Indira Gandhi
National Open University
School of Sciences

BBYET-141
CELL AND MOLECULAR BIOLOGY

Block

3

DNA-BLUEPRINT OF LIFE

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BLOCK 3 : DNA-BLUEPRINT OF LIFE

The Block 3 deals with the biological molecules-DNA, its structure and replication which play an important role in the life of all the living beings on earth. We have three units in this block which deal only with DNA.DNA is the genetic material in most of the organisms but in some viruses RNA is found to be the genetic material. We have described the molecular structure of DNA as a unit of biological information and unit of inheritance. In this block you will follow a logical step –by –step sequence from structure of DNA to the process by which the biological information contained in DNA is released to the cell when it is needed.

Unit 9 deals with the DNA as the main hereditary material in majority of organisms. We have described the amazing structure of DNA- the double helical structure with two helices which are coiled around each other spirally to form double helix. You will also study about how Watson and Crick determined the structure of deoxyribonucleic acid (DNA) and how Rosalind Franklin and Maurice Wilkins used X-ray diffraction technique to deduce the structure DNA .We have also discussed about RNA, which is a single stranded molecule and of three types -messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA). They are present in all organisms. The unit ends with the confirmation of DNA as genetic material which came from the experiments conducted by Frederick Griffith and later by Hershey and Chase. Biochemical evidences also suggested that DNA is the genetic material.

In Unit 10 we have discussed about the finer details of DNA structure, Watson and Crick double helix model and various types of DNA. Special reference has been given to organization of DNA in various cell types viz. bacteria, prokaryotes and eukaryotes. Chloroplast and mitochondria are major organelles found in plant cells. Chloroplasts help in carrying out photosynthesis while mitochondria supply energy within plant cells. Both chloroplasts and mitochondria possess their own genomes and are thought to have been originated from prokaryotes. However the present-day mitochondrial and chloroplast genomes have retained some of the characteristics of their ancestral prokaryotes.

Unit 11 deals with DNA replication or duplication in which DNA molecules are involved in formation of copies of molecules during cell division. It can be conservative, dispersive or semi-conservative. We have dealt with the DNA replication in prokaryotes and eukaryotes replication at length so that you can appreciates how precise, well defined and arduous processes are involved in the replication of DNA

Objectives

After studying this block, you should be able to:

- illustrate the types of genetic material found in living organisms; and endowed with the evidence about DNA as genetic material through transformation experiments;
- appreciate a detailed account of Griffith's and Avery *et.al.* experiments, along with the experiments by Hershey and Chase;
- provide a detailed description of structure of DNA given by Watson and Crick and type of DNA organization found in prokaryotes and eukaryotes ;
- enlist the major features of DNA found in chloroplasts and mitochondria; and
- explain DNA replication in prokaryotes and eukaryotes and enlist various enzymes involved in replication.

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UNIT 9

GENETIC MATERIAL

Structure

9.1 INTRODUCTION

This is the first unit of second volume. Here we will study about the structure of genetic material which is responsible for various activities of living organisms and passing of characters from one generation to other. Cells use different types of molecules to store diverse types of information to keep themselves alive.

Most of you are familiar with nucleic acids which are molecules of great importance to cell because of their role in storage, transmission and expression of genetic information. The two major nucleic acids i.e. DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) play an important role in storage of genetic information and expression of the information via protein synthesis. Both DNA and RNA differ in their chemical nature and functions they perform in the cell. In most of the cells, DNA is present within the nucleus. DNA acts as the genetic material in majority of living organisms. In some organisms such as viruses, RNA is the main hereditary material. In some organisms such as bacteriophages, both DNA as well RNA carry genetic information for the cells.

In this unit we are going to discuss the genetic material of the cell because it carries and stores fundamental information necessary for life of every living

organism. You will be studying about structure of DNA and RNA, their roles in organism and some experiments that provided the evidence that DNA is the hereditary material present in plants. This is an introductory unit for DNA structure.

Objectives

After studying this unit, you would be able to:

- describe the types of genetic material found in living organisms;
- describe the structure of DNA and RNA;
- provide evidence about DNA as genetic material through transformation experiments; and
- appreciate a detailed account of classical experiments by Griffith, Avery and Hershey-Chase.

9.2 TYPES OF GENETIC MATERIAL

Cells contain material or matter that controls inheritance of traits from one generation to the next. It is also able to convey its effect through the formation and functioning of the traits. This material is referred as genetic material. In genetic material, the hereditary information is present in coded form in genes. Thus the genetic material of an individual consists of several thousands of genes. The genetic material possess certain properties such as stability, expressed when required, replicates and is capable of being transmitted from parent to progeny without change. The replicated genetic material must be transferred from a cell to its daughter cells and from one generation to the next. In most of the living organisms, the genetic information is stored in the nucleotide sequences of DNA molecules. Both DNA and RNA carry the genetic information in living organisms. In most of the organisms, DNA is the genetic material, however in some viruses, bacteriophages, RNA has been found as the genetic material. It has been noted that in organisms where DNA is absent, RNA functions as genetic material.

Structure of Nucleic Acids (Polynucleotides)

Nucleic acid molecule is a long chain polymer (polynucleotide) that is composed of monomeric units, called nucleotides. These monomer units of nucleotide are joined by the formation of phosphodiester bonds (a diester bond is one that involves two ester bonds). These phosphodiester bonds are formed between any two neighboring nucleotides.

Each nucleotide consists of three components- nitrogen-containing bases, a five-carbon sugar, and a phosphate group. A phosphate group and nitrogen containing aromatic base is attached to five carbon sugar. The nitrogenous base is either a purine or a pyrimidine. The five-carbon sugar is either a ribose (in RNA) or a deoxyribose (in DNA) molecule. The phosphate is joined by a phosphoester bond to the 5´ carbon of the sugar. The base is attached at the 1´ carbon (Fig.9.1).

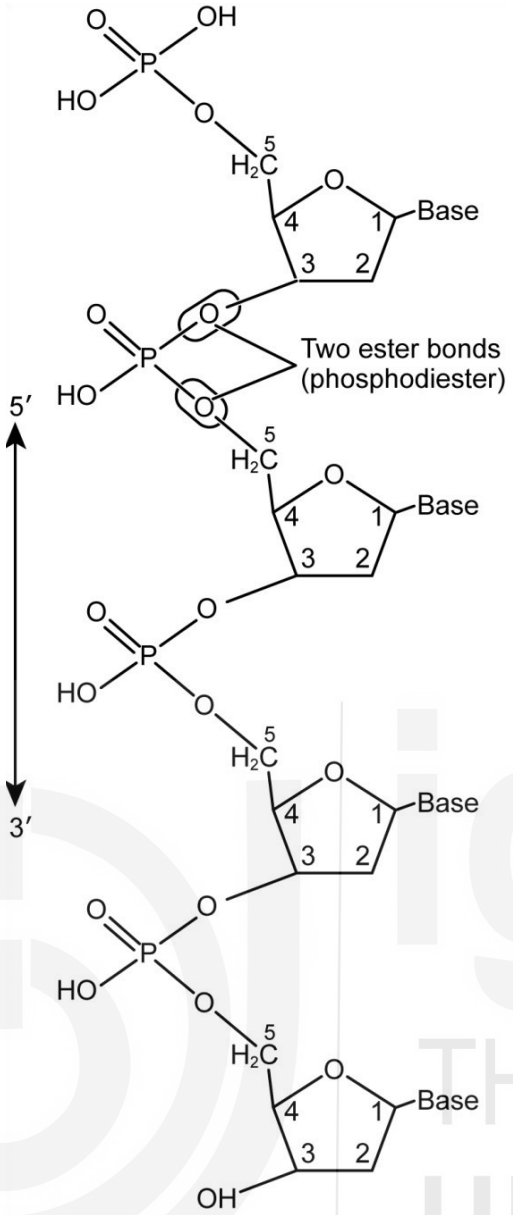


Fig. 9.1: Polynucleotide chain showing phosphodiester bonds.

If we remove phosphate group from the nucleotide, remaining base-sugar is called nucleoside. The purine or pyrimidine thus occurs as free base, the nucleoside or nucleotide (Fig.9.2).

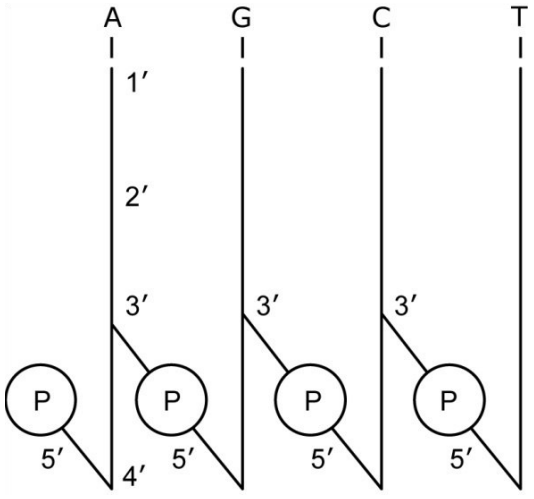


Fig. 9.2: A short-hand notation of a polynucleotide with four nucleotides (1´ to 5´ represent numbers of five carbon atoms of pentose sugar).

The phosphate group is attached by a phosphodiester bond to the 5′ carbon of one nucleotide becomes linked by a second phosphoester bond to the 3′ carbon of the next nucleotide. The condensation of H and OH groups coming from sugar and the phosphate results in linkage called phosphodiester bond. The polynucleotide is formed by such interactions. It shows the presence of 3′ hydroxyl group at one end and 5′ hydroxyl group at the other end.

9.3 DEOXYRIBONUCLEIC ACID (DNA)

As you know that DNA is the genetic material that gets inherited from parent to offspring. Most DNA is located in nucleus of the cell (called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (called mitochondrial DNA). DNA is organized structurally into chromosomes wound around nucleosomes as part of those chromosomes. Functionally, it's organized into genes that carry information for observable traits. Genes are small sections of the long chain of DNA. Gene is considered as functional unit of heredity. Genes possess instructions to make molecules called proteins (Fig.9.3).

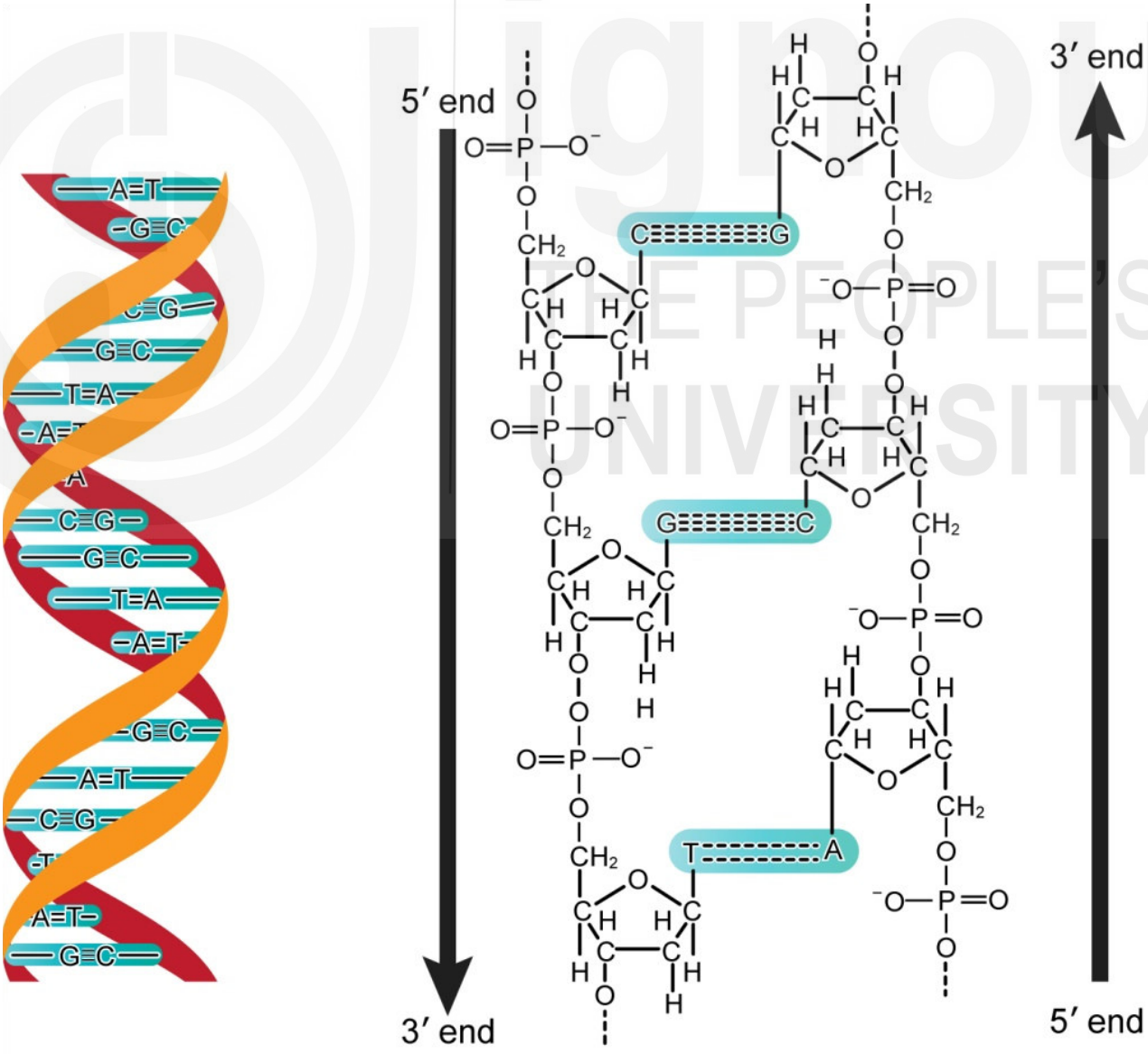


Fig 9.3: Double helix structure of DNA. Chemical structure for three base pair is shown.

DNA is made of 4 types of nucleotides which are arranged variously to form long chain molecules. The DNA molecule consists of two strands that wind around one another to form a shape known as a double helix. Each strand has a backbone made of sugar (deoxyribose) and phosphate groups. To each sugar is attached one of bases - adenine (A), cytosine (C), guanine (G), and thymine (T). The two strands are held together by hydrogen bonds between the bases; adenine (A) binds with thymine (T), and cytosine (C) binds with guanine (G). Each base is attached to a sugar molecule and a phosphate molecule. This pairing results in formation of base pairs. Base, sugar, and phosphate together form a nucleotide. Nucleotides are arranged in such a way that two long strands with their sugar –phosphate are on outside of the helix and their bases are on the inside. This spiral is called a double helix. You can imagine the structure of the double helix like a ladder, with base pairs running through the middle like rings and sugar and phosphate molecules along the outside. Helix is absolutely regular and it can be distinguished by a **major** and **minor** groove.

9.3.1 Discovery of DNA

DNA was first identified in the late 1860s by Swiss chemist Friedrich Miescher. Decades after Miescher's discovery, other scientists-Phoebus Levene, Erwin Chargaff, Albrecht Kossel, Maurice Wilkins and Rosalind Franklin carried out a series of researches that revealed additional details about the DNA molecule, including its primary chemical components and structure. The scientific foundation provided by these pioneers, provided preliminary information to Watson and Crick and they proposed that DNA molecule exists in the form of a three-dimensional double helix in 1953.

The apparent first observation of a substance later identified as nucleic acid was made by **Justus Liebig**, who in 1847 reported the presence of an acidic material in a filtrate obtained from beef muscle, which he then named "**inosinic acid**" (from the Greek word *inos*, fiber, and more specifically those of muscle). Twenty years later **Gregor Mendel** presented the results of his work on inheritance in pea plant, **Friedrich Miescher**, became interested in isolating and characterizing the chemical components present in the nucleus of a cell. He chose leukocytes (white blood cells) isolated from the pus coated surgical bandages. The extract obtained had chemical properties unlike any previously discovered molecule, which had higher phosphorus content. Miescher realized that he had discovered a new substance which he named as **"nuclein"**. Finally, in 1889, **Richard Altmann** succeeded in removing protein associated with nucleins and he named the remaining acidic material as "**nucleic acid**" (***Nucleinsäure*)**. This scientific achievement opened the gateway to investigate nucleic acids apart from their accompanying proteins.

Followed by **Albrecht Kossel**, **Altmann's** used several methods to determine the main chemical components of the nucleic acids. Kossel's found that hydrolysis of nucleic acids produced large quantities of phosphoric acid and specific sets of other molecules depending on the original material used as the source. Such compounds included **purine -adenine, guanine,** and

pyrimidine bases—thymine, uracil, and cytosine. By the end of the century, a variety of nucleic acids with different elemental compositions were known. Two main kinds of nucleic acids were distinguished i.e. the yeast nucleic acid, believed to be typical of plants (also called **"phytonucleic**"), and that obtained from the thymus gland ("thymonucleic", or occasionally "**zoonucleic**"), which was seen as particularly associated to animals. The main differences detected between these two types of nucleic acids (they were -RNA and DNA) were in composition regarding one of the pyrimidine bases (thymine in thymonucleic versus uracil in yeast).

Phoebus Levene, a Russian physician in collaboration with his student **J. A. Mandel** extracted DNA from the thymus. Later **Kossel** identified the base as "thymine." They concluded that linear complexes consist of one of a phosphoric acid, a carbohydrate, and a base that bind in such a way that they form a polyphosphoric acid. The base is probably bound to the sugar moiety in glycosidic form". **Levene** was the first to discover the order of the three major components of a single **nucleotide** (phosphate-sugar-base), carbohydrate component of DNA and RNA. **Levene** proposed that nucleic acids were composed of a series of nucleotides, and that each nucleotide was in turn composed of just one of four nitrogen-containing bases, a sugar molecule, and a phosphate group. He also proposed a **tetranucleotide structure**, in which the nucleotides were always linked in the same order (i.e., G-C-T-A-G-C-T-A and so on).

In 1962 **Watson, Crick, and Wilkins** jointly received the Nobel Prize in Physiology or Medicine for their determination of the structure of deoxyribonucleic acid (DNA). In 1953 **Rosalind Franklin** and **Maurice Wilkins** used X-ray diffraction technique called X-ray crystallography to figure out the structure of DNA. X-rays were beamed through crystals of the DNA molecule and photographic film was used to record the scattering of X rays. The DNA crystals resulted in a cross shape on the X-ray film which is typical of a molecule with a helix shape. They found that DNA molecules are helical with two periodicities along their long axis, a primary one of 3.4 Å and a secondary one of 34 Å.

Box 9.1: Unsung Hero Rosalind Elsie Franklin

Rosalind Elsie Franklin (Fig. 9.4) was born in London, England on 16^{nth} of April 1920. Rosalind Franklin was exceptionally bright and by the age of 15 she knew that she wanted to be a scientist. Her father actively discouraged her interest since it was very difficult for women to have such a career. However, with her excellent education from St. Paul's Girls' School which taught physics and chemistry to girls. Franklin entered Cambridge University in 1938 to study chemistry. When she graduated, Franklin was awarded a research scholarship to do graduate work. She spent a year in R.G.W. Norrish's lab without great success. Norrish recognized Franklin's potential but he was not very encouraging or supportive towards his female student. When offered the position as an assistant research officer at the British Coal Utilization Research Association (CURA), Franklin gave up her fellowship and took the job.

CURA being a young organization, was less firm on the way research was to be done. Franklin worked quite independently, a condition that suited her. Franklin worked for

CURA until 1947 and published a number of papers on the physical structure of coal. Then she moved to Paris for doing research work. An old friend introduced her to Marcel Mathieu who directed most of the research in France. He was quite impressed with Franklin's work and offered her a job as a "chercheur" in the Laboratoire Central des Services Chimiques de l'Etat. There she learned X-ray diffraction techniques from Jacques Mering.

In 1951, Franklin was presented with a 3-year research scholarship at King's College. Franklin began working as a research associate in the biophysics unit, where director John Randall used her expertise and X-ray diffraction techniques (mostly of proteins and lipids in solution) on DNA fibers. Here Maurice Wilkins was already using X-ray crystallography to try to solve the DNA problem at King's College. Studying DNA structure with X-ray diffraction, Franklin and her student Raymond Gosling made an amazing discovery: They took pictures of DNA and discovered that there were two forms of it, a dry "A" form and a wet "B" form. One of their X-ray diffraction pictures of the "B" form of DNA, known as Photograph 51, became famous as critical evidence in identifying the structure of DNA. The photo was acquired through 100 hours of X-ray exposure from a machine Franklin herself had refined. From this she deduced the basic dimensions of DNA strands, and that the phosphates were on the outside of what was probably a helical structure.

Fig 9.4: Rosalind Franklin (1920-1958).

John Desmond Bernal, United Kingdom's one of the most well-known and controversial scientists and a pioneer in X-ray crystallography, spoke highly of Franklin around the time of her death in 1958. "As a scientist Miss Franklin was distinguished by extreme clarity and perfection in everything she undertook," he said. "Her photographs were among the most beautiful X-ray photographs of any substance ever taken. Their excellence was the fruit of extreme care in preparation and mounting of the specimens as well as in the taking of the photographs."

She presented her data at a lecture in King's College that James Watson was attending. In his book *The Double Helix*, Watson admitted to not paying attention at Franklin's talk and not being able to fully describe the lecture and the results to Francis Crick. Watson and Crick were at the Cavendish Laboratory and had been working on solving the DNA structure. Franklin did not know Watson and Crick as well as Wilkins did and never truly collaborated with them. It was Wilkins who showed Watson and Crick the X-ray data Franklin obtained. The data confirmed the 3-D structure that Watson and Crick had theorized for DNA.

Despite her cautious and diligent work ethic, Franklin had a personality conflict with colleague Maurice Wilkins, one that would end up costing her greatly. In January 1953, Wilkins changed the course of DNA history by disclosing without Franklin's permission or knowledge her Photo 51 to competing scientist James Watson, who was working on his own DNA model with Francis Crick at Cambridge.

Upon seeing the photograph, Watson said, "My jaw fell open and my pulse began to race," according to author Brenda Maddox, who in 2002 wrote a book about Franklin titled *Rosalind Franklin: The Dark Lady of DNA*.

The two scientists did, in fact, use what they saw in Photo 51 as the basis for their famous model of DNA, which they published on March 7, 1953, and for which they received a Nobel Prize in 1962. Crick and Watson were also able to take most of the credit for the finding: When publishing their model in *Nature* magazine in April 1953, they included a footnote acknowledging that they were "stimulated by a general knowledge" of Franklin's and Wilkins' unpublished contribution, when in fact, much of their work was rooted in Franklin's photo and findings. Randall and the Cambridge laboratory director came to an agreement and both Wilkins' and Franklin's articles were published second and third in the same issue of *Nature*. Still, it appeared that their articles were merely supporting Crick and Watson's model.

Franklin left Cambridge in 1953 and went to the Birkbeck lab to work on the structure of tobacco mosaic virus. She published a number of papers on the subject and she actually did a lot of the work while suffering from cancer. She died from cancer in 1958. In the fall of 1956, Franklin discovered that she had ovarian cancer. She continued working throughout the following two years, despite having three operations and experimental chemotherapy. She experienced a 10-month remission and worked up until several weeks before her death on April 16, 1958, at the age of 37.

In 1962, the Nobel Prize in Physiology or Medicine was awarded to James Watson, Francis Crick, and Maurice Wilkins for solving the structure of DNA. The Nobel committee does not give posthumous prizes.

9.3.2 Structure of DNA

DNA was first crystallized in the late 70's -the 1953 X-ray data were from DNA fibers. So, the real "proof" for the Watson-Crick model of DNA came in 1982 after the B-form of DNA was crystallized and the X-ray pattern was solved.

In 1953 **J.D. Watson** and **F.H.C. Crick** (Fig. 9.5) proposed a double helical structure of DNA. According to them the two polynucleotide chains are connected by hydrogen bonds and running in opposite directions. In a DNA double helix two polynucleotide chains are coiled about the same axis in such a manner that they can separate from one another only be uncoiling. Bases are present in a plane at right angle to the long axis.

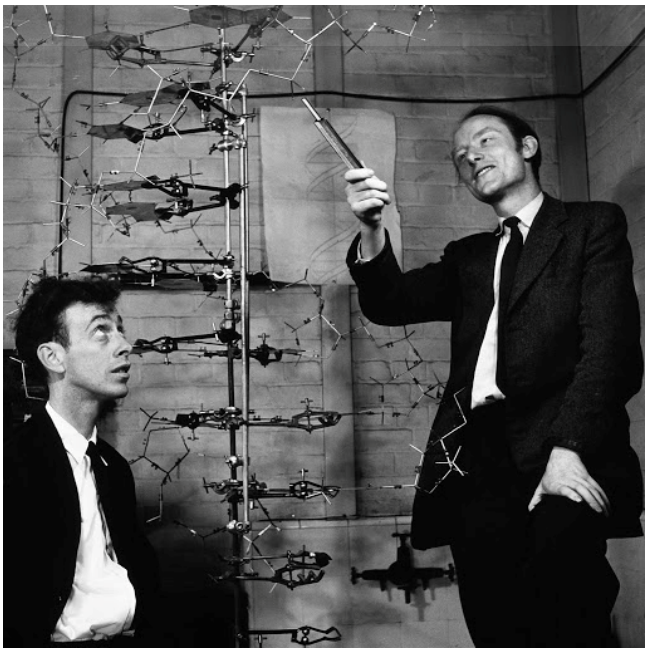


Fig 9.5: J.D. Watson and F.H.C. Crick

DNA is a stable molecule. Two polynucleotide chains which run in opposite directions have complementary base sequences.

You will realize that one most important feature to know that within the helix an adenine is always adjacent to thymine in the other strand and similarly guanine is always adjacent to cytosine in one polynucleotide. This means adenine is linked to thymine and cytosine to guanine. The distance between two base pairs is 3.4 Å and there are 10 base pairs in each turn. The molecule has a diameter of 20 Å in the molecule. Purines which are adenine and guanine need a little higher space as compared to two pyrimidines i.e. cytosine and thymine. When a pyrimidine always pairs with a purine, space could remain constant (Fig. 9.6). Normally they are written as A, G, C and T (Fig.9.7).

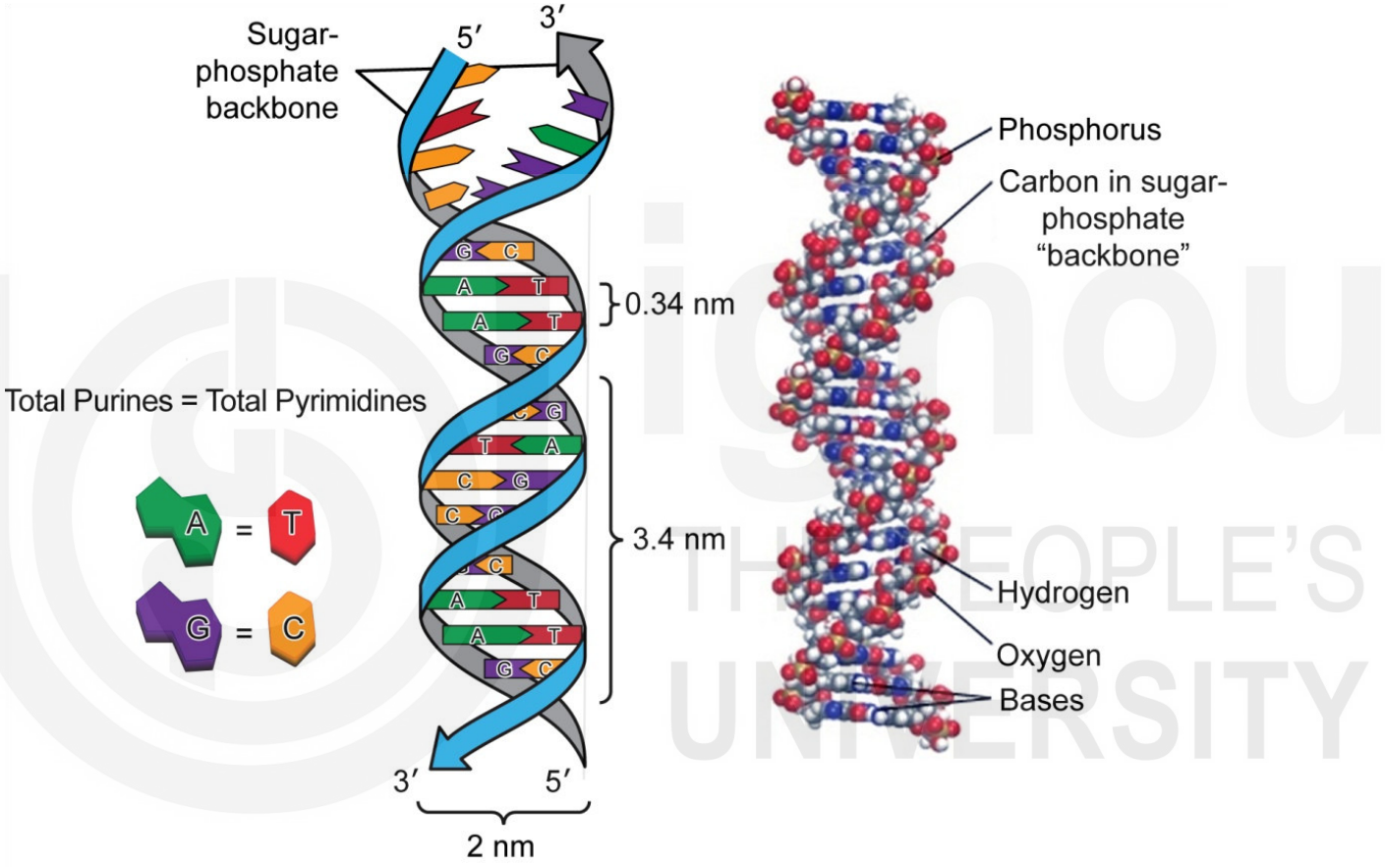


Fig. 9.6: A double helix model of DNA as proposed by Watson and Crick.

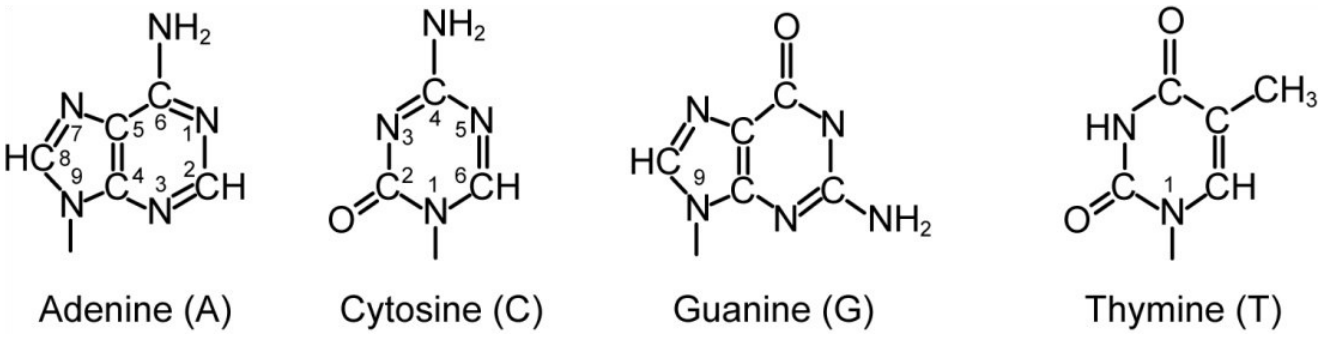


Fig. 9.7: Chemical structure of purines and pyrimidines.

Erwin Chargaff, an Austrian biochemist, expanded Levene's work and found additional information of the structure of DNA. Chargaff found that nucleotide composition of DNA varies among species. He concluded that almost all DNA-no matter what **organism** or tissue type it comes from but maintains certain

properties; even as its composition varies. Using paper chromatography, he found that the amount of adenine (A) is usually similar to the amount of thymine (T), and the amount of guanine (G) usually approximates the amount of cytosine (C). In other words, the total amount of purines (A + G) equals the total amount of pyrimidines (C + T) (known as "**Chargaff's rule**"). All DNA follow Chargaff's Rule that states that the total number of purines in a DNA molecule is equal to the total number of pyrimidines.

$$A+T=G+C$$

The analysis of DNA obtained from different organs of body of the same individual or from different individuals belonging to the same species showed no differences in the relative composition of different bases. The studies suggested that:

- Number of adenine molecules is equal to number of thymine molecules.
- Number of cytosine molecules is equal to number of guanine molecules.

Chargaff's rule may be represented as follow:

A=T: G=C; A+T= G+C

A+G=T+C

A+G/T+C=1; But

A+T ≠ G+C

i.e., A=T/G+C = constant

For example, this ratio is 0.4 for *Bacillus* whereas human DNA has the A:G ratio of 1.56

SAQ 1

a) Fill in the blanks:

i) DNA consists of …….. types of nucleotides.

ii) DNA is structurally organized into …….. .

iii) Justus Liebig isolated a substance from beef muscle and named it as …….. .

iv) Miescher discovered a new substance and named it as …….. .

b) Write the full form of the following:

A, G (purines) and C, T (pyrimidines)

9.4 RIBONUCLEIC ACID (RNA)

Generally RNA is formed from DNA during transcription. It carries genetical instructions that determine the sequence of amino acids in protein formed during translation. RNA is found as single stranded molecule. RNA consists of ribose nucleotides attached by phosphodiester bonds. The four bases present

in RNA, are adenine, guanine, uracil and cytosine. In RNA uracil is found instead of thymine. Chemical analysis also showed that polynucleotide chains of RNA contain ribose sugar instead of deoxyribose (Fig .9.8).

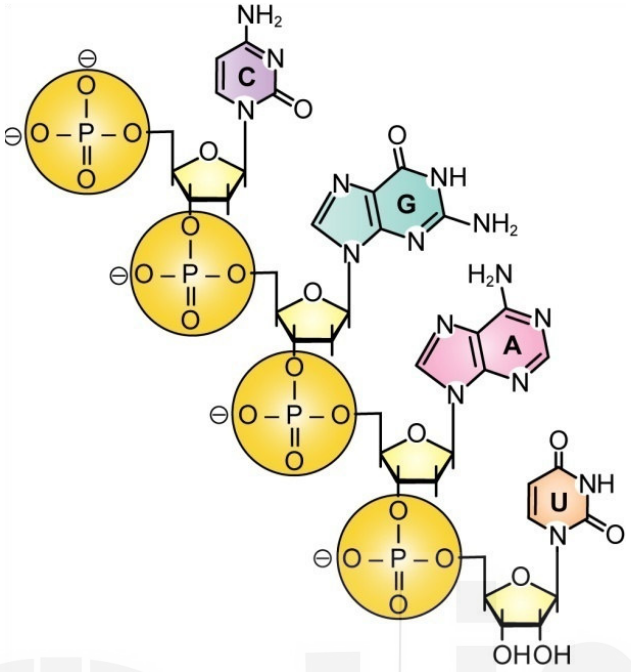


Fig: 9.8: Chemical structure of RNA.

The self-complementary sequences in the RNA strand base pair with bases present in the other strand (intrachain base-pairing) which results in folding of the ribonucleotide chain into complex structural forms consisting of bulges and helices (Fig.9.9). The three-dimensional structure of RNA is critical for its stability and function, allowing the ribose sugar and the nitrogenous bases to be modified in numerous different ways by cellular enzymes that attach chemical groups (e.g., methyl groups) to the chain. Such modifications facilitate the formation of chemical bonds between distant regions in the RNA strand, leading to complex contortions in the RNA chain, which further stabilizes the RNA structure. RNAs can also form complexes with molecules known as ribonucleoproteins (RNPs).

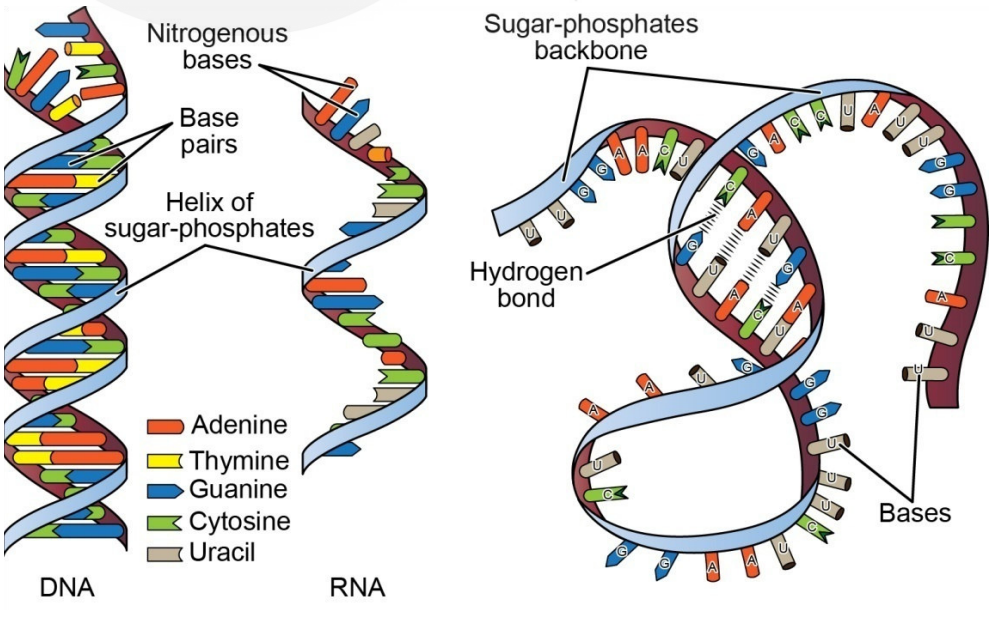


Fig 9.9: a) Comparison of DNA and RNA; b) Single strand of RNA.

Depending upon their presence in the plant and nature, RNA is of two types, namely genetic RNA and non-genetic RNA.

Genetic RNA- In most of the plant viruses, RNA is the genetic material. Such RNA contains information which is normally found in DNA. In other words, RNA has replaced DNA in such cases. The RNA present in these viruses could be single stranded or double stranded. In most of the bacteriophages, RNA is the genetic material (see Table 9.1). **Viroids** and **virusoids** are other two classes of small RNAs (~350 bases) found in plants. Viroids function independently without encapsidation by a protein coat, while Virusoids (also called **satellite RNAs**) are encapsidated by plant viruses packaged together with a viral genome. Virusoids cannot replicate independently, and need an association with the virus. The double stranded RNA follows the same rules of base pairing as DNA.

Non-genetic RNA- In some organisms, genetic information is contained in RNA which gets transmitted only through DNA. Although RNA is present in good quantity, it does not serve as a genetic material. This type of RNA is known as non-genetic RNA. Such a non-genetic RNA is synthesized on DNA template.

The non-genetic RNA species found in cells for specific functions include: i) **Anti-sense RNA** also called **mic RNA** (messenger RNA inhibiting complementary RNA) is synthesized sometimes on the strand complementary to the one used for mRNA synthesis. This is used for regulation of DNA synthesis and gene expression both *in vivo* and *in vitro;* ii) **HnRNA** is synthesized from split genes in eukaryotes.

Table 9.1: Different RNA viruses and the nature of RNA associated with them.

Virus	**Types of RNA**
Plant Viruses	
Mosaic virus (MV)	Single stranded
Wound	Double stranded
Animal Viruses	
Influenza virus	Single stranded
Rous sarcoma	Single stranded
Poliomyelitis	Single stranded
Reovirus	Double stranded
Bacteriophages	
MS2, F2, r17	Single stranded

On the basis of functioning, RNA has been classified into three types:

- Messenger RNA (mRNA),
- Transfer RNA (tRNA),
- Ribosomal RNA (rRNA)

Messenger RNA (mRNA) - This type of RNA carries genetic information contained in DNA from the DNA in the nucleus to ribosomes (sites of protein synthesis or translation). It constitutes a small fraction (5% - 10%) of total RNA present in the cell. It is synthesized in the cell nucleus and then transported out of the cell to facilitate protein synthesis and code sequencing on proteins. This type of RNA is rather short-lived. The molecular weight of this RNA ranges from 500 to 2000 kDa.

Ribosomal RNA (rRNA) - This is the most stable kind of RNA and is associated with the ribosomes. They constitute about 60 to 80% of the total RNA is the cell. rRNA is the part of the ribosomes. It catalyzes the synthesis of proteins. It plays a major part in the binding to mRNA, recruitment of tRNA, and catalysis of peptide bond formation between amino acids.

Transfer RNA (tRNA) - This is also known as soluble RNA (sRNA). It makes another small fraction (10-15%) of RNA. It is a small RNA chain of about 80 nucleotides. These are the smallest molecules of RNA and work as adapter molecules for carrying amino acid molecules to the site of protein synthesis. The non-coding RNA molecule acts as the intermediate between nucleotide and amino acid sequences.All these three types of RNA are found in all organisms (Fig. 9.10).

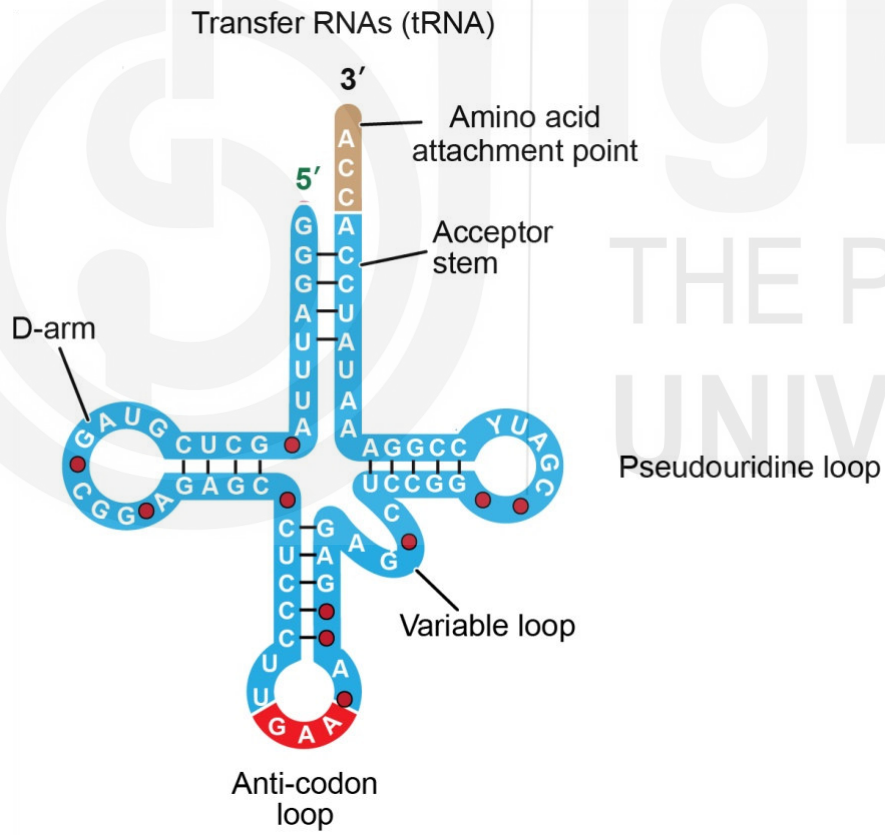


Fig 9.10: A clover-leaf model of t RNA.

Apart from above described RNA some other RNA molecules are also found in the cell.

Small Nuclear RNA (snRNA) mediates the processing of primary transcripts (Protein sequences) in the nucleus to produce functional elements which then are exported to the cytosol during DNA transcription.

Small nucleolar RNA (snoRNA) are small RNAs (about 60-300 nucleotides) found in the cell nucleolus that play a role in the synthesis of ribosomes.

MicroRNAs (miRNAs) is a small non-coding RNA that is single-stranded, containing only 22 nucleotides. These are found in plants, all animals, and some viruses and play a role in RNA silencing and post-transcriptional gene expression regulation.

You can comprehend the basic differences between DNA and RNA by going through Fig. 9.11.

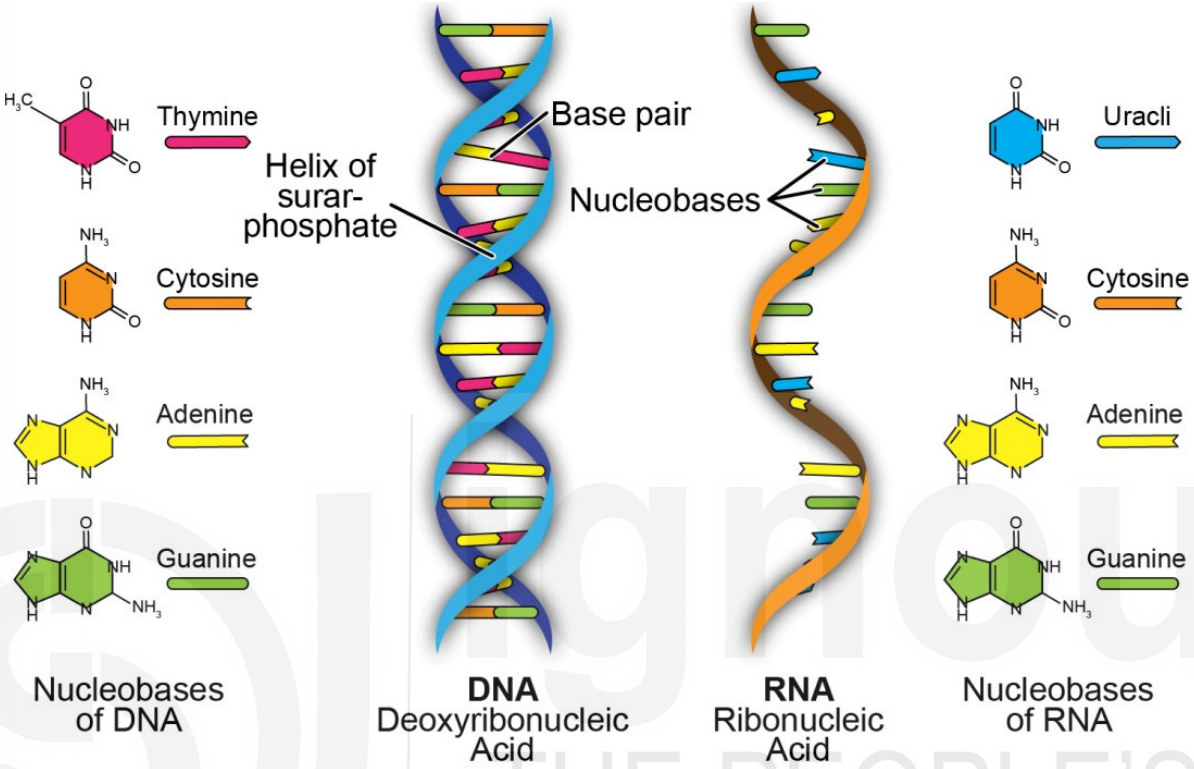


Fig.9.11: Comparison of DNA and RNA structure.

SAQ 2

a) State whether following statements are True or False:

i) The four bases present in RNA, are adenine, guanine, uracil and cytosine.

ii) RNA contain ribose sugar instead of deoxyribose.

iii) RNAs are unable to form complexes with protein molecules known as ribonucleoproteins (RNPs).

iv) Viroids and virusoids are other two classes of small RNAs (~350 bases) found in plants.

v) In most of the plant viruses, RNA is the genetic material.

b) Draw a fine structure of RNA.

c) Write the full form of the following:

i) snRNA

ii) snoRNA

iii) miRNA

9.5 TRANSFORMATION EXPERIMENTS FOR CONFIRMATION OF DNA AS GENETIC MATERIAL

By the early twentieth century, scientists knew that chromosomes contain genes, the bearers of hereditary information. They also knew that chromosomes are made of DNA and proteins. But it took the first fifty years of the twentieth century and many scientists to discover that DNA is the genetic material. The evidence for this is given below. A series of experiments were conducted in the 1920s which finally established that DNA is the genetic material. We are going to describe these experiments below.

9.5.1 Griffith's Experiment

You already know that Fredrich Miescher in 1898 isolated an acidic chemical from nuclei of pus cells which he termed as nuclein. In 1928, British physician Frederick Griffith while studying the pathogenic strain of bacterium called pneumococcus (causes pneumonia) discovered that the bacterium (*Streptococcus pneumoniae*) exists in two forms called **S strain** and the **R strain**. The S strain produces smooth and shiny colonies when grown on solid agar medium. This is because of mucous, polysaccharide coat secreted by the cell. The R strain lacks the ability to produce mucous coat, therefore produces colonies which are rough. When the S strain is injected into mice, the bacterium triggers a fatal pneumonia. The disease is caused because S strain had a polysaccharide coat which protects the bacterial cell from attack by the mouse's immune system. When R strain is injected into mice, the bacterium is not able to cause pneumonia. He suggested that since R stain lacks polysaccharide coat hence bacterial cell gets attacked by mouse's immune system. The injecting of live R- strain or dead S-strain alone did not caused disease in mice.

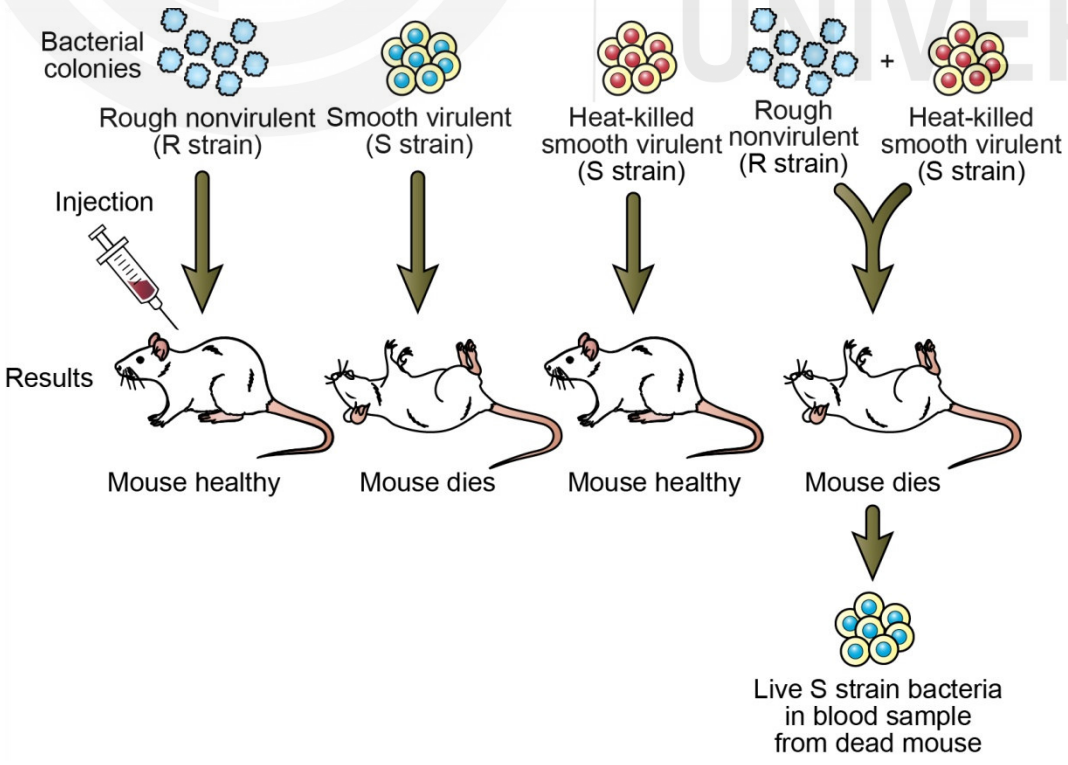


Fig 9.12: Griffith's bacterial transformation experiment.

The injection of heat killed S strain did not induce disease. The pneumonia was caused when mice was injected with a mixture of live R strain and dead S strain. Griffith autopsied animals that were injected with the mixture of live R and dead S strain. He concluded that non pathogenic strain somehow converted into pathogenic S strain by a substance present in the heat killed S strain. He called the phenomenon as **genetic transformation** (Fig. 9.12). The active substance responsible for inducing pathogenicity was termed as '**transforming principle'**. The chemical factor from heat killed S strain was able to cause heritable change of non-pathogenic R stain into pathogenic S strain.

9.5.2 Avery's Experiments

Later on Oswald Avery, Colin McLeod and Maclyn Mc Carty in 1944 pursued further investigations and provided evidence that the transforming substance in heat killed S strain of *Pneumococcus* was nucleic acid of DNA and not something residing in the cell wall. They separated and tested the molecules found in the debris of the dead S cells for transforming ability one by one. The tests showed that the polysaccharides themselves do not transform the rough cells. Therefore, the polysaccharide coat, although undoubtedly concerned with the pathogenic reaction, is only the phenotypic expression of virulence. Avery et al. found that only one class of molecules, DNA, induced the transformation of R cells. They deduced that DNA is the agent that determines the polysaccharide character and hence the pathogenic character. This gave the first confirmation and evidence that DNA carries the genetic information and is the main hereditary material found in living organisms.

9.5.3 Hershey-Chase Bacteriophage Experiment

Bacteriophages are the viruses that infect bacteria. Bacteriophage T2 infects bacterium ***Escherichia coli*** (*E. coli*). During infection, the virus attaches to the bacterial cell surface and injects material into the cell. The bacterial cell begins to produce thousands of new copes of the virus. The material injected into the bacterial cell carries the genetic information that guides the production of the virus. In 1952, Alfred Hershey and Martha Chase designed an experiment to know that T2 virus is made up of two kinds of molecules DNA and proteins (Fig 9.13). The proteins of the T2 virus contain elemental sulfur (present in amino acids methionine and cysteine) and not phosphorus, while the DNA contains phosphorus (present as sugar phosphate backbone) but not sulfur. They prepared two batches of T2 phage particles with different radioactive labelling. In one batch the phage proteins were labelled with the radioactive isotope ^{35}S and in the other batch the phage DNA was labelled with isotope ^{32}P. By radioactive labelling Hershey and Chase were able to trace the fate of protein and DNA during infection process.

They started the experiment by mixing the radioactive phage with intact bacterial cells. They allowed phage particles to attach to the bacterial cell surface and let them inject their genetic material into the cells. Then they removed protein coats from the surface of the bacterial cells by agitating the suspension in a blender and recovered the bacterial cells by centrifugation. They measured radioactivity in the supernatant liquid and in the pellet of bacteria at the bottom of the tube. The studies showed that most of the (65%) of the ^{32}P remained in the bacterial cells while the bulk of the ^{35}S was released

in the surrounding medium. Since ^{32}P labelled the viral DNA and ^{35}S labelled the viral protein, they concluded that DNA had been injected into the bacterial cells and functions as the genetic material of phage T2.

It is important to know that when the ^{32}P-labelled phages were used in the experiment, the majority of the radioactivity was found inside the bacterial cells, which indicates that the phage DNA has entered the cells. This ^{32}P can also be recovered from phage progeny. But when the ^{35}S-labelled phages were used in the experiment, most of the radioactive material was found in the phage ghosts, thus it indicates that the phage protein had never entered the bacterial cell. Thus it was concluded that DNA is the hereditary material and the phage proteins are simply structural packaging which is discarded after delivering the viral DNA to the bacterial cell.

When infected radioactively labelled bacteria were suspended in fresh liquid and incubated for longer time, the ^{32}P was transferred to some of the offspring phage particles. This gave the view that genes are made up of DNA.

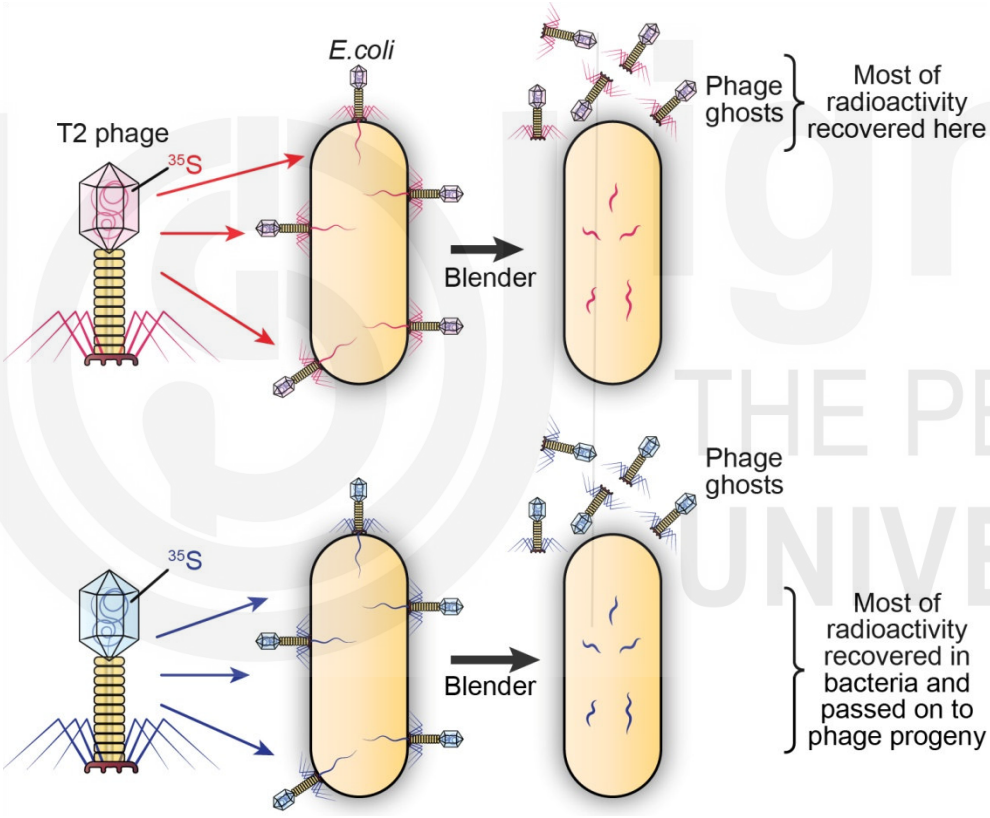


Fig 9.13: Experiment of Hershey and chase to demonstrate DNA as the hereditary material. a) The protein coat is labelled with radioactive sulfur (^{35}S), not found in DNA. b) The DNA is labelled with radioactive phosphorus (^{32}P), not found in protein. Only the ^{32}P is injected into the *E. coli,* indicating that DNA is the agent necessary for the production of new phages.

Apart from experimental evidence, biochemical evidence due to certain facts such as those given below confirmed that DNA is the genetic material.

i) Amount of DNA in every cell of an organism is the same.

ii) Amount of DNA in gametes is half that of DNA present in somatic cells.

iii) Amount of DNA is the same in all members of same species.

iv) Amount of DNA in a cell is constant and does not change by internal or external environment.

v) DNA or amount of genetic information is proportional to evolutionary complexity.

SAQ 3

a) State whether following statements are 'True' or 'False' for **Griffith experiment.**

i) In his experiment he discovered that the bacterium (*Streptococcus pneumoniae*) exists in two forms called S strain and the R strain

ii) In his experiment he found that R strain lacks the ability to produce mucous coat but can produce pneumonia.

iii) In his experiment he found that S strain has the ability to produce mucous coat but was unable to produce pneumonia.

iv) In his experiment he found that injecting of live R- strain or dead S-strain alone did not caused disease in mice.

b) Only through diagram show Hershey-Chase Bacteriophage experiment.

9.6 SUMMARY

- DNA acts as the genetic material in majority of living organisms except in some viruses where RNA is present as the main hereditary material. In some organisms such as bacteriophages, both DNA as well RNA carry genetic information for the cells.
- Nucleic acid molecule is a long chain polymer (Polynucleotide) composed of monomeric units called nucleotides. These monomer units of nucleotide are jointed by the formation of phosphodiester bonds. Each nucleotide consists of three components - nitrogen-containing bases, a five-carbon sugar, and a phosphate group. A phosphate group and nitrogen containing aromatic base is attached to five carbon sugar. The nitrogenous base is either a purine or a pyrimidine.
- DNA is a double helical structure with two helices of the nucleotide chains coiled around each other spirally to form double helix. The structure of the double helix is like a ladder, with base pairs running through the middle like rings and sugar and phosphate molecules along the outside.
- Levene was the first to discover the order of the three major components of a single nucleotide (phosphate-sugar-base), carbohydrate component of DNA and RNA. He proposed that nucleic acids were composed of a series of nucleotides. Watson and Crick determined the structure of deoxyribonucleic acid (DNA). Rosalind Franklin and Maurice Wilkins used X-ray diffraction technique to deduce the structure of DNA.

- RNA is a single stranded molecule. It consists of ribose nucleotides attached by phosphodiester bonds. The four bases present in RNA are adenine, guanine, uracil and cytosine. Three types -messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA) are present in all organisms.
- The confirmation of DNA as genetic material came from the experiments conducted by Frederick Griffith using the pathogenic strain of bacterium called *Streptococcus pneumoniae* and Hershey and Chase using bacteriophages particularly bacteriophage T2 that infects bacterium *Escherichia coli* (*E.coli*).
- Biochemical evidences also suggested that DNA is the genetic material.

9.7 TERMINAL QUESTIONS

1. Describe briefly the history of discovery of DNA.
2. What is the Chargaff rule? Discuss its importance.
3. Differentiate between genetic and non genetic RNA.
4. Describe the first experiment which confirmed that DNA carries genetic information.
5. How did Avery, McLeod and McCarty's experiment on bacterial transformation provide evidence for DNA being the genetic material?
6. Write short notes on the following:
   i) Types of RNA based on functioning
   ii) Structure of Nucleic acids
   iii) Genetic RNA.

9.8 ANSWERS

Self-Assessment Questions

1. a) i) 4
      ii) Chromosomes
      iii) inosinic acid
      iv) nuclein
   b) A=Adenine G= Guanine, C= Cytosine and T= Thiamine
2. a) i) True; ii) True; iii) False; iv) False; v) True
   b) Refer to Fig.9.10
   c) i) snRNA =Small Nuclear RNA
      ii) snoRNA= Small nucleolar RNA
      iii) miRNA =MicroRNAs

3. a) i) True; ii) False; iii) False; iv) True

   b) Refer to Fig 9.13

Terminal Questions

1. Refer to Subsection 9.3.1
2. Refer to Subsection 9.3.2
3. Refer to Section 9.4
4. Refer to Subsection 9.5.1
5. Refer to Subsection 9.5.2
6. i) Refer to Section 9.4

   ii) Refer to Section 9.3.2 and 9.4

   iii) Refer to Section 9.4

Acknowledgement

Fig.9.4 : Source: https://d2cbg94ubxgsnp.cloudfront.net/Pictures/480xAny/6/3/1/84631_feature-dna_fig2_300.jpg

Fig. 9.5 : Source: https://lh3.googleusercontent.com/proxy/VPBqjLBFhJg2Tc59r-ebeZqLaNc-

Fig. 9.10 : Source: https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fcdn.lecturio.com%2Fassets%2FTransfer-RNAs-tRNA-e1614263850807-1200x709.png&imgrefurl=

Fig.9.11 : https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fcdn.technologynetworks.com%

Fig. 9.12 : https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fdigitalcommons.rockefeller.edu%2Ftransforming-principle-dna%

Fig 9.13 : From: W. H. Freeman; 2000.DNA: The genetic material, An Introduction to Genetic Analysis. 7th edition. New York:

UNIT 10

DNA

Structure

10.1 INTRODUCTION

In the previous units you have studied that DNA is responsible for transfer of genetic information from an organism to its offsprings during cell division. You also know that within the nucleus, DNA present in the chromatin material possesses the genetic information. The details about various components of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) have been provided to you in the previous unit. The study carried out by Griffith, and later by Hershey and Chase proved that DNA is the main genetic material found in living organisms and carries the hereditary characters from one generation to another. The present unit discusses about the structure of DNA, its various forms and provides an elaborate account about organization of DNA in prokaryotes and eukaryotes. You will also be studying about the DNA present in mitochondria and chloroplasts in this unit.

Objectives

After studying this unit, you would be able to:

- provide a detailed description of structure of DNA given by Watson and Crick;
- describe various types of DNA;

- describe the type of DNA organization found in prokaryotes and eukaryotes; and
- enlist the major features of DNA found in mitochondria and chloroplasts.

10.2 STRUCTURE OF DNA

You have studied in previous unit that DNA is the genetic material found in nucleus of the cell. It is a double helical structure made up of two polynucleotide chains coiled around one another. Each strand of DNA has a backbone made of sugar (deoxyribose) and phosphate groups. The two strands are held together by bonds between the bases. Each base is attached to a sugar molecule and a phosphate molecule. This pairing between the bases of two strands leads to formation of base pairs. The structure formed by joining together of base, sugar and phosphate together is called a nucleotide. In a double helix, the base pairs are present in the middle while sugar and phosphate molecules are present towards the outer side.

10.2.1 Watson and Crick Double Helix Model

DNA is made up of nucleotides which possess three parts: a deoxyribose (5-carbon sugar), a phosphate group, and a nitrogenous base. The nitrogenous bases in DNA are of four types -adenine (A), guanine (G) which are double-ringed purines, and cytosine (C), thymine (T) which are small single-ringed pyrimidines. The phosphate group of one nucleotide bonds covalently with the sugar molecule of the next nucleotide. This results in formation of long chain of nucleotides. The sugar–phosphate groups form the backbone of each strand of DNA. The sugar-phosphate bonds in this backbone are called **phosphodiester bonds** (Fig. 10.1). The base pairs are held together by specific hydrogen bonds.

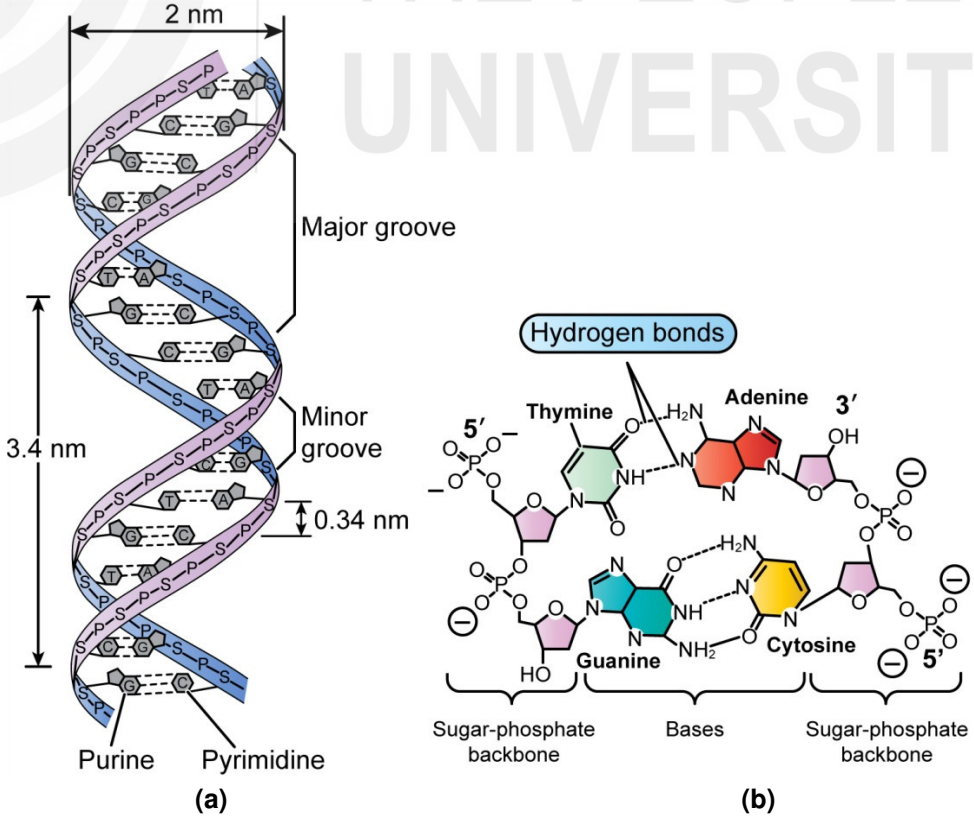


Fig. 10.1: Diagrammatic representation of a) DNA double helix; and b) sugar phosphate backbone formed in nucleotide.

The phosphate group is attached to the 5′ carbon of one nucleotide and the 3′ carbon of the next nucleotide. Hydrogen bonds formed between the bases assist in holding together the two strands in a DNA molecule. The two strands of DNA are twisted around each other to form a right-handed helix, called a double helix.

Pairing takes place between purine and pyrimidine bases. Adenine pairs with thymine and guanine pairs with cytosine. Adenine and thymine are complementary base pairs. Similarly cytosine and guanine are also complementary base pairs. This feature of DNA is known as **complementarity**. The quantity of adenine is equal to thymine and quantity of guanine is equal to cytosine in a DNA molecule. Adenine and thymine are connected by two hydrogen bonds, and cytosine and guanine are connected by three hydrogen bonds. The base sequence of one polynucleotide chain determines the base sequence of the opposite chain. The two chains are therefore considered to be complementary to each other.

These two strands are anti-parallel in nature. One strand has 3′ carbon of the sugar in the upward position, whereas the other strand will have the 5′ carbon in the upward position. The diameter of the DNA double helix remains uniform throughout the structure because a purine base always pairs with a pyrimidine base.

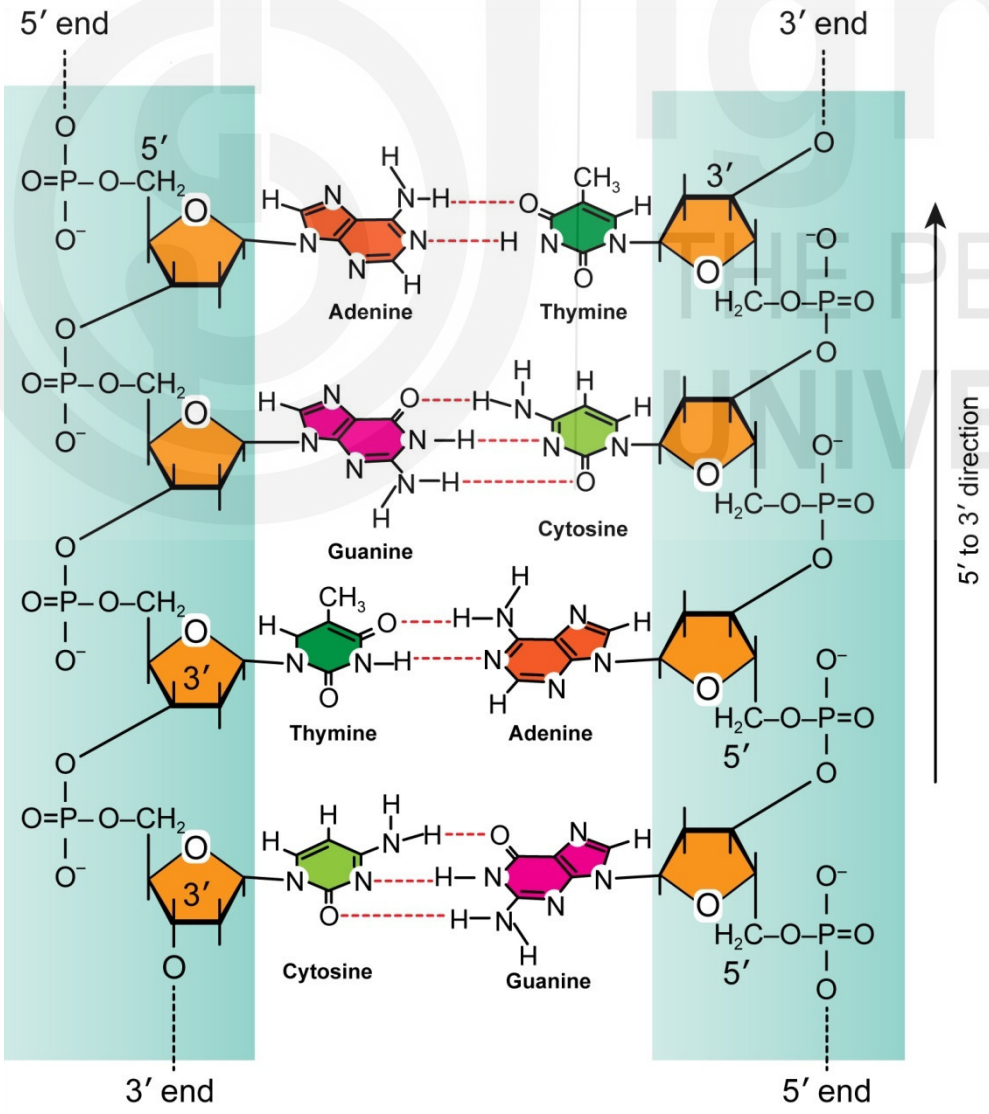


Fig. 10.2: Diagram showing bonding between the bases, sugar and phosphate groups present in DNA nucleotide strand.

The stacking of bases one on top of another contributes to the stability of the double helix. The van der Waals forces between the adjacent base pairs and the hydrophobic interactions between the bases stabilize the double helix. The two nucleotide strands are held together by weak associations between the bases of each strand. The backbone of each strand consists of a phosphate–deoxyribose sugar polymer held together by phosphodiester bonds (Fig. 10.2). These bonds also play a role in keeping the nucleotide chain organized.

Now we you can summarize the main features of the Watson - Crick Model of DNA as:

i) Two polynucleotide chains are coiled around a common axis. Both the nucleotide chains run in opposite directions. One end of a DNA strand has a 5´ phosphate and the other end has a 3´ hydroxyl group. The two strands are antiparallel.

ii) The sugar-phosphate backbones are present outside, while the purine and pyrimidine bases lie on the inside of the helix.

iii) In a helix, the bases are separated by distance of 3.4 Å. Ten bases have been found per turn (360^{o}) of helix helical structure. The base pairs are stacked about 0.34 nm apart.

iv) The diameter of the helix is 20 Å.

10.2.2 Double Helix

The structure of DNA shows presence of **major** and **minor** grooves. Each groove runs continuously along the entire length of the DNA molecule (Fig. 10.3).

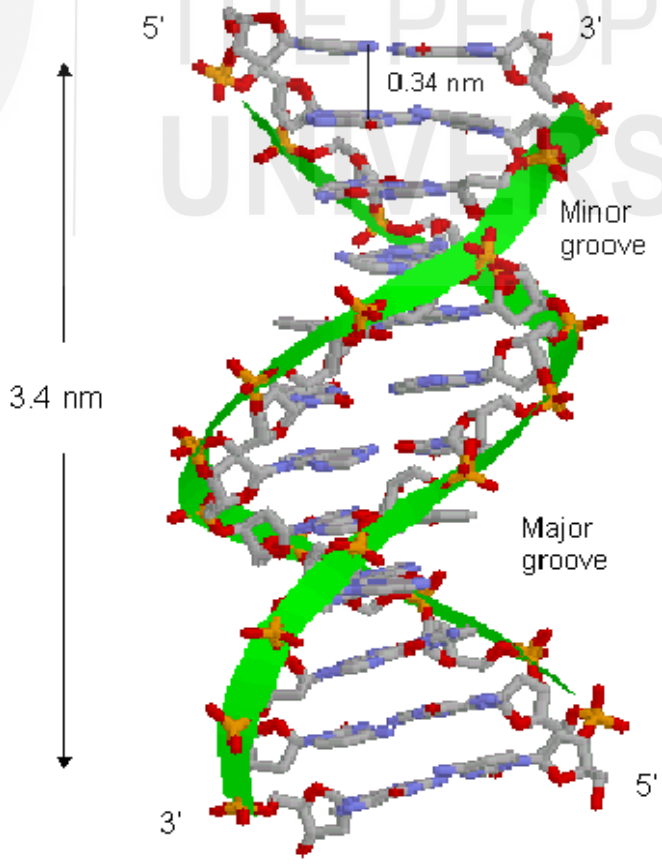


Fig 10.3: Presence of major and minor grooves in a DNA double helical structure.

The major and minor grooves are present opposite to each other. They arise from the antiparallel arrangement of the two backbone strands and are formed because the glycosidic bonds of a base pair are not diametrically opposite each other. The major groove is always on the same side for given base pair, while the minor groove lies close to the sugar moieties. Proteins bind to specific sequence of DNA in the major groove, while in the minor groove proteins bind DNA nonspecifically (such as chromatin proteins). The grooves play an important role in attachment of **DNA Binding Proteins** involved in replication and transcription.

A DNA structure in which two strands can be separated is defined as **paranemic**. The DNA strands show a side-by-side arrangement (Fig.10.4). The condition in which two strands are coiled around the same helical axis and look intertwined is referred as **plectonemic**. Intertwining prevents the two strands of DNA from separation.

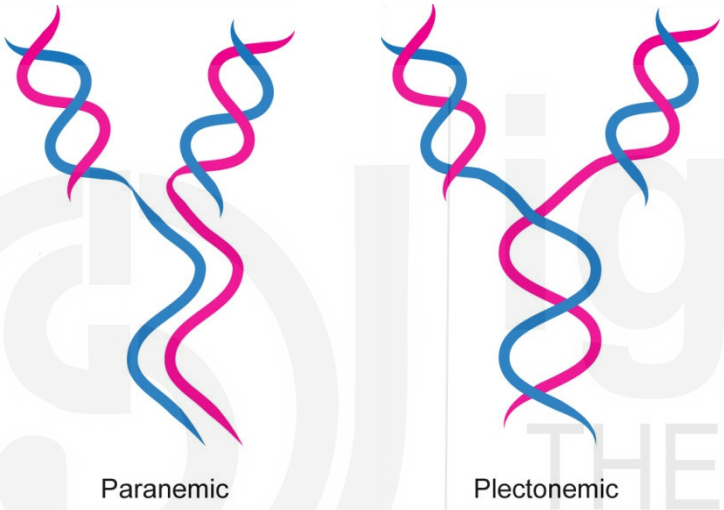


Fig. 10.4: Diagrammatic representation of paranemic (left) and plectonemic coil (right) structure of DNA.

SAQ 1

a) Fill in the blanks:

i) The sugar–phosphate groups form the ……..of each strand of DNA.

ii) The two strands of DNA are ……around each other to form a right-handed helix.

iii) In DNA both the nucleotide chains run in ……..directions.

iv) In a DNA molecule the quantity of adenine is ……to thymine and quantity of guanine is equal to cytosine.

v) In a helix, the bases are separated by distance of……...

vi) The major and minor grooves are present ……..to each other.

vii) Intertwining of the two strands of DNA ……....these strands from separation.

b) Define the given words:

i) Paranemic

ii) Plectonemic

iii) Complementarity

10.3 TYPES OF DNA

DNA is a double stranded structure. The bases present in one nucleotide chain show complementary pairing with those present in the other nucleotide chain. Besides this conventional structural organization, the DNA may also exist in alternate forms of double helical structures. The other forms of DNA show difference in features such as number of residues per turn and spacing of residues along the helical axis. Three major forms of DNA are A-form, B-form, and Z-form DNA (Fig. 10.5 and Table 10.1).

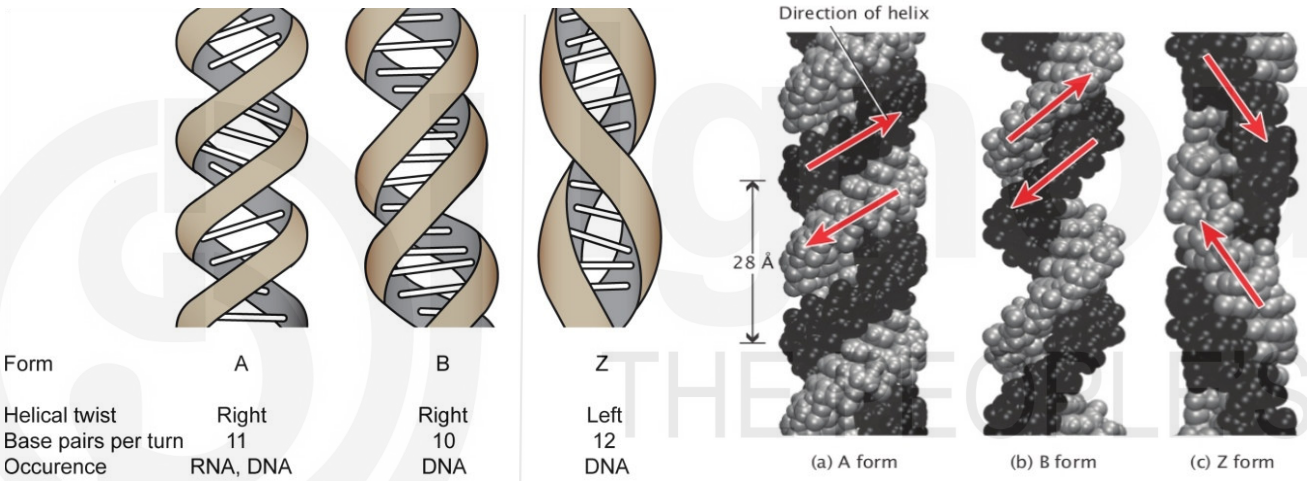


Fig. 10.5: a) Diagrammatic representation of various forms of DNA; b) realistic model of DNA showing different forms.

B-form DNA

The right handed Watson-Crick double helical structure is called B-DNA. In this type of DNA, two strands of DNA wound around the same axis. The two strands are held together by H-bonding between the bases. In this type of DNA, the major groove is wider than the minor groove. The distance between the base pairs is 0.34 nm. There are about 10 base pairs per turn and hence distance of 3.4 nm per turn is noted. B-DNA is the main form of DNA in cells.

A-form of DNA

This type of DNA has a right handed helical configuration which is shorter and thicker than B- DNA. It is created artificially by dehydrating B-DNA. It does not exist under normal conditions in a cell. In this type of DNA, minor groove is almost the same size of major groove.

Z-form of DNA

This type of DNA is a left-handed helical structure. The two strands show a zig-zag pattern in the sugar–phosphate backbone. The purine-pyrimidine

bases are present in an alternating sequence such as GCGCGC and different nucleotides conformations lead to a zig-zag pattern. It is generally noted in DNA regions that contain purine, pyrimidines or cytosines with extra methyl groups. This type of DNA is longer and thinner than B-DNA. The minor groove is deep and narrow, and the major groove is almost non-existent. Very less amount of DNA exists in this form.

Table 10.1: Comparison of B-form, A-form and Z-DNA.

	B-Form	A-Form	Z-Form
helix sense	Right Handed	Right Handed	Left Handed
base pairs per turn	10	11	12
vertical rise per bp	3.4 Å	2.56 Å	19 Å
rotation per bp	+36°	+33°	-30°
helical diameter	19 Å	19 Å	19 Å

Differences between A and B form of nucleic acid

The major difference between A-form and B-form of nucleic acid is in the conformation of the deoxyribose sugar ring. The second major difference is the placement of base-pairs within the duplex. In B-form, the base-pairs are almost centered over the helical axis. Double helix are antiparallel in both DNAs and both exhibit A=T and G=C pairing.

Differences between Z-DNA and B-DNA

i) Z-DNA has left-handed helical sense while B-DNA is right handed helical ii) phosphate backbone follows a zig-zag course in Z DNA while in B-DNA it is regular, iii) In Z-DNA, sugar residues of a polynucleotide chain have alternating orientation where repeating unit is a monoucleotide in contrast to dinucleotide found in B-DNA, iv) In Z-DNA, one complete helix has twelve base pairs while in B-DNA, one complete helix has only ten base pairs. v) Twelve base pairs are accommodated in one helix in Z-DNA, the angle of twist per repeating is 60º as against 36º in B-DNA which has ten base pairs (dinucleotide), vi) One complete helix is 45 Å in Z-DNA, while it is 34 Å in B-DNA (Table 10.2).

Table 10.2: Comparison of alternative forms of DNA

Parameter	A-DNA	B-DNA	Z-DNA	C- DNA
Helix sense	Right	right	Left	-
Base pair per turn	11	10	12	9.33
Axial rise(nm)	0.26	0.34	0.45	-
Helix pitch (°)	28	34	45	-
Base pair tilt (°)	20	-6	7	-
Twist angle (°)	33	36	-30	-
Diameter of helix (nm)	2.3	2.0	1.8	-
Rotation per bp	+ 3.27º (right handed)	+ 36.0º (right handed)	+ 30.0º (right handed)	+ 38.6º (right handed)
Helical diameter	25.5 Å	23.7 Å	18.4 Å	23.7 Å

Some other forms of DNA called D-form and E-form are also reported in some variants with only 8 and 7½ base pairs per turn respectively. These are found only in DNA molecules lacking guanine. Some other right-handed helices of DNA have been discovered. These include **C-, D, E,** and **P DNA**. **C DNA** is found under even greater dehydration conditions. The other two forms i.e. **D** and **E DNA** lack guanine in their base composition present in helices. The artificial stretching of DNA leads to change in conformation called **P- DNA** (named after **Linus Pauling)**. In some cases, three-stranded (triplex) structures are noted. These are called as H-DNA. In this type, some of the DNA unwinds and a single polynucleotide strand from one part of the molecule pairs with double stranded DNA from another part of the molecule. This happens because under certain conditions one base can simultaneously pair with two other bases. This type of DNA occurs in long sequences containing either purine bases or only pyrimidine bases.

SAQ 2

a) State whether following statements are True or False:

i) The left handed Watson-Crick double helical structure is called B-DNA.

ii) In B-form type of DNA, the major groove is wider than the minor groove.

iii) A-form type of DNA, does not exist under normal conditions in a cell.

iv) In A-form type of DNA, minor groove is almost the same size of major groove.

v) In Z-form type of DNA two strands show a zig-zag pattern in the sugar –phosphate backbone.

vi) Very large amount of Z-form type of DNA exists.

vii) In Z-form type of DNA the major groove is deep and narrow, and the minor groove is almost nonexistent.

b) Make a diagram to differentiate between A, B and Z-form of DNA.

10.4 ORGANIZATION OF DNA IN VARIOUS CELLULAR TYPES

You have studied about the structure of prokaryotic and eukaryotic cells in earlier units. The organization of nucleus and genetic material i.e. DNA is different in both prokaryotic and eukaryotic cells. The genomic DNA is organized in such a way so that it can carry out the essential functions such as DNA transcription, replication or recombination. The highly folded DNA in prokaryotes is present in nucleoid body while the eukaryotic DNA is present in a well developed nucleus. Bacterial nucleoid comprises of genomic DNA, RNA and associated proteins. The genomic DNA compaction starts from a nucleosome as a primary unit and is compacted 10^4-times more of that present in the nucleoid. DNA organization in eukaryotic chromatin has been studied extensively.

10.4.1 Prokaryotes

Prokaryotic cells do not possess a wall developed membrane-bound nucleus. The chromosomal double-stranded DNA consists of a single circular chromosome located in nucleoid present in the cytoplasm. The chromosome occupies about a third of the volume of nucleoid. The chromosome is tightly wound or supercoiled. Prokaryotes also contain other circular pieces of DNA called plasmids.

The bacterial genome shows coils fold over one another and form a condensed ball. The DNA is compressed through supercoiling (twisting of DNA) (Fig. 10. 6). This happens due to compaction of DNA after association with protein. The average density of supercoiling is 1 turn/100 bp. The nucleoid possesses 400 negatively supercoiled domains. Each domain consists of a loop of DNA. When DNA is twisted in the opposite direction of the double helix it is called as negatively supercoiled (Fig. 10.6). The twisting of DNA in the same direction as the double helix is referred as positively supercoiled. Most bacterial genomes show negative supercoiling during normal growth.

During 1980s and 1990s, researchers found that multiple proteins act together to fold and condense prokaryotic DNA. Numerous small protein molecules associate with the chromosomal DNA. These proteins help in folding of DNA to form a compact structure. Protein called HU is abundantly found in the nucleoid. This protein binds to DNA after getting associated with enzyme called *topoisomerase* I. This introduces sharp bends in the chromosome, generating the tension required for negative supercoiling. Binding of other proteins such as integration host factor (IHF) to specific sequences within the genome introduce additional bends. The folded DNA gets organized into supercoiled conformations that are wound around tetramers of the HU protein. After condensation of genome, enzymes such as *DNA topoisomerase* I*, DNA gyrase*, and other proteins help maintaining the supercoils. Protein named H-NS, is a maintenance protein which is basically a dimer of about 15.6-kDa polypeptides. It plays an active role in transcription by modulating the expression of the genes in the response to stimuli. Factor for inversion stimulation (FIS) is another maintenance protein present abundantly during exponential growth and regulates the expression of DNA *topoisomerase* I. In *E. coli*, enzyme *DNA gyrase uses* energy coming from hydrolysis of ATP to twist supercoils into DNA. Supercoiling contributes to the compaction of DNA so that it gets placed into a small portion of the cell. If supercoils are relaxed, DNA spreads out and appears as a single, replicating, circular DNA molecule.

The prokaryotic DNA (lack nuclear membrane) is not well separated from the ribosomes present within the cytoplasm, hence transcription and translation occur simultaneously in these organisms. In prokaryotic cell, nucleoid generally appears as an irregularly shaped mass but during transcription, small regions of the chromosome project out from the nucleoid into the cytoplasm, unwind and associate with ribosomes, thus allowing easy access by various transcriptional proteins. The nucleoids projections explain the change in shape of nucleoid during active growth. When transcription is over, the projections move back into the nucleoid forming a spherical shape (Fig 10.6).

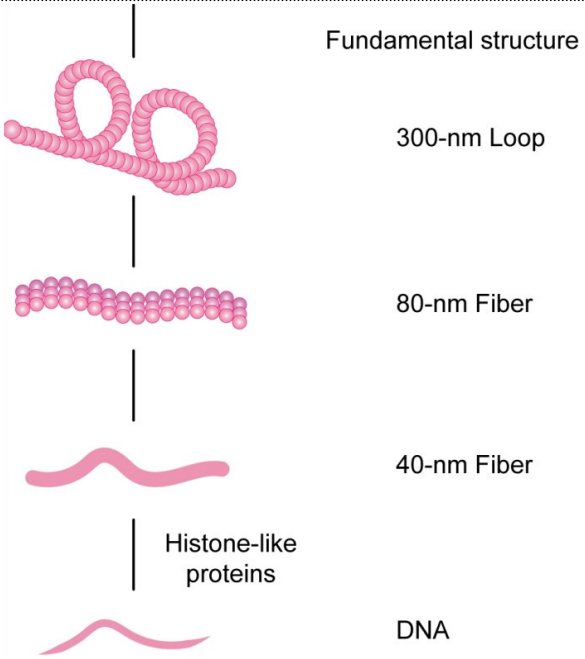


Fig.10.6: Structure of a nucleoid.

10.4.2 Eukaryotes

Each eukaryotic chromosome is made up of a single linear DNA molecule. Chromosomes are made of chromatin and proteins. Chromatin is a fibrous form of DNA that allows efficient packaging of DNA. The chromatin looks like beads on a string. The beads are called nucleosomes. A nucleosome is a segment of DNA associated with eight histone proteins (Fig. 10.7). Histones are the primary basic protein components of chromatin that associate with DNA. Association with histones makes DNA compact so that it can easily fit in the nucleus. Transcription is completed in the nucleus before the newly synthesized mRNA molecules get transported to the cytoplasm and undergo translation to form proteins.

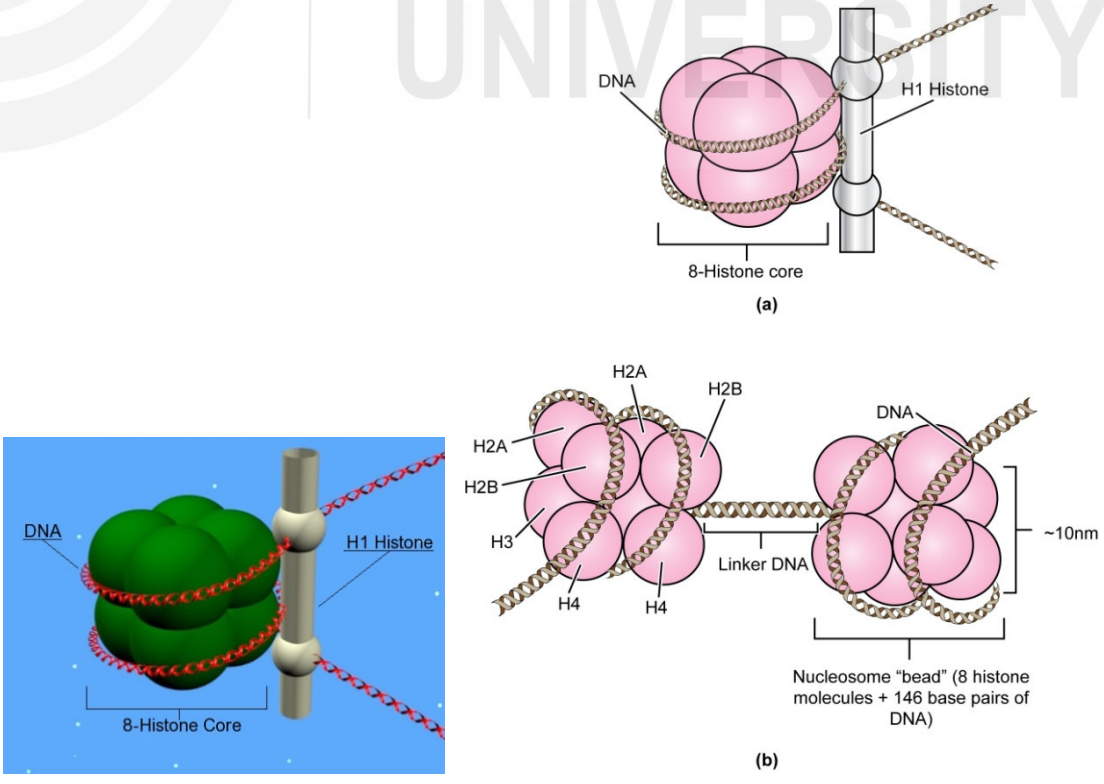


Fig. 10.7: Structure of Nucleosome.

DNA packaging

The cellular DNA is long and hence needs to be organized in a way that it fits easily into the nucleus. The basic structural unit of chromatin is the nucleosome. DNA is wrapped around proteins known as histones to form nucleosome. In 1974, **Kornberg** proposed that DNA and histones get organized into repeating units called nucleosome- a protein DNA complex (Fig. 10.7). The nucleosomes represent the first level of packaging. The DNA is tightly wrapped around the histone core. Each nucleosome consists of DNA wrapped around histone octamer. The core of histone octamer is made of two of each of four types of histones namely H_2A, H_2B, H_3 and H_4. X-ray crystallography studies proved that octameric histone core is a disk-shaped molecule made of interlocking histone subunits. These histone proteins are rich in positively charged basic amino acids, which interact with the negatively charged phosphate groups in DNA.

The core particle is wrapped around by 146 bp of DNA. The H_3 and H_4 histones from a subnucleosome particle that holds the main DNA loop, while the two H_2A and H_2B histones are associated with the DNA nucleotide sequence at either end of the loop. Lysine (basic amino acid) rich histones viz. H_2A and H_2B assist in binding to stretches of the DNA. Histone H_1 is also rich in lysine and is found to be associated with the spacer DNA. The core particle, the spacer DNA and H_1 together form a unit called mononucleosome. Two successive nucleosomes are connected to each other by means of linker DNA. Linker DNA is a short strand of DNA that is free of histones and joins the nucleosomes looking like beads. The linker DNA looks like a “string” (Fig. 10.8).

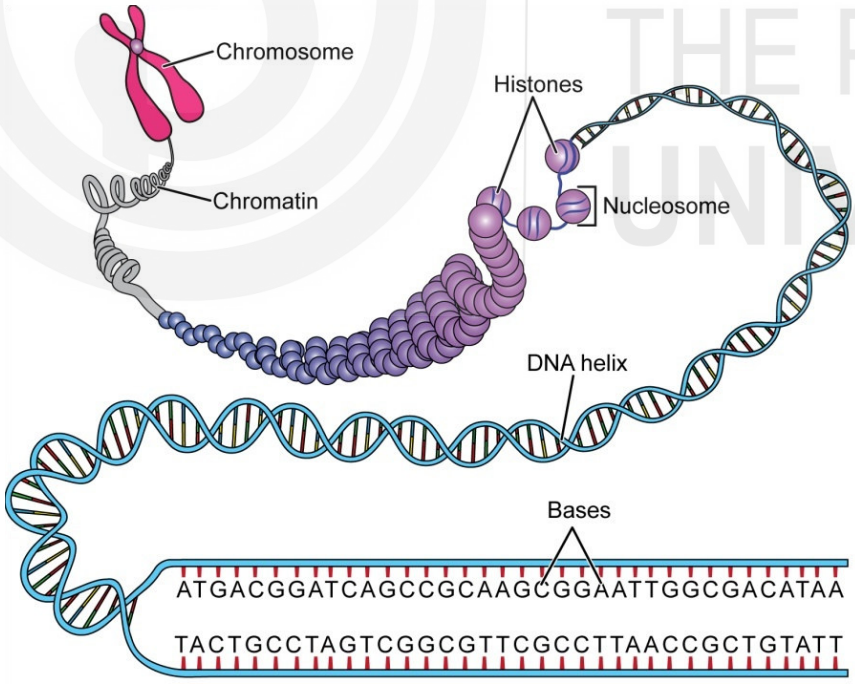


Fig. 10.8: Macrostructure of DNA. Strands of DNA are wrapped around supporting histones. These proteins are increasingly bundled and condensed into chromatin, which is packed tightly into chromosomes when the cell is ready to divide.

The length of linker DNA varies from few to 80 nucleotide pairs. The linker DNA is associated linker i.e. H_1 histone. The nucleosomes with DNA coiled around them, stack compactly over one another to form a fiber (30nm). The

fiber contains six nucleosomes per turn. Fiber is further coiled into a thicker and more compact structure. Further folding of DNA is caused by interaction of core histones with the histones of adjacent nucleosome and also with the H_1 histones. In condensed fibers, nucleosomes structure shows a spiral or solenoid arrangement with six nucleosomes per turn. Histone H1, is bound to the DNA on the inside of the solenoid. Each nucleosome associates with one H1 molecule. The 30-nm fiber has been predicted in solenoid model suggested by electron microscopic studies. Condensed chromatin is dynamic with regions partially unfolding and then refolding into a solenoid structure occasionally. High order of packaging starts at interphase stage. The chromatin fibers are further folded to from larger super coiled loops. These loops are called **domains** (Fig. 10.8). The diameter of these loops is 80-100nm. These loops are anchored to non-histone protein scaffolding also called nuclear matrix. Number of nucleotide base pairs per loop varies from 50,000 to 2,00,000. The metaphase chromosome appears to be composed of numerous loops giving a fuzzy appearance. At this stage, the chromosomes are at their most compact form and associated with scaffold proteins.

Eukaryotic chromosomes depict two distinct regions that can be distinguished by staining. A dark stained tightly packaged region that usually contains genes that are not active. This region is known as heterochromatin. It is found in the regions of the centromere and telomeres. A less dense region that usually contains genes which are active is referred as euchromatin (Fig. 10.9). Hypoacetylated chromatin assumes a more condensed structure than hyperacetylated chromatin.

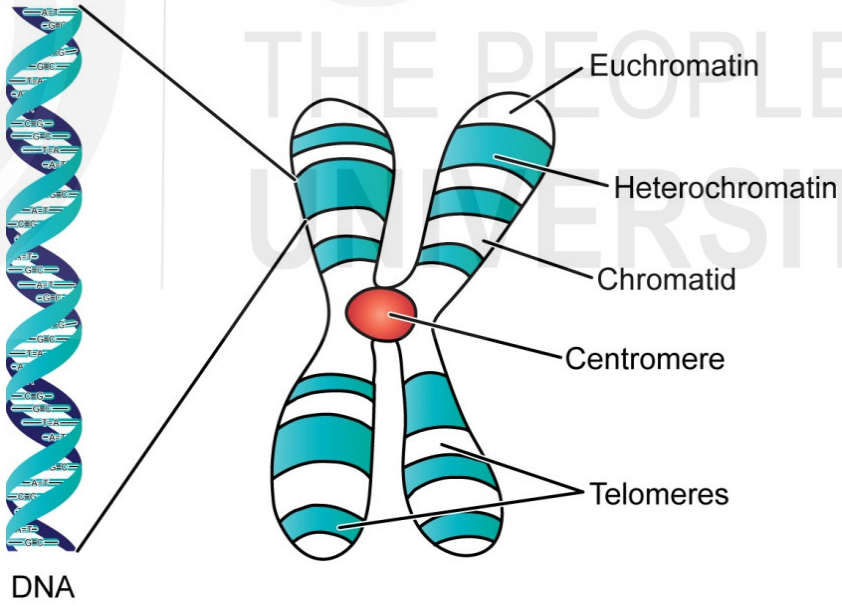


Fig. 10.9: Structure of chromosome.

Table 10.3: Prokaryotic versus Eukaryotic Chromosomes

Prokaryotic Chromosomes	Eukaryotic Chromosomes
A single circular chromosome is present.	Multiple linear chromosomes are present.
DNA can interact with the cytoplasm and chromosomes are condensed in the nucleoid via DNA supercoiling and the binding of various proteins.	Chromosomes are condensed in a membrane-bound nucleus via histones.

Transcription and translation occurs simultaneously.	Transcription occurs in the nucleus, and translation occurs in the cytoplasm.
Only one copy of each gene (i.e., they are haploid) is present.	Two copies of each gene (i.e., they are diploid) are present.
Nonessential genes are commonly encoded on extrachromosomal plasmids.	Extrachromosomal plasmids are not commonly present.
Operons are not found.	Some genomes are organized into operons.
Genomes are efficient and compact containing little repetitive DNA	Large amounts of noncoding and repetitive DNA is found.

10.4.3 Viruses

Viruses are small microscopic structures having DNA or RNA genomes. A virus particle known as a virion consist of nucleic acid surrounded by a protective coat of protein called a capsid. The capsid functions to protect the nucleic acid from the environment. In some viruses, capsid is surrounded by a membrane envelope. The shape of the capsid varies for various types. Most viruses have icosahedral or helical capsid structure (Fig. 10.10). In a helical virus, the nucleic acid coils into a helical shape and the capsid proteins wind around the inside or outside of the nucleic acid, forming a long tube or rod-like structure. The nucleic acid and capsid constitute the **nucleocapsid**. The protein that winds around the nucleic acid is called as nucleocapsid protein. The capsid length will be the size of the coiled nucleic acid.

In a helical capsid, one type of capsid protein is required. This protein subunit is repeated over and over again to form the capsid. This simple structure requires less free energy to assemble than a capsid composed of multiple proteins. Few viruses also show complex virion architecture. More nucleic acid can be packaged into the virion. In some viruses capsid is icosahedron, a geometric shape with 20 sides, each composed of an equilateral triangle. Icosahedral viruses increase the number of structural units in each face to expand capsid size. Icosahedron the most prevalent form of capsid shape found in viruses (Fig. 10.10).

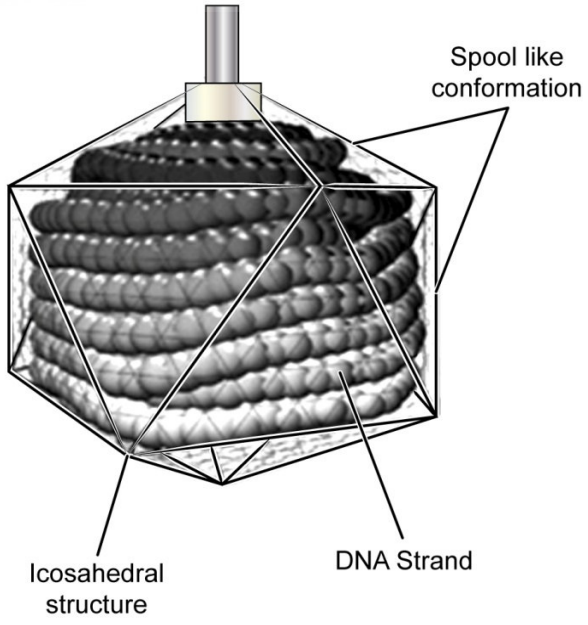


Fig. 10.10: Diagrammatic representation of DNA organization in virus.

In genomes of icosahedral viruses are packaged completely within an icosahedral capsid that acts as a protein shell. Capsid shows 2,3-5 rotational symmetry. A capsid is composed of proteins encoded by the viral genome. Virally coded protein units called protomers self-assemble to form the capsid. Capsid is about 50 nm wide. The simple virus has a single nucleic acid surrounded by a capsid having single type of proteins. Example- tobacco mosaic virus (TMV). In contrast complex viruses have cores that contain several nucleic acid molecules and their capsid has various kinds of proteins. Some viruses are surrounded by a membrane that is derived from the plasma membrane of the host cell in which virus particles are made and assembled.

A few viruses with **complex** architecture include Poxviruses, geminiviruses, and many bacteriophages. Poxviruses are the viruses that cause smallpox or cowpox and are large oval or brick-shaped particles 200-400 nm long. Inside the complex virion, a dumbbell-shaped core encloses the viral DNA. The plant-infecting geminiviruses also exhibit complex structure. These viruses are composed of two icosahedral heads joined together. **Bacteriophages (bacterial viruses** or **prokaryotic viruses)** are viruses that infect and replicate within bacteria. Many bacteriophages also have complex structure. They generally have an icosahedral head, containing the nucleic acid, attached to a cylindrical tail sheath that facilitates binding of the bacteriophage to the bacterial cell.

Viruses have **genomes** or genetic material that is composed of DNA *or* RNA. Viruses possess either single-stranded DNA (ssDNA) genomes or RNA genomes which can be single-stranded or double-stranded. A particular virus will only have one type of nucleic acid genome. The size of the virus particle and viral genome size varies significantly from virus to virus. A typical virus genome falls in the range of 7000–20,000 base pairs (bp). Smaller-sized virions hold less nucleic acid while some dsDNA viruses have very large genomes. Herpes viruses have genomes that are 120–200 kb.

DNA is packed in the head which is icosahedral in structure. The DNA inside the capsid is about 6.5 microns long. The DNA in the inner core region plane is organized in such a way so that capsid is able to accommodate more DNA. DNA is packed in an inverse-spool fashion. Inverse spool conformation of the genetic material found in virus has been confirmed through techniques of cryo-electron microscopy and image processing. The studies revealed that the duplex DNA genome of the T4 bacteriophage forms a highly condensed series of concentric layers, spaced about 2:36 nm apart. They follow the contour of the inner wall of the protein capsid. Electron microscopy also provided the evidence for the conformation of the packaged genome of phage T4.

DNA is packed into a protein shell called procapsid which is the characteristic of double-stranded (ds)DNA viruses, including bacteriophages, herpesviruses, and adenovirues. These procapsids are a few tens of nanometers in diameter. Viral genomes are several micrometers in length. Electron microscopy (EM) studies revealed that DNA gets packaged inside the small procapsid. High internal pressure exists inside the procapsid during DNA packaging. This

process requires lot of energy, hence this process is accomplished by packaging motors run by ATP hydrolysis through a DNA and *procapsid-dependent ATPase*. The DNA packaging motors possess an inherent abilitiy to recognize specific viral genomes in a pool of DNAs and to package them into such a tiny space against a large internal force.

A virus transports or releases a part of genetic material or genome (either RNA or DNA) inside the cell when it attacks or infects a host cell. Once assembly of the protein shell or capsid of a new virus is complete, the newly replicated genome is threaded inside, segment-by-segment.

Flash field model

This model explains the steps of packing of DNA in virus. Many molecular motors move unidirectionally along a DNA strand powered by nucleotide hydrolysis (Fig.10.11). The multimeric *ATPases* act as motors and possess more than one hydrolysis site. These motors generate the requisite force to process DNA. The nucleotide hydrolysis generates force based on electrostatic field. The electrostatic force explains the motion of *DNA helicases,* portal protein and the role of motor that bacteriophages use to pump the genome into their capsids. The tight packing in the virus is achieved by molecular motors. First the empty capsid with a protein complex, called the portal motor is synthesized. This motor grabs hold of the viral genome, a double strand of DNA, and pushes it into the capsid to complete assembly of the virus. The motor generates a strong force and compacts DNA about 6000 times its normal volume. The motor helps in overcoming DNA's resistance to bending, the electrostatic forces of repulsion encountered when pushing charged atoms close together, and the forces of entropy that make anything resist being constrained in a tight space. The molecular motor translocates the twisted strands of DNA (right) of the virus/bacteriophage into a protein capsid.

Binding of ATP to a hydrolysis site induces a conformational change that exposes a pair of negative and positive charge regions near the inner surface of the channel. The charged regions are oriented at an angle to the circumferential meridian. There is one charge pair region per nucleotide hydrolysis site, and that the axes of the charge pair regions are tilted with respect to the axis of the hole. The negatively charged phosphates spaced along the backbone of the DNA strand interact sequentially with the field of the charge pair regions as they "flash" on and off with the binding and hydrolysis of ATP. Each charge pair field gives the closest phosphate(s) an "electrostatic push" in the direction of the charge pair axis. The net effect of the charge pairs flashing on and off creates a sustained torsional and axial thirst. Note that the motor is an electrostatic machine that transduces strain fluctuations generated by nucleotide hydrolysis into a fluctuating electric field.

The DNA is packed in an Inverse Spool confirmation with DNA in the inner core region buckled out of the spooling plane; the capsid is able to accommodate more DNA at a lower density without suffering a high cost in bending energy.

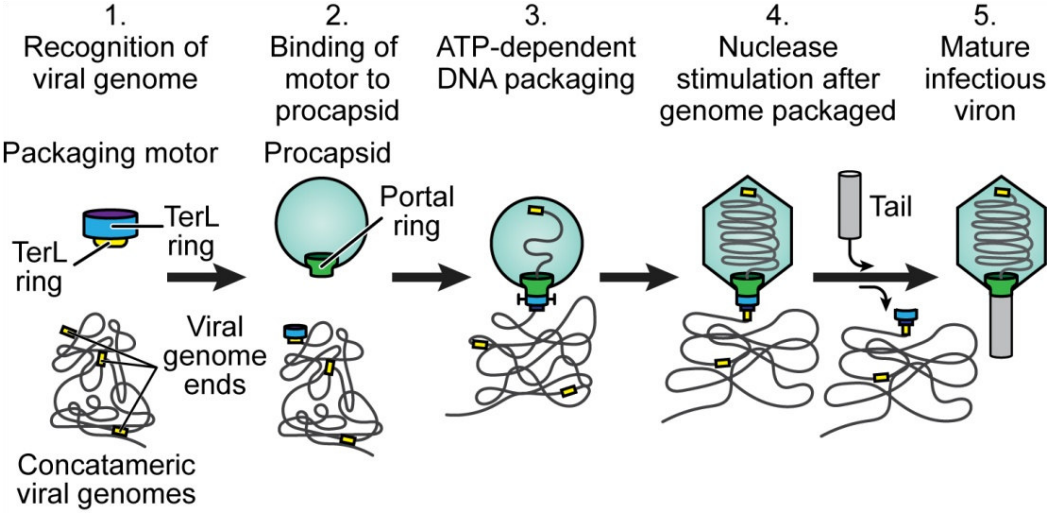


Fig. 10.11: Packaging of DNA into virus according to Flash Field Model.

SAQ 3

a) Fill in the blanks:

i) In prokaryotes chromosome occupies about a one…….. of the volume of nucleoid.

ii) The cellular DNA is long and hence needs to be organized in a way that it fits ……..into the nucleus.

iii) Eukaryotic chromosomes depict ……distinct regions that can be distinguished by staining.

iv) Poxviruses are the viruses that cause…… or …… .

v) In virus DNA is packed in the head which is ……in structure.

b) State whether the following statements are True or False:

i) DNA is packed into a empty protein shell called procapsid.

ii) The twisting of DNA in the same direction as the double helix is referred as positively supercoiled.

iii) A less dense region that usually contains genes which are active is referred as euchromatin.

iv) A particular virus will have several types of nucleic acid genome.

v) A virus releases a part of genetic material or genome (only RNA) inside the cell when it attacks or infects a host cell.

10.5 MITOCHONDRIAL AND CHLOROPLAST DNA

Chloroplast and mitochondria are major organelles found in plant cells. Chloroplasts help in carrying out photosynthesis while mitochondria supply

energy within plant cells. Both chloroplasts and mitochondria possess their own genomes. Small, circular DNA molecules are found in mitochondria and chloroplasts. Usually many copies of DNA are present in a single mitochondrion or chloroplasts. Sequencing technique showed chloroplast (cp) genome is about 120 to 160 kb in length and possesses high GC contents (30 to 40%). The gene content and structure of angiosperm cp genome is highly conserved. The mitochondrial genome (mt) is generally large and complex.

The prokaryotic ancestors of chloroplasts and mitochondria were endosymbionts whose genes became copied to the genomes of their cellular hosts. These copies gave rise to nuclear chromosomal genes that encode cytosolic proteins and precursor proteins that are synthesized in the cytosol for import into the organelle into which the endosymbiont evolved**.** The present-day mitochondrial and chloroplast genomes have retained some of the characteristics of their ancestral prokaryotes. They also have acquired new attributes during their evolution within eukaryotic cells. Like prokaryotic genomes, mitochondrial and chloroplast genomes neither bind with histone-like proteins nor show complex packaging into chromosome-like structures, as observed in eukaryotes. Unlike mitotic cell divisions observed in eukaryotic cells, mitochondria and chloroplasts undergo binary fission and equally separate their DNA into the daughter organelles as observed in prokaryotes. Mitochondrial and chloroplast genomes are much smaller with numbers of base pairs in the thousands with a few hundred genes. This genome size was developed in the course of evolution. During this phase, mitochondrial and chloroplast genomes were exported to the nucleus and this export of genes made them dependent on the nuclear genome for the supply of some proteins required for their biogenesis.

Chloroplast DNA

Most of the chloroplast DNA (cp) occurs as linear and branched molecules with defined ends and inversion isomers of IR (inverted repeat). The size of chloroplast genome is relatively small and contains slightly more than one hundred genes. The first chloroplasts genome studied was that of tobacco (*Nicotiana tabacum*). The chloroplast DNA or cpDNA is composed by a single large circular DNA molecule; typically 120,000-170,000 base pairs in length (Fig. 10.12). The mass is about 80-130 million daltons. Roughly half of the chloroplast genes produce either RNA molecules or polypeptides that are important for protein synthesis. The proteins produced by these genes are needed to carry out transcription and translation of their own genes. Half of the remaining genes produce polypeptides directly required for the biochemical reactions of photosynthesis. For example, enzyme *ATPase*, comprises nine different polypeptides. Six of these polypeptides are products of chloroplast genes, but the other three are products of nuclear genes that must be transported into the chloroplast to join with the other six polypeptides to make active *ATPase*. Another notable example is the enzyme *ribulose biphosphate carboxylase* (*RuBP carboxylase*), which is composed of two polypeptides. The larger polypeptide, called rbcL, is a product of a chloroplast gene, whereas the smaller polypeptide is the product of a nuclear gene.

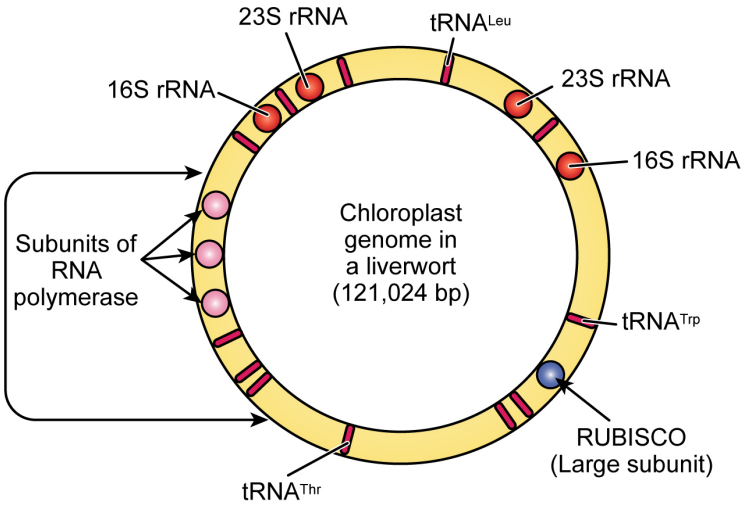


Fig 10.12: Diagram showing chloroplast DNA.

Chloroplasts sequences have proven useful in studies related to plant evolution, phylogeny and maternal inheritance characters. Genes present in chloroplast genomes have shown a great potential for phylogenetic markers. The discovery of genes that code for polypeptides required for photosynthesis has helped increase understanding of the biochemistry of photosynthesis. DNA sequences of many chloroplast genes are highly conserved. Because of this feature, chloroplast gene DNA sequences were used for reconstructing the evolutionary history of various groups of plants. Example- *rbcL* gene. It is one of the most conserved genes in the chloroplast genome (i.e. even distantly related plants will have a similar base sequence). Therefore, rbcL can be used to retrace the evolutionary history of groups of plants that are very divergent from one another. The *rbcL* gene, along with a few other very conservative chloroplast genes has been used to find out the origin of some of the group of major flowering plants.

Mitochondrial DNA

Plant mtDNA exist as a collection of linear DNA with combinations of smaller circular and branched molecules. Plant mitochondrial genomes are large ranging between 200-2,000 kbp in size. The increased size is due to repeated sequences, AT-rich non-coding regions, and large introns and non-coding sequences. Plant mitochondrial genomes also contain fairly significant amounts of relatively short nuclear and chloroplast genomic sequences that appear to have been integrated at some time during evolution. Recombination is the main mechanism driving replication of plant mtDNA. The genes of mtDNA encode for rRNAs, tRNAs, ribosome synthesis proteins, and oxidative phosphorylation proteins.

In majority of plants, mtDNA is observed as a collection of linear molecules in varying sizes. Lo *et al.* studied the structure of DNA in mung bean mitochondrial nucleoids and observed that plant mtDNA occurs as supercoiled molecules, sub-genomic open circles of variable size, linear molecules, and other highly complex structures. Nearly 50% DNA occurs as highly complex structures, about 30% in linear form, 15% as variable sized open circles, and 5% as supercoiled DNA molecules. Similar observations have been noted in tobacco cells.

The unique feature of plant mitochondrial genome is the presence of multiple large repeated regions. The plant mitochondrial genes contain mainly group II introns. Several mitochondrial tRNAs get transcribed from nuclear genes and imported into the mitochondrion (Fig.10.13).

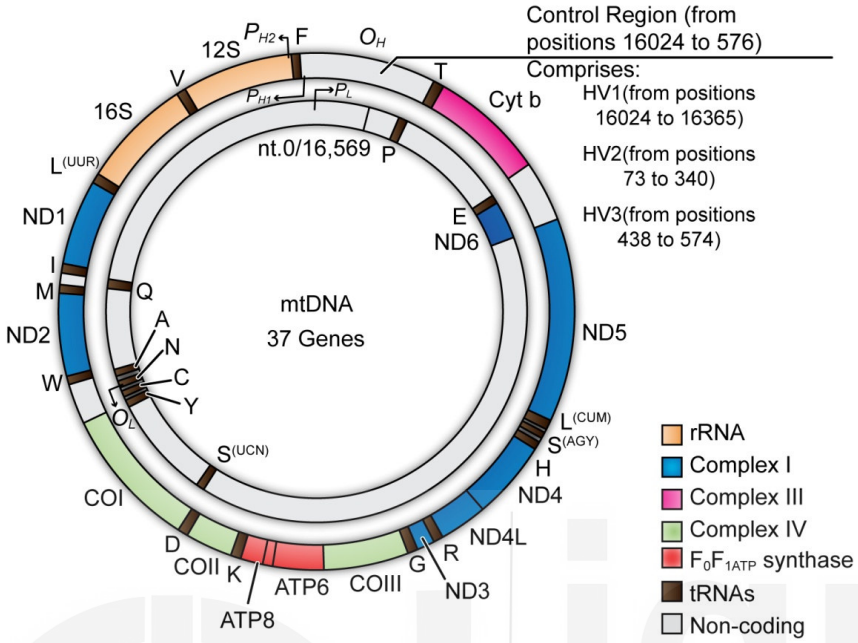


Fig. 10.13: Diagram showing mitochondrial DNA.

Plant mitochondrial genomes continuously take up exogenous DNA, lose portions of their own DNA and rearrange the order of their sequences. Unique rRNA genes have been found in plant mtDNA. The gene code for enzymes and proteins involved in oxidative phosphorylation, respiratory chain complexes, ribosomal proteins, transfer RNAs and ribosomal RNAs (Fig. 10.14). Recombination acts as an essential characteristic of plant mitochondrial genetic processes.

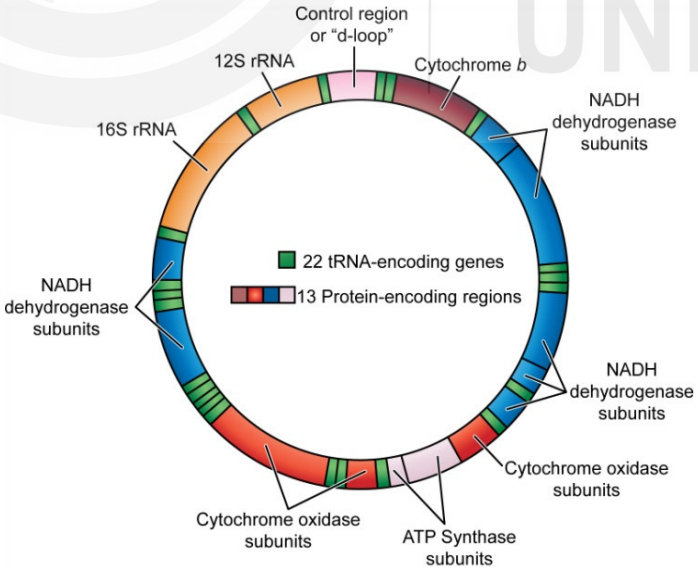


Fig. 10.14: Structure of mitochondrial DNA.

Over time, ancient mitochondria lost more and more of their DNA and its genes moved, got incorporated into the nuclear genome of the hosts they inhabited. The present day mitochondria and chloroplasts possess and maintain their own DNA, remnants of the past. This residual DNA encodes

only some of the genes required for respiration, photosynthesis, and other organelle functions. The proteins required for organelle function are encoded in the nucleus and transported to sites wherever required. These include the machinery required for DNA replication, gene transcription and some proteins and tRNAs needed for translation.

SAQ 4

a) Discuss how Chloroplasts sequences have proven useful in studies related to plant evolution.

b) Describe the unique features of mitochondrial DNA**.**

10.6 SUMMARY

- Genomic DNA in both bacteria and eukaryotes must be highly compacted in order to fit within cells.
- Bacterial chromosomes are circular DNA molecules. Bacterial DNA is highly supercoiled and associated with polyamines and low-molecular-weight basic proteins, which permit the DNA to fold tightly in the central portion of a bacterial cell.
- In eukaryotic cells, DNA is associated with about an equal mass of histone proteins in a highly condensed structure called chromatin. The building block of chromatin is the nucleosome, consisting of a histone octamer around which is wrapped about 146 bp of DNA. Chromatin appears as a 30-nm fiber made up of nucleosomes, the linker DNA between them, and histone H1a when visualized under electron microscope.
- Chromatin is visualized as beads on a string structure. The reversible acetylation and deacetylation of lysine residues in the N-termini of histones H2A, H2B, H3, and H4 controls how tightly DNA is bound by the histone octamer and affects the assembly of nucleosomes into the condensed forms of chromatin.
- Chloroplasts and mitochondria possess their own genomes**.** Small, circular DNA molecules are found in mitochondria and chloroplasts. Usually many copies of DNA are present in a single mitochondrion or chloroplasts**.** Mitochondrial and chloroplast genomes are much smaller with numbers of base pairs in the thousands with a few hundred genes. This genome size was developed in the course of evolution. Sequencing technique showed chloroplast (cp) genome is about 120 to 160 kb in length and possesses high GC contents (30 to 40%). The mitochondrial genome (mt) is generally large and complex.
- The present-day mitochondrial and chloroplast genomes have retained some of the characteristics of their ancestral prokaryotes. They also have acquired new attributes during their evolution within eukaryotic cells. Both mitochondrial and chloroplast genomes neither bind with

histone-like proteins nor show complex packaging into chromosome-like structures. Mitochondria and chloroplasts undergo binary fission and equally separate their DNA into the daughter organelles.

10.7 TERMINAL QUESTIONS

1. What is a nucleosome? Describe the structure of nucleosomes.
2. Write short note on packaging of DNA.
3. Compare between A, B, Z and C DNA.
4. Differentiate between prokaryote and eukaryote chromosomes.
5. Write a note on other less known type of DNA –forms found in nature.
6. Describe the packaging of DNA into virus with proper diagram.
7. Discuss the common features of chloroplast and mitochondrial DNA with proper diagram.

10.8 ANSWERS

Self-Assessment Questions

1. a) i) backbone
   ii) twisted
   iii) opposite
   iv) equal
   v) 3.4 Å
   vi) opposite
   vii) prevents

   b) i) Paranemic: A DNA structure in which two strands can be separated is defined as paranemic.

   ii) Plectonemic DNA strands are coiled around the same helical axis and look intertwined is referred as plectonemic.

   iii) Complementarity Adenine pairs with thymine and guanine pairs with cytosine. Adenine and thymine are complementary base pairs. Similarly cytosine and guanine are also complementary base pairs. This feature of DNA is known as complementarity.

2. a) i) False; ii) True; iii) True; iv) True
   v) True; vi) False; vii) False

   b) Refer to Section 10.3.

3. a) i) third
   ii) easily
   iii) two
   iv) smallpox or cowpox
   v) icosahedral

   b) i) True; ii) True; iii) True; iv) False; v) False

4. a) Refer to Section 10.5.

   b) Refer to Section 10.5.

Terminal Questions

1. Refer to Subsection 10.4.2.
2. Refer to Subsection 10.4.2.
3. Refer to Section 10.3.
4. Refer to Table 10.3.
5. Refer to Section 10.3.
6. Refer to Subsection 10.4.3.
7. Refer to Fig. 10.5.

Acknowledgements

Fig.10.3 : https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.researchgate.net%2Fprofile%2FBlessy-Mathew-4%2Fpublication%2F262175313%2Ffigure%2Ffig1%

Fig.10.6 : ttps://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.researchgate.net%

Fig. 10.6 b : Source: https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.nature.com%2Fscitable%2Fcontent%

Fig. 10.11 : Packaging of DNA into virus. https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.pnas.org%2Fcontent%2F112%2F29%2FE3792%2FF1.large.jpg&

Fig 10.12 : https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fwww.biology-pages.info%2FC%2FchlDNA.gif&imgrefurl=https%3A%2F%2Fwww.biology-pages.info%

Fig. 10.13 : https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fdfzljdn9uc3pi.cloudfront.net%2F2019%2F7314%2F1%

Fig. 10.14 : https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fupload.wikimedia.org%2Fwikipedia%2Fcommons%2Fthumb%2F3%2F3e

UNIT 11

DNA REPLICATION

Structure

11.1 INTRODUCTION

In the previous units you have studied about DNA. DNA carries all of the genetic information that is transmitted to the daughter cells after division. The knowledge of the structure of DNA enabled scientists to work out the detailed mechanisms of processes such as DNA replication and recombination. During replication, DNA makes a copy of itself by copying the genetic information. DNA replication is also referred as duplication. Before each division, duplication of DNA molecules takes place by replication resulting in formation of copies of molecules. DNA replication is the process by which DNA makes a copy of itself during cell division. In prokaryotes, a single, double-stranded DNA molecule is present in the form of a loop or circle. In

contrast there are several double-stranded linear DNA molecules present in the genome of eukaryotes. In this unit you will be studying about the process of DNA replication in detail.

Objectives

After studying this unit, you would be able to:

- explain DNA replication in Prokaryotes;
- describe DNA replication in Eukaryotes;
- enlist various enzymes involved in replication; and
- describe replication of linear and double stranded (ds) DNA.

11.2 TYPES OF DNA REPLICATION

Watson and Crick proposed the double helical structure of DNA with complementary base pairing. The structure of DNA suggested the possible mechanism for replication of DNA molecules. Three models of DNA replication were suggested namely conservative, dispersive and semi-conservative (Fig. 11.1). In **conservative** type of replication, double stranded molecule is conserved as such and a new copy of DNA is synthesized from the old molecule. In **dispersive** mode of replication, the old molecule disintegrates and two molecules are synthesized, while in **semiconservative** type of replication, the two strands separate from each other, maintain their integrity and synthesize its complementary strand from the pool of nucleotides (Fig. 11.1).

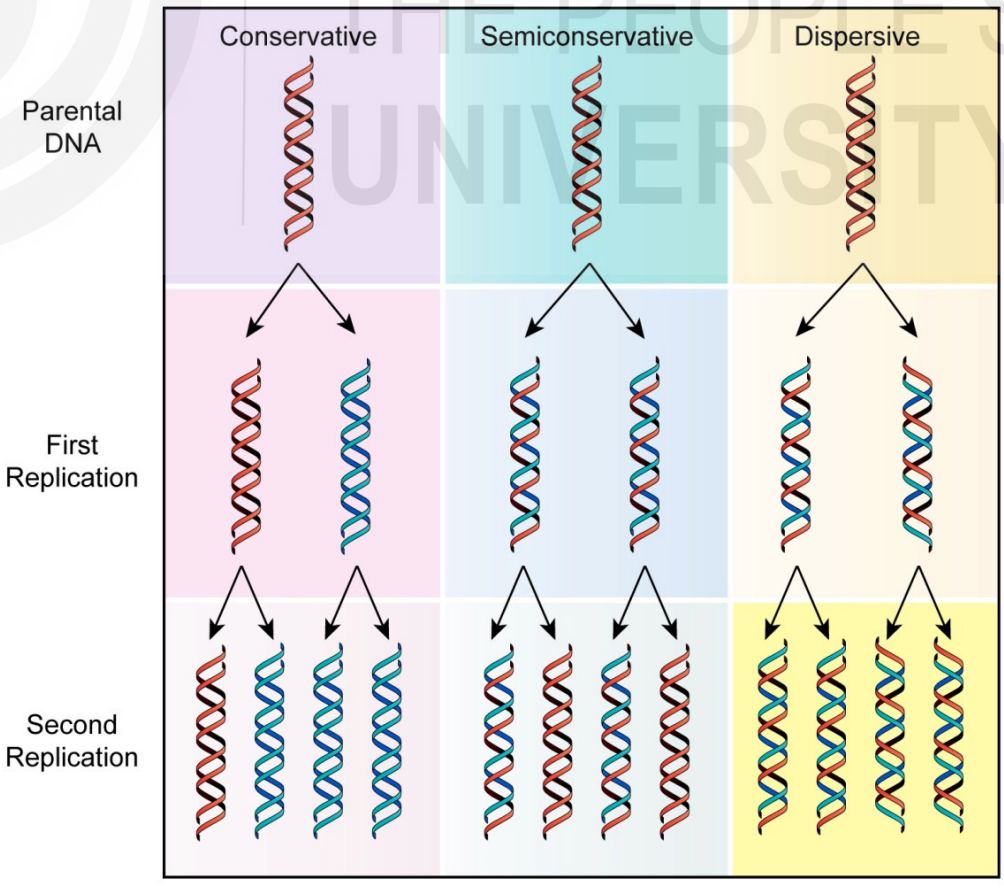


Fig.11.1: Diagram showing different modes of DNA replication.

The newly synthesized molecule would carry or conserve one of the two strands from the parent molecule and the other would be newly synthesized.

11.2.1 Semi Conservative Replication

Watson and Crick proposed that two complementary strands of the DNA double helix unwind and separate. Each strand guides the synthesis of a new complimentary strand. The sequence of bases in each parental strand is used as a template. The template guides the sequence of bases in the newly synthesized strand. This mechanism of DNA replication is called semi conservative replication. In this type of replication, half of the parental molecule is conserved. In 1958, Matthew Meselson and Franklin Stahl demonstrated that chromosome of *E. coli* replicate in a semiconservative manner.

Meselson-Stahl Experiment

Meselson and Stahl proved that DNA replication was semiconservative using nitrogen which is a major constituent of DNA. They grew *E. coli* in a medium containing $^{15}NH_4Cl$. The medium contained a heavy isotope of nitrogen, ^{15}N (^{14}N is the naturally abundant isotope). After growing the bacteria on ^{15}N containing medium for several generations, they found that DNA of cells had a high density (heavier). Meselson and Stahl transferred the bacteria grown on a ^{15}N medium to a medium containing only ^{14}N. The new DNA, replicated in the ^{14}N medium, showed density intermediate between those cells grown independently on light (^{14}N) and heavy (^{15}N) medium. Since the replication was semiconservative, double stranded DNA was synthesized in which one strand had ^{15}N DNA and the other strand was having ^{14}N DNA. If replication had been conservative, two strands have either heavy (^{15}N) or light (^{14}N) DNA (Fig.11.2). If the method of replication had been dispersive, various multiple-banded patterns would have appeared, depending on the degree of dispersiveness.

The density of the strands was determined using a technique known as **density-gradient centrifugation.** In this technique, a cesium chloride (CsCl) solution is spun in an ultracentrifuge at high speed for several hours. Eventually a density gradient is established in the tube with an increasing concentration of CsCl from top to bottom. DNA formed a band in the tube at the point where its density is the same as that of the CsCl. If DNA with different densities are added, then several bands are formed.

Meselson and Stahl took cells growing in the medium containing ^{15}N for several generations, washed them to remove the medium containing ^{15}N and transferred them to medium containing ^{14}N. The cells were allowed to grow in the presence of ^{14}N for varying periods of time. The DNA was extracted and analyzed using CsCl density gradient. The result indicated semi conservative mode of DNA replication. The DNA isolated from cells had a density half way between the densities of heavy and light DNA. The intermediate density is called as hybrid density. After two generations of growth in the medium containing ^{14}N, half of the DNA showed hybrid density (^{15}N-^{14}N) and the other half showed light density (^{14}N). The results proved the semiconservative replication in DNA.

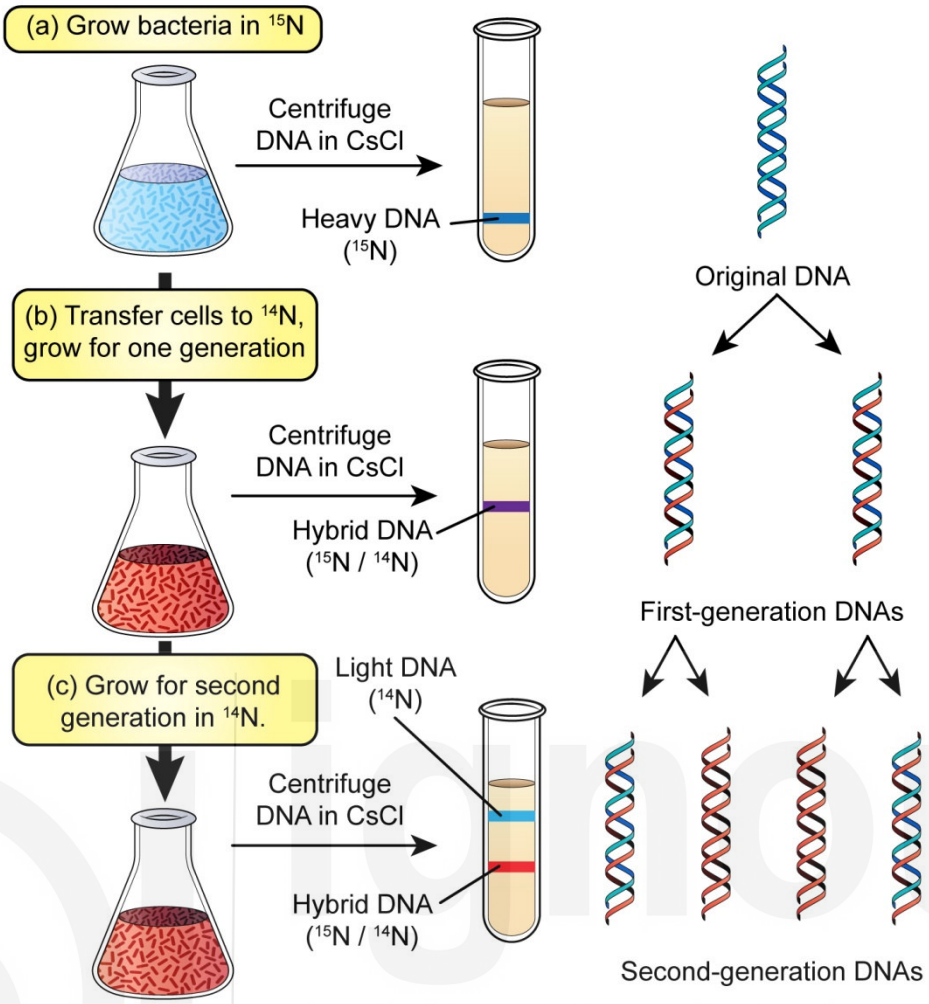


Fig. 11.2: Meselson-Stahl experiment.

DNA replication in eukaryotes is also semi conservative. This was demonstrated at the chromosome level through experiments carried out by J. Herbert Taylor, Philip Woods and Walter Hughes in 1957. They took root tip cells of *Vicia faba*. They grew the root tips for eight hours in a medium containing radioactive ^{3}H- thymidine. The root tips were washed and transferred to a non-radioactive medium containing alkaloid colchicines. Colchicine binds to microtubules and prevents the formation of functional spindle fibers. Therefore, the daughter chromosomes do not undergo the separation during anaphase. The numbers of chromosomes double per cell cycle. The doubling of chromosomes number per cell generation helped in determining number of times DNA duplication each cell has undergone subsequent to incorporation of radioactive thymidine. Taylor and his colleagues used technique of autoradiography to examine the radioactivity in the chromosomes of cells of the first metaphase, second metaphase and so on. In autoradiography technique, the radioactive isotopes are used in the cytological preparations of macromolecules. The radioactive atoms are exposed to photographic film. The radioisotopes are detected by exposing them to photographic emulsion that is sensitive to a low energy radiation. The emulsion contains silver halides that produce tiny black spots called silver grains when exposed to the charged particles emitted during the decay of radioisotopes. The silver grains visible on the film are counted and each grain of silver represents a radioactive decay. The radioactive decay provides an

estimate of the quantity of radioactive material present. The technique of autoradiography proved useful in studying DNA metabolism as DNA are specifically labelled by ^{3}H- thymidine. Thymidine is incorporated into DNA. Taylor and his colleagues concluded that chromosomal DNA in *V. faba* segregated in a semi conservative manner during each cell division.

11.3 MODELS OF REPLICATION

Various models have been proposed to explain the semi-conservative replication of DNA in viruses, bacteria and eukaryotes. You will not study these models in detail.

11.3.1 Theta (Ø) Model of Replication

In 1963**, J. Cairns** verified the semi conservative method of replication photographically using autoradiography technique. Cairns grew *E. coli* bacteria in a medium containing radioactive thymine, a component of one of the DNA nucleotides. As the DNA replicated, radio labelled tritium (^{3}H)/ tritiated thymidine got incorporated into the newly synthesized DNA at the place of thymine which is present opposite to adenine. Cairns extracted DNA from bacteria and placed it on photographic emulsion for a period of time. He examined the autoradiographs under the electron microscope. The study of the autoradiograph revealed that DNA of *E. coli* is circular and replicates maintaining the integrity of the circle (Fig. 11.3). The circular DNA does not break during the process of DNA replication but an intermediate **theta structure** is formed (similar in shape to the Greek letter theta).

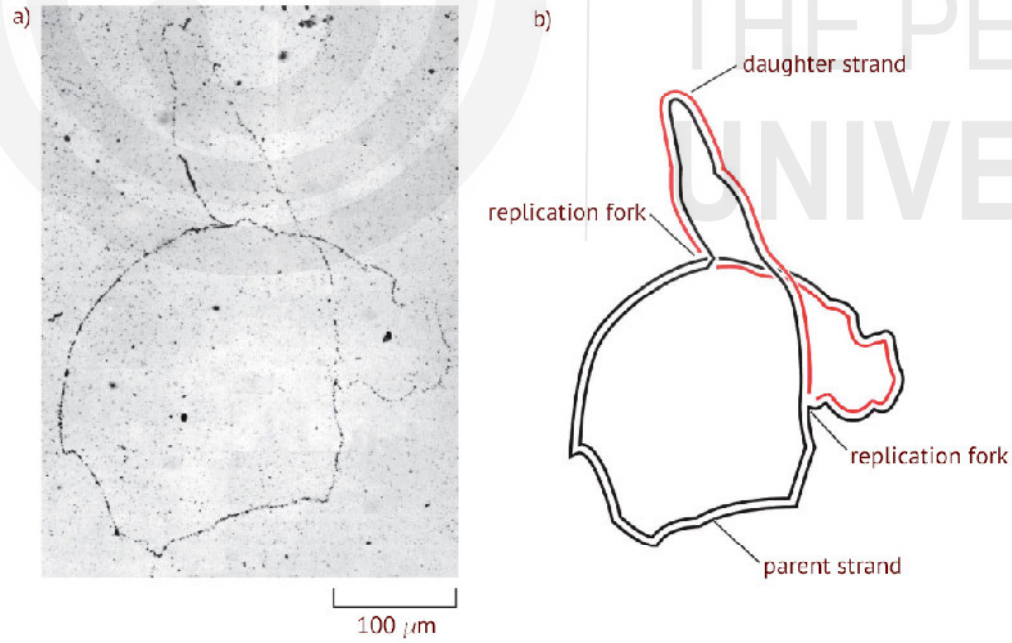
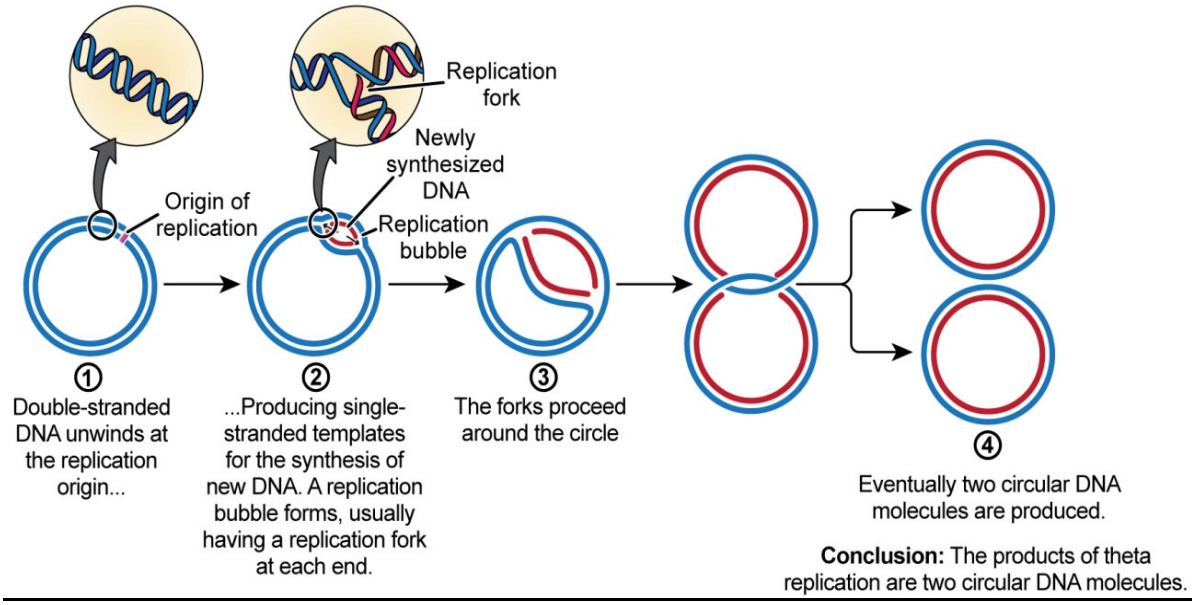


Fig. 11.3: Autoradiograph developed during Cairns experiment.

The replication initiates from the unique origin of replication (***ori C***) site, which is preceded by the formation of a **Y-shaped** structure called the **replication fork** of the DNA. The DNA is unwound at origin site, and replication proceeds in a semiconservative manner in the direction of the replication fork. Each ori C site shows two Y-shaped replication forks clearly indicating the bidirectional replication of DNA (Fig. 11.4).



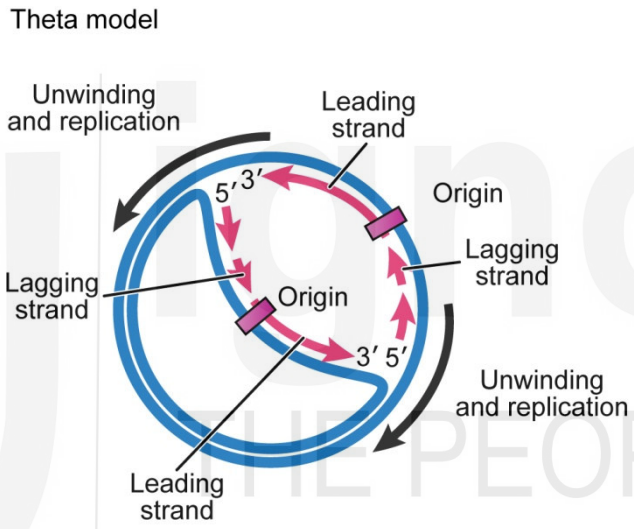


Fig. 11.4: Theta model of DNA replication.

Every newly synthesized DNA has one parental template strand and one daughter strand. The replication always initiates from a specific point in DNA. John Cairns established the existence of a site of initiation or origin of replication on the circular chromosome of *E. coli*. There is one unique origin per chromosome which controls the replication of whole chromosome. The single origin of replication, called *ori C*, in *E. coli* has been characterized. It has been found to be 245 nucleotide pairs long with two different conserved A: T rich repeat sequences. The region where the double helical structure opens for replication is called replication bubble/eye. In prokaryotes one bubble is seen. Each replication bubble has two replication forks moving and synthesizing DNA in two opposite directions. The replication of DNA in *E. coli* occurs at the time of cell division.

11.3.2 Uni and Bidirectional Replication

The replication in DNA can be **unidirectional** or **bidirectional. This depends** upon whether the replication from the point of origin proceeds only in one direction or proceeds in both the directions. Replication eye may appear in both the types unless the replication starts from one of the two ends of a linear DNA molecule. In **unidirectional replication,** one of the two ends of the

replication eye will be **stationary** and the other end will move with replication. On the other hand, in **bidirectional replication,** both ends will be moving and none will be stationary (Fig. 11.5). Distinction between unidirectional and bidirectional replication can be made by the study of autoradiographs If radioactively labelled nucleotides are used during DNA synthesis.

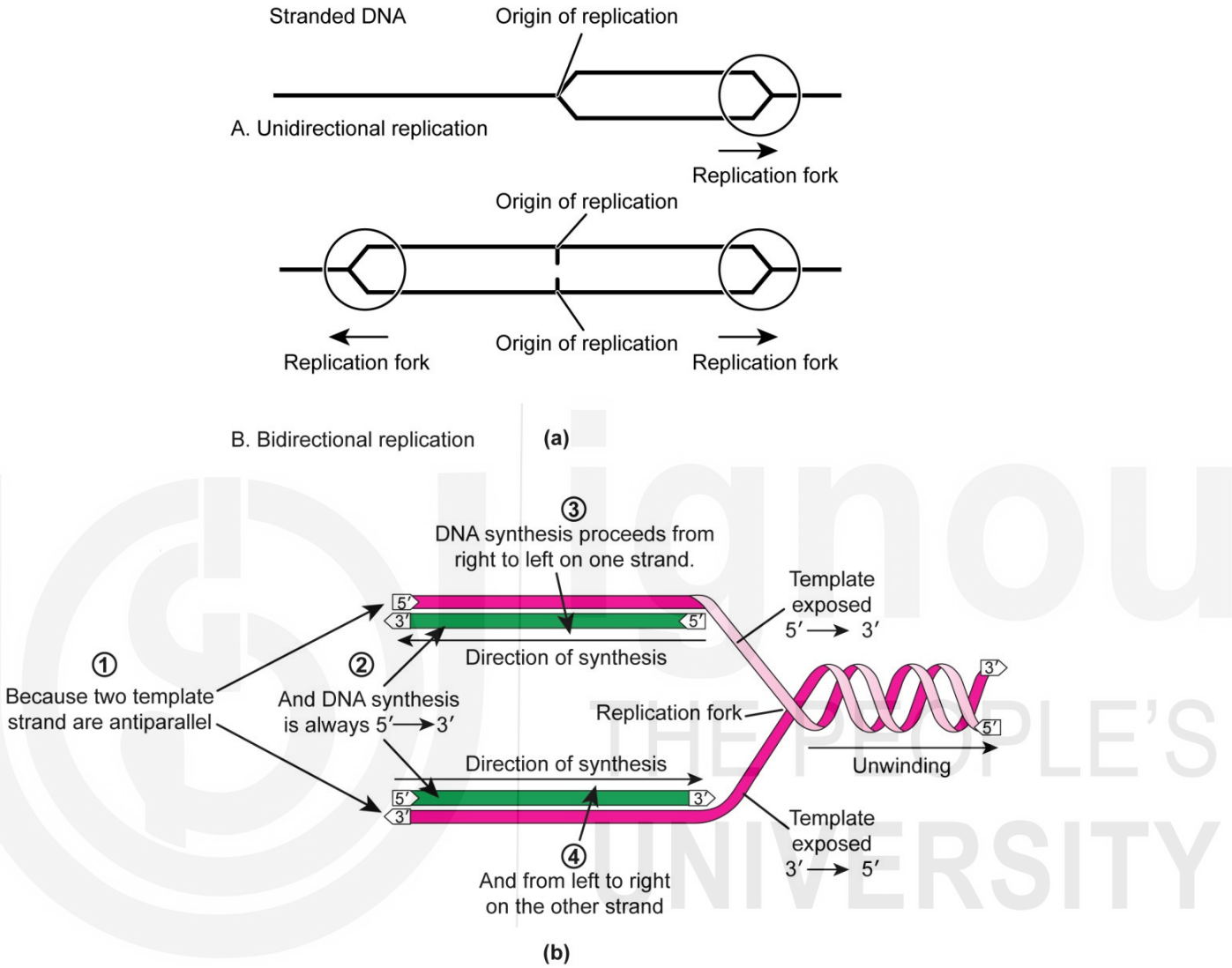


Fig. 11.5: a) Diagrammatic representation of the uni and bidirectional DNA replication; b) bidirectional DNA replication

The structure of bacterial chromosome was determined by John Cairns in 1963 with the help of autoradiography technique. He grew *E. coli* cells in medium containing ^{3}H –thymidine for varying period of time. He carefully collected chromosomes on membrane filters. These filters were stored in dark for a period of time to allow sufficient radioactive decay. The autoradiographs showed that chromosomes of *E. coli* are circular structures that exist as theta (θ) shaped intermediates during replication. The auto radiographs indicate that unwinding of two complementary strands of DNA and their semi conservative replication occur simultaneously or are closely coupled. The parent double helix rotates about 360^{o} to unwind each strand of helix to produce some kind of swivel (turn around a point or axis). The enzyme *topoisomerases* is responsible for producing transient breaks in the DNA that acts as a swivel for DNA unwinding and keep the DNA untangled. Enzyme *topoisomerases* provide the required axes of rotation during replication of circular DNA

molecules. The enzyme catalyzes the transient break in DNA molecules which provides an axis of rotation that allows segments of DNA on opposite sides to break. The replication of circular chromosome in *E. coli* is bidirectional starting from the unique origin of replication. Two replication forks move in opposite directions sequentially around the circular chromosome. Hence Cairns experiment suggested that replication of *E. coli* chromosome advances in both directions away from the origin of replication.

Box 11.1 : *Topoisomerase*

Enzyme *Topoisomerases* change the state of supercoiling in a DNA molecule. *Topoisomerases* were discovered by **Martin Gellert** and **James Wang**. They are of two types - type I or type II. *Topoisomerase I* make a nick in only one strand of the DNA molecule through which passes the intact strand whereas *Topoisomerase II* cuts both the strands of DNA creating a passage through which the loop of the other segment of helix passes**.** In *E. coli*, a *Type II Topoisomerase* called ***DNA gyrase*** is found (Fig. 11.6). The enzyme relieves the mechanical strain that builds up during replication in *E. coli*. The enzyme travels along the DNA ahead of the replication fork, removing the supercoils. *DNA gyrase* does this by cleaving both strands of the DNA duplex, passing a segment of DNA though the double stranded break to the other side, then sealing the cuts. The process is driven by energy released from hydrolysis of ATP. The movement of replication fork generates positive supercoils in the unreplicated portion of DNA ahead of the fork.

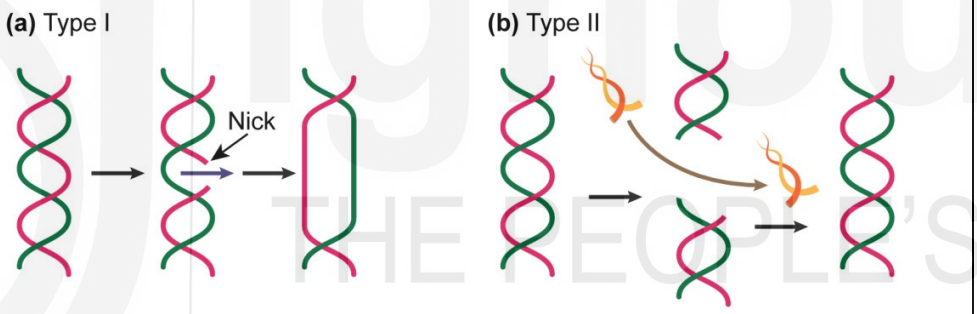


Fig. 11.6: Mechanism of *topoisomerase* action.

11.3.3 Rolling Circle Replication

Rolling circle type of replication is noted in many viruses. This type of replication is used by viruses to duplicate their genomes and bacteria to transfer DNA from donor cells to recipient cells during genetic exchange. It has also been noted in amphibians that amplify extrachromosomal DNAs carrying clusters of ribosomal RNA genes during oogenesis. Rolling- circle replication is a mechanism for replicating circular DNA molecules. The unique aspect of this replication is that one parental circular DNA strand remains intact and rolls or spins while serving as the template for the synthesis of a new complementary strand. The first step in rolling circle replication is the generation of a nick by sequence-specific endonuclease in one strand at the origin, producing 3´-OH and 5´-phosphate termini. The 5´ terminus is displaced from the circle as the intact template strand rolls about its axis. Covalent extension occurs at the 3´-OH of the cleaved strand. Since the circular template DNA may turn 360° many times, with the synthesis of one complete or unit length DNA strand during each turn, rolling circle replication generates single- stranded tails longer than the contour length of the circular chromosome. This type of replication can produce either single- stranded or

double- stranded progeny DNAs. Circular single – stranded progeny molecules are produced by site-specific cleavage of the single- stranded tails. This is followed by recircularization of the resulting molecule at the origin of replication (Fig. 11.7). The enzymes involved in the process are the same as those involved in θ- model of replication.

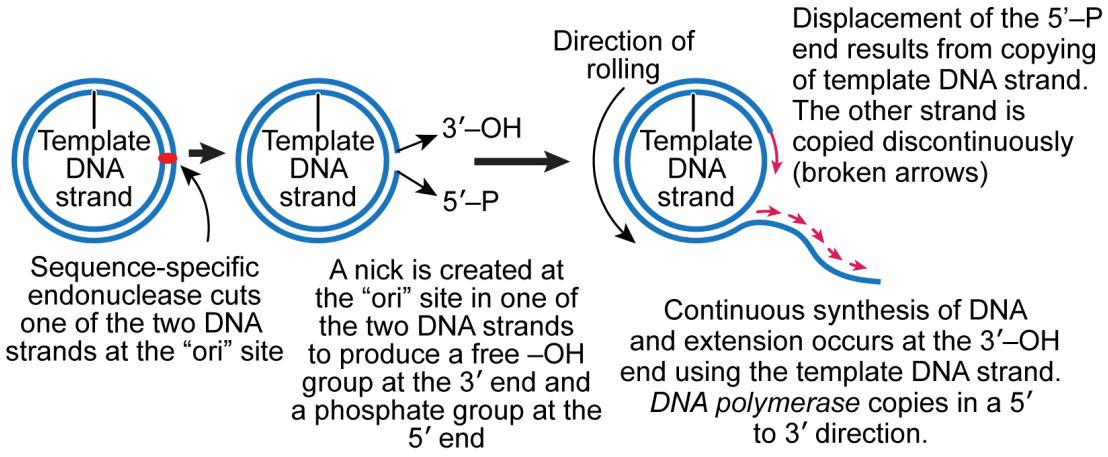


Fig.11.7: Rolling circle replication of DNA.

Box 11.2 : *Endonuclease*

> ***Endonuclease*** is a **restriction enzyme** produced by bacteria that cleaves double-stranded DNA at specific sites along the molecule. When a *restriction endonuclease* recognizes a sequence, it snips through the DNA molecule by catalyzing the hydrolysis (splitting of a chemical bond by addition of a water molecule) of the bond between adjacent nucleotides.

SAQ 1

a) Differentiate between:

i) conservative, semi conservative and dispersive type of DNA replication

ii) unidirectional and bidirectional replication

b) Write the functions of the following:

i) Replicon

ii) Endonuclease

iii) Topoisomerase

11.4 DNA REPLICATION IN PROKARYOTES

In prokaryotes such as *E. coli* DNA replication occurs in a semi conservative manner. This refers to a replication process in which two strands separate from one another, maintain their integrity and each strand synthesizes a new complementary strand from the pool of nucleotides. Studies have shown that bacteria undergo bidirectional, semi conservative mode of replication.

11.4.1 Initiation of Replication

The replication of DNA begins with unwinding of the double helix at sites called origin of replication. At these sites, the hydrogen bonds between the bases are broken and paired bases separate. The sites where the pair of replicated segments come together and join the non-replicated DNA are called as **replication fork.** In bacterial chromosome, DNA replication always begins at specific sites called the **origin.** Each origin controls the replication of a unit of DNA called a replicon. The bacteria have a single specific origin of replication (Fig. 11.8). The origin of replication in *E. coli* chromosome has been recognized as a specific sequence called ***ori* C**. The *ori C* is a 9 base pair (bp) sequence that is repeated four times within the region**.** At this site the proteins bind and initiate the process of replication. The replication fork is formed at this site. Replication fork is a site where the parental double helix undergoes strand separation and nucleotides are incorporated into newly synthesized complimentary strands. The replication proceeds outward from the origin in both the directions (bidirectional). Two replication forks move in opposite directions until they meet at a point across the circle from the origin where the replication is terminated. The newly synthesized strands detach form each other and move to the different cells.

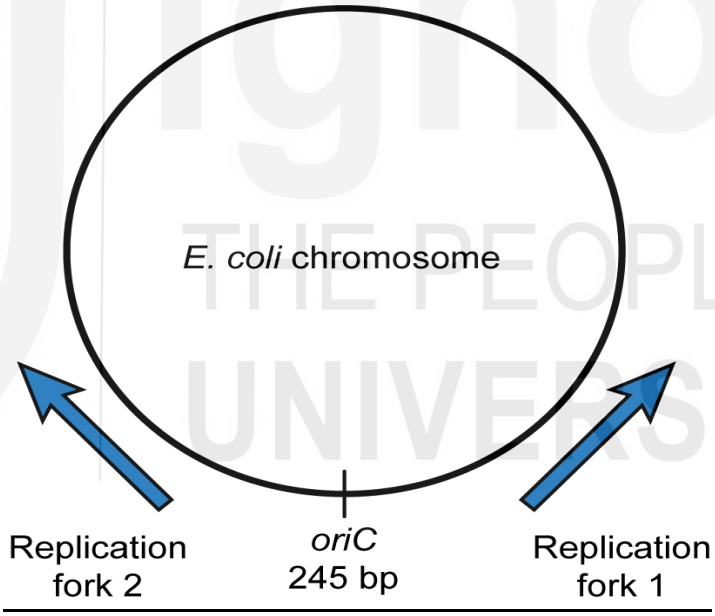


Fig. 11.8: Diagram showing origin of replication of DNA in prokaryotes at site *ori C*.

11.4.2 Unwinding of DNA

For replication, the strands from the DNA duplex need to be separated. The separation of the strands of a double helix is called unwinding. The separation of strands at one end is accompanied by rotation of entire fiber as it resists development of tension. The unwinding of DNA strands is accomplished by an enzyme *DNA helicase*. The enzyme *DNA helicases* bind to the double stranded DNA and help in separation of two strands (Box 11.3). Energy released by ATP hydrolysis is used in the process of unwinding. The hydrogen bonds that hold the two strands together are broken exposing the two separated single strands as DNA templates. *E. coli* has 12 different *helicases* that take part in various aspects of DNA metabolism. In addition to *helicase,*

unwinding the duplex and separation of strands require the assistance of a type of proteins called single stranded DNA binding proteins (SSB). The unwound section appears as a bubble and is known as **replication bubble**. As the strands unwind, enzyme *DNA gyrase* catalyses the formation of negative supercoils which helps in the process of unwinding of DNA. The enzyme prevents accumulation of twists by making temporary single strand nicks in the DNA.

Box 11.3: Role of Dna B *helicase* in DNA replication

One of the *helicases* that is the product of *dnaB* gene helps in unwinding of DNA during replication. The enzyme Dna B *helicase* consist of six subunits that form a ring shaped protein that encircle single DNA strand. Replication in *E. coli* begins when the multiple copies of DnaB (a 52KD protein) bind to the AT rich origin of replication i.e. *ori C* site and causes 45bp of DNA to separate into a single DNA strand. The DnaB *helicase* is then loaded on to the single stranded DNA of the lagging strand of *ori C* with the help of protein DnaC. The DnaB *helicase* then translocates in a 5′→ 3′ direction along with the lagging strand template, unwinding the helix as it proceeds. Unwinding of DNA by the *helicase* is aided by the attachment of single strand binding proteins (**SSB protein**). These proteins bind selectively to a single stranded DNA and the binding of these proteins keeps single stranded DNA in an extended state, preventing it from becoming rewound or damaged.

11.4.3 RNA Priming

As the two strands separate or unzip, the bases are exposed, and the enzyme *DNA polymerase II* moves at the point where synthesis will begin. The short segment of RNA that provides the necessary 3′ OH terminus for the initiation of *DNA polymerase* activity is called a **primer**. The primer is laid down complementary to the DNA template by an enzyme *RNA polymerase* or *Primase*. Hence we can say that synthesis of leading strand begins at the origin of replication is initiated by a *primase* (*RNA polymerase*). The DNA replication requires a template DNA strand to copy and a primer strand to which nucleotides can be added. The enzyme DNA *polymerase s*ynthesizes DNA in a 5′→3′ direction. The short RNAs synthesized by the *primase* at the 5′ end of the leading strand and the 5′ end of the each Okazaki fragment serve as the primer for the synthesis of DNA by *DNA polymerase*.

In bacteria, *primase* and *helicase* associate to form a protein complex called primosome. Primosome is involved in the synthesis of RNA primer sequences used in DNA replication. It moves along a DNA molecule with the help of energy of ATP. The *helicase* moves along the lagging strand template, while *primase* binds to helicase periodically and synthesizes short RNA primers that begin the formation of Okazaki fragments. The discovery that DNA gets synthesized as short fragments due to discontinuous replication was made by Reiji Okazaki. The initiation of Okazaki fragments on the lagging strand is carried out by primosome (Fig.11.9). As it proceeds, the *DNA helicase* unwinds the parental DNA double helix, and synthesizes RNA primers needed for the discontinuous synthesis of the lagging strand. *DNA primase* synthesizes RNA primers that are covalently extended with the addition of deoxyribonucleotides by *DNA polymerase III*. Single stranded DNA binding protein coats the unwound unreplicative DNA and keeps it in an extended state for *DNA polymerase* III. The RNA primers are replaced with DNA by

DNA polymerase I and the single strand nicks left by *polymerase I* are sealed by DNA *ligase.* The complete replication apparatus moving along the **Dnb A** molecule at replication fork is called **replisome.** The replisome contains *DNA polymerase* III holoenzyme, two catalytic cores one replicating the leading strand and the other replicating the lagging strand.

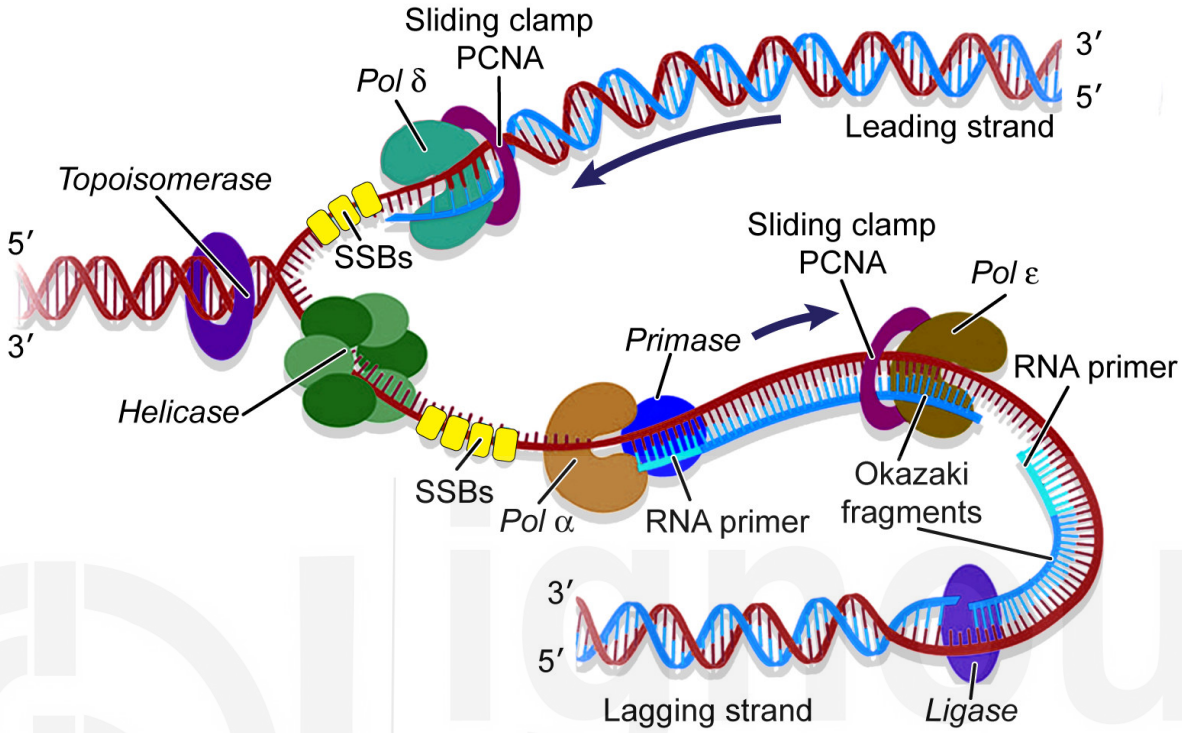


Fig. 11.9: Diagram showing priming during DNA replication in prokaryotes.

11.4.4 Elongation of DNA Chain

DNA Polymerase III is the enzyme mainly involved in DNA replication. It performs 5′→3′ *polymerase* function. It is also known as replicative *polymerase*. Specific activity of *DNA polymerase III* helps in extending the RNA primers. *DNA polymerase III* starts adding nucleotides one by one in a complementary manner i.e. A to T and G to C. The action of DNA *polymerase* is dependent on template. It reads the sequence of bases on the template strand and synthesizes the complementary strand. The template strand is always read in the 3′→5′ direction. The new DNA strand is synthesized in the 5′→3′ direction. DNA *polymerases III* also catalyzes the formation of hydrogen bonds between the new nucleotides and the nucleotides on the template strand. The enzyme also catalyzes the reaction between 5′ phosphate on a new nucleotide and free 3′ OH on the growing polynucleotide (phosphodiester bond), therefore the new DNA strands grow in 5′→3′ direction. The growth of the strand begins at 3′ OH group of the sugar and 5′ phosphate group of the newly synthesized nucleotide. DNA *polymerase* III helps in the synthesis of successive fragments for the lagging strand.

DNA strands are complementary and run antiparallel to each other. Only one new strand at the 3′ end of the template DNA grows continuously in the opposite direction because it is complementary to the template strand. The strand that is synthesized continuously is called the **leading strand** and the other strand, synthesized discontinuously in the form of small fragments, is called the **lagging strand** (Fig. 11.10). As a result of discontinuous replication,

short Okazaki fragments are synthesized**.** Okazaki fragments usually have 1000 to 2000 nucleotides in prokaryotes while in eukaryotes they are smaller in size ranging from 100 to 200 nucleotides. *DNA polymerase III* molecule synthesizes successive fragments of the lagging strand. The *polymerase III* molecule is recycled from the site where it has just synthesized one Okazaki fragment to the next site along the lagging strand template closer to the replication fork. At the new site, the *polymerase* attaches to the 3´-OH group of the RNA primer laid down by a *primase*.

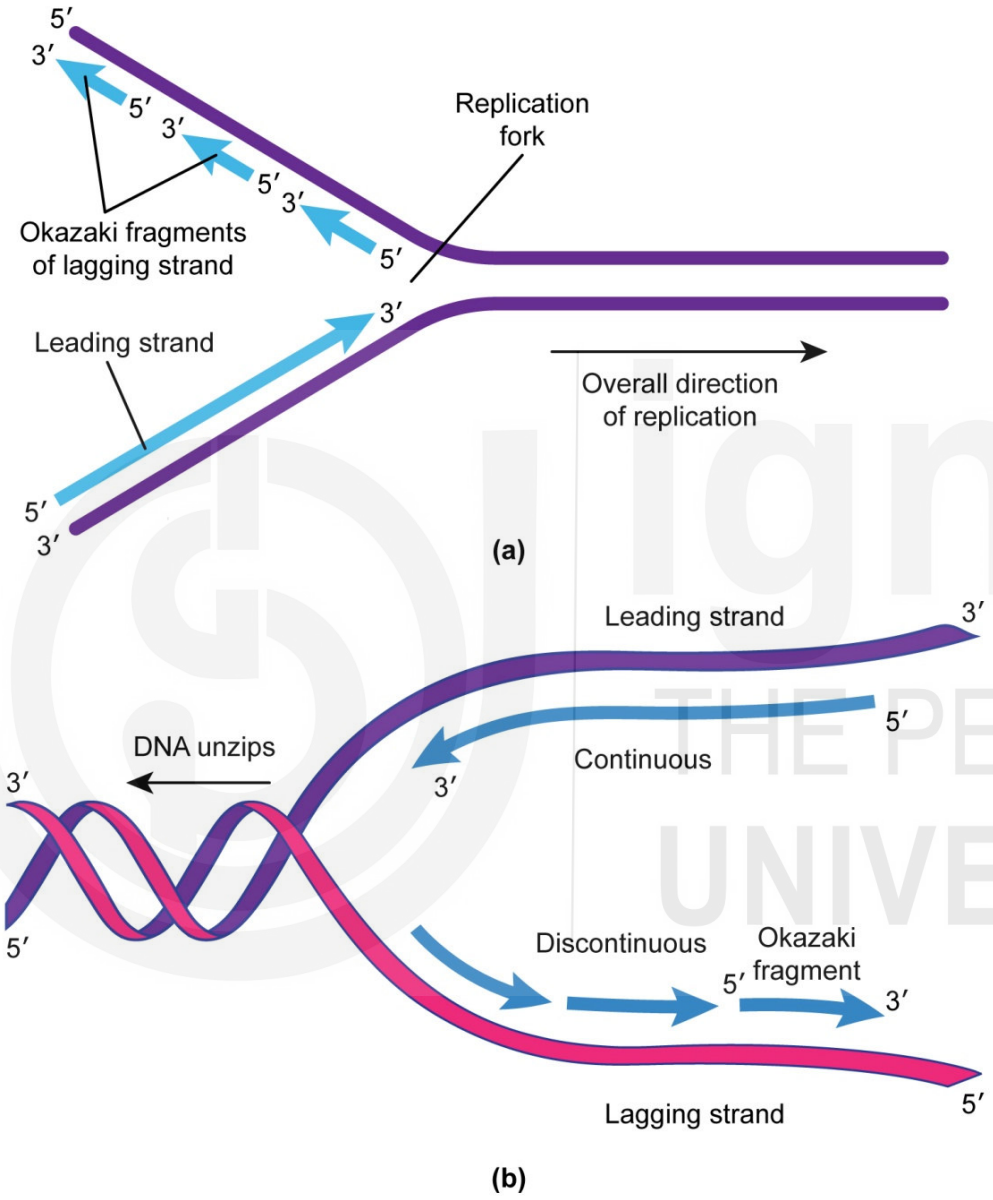


Fig. 11.10: Diagrammatic representation of the replication fork formed during DNA replication.

The primers associated with Okazaki fragments at its 5´end are removed and the resulting gaps are filled with DNA with the help of *DNA polymerase I* (Fig. 11.11). The ends of each fragment are later joined by DNA *ligase* to form a continuous strand (lagging strand). The enzyme joins the fragments together by forming the phosphodiester bonds. *DNA ligase* helps in sealing the gaps or nicks when RNA primer is removed.

Two *polymerases* move in opposite directions with respect to the linear axis of the DNA molecule. Once the *DNA polymerase III*, synthesizing an Okazaki

fragment on the lagging strand template, approaches the 5′ end of the previously synthesized Okazaki fragment, the lagging strand template is released and the *polymerase* begins work at the 3′ end of next RNA primer towards the replication fork (Fig.11.11).

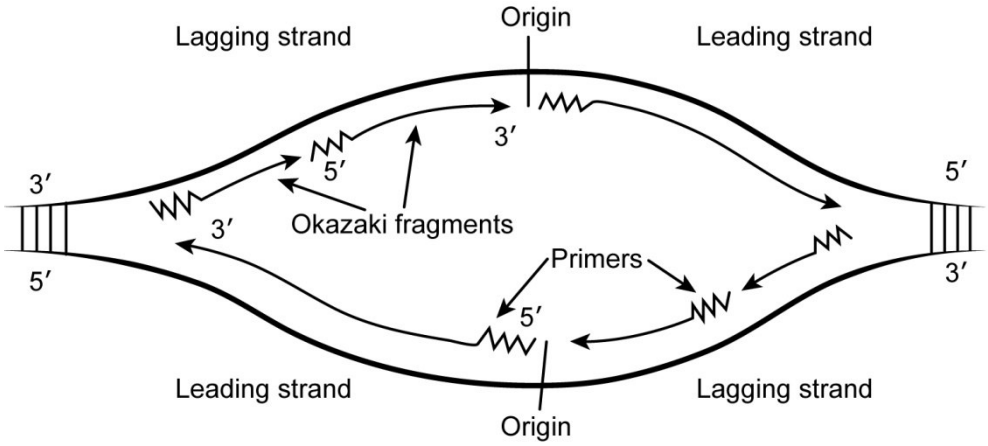


Fig. 11.11: Mechanism of DNA replication in prokaryotes.

Box 11.4: *DNA polymerase I*

DNA polymerase I was the first enzyme with the *polymerase* activity. It was isolated by Arthur Kornberg in 1960. The enzyme plays a role in 5′→3′ elongation (*polymerase* activity), 3′→5′ *exonuclease* (proof reading activity) and 5′→3′ *exonuclease* activity (DNA repair). These activities are confined to three active sites present on the large fragment of enzyme called Klenow fragment. *DNA polymerases* need to remain associated with the template to synthesize a continuous complementary strand and must get attached to the template to move from one nucleotide to the next. *DNA polymerase I* shows both 5′→3′ and 3′→5′ *exonuclease* activity. Being exonuclease *DNA polymerase* is able to degrade DNA polymers by removing one or more nucleotides from the end of the molecule.

Semidiscontinous replication

The newly synthesized strand is synthesized only in 5′→3′ direction. One strand i.e. the leading strand is synthesized continuously because its synthesis continues as the fork advances, while the other strand i.e. the lagging strand is synthesized discontinuously because the initiation of each fragment waits for the parental strand to separate and expose the additional template (Fig. 11.12).

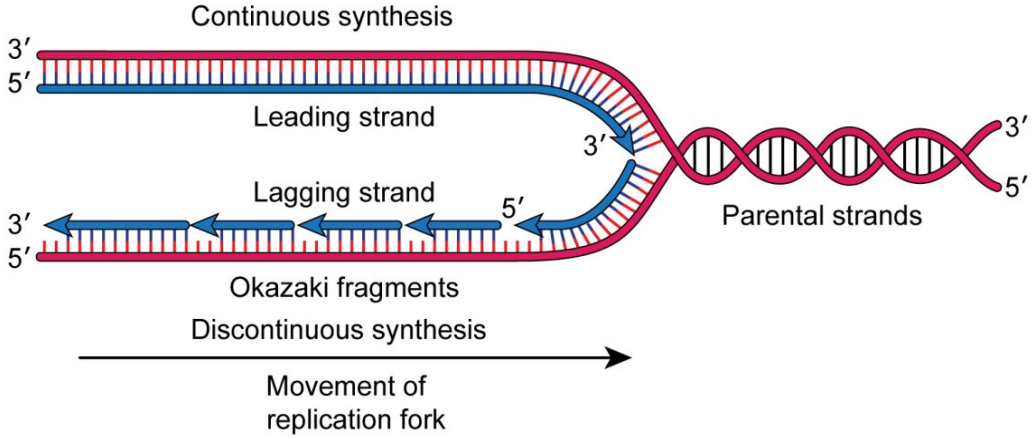


Fig. 11.12: Diagrammatic representation of Continuous and discontinuous type of DNA replication.

Since one strand is synthesized continuously and other discontinuously, the replication is called as semidiscontinuous. The leadings strand shows continuous replication by adding nucleotides to the growing end while the lagging strand shows discontinuous replication by adding short nucleotides segments to the growing end (Fig.11.13).

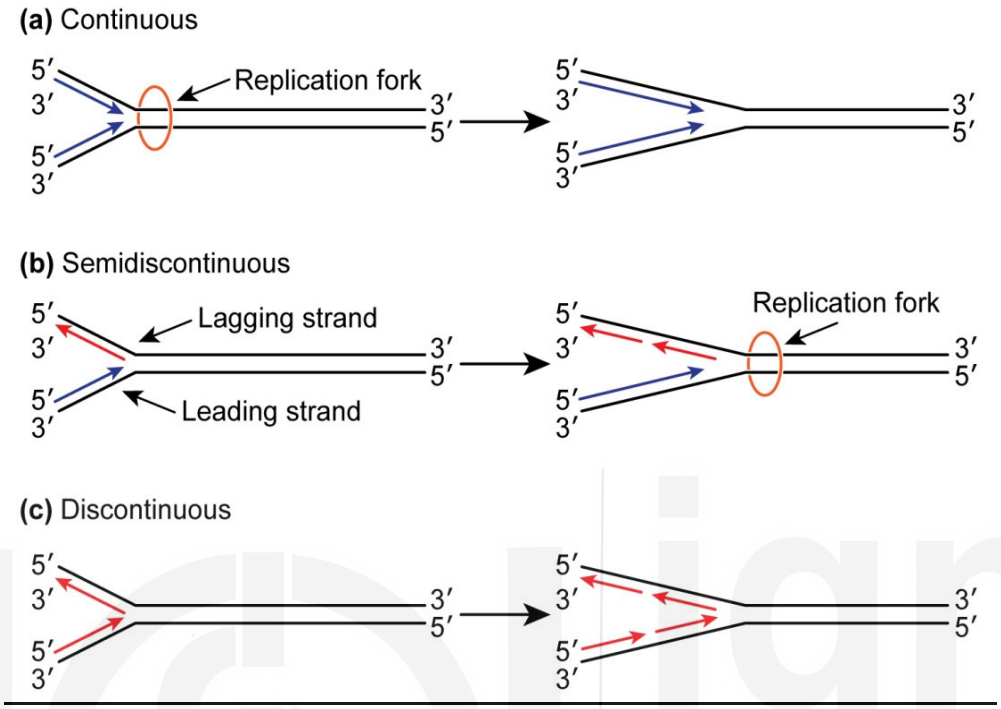


Fig. 11.13: Comparison of different types of DNA replication.

11.4.5 Proof Reading

During DNA duplication, one error occurs in every billion base pairs. This fidelity is necessary to minimize the occurrence of mutations, especially in large genomes. The problem of DNA replication is solved by a mechanism called proofreading. The process of proof reading involves scanning of termini of nascent DNA chains for errors and correcting them. The process is carried out by 3′→5′ *exonuclease* activity of *DNA polymerase*. This helps them to detect and excise a mismatched nucleotide in the 3′→5′ direction. When a template primer DNA has a terminal mismatch (an unpaired or incorrectly paired base or sequence of bases at 3′ end of primer), the 3′→5′ *exonuclease* activity of *DNA polymerase* clips off the unpaired base or bases. Once the mismatched nucleotide is removed, DNA synthesis proceeds in 5′→3′ direction. In *E. coli*, the proof reading function is carried out by ε subunit of *DNA polymerase II*.

Hence, high fidelity during DNA replication is based on the fact that *DNA polymerase* does not simply catalyze the incorporation of nucleotide at the right place, it also actively discriminates against incorporation of a mismatched base by adapting to the conformation of a correct base pair.

11.4.6 Termination of Replication

The meeting of DNA replication forks results in termination of DNA replication (Fig. 11.14). The formation of the replication fork stops when a protein called **replication terminator protein** binds to specific sites on a DNA molecule. It

has been found that proteins, *ter A* and *ter B* block the movement of replication forks advancing in the clockwise and anticlockwise direction at variable sites. Before completion of DNA replication, the enzymes are used to proof read the sequences to make sure the nucleotides are paired up correctly in a process called DNA repair. If any mistake occurs during the DNA replication, the enzyme *nuclease* removes the incorrect DNA. *DNA polymerase II* plays a role in DNA repair and filling up the gaps.

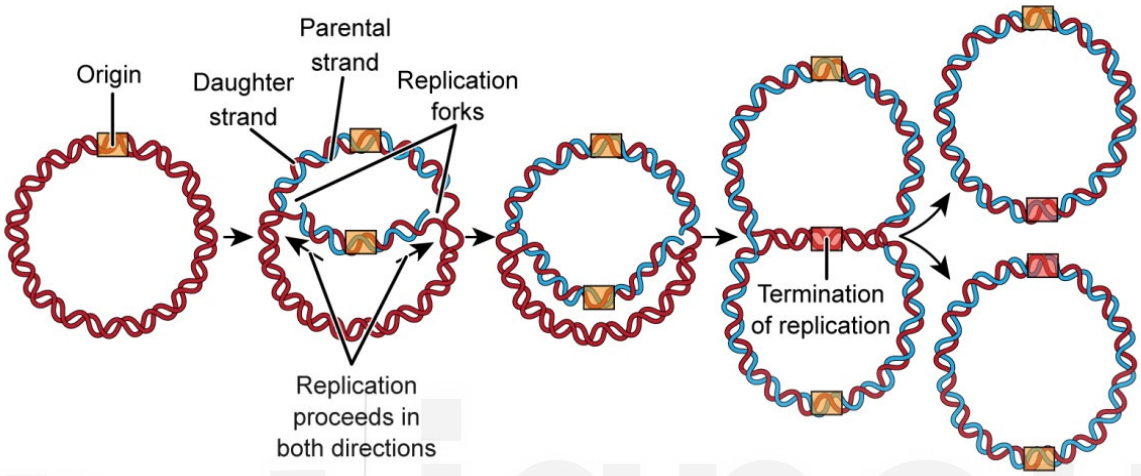


Fig.11.14: Diagrammatic representation of DNA replication in bacteria.

A series of enzymes are required in unwinding and separation of double stranded DNA molecule (Table 11.1).

Table 11.1: Role of different enzymes used in DNA replication.

Enzyme	Function in DNA Replication
DNA Helicase	Also known as helix destabilizing enzyme. Unwinds the DNA double helix at the Replication Fork.
DNA Polymerase	Synthesizes new duplex DNA strand by adding nucleotides in the 5′ to 3′ direction. Also performs proof-reading and error correction.
Primase	Provides a starting point of RNA (or DNA) for *DNA polymerase* to begin synthesis of the new DNA strand.
DNA Gyrase	A kind of topoisomerase that removes DNA supercoils ahead of replication fork.
Topoisomerase	Relaxes the DNA from its super-coiled nature.
Single-Strand Binding (SSB) Proteins	Bind to ssDNA and prevent the DNA double helix from re-annealing after DNA helicase unwinds it, thus maintaining the strand separation, and facilitating the synthesis of the nascent strand.
DNA clamp	A protein which prevents *DNA polymerases* from dissociating from the DNA parent strand during the process of replication.
DNA Ligase	Seals the gaps between the Okazaki fragments to create one continuous DNA strand
Telomerase	Lengthens telomeric DNA by adding repetitive nucleotide sequences to the ends of eukaryotic chromosomes.

Various steps involved in DNA replication in prokaryotes can be summarized as follows:

1. The double helix structure of the DNA molecule is unzipped. This process is carried out with the help of an enzyme *helicase* that breaks the hydrogen bonding between the complementary bases.
2. The separation of DNA strands into two single strands creates a 'Y' shaped replication 'fork'. Two single strands act as templates for making the new strands of DNA.
3. One of the strands is oriented in the 3′→5′ direction (towards the replication fork). This is referred as the leading strand. The other strand is oriented in the 5′→3′ direction (away from the replication fork). This is called as lagging strand. Because of the difference in orientation, the two strands replicate differently.
4. A fragment of RNA called Primer comes along and binds to the leading strand. The primer starts the DNA synthesis. *DNA polymerase* binds to the leading strand and moves ahead adding new complementary nucleotide bases to the DNA strand in the 5′ to 3′ direction (Fig. 11.15). This type of replication is called continuous.
5. Numerous RNA primers (synthesized by enzyme primase) bind at various points to the lagging strand. Fragments of DNA, called Okazaki fragments, are then added to the lagging strand also in the 5′→3′ direction. This type of replication is called discontinuous as the fragments will need to be joined up later (Fig. 11.15).
6. The bases are matched up (A with T, C with G) with the help of enzyme called exonuclease that removes the primer(s). The gaps are filled by complementary nucleotides. The new strand is proofread to make sure there are no mistakes in the new DNA sequence.
7. Enzyme called *DNA ligase* seals up the sequence of DNA into two continuous double strands. Hence after DNA replication, DNA molecule formed consists of one new and one old chain of nucleotides. Hence the DNA replication is described as semi-conservative.

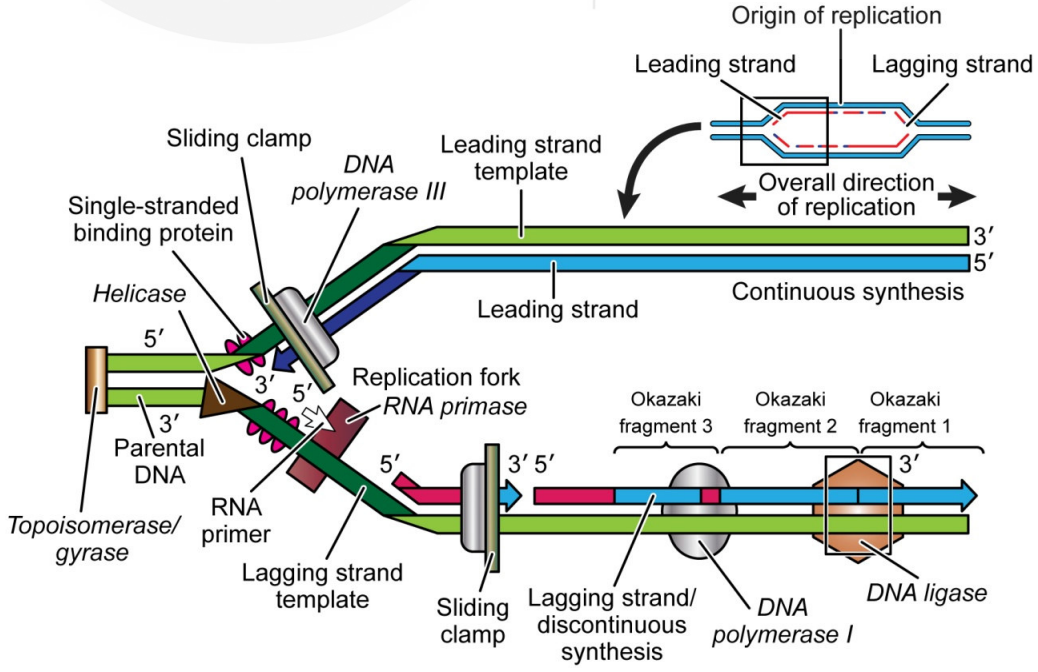


Fig. 11.15: An overview of DNA replication in prokaryotes.

SAQ 2

a) Fill in the blanks:

i) The site of origin of replication in prokaryotes such as *E. coli* has been recognized as…… .

ii) The process of separation of two strands of DNA is referred as …….. .

iii) …… enzyme helps in the unwinding of strands of a double helix DNA.

iv) Enzyme *DNA gyrase* induces formation of …… that helps in unwinding of DNA.

v) The short segment of RNA that provides the necessary 3´ OH terminus for the initiation of DNA replication is called ……...

vi) *Primase* provides a …….. segment for *DNA polymerase* to begin synthesis of new strand.

vii) …….. enzyme catalyzes the formation of hydrogen bonds between the new nucleotides and helps in elongation of DNA strand.

viii) The process that involves scanning of termini of nascent DNA chains for errors and correcting them is called as …….

b) Explain the following terms:

i) Primosome

ii) Okazaki fragments

iii) *Exonuclease* activity

11.5 DNA REPLICATION IN EUKARYOTES

The first experimental evidence for bidirectional replication in eukaryotic cells was obtained by autoradiography of labelled DNA molecules obtained from cultured mammalian cells. The studies revealed presence of clusters of active replicons each having two growing forks moving away from a central origin.

The DNA replication in eukaryotes occurs in a semi conservative manner. In a semi conservative mode, two complementary polynucleotide chains are synthesized on a template strand. The parental DNA strand undergoes cleavage and the two new double helices formed that carry old fragments and the newly synthesized DNA (Fig.11.16). Since the parental strands are dispersed into two newly synthesized helices it is called dispersive replication.

Though most aspects of DNA replication are similar in prokaryotes and eukaryotes but still some prominent differences are noted between them. Some of the major differences have been listed below:

- The eukaryotic cells have larger genomes and complex chromosomal structures.
- DNA synthesis takes place in a small portion of the cell cycle in eukaryotes. Replication occurs in the S phase of the cell cycle.

- DNA synthesis is not continuous as in prokaryotes.
- The large DNA molecules in eukaryotes take more time to replicate of each chromosome contained a single origin.
- The eukaryotic chromosomes contain multiple origins of replication. DNA replication is initiated at multiple origins in a coordinated manner.
- Two or more *polymerases* are used for replication of leading and lagging strands at replication fork.

Box 11.5: Autonomously replicating sequence (ARS)

The origin of replication sequences (ORIs) found in budding yeast is defined as autonomously replicating sequences (ARSs). These sequences initiate the process of replication. ARSs help in maintaining the stability of chromosomes and plasmids during genome replication. In yeast ARSs were identified by while maintaining the stable replication ability of plasmids. Replication timing and efficiency are the two key features of ARSs. The activation timing of ARS are controlled by chromatin environment and epigenetic modifications. ARS have been identified using DNA microarray technology and bioinformatics. The structural and functional properties of ARSs on yeast chromosomes are being explored. Researchers are also interested in knowing the effects of ARSs on gene silencing and expression of genes.

11.5.1 Multiple Replicons

In eukaryotes, very large sized chromosomes having multiple origins collectively control the replication. Replication origin sites in eukaryotes also have unique A:T rich sequences which facilitate the opening of double helix. In eukaryotic chromosomes several bubbles or eyes exist. Eukaryotes show many replicons. The multiple origins collectively control the replication of DNA molecule.

When the two replication forks move bidirectionally from a central origin, the DNA replication generally gets completed in 8.5 days as noted in *Drosophila melanogaster*. Faster replication is achieved by initiating the DNA synthesis at many origins of replication simultaneously. For faster replication of DNA, largest chromosome initiates replication at multiple sites (Fig.11.16). The first evidence of multiple origins in eukaryotic chromosomes came from the pulse labeling experiments with Chinese hamster cells. Joel Huberman and Arthur Riggs pulse labelled cells with ^{3}H- thymidine for a few minutes, extracted DNA and performed autoradiographic analysis of the labelled DNA. They observed tandem array of silver grains indicating that DNA had multiple origins of replication. The pulse labeling period was followed by a short interval of growth in non radioactive medium, the tandem arrays contained central regions of high grain density at both ends. The results indicate that replication is bidirectional. The tails of decreasing grain density result from the gradual dilution of the intracellular pools of ^{3}H- thymidine by ^{3}H- thymidine as replication fork move bidirectionally from central origin toward replication termini. A segment of DNA whose replication is under the control of one origin and two termini is called **replicon**.

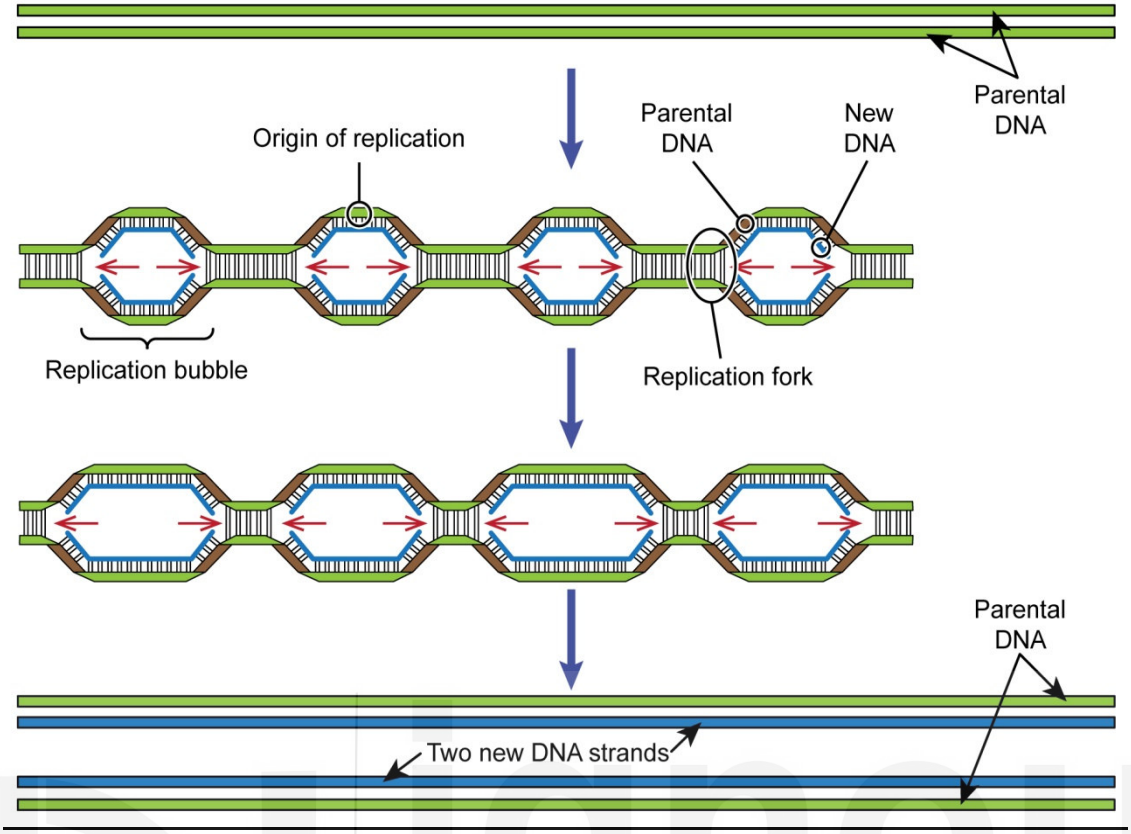


Fig.11.16: Diagrammatic representation of various origin of replication in eukaryotes.

11.5.2 Two or More *DNA Polymerases*

In prokaryotes **five** different forms of *DNA polymerases* are found viz. **I, II, III, IV, V** whereas in eukaryotes 1**5** different forms of *DNA polymerases* are found. These include **α, β, γ, δ, ε, ζ, η, θ, ι, κ, λ, μ, σ, REVI, TD**.

The unwinding of DNA strands requires DNA *topoisomerase* and DNA helicase as in prokaryotes. The unwound strands are kept in an extended state by a single strand DNA binding protein called replication protein A. The replication of chromosomes requires the presence of three DNA *polymerase-polymerase α* (*Pol α*), *polymerase δ* (*Pol δ*) and *polymerase ε* (*Pol ε*). All *polymerases* are present in each replication fork and each *polymerase* contains multiple subunits. The replisome in *E. coli* contains 13 proteins while those of yeasts and mammals contain 27 different polypeptides.

In eukaryotes, *Pol α* is required for the initiation of replication at origin and for priming of Okazaki fragments during discontinuous synthesis of the lagging strand. *Pol α* exists in a stable complex with DNA *primase*. The enzyme DNA *primase* synthesizes RNA primers which are extended by deoxyribonucleotides by *Pol α* to produce 30 nucleotides chain of RNA-DNA. These RNA-DNA primers are extended by *Pol δ*. This enzyme completes the replication of the lagging strand while Pol ε catalyzes the replication of the leading strand. Both *polymerases* δ and ε contain 3´→5´ *exonuclease* activity required for proof reading. These enzymes cannot remove RNA primers like *DNA polymerase I* found in *E. coli*. RNA primers are excised by nucleases, *ribonuclease H1* (which degrades RNA present in the RNA-DNA duplexes) and *ribonuclease FEN-1* (*F1 nuclease I*). *Polymerase δ* fills in the gaps and DNA *ligase* seals the nicks.

In prokaryotes, *Polymerase I, II, III* play a major role in DNA replication while in eukaryotes it is *polymerase* ***α, β, γ, δ, ε*** which play a crucial role in DNA replication and repair (Table 11.2).

Table 11.2: Role of different *DNA polymerases* found in eukaryotes

DNA polymerases	**Subunits**	**3´ to 5´ *exonuclease* activity**	**Function**
Alpha α	**4**	**No**	RNA/DNA primers, initiation of DNA synthesis
Delta δ	**4**	**Yes**	Lagging strand synthesis, DNA repair, proof reading
Epsilon ε	**4**	**Yes**	Leading strand synthesis, proof reading
Gamma γ	**2**	**Yes**	Mitochondrial DNA replication and repair/ editing
Beta β	**1**	**No**	Base-excision DNA repair
Eta η, zeta ζ, kappa κ, iota ι	**1,2,1,1**	**No**	Damaged/distorted (translesion; TLS) DNA synthesis
Theta θ, lambda λ, mu μ	**1,1,1**	**No**	DNA repair
Nu υ	**1**	**No**	Unknown
REV1	**1**	**No**	DNA repair

Box 11.6: *DNA polymerase III*

The active form of *DNA polymerase III is* known as **holoenzyme.** It is made up of ten unique polypeptide subunits. The largest subunit, **α**, along with subunits **ε** and **θ**, form a complex called the **core enzyme,** which imparts the catalytic function to the holoenzyme. Each enzyme contains two or three core enzyme complexes. Five subunits (**γ, δ, δ´, χ** and **υ**) are complexed together to form **slide clamp loader.** This pairs with the core enzyme and facilitates the function of a critical component of holoenzyme called the **β clamp or sliding DNA clamp.** Activity of **clamp loader** is driven by the energy of ATP hydrolysis. The **DNA clamp** resembles a doughnut which can open, close and encircle the unreplicated DNA helix (Fig.11.17). It keeps the *DNA polymerase* associated with the template DNA during polymerization of nucleotides.

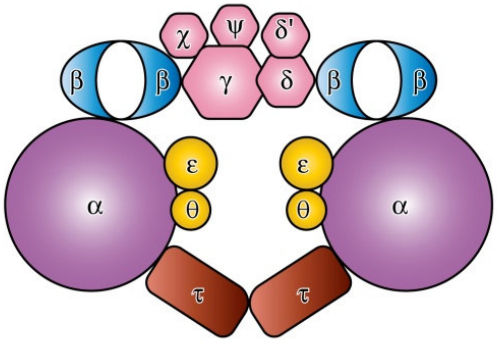


Fig.11.17: Structure of *DNA Polymerase III* holoenzyme.

11.5.3 Duplication of Nucleosomes at Replication Fork

As we know that DNA in eukaryotic chromosomes is packaged into bead like structures called nucleosomes, each of which contain 166 nucleotide pairs of DNA wound around an octamer of histone molecules. The nucleosome need to be disassembled so that the duplication of DNA packaged in a replisome takes place. A number of proteins play a role in the assembly and disassembly of nucleosomes during chromosome replication in eukaryotes. Two important ones are nucleosome assembly protein-I (Nap 1) and chromatin assembly factors (CAF-1). Nap-1 transports histones from their site of synthesis in the cytoplasm to the nucleus and CAF-1 carries them to the chromosomal sites of nucleosome assembly. CAF-1 delivers histones to the site of DNA replication by binding to proliferating cell nuclear antigen (PCNA) the clamp that tethers *DNA polymerase* δ to the DNA template.

11.5.4 *Telomerase* Enzyme

At the end of DNA replication, RNA primers are removed with the help of *DNA polymerase I.* When the primers are located at the end of the strand (telomeric ends) they get removed and single strand overhangs are formed. *DNA polymerases* cannot replicate the terminal DNA segment of lagging strand of a linear chromosome. As at the end of DNA molecule, no free 3´-OH (Primer) is available for polymerization of doxyribonucleotides**.** Because of this, in the next round of chromosome replication, shortened DNA strand serves as the template for the synthesis of new partner strand. The new DNA duplex lacks will lack the sequences corresponding to those of RNA primer from the previous round of replication. This result in loss of sequences and after successive rounds of replication, the chromosome shrinks from its ends.

The special structure of telomeres provides a mechanism for an RNA containing enzyme called ***telomerase*** which helps to prevent the shortening of chromosome ends. The enzyme *telomerase* was discovered by Elizabeth Blackburn and Carol Greider in 1985. In human chomosomes, telomeres contain tandemly repeat sequence TTAGGG. *Telomerases* recognizes G –rich telomere sequence on the 3´ overhang and extends it 5´→ 3´ one repeat unit at a time. *Telomerase* does not fill in the gap opposite the 3´ end of the template strand but simply extends the 3´ end of the template strand. The unique feature of *telomerase* is that it contains an inbuilt RNA template. The *telomerases* contain an integral RNA component which has a sequence complementary to the repeat sequence that makes it act as a template for a *reverse transcriptase* reaction (Fig. 11.18). The *telomerase* then moves to the new 3´ end once the repeat has been added and does it all again. Once several telomere repeat units are added by *telomerase*, *DNA polymerases* catalyze the synthesis of the complementary strand. Without *telomerase*, the linear chromosomes would become progressively shorter. If telomeres are not taken care off then with each round of cell division the DNA would become shorter. This is known as the **"end-replication" problem**. Abnormalities in telomere replication and malfunctioning of *telomerase* have been shown to result in several autosomal diseases resulting in premature ageing, cancers, bone marrow abnormalities, oesteoporesis, and many more.

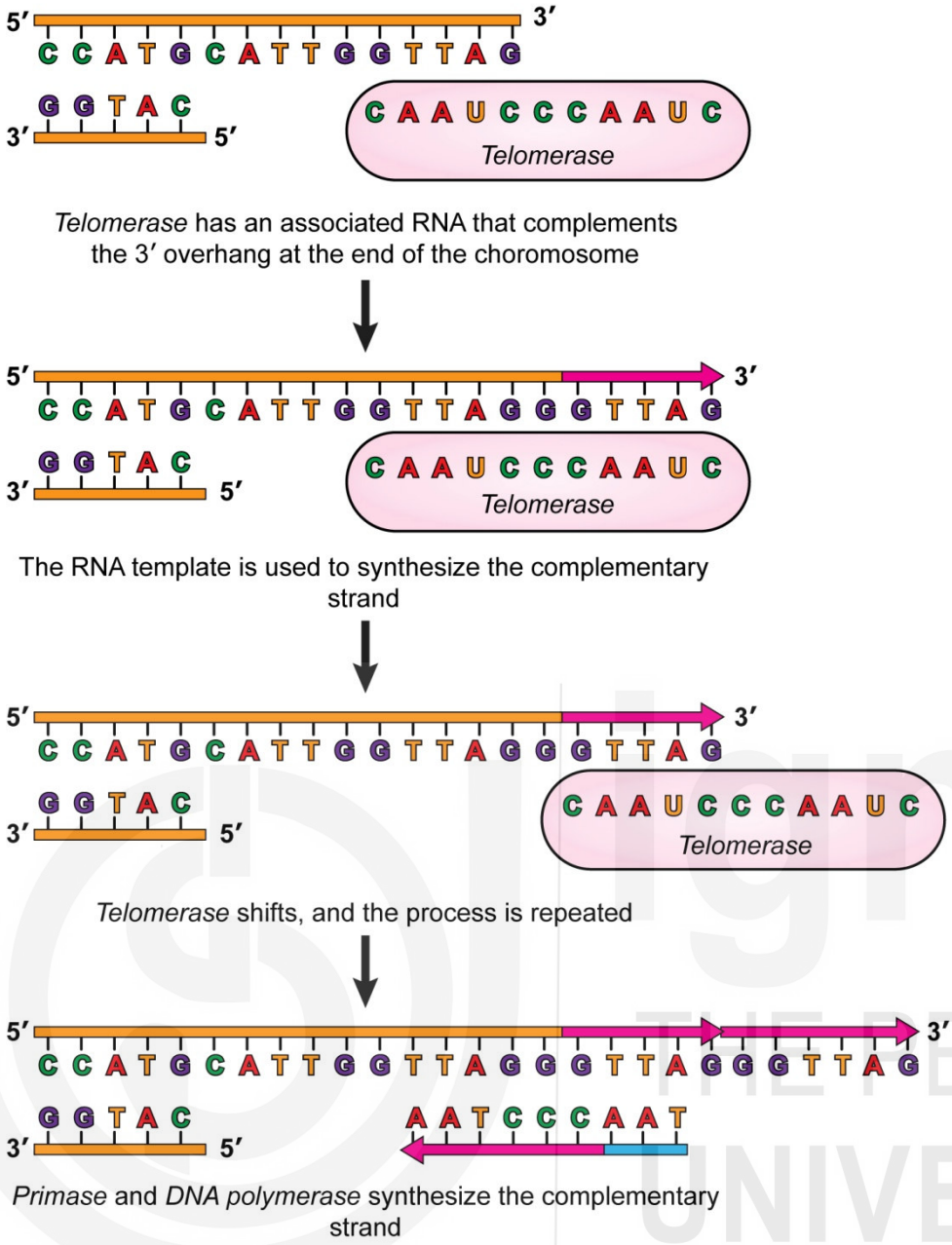


Fig 11.18: Diagrammatic representation of mechanism of *telomerase* action during DNA replication.

The process of DNA replication in eukaryotes can be summarized as follows:

1. DNA unwinds at the origin of replication.
2. *Helicase* opens up the DNA-forming replication forks; these are extended in both directions.
3. Single-strand binding proteins coat the DNA around the replication fork to prevent rewinding of the DNA.
4. *Topoisomerase* binds at the region ahead of the replication fork to prevent supercoiling (over-winding).
5. *Primase* synthesizes RNA primers complementary to the DNA strand.
6. *DNA polymerase III* starts adding nucleotides to the 3′-OH (sugar) end of the primer.
7. Elongation of both the lagging and the leading strand continues.

8. RNA primers are removed and gaps are filled with DNA by *DNA polymerase I*.

9. The gaps between the DNA fragments are sealed by *DNA ligase*.

During replication some problems are faced by eukaryotes. Some of these have been listed below:

- The chromosomes are linear in eukaryotes and they possess more amount of genetic material. A typical animal cell has 50 times more DNA than the bacterium. e.g. *E. coli* has 4.6 million base pairs, humans have 3000 million base pairs.
- Eukaryotes show high level of packaging in the form of nucleosomes (DNA wound around histones).
- *DNA polymerases* found in eukaryotes work slowly. More enzymes are required to speed up the process of replication.
- The replication initiates at multiple sites scattered some 30 to 300 kb apart.

Differences in DNA replication of prokaryotic and eukaryotic cells has been summarized in the table given below (Table 11.3).

Table 11.3: Comparative account of DNA replication in prokaryotes and eukaryotes.

DNA Replication in Prokaryotic Cells	DNA Replication in Eukaryotic Cells
Occurs in the cytoplasm	Occurs in the nucleus
There is a single origin of replication for each DNA molecule.	There are multiple origins of replication
Replication occurs at one point in each DNA molecule.	Replication at several points simultaneously in each chromosome.
Only one replication fork is formed in one DNA molecule.	Numerous replication bubbles are formed in one DNA molecule.
Both initiation and elongation is carried out by *DNA polymerase III*	Initiation is carried out by *DNA polymerase α* while elongation occurs with the help of *DNA polymerase δ* and ε.
DNA gyrase is required	*DNA gyrase* is not required.
Replication is very fast i.e. about 2000 base pairs/nucleotides are added per second.	Replication is slow i.e. about 100 nucleotides are added per second.
The Okazaki fragments are very long (1000-2000 nucleotides long)	The Okazaki fragments are short (100-200 nucleotides long)
RNA primer is removed by *DNA polymerase I*.	RNA primer is removed by *DNA polymerase β*.
DNA is circular, hence telomeres are not replicated	Telomeres present at the end of DNA are replicated

SAQ 3

a) State whether these statements are 'True' or 'False'.

i) The large DNA molecules in eukaryotes take more time to replicate.

ii) More than two *polymerases* are used for replication of leading and lagging strands at replication fork in eukaryotes.

iii) For faster DNA replication in eukaryotes, DNA synthesis is initiated at multiple sites.

iv) Five forms of *DNA polymerases* are found in eukaryotes.

v) In eukaryotes, *Polymerase* δ is the only enzyme that shows 3´ 5´ *exonuclease* activity required for proof reading.

b) Explain the role of *telomerase* in DNA replication.

11.6 SUMMARY

- DNA replication refers to duplication of DNA molecules resulting in formation of copies of molecules during cell division DNA replication is also referred as duplication.
- DNA replication can be conservative, dispersive or semi-conservative. In conservative type, one molecule is conserved as such and a new copy of DNA is synthesized from the old molecule. In dispersive type, the old molecule disintegrates and two molecules would be synthesized, while in semiconservative type of replication, the two strands separate from each other, maintain their integrity and synthesize its complementary strand from the pool of nucleotides.
- The prokaryotes show bidirectional, semiconservative mode of DNA replication. DNA replication always begins at specific sites on the bacterial chromosome called the **origin.** At this site the proteins bind and initiate the process of replication. The replication proceeds outward from the origin in both the directions. The pair of replicated segments come together and joins at site called replication fork**.** At this site, two strands get separated and nucleotides get incorporated into newly synthesized complimentary strands. The two replication forks move in opposite directions until they meet at a point across the circle from the origin where the replication is terminated.
- *DNA polymerases* are the enzymes that synthesize new DNA strands. The DNA strands serve as templates for the polymerization reaction. The strand provides the necessary 3´ OH terminus called as primer. The enzyme adds nucleotides to the 3´ hydroxyl terminus of an existing strand. The enzyme *DNA polymerase* synthesizes DNA in a 5´ to 3´ direction.

- The two strands are synthesized by different processes. One strand is synthesized continuously, it is called the leading strand. The other strand is synthesized discontinuously, it is called the lagging strand. Since one strand is synthesized continuously and other discontinuously, the replication is called as semidiscontinuous.
- DNA replication in eukaryotes is different from that in prokaryotes because eukaryotes s have larger genomes and complex chromosomal structures. Large DNA molecules in eukaryotes take more time to replicate. Eukaryotic chromosomes contain multiple origins of replication. Two or more *polymerases* are used for replication of leading and lagging strands at replication fork.
- *DNA polymerase III* begins to add deoxyribonucleotides, initiating DNA synthesis. *DNA polymerase III* efficiently synthesizes DNA in the direction of replication fork i.e. in 5´-3´ direction with the template showing 3´-5´ polarity. The leading strand is synthesized continuously while the lagging strand is synthesized discontinuously to form Okazaki fragments
- J. Cairns verified the semiconservative method of replication photographically using autoradiography technique. The examining of autoradiographs revealed that DNA o*f E. coli* is circular and replicates maintaining the integrity of the circle.
- Telomeres contain enzyme called *telomerase* which help to prevent the shortening of chromosome ends. *Telomerase* simply extends the 3´ end of the template strand. The unique feature of *telomerase* is that it contains an inbuilt RNA template. Integral RNA component of *telomerases* contain a sequence complementary to the repeat sequence that makes it act as a template for a *reverse transcriptase* reaction.

11.7 TERMINAL QUESTIONS

1. Describe Meselson and Stahl experiment to explain semi conservative mode of replication.
2. Rolling circle replication is noted in which organisms and how is it different from other modes of DNA replication?
3. Write a short note on ‘end-replication” problem’**.**
4. Summarize the various steps involved in the process of DNA replication in eukaryotes.
5. Compare the process of DNA replication in prokaryotes and eukaryotes.

11.8 ANSWERS

Self Assessment Questions

1. a) i) Refer to Section 11.2.

   ii) Refer to Subsection 11.3.2.

b) i) Refer to Subsection 11.2.1.

ii) Refer to Box 11.2.

iii) Refer to Subsection 11.3.2.

2. a) i) *ori* C

ii) unwinding

iii) *Helicase*

iv) negative supercoils

v) primer

vi) RNA segment

vii) *DNA polymerases III*

viii) proof reading

b) i) Refer to Subsection 11.4.3.

ii) Refer to Subsection 11.4.4.

iii) Refer to Subsection 11.4.5.

3. a) i) True; ii) True; iii) True; iv) False; v) False

b) Refer to Subsection 11.5.4.

Terminal Questions

1. Refer to Subsection 11.2.1.
2. Refer to Subsection 11.3.3.
3. Refer to Subsection 11.5.4.
4. Refer to Section 11.5.
5. Refer to Table 11.3.

Acknowledgment for Figures

Fig. 11.1 : Source:

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Fig. 11.3 : Source:
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Fig. 11.5 : Source: https://www.google.co.in/imgres?imgurl=https%3A%2F%2Fimage.slidesharecdn.com%2Freplication-111109072715-phpapp01%2F95%2Freplication-18-728.jpg

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