UNIT 13 REPLICATION AND TRANSCRIPTION

Structure

13.1 INTRODUCTION

The structure and chemistry of some bio-molecules were described in Units 2 to 5, with an introduction of their physiological role. Thus, carbohydrates (other then cellulose) and fats function mostly as energy sources. Cellulose is a structural polysaccharide. Similarly, many lipids are hormones, vitamins and components of biological membranes. Proteins are responsible for metabolic catalysis, stuctual, hormonal and several other functions. A basic characteristic of living cells is their ability to reproduce themselves and do so with a very high precision. This requires that some of the biomolecules must have the function of storage and transfer of information from a cell to its progeny. This information must be precisely copied prior to the cell division so that each daughter cell receives a copy each. A term "gene" was introduced to denote a then hypothetical molecular species which was responsible for the transfer of a particular characteristic from parent to the daughter cells, say ability to synthesise a particular protein like insulin. It has since been shown that DNA (and, in a limited number of cases, RNA) performs the functions attributed to genes. In this unit, we will study the evidence in support of this statement. We will also learn how DNA (i.e., a collection of genes) is duplicated, the process being called "Replication". In the living cells, the genetic information contained in DNA molecule is first "copied" ro "transcribed" in the form of specific RNAs, before it can be translated into the synthesis of various proteins. The process of transcription is very similar to that of replication and, therefore, will be described in this unit whereas translation will be dealt with in Unit 14. Since we will need the background information on the structure of nucleic Unit 4 again before procedding further with this Unit.

Objectives

After studying this unit, you should be able to,

- explain the evidence showing that DNA is the genetic material,
- describe the synthesis of new DNA using old DNA as a template (DNA replication) and
- describe the synthesis of RNA where DNA is employed as template.

13.2 DNA, THE GENETIC MATERIAL

It is now well known that DNA is the carrier of genetic information in all cellular life forms as well as in most viruses. Exceptions are the RNA-viruses or retro-viruses where this role is performed by RNA. However, a long time interval of about seventyfive years elapsed between the discovery and isolation of DNA from nuclei of the leucocytes by Friedrich Miescher (1869) and the recognition of their biological role. In this section, we will not

attempt recounting the long historical development. Instead, some key experiments and pieces of evidence will be described to demonstrate the genetic role of DNA.

13.2.1 Experiments of Griffits (1928) and Avery and coworkers (1944)

The virulent form of bacterium pneumococcus (*Diplococcus pneumoniae*) which causes pneumonia in animals grows as smooth colonies on agar plates, because it is covered (encapsulated) by a polysaccharide sheath or coating. The latter is required for recognising and interacting with the cells which it infects. A mutant pneumoococcus is known which lacks this polysaccharide coating and is non-virulent. This mutant grows as "rough" colonies on the agar plates. The two are referred to as the S (for smooth) and R (for rough) strains, respectively. Injecting live S cells into mice causes pneumonia and death of the animals. On the other hand, injecting live R cells of heat-killed S cells do not produce disease or death. Griffith (1928) injected a mixture of live R cells and heat killed S cells into mice and found that the animals became sick and died (Fig. 13.1). Morevoer, blood of dead mice was found to contain live and virulent S cells only. Thus the normally harmless R cells must have been transformed into virulent S cells and this transformation must have been brought about by some principle present in the heat-killed S cells. Lather (1944) Avery and coworkers found that the transforming principle was DNA. They showed that DNA of heat-killed S cells imparted new genetic properties, like virulence to the otherwise harmless R cells.

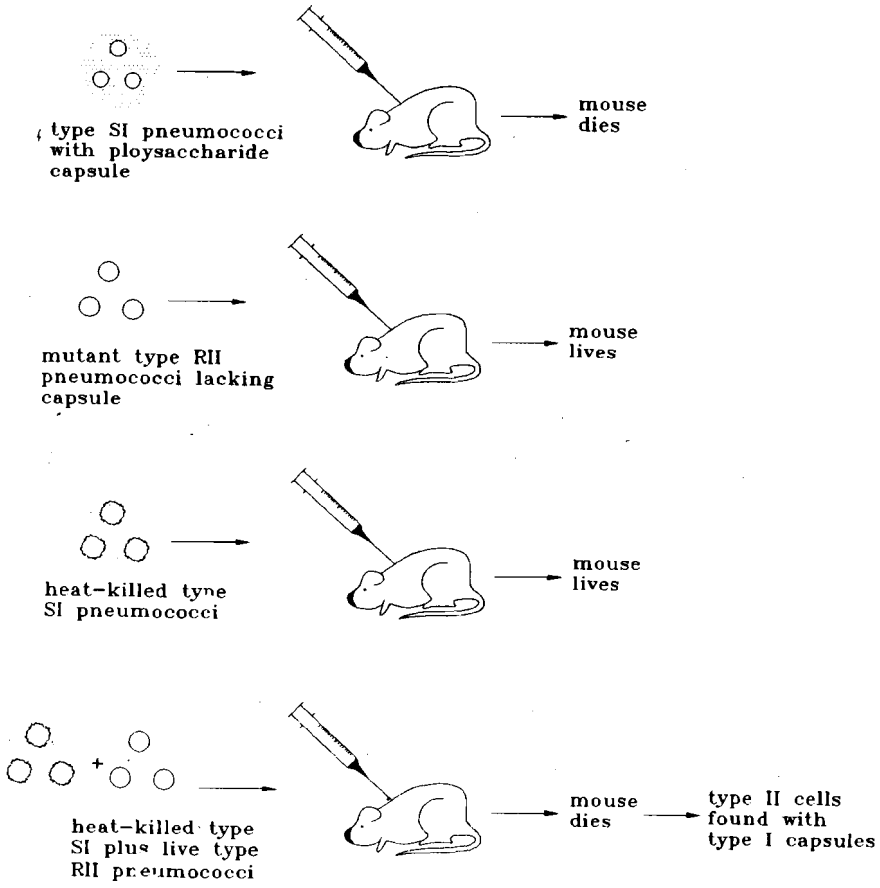


Fig. 13.1: Griffith's experiment demonstrating the effect of rough, smooth, heat-killed (smooth) and mixture of rough and heat-killed smooth strains of Pneumococci.

SAQ 1

Complete the following statement with one of the given choices

When Griffith took a mixture of heat-killed 'S' cells and live 'R' cells and injected into mice, the mice died because,.....

(a) 'S' cell had not been killed completely.

(b) polysaccharide capsule of 'S' cells had not been destroyed.

(c) Live 'R' cells can kill on being injected.

(d) Live 'R' cells had been transformed into 'S' cells by the presence of dead 'S' cells.

13.2.2 Hershey-Chase experiment (1952)

Virus which attack bacteria are called bacterio-phages. A phage is made up of a single length of double helical DNA core which is surrounded by a protein coat. The core opens into a hollow protein tube, called the tail, at the end of which there are tail fibres (Fig.13.2). When a phage infects a bacterial cell, the core DNA enters the cell but the protein coat remains outside and can be separated by agitation. Hershey and Chase (1952) confirmed this by the following experiment. They grew phage particles on *E. coli* in a medium contating nutrients with redio-active isotopes. ^{32}P and ^{35}S. Note that only nucleic acids contain phosphorus atoms and only proteins contain S-Containing amino acids (mathion and Cystein). A phage so grown will have ^{32}P-labelled DNA in the core and ^{35}S-labelled protein coat. When such a phage was allowed to infect *E.coli* Cells for some time and the culture was agitated the proteins coat fell off and most of the ^{35}S radioactivity was recovered in the medium and the bacterial cells contained most of ^{32}P radio activity (Fig.13.3). Further, about 30% of ^{32}P was recovered in the newly formed phage particles whereas less than 1% of ^{35}S could be so recovered. This experiment showed clearly that phage DNA alone carried its genetic information and was essential for the production of phage progency. Therefore, DNA must be the genetic material.

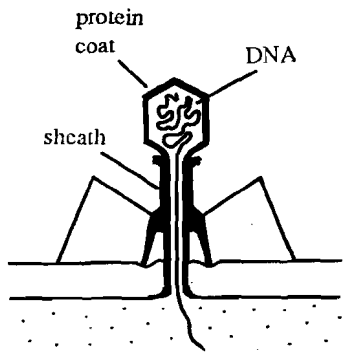


Fig. 13.2 : Bacterial viruses or bacteriophages of the type called "T phages" are composed of a single double-helical length of DNA inside a protein coat. There is a "tail" in the form of a hollow protein tube, surrounded by a sheath, with tail fibres which attach to the cell wall of the bacterium.

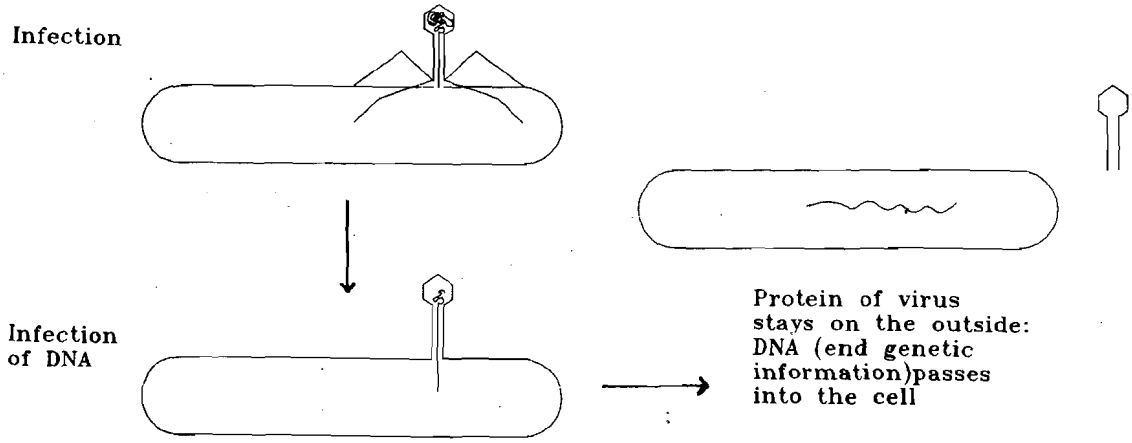


Fig. 13.3 : A Schematic representation of the Hershey and chase experiment.

SAQ 2

What is bacteria-phages?

13.2.3 DNA content of eukaryotic cells

In 1955, it was shown that the somatic cells of eukaryotes had twice the amount of DNA as compared to the corresponding germ cells. It may be recalled that two germ cells, one from each parent, combine to form a single cell from which all the somatic cells arise. In this way,

both the parents contribute equally to the genetic Make up, and therefore the hereditary characteristics each offspring.

Note that at the time of cell division, each daughter cell receives the complete genetic information from its parent and later passes it on to its progeny. Thus, the genetic material must be duplicated in the life time of a cell, so that is may be equally divided between the two daugher cells. Thus, a major characteristic of the genetic material is that it must be able to duplicated or be duplicated by the cell machinery. In the next section, we will see how the nuique double helical structure of DNA is well suited for duplication or as is commonly called "replication", i.e., producting or causing to produce another replica of itself.

SAQ 3

Write 'F' for false and 'T' for true for the following statements.

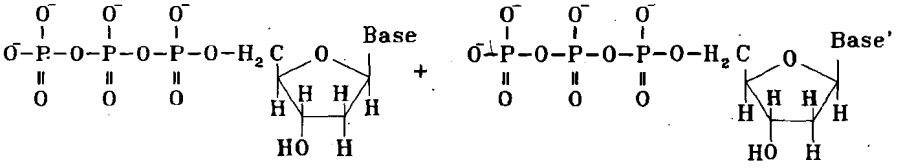
(a) Somatic cells of eukaryotes has equal amount of DNA as compared to corresponding germ cell. [ ]

(b) Two germ cells, one from each parent, combine to form a single cell. [ ]

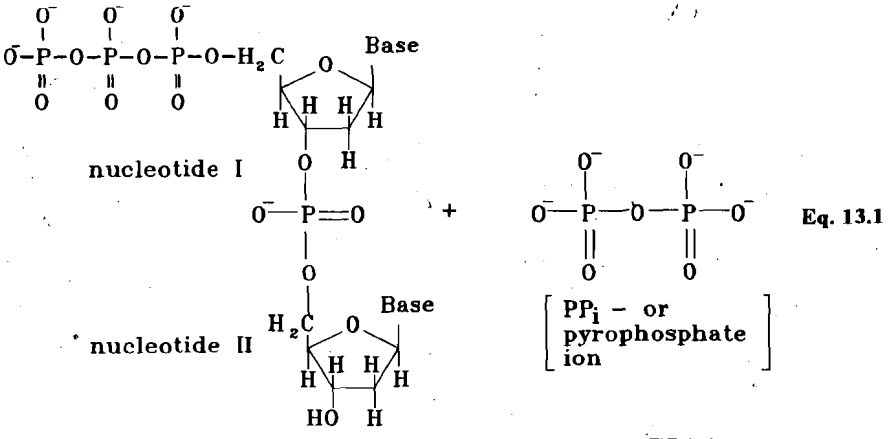
(c) The characteristic of the genetic material is not to duplicate. [ ]

13.3 DNA REPLICATION

DNA is synthesised in the cell from the four deoxyribonucleotide triphosphates, namely deoxyadenosine triphosphate (d-ATP), deoxy-guanosine triphosphate (d-GTP), deoxy-cytidine triphosphate (d-CTP) and deoxy-thynsidine triphosphate (d-TTP). Their structures ar shown in Fig. 13.4. These are linked to each other with the elimination of a pyrophosphate ion to form a 3′– 5′-phospho-diester-linkage as shown in Eq.13.1.



nucleotide I ⇌ nucleotide II



Eq. 13.1

The pyrophosphate ion is rapidly hydrolysed to phosphate ions (PPi) in the presence of the enzyme pyrophosphatase.

$$PP_i + H_2O \longrightarrow 2P_i$$

Since this hydrolysis is practically irreversible, it helps to drive there action of Eq. 13.1 to completion. Note that a poly-deoxy nucleotide built by repitition of this reaction will have two distinct ends, namely a 5′-end which will be towards the top in the structure depieted in Eq. 13.1 and a 3′-end which will be towards the bottom of the same structure. The biosynthesis of DNA always proceeds from the 5′-end to the 3′-end, i.e., the next incoming deoxy nucleoside triphosphate will react at the bottom of the structure in Eq. 13.1.

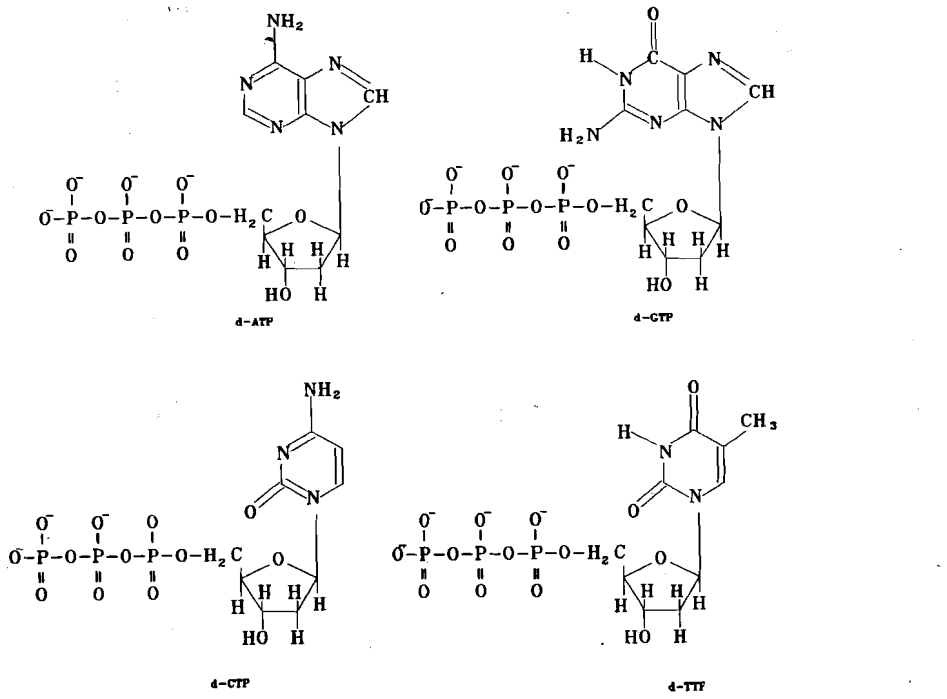


Fig. 13.4 Structures of deoxynucleoside triphosphate precursors of DNA

This brief description gives only the chemistry of the formation of DNA. However, replication of DNA requires that the bases (i.e., individual nucleotides) follow each other in a precise sequence. This fidality in DNA formation is achieved by virtue of highly specific base pairing or hydrogen bonding as described below.

Recall the unique double helical structure of DNA described in Unit 4, in which two poly-deoxynucleotide chains running in opposite directions are wound around a common axis in such a way that they cannot be separated without unwinding the helix. The bases of both the strands lie on the inside of the helix and their planes are perpendicular to the helix axis. Bases from the two strands form specific base pairs in which adenin of one strands lie alongside thymine of the other strand (A-T pair) and quanine and cytosine of different strand lie side by side (G-C pair). Specificity and stability of each bases pair is dur to to base-base hydrogen bonding (see Unit 4, Section 4.51). Such a structure provides the basis for replication of DNA; each strand can function as a template for the synthesis of the other because of the specificity of base pairing.

Let us represent a portion of DNA, which is to be replicated as a linear array of bases of the two strands as in Fig.13.5(a). The verticle lines in this figure represent the hydrogenbonds (two in A=T and three in G≡ C pair). If we imaging that the two strands of a small segment of DNA unwind and separate the strands can be represented as in Fig. 13.5(b). Nucleotide precursors (the four deoxy-nucleoside triphosphates shown in Fig. 13.4) can now align opposite these strands by virtue of specific base pairing as in Fig. 13.5(c). In this figure, the new bases are shown in bold face. The next logical step will be polymerisation of the precursors by a succession of reactions as shwon in Eq.13.1. This will give rise to two double stranded molecules (Fig.13.5(d)) which are identical to each other and to the starting parent DNA molecule. Note that this replication is "semi-conservative" in that each new, daughtere, DNa molecule has one old and one new strand. In actual synthesis, the precursors do not align in a row but are bound and joined one-by-one to a growing poly-nucleotide chain. The chemical reaction at each step is similar to that of Eq.13.1, except that I is not a single nucleotide but a growing polynucleotide chain. The selection of the particular precursor is based on the specificity of base pairing as described about. Further, the newly forming strand is always synthesised from the 5′ to the 3′ end, i.e., the newly incoming nucleoside triphosphate forms a phosphodiester linkage with the 3′-OH group of the growing poly nucleotide chain. Thus, the new chain will grow from right to left for the top pair and from left to rigth for the bottom pair of Fig. 13.5(d). This creates a peculiar problem which is discussed later in this section after a brief description of the enzymes involved in this process.

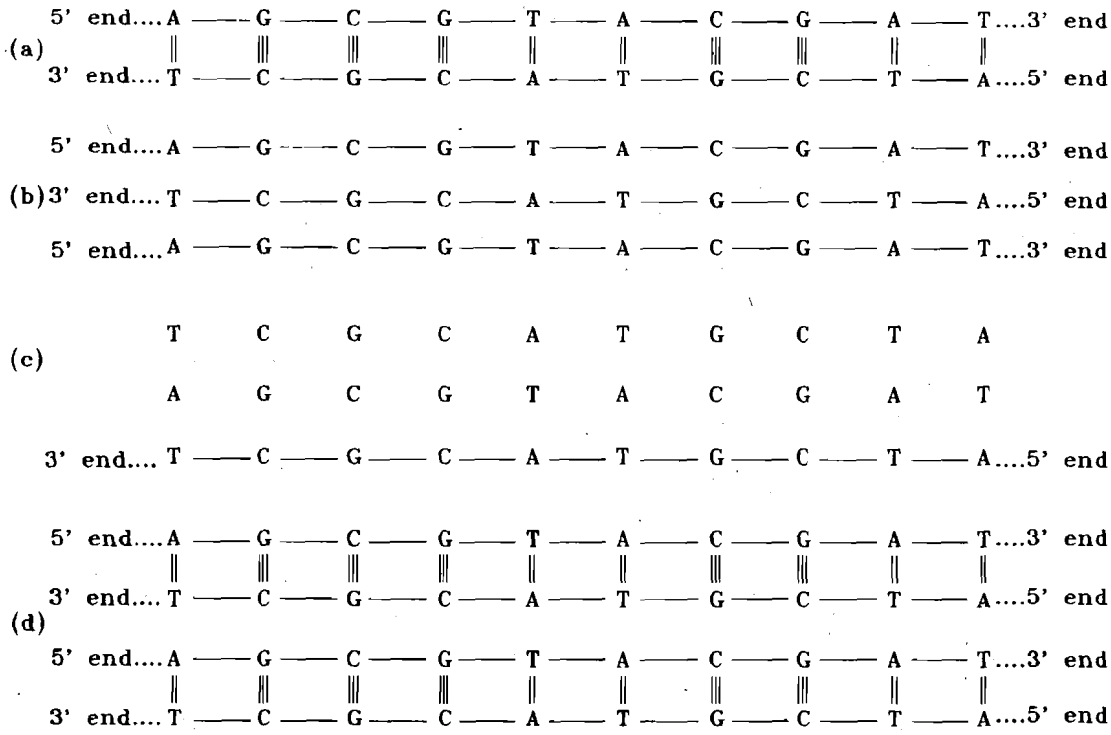


Fig. 13.5 : Diagramatic representation of the replication of DNA. (a) Double stranded parent DNA. The vertical lines represent the hydrogen bonds. (b) Separate strands of the parents DNA. (c) Alignment of the bases of the deoxy-nucleoside triphosphate precursors (bold face) along the two strands and (d) polymerisation of the precursors to give two identical daughter DNA molecules.

An important enzyme involved in DNA rreplication is DNA-polymerase. Three different DNA-polymerases are known. These are referred to as DNA-polymerase I, II and III. They all catalyse the reaction of Eq. 13.1 with I being substituted by a growing polynucleotide chain. DNA polymerases also possess some exonuclease activities which are important for "proof-reading" or "double checking" the correctness of the incoming nucleotide. For example, DNA-polymerase I has the 3′ → 5′ exonuclease activity, i.e., it can hydrolytically cleave and remove the newly incorporated nucleotide at this growing 3′ end of the polynucleotide chain. If a wrong nucleotide gets incorporated, it does not form proper hydrogen bonding with the base of the template strand. This inhibits the polymerase activity and the 3'-5' exonuclease activity excises (cuts) the wrong nucleotide after which the polymerase function resumes and DNA replication progresses further.

None of the DNA polymerases can initiate a new polynucleotide chain, i.e., I in Eq. 13.1 must be an oligo- or poly-nucleotide chain which is called a primer. DNA-polymerase I requires a length of RNA as a primer which is excised and removed later on.

As mentioned above, DNA replication always proceeds from the 5′ to the 3′ end. This is due to the reaction specificity of DNA-polymerases. As seen in Fig. 13.5, it is necessary then the two newly synthesised strands must proceed in opposite directions. It has been shown that when replication of one strand has progressed for some length, replication of the second strand commences in what is called the replication fork and proceeds in opposite direction (Fig.13.6). It can be easily understood that in the latter case, the replication must necessarily be discontinuous. A new fragment is initiated every time when the replication of the first strand (continuously replicated) has progressed somewhat further. The parent DNA is continuously being unwound as the replication progresses.

The fragments in the discontinuous strand are 1000-2000 nucleotides in length and are called Okazaki fragments. The strand which is synthesised continuously is called the leading strand and the one with discontinuous growth is referred to as the lagging strand. The latter is constituted by the Okazaki fragments. Subsequently these strands are covalently linked to each other by the action of another enzyme called DNA-ligase.

It may be noted that unwinding of the DNA double helix is an energy - requiring process. It also requires the presence of specific proteins, namely a helicase, rep proteins and a single strand binding (SSB) protein. Helicase and rep protein bind to specific sites in the DNA molecule and bring about unwinding of the double helix with the expenditure of two ATP

molecules per base pair. The SSB protein binds to the two separated strands thus preventing them from forming the double helix again.

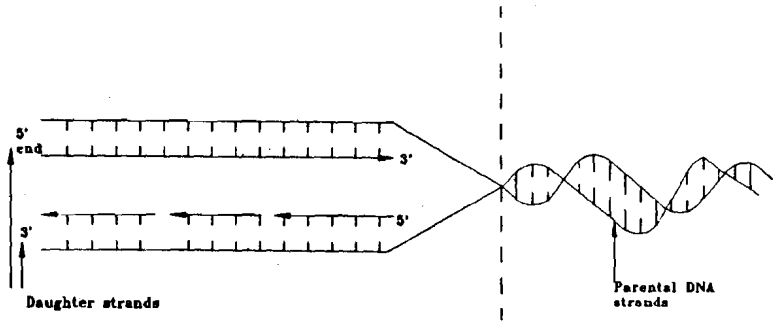


Fig. 13.6 : Replication fork, Replication of both the strands proceeds from 5' to the 3' end. In one of these the replication proceeds continuously (leading strand). In the other, short polynucleotide chains (1000-2000 bases) are synthesised in a discontinuous manner (lagging strand). The structure on the left hand side of the verticle border line is shown as two sets of parallel lines. Actually these represent two

It must be clear that specific base pairing is resoponsible for the exceedingly high precision of DNA-replication. The complimentarity between base sequences of the newly synthesised DNA strandand and its template was first demonstrated by hybridisation studies and later by direct base sequence determination. The error rate in DNA synthesis by DNa polymerase is negligibly small indeed. The same principle of base pairing is also the basis for DNA-directed synthesis of RNA, or transcription, which is described in the next section.

SAQ 4

What is Qkazaki?

13.4 RNA SYNTHESIS : TRANSCRIPTION

There are three types of RNAs in the cell, namely the ribosomal RNAs, transfer RNAs and messenger RNAs. They have similar chemical structure, i.e., they are all polynucleotides in which the individual nucleotieds are linked by phospho-diester linkages. However, they differ with respect to the size, secondary structure and function (see Units 4 and 14). They are all synthesised on DNA templates by an identical chemical reaction, which is similar to that of Eq. 13.1. This process is called **Transcription.**

The precursors in this case are ribonucleoside triphosphates, as against the corresponding deoxy analogues required for DNA. Further, thymine is not found in RNA. Instead, there is uracil. Thus, the precursors are adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and uridine triphosphate (UTP))(Fig.13.7).

The enzyme involved in RNA synthesis is called RNA-polymerase. In addition to the precursors (Fig. 13.7), Mg^{2+} and Mn^{2+} ions, it also requires double stranded DNA to serve as a template. The latter is unwound for transcription to take place. It is interesting to note that only one of the two strands of DNA is transcribed. This is in contrast to DNA synthesis where both the strands are replicated. Further RNA polymerase does not require any primer. There are specific sites on DNA where RNApolymerase can bind and initiate transcription. Similarly there are termination signals where RNA polymerase stops and releases the newly synthesised RNA. Some of these aspects are described in Unit 14, because they are closely related to the regulation of protein biosynthesis. Further, RNA polymerase does not possess any exonuclease activity.

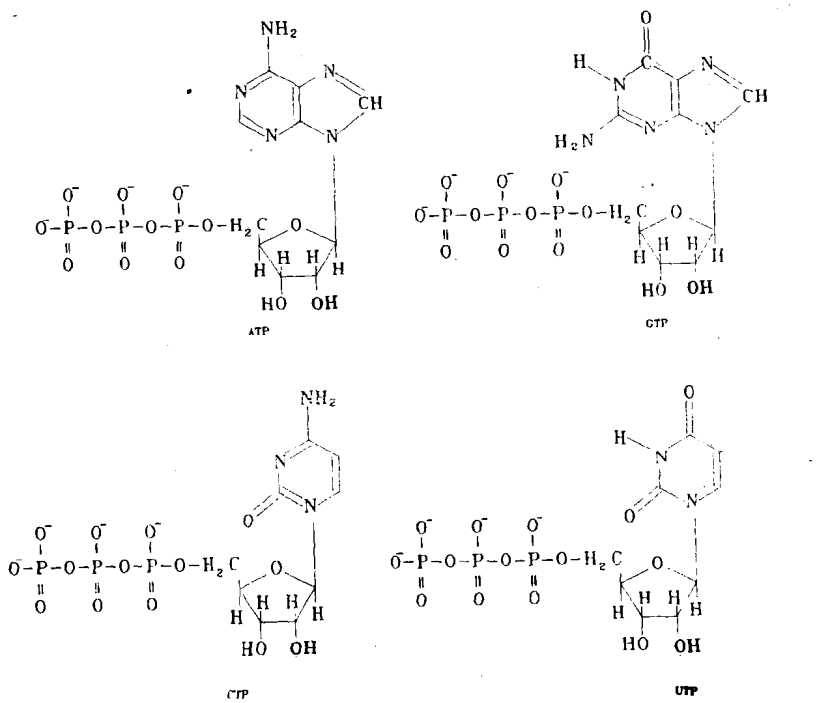


Fig. 13.7 : Structures ribonucleoside triphosphates, the precursors in RNA synthesis.

SAQ 5

How many types of RNAs are found in the cell ?

13.5 SIMILARITIES BETWEEN DNA-REPLICATION and RNA TRANSCRIPTION

1. Each process requires the corresponding nucleoside triphosphates as precursors.
2. In each case, the polynucleotide chain grows from the 5' to the 3' end side, i.e., the reaction involves a nucleophilic attack of 3'-OH grroup of the growing chain on the a-phosphate group (i.e., the one attached directly to the ribose moiety) of the incoming precursor (Eq.13.1).
3. In each case, the reaction is driven to completion by hydrolytic removal of pyrophosphate ion.
4. In each case, the base sequence in the product is determined by the spacificity of bas pairing with DNA template.

13.6 SUMMARY

DNA, i.e., poly-deoxynucleotide, is the genetic material for all cellular life and for most viruses. It has been shown that DNA isolated from a virulent strain of pneumococci mixed with a live non-virulent strain of the same bacteria can cause sickness (pneumonia) in experimental animals. Further, the blood of these animals showed the presence of live virulent bacteria only. This suggests that DNA of the virulent strain can transform a non-virulent into virulent one. Experiments with bacteriophages showed that the phage DNA above entered the infected bacterial cell. The empty protein sheath could be separated from the infected cell by simple agitation. The infected cell had all the informati required to produce complete infective phage particles. This information could have come from the phage DNA only.

DNA is replicated, i.e., its exact copies are produced, in the cell by virtue of the specificity of base pairing. Each strand of the existing double stranded DNA functions as a template for the synthesis of a new strand. Deoxy-ribonucleoside triphosphates are the precurrsors from which DNA is formed. The newly formed poly-deoxynucleotide chain grows from its 5' to the 3' end. The reaction is catalysed by a DNA-polymerase. For the addition of each nucleotide to the growing chain, a molecule of pyrophosphate ion is produced. hydrolysis of the latter drives the reacation to completion. Existing DNA is unwound by helicase and other proteins to produce short regions of single strand DNAP where DNA-polymerase binds. This enzyme cannot initiate a new chain and requires an RNA primer to which the new DNA chain gets attached. Subsequently, the RNA portion is removed. Both strands of DNA are replicated simultaneously in opposite directions. One of the new strrands is continuously synthesised (the leading strand). The other strand is synthesised as short (1000-2000 nucleotide long) fragments (lagging strand) which are still held by their template. These are subsequently joined to each other by the action of DNA-Ligase. On completion of the entire process two molecules of double stranded DNA are prroduced which are identical to each other and to the starrting DNA. Replication of DNA is said to be "semiconservative", because each new (daughter) DNA molecule contains one old (parental) strand and one newly synthesised strand.

All cellular RNAs are produced by using one or the other strand of double strranded DNA as a template. Neucloside triphosphates are the precursors and the enzyme catalysing the synthesis (Transcription) is called RNA-polymerase. Chemically, the reaction is similar to that in DNA-replication. The base sequence of newly synthesised RNA is dictated by that in DNA template and the precursors get selected by virtue of specific base pairing as in DNA replication. In RNA synthesis, only one strand of DNA is transcribed.

13.7 TERMINAL QUESTIONS

1. In hershey and chase experiment why did the radioactivity pass into the bacterial cell only from DNA labelled bacteriophages?
2. What is the direction of RNA synthesis?
3. Whether exonuclease activity is present in RNA polymerase.
4. How the bases are paired in a DNA?
5. What is the difference between the bases of DNA and RNA?
6. Which enzyme is involved in DNA Replication?
7. What are the similarities between DNA Replication and Transcription?

13.8 ANSWERS

Self ASsessment Questions

1. d
2. Virus which attack bacteria are called bacteriophages.
3. (a) -F; (b) -T; (c) -F
4. The fragments in the discontinuous strrand are 1000-2000 nucleotides in length and are called Okazoki.
5. Three type: Transfer RNA, Ribosomal RNAs and Messenger RNAs.

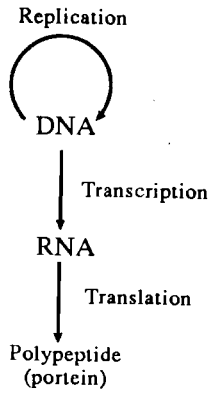
Terminal Questions

1. The radioactivity passed into the bacterial cell from DNA labelled bacteriophages only because it acts as the genetic material and carries information for the synthesis of new bacteriophages. Bacteriophages protein rremains outside the bacterial cell.
2. It is from 5' to 3'
3. No, exonuclease acataivity is not present in RNA polymerase.
4. Adenin of one strand lies alongside thymine of the other strrand (A-T pair) and guanine and cytosine to different strands lie side by side (G-C pair).
5. The thymine is replaced by Urracil in RNA.
6. DNA polymerase.
7. Please see section 13.5.

UNIT 14 PROTEIN BIOSYNTHESIS

Structure

14.1 INTRODUCTION



Genetic information is stored as the sequence of bases in DNA, rewritten into the sequence of nucleotides in RNA, and finally expressed as the sequence of amino acids in a polypeptide.

In the units 4 and 5, you have learnt about nucleic acids and proteins. You must be familiar with their constituents, structures and functions. In this unit, we will learn how proteins are synthesised in the living organisms and the role of nucleic acids.

DNAs i.e. the genes, contain coded instructions for specific sequences of amino acids in various proteins. These are first "copied", i.e. transcribed, in the form of specific sequences of bases in a special RNA, called messenger RNA or mRNA. The latter combines with ribosomes where the coded instructions are decoded, i.e. "**translated**" into amino acid sequence in proteins with the help of another type of RNA, called transfer RNA or tRNA. There are a large number of different tRNAs, each specific for one amino acid. These carry the amino acids to the ribosomes and align them properly in accordance with the coded message on mRNA. This is achieved by specific interaction of some bases on tRNA which form base-pairs with those on the mRNA. Relationship between the base sequence on mRNA and the amino acides specified by them is called **genetic code**. The RNA specific synthesis of polypeptides and proteins is called "**translation**". This process is complex and requires the participation of a large number of different macromolecules.

In this Unit, we will first give an overview of protein synthesis and then discuss the various stages in some detail. We will also briefly study how this process is regulated according to cell needs. This will be followed by a description of the molecular basis of the action of antibiotics. At the end we will study the biochemistry of cancer. You are advised to go through Unit 4 again before proceeding on with this unit.

Objectives

After studying this unit, you should be able to :

- describe the genetic code,
- explain the role of ribosomes,
- list the different steps in protein biosynthesis and describe their significance in the process,
- describe different factors and constraints governing the protein synthesis in a cell,
- explain the principle of antibiotic drug action, and
- describe cellular basis of cancer.