Chapter 19

# CHROMOSOME AND DNA

## STUDENTS'LEARNING OUTCOMES

**After studying this chapter, the students will be able to:**

- Annotate the detailed structure of a chromosome.
- Narrate the experimental work of Griffith and Hershey-Chase, which proved that DNA is the hereditary material.
- Describe the three models proposed about the mechanism of DNA replication.
- Describe the events of the process of DNA replication.
- Describe DNA stability and variability as two characters of the replicating DNA molecule.
- Describe the characteristics of genetic code (universal, triplet, non-overlapping, degenerate, has no punctuation).
- Differentiate between the terms genetic code and codon.
- Explain the mechanism of transcription.
- Explain why the length of transcribed mRNA molecule (in Eukaryotes) shortens as it enters the cytoplasm for translation.
- Describe the mechanism of protein synthesis.
- State the difference between protein synthesis in prokaryotes and eukaryotes.
- Suggest possible ways in which the synthesized protein can be used within or outside a cell that synthesized it.
- State the importance of the regulation of gene expression.
- Describe the negative control of gene expression by repressor proteins.
- Describe the positive control of gene expression by activator proteins.
- Define mutation and identify various sources of mutation.
- Differentiate between natural and induced mutations and mutagens.
- Justify most mutations are harmful.
- Rationalize that mutations might be a contributing factor towards evolution.
- Describe the symptoms, causes and possible available treatments of some of the
- chromosomal mutations. (Down's, Klinefelter's and Turner's syndrome).
- Describe the symptoms, causes. and possible available treatments of some of the gene mutations.

You have the basic knowledge of chromosomes and DNA. You know that genes are specific segments of DNA which carry information for the making of a protein. This chapter aims the detailed study of genes. We will also study the metabolic processes involved in gene working.

## 19.1- Detailed Structure of Chromosome

The structure and chemical composition of chromosomes are different in prokaryotic and eukaryotic cells. In this section, we will study chromosomes in eukaryotic cells.

**Tidbit**

Chromosomes were discovered by a German embryologist, **Walther Flemming** in 1882. He discovered chromosomes in the rapidly dividing cells of salamander larvae. The name "chromosome" was proposed by German anatomist, **Waldeyer-Hartz** in 1888. "Chromosome" literally means coloured bodies.

Chromosomes are thin, thread-like structures. They are made of DNA and histone proteins. The number and shape of chromosomes are different in each species. This complete set is called the **karyotype**. In body cells (somatic cells) of diploid organisms, chromosomes occur in pairs. These pairs are called homologous chromosomes. Gametes (sex cells) have half the number of chromosomes. These chromosomes are not in pairs.

When a cell is not dividing (interphase), its chromosomes are not visible as distinct structures. They remain uncoiled and form a fine network, called **chromatin**. When a cell prepares to divide, the DNA starts to coil. It wraps around small units of histones. These units then coil further into highly condensed form. With more coiling and condensation, the structure becomes thicker and shorter. Finally, it forms compact thread-like structures i.e., chromosomes.

### 19.1.1- Morphology of Chromosomes

During interphase, the duplication of chromatin also occurs. It involves the replication of DNA and synthesis of new histone proteins. After duplication, each chromosome consists of two identical strands called **chromatids**. These two strands are known as sister chromatids. They are attached to each other at a constricted region called the **centromere**. The centromere is a specific DNA sequence of about 220 nucleotides. A disc-shaped protein structure, called the **kinetochore**, is attached to the centromere. It serves as the point where chromosomes attach to the mitotic spindle during cell division.

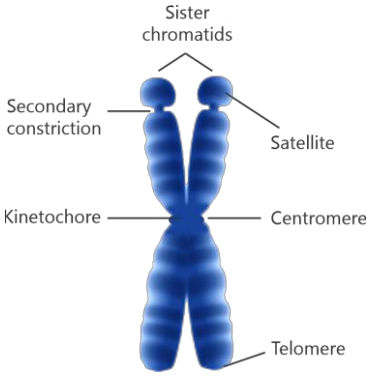



**Figure 19.1: Morphology of a duplicated and condensed chromosome**

In some chromosomes, there may be another constriction, called **secondary constriction** or **Nuclear Organizer Region (NOR)**. It makes nucleoli during interphase. The end of chromosome beside secondary constriction is knob-like. It is called **satellite**. This

region has highly repetitive DNA sequences called junk DNA. The terminal ends of chromosomes are called **telomeres**. They prevent the two chromosomes to attach with each other from their ends.

The position of centromere is different in different chromosomes. The chromosomes in which centromere is at the centre are called **metacentric** chromosomes. The ones in which centromere is located slightly away from centre are **sub-metacentric** chromosomes. In **acrocentric** chromosomes, centromere is located near the end, and in **telocentric** chromosomes, centromere is located at the end.

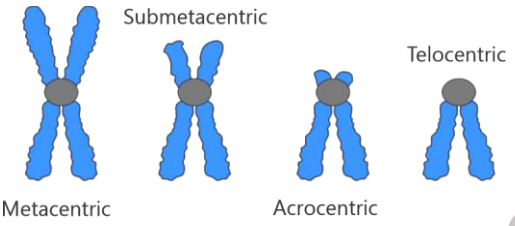


Figure 19.2: Types of chromosomes on the basis of location of centromere

## 19.1.2- Chemical Composition and Organization of Chromosome

A chromosome is made up of **40% DNA** and **60% proteins (histones)**. Both chromatids have identical DNA molecules. The DNA in an average human chromosome is about 5 cm long and consists of about 140 million nucleotides. DNA is a negatively charged due to the presence of phosphate groups. It has strong affinity to **histone** proteins, which are positively charged due to the abundance of basic amino acids such as arginine and lysine. The following are the levels of the organization of chromosomes.

### 1- Nucleosome String

After replication, approximately every 146 nucleotides of DNA (2 nm thick) wrap twice around a histone octamer (set of 8 histone molecules i.e., two copies each of the histones H2A, H2B, H3, and H4). So, aggregations called **nucleosomes** are formed. Nucleosomes are connected by about 80 nucleotide long **linker DNA**. The fifth type of histone i.e., H1 called **linker histone** is present at the base of the nucleosome near the DNA entry and exit points. It helps in the winding of the chain of nucleosomes. All nucleosomes (without linker histone) resemble beads on a string called nucleosome string (11 nm thick).

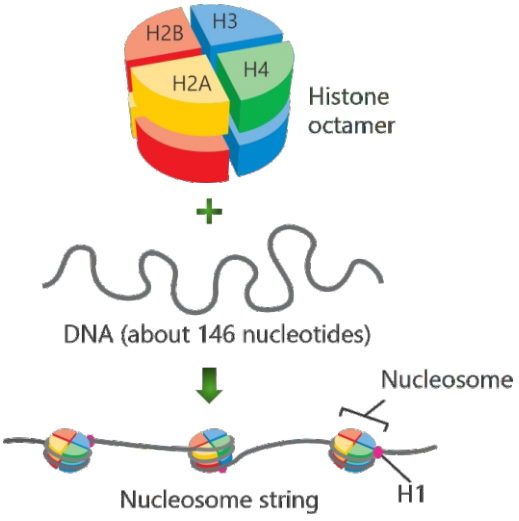


Figure 19.3: Nucleosome string

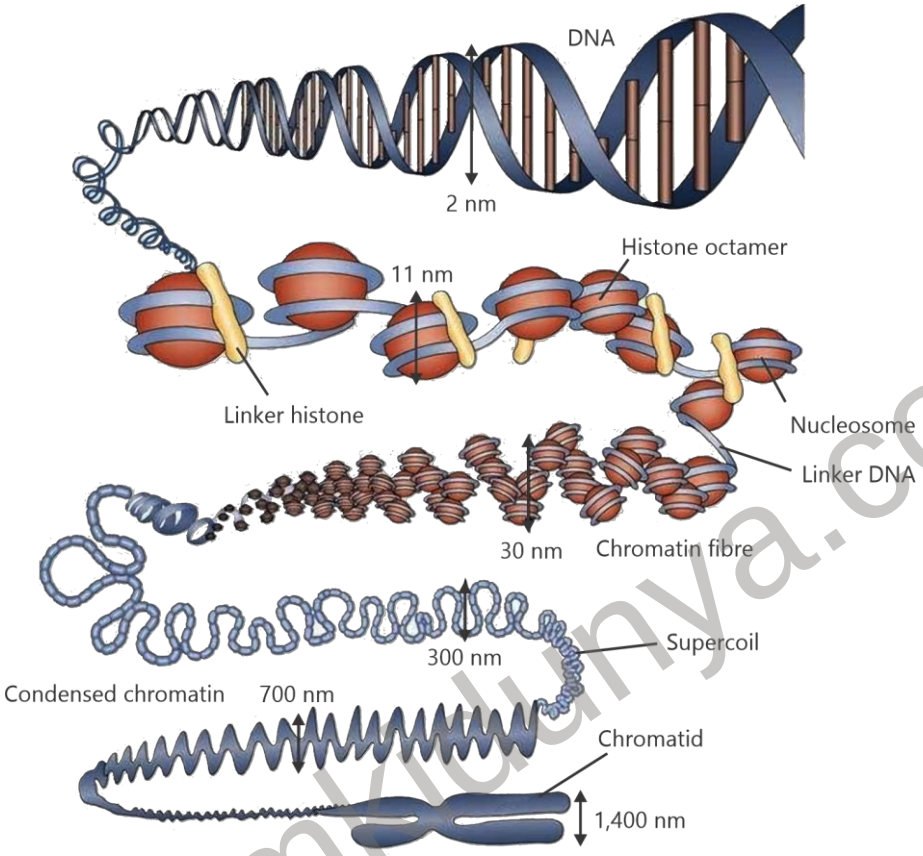



Figure 19.4: Organization of chromosome

## 2- Chromatin Fibre

In the early stages of cell division, the nucleosome string again coils about its axis. Tightly packed (condensed) nucleosomes make a thicker fibre called chromatin fibre (30 nm).

**Tidbit**

There are two regions in chromatin. The inactive portions of chromatin produce dark bands and are called **heterochromatin**. Whereas active portions of chromosomes are called **euchromatin**.

## 3- Chromatids

The chromatin fibre folds upon itself to make a supercoil (300 nm thick). The supercoil further coils and condenses and takes the shape of chromatid (700 nm thick).

## 19.1.3- The Concept of Genes and Alleles

In 1909, a Danish botanist **Wilhelm Johannsen** introduced the term "gene" for the basic unit of heredity. It was found that a gene is "a short segment of DNA which encodes the sequence of amino acid of a particular polypeptide". The whole DNA molecule present in a chromosome does not contain message for sequencing amino

acids (preparing proteins). Rather, short segments of DNA, called genes, encode proteins. The locations where a gene is present on chromosome is called its **locus** (plural: loci).

On a homologous chromosome the alternate forms of a gene i.e., **alleles** are present on the same locus. For example, if a chromosome has the allele for 'eye colour' at a particular locus, its homologous chromosome will carry the allele of same character at the same locus.

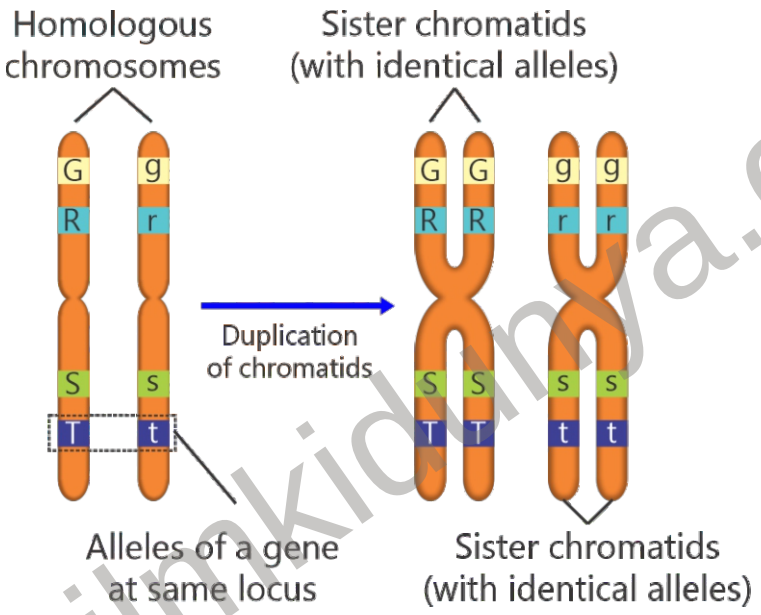



Figure 19.5: Alleles on homologous chromosomes

## 19.2- DNA AS THE HEREDITARY MATERIAL

After the confirmation of the following facts;

- Genes are the units of inheritance,
- Genes are located on chromosomes, and
- Chromosomes are made of DNA and proteins,

scientists tried to determine whether genes are made of DNA, or protein or both. It took almost 30 years (1920s – 1950s) to solve this question.

**Information**

A molecule that serves as genetic material must have certain characteristics. For example:

- It must be able to code for the sequence of amino acids in proteins.
- It must be able to replicate itself prior to cell division.
- It must be in the nucleus of eukaryotic cells.
- It must be able to change over time to account for evolution.

Only one molecule i.e., DNA fulfils all these requirements.

## 19.2.1- Griffith's Experiment

In 1928, British biologist, **Fredrick Griffith**, worked on a pathogenic bacterium *Streptococcus pneumoniae*. He observed two strains of this bacterium;

**"S-type"** strain had a polysaccharide coat and its colony appears "smooth".

**"R-type"** strain lacked polysaccharide coat and forms a "rough" colony.

When Griffith injected S-type bacteria into mice, they proved virulent (lethal). The mice developed pneumonia and died. But, when he injected R-type bacteria in mice, the mice showed no effects. Griffith thought that the virulent effect of S-type might be due to its polysaccharide coat which protects it from host's immune system. Then, he injected heat-killed S-type bacteria into mice, they remained healthy. To further disclose the reasons of virulence, he injected a mixture of heat-killed S-type (virulent, but killed) and alive R-type (non-virulent) into mice. He expected that the mice should not develop the disease. But the mice developed pneumonia and died. When he observed the blood of these dead mice, he found many alive S-type bacteria in the blood of dead mice.

Griffith concluded that the information for making polysaccharide coat and virulence had passed from the heat-killed S-type bacteria to the alive R-type. So, R-type bacteria made polysaccharide coat and transformed into virulent S-type. He called this process as **transformation** i.e., the transfer of genetic material from one organism to another to change the genetic makeup of the recipient organism. Griffith could not find the chemical nature (DNA or protein or both) of the material that was transferred from dead S-type to the alive R-type bacteria.

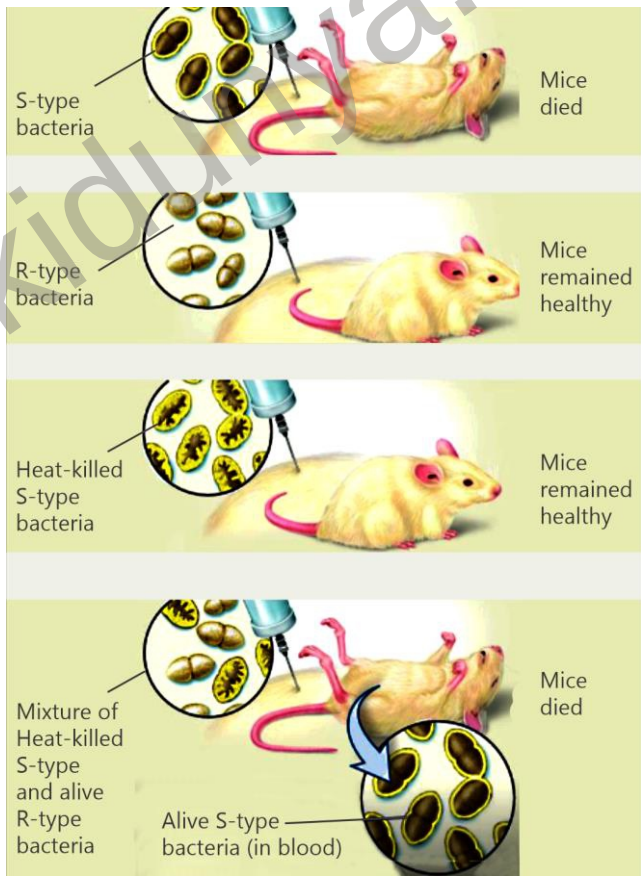



**Figure 19.6: Griffith's experiment**

**Information**

In 1944, three scientists, Avery, MacLeod and McCarty discovered the transforming principle. They removed proteins from the dead S-types bacteria and mixed them with alive R-type. When they injected this mixture into mice, they developed disease and alive S-type bacteria were found in them. It means that proteins are not responsible for transforming R-type into S-type.

Then, they removed all DNAs from dead S-type. They mixed S-type (without DNA) with alive R-type and injected into mice. They observed that no transformation had occurred this time as the mice remained healthy.

They concluded that "DNA is the fundamental unit of the transforming principle".

## 19.2.2- Hershey-Chase Experiments

Alfred Hershey & Marha Chase

In 1952, two American biologists, Alfred **Hershey** and Martha **Chase**, conducted experiments to confirm that DNA is genetic material. Their main experiment was with bacteriophages (viruses that attack bacteria).

**Background Information:** Bacteriophages consist of a core (made of DNA or RNA) and a protein coat. When a lytic bacteriophage infects a bacterium, it binds to the bacterial surface and then injects some material into the cell. The material is hereditary in nature because it directs the production of new bacteriophages within the bacterium. The bacterial cell eventually ruptures, or lyses and new bacteriophages are released.

**Question:** Which material (DNA/RNA, protein, or both) bacteriophages inject into bacterial cells that produce new bacteriophages?

**Experiments:** Hershey and Chase used a bacteriophage that contained DNA rather than RNA. They labelled the DNA and protein of bacteriophage with different radioactive isotopes. The isotopes would serve as **Tracers**. For this purpose.

- Some bacteriophages were grown on a medium containing an isotope of sulphur ($^{35}S$). The isotope was incorporated into the amino acids of the protein coats of new bacteriophages.
- Some bacteriophages were grown on a medium containing an isotope of phosphorus ($^{32}P$). The isotope was incorporated into the phosphate groups of the DNA of new viruses.

They prepared 2 setups.

1. In setup 1, they allowed bacteriophages with $^{35}S$ labelled protein to infect bacteria.

2. In setup 2, they allowed bacteriophages with $^{32}P$ labelled DNA to infect bacteria.

After some time, the infected bacterial cells and medium of both setups were separated by using centrifugation technique. The bacterial cells and media were analysed for the presence of radioactivity.

- In setup 1 (with bacteriophages containing $^{35}S$ labelled protein), they found radioactivity in the medium and not in the bacterial cells.
- In setup 2 (with bacteriophages containing $^{32}P$ labelled DNA), radioactivity was observed in bacterial cells and not in the medium.

These observations showed that during infection, $^{32}P$ labelled DNA of bacteriophages entered into the bacterial cells and directed the making of new bacteriophages. While, $^{35}S$ labelled protein coat remained outside.

Based on these observations, Hershey and Chase claimed that the DNA of bacteriophages, not the protein, was responsible for directing the production of new bacteriophages.

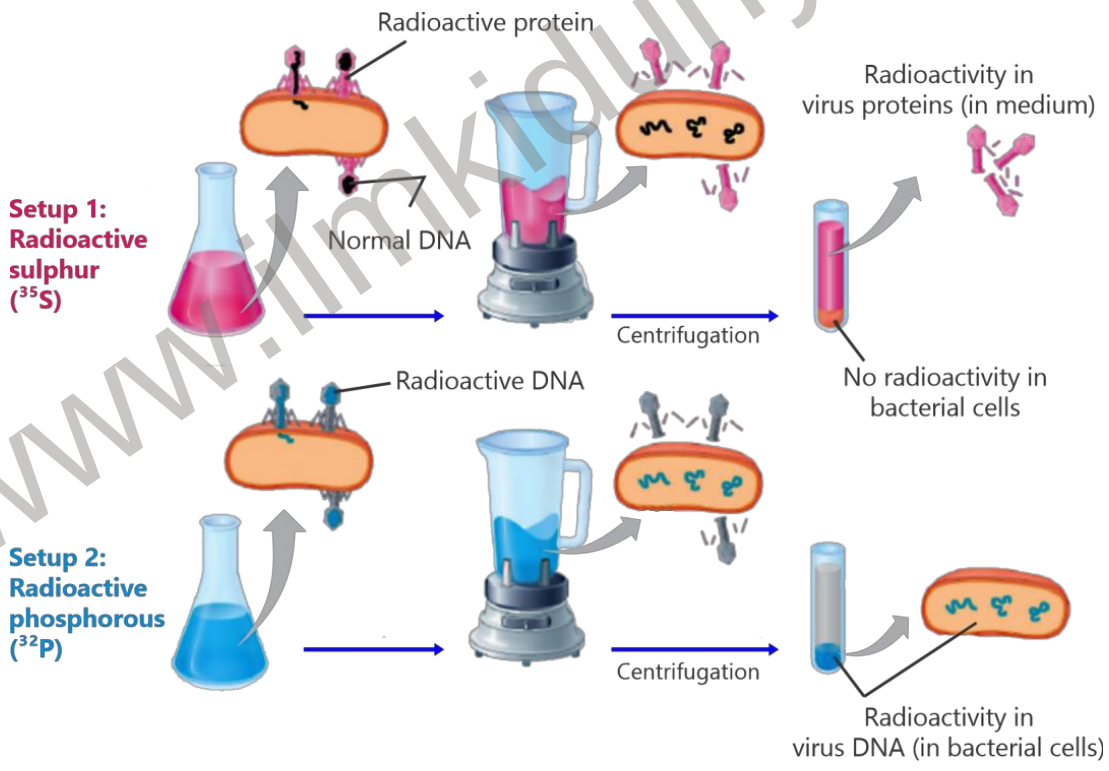



Figure 19.7: Hershey-Chase experiment

## 19.3- DNA REPLICATION

DNA replication is the process in which the molecule of DNA makes its identical copy (replica). DNA is a double-helix molecule (made of two strands). So, it makes copies of both of its strands.

**Recalling:**
The DNA present in chromatids replicates so that chromatids can duplicate. It happens before a cell divides i.e., during the S-phase of interphase.

### 19.3.1- Models Proposed for the Mechanism of DNA Replication

Three models were proposed for the basic mechanism of DNA replication.

**1- Conservative model:** According to this model, the double-helix of parent DNA remains intact. This double-helix generates new double-helix which completely consists of new material. So, after replication, when two daughter DNAs are produced;

- 1 daughter DNA contains both strands of parent DNA, and
- 1 daughter DNA contains both new strands.

**2- Semi-conservative model:** According to this model, the double-helix of parent DNA is broken. It means that two strands of parent DNA separate. Each of these strands acts as a template for the synthesis of a new strand. So, after replication;

- Both daughter DNAs consist of one strand of parent DNA and one new strand.

**3- Dispersive model:** According to this model, parent double-helix DNA is broken and dispersed into nucleotides. Then, each nucleotide combines with new nucleotides to generate the four strands of daughter DNAs. So, after replication;

- Both daughter DNAs contain strands which are mixtures of old and new nucleotides.

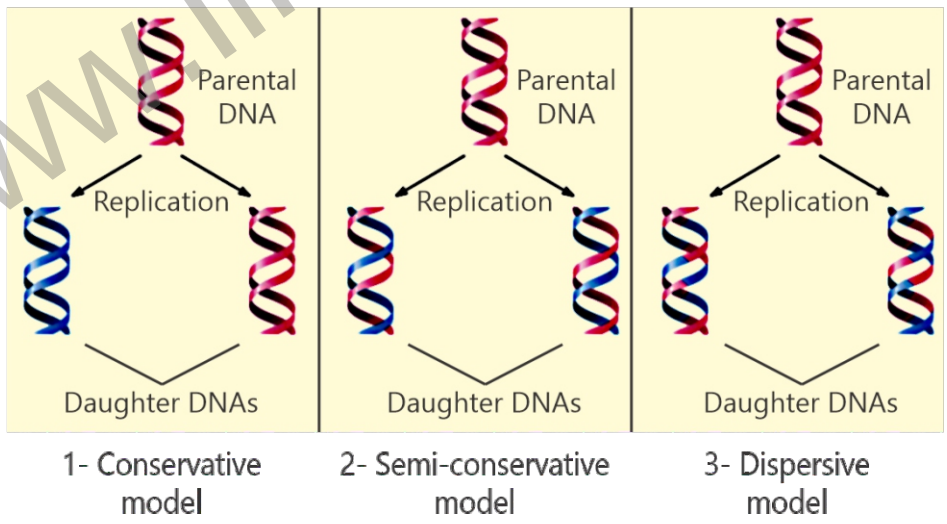



Figure 19.8: Three models of DNA replication

## 19.3.2- Meselson-Stahl Experiment

In 1958, two American biologists, Mathew **Meselson** and Franklin **Stahl**, evaluated the three models of DNA replication. They proved that the DNA replication is according to semi conservative model.

### Experiment

They grew bacteria in a medium containing heavy isotope of nitrogen i.e., $^{15}N$. The next generation of the bacteria incorporated $^{15}N$ in the nitrogen-bases of DNA instead of normal $^{14}N$. So, after many generations, the DNA of these bacteria was denser than that of normal bacteria. Then, they transferred the new bacteria (with $^{15}N$ in DNA) from the $^{15}N$ medium to a medium that contained $^{14}N$. They thought that now these bacteria will make new DNA by using $^{14}N$ from the medium.

When bacteria divided and increased in number, Meselson and Stahl collected the new DNAs at different intervals. They checked heavier DNA (with $^{15}N$) and lighter DNA (with $^{14}N$). For this purpose, they separated the heavier and lighter DNAs in the following steps.

1. They dissolved the collected DNA in aqueous solution of cesium chloride and conducted the ultracentrifugation of the solution.
2. The ultracentrifugation created a gradient of cesium density (cesium with more density settled at bottom). DNA strands of different densities were also separated during this ultracentrifugation.
3. Each DNA strand settled at the position where its density was exactly matching the density of the cesium there. The denser DNA with $^{15}N$ migrated further down as compared to the lighter DNA with $^{14}N$.

### Observations after Ultracentrifugation

a. First sample was collected at 0 minutes i.e., before any replication. All sample made single sediment of heavy density. It means that all DNA was dense (with $^{15}N$).
b. Second sample was collected at 20 minutes i.e., after the bacteria had completed first DNA replication in $^{14}N$ medium. The sample made a single sediment of intermediate density. It means that all DNA has intermediate density i.e., between $^{14}N$-DNA and $^{15}N$-DNA.
c. Third sample was collected after 40 minutes when bacteria had completed the second replication. In this sample, DNA made two sediments i.e., one intermediate and one light (equal to that of $^{14}N$-DNA).

**Tracing radio-isotope labelled DNA in the progeny**

An experiment in which a radio-isotope labelled DNA is traced in the progeny of an organism is called a **"pulse-chase experiment."** The experiment has two stages:

- In "pulse" stage, the organism is exposed to the radio-isotope for a brief time. During this step, the organism incorporates radioisotope into its DNA.
- In "chase" stage, the labelled DNA is observed over time by measuring the radioactivity of DNA.

## Explanation of Meselson and Stahl Experiment

a. DNA collected before any replication was heaviest because it had both strands made of $^{15}N$

b. DNA collected after first replication was intermediate in density because each daughter DNA was a hybrid i.e., one strand was made of $^{14}N$ and one of $^{15}N$.

c. DNA collected after second replication, formed two sediments (one intermediate and one lighter) because each parent DNA contributed one heavy strand to form another hybrid DNA and one light strand to form a light DNA (containing both $^{14}N$ strands).

On the basis of these results, they claimed that DNA replicates in a semiconservative manner.

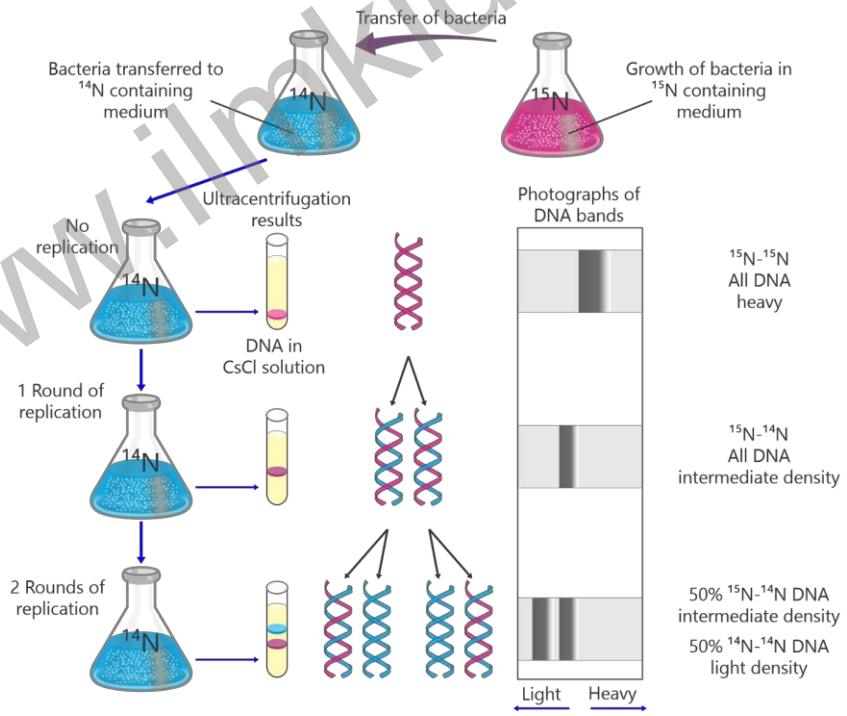



Figure 19.9: Results of Meselson-Stahl experiment

## 19.3.3- Mechanism of DNA Replication

The basic mechanism of DNA replication is similar in all organisms. The following steps refer to replication in prokaryotes, but eukaryotes also adopt almost the same mechanism.

### 1- Opening up the DNA double helix

The replication of DNA begins at one or more sites on DNA molecule where there is a specific sequence of nucleotides called a **replication origin site**. In prokaryotes, there is only one replication origin site. But in eukaryotes, there are many replication origin sites along the length of DNA.

i- **Initiator protein** binds to the replication origin site and initiates the process.

ii- At the origin site, the "unwinding" enzymes called **helicases** break the hydrogen bonds between the nucleotide-pairs (base-pairs) of two strands. The stands separate a little and make two Y-shaped structures called **replication forks** (collectively called a replication bubble).

iii- Due to unwinding of the DNA helix, supercoils are produced ahead of the replication fork.

iv- The enzyme **gyrase** (or topoisomerase) attaches ahead of the replication fork. It prevents supercoiling again.

v- **Single-strand binding proteins** bind to single strands and prevent them from re-joining and rewinding.

vi- The two replication forks move away from each other so that replication can occur at both forks. Both single strands act as templates for the next steps.

### 2- Building a primer

On the exposed strands, new DNA cannot be formed unless some nucleotides are already arranged on template. For this purpose, an enzyme **primase** attaches some RNA nucleotides in front of each template strand at replication origin site. This short fragment of 5-10 RNA nucleotides is called **primer**.

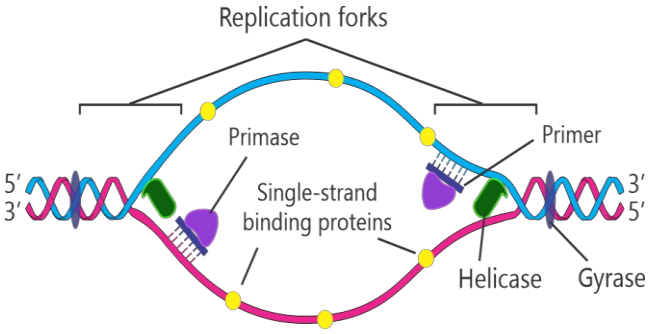



**Figure 19.10: Replication – opening up of strands and primer attachment**

## 3- Assembling complementary strands (Extension)

### The Main Builder: DNA Polymerase III

The enzyme DNA polymerase adds new nucleotides along both template strands. In prokaryotes, there are DNA polymerase I, polymerase II and polymerase III. But, **DNA polymerase III** is the main enzyme. It grabs free floating nucleotides and matches them to the exposed template strands (A with T, and C with G).

**For Information**
DNA polymerase I provides a support to polymerase III by replacing the primers at the end of replication. Polymerase II is involved in the repairing process of DNA damages during the life time of a cell.

### The Raw Materials

The units used for synthesizing new strands are Deoxyribonucleoside triphosphates (dATP, dCTP, dGTP, dTTP). These molecules have three phosphate groups. As DNA Polymerase III attaches a nucleotide to the strand, it breaks off two phosphates. This action releases energy which is used for the building process.

**Tidbit**
Why an RNA primer, rather than DNA? Starting chains on exposed templates introduces many errors; RNA marks this initial stretch as "temporary". It makes easy to excise this error-prone later.

### One-Way Traffic (5′ to 3′ Direction)

DNA Polymerase III can only add a new nucleotide to the 3′ end of an existing one. First, it adds a nucleotide at 3′ end of primer and then continues adding nucleotides in 5′ to 3′ direction. Because the two original DNA strands run in opposite directions, the enzyme must handle them differently.

### Building the Leading Strand

One unit of the enzyme attaches to the template strand that runs toward the opening "fork." Because it is moving in the same direction the DNA is unzipping, it can add nucleotides in one continuous 5′ to 3′ chain. This smoothly growing daughter strand is called the **Leading Strand**.

**Tidbit**
The leading strand can be extended from one primer alone, whereas the lagging strand needs a new primer for each Okazaki fragment (named for the Japanese scientist who discovered them).

### Building the Lagging Strand

The second unit of the enzyme works on the other template strand. However, to maintain the 5′ to 3′ direction, it must move away from the replication fork. As the DNA unzips further, the enzyme has to "jump back" to a new primer to start a new

segment. So, this daughter strand grows discontinuously away from the replication fork. It makes short, separate pieces of DNA called **Okazaki fragments**. Because this strand is built in "stops and starts" rather than one long piece, it is called the **lagging strand**.

## 4- Termination of replication

The termination of replication occurs at specific sequence of nucleotides called **termination site**. The region of DNA between the origin site and termination site is termed as a replication unit or **replicon**. During termination, DNA polymerase I removes the RNA primer and fills in the gap with DNA nucleotides. It also fills any gaps between Okazaki fragments. The enzyme **DNA ligase** joins the Okazaki fragments of the lagging strand.

**For Information**

During replication, one strand of the original DNA is conserved, while the daughter strand is synthesized anew. It means that half of the original DNA molecule is conserved in each new generation of DNA.

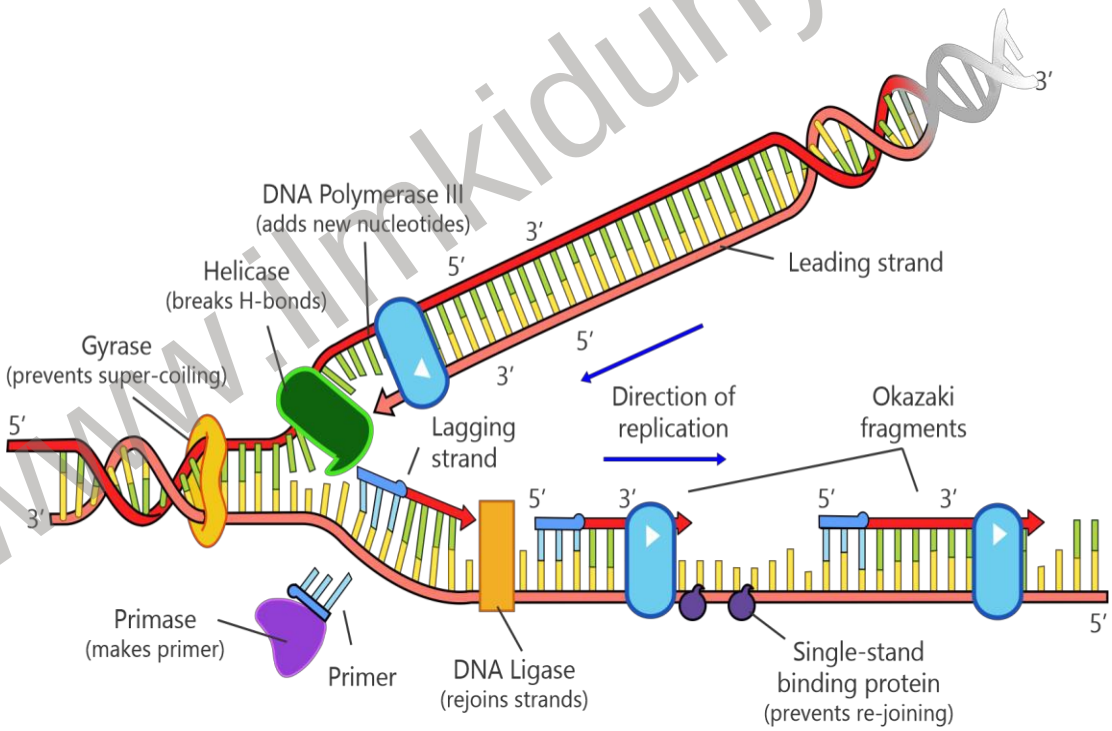



Fig. 19.11 Process of DNA replication

## Difference between DNA Replication in Prokaryotes and Eukaryotes

**Location:** In prokaryotes, replication occurs in the cytoplasm (as they do not have nucleus). In eukaryotes, nucleus is the site for DNA replication.

**Polymerases:** In prokaryotes, DNA Polymerase I, II, and III work in replication. In eukaryotes, there are at least 14 polymerases (such as alpha, delta, and epsilon).

**Initiation and termination sites:** In prokaryotes, DNA has a single origin site and a single termination site. In eukaryotes, there are multiple origin and termination sites on each chromosome. DNA replication starts at each origin site simultaneously. Thus, each chromosome has several replicons.

**Okazaki fragments:** In prokaryotes, the Okazaki fragments are longer (1000 to 2000 nucleotides). In eukaryotes, the Okazaki fragments are short (between 100 and 200 nucleotides).

**Speed:** Prokaryotes replicate their small DNA very quickly. Eukaryotes have massive amounts of linear DNA. To compensate for this, eukaryotes start replication at thousands of different origin sites along a single chromosome at the same time.

**For Information**

Biotechnologists use DNA replication in new technologies. For example;

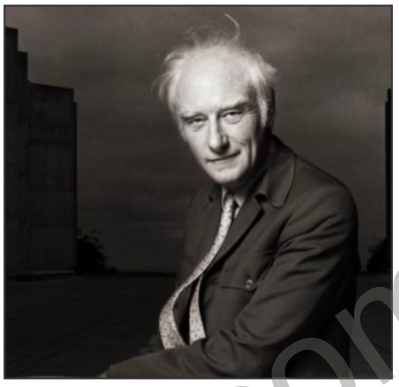
- In Polymerase Chain Reaction (PCR), scientists conduct replication process to make billions of copies of a specific DNA.
- In recombinant DNA technology, scientists cut DNA from different sources. Then, they conduct DNA replication to amplify the DNA molecules.
- In genome sequencing, scientists use DNA replication to make multiple copies of the genome, which are then sequenced to know the genome sequence.
- In diagnostic studies, scientist replicate specific sections of patient's DNA. Then, they search changes or mutations in DNA to know the presence of a genetic disorder.

## Stability and Variability of DNA

These are two important properties of a replicating DNA. “Stability” means the ability of DNA to be copied with accuracy. DNA Polymerase III (and eukaryotic polymerases) have a "proofreading" ability to ensure that the sequence of nucleotides is maintained. This consistency is vital because it maintains the specific traits of an organism. It also ensures that cells function correctly.

While stability is the rule, “variability” is an exception. Occasionally, a permanent change in the DNA sequence occurs, known as a **mutation**. These mutations can be caused by errors during replication. Environmental factors can also cause mutations. Variability is the primary source of new traits. It allows populations to adapt to changing environments and is the raw material for evolution.

## 19.4- Gene Expression



Francis Crick

You know that a gene is a specific sequence of nucleotides along the DNA strand which directs the synthesis of a polypeptide (protein). In other words, a gene expresses itself by making a protein or an enzyme that controls the development of a specific trait.

All organisms use the same basic mechanism of expressing genes. English biologist, Francis Crick used the term **"Central Dogma"** for this mechanism. The central dogma states that information passes from the genes (DNA) to an RNA copy of the gene, and the RNA copy directs the sequential assembly of a chain of amino acids. In simple words, the central dogma is;

DNA → RNA → Protein

It means that the flow of genetic information is one-way. Once the information has gone into a protein, it never goes back.

The central dogma consists of two steps i.e., transcription (synthesis of RNA from DNA) and translation (synthesis of a protein by using the information present in RNA).

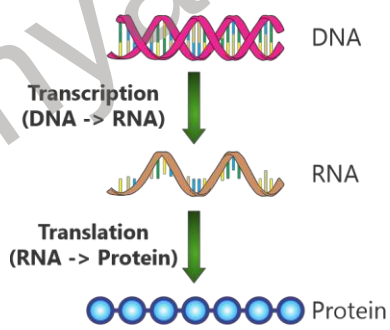



Figure 19.12: Central dogma

### 19.4.1- Genetic Code

After the discovery of the structure of DNA (in 1953) and the idea of central dogma (in 1957), the essential questions were;

- How can DNA, a substance containing only four different nucleotides, store genetic information for making thousands of specific proteins?
- If DNA has genetic information in the form of a genetic code, what is the nature of this code?
- If genetic code is in the form of the order of nucleotides in a DNA, how does it specify the order of amino acids in a polypeptide?

These questions started a race among biologists. The following were the main achievements, in this regard.

#### Work of Francis Crick

In 1961, Francis **Crick** and his colleagues presented an idea; "the genetic code consists of a series of blocks of information called **codons**. Each codon specifies one amino acid of the polypeptide to be synthesised". They further thought that;

- If a sequence of single nucleotides specifies a single amino acid, only four different amino acids could be encoded.
- Even a sequence of 2 nucleotides would make 16 (4 x 4) codons and they would specify 16 amino acids.

**For Information**
The information in DNA for the synthesis of proteins is called as genetic code. The genetic code consists of a series of codons consisting of 3-nucletotide.

They reached to a conclusion that one codon should be a sequence of 3 nucleotides. This 3-nucleotide sequence specifies a particular amino acid. The 4 nucleotides can be arranged in 64 (4 x 4 x 4) different combinations which are more than enough to code for the 20 amino acids.

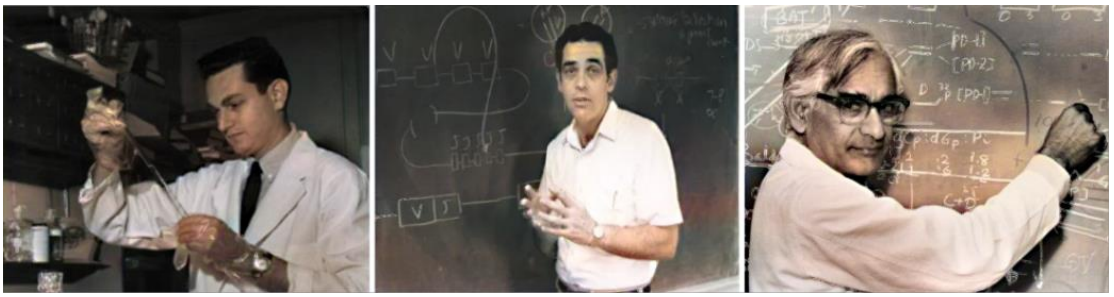
According to the conclusions of Crick, the genetic code consists of continuous sequences of three nucleotides. In other words, it is a **triplet code**. When a gene works, the codons present on the DNA are transcribed in the form of complementary codons of mRNA. So, the TAC codon on DNA would mean AUG codon on mRNA. In general, the term "codon" refers to the codon of mRNA. However, in some cases, the term "codon" may also be used to refer to codons of DNA, especially when discussing mutations in DNA.

## Work of Marshall Nirenberg, Philip Leder and Har Gobind Khorana

In 1961, the American biochemist, Marshall **Nirenberg** made a poly-U mRNA. This mRNA consisted of uracil nucleotides only. He added this poly-U mRNA to the cytoplasm of bacteria. He observed that the bacteria produced a polypeptide consisting of many phenylalanine amino acids. He concluded that the codon UUU specifies phenylalanine. Nirenberg and another American geneticist Philip **Leder** tested all triplet codons. They determined the amino acids specified by 47 codons. Har Gobind **Khorana** (a scientist who completed his studies in Government College, Lahore) determined the amino acids specified by the remaining 17 triplet codons.

Out of the total 64 codons, three codons (UGA, UAG, and UAA) are **stop codons** or **nonsense codons**. If any of these is present at the end of mRNA, it does not specify any amino acid and stops the protein synthesis. The codon AUG acts as **start codon**. It is present at the beginning of all genetic codes. It specifies the amino acid methionine.

The 1968 Nobel Prize in Physiology & medicine awarded to Nirenberg (work on genetic code), Har Gobind Khorana (synthesis of nucleic acids), and Robert Holley (discovering the chemical structure of transfer-RNA).



(Left to Right)- Marshal Nirenberg, Philip Leder, Har Gobind Khorana

## 19.4.2- Characteristics of Genetic Code

**1- Genetic code is universal:** The genetic code is same in almost all organisms. For example, AGA specifies arginine in bacteria, in humans and all other organisms. Due to this characteristic, genes can be transferred among organisms.

**For Information**
The exceptions to the universality of the genetic code demonstrate that while the standard genetic code is widely used, there is still room for variation and adaptation in the genetic code.

However, there are a few exceptions to this universality. For example, genetic code in the DNA of mitochondria is slightly different from the genetic code in the DNA of chromosomes. It reflects the independent evolutionary history of mitochondria. Similarly, some organisms (e.g., ciliates) and viruses (e.g., bacteriophages) use different genetic codes for the same amino acids.

**2- Genetic code is triplet:** The genetic code in DNA is a sequences of three nucleotides (codons). Due to this characteristic, the amount of information present in DNA balances the information required to specify 20 kinds of amino acids.

**Analyse & Interpret**
Interpret how many types of t-RNA molecules are necessary for a living cell, if the genetic code is a triplet code.

**3- Degeneracy of genetic code:** Degeneracy of genetic code means that multiple codons can specify the same amino acid. For example, four different codons (AUG, GUG, UUG, and CUG) code for the same amino acid i.e., methionine. Characteristic is important for mutation tolerance and evolutionary flexibility.

**4- A codon does not have punctuations:** A codon is read continuously. It means that it is without punctuation (gaps) between the three-nucleotides of a codon.

**5- Genetic code is non-overlapping:** It means that each triplet codon is read independently of the adjacent codons. For example, the series of AUGAGCGCA codons cannot be read as AUG/UGA/GAG etc. These will only be read as AUG/AGC/GCA.

**Table: Codons (on mRNA) and their specific amino acids (or stop functions)**

| FIRST NUCLEOTIDE | SECOND NUCLEOTIDE U | | SECOND NUCLEOTIDE C | | SECOND NUCLEOTIDE A | | SECOND NUCLEOTIDE G | | THIRD NUCLEOTIDE |
|---|---|---|---|---|---|---|---|---|---|
| U | UUU | Phenylalanine | UCU | Serine | UAU | Tyrosine | UGU | Cysteine | U |
| | UUC | | UCC | | UAC | | UGC | | C |
| | UUA | Leucine | UCA | | UAA | Stop | UGA | Stop | A |
| | UUG | | UCG | | UAG | Stop | UGG | Tryptophan | G |
| C | CUU | Leucine | CCU | Proline | CAU | Histidine | CGU | Arginine | U |
| | CUC | | CCC | | CAC | | CGC | | C |
| | CUA | | CCA | | CAA | Glutamine | CGA | | A |
| | CUG | | CCG | | CAG | | CGG | | G |
| A | AUU | Isoleucine | ACU | Threonine | AAU | Asparagine | AGU | Serine | U |
| | AUC | | ACC | | AAC | | AGC | | C |
| | AUA | | ACA | | AAA | Lysine | AGA | Arginine | A |
| | AUG | Methionine (Start) | ACG | | AAG | | AGG | | G |
| G | GUU | Valine | GCU | Alanine | GAU | Aspartate | GGU | Glycine | U |
| | GUC | | GCC | | GAC | | GGC | | C |
| | GUA | | GCA | | GAA | Glutamate | GGA | | A |
| | GUG | | GCG | | GAG | | GGG | | G |

## 19.5- TRANSCRIPTION

It is the first step of gene expression. During transcription, a complementary RNA is synthesised from DNA. Only one strand of DNA, called the **template strand**, is transcribed. The mRNA synthesised in transcription is complementary to the template strand of DNA. The other strand of DNA that is not transcribed is called the **coding strand** because it has the same sequence as the transcribed mRNA, except T takes the place of U. The following are the main phases of the transcription of mRNA.

**For Information**
The template strand of DNA is also known as the antisense (–) strand and coding strand as the sense (+) strand.

### 1- Initiation Phase

In this process, no primer is needed. An enzyme RNA polymerase binds at template DNA at a special nucleotides sequence. This sequence is called **promoter**. It is not itself transcribed by the enzyme. For example;

- In prokaryotes, a promoter **TTGACA** is present 35 nucleotides before the start point of gene. So, it also called –35 sequence.
- In prokaryotes, another promoter **TATAAT** is 10 nucleotides before the start point of gene. It is also called –10 sequence.

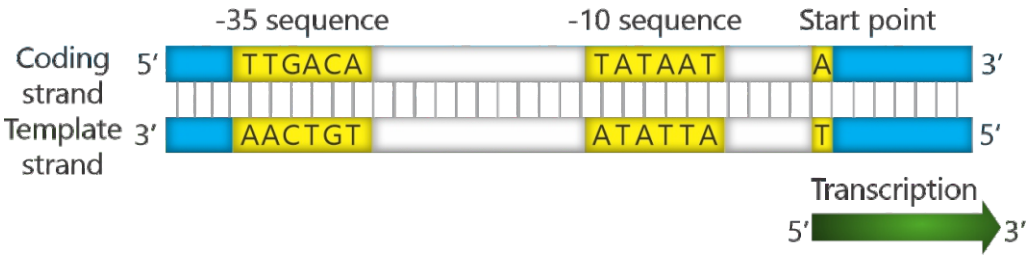



Figure 19.13: Promoters in prokaryotes

- In eukaryotes, a promotor **TATAAA** (called **TATA box)** is 25 nucleotides before the start point. It is also called –25 sequence.
- In eukaryotes, a promoter **GGCCAATCT** (called **CAAT box)** is 70 nucleotides before the start point. It is also called –70 sequence.

**For Information**
Prokaryotes have only one RNA polymerase, which synthesises all 3 kinds of RNA. Eukaryotes have three different RNA polymerases: RNA polymerase I synthesizes rRNA in the nucleolus; RNA polymerase II synthesizes mRNA; and RNA polymerase III synthesizes tRNA.

RNA polymerase consists of five subunits:

- two **α subunits** bind with regulatory proteins,
- a **β′ subunit** binds with template DNA,
- a **β subunit** binds with RNA nucleotides, and
- a **σ subunit** recognizes the promoter and initiates synthesis.

When RNA polymerase attaches to promoter, its **σ subunit** is removed. The other four subunits (core enzyme) catalyse the process. They unwind the DNA helix. They breakdown nucleotide-pairs (base-pairs). It results in the formation of a bubble-like structure, the **transcription bubble**. The assemblage of RNA chain occurs in this bubble.

## 2- Elongation Phase

Inside the transcription bubble, RNA polymerase begins making a chain of complementary ribonucleotides in front of template DNA strand. It is done in 5′ to 3′ direction. It usually starts with A or G ribonucleotide. Here, the nucleotides available for attachment are in the form of ribonucleoside triphosphates (rNTP). When an rNTP is added, its two phosphates detach with the release of energy, and ribonucleotide is attached to the chain.

The first 12 bases of new mRNA strand make a temporary helix with template DNA strand. Due to it, the position of the 3′ end of the mRNA remains stable. So, it can receive an incoming ribonucleotide. The RNA-DNA helix rotates each time a nucleotide is added. So, the 3′ end of RNA stays at the catalytic site of RNA polymerase. The

transcription bubble moves down the DNA. The growing mRNA strand protrudes from the bubble. After the transcription bubble passes, the transcribed DNA is re-wound.

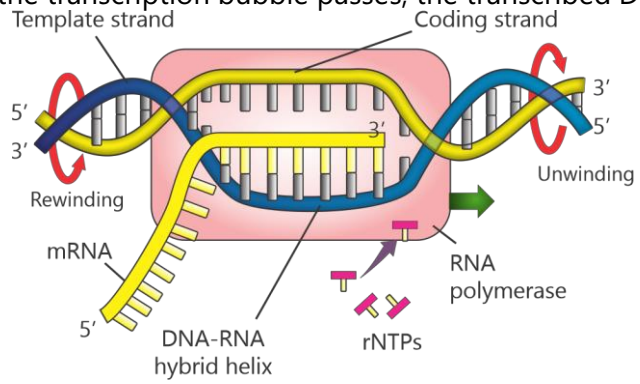



Figure 19.14: Elongation phase of transcription

## 3- Termination Phase

At the end of a gene on template DNA. There is a stop codon. After this stop codon, there is a series of **stop region** in the form of GC nucleotide-pairs (base-pairs) followed by a series of AT pairs. When these nucleotide-pairs are transcribed into mRNA, hydrogen bonds are formed within the strand of mRNA. This intra-strand nucleotide-pairing (base-pairing) creates a loop called **GC hairpin** with a tail of four or more U ribonucleotides. The GC hairpin causes the RNA polymerase to pause. The pairing of four U of mRNA of with A of DNA is the weakest. It cannot hold the RNA-DNA hybrid strands together during the long pause. So, the RNA strand dissociates from DNA stand within the transcription bubble, and transcription stops.

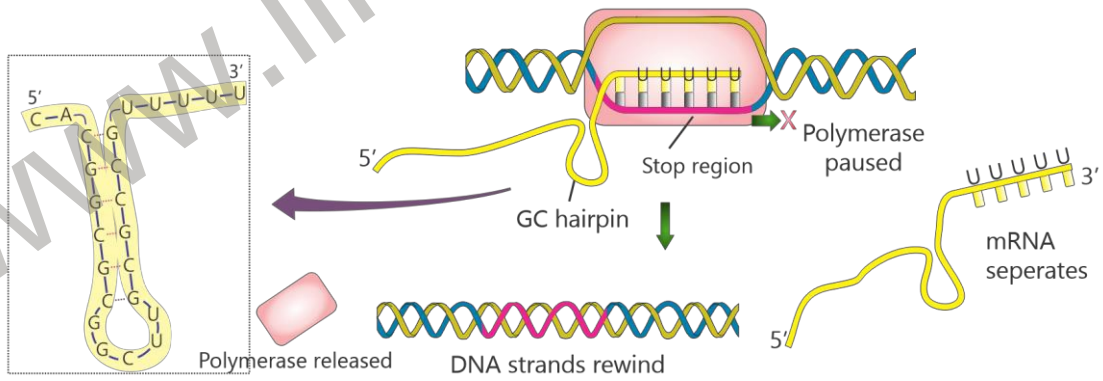



Figure 19.15: Termination phase of transcription

## Post-Transcriptional Modifications

As the prokaryotes have no nucleus and transcription happens in cytoplasm, their new mRNA does not need to travel anywhere to start translation. On the other

hand, in eukaryotic cells the new mRNA has to travel from nucleus to cytoplasm. For this journey, the new mRNA undergoes the following modifications.

**1- Addition of 5′ Cap:** The first nucleotide (at the 5′ end of mRNA) is A or G. At the terminal 5′ end of A or G a methylated GTP is attached. This bond is formed between 5′end of terminal A or G and 5′end of GTP. This structure is called a 5′ cap. It protects the mRNA from nucleases and phosphatases during its journey.

**2- Addition of 3′ Poly-A tail:** At the 3′end of mRNA, a small chain of about 250 A nucleotides is added. It is called 3′ poly-A tail. It protects the mRNA transcript from degradation by nucleases.

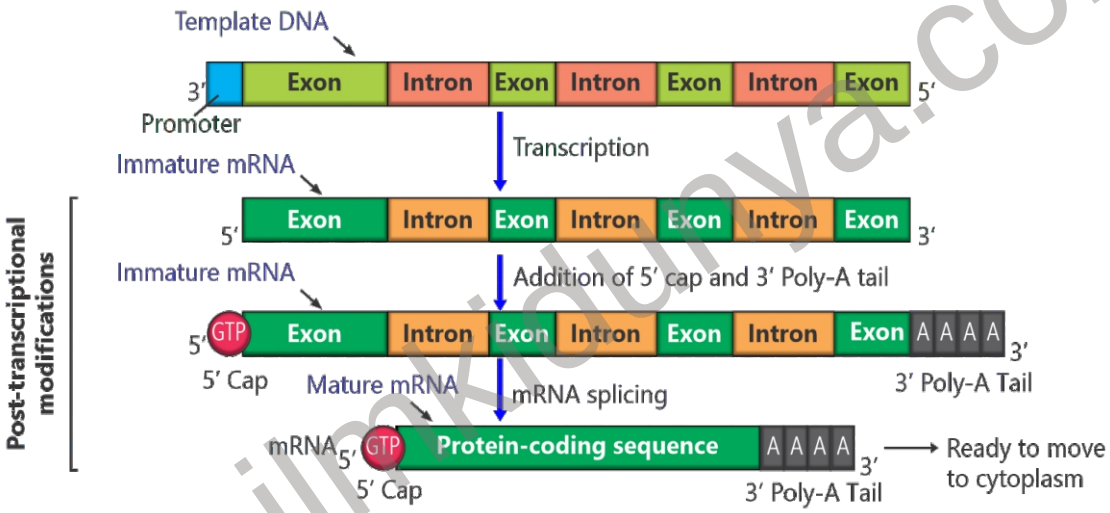



Figure 19.16: Post-transcriptional changes in eukaryotes

**3- Splicing:** The new mRNA is immature because it contains introns (regions of DNA or mRNA that do not code for proteins) and exons (regions of DNA or mRNA which are "expressed" or translated into a protein). mRNA splicing is the process by which introns are removed from the immature mRNA. After splicing, the remaining exons are joined. So, the mature RNA consists of coding segments (gene) only.

## 19.6- TRANSLATION

In translation or protein synthesis, the information present in an mRNA transcript is used to direct the sequence of amino acids to produce a polypeptide.

## Activation of Amino Acids

Before protein synthesis, the assemblage units are prepared. Specific tRNAs attach with specific amino acids and make **aminoacyl-tRNAs (aa-tRNA)**. The enzyme **aminoacyl-tRNA synthetase** carries out this reaction. There are 20 kinds of this enzyme. Each kind binds to a specific amino acid. It also recognizes and binds to the tRNA with anticodons for that amino acid. The aa-tRNA delivers the amino acid to the ribosome for incorporation into the polypeptide chain.

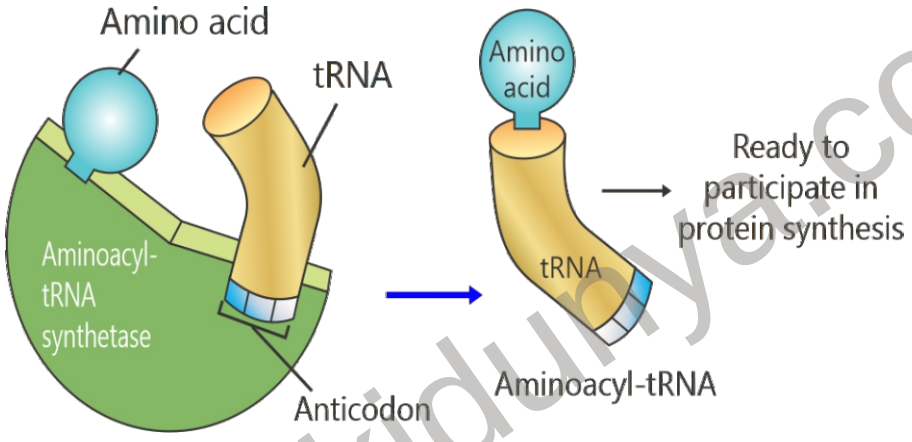



Figure 19.17: Activation of amino acids

## 1- Initiation Phase

In this phase, an **initiation complex** is formed in the following steps:

1. The aa-tRNA carrying the first amino acid binds to the smaller ribosomal subunit. An enzyme called **initiation factor** controls this binding. In prokaryotes, the first amino acid is modified methionine i.e., N-formyl-methionine. While in eukaryotes, it is non-modified methionine
2. Another initiation factor attaches the 5′ end of mRNA with smaller ribosomal subunit. It positions the anticodon of aa-tRNA over the first codon (AUG) of mRNA.
3. The larger ribosomal subunit is also placed upon smaller subunit.

The **initiation complex** formed in this way consists of both ribosomal subunits, first aa-tRNA, mRNA, and the enzyme.

The site of ribosome where first aa-tRNA is placed consists of;

- A **Peptidyl site** (P site) – where peptide bonds will form between amino acids.
- An **aminoacyl site** (A site) – where next aa-tRNA will bind.

- An **exit site** (E site) – where empty tRNAs will exit the ribosome.

With respect to mRNA, the three sites are oriented in 5′ to 3′ as E-P-A, because ribosomes move toward the 3' end of mRNA.

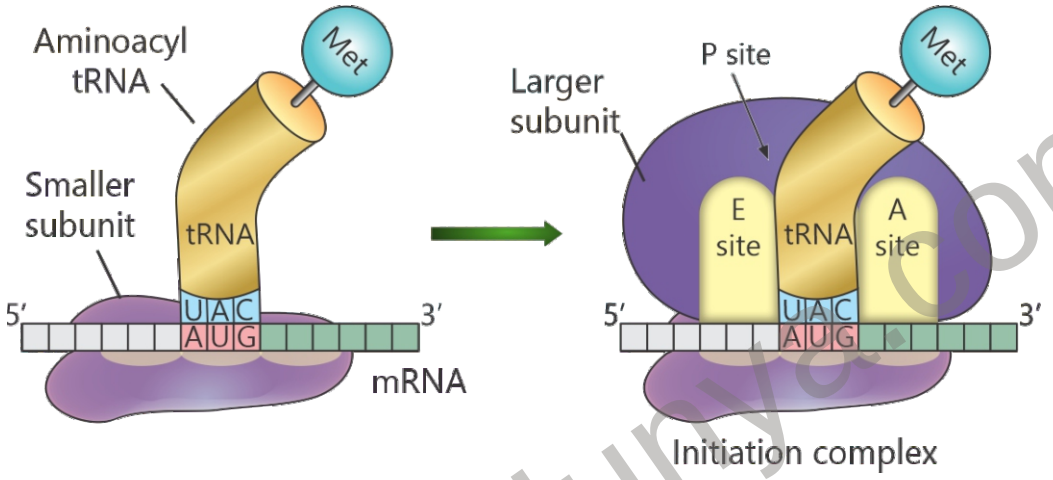



Figure 19.18: Initiation phase of translation

## 2- Elongation Phase

In the initiation complex, the P-site of ribosome is occupied by first aa-tRNA (with its anticodon matching the mRNA codon). The next codon of mRNA is exposed at A site. Elongation happens in the following steps;

1. The next aa-tRNA with matching anticodon and matching amino acid binds to A site with the help of an enzyme, the **elongation factor**.
2. Another enzyme **peptidyl transferase** removes methionine from first aa-tRNA present on P site and attaches it by a peptide bond to the amino acid of the second aa-tRNA present at A site.
3. The ribosome moves three nucleotides along the mRNA in 5′ - 3′ direction. This movement is called **translocation**.
4. As a result of the previous step, the aa-tRNA carrying the chain of two amino acids is shifted from A site to P site. The new codon of mRNA is also exposed at A site. Moreover, the empty tRNA comes to E site to leave the ribosome.
5. These steps are repeated again and again until the stop codon is reached at A site.

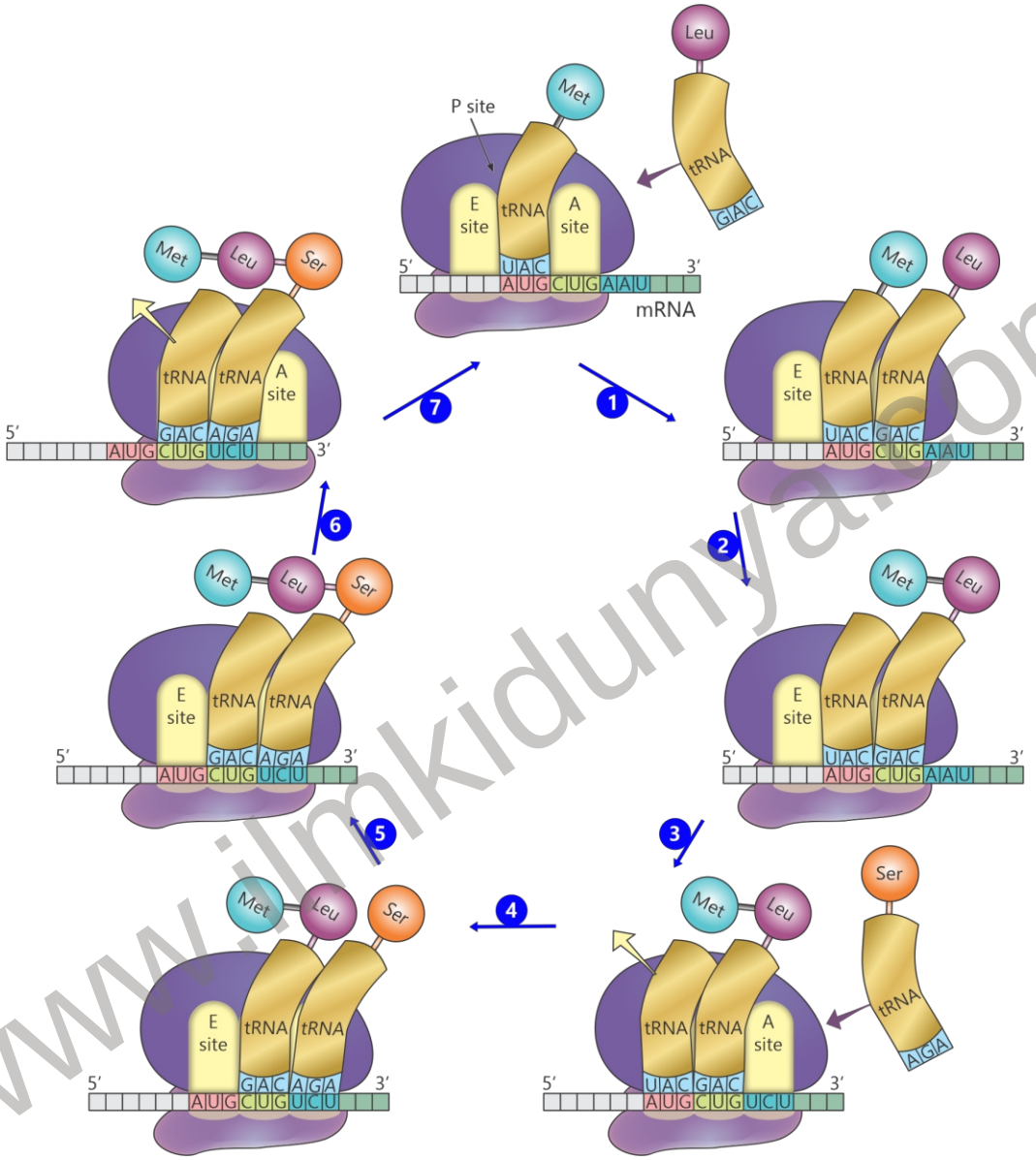



**Figure 19.19: Elongation phase of translation (1- second aminoacyl-tRNA binds to A site; 2- first amino acid is removed from first tRNA and attached to the amino acid of the second aminoacyl-tRNA; 3- ribosome moves three nucleotides in 5′ - 3′ direction; 4- third aminoacyl-tRNA binds to A site; 5- chain of first two amino acids is removed from the tRNA at P-site and attached to the amino acid of the third aminoacyl-tRNA; 6- ribosome again moves three nucleotides and next codon of mRNA is exposed at A site; 7- the process is repeated for all codons)**

### 3- Termination Phase

When a stop codon is exposed at A site, no aminoacyl-tRNA comes to A-site (because there is no anticodon of the stop codons). Stop codon at A site is recognized by a protein called **release factor**. It releases the polypeptide from the tRNA present at P site. In this way, the process of translation is terminated. The tRNA is also released from ribosome and ribosomal subunits separate from the mRNA.

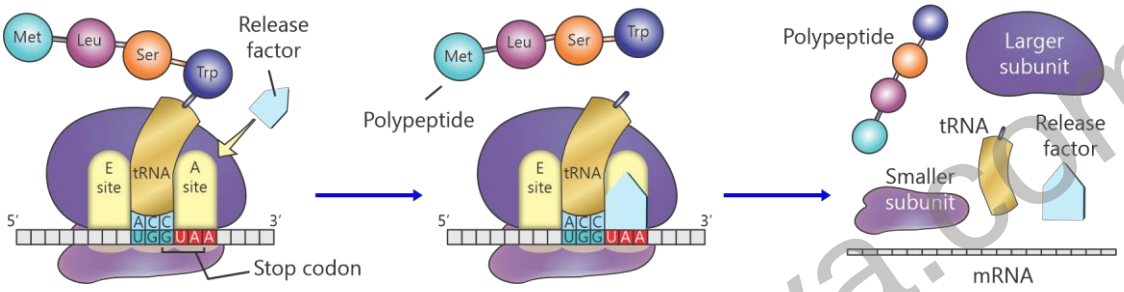



Figure 19.20: Termination phase of translation

### 19.6.1- Difference between Translation in Prokaryotes and Eukaryotes

| | Prokaryotes | Eukaryotes |
|---|---|---|
| 1 | Prokaryotic genes lack introns. | Most eukaryotic genes possess introns. |
| 2 | After transcription, the mRNA immediately undergoes translation. There is no post-transcriptional modification in mRNA. | The mRNA is modified before its translation: introns are cut out; a 5′ cap is added; and a 3′ poly-A tail is added. |
| 3 | Translation occurs in cytoplasm. | Translation occurs in cytoplasm as well as in rough endoplasmic reticulum. |
| 4 | Ribosomes are smaller in size (70S). | Ribosomes are larger in size (80S). |
| 5 | The initial amino acid is modified i.e., N-formyl methionine. | The initial amino acid is methionine. It is not modified. |

## 19.7- Regulating Gene Expression

All of the genes in an organism are not expressed simultaneously. Instead, different genes are expressed at different times. Similarly, all types of cells contain the same genome but all genes are not turned ON in all cells. Instead, specific genes are turned ON in each cell type. Turning the genes ON and OFF and the timing of their expression are controlled by **regulator proteins** and the process is called regulation of gene expression.

## Importance of Regulation of Gene Expression

Regulation of gene expression enables the cells to respond to environmental conditions and to prepare protein when needed. In multicellular organisms, gene regulation is the basis for cellular differentiation and morphogenesis. It leads to the creation of different cell types. In different cell types, the genome is the same but different genes are expressed according to the type.

## Methods of Regulation of Gene Expression

Regulation of gene expressions occurs at several levels. For example, at transcription level, the rate of transcription is regulated. At post-transcriptional level, the modification of mRNA is controlled. At translation level, the rate of translation is regulated. At post-translational, the modification of protein (its folding, stability etc.) is regulated. The important methods of regulation of gene expression include;

### 1- Regulation at Transcriptional Level

The genes have special regulatory sequences. Specific regulatory proteins bind to these sequence. These proteins can take two types of actions;

a. **Negative gene regulation:** Some regulatory proteins, called **repressors** bind to some pieces of DNA, called **operators**, present near the promoter. Here, the repressors block RNA polymerase and stop or reduce transcription.

b. **Positive gene regulation:** Some regulatory proteins, called **activators (or inducers)** bind to the operators. Here, the activators start transcription or increase its speed by helping in the binding of RNA polymerase to the promoter.

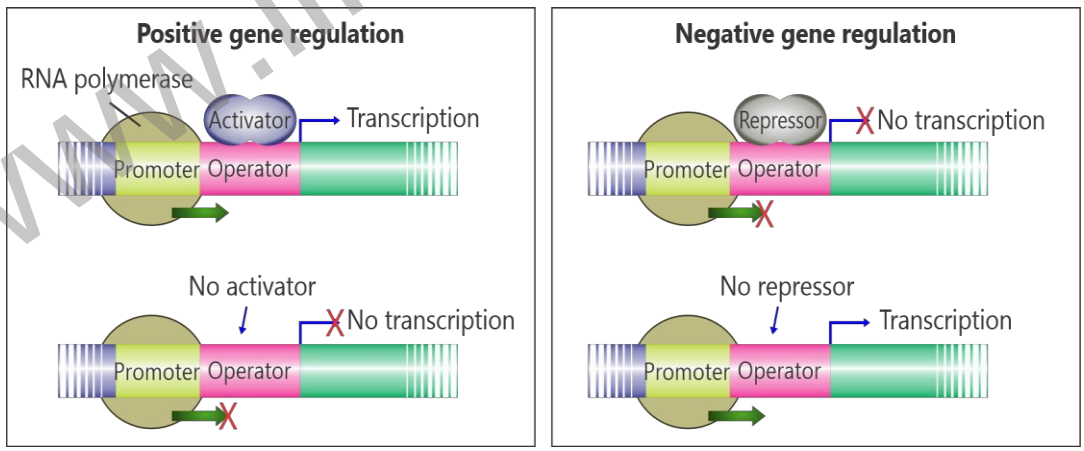



Figure 19.21: Positive and negative gene regulation by regulatory protein

### 2- Regulation during Splicing

In eukaryotes, mRNA is modified before its export to cytoplasm. Genes can also be regulated (tuned ON or OFF) during mRNA splicing (removal of introns and joining the exons). Specific regulatory proteins in specific cell types control splicing. These proteins remove the pieces of mRNA (introns or exons) in different ways. So, mRNAs of different lengths are produced. It results in the production of more than one polypeptide from a single gene.

**Tidbit**

Liver cells express specific genes which make the enzyme alcohol dehydrogenase. This enzyme breaks alcohol down into a non-toxic molecules. The neurons of brain also have these genes but they keep these genes unexpressed or "turned OFF".

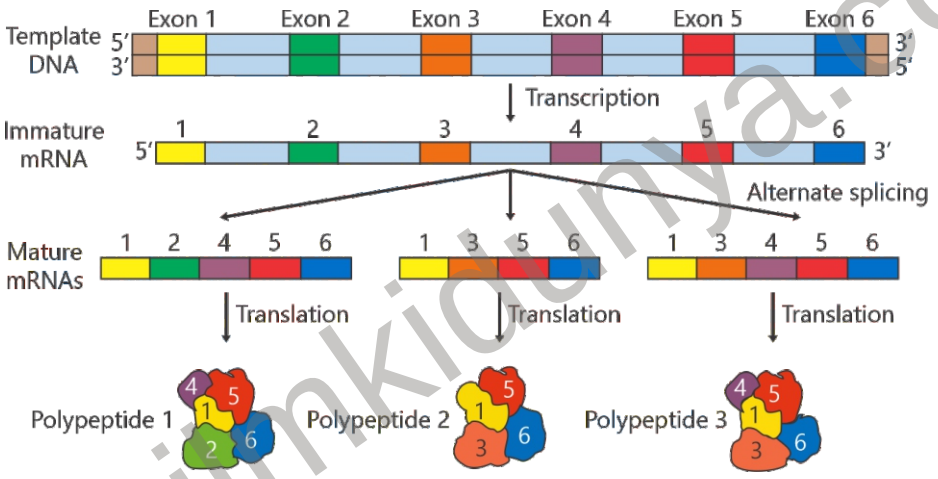



Figure 19.22: Alternate splicing and different gene products

## 19.8- MUTATIONS

A change in the content of genetic message is called mutation. Mutations may range from small (e.g., a change in a single or few nucleotides) to large (e.g., a change in a large segment or whole chromosome). There may be natural mutations and induced mutations.

**Natural mutations** occur spontaneously as a result of sequencing errors or DNA damage during replication. These mutations are a part of the process of evolution and can result in beneficial, neutral, or harmful effects on organism.

**Mutations – Contributors in Evolution**

The ultimate sources of all genetic variation are mutation are recombination. These processes create new DNA sequence (allele) and combination of alleles. The new alleles form new traits which may be advantageous, harmful or neutral. Natural selection acts upon the variations created by new traits and the allelic frequencies of advantageous traits increase. It leads of evolution in the long run.

**Induced mutations** are caused by external factors such as exposure to mutagen substances or radiation. Such mutations can also result from exposure to chemicals in the environment, including certain pesticides and industrial pollutants, or from medical treatments such as chemotherapy. Induced mutations can have serious health effects on an organism.

**For Information**
The agents that cause mutations are called mutagens while the organism or cell in which mutation occurs is called mutant.

On the basis of the size of mutations, we can classify them in two main types i.e., point mutations and chromosomal mutations.

## 19.8.1- Point Mutations

The mutations involving the change in only one or a few nucleotides, are called point mutations. The following are the common examples:

1. **Substitutions:** These mutations involve exchange of a single nucleotide for another e.g., during replication, A may be substituted with G or C is substituted with T.
2. **Insertions**: It is the addition of one or more extra nucleotides into the DNA. These mutations usually occur due to errors during replication.
3. **Deletions:** Such mutations remove one or more nucleotides from the DNA.

**For Information**
**Gene mutation** is a general term for any change within the chemical structure of a single gene. It is a change at the "molecular level" (the nucleotide sequence) rather than at the "chromosomal level" (large chunks of DNA). **Point mutation** are the most common gene mutation.

| | |
|---|---|
| **Normal DNA** | TAT CAT CCT AAG GTA |
| Protein | Tyr–His–Pro–Lys–Val |
| **Substitution** | TAT CAT CGT AAG GTA |
| Protein | Tyr–His–Arg–Lys–Val |
| **Insertion** | TAT CAT CGC TAA GGT A |
| Protein | Tyr–His–Arg–Stop |
| **Deletion** | TAT CTC TAA GGT A |
| Protein | Tyr–Leu–Leu–Arg– ··· |

Figure 19.23: Point mutations

### Sources of Point Mutations

**1- Physical Damage to DNA**

**Ionizing Radiation:** High-energy forms of radiation, such as X rays and gamma rays, are highly mutagenic. Such radiation change atoms into free radicals which may break phosphodiester bonds of DNA strand.

**Ultraviolet Radiation:** DNA strongly absorbs UV radiation. When two adjacent pyrimidine nucleotides (C and T) absorb UV radiation, a double covalent bond forms between them. It can result in complete replication blockage.

**2- Mutagen Chemicals**

Many point mutations are caused by mutagen chemicals. Some of these chemicals resemble nucleotides and are incorporated into DNA. Such DNA cannot be

properly transcribed. Some chemicals remove the amino group from adenine or cytosine, causing them to mis-pair; and some chemicals add hydrocarbon groups to nucleotides, also causing them to mis-pair. Such mutagen chemicals are commonly used in laboratories, and sometimes released into the environment (e.g., mustard gas).

**3- Spontaneous Point Mutations**

Many point mutations occur spontaneously. Sometimes nucleotides spontaneously shift to alternative positions. During replication, DNA polymerase pairs a different nucleotide with the shifted nucleotide. Sometimes, the nucleotide sequences misalign when homologous chromosomes pair. It causes a portion of one strand to loop out. The cell cuts out the loop and it results in a deletion of several hundred nucleotides from one of the chromosomes.

**Minor Mutations in Humans**

**Single nucleotide polymorphisms (SNPs):** These mutations involve a single nucleotide. These are very common and can be harmless or have a subtle effects. For instance, a single base change in a gene can make a person unable to digest milk (lactose).

**Insertions and deletions:** These mutations add or remove one or more nucleotides. For example, the common cause of **Cystic Fibrosis** is a deletion of just three nucleotides in a gene. This deletion causes a specific amino acid to be left out, resulting in a protein that doesn't fold correctly, leading to lung and digestive issues.

**Silent mutations:** These mutations do not change the amino acid sequence of protein, for example, a DNA code changes from GGC to GGU. Because of the "degeneracy" of the genetic code, both of these codes translate to the same amino acid: Glycine.

**Missense mutations:** These mutations change only one amino acid, which can affect protein function. For example, a single nucleotide change (A to T) in the gene for haemoglobin causes one amino acid (Glutamic acid) to be replaced by another (Valine). This single "wrong" amino acid causes red blood cells to lose their round shape and causes sickle cell anaemia

**Nonsense mutations:** These mutations create a premature stop codon in the protein sequence. It results in shortened and non-functional proteins. For example, in Duchenne Muscular Dystrophy, a nonsense mutation creates a premature stop signal in the middle of the gene. The resulting protein is too short and cannot function, leading to progressive muscle weakness.

**Why are most mutations harmful?**

Most mutations disrupt the normal functioning of genes and prove harmful. For example;

- A mutation that changes a single amino acid in the haemoglobin protein can lead to sickle cell anaemia, a debilitating and potentially life-threatening disease.
- Mutations can also disrupt gene regulation and can alter developmental processes and contribute to the development of various diseases, such as cancer.
- Some mutations can create non-functional or unstable proteins that can be harmful to the organism e.g., phenylketonuria.

## Genetic Disorders Caused by Point Mutations

The disorders that result from a change in the DNA sequence can be inherited in an autosomal dominant, autosomal recessive, or X-linked manner. The following are two important genetic disorders caused by single-gene mutations.

### 1- Sickle Cell Anaemia

Sickle cell anaemia is due to a recessive gene of abnormal β chain of haemoglobin. This gene is present on chromosome 11. The recessive gene has a point mutation in which the DNA nucleotide T is substituted by A. So, the codon CTC of glutamic acid is converted to CAC, which is for valine. If a child receives both recessive (mutant) alleles, he produces abnormal β chains (with glutamic acid replaced by valine). The abnormal haemoglobin carries lesser than normal oxygen. Due to this abnormal haemoglobin, RBCs also become sickle shaped. Major symptoms of sickle cell anaemia include pain, fatigue, shortness of breath, jaundice, and an increased risk of infections.

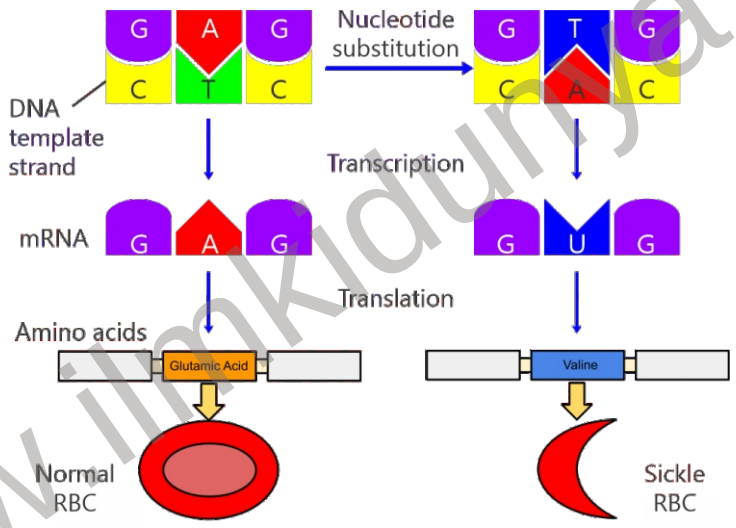



Figure 19.24: Point mutation, causing sickle cell anaemia

The treatments for sickle cell anaemia include pain management with nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids etc. Blood transfusions can help to increase the number of healthy RBCs. Bone marrow transplants can provide a long-term cure. Regular monitoring and preventative measures such as vaccinations and antibiotics can help to prevent complications.

### 2- Phenylketonuria

It is a genetic metabolic disorder. A normal gene present on chromosome 12 produces an enzyme of liver called **phenylalanine hydroxylase** (PAH). This enzyme coverts amino acid phenylalanine into tyrosine. Point mutation (e.g., substitution or

deletion of a nucleotide) makes the allele recessive. If a child inherits both recessive alleles, PAH enzyme is not produced properly and its activity is reduced. As a result, phenylalanine can build up to toxic levels in the blood and other tissues. This condition is called phenylketonuria (PKU).

Major symptoms include intellectual disability, seizures, behavioural problems, mental disorder, microcephaly (smaller head size), and musty odour in breath, skin, and urine. Excessive phenylalanine can also cause brain damage.

Individuals with PKU have to avoid foods that are high in phenylalanine (e.g., meat, dairy products, eggs, and some grains). The blood phenylalanine levels are monitored regularly to adjust the diet. Some medications contain synthetic PAH enzyme. These are used to lower blood phenylalanine levels. Patients are also given nutritional supplements to ensure they are getting enough vitamins and minerals that may be lacking in the low-phenylalanine diet.

**Possible ways to treat genetic diseases**

**Gene therapy:** It involves correcting or replacing the mutated gene with a functional gene.

**Cell therapy:** For example, in type 1 diabetes, the pancreatic beta cells that produce insulin do not function. Cell therapy involves replacing these cells with healthy ones through transplantation.

**Pharmacogenomics:** This involves tailoring drug treatments to an individual's genetic makeup. By analysing a patient's DNA, doctors can identify the best drug for them.

**Genetic counselling:** For individuals with a family history of genetic diseases, genetic counselling can provide valuable information and support. This includes genetic testing, education on the disease, and guidance on family planning and reproductive options.

## 19.8.2- Chromosomal Mutations

These are large-scale mutations and involve changes in the position of genes, or changes in the structure or number of chromosomes. Such mutations are usually spontaneous. The following are the types of chromosomal mutations.

### 1- Changes in Chromosome Structure

**Chromosomal Rearrangements:** Chromosomes can undergo physical alterations. Such changes may involve deletions, duplications, inversions, and translocations. Usually, these changes are caused by a breakage in the DNA double helices at two different locations. In translocations, a segment of one chromosome becomes part of another chromosome. In inversions, the orientation of a portion of a chromosome is reversed.

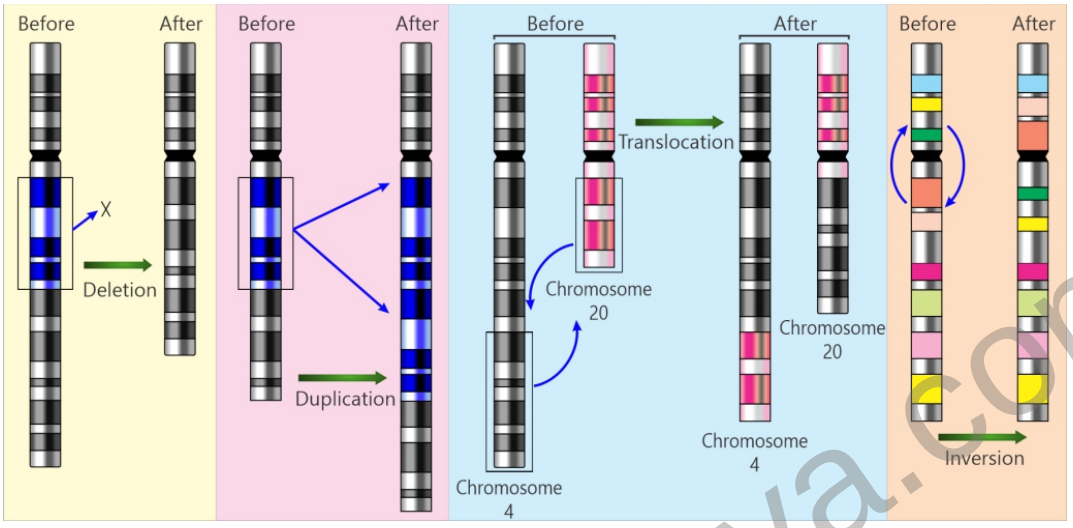



Figure 19.24: Chromosomal rearrangements

**Insertional Inactivation:** Many small segments of chromosomes can move from one location to another in the genome; These mobile bits of DNA are called **transposons** or **jumping genes**. Transposons select their new locations at random. Some transposons are able to be inserted into genes and inactivate the genes. This form of mutation is common in nature. Many human gene disorders are the result of transposition. For example, a human transposon gets inserted into clotting factor IX gene on X chromosome. It places a stop codon in the middle of the gene. It results in X-linked haemophilia.

## 2- Changes in Chromosome Number (Chromosomal Aberrations)

Occasionally, sister chromatids fail to separate properly in meiosis, leading to the gain or loss of a chromosome in a gamete. This condition, called **non-disjunction**, can result in individuals with severe abnormalities if the affected gamete forms a zygote. Nondisjunction can happen in autosomes or in sex chromosomes. The following are the major abnormalities that arise due to non-disjunction.

**Tidbit**

There is a much greater chance of non-disjunction to accumulate over time in eggs than in sperm.

The reason is that all of the future eggs have developed to the point of prophase I by the time a female is born. It means that when she has children, her eggs are as old as she is. In contrast, men produce new sperm daily. Therefore, the age of mother is more critical than that of father.

## 1- Down Syndrome

In this developmental defect, the total chromosome number is 47 (2n+1). It was first described in 1866 by J. Langdon **Down**; that's why it is called Down syndrome (formerly "Down's syndrome"). About 1 in every 750 children have Down syndrome. It is caused by non-disjunction in the autosome pair 21. This non-disjunction can happen during the formation of egg in women. The 21st chromosomal pair fails to segregate properly during meiosis and results in the formation of an egg having 24 chromosomes. The fertilization of such egg by a normal sperm produces a child (male or female) with **trisomy** having 47 chromosomes.


Figure 19.25: Karyotype of Down syndrome

Its symptoms include mental impairment, stunted growth, increased skin on back and neck, flat facial features, small head, short neck and limbs, upward slanting eyes, small ears, poor muscles, a protruding tongue, and heart defects. Males with Down syndrome are usually sterile, while females have lower rates of fertility relative to normal females.

Proper management of affected children can improve the quality of life of people with Down syndrome e.g., screening for common problems, medical treatment where indicated, a good family environment, and special education and proper care.

**Tidbit**

If an XX egg fuses with an X sperm, the female child will have 47 chromosomes with XXX sex chromosomes. This Trisomy in X is known as triple X syndrome. Such females show symptoms of learning disabilities, tall stature, and wide-spaced eyes. They are fertile and puberty starts at normal age.

## 2- Klinefelter Syndrome

If non-disjunction of X chromosome occurs during meiosis in mother, the egg may have both X chromosomes (XX) or may have no sex chromosome (designated as O). If an egg with XX chromosomes is fertilized by a normal sperm, the offspring will have **trisomy** (XXY) in sex chromosomes and the total number of chromosomes will be 47 (2n+1 or 44+XXY). Such non-disjunction may also occur in father during gamete

formation. It can result with a sperm having both X and Y chromosomes i.e., XY. If such sperm fertilizes a normal egg (X) it may result in XXY chromosomes in child. This condition (XXY) is known as Klinefelter syndrome (named after American physician Harry **Klinefelter** who first described it in 1942).

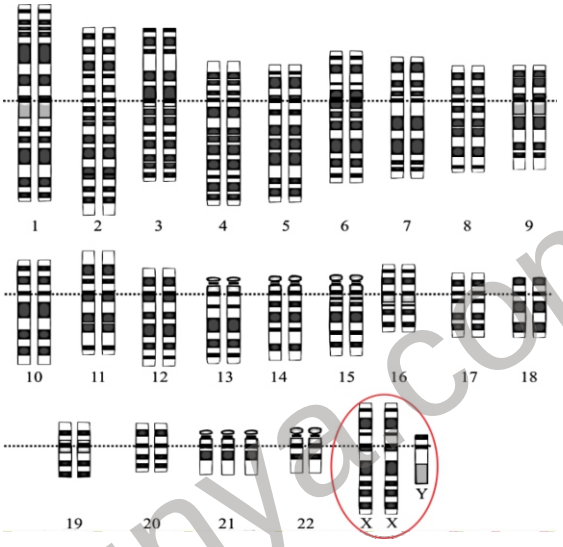



**Figure 19.26: Klinefelter's syndrome karyotype**

The affected children grow to sterile males with many female body characteristics (e.g., less body hair, breast growth), small testicles, and abnormal body proportions (long legs, short trunk, and shoulder equal to hip size). Their voices may not be as deep. The treatments include giving testosterone (to compensate testosterone deficiency at onset of puberty), and surgical removal of breast. Behavioural therapy is given to help in language disorders, difficulties at school, and socialization.

## 3- Turner Syndrome

If an egg with no X chromosome (O) fuses with a normal Y sperm, the resulting OY zygote fails to develop further because humans cannot survive without the genes on X chromosome. On the other hand, if an O egg fuses with a normal X sperm (or a O sperm fuses with a normal X egg), the zygote develops into female baby with 45 chromosomes (XO or 2n-1 or 44+XO) i.e., **monosomy** in sex chromosomes. The affected female is sterile and the condition is called Turner syndrome (American endocrinologist Dr. Henry **Turner** first described it in 1938).

The major symptoms include short stature, webbed neck, immature sex organs and infertility, low mental abilities, and small chin and jaw. Turner syndrome is also associated many health problems, such as liver and kidney issues, obesity, diabetes, and hypertension. Turner syndrome occurs roughly once in every 5000 female births.

Growth hormone treatment can improve growth. The affected female can have adult height, if growth hormone treatment is started early in childhood. Estrogen replacement therapy is used for the development of secondary sexual characteristics.

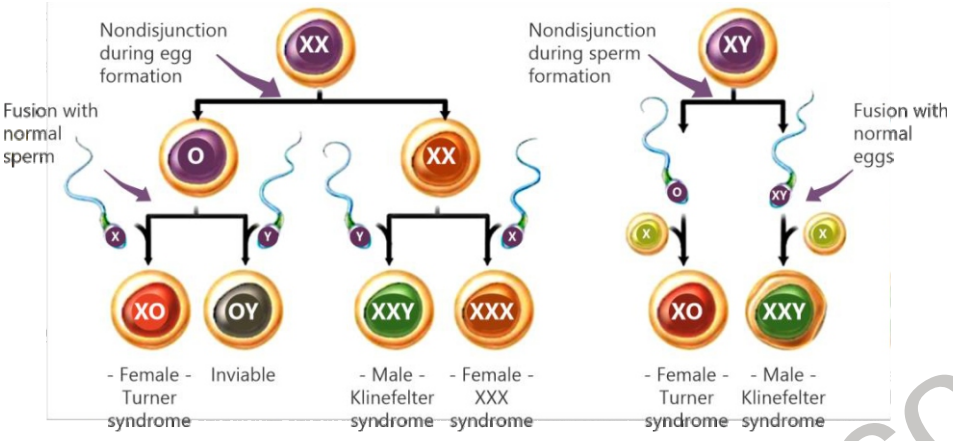



**Figure 19.27: Nondisjunction of sex chromosomes**

**Tidbit**

If a zygote receives one less autosome, it cannot complete development. Most of the zygotes with an extra autosome also do not survive. However, the chromosome pairs 13, 15, 18, 21, and 2 can have three chromosomes and the individual can survive for a time. The presence of an extra chromosome in pairs 13, 15, or 18 causes severe developmental defects, and infants with such a genetic makeup die within a few months.

In contrast, individuals who have an extra copy of chromosome 21 or 22, usually survive to adulthood. In such individuals, the maturation of the skeletal system is delayed, so they generally are short and have poor muscles. Their mental development is also affected.

## EXERCISE

### SECTION 1: MULTIPLE CHOICE QUESTIONS

16. Which of the following looks like beads on a string?

(a) Chromosomes (b) Chromatin
(c) Nucleosomes (d) Heterochromatin

17. The most convincing proof that DNA is the genetic material was provided by;

(a) Mendel (b) Morgan
(c) Hershey & Chase (d) Meselson and Stahl

18. Degeneracy of genetic code means that;

(a) A codon consists of 3 nucleotides
(b) Multiple codons can specify the same amino acid
(c) Same codon can specify multiple amino acids
(d) Each codon is read independently of the adjacent codons

19. How many codons are needed to specify for five amino acids?
(a) 3 (b) 5 (c) 15 (d) 30

20. Which of the following is NOT involved in the initiation of replication?
(a) DNA ligase (b) DNA gyrase
(c) single-stranded binding protein (d) Primase

21. Why DNA polymerase synthesises the leading and lagging strands of DNA in different manners?
(a) The origins of replication occur only at the 5′ end
(b) DNA ligase works only in the 3′ to 5′ direction
(c) DNA polymerase can only work on one strand at a time
(d) DNA polymerase can join new nucleotides only to the 3′ end of strand

22. Synthesis of a new DNA strand usually begins with:
(a) RNA primer (b) DNA primer
(c) DNA ligase (d) Okazaki fragment

23. The elongation of the leading strand during DNA synthesis;
(a) Progresses away from the replication fork
(b) Occurs in the 3′ – 5′ direction
(c) Produces Okazaki fragments
(d) Depends on the action of polymerase

24. Which of the following enzymes remove supercoiling in replicating DNA ahead of the replication fork?
(a) DNA polymerases (b) Helicases
(c) Primases (d) Topoisomerases

25. Which enzyme joins the bits of DNA?
(a) DNA polymerase (b) DNA ligase
(c) Endonuclease (d) Primase

26. Which of these subunits of RNA polymerase is required to initiate transcription?
(a) Alpha (α) (b) Sigma (σ)
(c) Omega (ω) (d) Beta (β)

27. When and where does the splicing of mRNA occur?
(a) Before transcription in nucleus (b) After transcription in nucleus
(c) After transcription in cytoplasm (d)After mRNA attachment with ribosome

28. What happens in mRNA splicing?
(a) Introns and exons are removed from the gene
(b) Introns and exons are joined together
(c) Exons are removed and introns are joined together
(d) Introns are removed and exons are joined together

29. Which of these happens first during protein synthesis?
    (a) Peptide bond formation
    (b) Binding of aminoacyl t-RNA with ribosome
    (c) Translocation
    (d) Binding of release factor with ribosome
30. The synthesis of protein chains is initiated with the amino acid;
    (a) Arginine (b) Methionine (c) Serine (d) Valine
31. The main function of tRNA is to:
    (a) Bind the mRNA with the smaller ribosomal subunit
    (b) Form a portion of ribosomes
    (c) Bind the complementary RNA and DNA strands together
    (d) bring amino acids from the cytoplasm to the ribosomes?
32. Ultraviolet radiation can cause mutations by;
    (a) Making double covalent bonds between adjacent pyrimidine bases
    (b) Breaking phosphodiester bonds in DNA strands
    (c) Adding nucleotides in NA
    (d) Substituting nucleotides in DNA
33. Phenylketonuria is a genetic disorder with a defect in the metabolism of:
    (a) Vitamin (b) Glucose (c) Amino acid (d) Lipid
34. The condition sickle cell anaemia is due to;
    (a) Deletion type point mutation
    (b) Substitution type point mutation
    (c) Insertion type point mutation
    (d) Chromosomal rearrangement

## SECTION 2: SHORT QUESTIONS

17. What are the four types of chromosomes on the basis of position of centromere?
18. What is chromatin fibre? How is it formed during condensation of chromatin?
19. Briefly describe the concept of gene and gene locus.
20. State the functions of DNA polymerase I, II and III.
21. State the central dogma of gene expression.
22. What do you mean by "degeneracy of genetic code"?
23. Enlist some commonly occurring minor mutations in human.
24. Differentiate between:
    - Histone and nucleosome
    - Heterochromatin and euchromatin
    - Conservative and semi-conservative model of DNA replication
    - DNA helicase and DNA gyrase
    - DNA polymerase I and II

- Leading strand and lagging strand of DNA
- Translation and transcription
- Genetic code and codon
- Codon and anticodon
- Gene and allele
- Intron and exon
- Start codon and stop codon
- Non-sense codon and sense codon
- Repressor and activator proteins
- Point mutation and chromosomal mutation
- Down syndrome and Klinefelter syndrome
- Klinefelter syndrome and Tuners syndrome

## SECTION 3: LONG QUESTIONS

15. Annotate the detailed structure of a chromosome.
16. Explain the concept of alleles as the alternative forms of a gene.
17. Narrate the experimental work of Griffith that proved that DNA is the hereditary material.
18. Narrate the experimental work of Hershey and Chase that proved that DNA is the hereditary material.
19. Describe the three models proposed about the mechanism of DNA replication.
20. Narrate the work of Meselson and Stahl to justify the semi-conservative replication.
21. Describe the events of the process of DNA replication.
22. Explain the mechanism of transcription.
23. Describe the mechanism of protein synthesis.
24. Describe the difference between protein synthesis in prokaryotes and eukaryotes.
25. Describe the importance of the regulation of gene expression.
26. Describe the negative and positive control of gene expression.
27. Explain the post-transcriptional modification of mRNA.
28. Write a note on point mutations and their sources.
29. Describe the symptoms, causes and treatments Down syndrome and Klinefelter syndrome.
30. Describe the symptoms, causes and treatments Turner syndrome.
31. Describe the symptoms, causes and treatments of Sickle cell anaemia and phenylketonuria.

## INQUISITIVE QUESTIONS

7. DNA is tightly wrapped around histone proteins. How can replication enzymes read the code if it is buried inside a coil?

8. Our genetic code uses three-letter "words" to create 20 amino acids. Could a complex human exist if the code used only two-letter words?
9. DNA Polymerase III is a highly efficient proof-reader that fixes its own mistakes. What happens to a cell if the gene for the "proof-reader" enzyme itself gets a mutation?
10. Why the length of transcribed mRNA molecule (in eukaryotes) shortens as it enters the cytoplasm for translation?
11. How many types of tRNA molecules are necessary for a living cell, if the genetic code is a triplet code?
12. After isotopic labelling of the starting DNA with15N, Meselson and Stahl continue their experiment to at least two rounds of replication instead of stopping the experiment after only one round of replication. Justify their decision to continue to at least two replications.