the fat is low. A high saponification number shows that the fat is composed of short chain fatty acids (low molecular weight). Thus, the fat contains mostly short chain fatty acids, with a low degree of unsaturation.

2) Fatty acids are of two types, saturated and unsaturated. Most naturally occurring fatty acids have 16-20 carbon atoms but short chain fatty acids are also present occassionally. The naturally occurring unsaturated fatty acids are alwlays of the ***cis*** configuration, and PUFAs are generally present in vegetable fats. Saturated fatty acids are often found in animal fats.

3) The plasma lipoproteins are distinguished on the basis of their composition and density into four types, namely, chylomicrons, VLDL, LDL and HDL. Lipoproteins represent the principal form, in which the lipids are transported to tissues by plasma. The protein portion (apolipoproteins) of the complex are recognised by tissue specific receptors, wherein they are further utilised.

4) The eicosanoids are 20 carbon atom lipids derived from the tetraenoic acid, arachidonic acid. They include prostaglandins, thromboxanes and leukotrienes. They generally have profound effects, such as smooth muscle contraction, mediation of hormonal effects, increase in blood pressure, blood clotting and inflammatory responses.

5) Sphingolipids are also phospholipids, except that the alcohol in the former is the C_{18} amino alcohol, sphingosine, whereas the latter term also includes phosphoglycerides derived from the trihydroxy alcohol, glycerol. The phosphate group in both lipids is on the primary alcohol group. The fatty acids in phospholipids are esterified to the two alcohol groups of glycerol, while in sphingolipids they are attached by an amide bond, to the amino group of sphingosine.

6) Singer and Nicolson model for biomembranes proposes that proteins are dispersed in the phospholipid bilayer as a mosaic. While the exterior of the bilayer sheet is hydrophilic, the interior is hydrophobic, so that water soluble molecules cannot freely pass through the bilayer. The lipid and protein molecules are capable of lateral movement within the plane of the bilayer. The different proteins present in the membrane carry out both active and passive (facilitated) transport of molecules.

7) Essential fatty acids are those fatty acids that contain more than one double bond and cannot be synthesised by the body. Arachidonic, linolenic and linoleic acids are important polyunsaturated fatty acids that are required by animals and humans for proper nutrition. They are precursors of an important class of lipids, namely the eicosanoids.

8) Membrane fluidity is influenced by the lipid composition, primarily by cholesterol and unsaturated fatty acid content of the membranes. Because of the ***cis*** double bonds in unsaturated fatty acids, a bend is produced in the molecule and the highly ordered packing of fatty acids in the bilayer is disrupted, leading to increased fluidity.

9) Membrane proteins could be both peripheral and integral. These proteins perform several functions such as, enzymic, receptor, transport, cell communication and energy transduction.

UNIT 4 : NUCLEIC ACIDS

Structure

4.1 Introduction
Objectives

4.2 Biological Role of Nucleic Acids

4.3 Structure of Nucleic Acids
Nucleosides and Nucleotides

4.4 Ribonucleic Acids (RNA)
Ribosomal RNA (rRNA)
Messenger RNA (mRNA)
Transfer RNA (tRNA)

4.5 Deoxyribonucleic Acids (DNA)
What Holds the Double Helix Together?
DNA Denaturation
Replication of DNA

4.6 Synthesis of RNA (Transcription)

4.7 Genetic Defects

4.8 Summary

4.9 Terminal Questions

4.10 Answers

4.1 INTRODUCTION

In Unit 3, we described lipids, which are a class of biomolecules grouped together mainly on the basis of common solubility properties. You also learnt about the structure of biomembranes. You will recall that membranes are an important part of cell structure, and are vital for living organisms. In Unit 4, we shall study another type of vital biomolecule, namely the nucleic acids. These biomolecules, like polysaccharides, are biopolymers, and are important components of living organisms. In this unit you will learn about the biological importance of nucleic acids. We shall also describe nucleotides, which are the building blocks of nucleic acids. Besides, you shall learn about the types of nucleic acids and the helical structure of DNA. Nucleic acids (RNA) play an important role in the biosynthesis of proteins and polypeptides, and Unit 5 shall deal with the study of proteins. These are complex biomolecules, constituting half the solid mass of a cell. We shall describe their structural organisation first, and then you will learn about their role as biocatalysts in Unit 6.

Objectives

After studying this unit, you should be able to:

- explain the functions of nucleic acids,
- describe the structure of nucleic acids,
- distinguish between ribonucleic acids and deoxyribonucleic acids,
- describe the role of various types of RNAs, and
- explain the double helical structure of DNA.

4.2 BIOLOGICAL ROLE OF NUCLEIC ACIDS

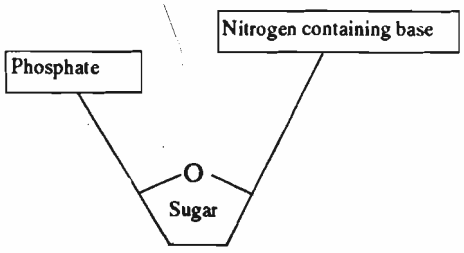
Nucleic acids are macromolecules which are present in the living cells, either in a free state or combined with proteins, as nucleoproteins. You will recall from Unit 1 that nucleic acids constitute about 7% of the dry weight of a cell. You might have wondered sometimes as to why humans beget only humans or for that matter, why a horse begets a horse, an *E.Coli* cell multiplies into *E.Coli* cells only and so on. One could simply ask, what are the factors behind heredity? The key to all these questions is a type of nucleic acid known as deoxyribonucleic acid (DNA). You will recall that DNA is present in the chromosomes and is the genetic material that is passed on from one generation to another. It contains the specific information about each plant or animal, and indeed what each cell in a living thing can do. That is why it is sometimes referred to as the "thread of life".

We may also wonder as to how a protein molecule is formed in the organism with the precise amino acid sequence, so that it is physiologically active. Every organism requires proteins and nearly every reaction in a living organism is catalysed by enzymes, which are also proteins. Enzymes are highly specific in their action. During reproduction, each organism transmits to its offspring the capability to produce the specific sets of enzymes, that are unique to that organism. This information is not passed on in the form of prepared enzymes, but is coded in the form of information on nucleic acids. These in turn effect and control the synthesis of different proteins from amino acids.

As we mentioned earlier, each cell in a living organism carries nucleic acids. In the cell, they are held in the ribosomes and in the nucleus. In the next section we shall describe the structure of these vital biomolecules. You will study about the different structural units present in these molecules and what type of linkages hold these units together.

4.3 STRUCTURE OF NUCLEIC ACIDS

Nucleic acids are polynucleotides i.e., they are polymerised chains of nucleotides, joined by phosphodiester linkages spanning from the 5′ position of one nucleotide to the 3′ position of the adjacent nucleotide. The nucleic acids, thus, consist of repeated 5′ → 3′ phosphodiester bonds, linking numerous monomer nucleotide units. The nucleotides in turn, are composed of one unit each of phosphate, a sugar and a heterocyclic base, as illustrated below:



A Nucleotide

You will study in detail about nucleotides in subsection 4.3.1. We have shown a polynucleotide chain in Fig. 4.1.

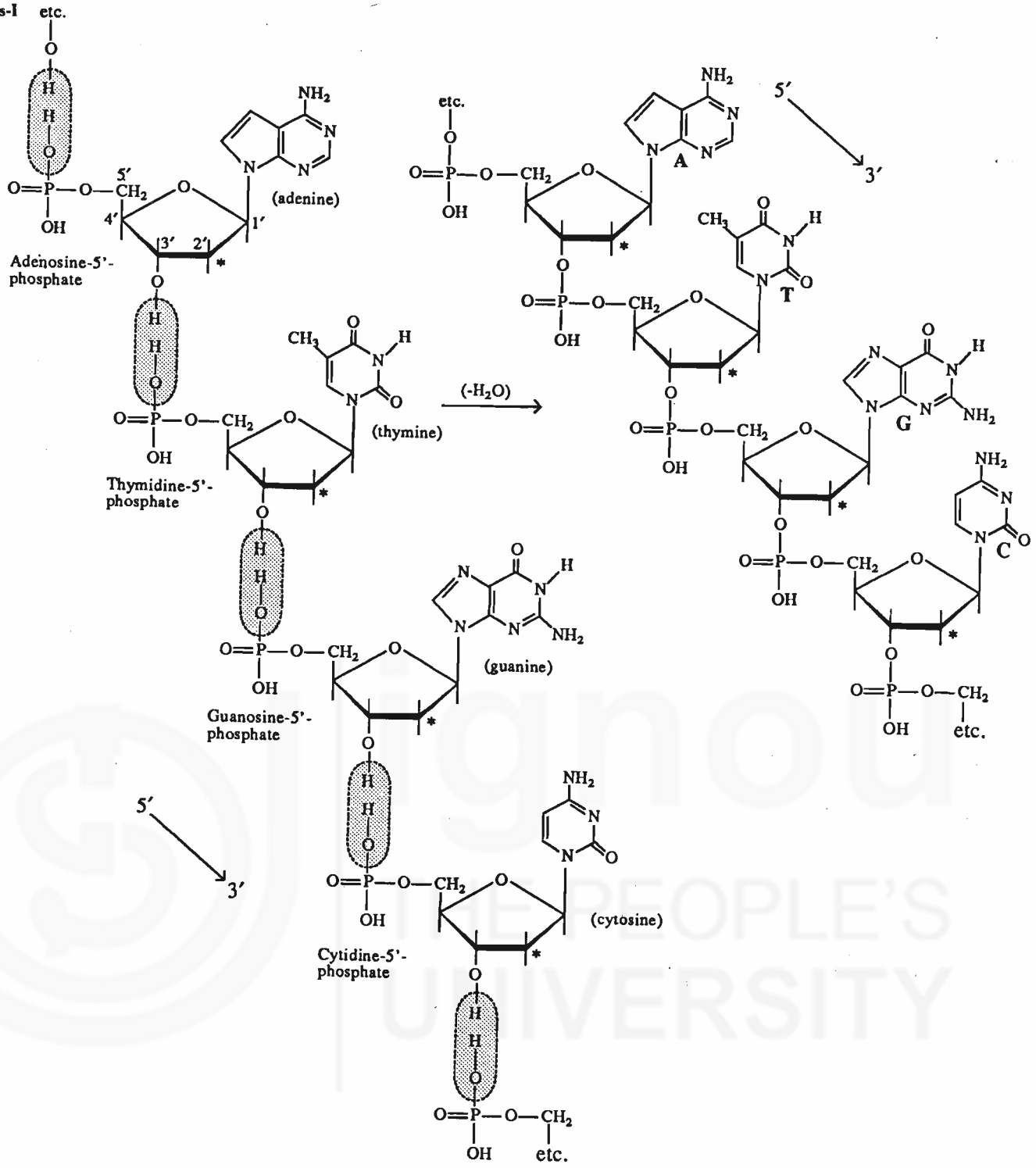
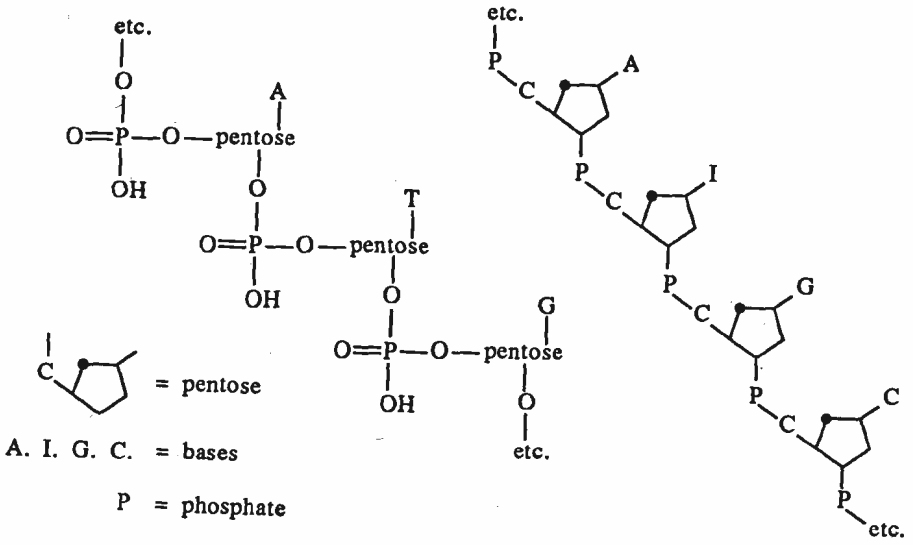


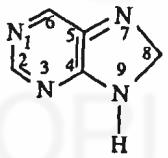
Fig. 4.1 : **The relationship of a nucleic acid chain to its nucleotide monomers. On the right is a short section of a DNA strand. On the left are its constituent nucleotide monomers. The asterisks on the pentose units identify the 2′ positions of these rings where there would be another OH group if the nucleic acid were RNA (assuming that uracil also replaced thymine). The designation 5′ → 3′ means that the complete strand would have a phosphate ester group on C – 5′ of the first pentose unit and an unesterified C – 3′ OH on the other end. Further the sequence of bases is written from the 5′ end to the 3′ end. Thus the sequence shown above would be ATGC, and not as CGTA**

After studying the arrangement of monomer units, i.e., the nucleotides, you would observe that the pattern for the backbone of nucleic acids is, thus, alternating phosphate and sugar units. The sugar in this case is a pentose molecule. It is this pentose sugar which is attached to a different nitrogenous base. The distinctiveness of nucleic acids lies in the sequence of the bases and the length of the backbone. The backbone holds the system together, while the **sequence of bases carries the genetic information.** To make the understanding of nucleic acids easy and clear to you, we have represented a segment of nucleic acid in a simple way below:

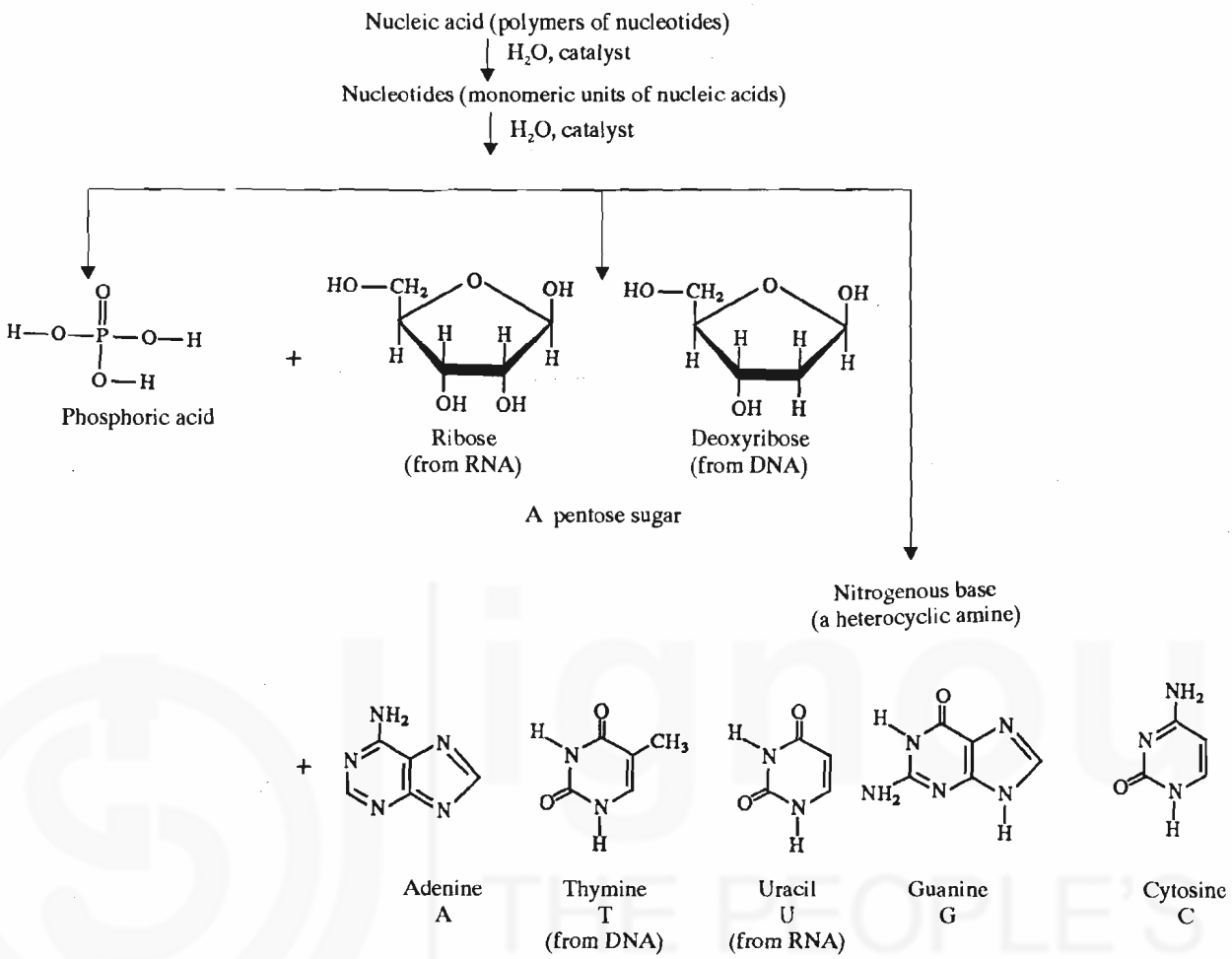


Condensed structures of nucleic acids

By now you must have realised that a large and complex looking nucleic acid molecule, can be considered as a polynucleotide, where a phosphodiester link is formed between the 5′ position of one nucleotide and the 3′ position of the adjacent nucleotide. By convention, the sequence of nucleic acid is written from 5′ → 3′ direction, the 5′ terminus always being to the left. Thus, a pentaribonucleotide, AUGCG, means that adenosine (A) is the 5′ terminal and guanosine (G) is the 3′ terminal. This is obviously different from GCGUA, as shown below:



Nucleic acids are colourless solids. They are of two types, **ribonucleic acids** (RNA) and **deoxyribonucleic acids** (DNA). Both the biopolymers, comprise of repeating monomer nucleotide units. The monomer units in both, RNA and DNA, are composed of a pentose sugar, a nitrogenous base, i.e., a pyrimidine or a purine, and a phosphate group:



Constituents of nucleic acids

As a convention, the carbon atoms of the pentoses are numbered with a dash or prime, to distinguish them from the atoms makingup the nitrogenous bases.

You would observe that RNA and DNA have certain common constituents. These are the phosphate groups, the purine bases, namely adenine (A) and guanine (G), and the pyrimidine base, cytosine (C). However, the major difference between RNA and DNA is that the former contains the pentose sugar, ribose, whereas DNA contains 2-deoxyribose as the pentose sugar. Besides this, base uracil(U) is found only in RNA, and the base thymine (T) is present in DNA only. We shall describe in detail RNA and DNA in Sections 4.4 and 4.5. Let us first study more about nucleotides.

SAQ 1

Tick [√] mark the appropriate statement.

Nucleic acids are important because they

a) are complex and large molecules [ ]

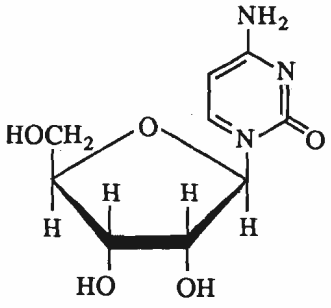
b) take part in ion exchange [ ]

c) give stability to the cell [ ]

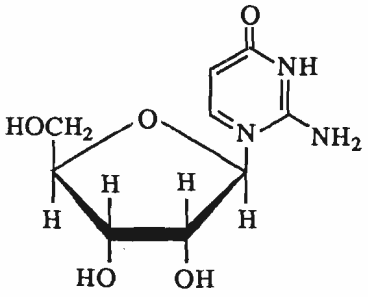
d) are the genetic material [ ]

4.3.1 Nucleosides and Nucleotides

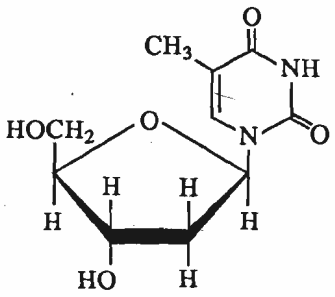
When a nitrogenous base, whether purine or a pyrimidine, is covalently linked to a pentose sugar (ribose or deoxyribose), the resulting molecule is known as a **nucleoside**. The bond formed in a nucleoside is between C-1, i.e., 1′, of the pentose sugar and either N-1 of the pyrimidine base or N-9 of the purine base. Depending upon the nature of the pentose involved i.e., ribose or deoxyribose, the nucleosides formed are termed as **ribonucleosides** or **deoxyribonucleosides**. The common ribonucleosides are adenosine, guanosine, cytidine and uridine. Similarly, deoxyribonucleosides are named as deoxyadenosine (dA), deoxythymidine (dT), etc.



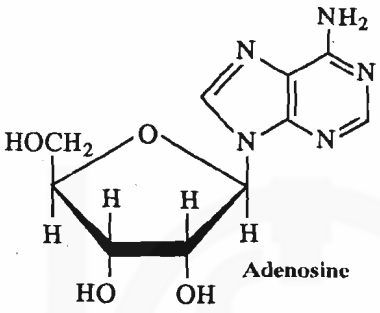
Cytidine



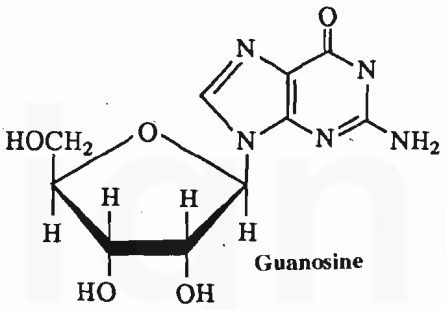
Uridine



2-Deoxythymidine



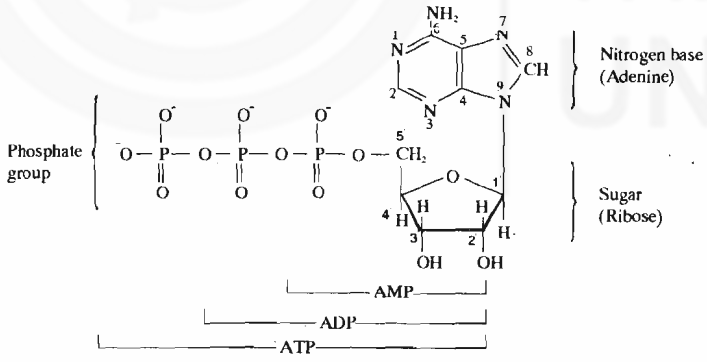
Adenosine



Guanosine

Nucleosides

When a phosphate group is attached to a nucleoside, the resulting molecule is known as a **nucleotide**. The most common site for the formation of a phosphoester linkage is the 5′ position of the ribose or deoxyribose molecule:



Nucleotides

One or up to a maximum of three phosphate groups can be attached to the C-5 position of the pentose, resulting in the formation of mono-, di- and triphosphonucleosides. The first phosphate group is attached by an ester linkage but the next two phosphate groups are attached by phosphoanhydride (i.e. pyrophosphate) linkages. Although, by definition, nucleotides are nucleoside phosphates, some other phosphates, like pyrophosphates, polyphosphates, and the phosphate of *N*-glycosides of heterocyclic bases in general, are also included in the definition of the nucleotides. The attachment of phosphoric acid to the pentose of the nucleoside may be at 2′, 3′ or 5′ in the ribose molecule and at 3′ or 5′ in the deoxyribose molecule. Enzymatic hydrolysis of nucleic acids gives rise to 3′ or 5′-

phosphates, suggesting that in these compounds the phosphate link must be at 3′ and 5′ positions.

A number of nucleotides are found in the cell in free form. They perform diverse functions. In addition to their role as the basic structural units of nucleic acids, they also serve as coenzymes, participate in biosynthetic reactions and play an important part in the transport of energy from energy releasing reactions to energy requiring reactions. Adenosine triphosphate (ATP), guanosine triphosphate (GTP) and uridine triphosphate (UTP) are some of the energy carrying nucleotides.

You will find the chemical structure of GTP in subsection 1.5.7.

SAQ 2

Tick [√] mark the compound which is not a constituent of nucleic acids.

a) ribose [ ]
b) nitrogenous base [ ]
c) phosphate residue [ ]
d) amino acid [ ]

After describing the nucleotides in the preceding section, let us now explain in detail about the two types of nucleic acids, namely RNA and DNA.

4.4 RIBONUCLEIC ACIDS (RNA)

As we stated earlier, RNAs are polymers of ribonucleotides, having a 5′ → 3′ phosphodiester linkage. RNAs almost always have a linear structure. The molar proportions of the heterocyclic nitrogenous bases present in RNA, i.e. adenine, guanine, uracil and cytosine, vary considerably and these are not equimolar as thought earlier. With the exception of a few viral RNA molecules, RNA consists of a single strand of nucleic acid. The synthesis of proteins and polypeptides in a living cell is controlled together by DNA and RNA (Fig. 4.2).

You will study protein biosynthesis in Unit 14.

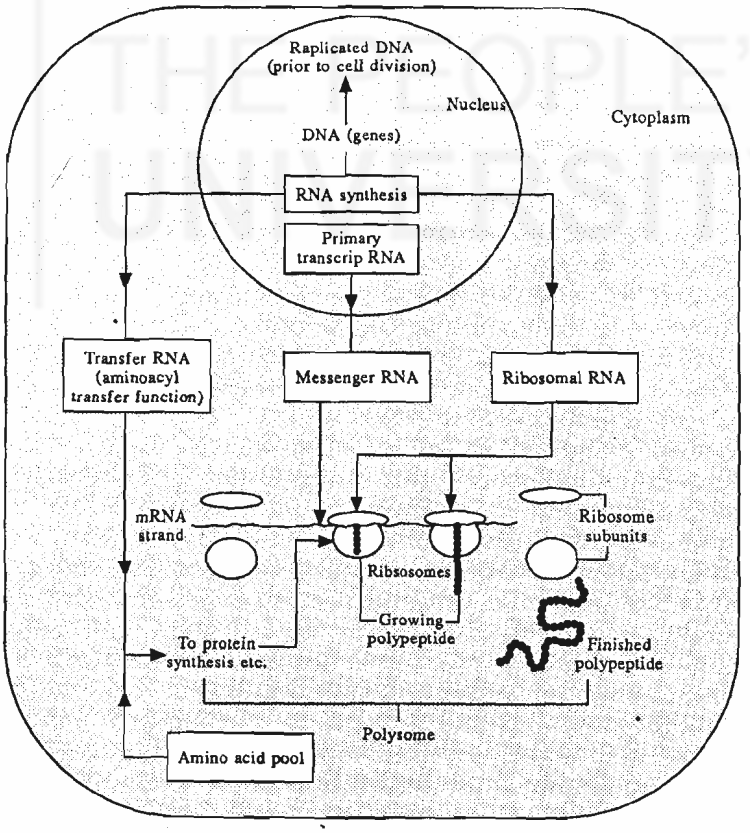


Fig. 4.2: Diagrammatic representation of the relationship of nuclear DNA to different RNAs, and to the synthesis of polypeptides

The RNAs, in fact, are the "middle man" of informational transfer. Depending upon the different roles they play in the biosynthesis of proteins and polypeptides, RNAs have been classified into three main categories, namely ribosomal RNA (rRNA), messenger RNA (mRNA), and transfer RNA (tRNA). Let us study more about these three types of ribonucleic acids.

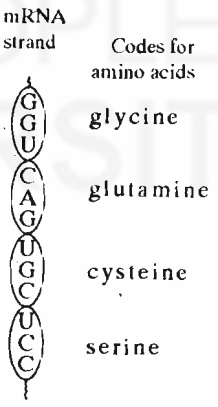
4.4.1 Ribosomal RNA (rRNA)

These are the most abundant RNAs and are present in the ribosomes in a cell. The ribosomal RNA account for 66% of the total RNA, which indicates their important structural role. These are integral part of ribosomes and cannot be removed without disrupting the ribosomal function. The rRNA are single stranded. The G to C and A to U ratio is unequal unlike that of the DNAs, where the base pairs A:T and G:C occur in equal ratio. Nonetheless, the formation of hydrogen bonds between base pairs results in formation of the hairpin type turns (as found in tRNAs also). The collective result of the hydrogen bonding and the single stranded nature of rRNA gives it a peculiar/irregular three-dimensional shape. It is precisely because of this reason that X-ray structure analysis is much more distorted in case of rRNA. The base sequencing is the only way to explore its structure. However, the very long chain (1520-3000 nucleotide) does not render the process feasible. Inspite of all these facts microbiologists have been trying to unfold the mystery of rRNA structure and tentative sequences are known for some *E.Coli* rRNA chains. But still we do not have any concrete idea of the rRNA three-dimensional structure. The precise roles of rRNA are not known, but they are sites of polypeptide synthesis and they form complex(es) with messenger RNA.

4.4.2 Messenger RNA (mRNA)

Messenger RNAs are much shorter than the ribosomal RNA and are the active RNA in the polypeptide synthesis.They derive their name, as they carry the 'message' from the coded DNA and execute /guide the synthesis of polypeptides. Messenger RNA is complementary to the gene, and the triplet of the bases in it is the code for a particular amino acid, and is called a **codon**. The sequence of the codon on the mRNA backbone guides the synthesis of the particular polypeptide. The messenger RNAs differ greatly in their chain length and molecular weight, as reflected by a number of different polypeptides of different length and molecular weight synthesised by a cell. These variations are also attributed to the unequal length of sequence that does not code for any amino acid, but controls synthesis of specific proteins. These base sequences, known as **leader sequences**, are present at the 5′ end of the mRNA. Messenger RNA is degraded rapidly in the cell, so fresh supplies must be made as and when needed. Messenger RNA is synthesised from the DNA by an enzyme called RNA polymerase and **genetic code** is transcribed from the gene (DNA molecule) onto the mRNA. This process of synthesis of mRNA is called **transcription**. We shall briefly discuss about it in Section 4.6.

A three base sequence is needed to code for each amino acid. This three base sequence on mRNA is known as a **condon**:



Each tRNA also carries a unique three-base sequence, complementary to codons on a mRNA, and is known as **anticoudon**.

4.4.3 Transfer RNA (tRNA)

Transfer RNA takes part in the amino acid transport system in the cell, that carries the individual amino acids to the site of the synthesis of proteins (polypeptides). They are small molecules, each having 60-95 nucleotides. They are single stranded, but complementary base pairing, adenine:uracil and guanine:cytosine, is so extensive in tRNA that this adopts a unique clover-leaf like structure (Fig. 4.3a).

It was found that the basic three dimensional arrangement is the same for all tRNA molecules (Fig. 4.3b). One of the most striking feature of tRNA base sequence is

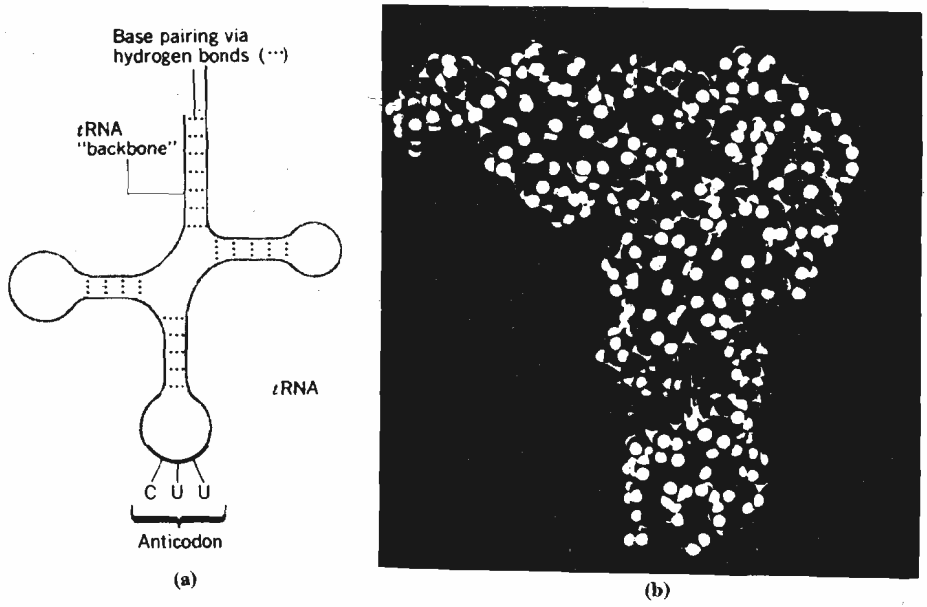
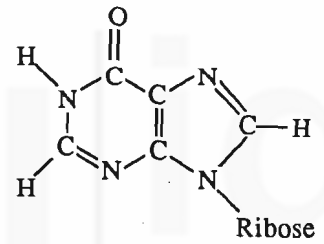
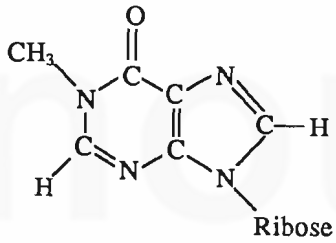


Fig. 4.3: Transfer RNA (tRNA). (a) This is a highly schematic representation to highlight the occurrence of double- stranded regions due to extensive base pairing. (b) The model of tRNA for phenylalanine. Its anticondon occurs at the tip of the base, and the place where the phenylalanyl residue can be attached is at the upper left point

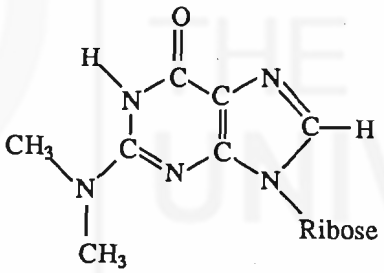
the occurence of unusual bases, i.e. bases other than A, G, C or U. These unusual bases are shown below:



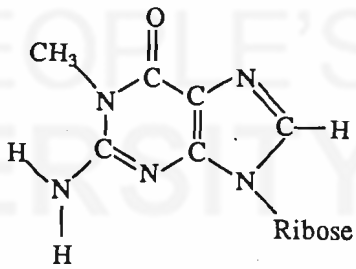
Inosine



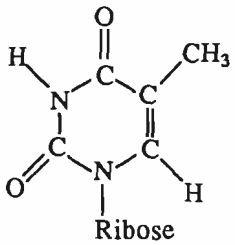
1-methylinosine (Mel)



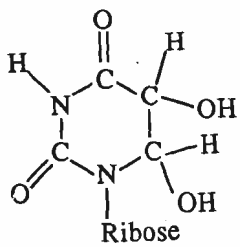
N^2-dimetylguanosine (DiMeG)



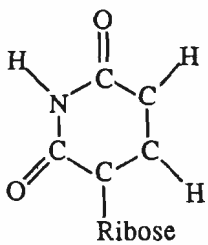
1-methyguanosine (MeG)



Ribothymidine (T)



Dihydrouridine (DiHU)



Pseudouridine (ψ)

These unusual bases arise from the enzymatic modifications of the existing polynucleotides. The role of these unusual bases is not yet fully understood. Many of these bases can form complementary base pairs and cause disruption in the three. dimensional structure of the tRNA, thereby, exposing the keto and amino groups, which can form secondary bonds with mRNAs, ribosomes or enzymes.

One might wonder how tRNA is able to bring the particular amino acid to the polypeptide assembly site. In fact it acts as an interpretor, like the one needed in a

talk between two persons, who do not speak the same language. The interpretor communicates between the two, thus, enabling them to understand each other. tRNA is able to work with two "languages", the genetic code and the polypeptide. The genetic language is expressed in codons and it is able to recognise a codon as it carries a complementary triplet of bases, called the **anticodon** (Fig. 4.3). Another part of a tRNA molecule, with an OH group at the 3′-end ribose unit, can attach a particular aminoacyl unit by an ester bond. Thus a tRNA molecule having a particular amino acid, which in turn is decided by the anticodons, is brought into alignment with the codon of mRNA at a polysome polypeptide synthesis site. This process is called **translation**. You should note that the unique series of codons allow the polypeptide chain to grow only with an equally unique sequence of amino acid residues, as pairing between the codons and anticodons can permit only one sequence.

Let us now describe the DNA molecules in detail.

4.5 DEOXYRIBONUCLEIC ACIDS (DNA)

As mentioned before, DNA is the polymer of deoxyribonucleotides, with a structure similar to that of RNA, except that the pentose unit is 2′-deoxyfuranoribose as compared to the furanoribose in RNAs, and the four nitrogenous bases are adenine (A), thymine (T), guanine (G) and cytosine (C), replacing uracil by thymine. DNA is the fundamental unit of heredity.

DNA has a pronounced secondary structure, consisting of two independent poly-deoxyribonucleotide strands, coiled round a common axis and associated together by hydrogen bonds. This structure of DNA was proposed in 1953 by Francis Crick and Johnson Watson (known as **Crick & Watson Model**) and involves two complementary DNA strands, twisted into a right handed helix. The purine and pyrimidine bases are on the inside of the helix, whereas the phosphate and deoxyribose moieties are on the outside (Fig. 4.4).

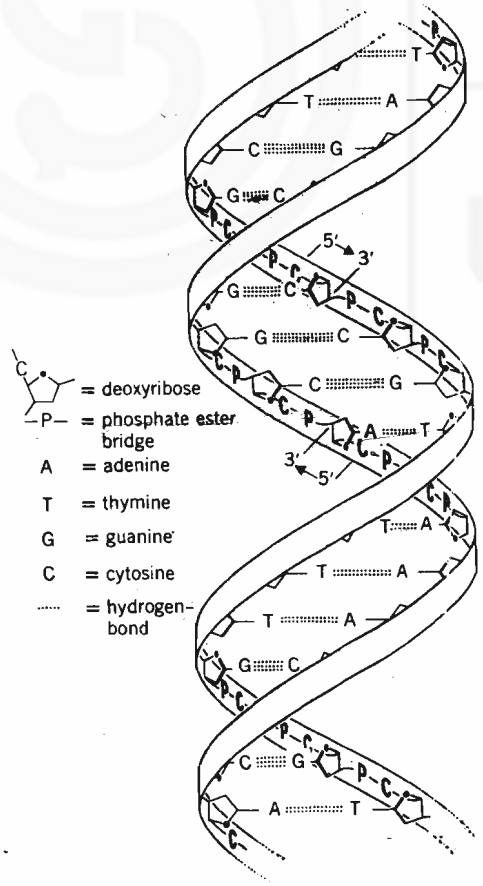


Fig. 4.4 : A schematic representation of the DNA double helix. The two spiraling strands coiled around a common axis are held together by hydrogen bonds between bases on the opposite strands

You should note that all the bases are heterocyclic rings and have planar structures. Their planes lie perpendicular to the axis of the helix and the planes of the deoxyribose molecules are nearly at right angles to those of the bases. The system resembles a spiral staircase in which the steps, which are perpendicular to the long axis of the spiral, consist of the hydrogen bonded base pairs. Hydrogen bonds between the pairs are centered around the long axis. The idea of complimentary strand originated from the finding that the pairs of bases, namely A & T and G & C, always occur in a 1:1 molar ratio. It suggested that A pairs with T and G pairs with C between the strands, as shown below (Fig. 4.5):

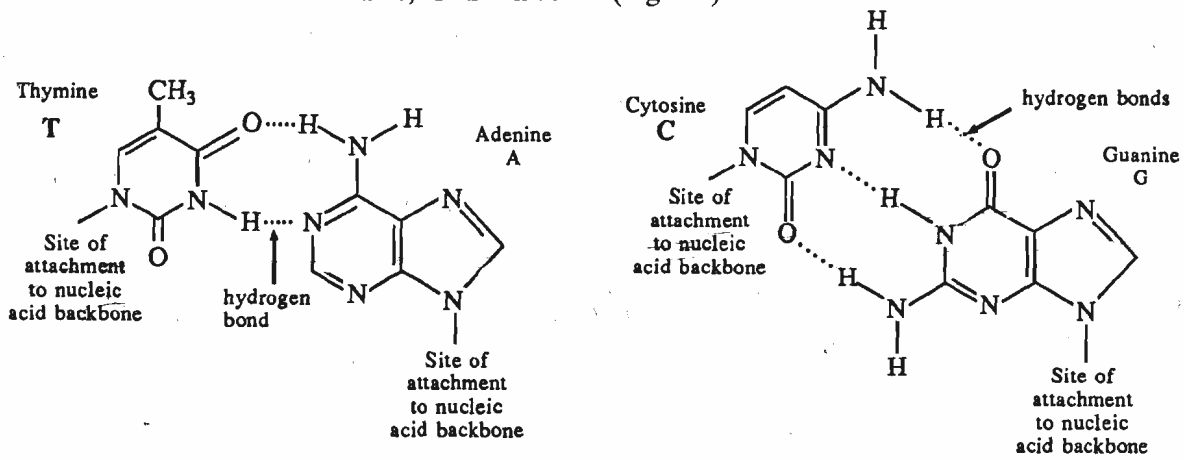


Fig. 4.5 : Hydrogen bonding between base pairs. (a) Thymine (T) and adenine (A) form one base pair with two hydrogen bonds. (b) Cytosine (C) and guanine (G) form another base pair with three hydrogen bonds. Adenine can also base pair to uracil (U) in a similar manner as to thymine

The diameter of the helix is 1.8nm (or 18Å). Adjacent bases are separated by 0.34nm (3.4Å) along the helix and rotated by 36°. Thus the helical stucture is repeated after ten nucleotide residues on each chain, or every 3.4nm (34Å). The X-ray diffraction data of DNA can be explained by another model that requires a slight adjustment in the angle of rotation between the adjacent base pairs. It has been proved that the average number of base pairs per turn is 10.4, instead of 10 as predicted by the classical B-model (Watson & Crick model). Because of this observation, the single structure for the DNA double helix has been replaced by families of structures showing some variations in the number of nucleosides per turn and in the distance between the adjacent repeating units.

There are three forms which are known for DNA molecules, namely, A, B and C forms (Table. 4.1).

Table 4.1 : Characteristics of different forms of DNA double helix

Helix type	Base pairs per turn	Rotation per base pair (deg)	Vertical Run per base pair (nm)	Helical diameter (nm)
A	11	+32.7	0.256	2.3
B	10	+36.0	0.338	1.9
C	9.33	+38.6	0.332	1.9
Z	12	- 30.0*	0.371	1.8

(* -ve rotation for the left handed helix)

A-form has got more base pairs than the average, i.e. 11. The bases do not lie perpendicular to the helical axis, but instead are tilted. The A form resembles the DNA-RNA hybrid in conformation. This form appears at 75% relative humidity and requires the presence of sodium, potassium or caesium ions.

B-form is the famous Watson & Crick model. It is the form that is considered to prevail in the living cells. It requires high relative humidty (92%) and solutions of low ionic strength.

C-form contains less number of base pairs per turn than the B-DNA. It occurs at 66% relative humidity, and in presence of lithium ions.

Other forms are also known to exist, such as, the **D-form** and **E-form** which have 8 and 7.5 base pairs perturn, respectively and also lack guanine. The **Z-form** is the most interesting form as it is the only form that is a left handed helix, in contrast to others. It contains highest humber of base pairs per turn. It possesses the least twisted structure, is very skinny and the sugar-phosphate backbone follows the zig zag path along the helix (hence the name Z-DNA), unlike the smooth curve in other forms of DNA double helix (Fig 4.6).

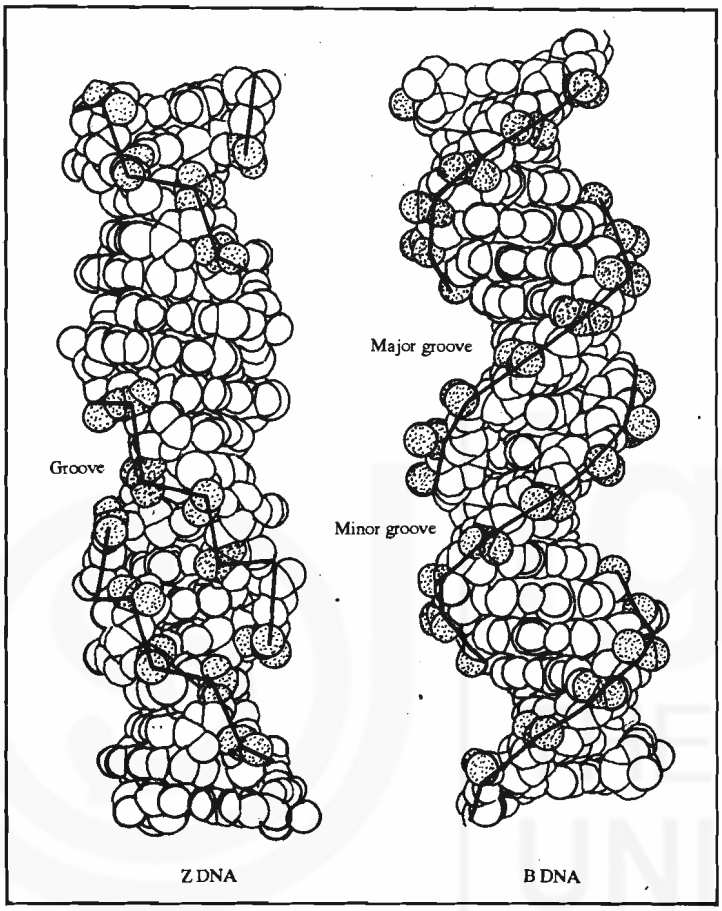


Fig. 4.6: B-DNA and Z-DNA present different double helical structures for DNA

The double helix has two grooves, one major and one minor (Figs. 4.4 and 4.6). These are binding sites for proteins. The grooves are also sites for the initial molecular interactions of certain drugs, antibiotics, carcinogens and poisons. The important features of the double helix are:

- The two chains run in opposite directions, i.e. they are antiparallel.
- The two strands of the helix are complementary as per the requirements of base pairing. Thus, the two strands may have different base sequences, but the base sequence of one determines precisely the sequence of the other strand by virtue of the specificity of base pairing.
- Adenine always pairs with thymine and guanine always pairs with cytosine.

These double helices further twist and coil into superhelices as it is necessary for the DNA to fit into the cell's nucleus. A typical human cell nucleus, for example is only about 10^{-7}m across, but if all its DNA double helices were stretched out, they would measure 1m end to end.

We shall now explain how the two strands of the double helix are held together.

SAQ 3

Fill in the blanks with appropriate words in the following statements:

The number of bases constituting nucleic acids is ..... and base characteristic of RNA but not found in DNA is .....

4.5.1 What holds the double helix together?

Specific hydrogen bonding between the base pairs A:T and G:C is responsible for the unique relationship between the base sequences of the two strands of the double helix. However, it contributes little to the stability of the double helical structure. The latter is mostly attributed to the interaction of bases with each other on being stacked one above the other in the interior of the helix. Note that all the bases are aromatic heterocyclic compounds with limited solubility in water and a strong tendency for interacting with each other. In the DNA double helical structure, these bases are stacked on top of each other. The forces holding them together have sometimes been referred to as hydrophobic interactions. These may be similar to but not entirely identical with the hydrophobic interactions responsible for the spontaneous formation of biomembrane lipid bilayers (Unit 3, subsection 3.4.2) and for imparting unique structures to proteins (Unit 5, subsection 5.4.3). Therefore, it is more common to refer to them as "stacking interactions".

As with proteins, the secondary structure of DNA (double helix) is disrupted on heating. Let us now study the effect of temperature on this structure.

4.5.2 DNA denaturation

On heating beyond a certain characteristic temperature, the double helical structure of DNA collapses and the two strands are separated (**denaturation**). This is accompanied by changes in several physical properties, e.g. a sharp decrease in viscosity and an increase in light absorbance in the ultraviolet region (260 nm). The progress of denaturation may be monitored with the help of any of these properties. The increase in UV absorbance (hyperchromicity) is most commonly employed for this purpose. Effect of temperature on absorbance of a DNA preparation is shown in Fig. 4.7. The mid-point of the temperature range over which the change takes place is called the **melting temperature**, denoted by T_m.

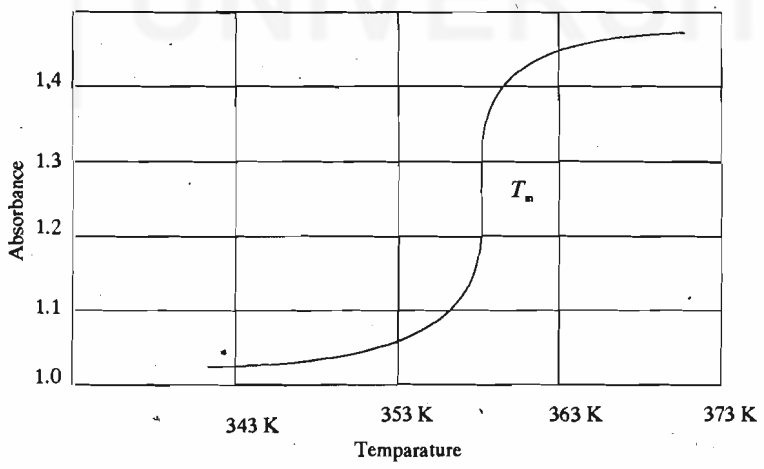


Fig. 4.7 : The denaturation of DNA, which is followed by an increase in optical density, is described by T_m

The two strands remain separated if the solution is cooled too rapidly. However, if the cooling is done gradually the two strands often get united to form the original double helical structure. This process is called **renaturation** (Fig 4.8). Very high pH also sometimes causes denaturation.

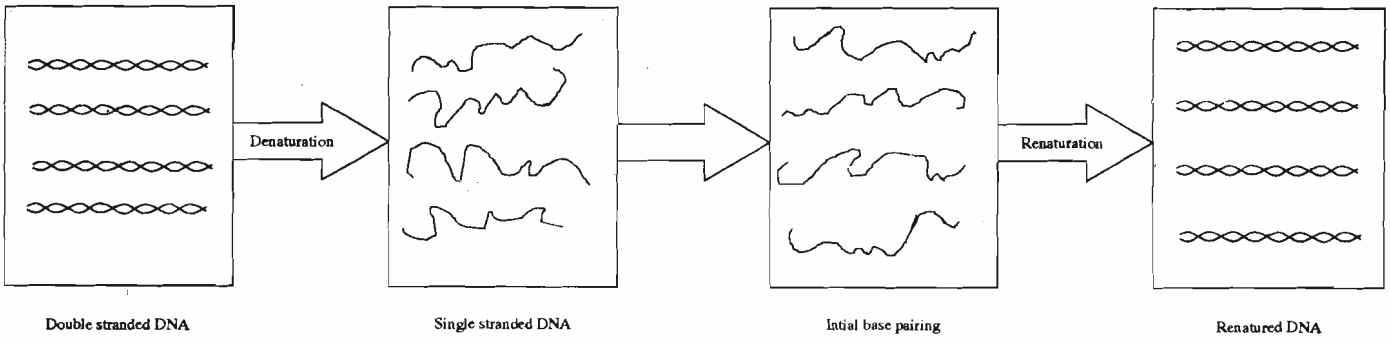


Fig. 4.8 : Denatured single strands of DNA can renature to the double stranded form

In the next subsection we shall briefly study the replication of DNA molecules.

4.5.3 Replication of DNA

Cell division takes place throughout the life of an organism. During cell division,

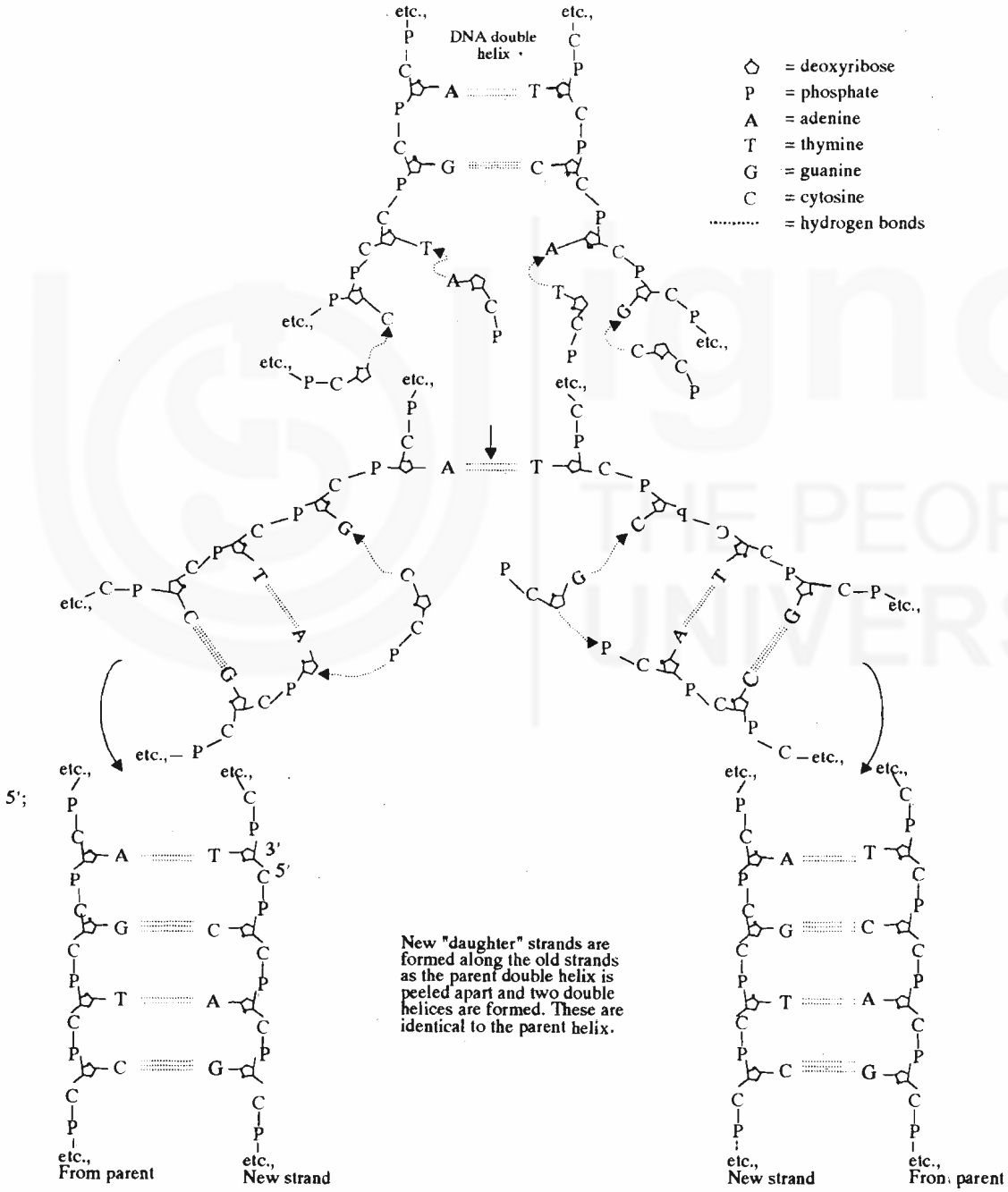


Fig. 4.9 : Replication of DNA. The two new strands at the bottom are replicas of the original strand shown at the top

new daughter cells are produced, having complement of DNA identical in amount and structure, particularly in terms of base sequence, to that of the parent cell. So formation of DNA is a continuous process to meet the demands of everforming new cells. This requirement is met by a process called **DNA replication**. In this process, the initiation is made by destabilisation of the DNA, which is followed by unwinding of the double helix longitudinally. The split strands then serve as templates for DNA polymerase enzymes, which condense deoxyribonucleotide-5′ -triphosphate (dATP, dCTP, dGTP and dTTP) together (Fig. 4.9).

The bases are inserted in the 5′ → 3′ direction, two high energy-phosphodiester bonds are consumed and phosphates are eliminated. The newly formed DNA, called "daughter" DNA, is complementary in base sequence to the template DNA i.e., the strand of parent DNA, as base pairing between A:T and G:C takes place. Both DNA strands are replicated and the new double helix consists of one template, i.e., the parental strand and the other a new "daughter" strand. The template DNA is not replicated as a long unbroken DNA strand, but instead DNA replication is a discontinuous process. The replicated short fragments of DNA, of only a few hundred nucleotides long, are joined together by another enzyme, namely DNA ligase.

The energy for this process is provided by the concomitant breakdown of NAD^+ in bacteria, whereas ATP serves the purpose in viruses and the eukaryotes. The process involves the formation of an activated or high energy form of DNA ligase, irrespective of energy source, i.e., ATP or NAD^+. The process requires the concerted activities of many enzymes and controlling factors.

Let us now briefly describe the synthesis of RNA molecules also.

4.6 SYNTHESIS OF RNA (TRANSCRIPTION)

In a cell, the synthesis of RNA is carried out by an enzyme, called RNA polymerase. The process of RNA synthesis is called **transcription** and is similar to DNA replication, but there are some important differences. As in DNA replication,the RNA synthesis is also preceded by unwinding or destabilisation of

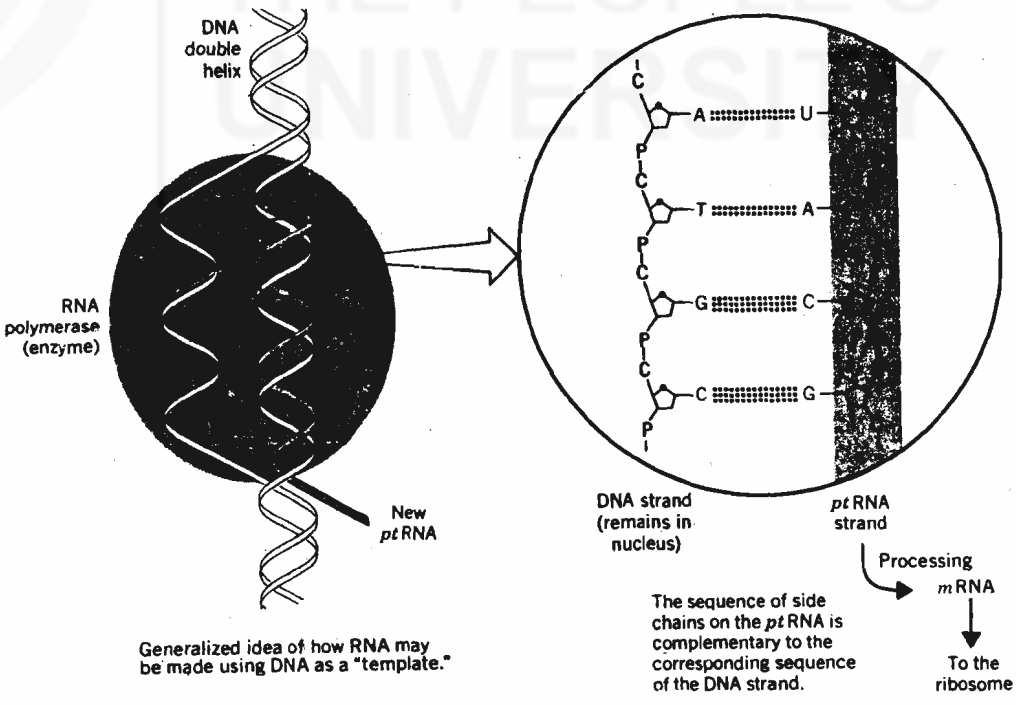


Fig. 4.10 : DNA-directed synthesis of pt RNA (primary transcript RNA) in the nucleus of a cell in a higher organism. The enzymes that catalyse this step are represented by the shaded area

parent DNA. However, only one strand of DNA acts as a template for transcription. In addition, active or expressed genes need not necessarily be all on the same DNA strand. RNA-polymerase is directed to the appropriate transcribing strands with remarkable accuracy. This enzyme catalyses the stepwise condensation of the ribonucleotide-5′-triphosphate (ATP, GTP, CTP and UTP). The sequence of the bases in RNA is dictated by the base sequence of the template DNA by virtue of the specificity of base pairing. The base sequence in the newly formed RNA is complementary to that of the DNA template, dC:G, dG:C, dT:A and dA:U (Fig. 4.10).

Transcription is a continuous process in which RNA is transcribed in 5′ → 3′ direction without any break. The product RNAs are very long strands containing thousands of nucleotides. The DNA template is conserved and neither strand appears in the resultant RNA. Both DNA strands in the template are necessary for accurate transcription, however, the role played by the inactive strand is not clear.

SAQ 4

Tick [√] mark the following statements as true or false

a) The phosphodiester linkage in nucleic acid spans positions 5′ and 3′ of neighbouring nucleotides. [True/False]

b) In DNA, base pairing takes place between Adenine:Thymine and Guanine:Cytosine [True/False]

c) Replication of DNA is catalysed by DNA polymerase [True/False]

d) G:C and A;T ratios are unity in DNA [True/False]

Let us briefly describe the impact of defective genes in the living organisms.

4.7 GENETIC DEFECTS

In the modern era, clinical genetics is one of the most rapidly developing fields in medicine and there are a number of reasons for it, the main being the enormous explosion of knowledge in basic genetics. It can be said that a defective gene makes a defective enzyme. About 2500 diseases in human beings are caused directly or indirectly by flawed genes. Cystic fibrosis, diabetes mellitus, hypertension are a few examples of diseases caused by flawed genes.

An understanding of the true nature of human genetic diseases requires a more detailed knowledge of the structure and functions of human genes, besides a deeper understanding of the current techniques of analysis. The technique of genetic engineering is already being used to locate the chromosomes that hold the defective genes for a number of neurological disorders. In USA, a very ambitious programme of characterising the whole human genome has been going on. If successfully accomplished, the information would be useful in planning gene therapy and man will some day be able to correct the genetic disorders.

Can DNA be modified

Today a fascinating new field of recombinant DNA technology has emerged and captured the attention of many minds. At the heart of this technology lies the ability to incorporate DNA sequences from one organism into another, the operation being called "cloning". Once any particular segment of DNA has been cloned, its properties can be characterised more easily. Pro- insulin was the first protein whose gene sequence was determined. Its importance lies in its ability to cause manufacture of insulin by the host organism (recipient of the cloned gene). This can be given to diabetic patients. A clot dissolving enzyme called tissue plasminogen

activator or tPA has been geneticaly engineered for use in reducing the damage to heart tissue, following a sudden heart attack. The small pox vaccine is being remodelled to provide altered forms that might give immunity against many other diseases, ranging from malaria to influenza. The list of potential applications of genetic engineering to health problems grows yearly. You shall study more about genetic engineering in Unit 15.

Thus, significance of nucleic acids lies not only in understanding the mystery of life, but also in applying this knowledge for a better life and a better world.

4.8 SUMMARY

- Nucleic acids are large and complex biomolecules. They are found in the nucleus of the living cell, and on the ribosomes in the cytoplasm. They are responsible for the heredity and are answers to many querries about life.
- There are two types of nucleic acids, deoxyribonucleic acids (DNA) and ribonucleic acids (RNA).
- Nucleic acids are polynucleotides, and the individual nucleotide monomers are linked to each other by a $5' \rightarrow 3'$ phosphodiester linkage. The nucleotides, in turn, are composed of a nitrogenous base, a pentose sugar (in furanose form) and a phosphate unit. The combination of pentose sugar and the nitrogenous base is known as a nucleoside. Nucleotides are thus phosphate esters of nucleosides generally at the 5′ position of the sugar.
- DNA is the universal genetic material. All forms of life in our world owe their origin and existence to them. The DNAs contain four nitrogenous bases-two purines, namely adenine(A) & guanine (G), and two pyrimidines, namely cytosine (C) & thymine (T). The pentose sugar in DNA is deoxyribose.
- The molecular backbone of the DNA molecule is thus a series of deoxyribose sugar units, joined together by phosphodiester bonds. The bases project from this backbone.
- The sequence of bases and the length of the molecular backbone constitute the distinctiveness of each DNA. The genetic information is coded usually in form of triplets of contiguous bases.
- The DNAs in cell nuclei exist in the form of right handed double helices with bases projecting into the interior and hydrogen bonds being established between a base on one strand to that on the other strand. Base pairing always takes place between A & T and C & G. Adenine and thymine are held together by two hydrogen bonds, whereas guanine and cytosine are held by three hydrogen bonds. There are other interactions called hydrophobic and stacking interactions, which are mainly responsible for the stability of the double helix.
- DNAs can help in generating a new DNA molecule, exactly the same as itself, by a process called **DNA replication**, where each strand on the parent DNA is replicated. The newly formed DNA strands are complementary to the parent DNA strands, and to each other. DNA also help to synthesise ribonucleic acids by a processs called **transcription** which is similar to DNA replication.
- Ribonucleic acids are the other nucleic acids consisting of nitrogenous bases-adenine, guanine, cytosine and uracil. In RNA the base, uracil replaces thymine, which is present only in DNA. Also, the pentose sugar in RNA is ribose, instead of deoxyribose. The RNAs thus consist of polyribonucleotides in which mononucleotides are attached to each other by $5' \rightarrow 3'$ phosphodiester bonds.
- There are three kinds of RNAs depending upon the role played by them in the polypeptide synthesis. These are ribosomal RNA (rRNA), messenger RNA

(mRNA) and transfer RNA (tRNA). The RNA's usually exist as a single linear strand. The rRNAs are present in the ribosome and form the site of polypeptide synthesis. mRNA carries the genetic information in the form of condons from DNA in the cell nucleus to the site of polypeptide synthesis. The tRNAs are the smallest of all the RNAs. Their function is to carry the aminoacyl units (activated amino acids) to the polypeptide assembly sites.

- Many diseases in human beings are due to defective genes. Attempts are being made to modify DNA sequences, so that man is able to combact these diseases.

4.9 TERMINAL QUESTIONS

1) What are the principal constituents of nucleic acids?

2) What are nucleosides and nucleotides?

3) What are the major differences between RNA and DNA?

4) Give a brief description of the DNA double helical structure. What are the forces that account for the stability of the double helix?

5) Name the different types of RNA and briefly describe their functions.

4.10 ANSWERS

Self Assessment Questions

1) d

2) d

3) four, uracil

4) (a) True; (b) True; (c) True; (d) True

Terminal Questions

1) Nucleic acids are composed of nitrogenous bases, a pentose sugar and phosphate groups. These three groups are composed into nucleotides, which are, inturn, joined by 5′ → 3′ phosphodiester links into a polynucleotide chain. The nitrogenous bases present in nucleic acids are adenine, guanine, thymine, cytosine and uracil. Ribose and 2-deoxyribose are the pentose sugars found in nucleic acids.

2) The compound formed by a combination of a nitrogenous base, whether a purine or a pyrimidine, with a pentose sugar, through a covalent bond between C-1 of sugar and N-1 of pyrimidine base or N-9 of purine base is known as a nucleoside. The pentose sugar involved can be either ribose or deoxyribose. Accordingly, the nucleoside is known as a ribonucleoside or a deoxyribonucleoside. Nucleotides are nucleoside phosphates, i.e., they are phosphate esters of pentoses to which nitrogenous bases are attached. The phosphate links are generally at 3′/5′ positions.

3) The pentose sugar in RNA is ribose, where as in DNA it is deoxyribose. Besides, RNAs do not have thymine and DNAs do not have uracil. Further DNA consists of a double helix, which is composed of two complementary nucleic acid strands. However, RNA is usually a single strand of nucleic acid.

4) The double helical structure of DNA, as proposed by Crick & Watson, consists of two complementary nucleic acid strands, which are twisted into a right handed helix. The nitrogenous bases lie on the inside of the helix, whereas the phosphate and sugar groups are on the outside of the helix. (This

has been depicted in Fig. 4.4). This model can be compared to a spiral staircase, in which the steps are perpendicular to the long axis of the spiral. In the above model, the plane of the nitrogenous bases are also perpendicular to the helix axis. In the double helix hydrogen bonding takes place between adenine:thymine, and guanine:cytosine of the two complementary strands. Hydrophobic stacking interactions among bases account for the stability of the double helix.

5) The RNAs are of three types, namely, ribosomal RNA (rRNA), messenger RNA (mRNA) and transfer RNA (tRNA). mRNAs, which are synthesised in the cell nucleus whenever needed, migrate to the ribosomes and provide information for sequencing of amino acids in protein synthesis. rRNAs, which are integral parts of ribosomes, combine with mRNA to form the sites of polypeptide synthesis. tRNAs, which happen to be the smallest RNA molecules capable of easy movement within the cell, carry amino acid molecules to the ribosomes for protein synthesis.

Further Readings

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2) *Outlines of Biochemistry* (4th ed.)
Eric E. Conn and P.K. Stumpf
Wiley Eastern Limited, New Delhi
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3) *Principles of Biochemistry*
Albert L. Lehninger
CBS Publishers and Distributors, Delhi
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Besides the above books which will be provided in your study centres, we also suggest that you may consult the following books which may be available to you from other sources.

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2) *Biochemistry* (2nd ed.)
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