bases, there would be 4 amino acid molecules in a chain. Experimental evidence is in favour of non-overlapping nature.



- iv. **Genetic code is commaless** : There is no gap or punctuation mark between successive/ consecutive codons.
- v. **Genetic code has degeneracy** : Usually single amino acid is encoded by single codon. However, some amino acids are encoded by more than one codons. e.g. Cysteine has two codons, while isoleucine has three codons. This is called degeneracy of the code. Degeneracy of the code is explained by Wobble hypothesis. Here, the first two bases in different codons are identical but the third one, varies.
- vi. **Genetic code is universal** : By and large in all living organisms the specific codon specifies same amino acid. e.g. codon AUG always specifies amino acid methionine in all organisms from bacteria up to humans.
- vii. **Genetic code is non-ambiguous** : Specific amino acid is encoded by a particular codon. Alternatively, two different amino acids will never be encoded by the same codon.
- viii. **Initiation codon and termination codon**: AUG is always an initiation codon in any and every mRNA. AUG codes for amino acid methionine. Out of 64 codons, three codons viz. UAA, UAG and UGA are termination codons which terminate/ stop the process of elongation of polypeptide chain, as they do not code for any amino acid.
- ix. **Universal** : Usually in all organisms the specific codon specifies same amino acid.
- x. **Codon and anticodon** : Codon is a part of DNA e.g. AUG is codon. It is always represented as 5' AUG 3'. Anticodon is a part of tRNA. It is always represented as 3'UAC 5'.

- It is possible to predict sequence of codon on mRNA by studying the sequence of amino acids in a polypeptide chain.

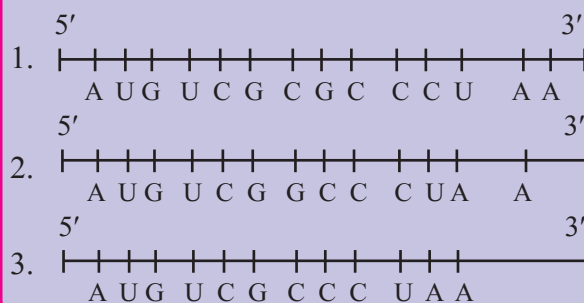


### Activity :



m-RNA

Consider given m-RNA strand which has undergone mutation and lost nucleotides A, C, and G sequentially. Resultant mRNA is represented by 1, 2 and 3. With the help of checker board of amino acids, explain the changes in amino acid sequence that will occur due to such mutation.



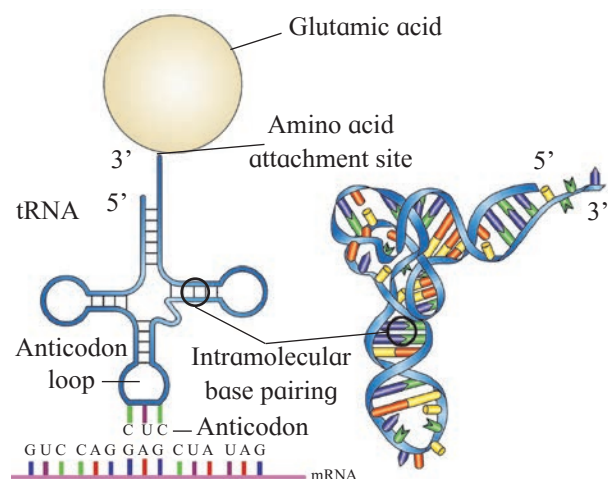
### Mutations and Genetic Code:

Mutation is a phenomenon in which sudden change in the DNA sequence takes place. It results in the change of genotype (i.e. character). Along with recombination, mutation is raw material for evolution as it also results in variations. During mutation, possibility of loss (deletion) or gain (insertion/ duplication) of a segment of DNA results in alteration in the chromosome. Mutation can also occur due to change in a single base pair of DNA. This is known as point mutation. Eg. Sickle cell anaemia (Refer to earlier chapter). Deletion or insertion of base pairs of DNA causes frame – shift mutations or deletion mutation. Insertion or deletion of one or two bases changes the reading frame from the point of insertion or deletion. Insertion or deletion of three or multiples of three bases (insert or delete) results in insertion or deletion of amino acids and reading frame remains unaltered from that point onwards.

### t-RNA- the adapter molecule:

Scientists considered that there has to be a mechanism in which t-RNA will read the

codon and also simultaneously binds with the amino acid as amino acid does not have any special capacity to read the codon. So t-RNA is considered as an adapter molecule. This role of tRNA was understood much later.



**Fig. 4.14: t-RNA - the adapter molecule**

Cloverleaf structure (2 dimensional) of t-RNA possess an anticodon loop that has bases complementary to the codon. It is called **anticodon**. It shows amino acid acceptor end (3' end) having unpaired CCA bases (i.e. amino acid binding site) to which amino acid binds. For every amino acid, there is specific t- RNA. Initiator t-RNA is specific for methionine. There are no t-RNA's for stop codons. In the actual structure, the t-RNA molecule looks like inverted L (3 dimensional structure).



#### Can you tell?

Why t-RNA is called as adapter molecule?



#### Can you recall?

1. Name different types of RNAs.
2. Name the site of protein synthesis.
3. Which molecule carries information of protein synthesis from gene?
4. Which molecule carries amino acid from cytoplasm to ribosome?

## B. Translation - protein synthesis :

Translation is the mechanism in which codons of mRNA are translated and specific amino acids in a sequence form a polypeptide on ribosomes. All types of proteins are synthesised by the cell, within itself (i.e. intracellularly).

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

- i. Amino acids form raw material for protein synthesis. About 20 different types of amino acids are known to form proteins. These are available in the cytoplasm.
- ii. DNA controls synthesis of proteins having amino acids in specific sequence. This control is possible through transcription of m-RNA. Genetic code is specific for particular amino acid.
- iii. RNAs serve as intermediate molecules between DNA and protein.
- iv. Ribosomes serve as site for protein synthesis. Each ribosome consists of large and small subunits. These subunits occur separately in cytoplasm. Only during protein synthesis, these two subunits get associated together due to  $Mg^{++}$  ions.

A ribosome has one binding site for m-RNA and 3 binding sites for t-RNA. They are P site (peptidy t-RNA site), A site (aminoacyl – t-RNA site) and E site (exit site). Only first t- RNA- amino acid complex, directly enters P site of ribosome.

In Eukaryotes, a groove is present between two subunits of ribosomes. It protects the Polypeptide chain from the action of cellular enzymes and also protects mRNA from the action of nucleases.

### Mechanism of translation (i.e. synthesis of polypeptide chain) :

**It involves three steps :**

- i. Initiation, ii. Elongation, iii. Termination

## 1. Initiation of Polypeptide chain :

- Activation of amino acids is essential before translation initiates for which ATP is essential. Small subunit of ribosome binds (attaches) to the m-RNA at 5' end. Initiator codon, AUG is present on m-RNA which initiates the process of protein synthesis (translation). Initiator t- RNA binds with initiation codon (AUG) by its anticodon (UAC) through hydrogen bonds. It carries activated amino acid methionine (in Eukaryotes) or formyl methionine (in prokaryotes).
- Now the large subunit of ribosome joins with the smaller subunit, that requires  $Mg^{++}$  ions.
- Initiator charged t-RNA (with activated amino acid methionine) occupies the P-site of ribosome and A- site is vacant.

## 2. Elongations of polypeptide chain :

During this process, activated amino acids are added one by one to first amino acid (methionine). Amino acid is activated by utilising energy form ATP molecule. This amino acid binds with amino acid binding site of t-RNA- This results in formation of t-RNA- amino acid complex.

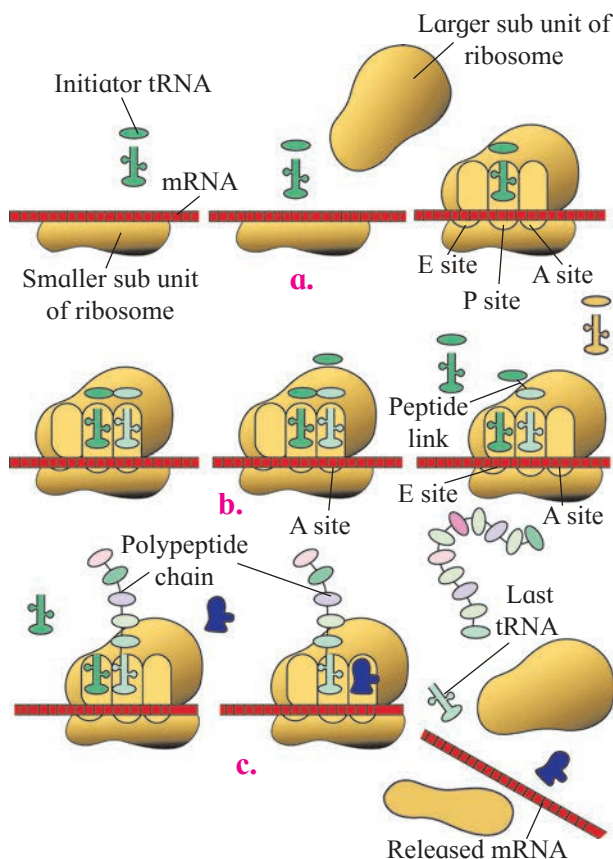
Addition of Amino acid occurs in 3 Step cycle -

- Condon recognition- Amino acyl t- RNA molecule enters the ribosome at A-site. Anticodon binds with the codon by hydrogen bonds.
- Amino acid on the first initiator t-RNA at P-site and amino acid on t-RNA at A-site join by peptide bond. Here enzyme Ribozyme acts as a catalyst. At this time first tRNA at 'P' site is kicked off.
- Translocation- The t- RNA at A-site carrying a dipeptide at A-site moves to the P-site. This process is called **translocation**. In translocation, both the subunits of ribosome move along in relation to tRNA and mRNA. Hence,

tRNA carrying dipeptide now gets positioned at 'P' site of ribosome, making 'A' site vacant. At this site, then next charged tRNA molecule carrying amino acid will be received. During this process, first uncharged tRNA is discharged from E-site.

This process is repeated as amino acids are added to Polypeptide. It takes less than 0.1 second for formation of peptide bond.

Third charged t-RNA with its amino acid, arrives at A-site of ribosome. Anticodon and codon bind by hydrogen bond. Polypeptide bond is formed. Second t-RNA is discharged from P-site to E-site and leaves the ribosome. So the events like arrival of t-RNA- amino acid complex, formation of peptide bond, ribosomal translocation and removal of previous tRNA, are repeated. As ribosome move over the m-RNA, all the codons on mRNA are exposed one by one for translation.



**Fig. 4.15 : Translation Protein synthesis**  
**a. Initiation, b. Elongation, c. Termination**

### 3. Termination and release of polypeptide:

At the end of m-RNA, there is a stop codon (UAA/ UAG/ UGA). It is exposed at the A-site. It is not read and joined by anticodon of any t-RNA. The release factor binds to the stop codon, thereby terminating the translation process. The Polypeptide is now released in the cytoplasm.

Two subunits of Ribosome dissociate and last tRNA is set free in the cytoplasm.

m-RNA also has some additional sequences that are not translated and are referred as **untranslated regions** (UTR). The UTRs are present at both 5'-end (before start codon) and at 3'-end (after stop codon). They are required for efficient translation process.

Finally mRNA is also released in the cytoplasm. It gets denatured by nucleases immediately. Hence mRNA is short-lived.



#### Can you tell?

1. Enlist different steps of protein synthesis.
2. Name the initiator codon of protein synthesis.
3. Explain in brief the process of initiation during protein synthesis.
4. Name three binding sites of ribosome.
5. What is translocation?

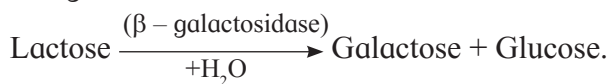
### 4.6 Regulation of gene expression:

It is the multistep process by which a gene is regulated and its product is synthesized. Thus, gene expression results in the formation of a Polypeptide. Gene expression process is regulated at different levels.

In eukaryotes, the regulation can be at different levels like-

1. Transcriptional level (formation of primary transcript)
2. Processing level (regulation of splicing)
3. Transport of m-RNA from nucleus to the cytoplasm.
4. Translational level.

Genes of a cell are expressed to perform different functions. For eg. An enzyme beta galactosidase is synthesised by *E.coli*. It is used for hydrolysis of lactose into galactose and glucose.



If *E.coli* bacteria do not have lactose in the surrounding medium as a source of energy, then enzyme  $\beta$ -galactosidase is not synthesised. So, it is the metabolic or physiological or environmental conditions that regulate expression of genes. The development and differentiation of embryo into an adult organism, is also a result of the coordinated regulation or expression, of several sets of genes.

Now one has to understand and know the mechanism by which the organisms regulate gene expression in response to changes in the environment. If so, whether single mechanism exists for regulation of the expression of different genes/ sets of genes or different genes are regulated by different mechanisms.

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



#### Do you know ?

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



#### 4.7 Operon concept :

It is a transcriptional control mechanism of gene regulation. Francois Jacob and Jacques Monod (1961) explained that metabolic pathways are regulated as a unit.

For example in *E.coli*, when lactose sugar is provided to the culture medium, cell induces production of three enzymes necessary for digestion of lactose. The enzymes are :

- i.  **$\beta$ -galactosidase** : Digests lactose into galactose and glucose.
- ii.  **$\beta$ -galactoside permease** : Permits lactose molecules to enter into the cell.
- iii. **Transacetylase ( $\beta$ -Galactoside acetyltransferase)** : Transfers an acetyl group from acetyl CO-A to galactoside.

Synthesis of these three enzymes, is controlled by a long segment of DNA known as **Operon**. It consists of an operator site O and three structural genes Z, Y and A. The action of structural genes is regulated by operator site with the help of a **repressor protein**. Repressor protein is produced by the action of gene i (inhibitor) known as **regulator gene**.

The gene expression depends on whether operator is switched on or switched off.

If the operator is switched on, the three genes z, y and a are transcribed by RNA Polymerase into a single m-RNA. Each structural gene is generally known as **cistron** and the transcribed long m-RNA covering various cistrons is known as **Polycistronic**.

**Switching on or switching off** of the operator is achieved (accomplished) by a protein called **repressor**. When this protein is attached to the operator and blocks it, the switch is turned off and structural genes are not expressed.

#### Lac operon :

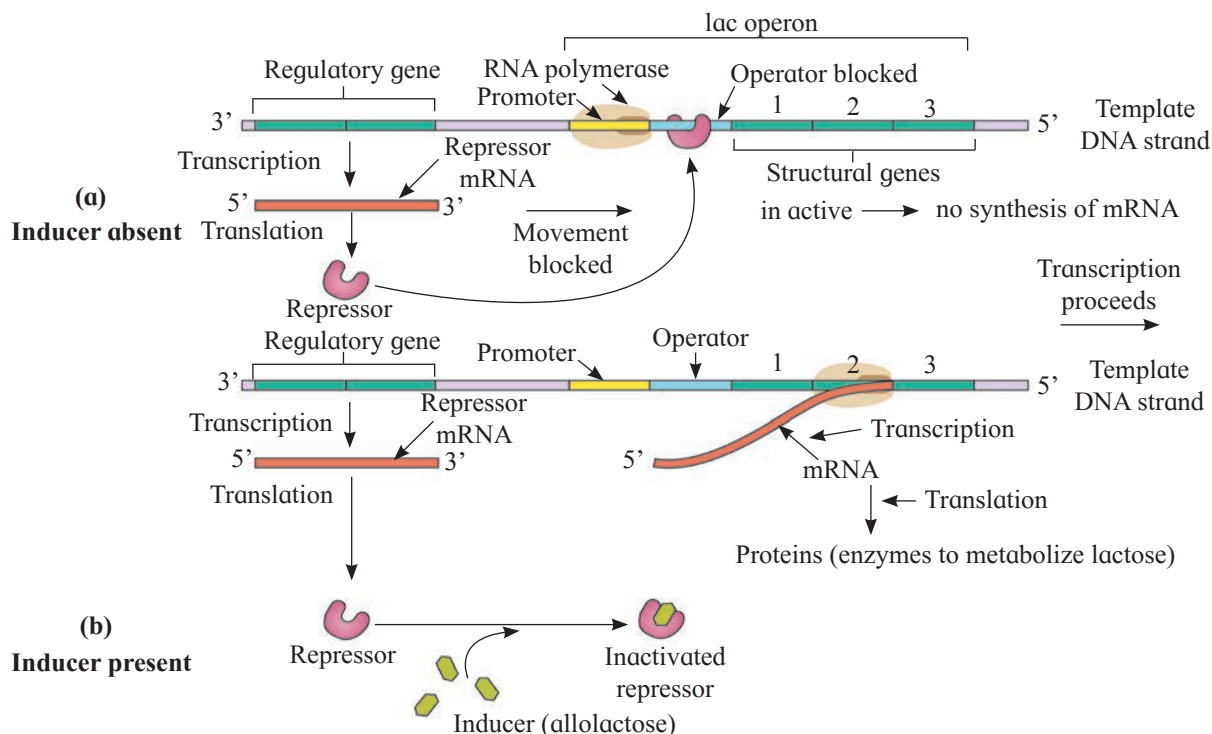
Lactose or lac operon of *E.coli* is inducible operon. The operon is switched on when a chemical inducer- lactose is present in the medium.

#### Lac operon consists of following components :

1. Regulator gene (repressor gene)
2. Promoter gene
3. Operator gene
4. Structural genes
5. Inducer - It is not a component of operon.

1. **Regulator gene** : This gene controls the operator gene in cooperation with an inducer present in the cytoplasm. Regulator gene precedes the promoter gene. It may not be present immediately adjacent to operator gene. Regulator gene produces a protein called **repressor protein**. Repressor binds with operator gene and represses (stops) its action. It is called **regulator protein**.
2. **Promoter gene** : This gene precedes the operator gene. It is present adjacent to operator gene. The promoter gene marks the site at which the RNA Polymerase enzyme binds. When the operator gene is turned on, the enzyme moves over the operator gene and transcription of structural genes starts. Promoter gene base sequence determines which strand of DNA acts a template.
3. **Operator gene** : It precedes the structural genes. This controls the functioning of structural genes. It lies adjacent to the Structural genes. When operator gene is turned on by an inducer, the Structural genes produce m-RNA. Operator gene is turned off by a product of repressor gene.
4. **Structural gene** : When lactose is added to the *E.coli* culture, the structural genes catalyse (produce) m-RNA which in turn produces polypeptides, on the ribosomes.

The polypeptides formed, act as enzymes to catalyse lactose in the cell. There are 3 structural genes in the sequence lac-Z, lac-Y and lac-A. Enzymes produced are  $\beta$ -galactosidase,  $\beta$ -galactoside permease and transacetylase respectively.



**Fig. 4.16 : Working of Lac Operon**

**5. Inducer :** It is a chemical in the cytoplasm (allolactose) which inactivates the repressor. When lac operon is switched on, then inducer joins with repressor protein preventing the binding of repressor to the operator gene. So the Operator gene is free and now enzyme RNA polymerase can move from promoter to structural genes via operator gene.

#### Role of lactose :

A few molecules of lactose enter into the cell by an enzyme permease. A small amount of this enzyme is present even when operon is switched off. A few molecules of lactose, act as inducer and bind to repressor. This repressor – inducer complex fails to join with the operator gene, which is then turned on. Structural genes produce all enzymes. Thus, lactose acts as an inducer of its own break down. When the inducer level falls, the operator is blocked again by repressor. So structural genes are repressed/ inactivated again. This is negative feedback.



#### Use your brain power

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



#### Can you tell?

1. What is the role of a repressor gene?
2. Name the different structural genes in sequence of lac operon.
3. Which molecule does act as inducer molecule in lac operon?
4. In which condition, lac operon is switched off?



#### Internet my friend

Find out information about Trp-operon, Ara-operon, His-operon, Val-operon.

#### 4.8 Genomics:

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

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

The sequencing of yeast, *Drosophila* and mouse genome was done in order to facilitate comparative studies between humans and other organisms commonly used for genetic studies, in laboratory. Several additional genomes are now either actively being sequenced or strongly considered for sequencing. These include several microbes, bee, tomato and other crops. **Genomics study may be classified into two types:**

- a. **Structural genomics:** It involves mapping, sequencing and analysis of genome.
- b. **Functional genomics:** It deals with the study of functions of all gene sequences and their expression in organisms.

#### Application of genomics:

Structural and functional genomics is used for different purposes in the improvement of crop plant, human health and livestock. The knowledge and understanding acquired from genomics research can be applied in a number of different sectors, including medicine, biotechnology and social sciences. It helps in the treatment of genetic disorders through gene therapy.

- Genomics is used in agriculture to develop transgenic crops having more desirable characters.
- Genetic markers developed in genomics, have applications in forensic analysis.
- Genomics can lead to introduce new gene in microbes to produce enzymes, therapeutic proteins and even biofuels.

#### 4.9 Human Genome Project :

The human genome project was initiated in 1990 under the International administration of the Human Genome Organization (HUGO). This project was co-ordinated by the US department of Energy and National institute of health.

international partners in the United Kingdom, France, Germany, Japan, India and China. The

Human Genome Project formally began in 1990 and was completed in 2003. The human genome project is a multinational research project to determine the genomic structure of humans. **The main aims of project are –**

- I. Mapping the entire human genome at the level of nucleotide sequences.
- II. To store the information collected from the project in databases.
- III. To develop tools and techniques for analysis of the data.
- IV. Transfer of the related technologies to the private sectors, such as industries.
- V. Taking care of the legal, ethical and social issues which may arise from project.

HGP (Human Genome Project) was closely associated with rapid development of a new area in biology, called **Bioinformatics**. The work of human genome project has allowed researchers to begin to understand the blueprint in building and constructing the human genome. As researchers learn more about the functions of genes and proteins, this knowledge will have a major impact in the fields like Medicine, Biotechnology and the Life sciences. Therefore HGP is very important.

Human Genome Project was to provide a complete and accurate sequence of the 3 billion DNA base pairs that make up the human genome and to find out the estimated number of human genes. Now about 33000 genes have been estimated to be present in humans.

The project was also aimed to sequence the genomes of several other organisms such as bacteria e.g. *E.coli*, *Caenorhabditis elegans* (a free living non-pathogenic nematode), *Saccharomyces cerevisiae* (yeast), *Drosophila* (fruit fly), plants (rice and *Arabidopsis*), *Mus musculus* (mouse), etc. Complete genome sequences of these model organisms will be useful for comparative studies that will allow researchers to study gene functions in these organisms.

**Table 4.17 : Comparative genome sizes of humans and other models organisms.**

Organism	Chromosome number	Estimated gene number	Estimated size (base pairs)
Human ( <i>Homo sapiens</i> )	46	33,000	3 billion
Mouse ( <i>Mus musculus</i> )	40	25,000	2.9 billion
Fruit fly ( <i>Drosophila melanogaster</i> )	8	13,000	165 million
Plant ( <i>Arabidopsis thaliana</i> )	10	25,000	157 million
Roundworm ( <i>Caenorhabditis elegans</i> )	12	19,000	97 million
Yeast ( <i>Saccharomyces cerevisiae</i> )	32	6000	12 million
Bacteria ( <i>Escherichia coli</i> )	1*	4400	4.6 million

The secret of our complexity may lie not in the number of our genes but how we use them. It will lead to the understanding of gene structure and function in other species. Since we possess many of the genes same as these of flies, round worms and mice, such studies will lead to a greater understanding of human evolution.



#### Use your brain power

1. What have we learnt from the Human Genome Project?
2. Why is HGP important?



#### Can you tell?

Do different organisms have the same DNA?

### 4.10 DNA Fingerprinting:

Genes present on chromosomes are responsible for determining characters of the organism as well as for inheritance of characters. Due to recombination of paternal and maternal genes, we differ from our parents. Differences also arise due to infrequent mutations that occur during gamete formation (cell division). Due to all these factors, every individual has its unique genetic make-up, which may be called its **Fingerprint**. The technique developed to identify a person with the help of DNA restriction analysis, is known as **DNA profiling** or **DNA fingerprinting**. The technique of finger printing was first given by British geneticist, Dr. Alec Jeffreys in 1984.

DNA fingerprinting technique is based on identification of nucleotide sequence present in this wonder molecule. About 99.9% of nucleotide sequence in all persons, is same. Only some short sequences of nucleotides differ from person to person. In the population, every person shows unusual sequences of 20-100 base pairs, which are repeated several times. They are termed as Variable Number of Tandem Repeats (VNTRs).

The length of the regions having VNTRs is different in each individual and hence is the key factor in DNA profiling. Steps involved in DNA finger printing are as follows:

1. **Isolation of DNA:** The DNA must be recovered from the cells or tissues of the body (host). Only small amount of tissue like blood, hair roots, skin, etc. is required.
2. **Restriction digestion:** The isolated DNA is treated with restriction enzymes. The restriction enzymes cut the DNA into small fragments having variable lengths. This phenomenon is called **Restriction Fragment Length Polymorphism (RFLP)**.
3. **Gel electrophoresis:** The DNA samples are loaded for agarose gel electrophoresis under an electric influence. The DNA fragments, which are negatively charged move to the positive pole. The movement of these fragments depends on length of the fragments. This results in formation of bands. dsDNA splits into ssDNA by alkali treatment.



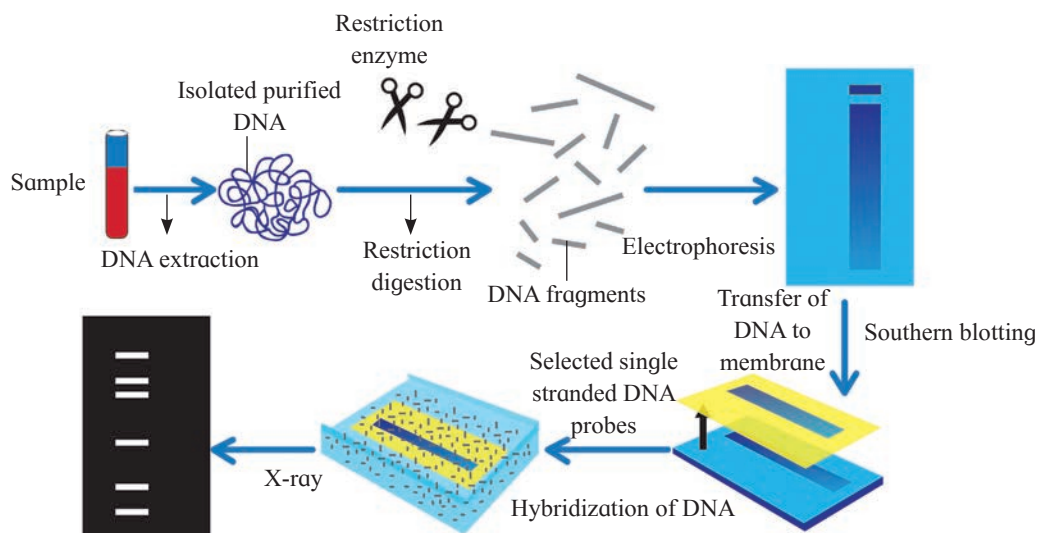
4. **Southern blotting:** The separated DNA fragments are transferred to a nylon membrane or a nitrocellulose filter paper by placing it over the gel and soaking them with filter paper overnight.
5. **Selection of DNA probe:** A known sequence of single-stranded DNA is prepared. It is called DNA Probe. DNA Probe is obtained from organisms or prepared by cDNA preparation method. The DNA probe is labelled with radioactive isotopes.
6. **Hybridization:** Probe DNA is added to the nitrocellulose filter paper containing host DNA. The single-stranded DNA probe pairs with the complementary base sequence of the host DNA strand. As a result DNA-DNA hybrids are formed on

the nitrocellulose filter paper. Remaining single stranded DNA probe fragments are washed off.

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

#### Application of DNA fingerprinting

1. In forensic science, DNA finger printing is used to solve problems of rape and some complicated murder cases.
2. DNA finger printing is used to find out the biological father or mother or both, of the child, in case of disputed parentage.
3. DNA finger printing is used in pedigree analysis in cats, dogs, horses and humans.



**Fig. 4.18 : DNA Fingerprinting**



#### Know the scientists



**Dr. Lalji Singh**  
(1947 - 2017)

Father of DNA Fingerprinting in India. He was instrumental in making DNA fingerprinting mainstream in India, for research and its forensic applications. He obtained DNA probe from Y chromosome of female banded krait snake (in this snake female has XY and male has YY chromosome). The unique segment obtained from this chromosome is, banded krait minor (BKM - DNA). It was used to develop probe for the Indigenous DNA fingerprinting technique.

**Contributions of Dr. Lalji Singh:** i. He installed several dedicated laboratories on aspects of genetics such as population biology, structural biology and transgenic research. ii. His work in the field of DNA fingerprinting technology, contributed for, wildlife conservation, forensics, evolution and phylogeny. iii. Established Centre for DNA Fingerprinting and Diagnostics (CDFD) in late 1990s- making it nodal centre for DNA fingerprinting and diagnostics for all species and several diseases. iv. Founded Laboratory for Conservation of Endangered Species (LaCONES).



### **Activity :**

**Prepare physical model of DNA molecule (Watson-Crick model)**

**Requirements :**

**Labelled Diagram :**

**Functions :**

## Exercise

### Q. 1 Multiple Choice Questions

- Griffith worked on .....  
a. Bacteriophage      b. *Drosophila*  
c. Frog eggs            c. *Streptococci*
- The molecular knives of DNA are .....  
a. Ligases                      b. Polymerases  
c. Endonucleases          d. Transcriptase
- Translation occurs in the .....  
a. Nucleus                      b. Cytoplasm  
c. Nucleolus                  d. Lysosomes
- The enzyme required for transcription is .....  
a. DNA polymerase  
b. RNA polymerase  
c. Restriction enzyme  
d. RNAase
- Transcription is the transfer of genetic information from .....  
a. DNA to RNA  
b. tRNA to mRNA  
c. DNA to mRNA  
d. mRNA to tRNA
- Which of the following is **NOT** part of protein synthesis?  
a. Replication                  b. Translation  
c. Transcription              d. All of these
- In the RNA molecule, which nitrogen base is found in place of thymine?  
a. Guanine                      b. Cytosine  
c. Thymine                      d. Uracil
- How many codons are needed to specify three amino acid?  
a. 3                                  b. 6  
c. 9                                  d. 12
- Which out of the following is **NOT** an example of inducible operon?  
a. Lactose operon  
b. Histidine operon  
c. Arabinose operon  
d. Tryptophan operon

- Place the following event of translation in the correct sequence  
i. Binding of met-tRNA to the start codon.  
ii. Covalent bonding between two amino acids.  
iii. Binding of second tRNA.  
iv. Joining of small and large ribosome subunits.  
A. iii, iv, i, ii                      B. i, iv, iii, ii  
C. iv, iii, ii, i                      D. ii, iii, iv, i

### Q. 2 Very Short Answer Questions:

- What is the function of an RNA primer during protein synthesis?
- Why the genetic code is considered as commaless?
- What is genome?
- Which enzyme does remove supercoils from replicating DNA?
- Why are Okazaki fragments formed on lagging strand only?
- When does DNA replication take place?
- Define term- codon and codogen.
- What is degeneracy of genetic code?
- Which are the nucleosomal 'core' histones?

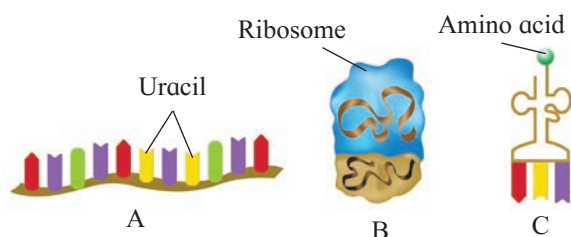
### Q. 3 Short Answer Questions:

- Write short note on DNA packaging in eukaryotic cell.
- Enlist the characteristics of genetic code.
- Write a note on applications of DNA finger printing.
- Explain the role of lactose in 'Lac Operon'.

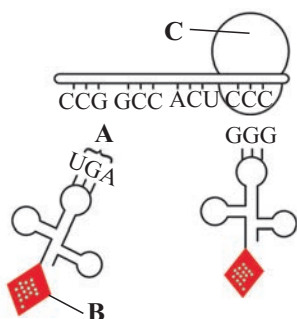
### Q. 4 Short Answer Questions:

- Write a note on Human genome project (HGP).

- Describe the structure of 'Operon'.
- In the figure below A, B and C are three types of \_\_\_\_\_.



- Identify the labeled structures on the following diagram of translation.



Part A is the \_\_\_\_\_.

Part B is the \_\_\_\_\_.

Part C is the \_\_\_\_\_.

- Match the entries in column I with those of column II and choose the correct answer.

Column I	Column II
A. Alkali treatment	i. Separation of DNA fragments on gel slab
B. Southern blotting	ii. Split DNA fragments into single strands
C. Electrophoresis	iii. DNA transferred to nitrocellulose sheet
D. PCR	iv. X-ray photography
E. Autoradiography	v. Produce fragments of different sizes
F. DNA treated with REN	vi. DNA amplification

### Q. 5 Long Answer Questions:

- Explain the process of DNA replication.
- Describe the process of transcription in protein synthesis.
- Describe the process of translation in protein synthesis.
- Describe the 'Lac-operon'.
- Justify the statements. If the answer is false, change the underlined word(s) to make the statement true.
  - The DNA molecule is double stranded and the RNA molecule is single stranded.
  - The process of translation occurs at the ribosome.
  - The job of mRNA is to pick up amino acids and transport them to the ribosomes.
  - Transcription must occur before translation may occur.
- Guess (i) the possible locations of DNA on the collected evidence from a crime scene and (ii) the possible sources of DNA.

Evidence	Possible location of DNA on the evidence	Sources of DNA
e.g. Eyeglasses	e.g. Ear pieces	e.g. Sweat, Skin
Bottle, Can, Glass	Sides, mouthpiece	----- ----
-----	Handle	S w e a t , skin, blood
Used cigarette	Cigarette butt	-----
Bite mark	-----	saliva
-----	Surface area	Hair, semen, sweat, urine

**Project :** Collect information about B and Z forms of DNA. Sketch the diagrams and write the differences between these two forms.