

VOL.**2****LIPIDS, NUCLEIC ACIDS AND
METABOLISM**

BLOCK 1**LIPIDS AND NUCLEIC ACIDS****7**

BLOCK 2**BIOENERGETICS AND METABOLISM****93**

Block

4

LIPIDS AND NUCLEIC ACIDS

UNIT 10

Lipids I

7

UNIT 11

Lipids II

26

UNIT 12

Nucleic Acids

44

UNIT 13

Replication and Transcription

71

Course Design Committee

Prof. K.K. Arora, Dept. of Chemistry, Zakir
Husain College, University of Delhi, Delhi

**School of Sciences,
IGNOU, New Delhi 110068**

Dr. Sunita Joshi (Retd.), Dept. of
Biochemistry, Daulat Ram College,
University of Delhi, Delhi

Prof. M.S. Nathawat
Prof. Sunita Malhotra
Prof. B.I. Fozdar

Dr. Bhupinder Mehta, Dept. of Chemistry,
Swami Shraddhanand College, University
of Delhi, Delhi

Prof. Javed A. Farooqi
Prof. Sanjiv Kumar
Prof. Lalita S. Kumar
Prof. Kamalika Banerjee

Block Preparation Team

Prof. Bharat Inder Fozdar
School of Sciences, IGNOU

Prof. Seemi Bashir Ahmed (Editor)
Department of Chemistry,
Jamia Millia Islamia, New Delhi

Prof. Javed A. Farooqi, Ex. Consultant
School of Sciences, IGNOU

Course Coordinator:

Prof. Lalita S Kumar

Print Production

Mr. Rajiv Girdher
AR (P), MPDD, IGNOU

Mr. Hemant Kumar
SO(P), MPDD, IGNOU

Acknowledgements: Sh. Sarabjeet Singh for CRC preparation
Sh. Deepak Kumar
Sh. Ajit Kumar for the Art Work
Material partially adapted from the Biochemistry Course (CHE-09).

November, 2021
@ Indira Gandhi National Open University, 2021

ISBN:

All rights reserved. No part of this work may be reproduced in any form, by mimeograph or any other means, without permission in writing from Indira Gandhi National Open University.

Further information on Indira Gandhi National Open University courses may be obtained from the University's office at Maidan Garhi, New Delhi-110068 or IGNOU website www.ignou.ac.in

Printed and published on behalf of Indira Gandhi National Open University, New Delhi by the Registrar, MPDD, IGNOU.

Printed at:

BLOCK 4: LIPIDS AND NUCLEIC ACIDS

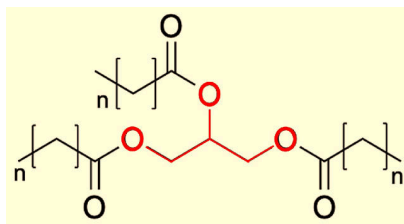
This block contains four units (Unit 10-13).

Unit 10 and Unit 11 deal with lipids. Lipids have a variety of structures and functions. They form the framework for the structure and function of living cells. In Unit 13 we shall describe a vital biomolecular, namely the nucleic acids. Unit 13 deals with replication of DNA, transcription of RNA and protein biosynthesis (translation). In this unit first we will briefly discuss about the discovery of DNA and its nature for storing genetic information. We will also study the synthesis of DNA (replication) and synthesis RNA (transcription) using old DNA as template. Finally we will discuss protein synthesis (translation).

Expected Learning Outcomes

After studying this block, you should be able to:

- classify lipids and describe their main functions as well as the structure of biomembrane;
- explain the structure and functions of nucleic acid;
- describe genetic code;
- explain the evidence showing that DNA is genetic material;
- describe DNA replication and synthesis of RNA, where as DNA is employed as template; and
- list the different steps in protein synthesis.



UNIT 10

LIPIDS I

Structure

10.1	Introduction	10.7	Essential Fatty Acids
	Expected Learning Outcomes	10.8	Properties of Fatty Acids
10.2	What are Lipids?		Physical Properties
10.3	Important Functions of Lipids		Chemical Properties
10.4	Classification of Lipids	10.9	Fats and Oils
10.5	Fatty Acids	10.10	Waxes
	Nomenclature of Fatty Acids	10.11	Summary
10.6	Types of Fatty Acids	10.12	Terminal Questions
	Saturated Fatty Acids	10.13	Answers
	Unsaturated Fatty Acids		
	Fatty Acids Containing Functional Groups other than Carboxylic Group		

10.1 INTRODUCTION

In the previous blocks you have learnt about biomolecules viz., carbohydrates, proteins and enzymes. This unit and Unit 11 deal with another very important biomolecules, viz., lipids. Lipids have a variety of structures and functions. They form the framework for the structure and functions of living cells.

Lipids are a wide group of compounds that include fatty acids, fats, oils, waxes, phospholipids, steroids and some vitamins. In this unit our focus will be on simple lipids which include fatty acids, fats, oils and waxes. Lipids are very important class of organic compounds and carry out multiple biological functions; therefore, first we will discuss their biological importance. Next section of this unit deals with the classification of lipids. After that we will take up fatty acids. Fatty acids are the simplest lipids which are commonly present in different types of lipids. They are carboxylic acids with long hydrocarbon chains. Besides the usual fatty acids, there are fatty acids containing functional groups other than carboxylic group. These include cyclopropene, hydroxyl, epoxy and keto fatty acids. There are some fatty acids which cannot be synthesised in our body but these are essential for our system, therefore,

we will study these acids in more details. In this unit we will also learn the physical and chemical properties of fatty acids as well. In the last section, there is a brief discussion on fats, oils and waxes. In Unit 11 we will describe complex lipids and derived lipids. These lipids along with proteins not only form biological membrane but also act as signaling molecules.

Expected Learning Outcomes

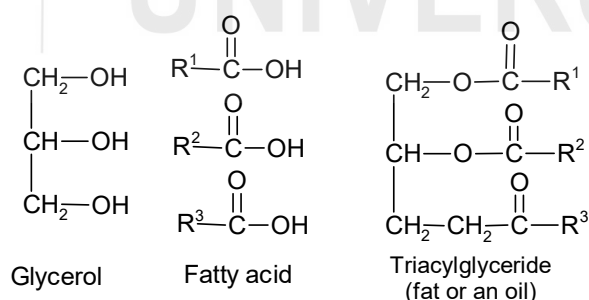
After studying this unit, you should be able to:

- ❖ define and describe lipids;
- ❖ explain the biological importance of lipids;
- ❖ list the different classes of lipids;
- ❖ define and describe fatty acids;
- ❖ describe the types of fatty acids;
- ❖ explain the importance of essential fatty acids;
- ❖ list the physical and chemical properties of fatty acids; and
- ❖ describe fats, oils and waxes.

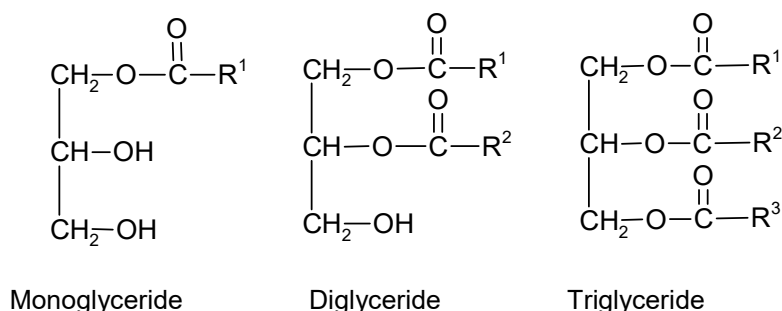
10.2 WHAT ARE LIPIDS?

The word *lipid* comes from the Greek *lipos*, which means "fat."

According to one definition lipids are organic compounds that contain hydrogen, carbon, and oxygen atoms, which form the framework for the structure and function of living cells. They are relatively insoluble in water and readily soluble in nonpolar organic solvents such as ether, chloroform and benzene. The term "lipid" is sometimes used as a synonym for fats. Fats are a subgroup of lipids, called triglycerides also called triacylglycerols. Triglycerides are compounds in which the three OH groups of glycerol are esterified with fatty acids.



Fats may also exist in the form monoglycerides (monoacylglycerol), diglycerides (diacylglycerol) or triglycerides (triacylglycerol). The structures of these molecules are given below:



Lipids make up the building blocks of living cells. Fats, Oils, waxes, steroids, certain vitamins (such as A, D, E and K) are some of the examples of lipids. In the human body, these molecules can be synthesized in the liver. Lipids are important constituents of oil, butter, milk, cheese, fried foods and also of some red meats. Being a constituent of cell membrane, lipids form a mechanical barrier dividing a cell from the external environment. You will study in detail about the structure and role of lipids in cell membrane in Unit 11.

10.3 IMPORTANT FUNCTIONS OF LIPIDS

Lipids constitute a very heterogeneous group of biological compounds. They include such diverse compounds as fatty acids, acylglycerols, phospholipids, steroids, terpenes and prostaglandins. Triglycerides, the most predominant form of natural lipids, serve as important dietary constituents, because of their high calorific value (9.3 kcal/gm or 38.8 kJ g^{-1}) as compared to proteins or carbohydrates (4.1 kcal/gm or 17.1 kJ g^{-1}). Phospholipids are essential structural components of all biological membranes and serve to compartmentalize the cells. Some lipids are also important precursors of hormones and vitamins. The cell also synthesises prostaglandins from lipid molecules. Prostaglandins act as defenses against many sorts of changes in the cell.

There are three main functions of lipids in human body.

- i) At the cellular level, these act as signaling molecules,
- ii) These form the structural components in cell membranes and
- iii) Lipids store energy in the body.

Lipids are found in different parts of a human body i.e., cell membranes, blood cells, and the brain.

High percentage of lipids in human body may cause diabetes, hypothyroidism, alcoholism, kidney disease, liver disease and stress. In some people, certain medicines, such as birth control pills, steroids and blood pressure medicines can cause high lipid levels. Deficiency of fat may lead to dermatitis. "Dermatitis" is a general term used to describe inflamed skin.

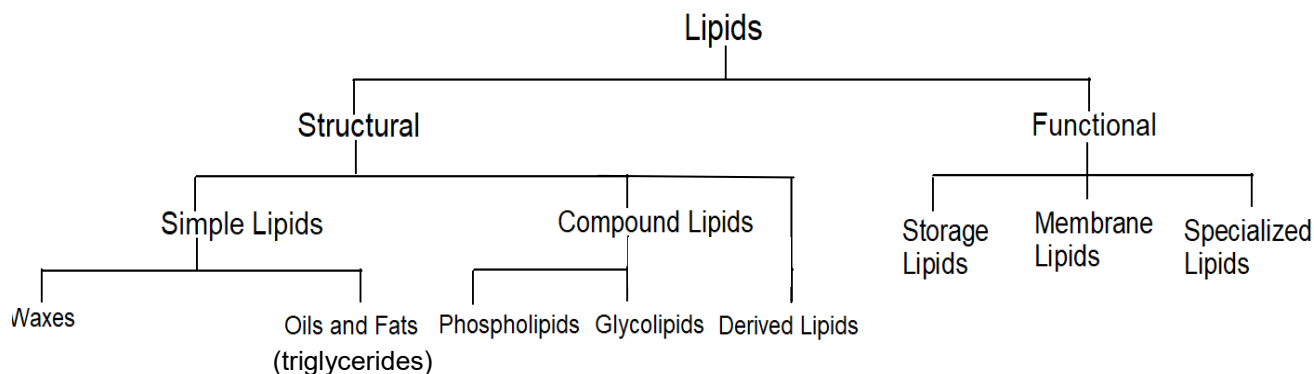
We shall now describe the general classification of lipids and then learn in detail about the simple lipids. Before proceeding further let us try to answer the following SAQ.

SAQ 1

What are the important functions of lipids?

10.4 CLASSIFICATION OF LIPIDS

Lipids are classified both on the basis of their chemical structure as well as function these perform. The classification of lipids is depicted in the following chart.



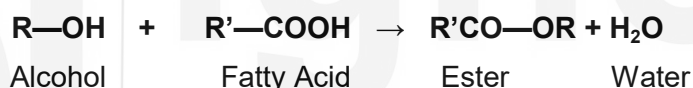
A brief description of all the classes of lipids is given in the following paragraphs.

A) Structural Classification

On the basis of chemical structure, lipids are divided into three classes: simple lipids, compound lipids and derived lipids.

i) Simple Lipids

Simple lipids are formed by the reaction of alcohols with fatty acids. Fatty acids are carboxylic acid with long hydrocarbon chain. The general reaction can be represented as follow:



Based on the type of alcohol molecule, simple lipids are of two types: triglycerides and waxes. Triglycerides contain glycerol, a three carbon alcohol to which three fatty acid chains are bonded whereas waxes contain alcohol of relatively longer chain (generally C_{16} - C_{36}).

ii) Compound Lipids

Compound lipids are formed by modification of simple lipids such as addition of phosphate, sulfate, and carbohydrate or protein group. These include phospholipids, glycolipids and lipoproteins.

iii) Derived Lipids

Derived lipids are obtained by the modification, condensation or hydrolysis of simple and compound lipids. These include fatty acid derivatives, glycerol, steroid hormones and lipid-soluble vitamins.

B) Functional Classification:

Different structural classes of lipids have been grouped into following three major groups based on their functions. These are **storage lipids, membrane lipids and specialized lipids**.

i) Storage Lipids

Storage lipids serve as the principal form of energy reserves in the animals and plants. These include triglycerides and waxes.

ii) Membrane Lipids

These constitute the major structural components of cell membranes and include phospholipids, glycolipids, sphingolipids and sterols.

iii) Specialized Lipids

This group includes lipids which are present in relatively smaller amounts but play specialized functions such as enzymes, cofactors, electron carriers, light absorbing pigments, hormones and intracellular messengers or signals. These include eicosanoids, steroid hormones, fat soluble vitamins A, D, E, K and conjugated dienes.

Classification of lipids based on their structure is a more meaningful for chemist to understand their chemistry. Therefore, units in this block are organized accordingly. In this unit, we begin our discussion on building blocks of most of the lipids that is fatty acids with reference to their chemical structure, physical and chemical properties.

SAQ 2

What are simple lipids?

10.5 FATTY ACIDS

As mentioned earlier, fats are found in animals while oils are mainly present in plant seeds and sometimes in fish. Fats and Oils are present as triglycerides (or triacylglycerols) of fatty acids. The fatty acids are the simplest forms of lipids. Fatty acids are long aliphatic hydrocarbon chains with a carboxylic acid group at one end and methyl group at the other end. The carboxylic end of the chain is called alpha (α) end and methyl end as omega (ω) end.

Generally, more than 95% oils and fats are triglycerides with little amount of monoglycerides, diglycerides and free fatty acids. The seed oils constitute the largest source of edible and non-edible oils. According to the world estimates, natural fats are used in three ways, edible (78%), making of soaps (13%) and remaining (9%) used for other purposes. Fats provide essential fatty acids, which are not made by the body and must be obtained from food.

The fatty acids which occur in natural fats contain generally even number of carbon atoms. The chain length varies from 4 to 32 carbon atoms and from zero to six double bonds. Over 500 fatty acids have been discovered and the number is increasing rapidly. Of these, most commonly occurring acids, in the oils, are lauric, myristic, palmitic, oleic, stearic, linoleic and linolenic. Fats and oils have combinations of saturated and unsaturated fatty acids.

Besides the usual fatty acids, seed oils may contain a wide spectrum of unusual fatty acids not found in animal kingdom. These include acetylenic, conjugated, allenic, cyclopropene, cyclopropane, cyclopropentane, cyclopentene, furan, epoxy, hydroxy and keto acids.

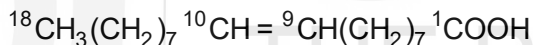
SAQ 3

Fill in the following blanks:

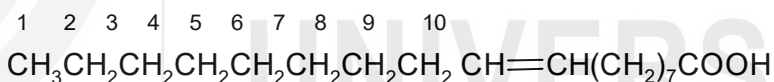
- The carboxylic end of the chain is called end and methyl end as end.
- The main sources of oils are and

10.5.1 Nomenclature of Fatty Acids

Fatty acids are generally named after the parent hydrocarbon, with **-oic** being substituted for the final **-e** in the name of the hydrocarbon. Thus, saturated fatty acids end in **-anoic** acids, as in octanoic acid ($\text{CH}_3(\text{CH}_2)_6 - \text{COOH}$). Similarly unsaturated fatty acids with double bonds end in **-enoic** acid, as an octadecenoic acid ($\text{CH}_3(\text{CH}_2)_7\text{-CH}=\text{CH}(\text{CH}_2)_7 - \text{COOH}$, oleic acid). The carbon atoms numbered from the carboxyl carbon, which is designated as carbon atom no. 1. The carbon atom adjacent to the carboxyl carbon is known as β -carbon, and the terminal methyl carbon is known as the ω -carbon or n -carbon atom. For indicating the number and position of the double bond in unsaturated fatty acids, various conventions are used. For example, Δ^9 indicates a double bond is between carbon atoms 9 and 10 in the hydrocarbon chain. Similarly, ω -9 indicates a double bond on the 9th carbon atom, counting from the carbon ω -carbon. We shall illustrate this by taking oleic acid, as an example. A number of 18:1; 9 or 18:1; Δ^9 would suggest an 18-carbon chain with a double bond at C-9, counting from the carboxyl carbon.



The oleic acid molecule can also be presented by ω -9, C18:1 or n -9, C 18:1



In this case the carbon no. 9 is counted from the ω -carbon. The digit 1 after 18 in both the cases, denotes one unsaturated bond in the molecule. We have presented the systematic names, common (trivial names, structure and occurrence of the more frequently occurring saturated and unsaturated fatty acids in Tables. 10.1 and 10.2, respectively.

10.6 TYPES OF FATTY ACIDS

Fatty acids possess an even number of carbon atoms and are mostly unbranched. There are mainly three types of fatty acids:

- Saturated fatty acids
- Unsaturated fatty acids
- Fatty acid containing functional groups other than carboxylic Group

10.6.1 Saturated Fatty Acids

As the name suggests saturated fatty acids contain only single bonds and do not contain any double bond. The saturated fatty acids have higher melting

points compared to unsaturated fatty acids of the corresponding carbon chain, due to their ability to pack their molecules together. The following Table (10.1) gives examples of some common saturated fatty acids.

Table 10.1: Structure, name and common sources of saturated fatty acids

Structure	Short form	Systematic name	Common name	Occurrence	Melting point (°C)
$\text{COOH}(\text{CH}_2)_6\text{CH}_3$	8:0	Octanoic acid	Caprylic acid	Milk fat	12.7
$\text{COOH}(\text{CH}_2)_8\text{CH}_3$	10:0	Decanoic acid	Capric acid	Milk and some seeds	29.6
$\text{COOH}(\text{CH}_2)_{10}\text{CH}_3$	12:0	Dodecanoic acid	Lauric acid	Most milk fats, cinnamon, palm kernel, coconut oils	42.2
$\text{COOH}(\text{CH}_2)_{12}\text{CH}_3$	14:0	Tetradecanoic acid	Myristic acid	Cow's milk, nutmeg, palm kernel, coconut oils, butter	52.1
$\text{COOH}(\text{CH}_2)_{14}\text{CH}_3$	16:0	Hexadecanoic acid	Palmitic acid	Most abundant saturated fatty acid in plants, animals and micro-organisms, highest concentration in palm oil	60.7
$\text{COOH}(\text{CH}_2)_{16}\text{CH}_3$	18:0	Octadecanoic acid	Stearic acid	Second most abundant, highest in ruminant fats, vegetable oils such as, cocoa butter, meat, hydrogenated fats	69.6
$\text{COOH}(\text{CH}_2)_{18}\text{CH}_3$	20:0	Eicosanoic acid	Arachidic acid	Peanut and fish oil	75.4

Saturated fatty acids are found in animal products such as butter, cheese, whole milk, ice cream, cream, and fatty meats. They are also found in some vegetable oils like coconut, palm, and palm kernel oils. Palmitic and stearic acids are the most common saturated fatty acids. Excess intake of saturated fat can raise one's blood cholesterol and increase the risk of developing coronary artery disease. These are the biggest dietary cause of high LDL levels ("bad cholesterol"). Saturated fat should be limited to 10% of calories.

10.6.2 Unsaturated Fatty Acids

The fatty acids containing carbon-carbon double bonds are classified as unsaturated. Most of unsaturated fatty acids have double bonds although triple bonds have also been found occasionally. Fatty acids with one double bond are called monoenoic or monounsaturated fatty acids (**MUFA**) and those with two or more double bonds are called polyenoic or polyunsaturated fatty acids (**PUFA**).

All the fatty acids found in human body are generally *cis* fatty acids, with the exception of retinoic acid present in the eye which is a *trans* fatty acid.

Most (but not all) liquid vegetable oils are unsaturated. (The exceptions include coconut, palm, and palm kernel oils). Oleic and linoleic acids are the most common unsaturated fatty acids

Some common unsaturated fatty acids are given in Table 10.2.

Table 10.2: Common unsaturated fatty acids

Structure	Short form	Systematic name	Common name	Type of (ω) fatty acid	Occurrence	Melting point ($^{\circ}\text{C}$)
Monoenoic Acid (Fatty acids with one double bond. Also called monounsaturated)						
$\text{COOH}(\text{CH}_2)_7\text{HC}=\text{CH}(\text{CH}_2)_5\text{CH}_3$	16:1(Δ^9)	cis-9-Hexadecenoic acid	Palmitoleic acid	ω -7	In nearly all animal fats.	1- 0.5
$\text{COOH}(\text{CH}_2)_7\text{HC}=\text{CH}(\text{CH}_2)_7\text{CH}_3$	18:1(Δ^9)	cis-9-Octadecenoic acid	Oleic acid	ω -9	Olive oil, possibly the most abundant monounsaturated fatty acid in plants and animals.	16
$\text{COOH}(\text{CH}_2)_7\text{HC}=\text{CH}(\text{CH}_2)_7\text{CH}_3$	18:1(Δ^9)	trans-9-Octadecenoic acid	Elaidic Acid	ω -9	Hydrogenated and ruminant fats.	44
Polyenoic Fatty acids (fatty acids with two or more double bonds. Also known as polyunsaturated)						
Dienoic Acid (Fatty acids with two double bonds)						
$\text{COOH}(\text{CH}_2)_7\text{HC}=\text{CHCH}=\text{CH}(\text{CH}_2)_4\text{CH}_3$	18:2($\Delta^{9,12}$)	all-cis-9, 12-Octadecadienoic acid	Linoleic acid	ω -6	Corn, peanut, cottonseed, soybean, grape seed oil	-5
Trienoic acid (Fatty acids with three double bonds)						
$\text{COOH}(\text{CH}_2)_7\text{HC}=\text{CHCH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}_3$	18:3($\Delta^{9,12,15}$)	all-cis-9, 12, 15-Octadecatrienoic acid	* α -Linolenic acid	ω -3	Linseed (flaxseed)oil.	-11
$\text{COOH}(\text{CH}_2)_4\text{HC}=\text{CHCH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_4\text{CH}_3$	18:3($\Delta^{6,9,12}$)	all-cis-6,9,12-Octadecatrienoic acid	* γ -Linolenic acid	ω -6	oil of evening primrose, borage oil; minor fatty acid in animals	
Tetraenoic acid (Fatty acids with four double bonds)						
$\text{COOH}(\text{CH}_2)_3\text{HC}=\text{CHCH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_4\text{CH}_3$	20:4($\Delta^{5,8,11,14}$)	all-cis-5,8,11,14-Eicosa tetraenoic acid	Arachidonic acid	ω -6	animal fats and peanut oil, liver fat	-49.5

* indicates essential fatty acid.

SAQ 5

Write the structure of:

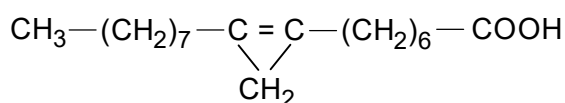
- Lauric acid
- Eicosanoic acid
- Palmitic acid
- Stearic acid
- Tetradecanoic acid

10.6.3 Fatty Acids Containing Functional Groups other than Carboxylic Group

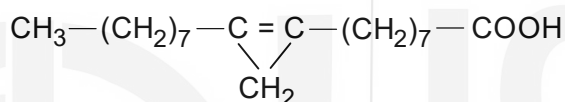
Besides the usual fatty acids, seed oil may contain a wide spectrum of fatty acids that are not found in animal kingdom, i.e. containing functional groups other than carboxylic acid group. These include acetylenic, conjugated, cyclopropene, hydroxyl, epoxy, furan and keto fatty acids. In this course we will discuss few of them.

Cyclopropenoid Fatty Acids

Fatty acids containing cyclopropenoid group is known as cyclopropenoid fatty acids. These acids are of two types malvalic acid and Sterculic acid. Both malvalic and sterculic acids usually occur together and sometimes accompanied by small amount of their respective di-hydro derivatives.



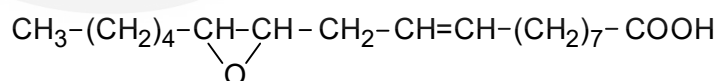
Malvalic acid



Sterculic acid

Epoxy Fatty Acids

Epoxy fatty acids occur as metabolites of polyunsaturated fatty acids. Epoxy group is present in a number of long chain fatty acids. The natural species are generally C₁₈ compounds either saturated or unsaturated, For example 9,10-epoxystearic, 9,10-epoxyoctadec-12-enoic acids (coronaric acid), and 12,13-epoxyoctadec-9-enoic acid (vernolic acid). These fatty acids are commonly used to make paints and coatings. Vernolic acid is the best known epoxy acid from natural sources.

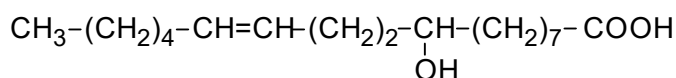


12,13-epoxyoctadec-9-enoic acid (Vernolic acid)

A C₂₀ epoxy fatty acid and *cis*-14,15-epoxy-*cis*-11-eicosenoic acid (alchornoic acid) has been found in some seed oil.

Hydroxy Fatty Acids

Some fatty acids contain hydroxyl group. The most common hydroxy fatty acids are ricinoleic acid (12-hydroxy-9-*cis*-octadecenoic acid) and isoricinolic acid (9-hydroxyoctadec-*cis*-12-enoic acid). Dihydroxy acids, such as 9,10-dihydroxy-octadecanoic acid and higher homologues, can be minor components of several seed oils.



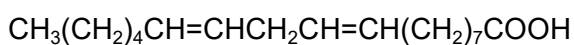
9-Hydroxyoctadec-*cis*-12-enoic acid

Keto Fatty Acids

Other than hydroxy fatty acids, Keto fatty acids are often reported as products of oxidation of fatty acids. However, analogues of some of the common plant hydroxy fatty acids are occasionally found, for example 13-oxo-trans-9, trans-11-octadecadienonic acid, an analogue of coriolic acid.

10.7 ESSENTIAL FATTY ACIDS

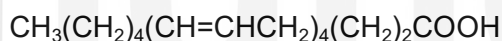
Most of the fatty acids can be synthesized in our body. However, there are some which cannot be synthesized by the mammals and therefore, need to be taken in the diet to fulfill their daily requirement. It is because mammals lack enzymes that can introduce double bonds beyond 9th position. Such fatty acids are known as **essential fatty acids**. Linoleic, linolenic and arachidonic acids are examples of essential fatty acids.



Linoleic acid,



Linolenic acid



Arachidonic acid

If human body doesn't have enough essential fatty acids it may have symptoms like: dry, scaly, flaky, dull, or bumpy skin. Dry, brittle, or lackluster hairs are another symptom of lack of essential fatty acids.

Fatty acids which can be synthesized in the body and need not necessarily to be taken in the diet are called **non-essential fatty acids**, for example, oleic acid. However, these terms in no way imply that non-essential fatty acids are less important than essential fatty acids. Both types are equally important for normal functioning of the body.

10.8 PROPERTIES OF FATTY ACIDS

Here we will first discuss the physical properties then chemical properties of fatty acids.

10.8.1 Physical Properties of Fatty Acids

Let us discuss some physical properties of fatty acids in correlation to their structures.

i) Solubility

Fatty acids are made up of long aliphatic hydrocarbon chains which make them **nonpolar**; however carboxylic acid group at one end of the chain makes it slightly polar. In general, fatty acids are more soluble in organic/nonpolar solvents such as chloroform and ether. Solubility in water and

other polar solvents decreases with increase in chain length, e.g. butyric acid, a short chain fatty acid of four carbons is soluble in water in all proportions. Caproic acid with six carbon chain length is slightly soluble in water and higher fatty acids are practically insoluble in water.

ii) Melting Point

Melting point of a fatty acid depends on two factors:

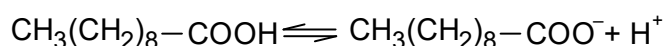
a) Degree of unsaturation and b) Length of chain

a) **Degree of unsaturation:** Saturated fatty acids have fully extended chains in zigzag form, which can fit very well with other chains; however unsaturated fatty acid chains are bent wherever there is a *cis* double bond. Higher the number of double bonds, higher is the number of bends; which makes it difficult for the chains to pack compactly. Also they have fewer van der Waal's interactions. For example, stearic acid (69.6° C) has higher melting point than oleic acid, which is an unsaturated fatty acid (16° C), although both are of same length (Tables 10.1 and 10.2).

It is because of this arrangement, lipids that are rich in saturated fatty acids, have higher melting point and exist as solids at room temperature (e.g., coconut oil), whereas, those containing unsaturated fatty acids have lower melting point and exist as liquid at room temperature (e.g., olive oil). To observe the effect of increase in number of double bonds on decreasing melting points, you may compare the melting points of oleic (13.4° C), linoleic (1° C) and linolenic (-11° C) acids from the Tables 10.2 and 10.3. Further note that *cis* isomer of a fatty acid has lower melting point than the *trans* isomer. The melting point of oleic acid is 16° C and that of elaidic acid is 44° C.

b) **Length of chain:** Fatty acids with longer chains have higher melting point than the ones having shorter chain length. For example, saturated fatty acids of eight carbon atoms are liquids at room temperature while those containing more than ten carbons are solids at room temperature.

iii) **Ionization:** Fatty acids are weak acids; therefore, do not undergo complete ionization of the carboxylic group. Their pK_a value is approximately 4.5. In general, fatty acids are neutral above pH 4.5 and charged below pH 4.5.



Hence at physiological pH, the carboxyl group is readily ionized, rendering a negative charge onto the fatty acids.

10.8.2 Chemical Properties

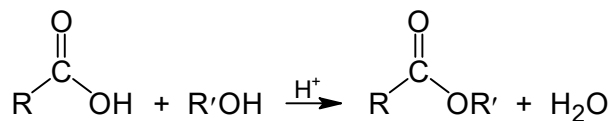
Chemical reactions of fatty acids: Fatty acids undergo four major reactions i.e., esterification, hydrogenation, halogenation and oxidation.

i) Esterification of fatty acids:

Hydrophilic (*hydro*-water; *philic*- loving) molecules loves to be in water or other polar solvents.

Hydrophobic (*hydro*-water; *phobic*- hate) molecule shies away from water and interacts only with molecules of its own kind.

Fatty acids rarely occur as free fatty acids and undergo esterification reaction typical of carboxylic acids. They react with alcohols to form esters and water.



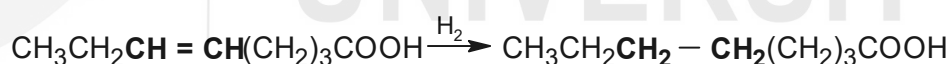
It is the esterified form of fatty acids which constitute major component of various lipids.

ii) Hydrogenation

Hydrogenation means addition of hydrogen. Unsaturated fatty acids get hydrogenated across the double bonds in the presence of catalyst such as nickel to yield saturated fatty acids. Hydrogenation of oils yield hardened oil/fat (such as hard butter) and on partially hydrogenation the oils are only partly hardened.

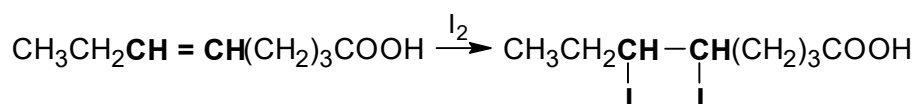
These hard fats can raise LDL levels. They can also lower HDL levels ("good cholesterol"). The *trans* fatty acids are found in fried foods, commercial baked foods (donuts, cookies, crackers) and processed foods. These fats may have some side effects. Hydrogenated oils or fats contain high levels of *trans* fatty acids, which are linked to **heart disease** and cancer. A large intake of polyunsaturated fat is also risky and it may increase the risk for some types of cancer. However, reducing daily fat intake is not a guarantee against developing cancer or heart disease.

The phenomenon of hydrogenation has been used in a controlled manner for preparation of margarine and processed cheese by conversion of unsaturated fatty acids of oils to the saturated ones resulting in solidification.



iii) Halogenation of Unsaturated Fatty Acid

Just like addition of hydrogen across unsaturated double bonds, halogens such as iodine can add across the double bonds to form dihalogenated fatty acids. The following reaction shows the addition of iodine across the unsaturated bond. The process is known as **iodination**.



This property of fatty acids has been used to assess the extent of unsaturation in the lipids. Higher the unsaturation in fatty acids, higher will be amount of iodine consumed by it for addition across the unsaturated bonds. This parameter is known as **iodine number** and is defined as **number of gram of iodine required to saturate 100 grams of the lipid**.

iv) Oxidation

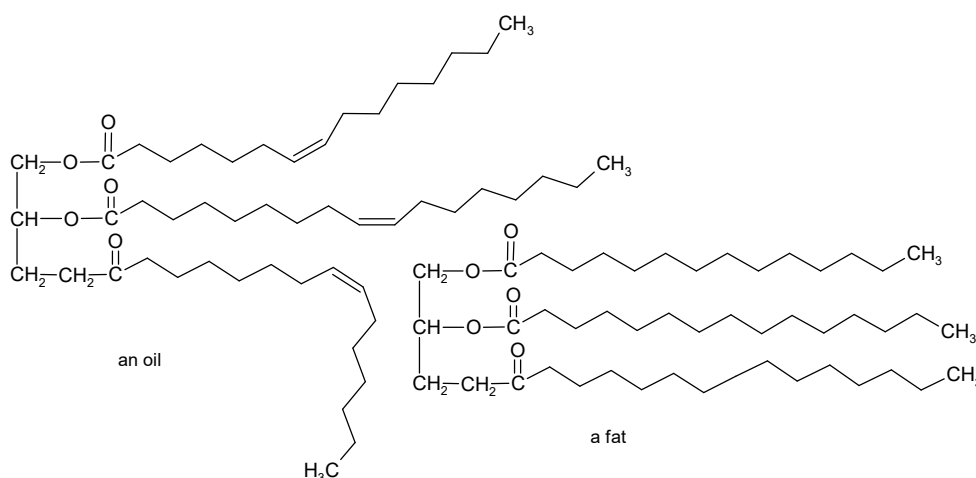
Unsaturated fatty acids can spontaneously react with atmospheric oxygen and get oxidized. The process of oxidation results in formation of fatty acid peroxides, epoxides or aldehydes which give unpleasant smell and taste to lipids. Such lipids are said to be rancid and are toxic and unsuitable for consumption. Degree of rancidity can be assessed by **acid number which is defined as the number of milligrams of KOH required to neutralize free fatty acids in a gram of fat**. It is different from saponification number as it takes into account both free fatty acids as well as those present as esters in a fat. For a lipid which is properly processed and stored has low acid number. Rancidity can be prevented with the help of antioxidants such as vitamin E.

10.9 FATS AND OILS

Fatty acids are the building blocks of the fat in our bodies and in the food we eat. During digestion, the body breaks down fats into fatty acids, which can then be absorbed into the blood. Fatty acids serve as energy for the muscles, heart, and other organs, as building blocks for cell membranes, and as energy storage for the body. Healthy skin and hair are maintained by fat. Fat helps the body absorb and move the vitamins A, D, E, and K through the bloodstream. Fats and oils are mainly triglycerides.

Triglycerides that are solids or semisolids at room temperature are called **fats**. They are usually obtained from animals and are composed largely of triglycerides with either saturated fatty acids or fatty acids with a double bond. The saturated fatty acids are packed closely together, giving the triglycerides relatively high melting points, causing them to be solids at room temperature.

Liquid triglycerides are called **oils**. Oils typically come from plant products such as mustard, sunflower, corn, soybeans, olives, and peanuts. They are composed primarily of triglycerides with unsaturated fatty acids that cannot be packed tightly together due to their zigzag structures; therefore, they have relatively low melting points, causing them to be liquids at room temperature (see representative structures of fats and oil below).



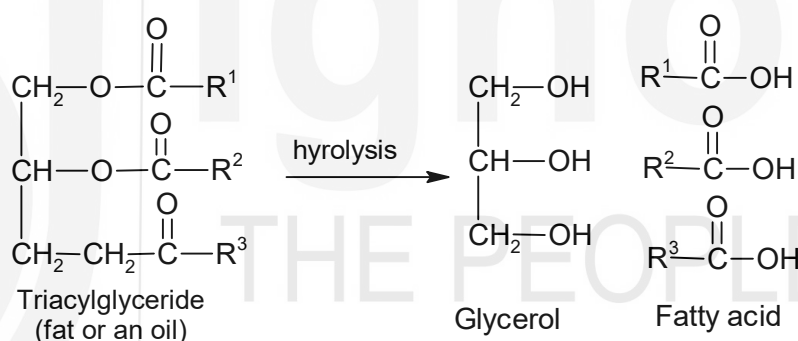
With the increase of temperature, the fat changes into oil. If the temperature continues to increase beyond the melting point to the point where some smoke becomes evident, the molecular structure of the oils will change and a number

of toxic molecular isomers will be produced in the oil. The temperatures where this damage is done to our fats and oils are about half the temperatures reached in the refining and hydrogenation processes. Thus, these processes routinely destroy all the nutritional value of our fats and oils. Oils contain a higher percentage of unsaturated fatty acids than fats. Fats from animal sources tend to be solids and fats from vegetable sources tend to be liquids. Thus, fats are often referred to as "animal fats" and "vegetable oils."

Pure oils and fats are colourless, odourless and tasteless. They are insoluble in water but can form emulsions in the presence of emulsifying agents, such as soaps and bile salts. This process of emulsification usually occurs during the digestion of fats and oils in the small intestine, in which bile salts aid in emulsification process. Let us now examine the effect of hydrolysis on acylglycerols.

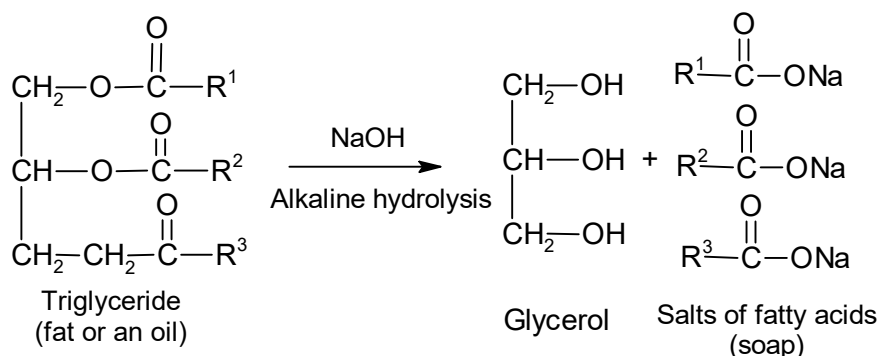
Hydrolysis of Acylglycerols

When a triglyceride is subjected to hydrolysis, the ester bonds are hydrolysed. Three molecules of fatty acids and one molecule of glycerol are obtained. This reaction can be catalysed by acids, alkalies or enzymes (lipases):



Lipases are secreted along with digestive juices in an animal and are responsible for digestion of fats in the stomach.

Alkaline hydrolysis of a fat is also called **saponification**, since the fatty acids liberated would be present as their salts, i.e. soaps. The soaps are having excellent cleansing properties.



Lipids are sometimes classified as **saponifiable** and **non-saponifiable** based on their ability to release salts of long fatty acids on alkaline hydrolysis. Thus, monoglycerides, diglycerides and triglycerides are saponifiable lipids. An oil or

fat can be characterised by the saponification number, which is the weight in milligrams of KOH required to completely saponify one gram of fat or oil. This is a measure of the fatty acid component in a fat and a higher value indicates presence of mostly short chain fatty acids. Thus, the **saponification number** provides information about the average chain length and molecular mass of the fatty acids in a fat. It varies inversely with the chain length of the fatty acids. The shorter the average chain length of the fatty acids, the higher is the saponification number. It is so because more number of short chain fatty acids are present in 1 gram of fat compared to that of long chain fatty acids. This results in availability of more acid groups for saponification in a fat containing short chain fatty acids as compared to fat with longer chain length for the same weight i.e., 1 gram.

Mechanism of soap action - Triglycerides are non polar molecule as polar groups of both glycerol (OH) and fatty acid chains (COOH) are occupied in making ester bonds. However, soap is amphipathic molecule which means that it has both polar and nonpolar ends (fatty acid chain). Polar head is constituted by the ionizable carboxylic group (COO^+Na^-). This part is hydrophilic. Nonpolar end is the hydrocarbon chain of the fatty acid which is hydrophobic (Fig. 10.1).

When such molecules are present in water or other such polar solvent, they form **micelles**. They arrange themselves in such a way so that nonpolar chains are close together with least contact with water and polar heads are exposed to water.

Catalysts used during hydrogenation of oils containing unsaturated fatty acids convert some of the *cis* fatty acids to *trans*. Elaidic acid is generally formed. *Trans* fatty acids are harmful and have been associated with the cardiovascular diseases.

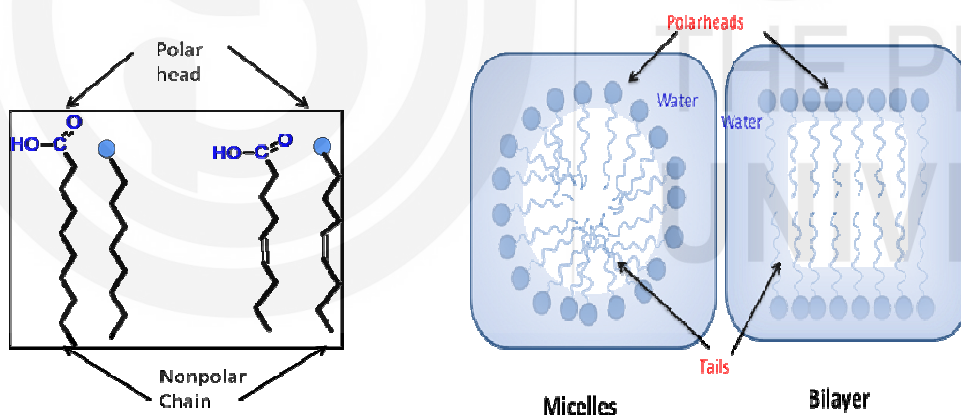
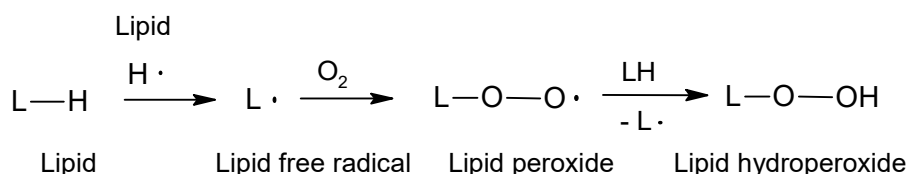


Fig. 10.1: Cleansing action of soap involves entrapment of grease and dirt in the nonpolar part of micelles which are then washed away with water as the polar part is in contact with water. The phenomenon of entrapping grease or oily substances into micelle formed by soap is known as emulsification.

The unsaturated fatty acid present in an oil or a fat can be catalytically reduced in the presence of hydrogen (hydrogenation). They can also be halogenated with bromine or iodine. This addition of halogen (**iodine number**) is used to determine the degree of unsaturation of an oil or a fat. Iodine number, of a fat is defined as the weight in grams of iodine that can be taken up by 100 grams of fat or oil. Higher the iodine number, greater is the degree of unsaturation present in the fat. Oils with higher iodine number are thus preferred, as their nutritional value is higher. The iodine number of coconut oil

In nature, partial hydrogenation of some of the unsaturated fats ingested by ruminants takes place by bacteria in the rumen. Therefore, milk fat, dairy products, beef and mutton fat also contain *cis* and *trans* fatty acids such as vaccenic acid.

is about 8, while that of butter fat is about 36, compared to 81 for olive oil, 93 for groundnut oil and 145 for safflower oil. Another property of unsaturated fats is their easy oxidation. Many oils and fats when allowed to stand in contact with air, become rancid and develop unpleasant odours. This is attributed to the oxidation of the double bonds, yielding short chain aldehydes and keto acids. This oxidative rancidity of oils and fats can be prevented by the addition of antioxidants, like vitamin E. Rancidity of fats can also occur due to partial hydrolysis of the ester linkage, giving rise to free fatty acids. The process of autooxidation of fats, called peroxidation, can also occur within the body due to free radicals that are generated during metabolism. Lipid peroxidation can become a chain reaction, so that further peroxidation is continued:



Now you can able to difference between lipids and fats. The main difference between lipids and fats is that lipids are a broad group of biomolecules whereas fats are a type of lipids. Fat is stored in the adipose tissue and under the skin of animals. It is mainly used as an energy-storage molecule in the body.

SAQ 6

Fill in the following blanks:

- Fat are solid or semisolid at temperature.
- Oils are present as of fatty acids.
- is a general term to describe inflamed skin.
- Lipids are insoluble in water and soluble in solvents.

10.10 WAXES

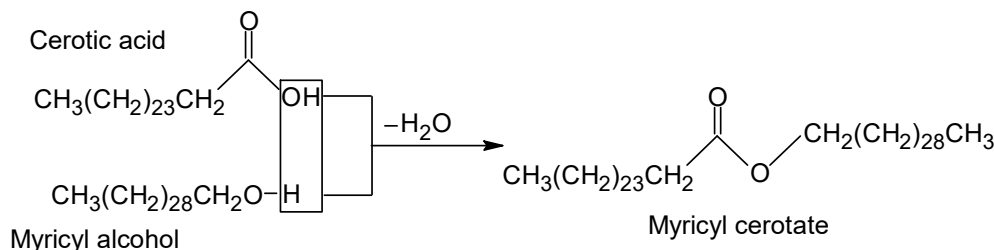
Waxes are formed when a fatty acid undergoes esterification with a monohydric alcohol of high molecular weight. Waxes are also esters of long chain saturated or unsaturated fatty acid ($\text{C}_{14}\text{-C}_{36}$). However, instead of glycerol, they contain long chain ($\text{C}_{16}\text{-C}_{30}$) alcohols. They have higher melting point than triglycerides. Waxes are the major energy storage form in planktons, which serve as food for marine organisms such as whale, herring, salmon.

Waxes are different from paraffin wax, which is a complex mixture of high molecular weight hydrocarbons.

Waxes are widespread in nature, as many of them serve a protective function on fruit and leaf surfaces. Being highly insoluble in water, and having no double bonds in their hydrocarbon chain, they are chemically inert and thus protect leaves from water loss. In vertebrates, waxes secreted by cutaneous glands provide a protective coating on the skin and hair to keep them lubricated and waterproof. Birds, particularly waterfowl, secrete waxes to make their feathers water-repellent. The leaves of many plants such as rhododendron and calotropis are shiny because of the deposition of protective waxy coating.

Waxes find important use in pharmaceutical and cosmetic industry. Lanolin from lamb's wool, beeswax, carnauba wax from a Brazilian palm tree is used in lotions, ointments and polishes. Following equation gives the structure of myricyl cerotate present in beeswax and carnauba wax.

In the early 1960s India used to be an exporter of oilseeds, but now due to the growth of population, large import of edible oils and seeds is made for domestic consumption.



10.11 SUMMARY

- Lipids are organic compounds that contain hydrogen, carbon, and oxygen atoms, which forms the framework for the structure and function of living cells.
- Lipids are sparingly soluble in water and polar solvents. They are generally soluble in organic solvents such as ether and chloroform.
- Lipids are classified on the basis of similarity in chemical structure and the functions they perform. Structurally they are classified as simple, compound and derived lipids.
- Lipids are important constituents of oil, butter, milk, cheese, fried foods and also in some red meats.
- There are three main functions of lipids in human body. i) At the cellular level, it act as signalling molecules, ii) it forms the structural component in cell membranes and iii) lipids also store energy in the body.
- Lipids are found in i) cell membranes, ii) blood cells, and in the brain.
- Fatty acids are long aliphatic hydrocarbon chains with a carboxylic acid group at one end and methyl group at the other end.
- Palmitic and stearic acids are the most common saturated fatty acids.
- The saturated fatty acids have higher melting points compared to unsaturated fatty acids of the corresponding carbon chain, due to their ability to pack their molecules together
- Besides the usual fatty acids, some fatty acids contain functional groups other than carboxylic acid group, e.g. cyclopropene, hydroxyl, epoxy, furan and keto fatty acids.
- There are some fatty acids which cannot be synthesized by the human body and therefore, need to be taken in the diet to fulfill their daily requirement. Such fatty acids are known as **essential fatty acids**. Linoleic and arachidonic acids are examples of essential fatty acids
- Triglycerides constitute the storage form of fatty acids in animals and plants.
- Fats and oils are nothing but triglycerides which occur as solid and liquid, respectively at room temperature.
- Fats generally exist in the form monoglycerides, diglycerides or triglycerides.

- Waxes are the esters of long chain saturated or unsaturated fatty acid chains (C_{14} - C_{36}). However instead of glycerol, they contain long chain (C_{16} - C_{30}) alcohols.
- Important derivative of fatty acids are fats and oil. They are triglycerides which on alkaline hydrolysis produce soaps and glycerol.
- Waxes are the major energy storage form in planktons, which serve as food for marine organisms such as whale, herring, and salmon. They also provide water proof, protective coating on skin, hair and feathers of many animals, birds and plants.

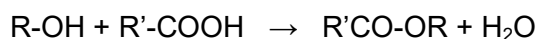
10.12 TERMINAL QUESTIONS

1. Give the IUPAC name of the following fatty acids
 - i) Oleic acid
 - ii) Palmitoleic acid
 - iii) α -Linoleic acid
 - iv) Arachidonic acid
2. Define i) Saponification value ii) Acid number and iii) Iodine number
3. Fill I the following blanks:
 - i) **Waxes** are formed when a fatty acid undergoes----- with a monohydric alcohol
 - ii) Waxes are ----- of long chain saturated or unsaturated fatty acid chains
 - iii) The ----- fatty acids are found in fried foods
 - iv) In vertebrates, waxes secreted by ----- glands
 - v) Degree of rancidity can be assessed by -----
4. A fat has low iodine number and a high saponification number. What do you infer from that?
5. What are the different types of naturally occurring fatty acids?

10.13 ANSWERS

Self-Assessment Questions

1. Most important function lipids perform is as the building blocks of cellular membranes. Other functions include energy storage, insulation, cellular communication and protection.
2. Simple lipids are esters of carboxylic acids having long unbranched hydrocarbon chain termed as fatty acids.

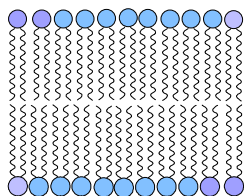


3. i) alpha (α) and omega (ω)

- ii) plants seeds and fish.
5. i) $\text{COOH}(\text{CH}_2)_{10}\text{CH}_3$; ii) $\text{COOH}(\text{CH}_2)_{18}\text{CH}_3$; iii) $\text{COOH}(\text{CH}_2)_{14}\text{CH}_3$;
iv) $\text{COOH}(\text{CH}_2)_{16}\text{CH}_3$; v) $\text{COOH}(\text{CH}_2)_{12}\text{CH}_3$
6. 1. i) room ii) triglycerides iii) Dermatitis iv) organic

Terminal Questions

1. i) *cis*-9-Octadecenoic acid, $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
ii) *cis*-9-Hexadecenoic acid, $\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
iii) α -*cis*-9, 12, 15-Octadecatrienoic acid,
 $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
iv) α -*cis*-5,8,11,14-Eicosatetraenoic acid,
 $\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
2. i) Saponification value: It is the number of milligrams of KOH required to saponify 1 gm of fat. The saponification number, thus, provides information about the average chain length of the fatty acids in the fat. It varies inversely with the chain length of the fatty acids.
ii) Acid number: It is the number of milligrams of KOH required neutralizing free fatty acids in a gram of fat. Thus, this number gives an idea about the number of free fatty acids present in a lipid sample. For a lipid which is properly processed and stored has a low acid number.
iii) Iodine number: It is the number of gram iodine required to saturate 100 grams of the lipid. Higher the number of unsaturated fatty acids in a lipid, higher is its iodine number.
3. i) esterification; ii) esters, iii) acid number; iv) cutaneous; v) *trans*
4. The iodine number of a fat indicates the degree of unsaturation in it. A low iodine number of a fat, thus, indicates that the unsaturated fatty acid content of 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.
- 5) 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 occasionally. The naturally occurring unsaturated fatty acids are always of the *cis* configuration, and **PUFAs** are generally present in vegetable fats. Saturated fatty acids are often found in animal fats.



UNIT 11

LIPIDS II

Structure

11.1	Introduction	11.6	Prostaglandins
	Expected Learning Outcomes	11.7	Structure of Biomembrane
11.2	Biological Importance of Lipids		Composition and Biomembranes
11.3	Phospholipids		Membrane Structure
11.4	Glycolipids		Functions of Biomembranes
	Lipoproteins	11.8	Summary
11.5	Steroids	11.9	Terminal Questions
	Steroid hormones	11.10	Answer

11.1 INTRODUCTION

In Unit 10 we have introduced you with another class of biomolecules, lipids. Therein we described that these molecules are extremely hydrophobic in nature, are essential constituents of all living cells. We mentioned that these molecules also store energy for the cell, and, in addition, perform some other functions also. In that unit our focus was mainly on the classification of the lipids and the chemistry of simple lipids.

This unit is in the continuation to Unit 10, here our focus will be mainly on complex lipids and derived lipids. Lipids in these classes include phospholipids, glycolipids and sulpholipids, sphingolipids, steroids, steroid hormones and prostaglandins. These lipids along with proteins not only form biological membrane but also act as signaling molecules. You will recall that we briefly introduced you to the structure of membranes in Unit 1. You learnt that lipids form part of the structure of cell membranes. In this unit you shall also learn more about the structure and function of biomembranes. In the next unit we will describe nucleic acids. Like carbohydrates, nucleic acids are biopolymers and are important components of the cell.

Expected Learning Outcomes

After studying this unit you should be able to:

- ❖ explain biological functions of lipids;
- ❖ discuss importance of glycolipids;
- ❖ describe structural features of different types of phospholipids and their derivatives;
- ❖ classify sphingolipids and explain their biological importance;
- ❖ explain structure and importance of steroids, steroid hormones and prostaglandins; and
- ❖ describe the role of lipids in the formation and functioning of biomembranes.

11.2 BIOLOGICAL IMPORTANCE OF LIPIDS

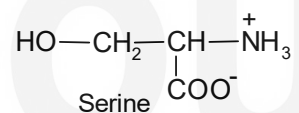
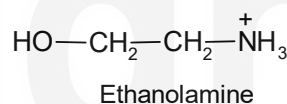
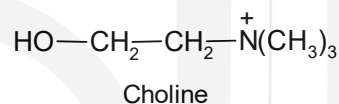
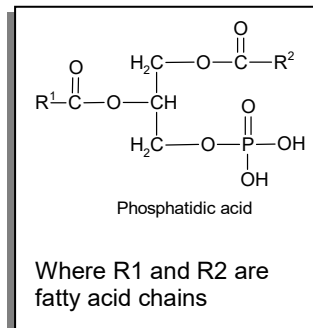
We have briefly described biological importance of simple lipids in earlier unit. Now our focus will be on the biological importance of complex lipids and derived lipids. The main functions of these classes of lipids are to provide structure support to cell and cell organelles, and act as signalling molecules. Phospholipids are essential structural components of all biological membranes and serve to compartmentalise the cells. They are important constituents of lipid bilayer in cell membranes. These molecules are amphipathic thus have both hydrophilic region and hydrophobic regions. Because of this nature biological membranes provide a selective barrier. They allow some compounds to pass, but prevent others. In that way membranes are able to regulate the internal chemical composition of the cells and its organelles. Some phospholipids are used as emulsifying agents in food industry and some are used in skin care products. Phospholipids are important constituents of plasma lipoproteins. They are essential for blood clotting, as they provide the platelet activating factor (PAF). Some complex lipids are also important precursors of hormones, vitamins and prostaglandins. These categories of lipids function as signalling molecules or cellular messengers. For example steroids hormones and prostaglandins act as signalling molecules and modulating several functions including metabolism, reproduction, blood pressure, body temperature etc.. Prostaglandins also act as defence against many sorts of changes in the cell.

In Unit 10, we have already described the general classification of lipids and learnt in detail about the various types of simple lipids such as oils and fats. Therein we have also briefly defined complex lipids derived lipids. Let us recall the classification of complex and derived lipids. Just like simple lipids complex lipids are also esters of fatty acids with alcohol. However, in addition, they contain other groups, such as a phosphate group or a carbohydrate moiety. Complex lipids are further classified in two main groups that are glycolipids and phospholipids. Derived lipids are obtained from the above mentioned groups by hydrolysis and include terpenoids, steroids, steroid hormones, lipid soluble vitamins and prostaglandins.

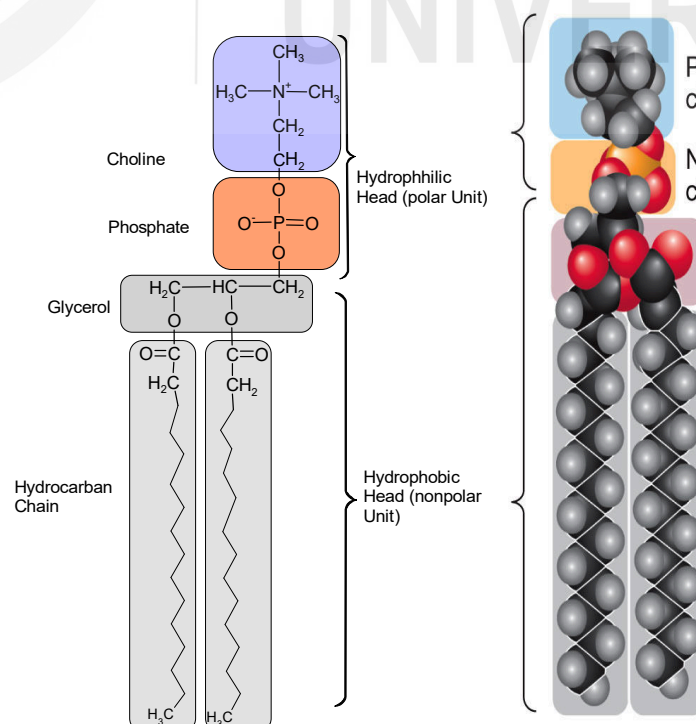
We shall now describe some of the important types of lipids and examine their structural features and importance.

11.3 PHOSPHOLIPIDS

Phospholipids are the major lipid components of all biological membranes, about which you will know more in Section 11.6. Phospholipids are so named because they contain a phosphate group. In addition, glycerol, fatty acids and nitrogenous bases are the key components of these molecules. Phospholipids are **Phosphoacylglycerols** (also called **phosphoglycerides**). They are similar to triacylglycerols except that a terminal OH group of glycerol is esterified with phosphoric acid rather than with a fatty acid. Phosphatidic acids are the simplest phosphoacylglycerols (see Fig. in margin). These are present only in small amounts in membranes. The most common phosphoacylglycerols in membranes have a second phosphate ester linkage. These phosphoglycerides may be considered as derivatives of phosphatidic acid, in which the phosphate group of phosphatidic acid is generally esterified with the hydroxyl of a suitable alcohol such as ethanolamine, choline, and serine.



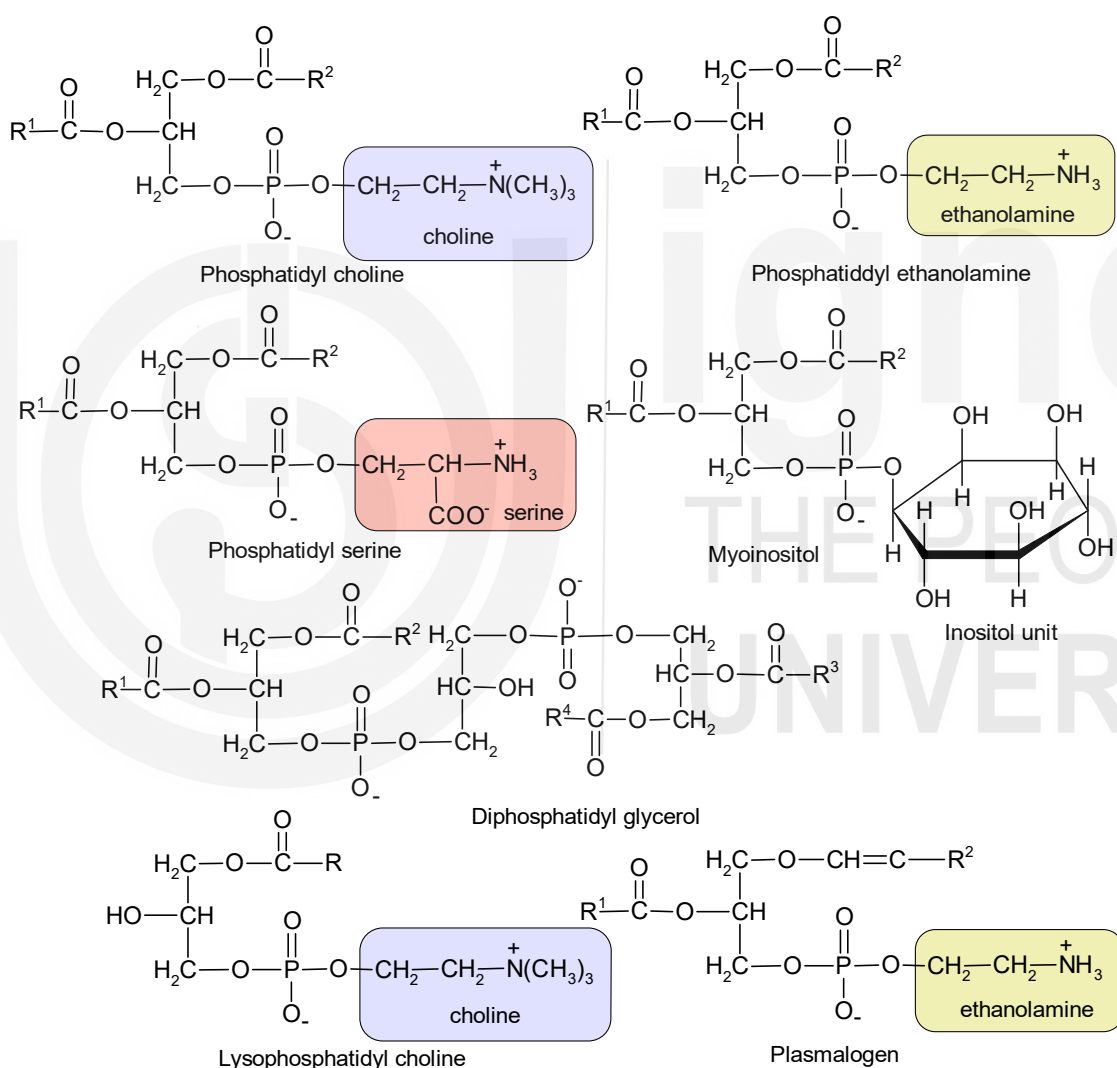
These substituted alcohols are highly polar in nature and further enhance polar nature of phosphate group. These structural features render these lipids **amphipathic** molecules, since they possess both polar phosphate groups (heads) and nonpolar hydrocarbon groups (tails). A representative structure of phospholipid is shown in see Fig. 11.1. Head of these molecules behave as hydrophilic and tail as hydrophobic.



The Phospholipids are further classified on the basis of the nature of polar substituted alcoholic group. They include (a) phosphatidic acid and phosphatidyl glycerols, (b) phosphatidyl choline, (c) phosphatidyl ethanolamine, (d) phosphatidyl inositol, (e) phosphatidyl serine, (f) lysophospholipids, (g) plasmalogens, and (h) sphingomyelins.

All these molecules are phosphoglycerides, except sphingomyelins, which do not contain glycerol but a complex amino alcohol, namely sphingosine.

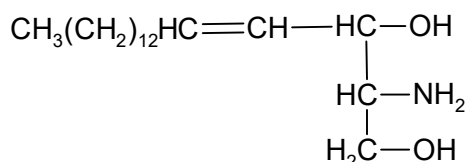
Phospholipids form membranes by arranging themselves in a **lipid bilayer** (details of lipid bilayer is given in Sec. 11.7). In lipid bilayer, polar heads of the phospholipids are on the outside of the bilayer, and the fatty acid chains form the interior of the bilayer. These phospholipids are not only important component of cell membranes but also carry out other functions. The structures of some of important phospholipids are shown below:



Phosphatidyl ethanolamines are also called **cephalins**, and phosphatidyl cholines are called **lecithins**. Cephalins are found in most cell membranes, and are particularly abundant in brain tissue. They are also found in blood platelets, and play a role in blood clotting. Lecithin is the most abundant phospholipid in cell membranes, and represents a large portion of the body's stores of choline, which is important in transmission of nerve impulses. Cephalins and lecithins are also used as emulsifying agents. Diphosphatidyl glycerol or cardiolipin is a major phospholipid present in mitochondrial membranes. Phosphatidyl inositol is also an important constituent of cell

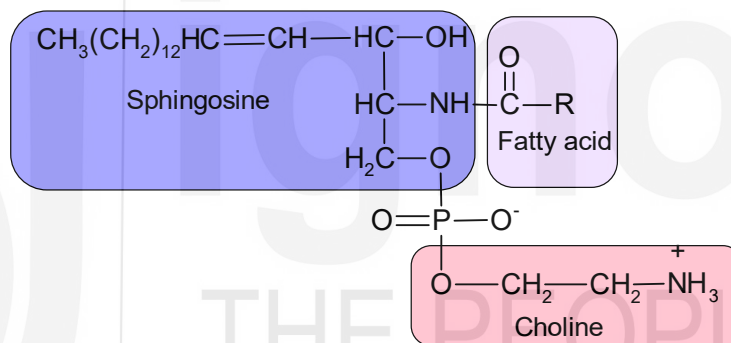
membranes and its 4, 5 bisphosphate acts a second messenger, and is important in cell signalling mechanism. Plasmalogens are phospholipids containing an, α , β unsaturated alcohol in an ether linkage rather than a fatty acid in is ester linkage on C-1 of glycerol 3-phosphate. Plasmalogens contain either ethanolamine, choline or serine and constitute as much as 10% of the phospholipids in brain and muscle.

Sphingomyelins are phospholipids that do not contain glycerol but instead contain the complex amino alcohol, sphingosine.



Sphingosine

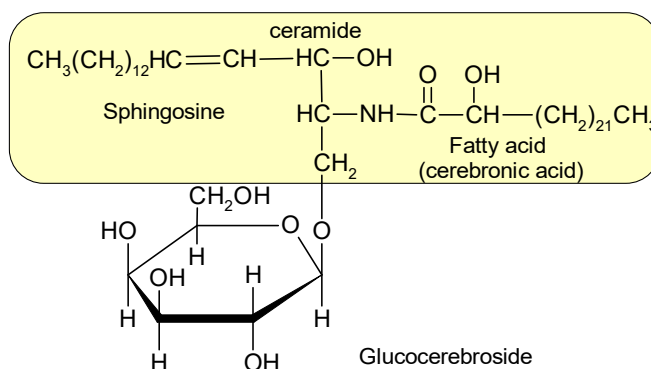
In the sphingomyelins, a choline unit is attached to sphingosine through a phosphate group, along with a single fatty acid attached to the nitrogen atom of sphingosine via an amide linkage. The structure of a sphingomyelin is shown below:



Sphingomyelin

Sphingomyelins are found in large quantities in brain and nerve tissue. The membrane sheath that surrounds and electrically insulates many nerve cell axons is particularly rich in sphingomyelins. On hydrolysis of sphingomyelin, a fatty acid, phosphoric acid, choline and sphingosine are formed. The combination of sphingosine and fatty acid is known as ceramide, a structure also found in glycolipoids. Another derivative of ceramides is cerebrosides. In cerebrosides, the terminal primary OH group of sphingosine is bonded to a sugar residue. Sphingomyelins are phospholipids because they contain a phosphate group. Ceramides and cerebrosides, on the other hand, are not phospholipids.

Ceramides help to restore the skin's barrier function and lock in moisture. They are used in moisturisers and in anti-aging cream.



Glucocerebroside

SAQ 1

Name the products which are formed on the hydrolysis of sphingomyelin.

SAQ 2

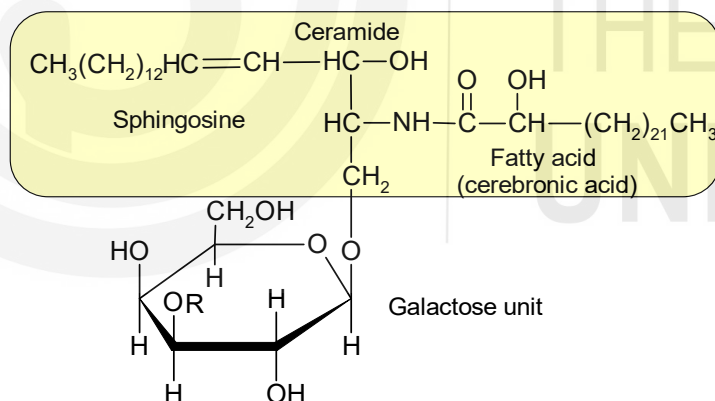
Name the structure components of following phospholipids:

(a) phosphatidyl choline, (b) phosphatidyl ethanolamine, (c) phosphatidyl inositol, (d) phosphatidyl serine, (e) sphingomyelins

We shall describe an important member from the **other-complex lipids group**, namely, glycolipids.

11.4 GLYCOLIPIDS

Glycolipids are widely distributed in most tissues of the body and particularly in the nervous tissue, such as the brain. In the plasma membrane they contribute to cell surface carbohydrates, which function as cell membrane receptors, and are involved in recognition mechanism. The major glycolipids of the animal tissues are the **glycosphingolipids**. They contain ceramide and one or more sugars and fatty acid residues which are attached to a sphingosine. Two simple examples are galactosylceramide and glucosylceramide, and the structure of the former is shown below:

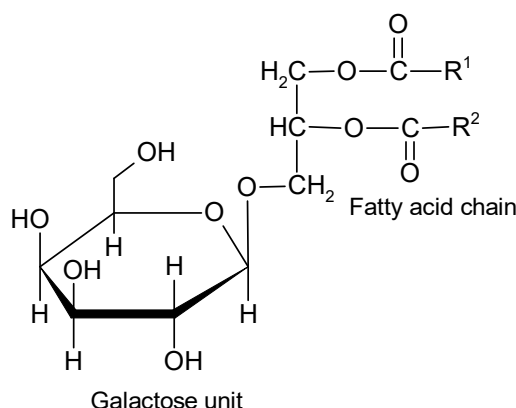


Structure of galactosylceramide (galactocerbroside, R=H), and sulpho galactosylceramide (a sulphatide, R = SO₃⁻)

Galactosylceramide has a characteristic 24 carbon fatty acids, as a constituent part. You would observe from the above structure that the sugar molecule can be sulphated in some cases. These sulphated glycolipids are known as **sulphatides**. Some highly complex glycosphingolipids are derived from glucosylceramide, and are called **ganglioside**. These are present in nerve tissues.

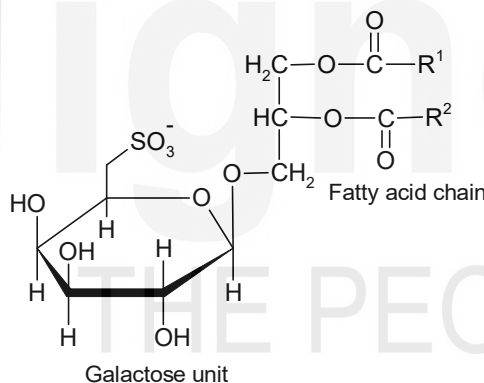
Another type of glycolipids is glyceroglycolipids. In these cases the sugar and fatty acid residues are attached to a glycerol molecule. Glyceroglycolipids include the galactolipids and the sulpholipids. Both these lipids are part of chloroplast membranes and play important roles in plant development and

photosynthesis. Galactolipids have one or two galactose residues linked to terminal of diacylglycerols by a glycosidic linkage. A specific example of a galactolipids: monogalactosyldiglyceride (MGDG).



Monogalactosyldiglyceride (MGDG)

Sulpholipids contain a sulphonated glucose residue attached to diacylglycerol by a glycosidic linkage. A specific example of a galactolipids: Sulphoquinovosyl diacylglycerol (SQDG):



Sulphoquinovosyl diacylglycerol (SQDG)

SAQ 3

Draw the structure of MGDG and SQDG.

We shall describe an important member from the **other-complex lipids group**, namely, lipoproteins.

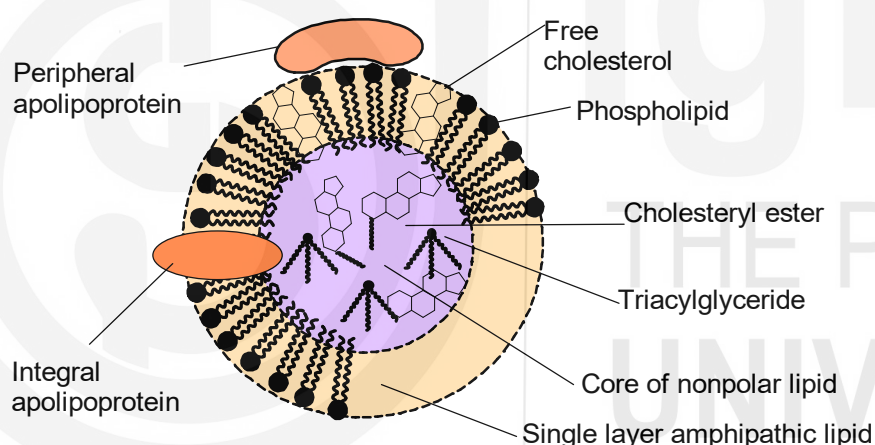
11.4.1 Lipoproteins

Lipids, being insoluble in water, cannot be transported in the free form in circulating blood plasma. Hence they are transported in association with proteins. Such protein complexes with lipids are called **lipoproteins**. Lipoproteins are water miscible, and consist mainly of triacylglycerols, phospholipids, cholesterol, and one or more protein compounds called the **apolipoproteins**. Depending upon the composition and density of the lipoproteins four major groups of lipoproteins have been identified. They are the chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). The composition of some of these different lipoproteins is shown in Table 11.1.

Table 11.1 : Source and Composition of Some Lipoproteins

Source	Type of Lipoprotein	Molecular weight	Percentage of components (%)			
			Protein	Phospholipid	Cholesterol (free+ester)	Triacyl glycerol
Blood serum	Chylomicron	$10^9 - 10^{10}$	2	7.5	10	80
	Very low density	$(5-10) \times 10^6$	8	19	18	55
	Low density	2×10^6	21	28	41	10
	High density	$(1-4) \times 10^5$	58	25	12	6
Egg yolk	β -Lipopitellin	4×10^5	78	12	1	9
Milk	Low density	4×10^6	13	52	0	35

The apolipoprotein content of HDL is as high as 60% while in that of chylomicrons it is as low as 2%. We have illustrated the generalised structure of a typical lipoprotein in Fig. 3.2.

**Fig. 11.2: Generalised structure of a lipoprotein**

Chylomicrons and VLDL consists of a lipid core of mainly nonpolar triacylglycerols and cholesterol ester, surrounded by a single layer of phospholipids and cholesterol molecules. The apolipoprotein is present either on the surface of the lipid core or it could be an integral part of the complex. Free fatty acids are released in the tissues from the lipoproteins, by the action of enzyme **lipoprotein lipase**. Dietary triglycerides are partially hydrolysed in the small intestines mostly to 2-acylglycerols which are absorbed together with the free fatty acids in an emulsified form. These are reconverted into triacylglycerols and transported to liver as part of chylomicrons. In the liver they are processed into other higher density lipoproteins. They are then transported to the extra hepatic tissues. Different lipoproteins are recognised by different tissues by specific receptors found in the cells. Various abnormalities of lipid metabolism occur at the sites of production and utilisation of lipoproteins, causing various hypo or hyperlipoproteinemias. Hypercholesterolemia and atherosclerosis are two such disorders seen in humans.

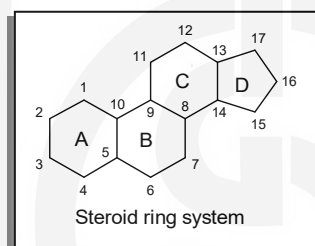
SAQ 4

Tick [✓] mark the statement which correctly describes the function of lipoproteins.

- | | |
|--|-------|
| a) Lipid solubilisation | [] |
| b) Increase in density of lipoproteins | [] |
| c) Stability of lipids | [] |
| d) Carrier of lipids | [] |

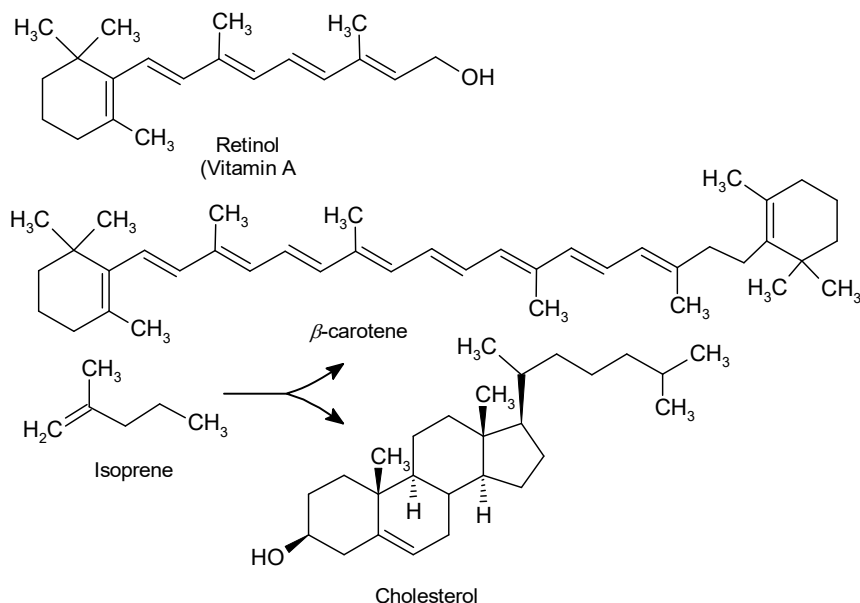
We shall now describe some members from the **derived lipid** group. They are also called non-saponifiable lipids *i.e.*, cannot be hydrolyzed by heating with alkali to yield soaps.

11.5 STEROIDS



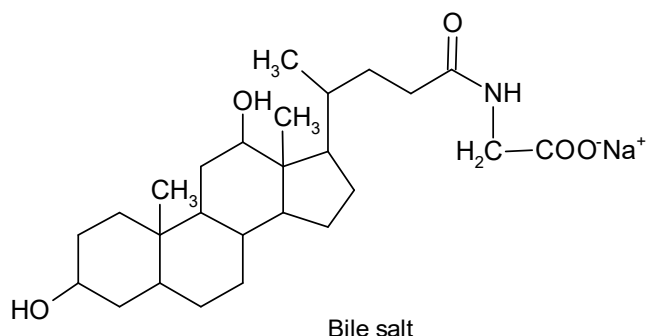
Steroids are the cyclic lipids which lack fatty acid chain and therefore, are non-saponifiable. They are derived from **four** fused and fully saturated rings called **sterane**. This system consists of 3 cyclohexane rings (A, B and C) fused in nonlinear or phenanthrene manner and a terminal cyclopentane ring (D). The sterane ring along with the conventional numbering of the carbon atoms is shown margin. Molecules containing sterane ring are known as sterols.

One of the commonly known steroids is cholesterol, which is widely distributed in body cells and is a major constituent of the plasma membrane and plasma lipoproteins. Terpenes are another large group of lipids which are made up of repeating “isoprenoids units” are the precursor of steroids. Terpenes molecules are derived by the condensation of isoprene units, which gives rise to such important compounds as carotenoids, from which vitamin A is derived, and cholesterol, from which several steroid hormones, bile acids and vitamin D are derived.



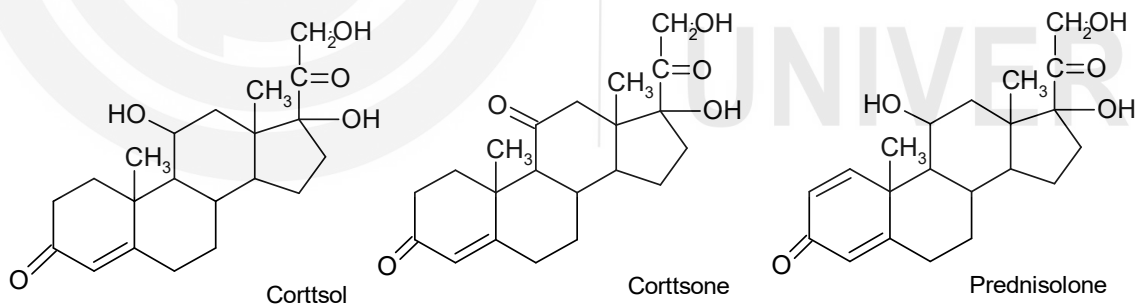
Bile Salts

Bile is a yellowish brown or green fluid produced in the liver and stored in the gall bladder. Bile salts act like soaps and other emulsifiers: they contain both polar and nonpolar regions, helping to break fats in foods into smaller pieces, so that food is hydrolysed more easily.

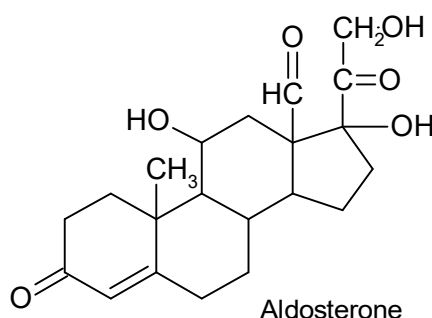


11.5.1 Steroid Hormones

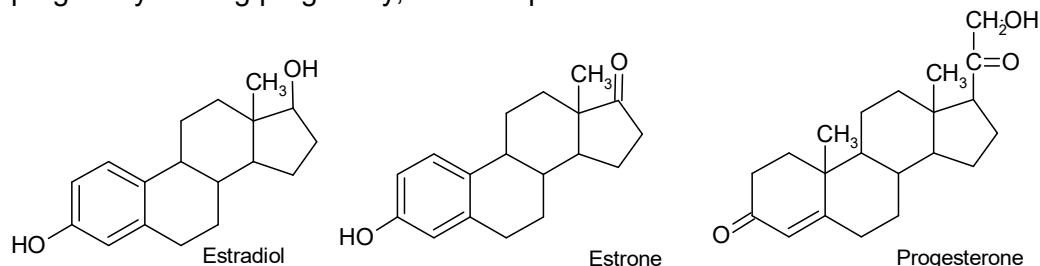
Hormones are chemicals released by cells or glands in one part of the body that send out messages that affect cells in other parts of the body. Many hormones are steroids. The steroid hormones can be divided into five classes: glucocorticoids, mineralocorticoids, androgens, estrogens, and progestins. Glucocorticoids and mineralocorticoids are synthesized in the adrenal gland (located on the top of the kidney) and are collectively known as **adrenal corticosteroids**. All adrenal corticosteroids have an oxygen at C-11. Glucocorticoids, as their name suggests, are involved in glucose metabolism, as well as in the metabolism of proteins and fatty acids. Glucocorticoids such as cortisol and its derivatives, cortisone and prednisolone (synthetic) are powerful anti-inflammatory drugs used to treat arthritis and asthma.



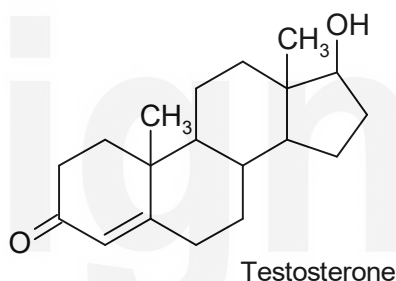
Mineralocorticoids regulate absorption of Na^+ , Cl^- and HCO_3^- ions by the kidneys. Any abnormal change leads to increase in blood pressure. It also regulates the retention of water in the body. Aldosterone is an example of a mineralocorticoid.



Estradiol and estrone are female sex hormones known as **estrogens**. They are secreted by the ovaries and are responsible for the development of female secondary sex characteristics. They also regulate the menstrual cycle. Progesterone is the hormone that prepares the lining of the uterus for implantation of a fertilized ovum and is essential for the maintenance of pregnancy. During pregnancy, It is also prevents ovulation.



Testosterone is male hormone and is produced in the testes. It regulates the production of sperm and aid in the development of male secondary sex characteristics.



You may have noticed that although the various steroid hormones have remarkably different physiological effects, but their structures are quite similar.

As mentioned earlier, prostaglandins are a class of derived lipids. These act as defences against many sorts of changes in the body. Let us learn more about these molecules.

SAQ 5

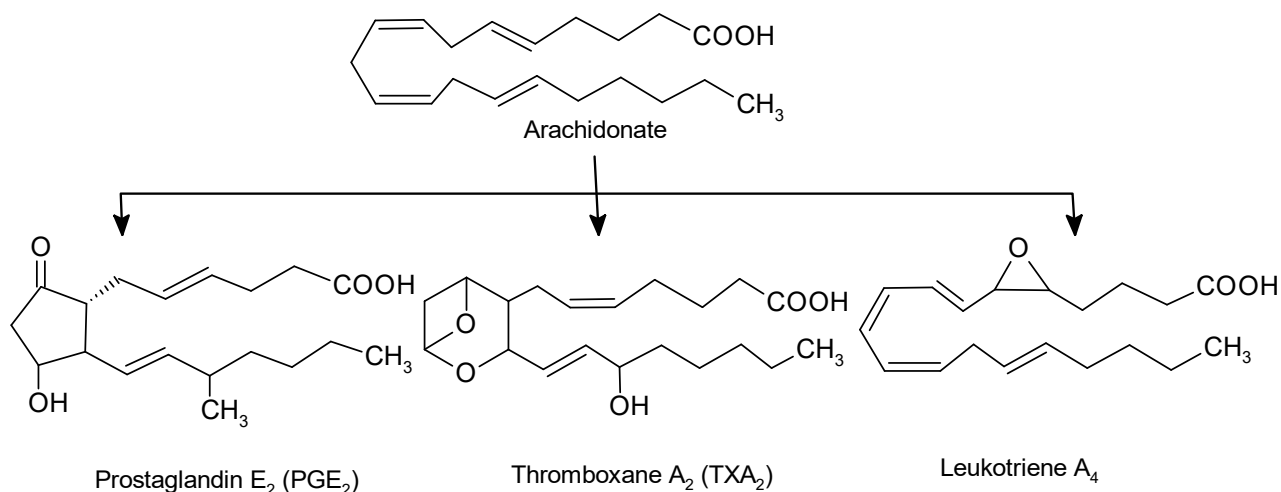
Name the steroid hormone which is responsible for the retention of water in our body.

SAQ 6

Draw the structure of three female sex hormones.

11.6 Prostaglandins

Poly unsaturated fatty acids (PUFAs), which we described in earlier unit, are essential for animals and human nutrition, because they are precursors to a class of compounds collectively called **eicosanoids**. These comprise prostaglandins, thromboxanes and leukotrienes. They are derived from arachidonic acid, which contains four double bonds. The structures of these three eicosanoids are depicted below:



Prostaglandins have a cyclopentane ring and are so called because they were first isolated from the secretions of the prostate gland. They exist in all tissues and act as local hormones, and exert important physiological and pharmacological effects. Some important actions of prostaglandins are their effects on blood pressure, blood clotting, gastric secretions, contraction of the uterine muscles and reproduction. Thromboxanes, first discovered in platelets, have a six membered ether ring called the oxane ring, and are derived from prostaglandins. They have potent effects on arterial walls of blood vessels and are implicated in several cardiovascular disorders. Leukotrienes, the third group of eicosanoids, first found in leukocytes, are characterised by the presence of three conjugated double bonds. Leukotrienes cause vascular permeability. They attract and activate leukocytes and are important regulators of many diseases involving inflammatory reactions, such as asthma.

For their work on prostaglandins, **Sune Bergström**, **Bengt Ingemar Samuelsson**, and **John Robert Vane** shared the 1982 Nobel Prize in medicine.

In the preceding section you learnt about the various types of lipids. Now we shall discuss the role of some of the lipids in the formation of biomembranes. You will recall from Unit 1 that membranes control the chemical environment of the space they enclose, by keeping out certain compounds and selectively transporting other molecules through the membrane. The chemical composition of the membranes allows cell to cell recognition. In addition, membranes contain receptors for many hormones. Let us now learn more about membranes.

SAQ 7

Match the lipid functions in I with appropriate lipids in II.

- | I | II |
|--------------------------|--------------------|
| a) Protection | a') Isoprenoids |
| b) Muscle contraction | b') Leukotrienes |
| c) Precursor | c') Prostaglandins |
| d) Inflammatory response | d') Waxes |

11.7 STRUCTURE OF BIOMEMBRANES

You will recall from Unit 1 that all living cells are enveloped by a surface plasma membrane, composed primarily of lipids and proteins. Because of its diverse functions, the plasma membrane plays a central role in cellular biology. Even cellular organelles, such as mitochondria, nucleus, lysosomes and chloroplasts are bound by membranes. The membranes thus give individuality to cells separating them from the environment and permitting compartmentalisation of cellular functions. You will learn about the composition, structure and functions of membranes in the next subsections.

11.7.1 Composition of Biomembranes

As we mentioned earlier, membranes are mainly composed of lipids and proteins. Although most membranes contain approximately equal amounts of proteins and lipids, their ratio varies depending upon the tissue or organelle. Protein and lipid composition of several membranes is given in Table 11.2.

Table 11.2 : Lipid and protein composition of some membranes

Membranes	% Dry weight	
	Protein	Lipid
Myelin	18	79
Human RBC	49	43
Retinal rods	51	49
Mitochondria (Outer)	52	48
Mitochondria (inner)	76	24
Gram positive bacteria	75	25

The erythrocyte and retinal rod membranes have a protein to lipid ratio of 1:1, while in the myelin membrane of nerve cells it is 1:4, and the inner mitochondrial membrane has a ratio of 3:1. Phospholipids are the most abundant lipid material in membranes, and in addition small amounts of cholesterol and glycolipids are also present. The predominant phospholipids of membranes are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin.

Cholesterol is found exclusively in eukaryotic membranes and is absent in bacterial membranes. In contrast to the relatively few kinds of lipids in membranes, many different kinds of proteins are present. These proteins are mainly involved in various cellular functions, such as, enzymic, transport, receptor and other specialised functions.

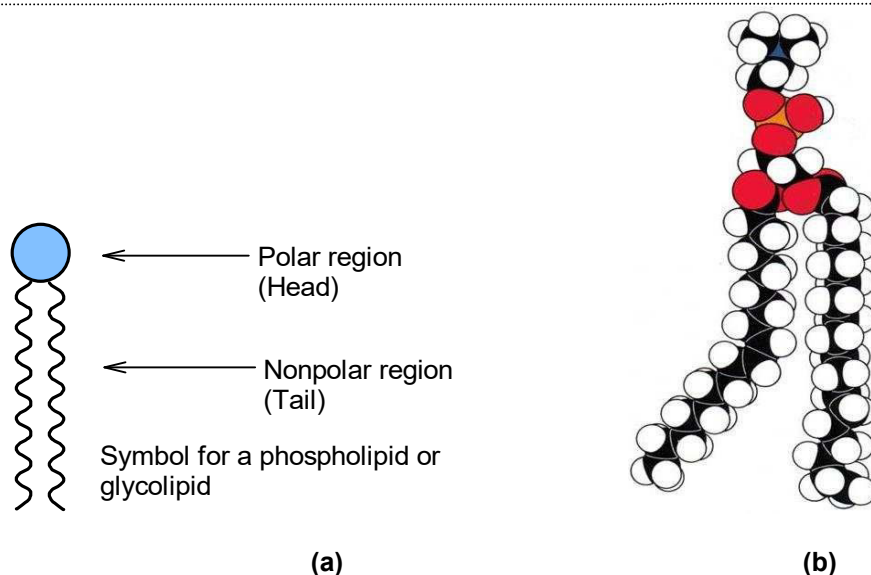


Fig. 11.3: (a) symbolic representation of phospholipids; (b) Space filling model of a phosphatidyl choline molecule.

11.7.2 Membrane Structure

We have briefly described the membrane structure in Unit 1. In this subsection you will learn some more details about membrane structure. The structure of the membrane is intimately associated with the amphipathic nature of phospholipids, which constitute the major component of the membrane. The amphipathic nature of the membrane lipids makes them organise themselves into stable structures at a water phase.

The polar head groups and nonpolar tails may be represented as shown in Fig.11.3 above, and because of this character they organise themselves into unique assemblies as illustrated in Fig. 11.4.

A micelle is a structure wherein the hydrophobic regions are shielded from water, while the hydrophilic polar groups are exposed to the aqueous environment. The phospholipids more often form a “lipid bilayer”, and satisfy the thermodynamic requirements of amphipathic molecules in an aqueous environment.

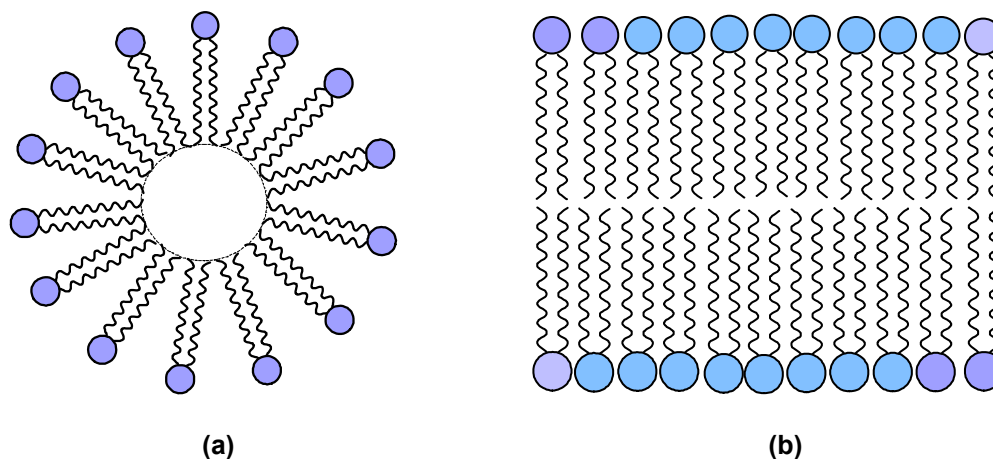


Fig. 11.4 : (a) A phospholipid micelle and (b) a phospholipid bilayer seen in cross section. Phospholipid molecules form such structures spontaneously in water.

The lipid bilayer, recognised nearly 60 years ago by Gorter and Grendel, exists as a sheet in which hydrophobic region of the phospholipids are protected from the aqueous environment, while the hydrophilic regions are exposed to water. The lipid bilayer provides one of the essential properties to the membrane, in that it acts as a barrier to the entry of water soluble molecules, since they are insoluble in the hydrophobic core of the bilayer. You will recall from Unit 1 that one of the most widely accepted model of the membrane is the one proposed by S.J. Singer and G.L. Nicolson in 1972 called the “**fluid mosaic model**”. According to this model, the membranes are a two-dimensional oriented solution of integral proteins (or lipoproteins) in the viscous phospholipid bilayer solven. The membrane phospholipids are arranged in a bilayer in which proteins are dispersed. Fig. 11.5 shows a diagrammatic representation of the model of a membrane.

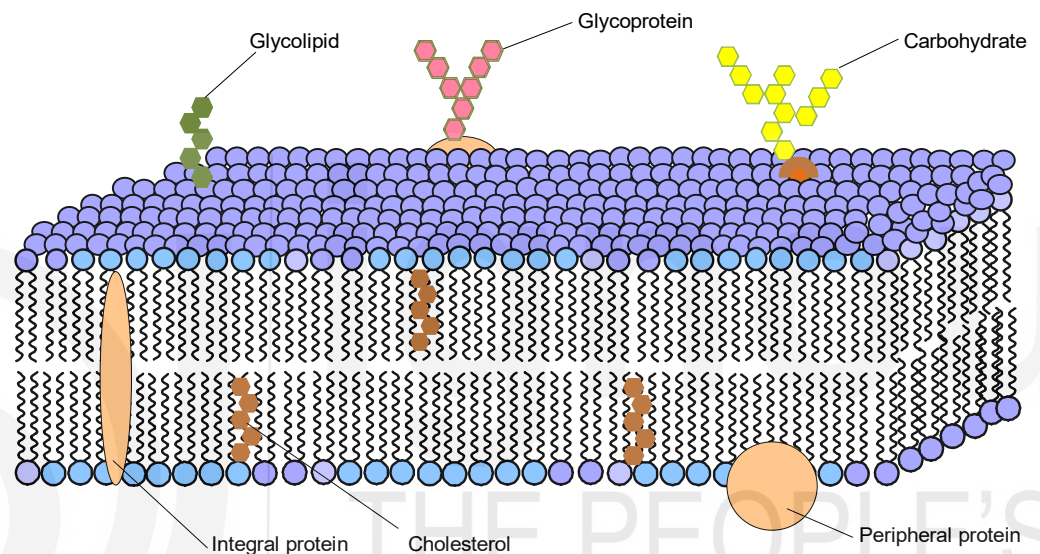


Fig. 3.5 : Fluid mosaic model of biomembranes

The membrane is a quasi fluid structure and the different components are held merely by noncovalent interactions. The lipid and protein molecules are free to perform lateral movements within the overall bilayer. The fluidity of the membrane is regulated by the cholesterol content, and the unsaturated fatty acid content of the phospholipids. The unsaturated fatty acid residues disrupt the highly ordered packing of fatty acid chains, and thus increase fluidity. This is illustrated in Fig. 3.6 in the space filling models of C_{18} saturated and unsaturated fatty acids.

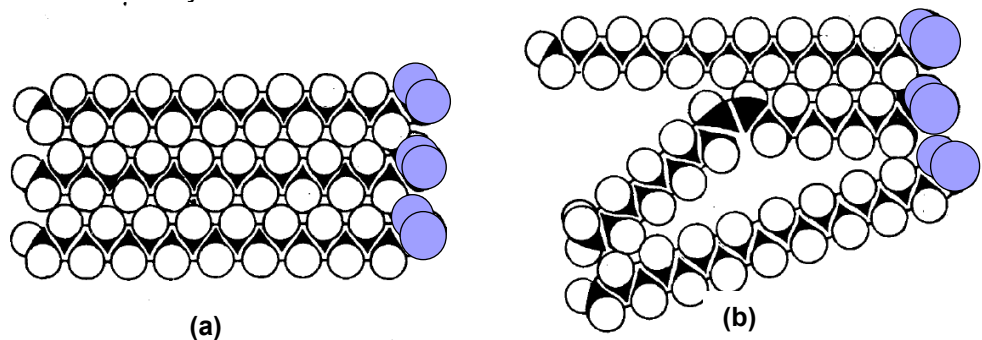


Fig. 3.6 : The highly ordered packing of fatty acid chains is disrupted by The presence of cis double bonds. These space filling models show the packing of (a) three molecules of stearate (C_{18} , saturated) and (b) a

molecule of oleate (C₁₈, unsaturated) between two molecules of stearate.

11.7.3 Functions of Biomembranes

While the cell membrane is responsible for many of the functions associated with the cell, some of the main and distinguishing functions are as follows:

- a) The cell membrane forms a selective barrier between cells and their environment, and regulates the entry and exit of substances across the cell membrane. Thus, it is responsible for the maintenance of the internal environment, whose composition is strikingly different from that of outside. This is accomplished by the presence of specific transport protein and transport channels that act as selective gates for the entry and exit of molecules. While some substances move by passive diffusion, other molecules like glucose, are transported by facilitated diffusion. These mechanisms do not require energy. Some substances e.g., most of the biomolecules are transported against a concentration gradient by active transport, a process that needs energy, as in the absorption of amino acids across the intestines.
- b) The cell membrane is also responsible for cell-cell communication and recognition. The cell surface proteins function as receptors recognising specific molecules such as hormone and other second messengers.
- c) Membranes also carry out specialised functions, such as membranes of the mitochondria and the chloroplast, which act as energy transducers. Retina rod cell membrane proteins, for instance, act as photoreceptors.

SAQ 8

Make appropriate corrections in the following statements and write the correct versions:

- a) Proteins exhibit free mobility in the lipid bilayer.
 - b) Lipid bilayers and micelles are formed by free fatty acids in water.
 - c) Membranes possess specific pores for transport of molecules.
 - d) Cholesterol is exclusively found in bacterial membranes.
-

11.8 SUMMARY

- Phospholipids are lipids that contain a phosphate group. They are differing from triacylglycerols in as the terminal OH group of glycerol is esterified with phosphoric acid instead of a fatty acid.
- Phospholipids form membranes by arranging themselves in a **lipid** bilayer. Sphingolipids contain sphingosine instead of glycerol.
- Lipids are transported in plasma as lipoproteins, which are protein lipid complexes. Lipoproteins vary in their size and composition, and are classified into different types. Defects in the synthesis and utilisation of

various lipoproteins lead to disorders, such as atherosclerosis and hypo or hyper lipoproteinemias.

- Hormones are chemical messengers. Many hormones are **steroids**. All steroids contain a tetracyclic ring system.
- The most abundant member of the steroid family in animals is cholesterol, it is an important precursor to various physiologically important biomolecules, such as vitamins and hormones.
- Phospholipids are the most important lipid constituents of biomembranes and their amphipathic character contributes to most of the properties of the membranes. The protein component of the membranes gives them their individuality. They are important in recognition mechanisms and carry out specific transport functions. Phospholipids are especially abundant in nervous tissue, such as the brain.

11.9 TERMINAL QUESTIONS

1. Name the different type of lipoproteins found in plasma. What is their role?
2. What are eicosanoids and what are their physiological activities?
3. What are the differences between a sphingolipid and a phospholipid?
4. What is the fluid mosaic model and how does it explain the membrane permeability?
5. What are the characteristics that affect membrane fluidity?
6. Name some important functions of membrane proteins.

11.10 ANSWERS

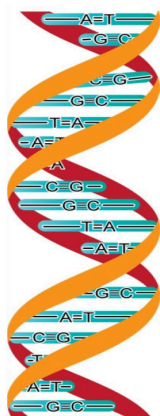
Self-Assessment Questions

1. On hydrolysis of sphingomyelin, a fatty acid, phosphoric acid, choline and sphingosine are formed.
2. a) Phosphatidic acids + choline, (b) Phosphatidic acids + ethanolamine, (c) Phosphatidic acids + inositol, (d) Phosphatidic acids + serine, (e) Sphingosine + phosphate group + choline.
3. See Sec. 11.4.
4. d)
5. Aldosterone
6. See Sec. 11.5.
7. a) d'; b) c' c) a' d) b'

8. a) Proteins exhibit lateral mobility in the lipid bilayer.
- b) Lipid bilayers and micelles are formed by phospholipids.
- c) Membranes possess specific carrier proteins for transport of molecules.
- d) Cholesterol is exclusively found in eukaryotic membranes.

Terminal Questions

1. 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.
2. 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.
3. Sphingolipids are also phospholipids, except that the alcohol in the former is the C₁₈ 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.
4. 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.
5. Membrane fluidity is influenced by the lipid composition, primarily by cholesterol and unsaturated fatty acid content of the membrane. 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.
6. Membrane proteins could be both peripheral and integral. These proteins perform several functions such as, enzymic receptor, transport, cell communication and energy transduction.



UNIT 12

NUCLEIC ACIDS |

Structure

12.1	Introduction	DNA Denaturation
	Expected Learning Outcomes	DNA Polymorphism
12.2	Biological Role of Nucleic Acids	12.6 Ribonucleic Acids (RNA)
12.3	Components of Nucleic Acids	Ribosomal RNA (rRNA)
	Nucleosides and Nucleotides	Messenger RNA (mRNA)
		Transfer RNA (tRNA)
12.4	Formation and Representation of Polynucleotides	12.7 The Genetic Code
12.5	Deoxyribonucleic Acids (DNA)	12.8 Summary
	What Holds the Double Helix Together?	12.9 Terminal Questions
		12.10 Answers

12.1 INTRODUCTION

In Unit 10 and 12, 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 12, 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 acid and the helical structure of **deoxyribonucleic acid** (DNA). **Ribonucleic acid** (RNA) plays an important role in the biosynthesis of proteins and polypeptides, and Unit 13 shall deal with the study of replication DNA and transcriptions of RNA.

Expected Learning Outcomes

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;
- ❖ explain the double helical structure of DNA;
- ❖ describe the role of various types of RNAs; and
- ❖ describe genetic code.

12.2 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 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”.

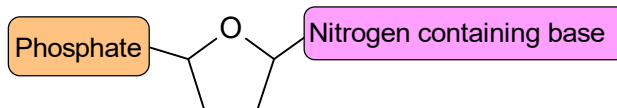
We may also wonder as to how a protein molecule is formed in the organism with the precise amino acid sequence, so that it is physiologically active. Every organism requires proteins and nearly every reaction in a living organism is catalysed by enzymes, which are also proteins. Enzymes are highly specific 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 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.

12.3 COMPONENTS 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 next subsection. We have shown a polynucleotide chain in Fig. 12.1.

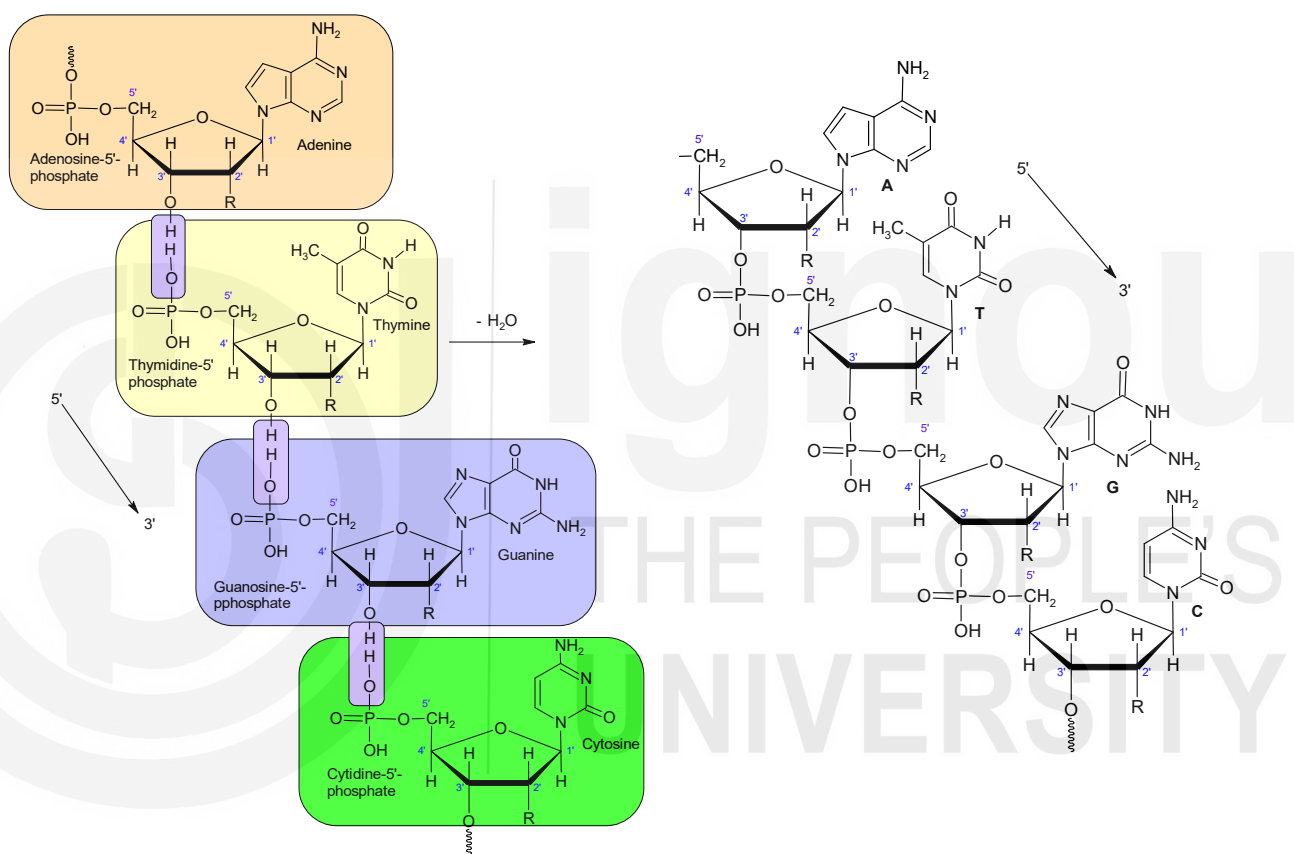
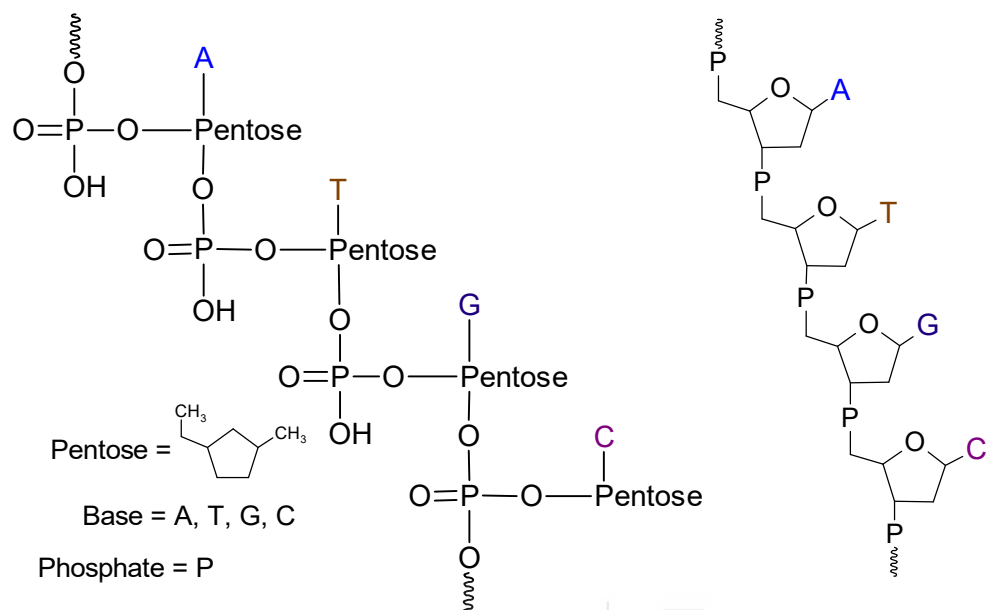


Fig. 12.1 : The relationship of a nucleic acid chain to its nucleotide monomers. On the right is a short section of a DNA strand. On the left its constituent nucleotide monomers. The R on the pentose sugar (at 2' position) represents H for DNA and OH for RNA (assuming that uracil also replaced thymine). The designation 5'→3' means that the complete strand would have a phosphate ester group on C-5' of the 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.

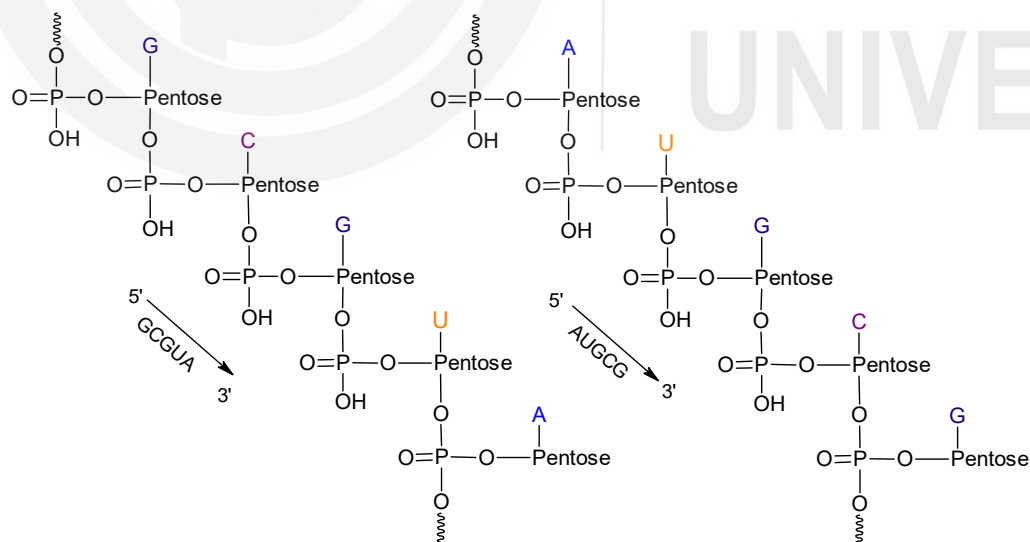
After studying the arrangement of monomer units, i.e., the nucleotides, you would observe that the pattern for the backbone of nucleic acids is, thus, alternating phosphate and sugar units. The sugar in this case is a pentose molecule. It is this pentose sugar which is attached to a different nitrogenous 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:

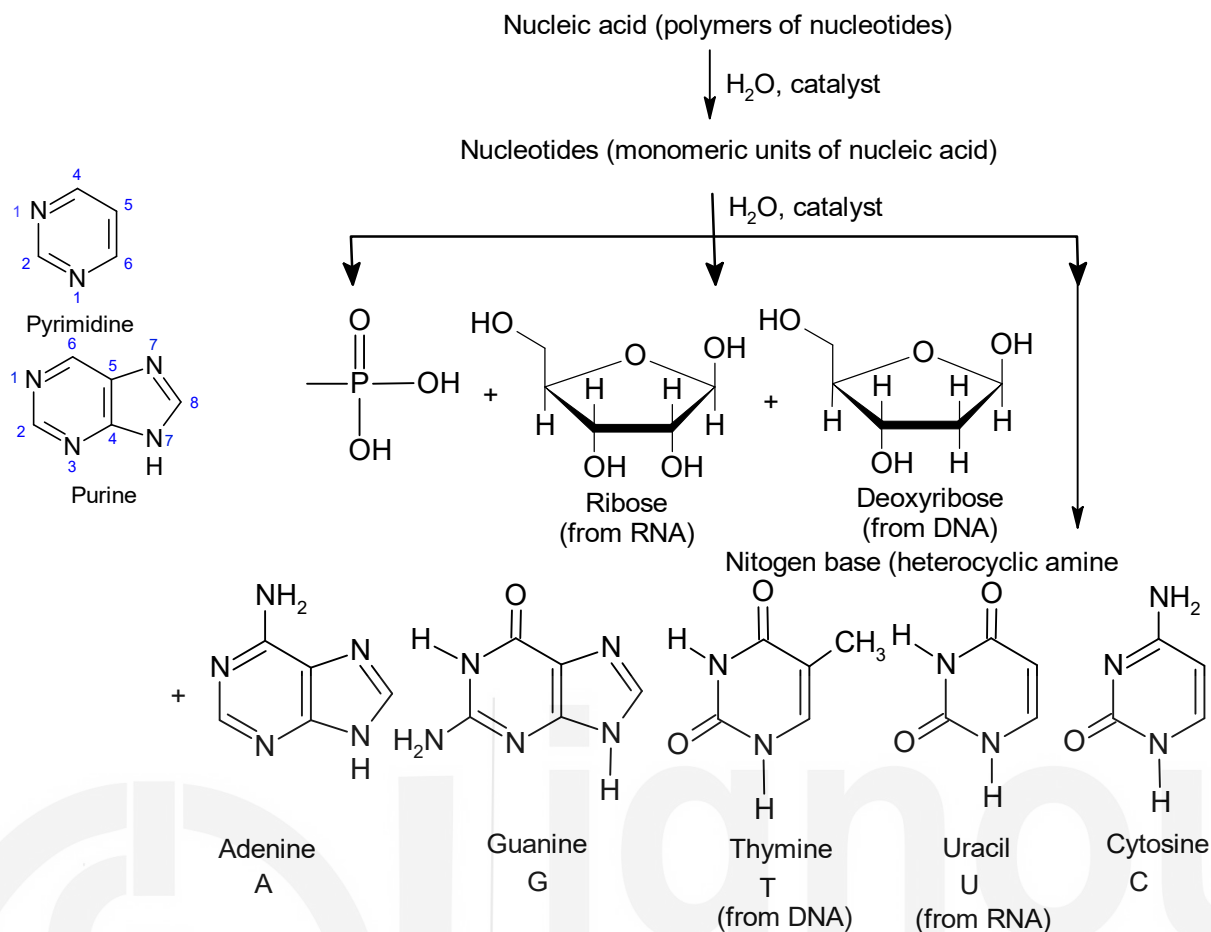


Condensed structures of nucleic acids

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 acids 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. There are of two types, **ribonucleic acids** (RNA) and **deoxyribonucleic acids** (DNA). Both the biopolymers, comprises of repeating monomer nucleotide units. The monomer units in both, RNA and DNA, are composed of a pentose sugar, a nitrogenous base and phosphate. There are five bases: two are substituted purines called purine bases: adenine and guanine, and three are substituted pyrimidines called pyrimidine bases: cytosine thymine and uracil.



As a convention, the carbon atoms of the pentose sugars are numbered with a dash or prime, to distinguish them from the atoms marking of 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 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 12.5 and 12.6. 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 | [] |
| b) take part in ion exchange | [] |
| c) give stability to the cell | [] |
| d) are the genetic material | [] |

12.3.1 Nucleosides and Nucleotides

When a nitrogenous base, whether substituted purine or substituted 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. In table 12.1 we have given base names and their corresponding nucleoside and nucleotides names.

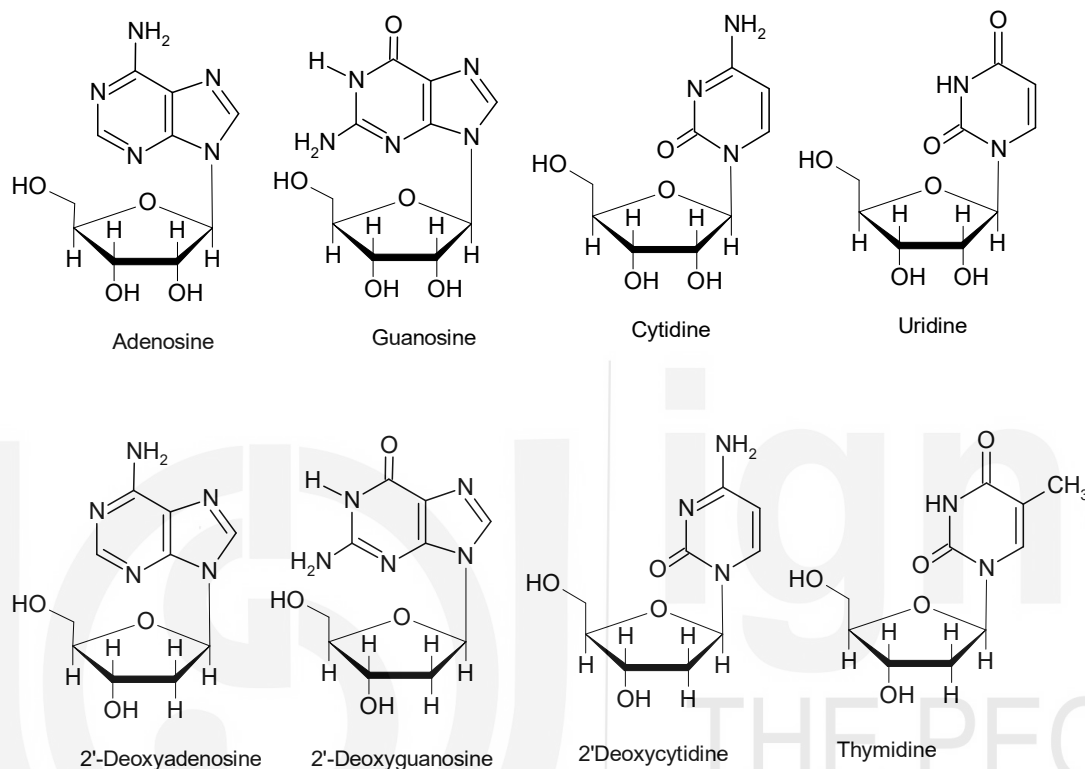
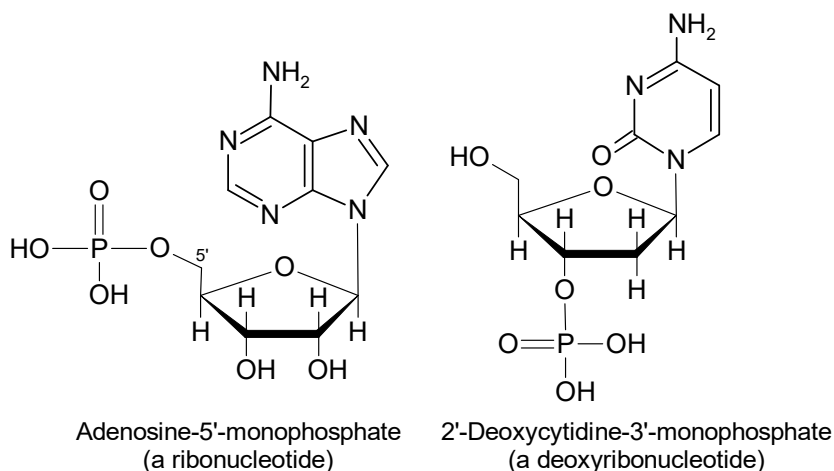


Table 12.1 The names of the bases, the nucleosides, and the nucleotides

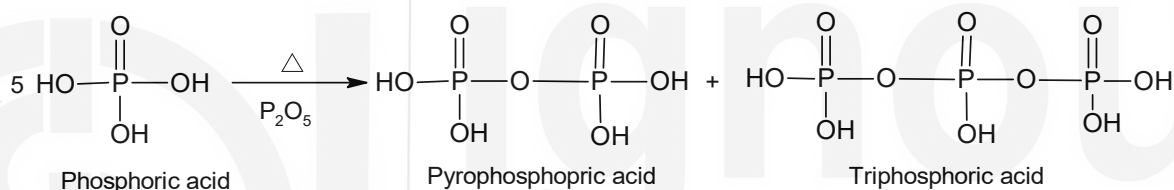
Base	Nucleoside		Ribonucleotide	Deoxyribonucleotide
Adenine	Adenosine	2'-Deoxyadenosine	Adenosine-5'-phosphate	2'-Deoxyadenosine-5'-phosphate
Guanine	Guanosine	2'-Deoxyguanosine	Guanosine-5'-phosphate	2'-Deoxyguanosine-5'-phosphate
Cytosine	Cytidine	2'-Deoxycytidine	Cytidine-5'-phosphate	2'-Deoxycytidine-5'-phosphate
Thymine	—	Thymidine	—	Thymidine-5'-phosphate
Uracil	Uridine	—	Uridine-5'-phosphate	—

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' or 3' positions of the ribose or deoxyribose

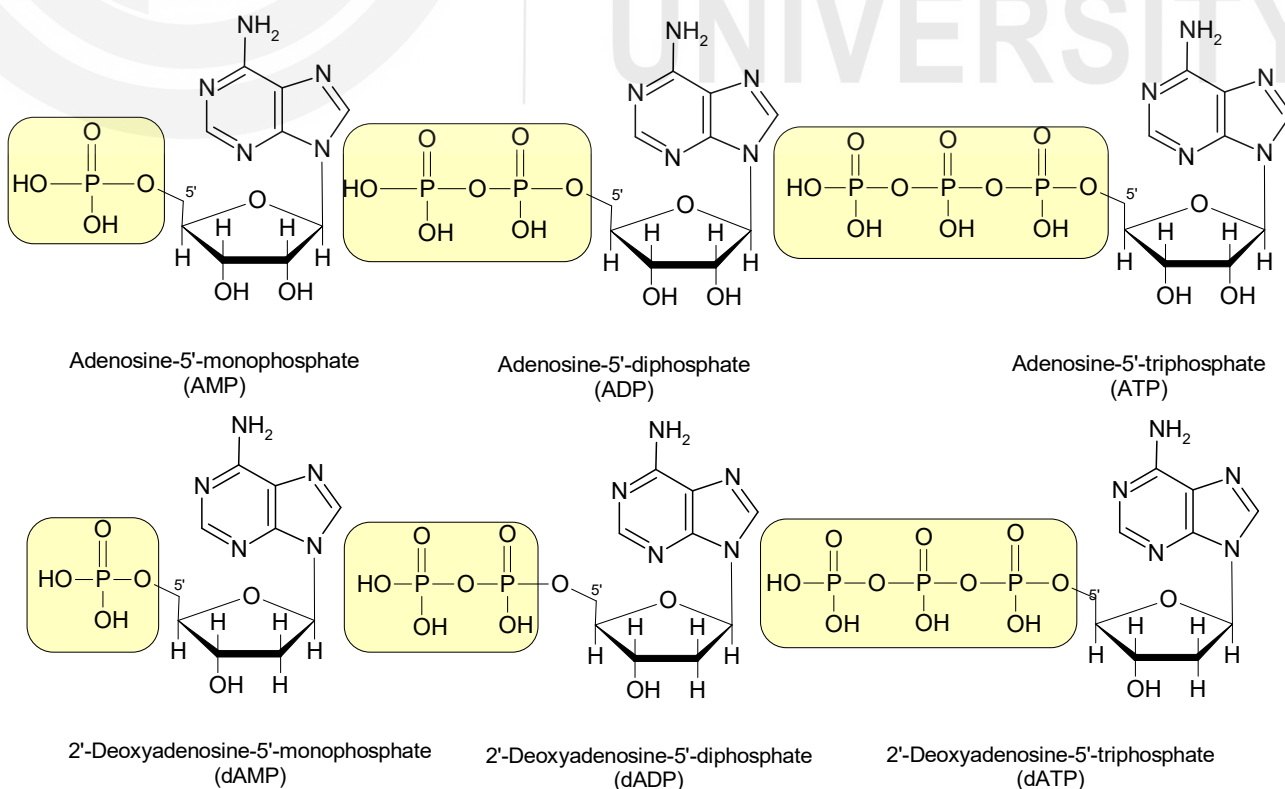
molecule. The nucleotides of RNA—where the sugar is D-ribose—are more precisely called **ribonucleotides**, whereas the nucleotides of DNA—where the sugar is 2'-deoxy-D-ribose—are called **deoxyribonucleotides**.



When phosphoric acid is heated with P_2O_5 it loses water, forming a phosphoanhydride called pyrophosphoric acid. Triphosphoric acid and higher polyphosphoric acids are also formed.



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 triphosphonucleotides. The first phosphate group is attached by an ester linkage but the next two phosphate groups are attached by phosphoanhydride (i.e. pyrophosphate) linkage.



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. As mentioned earlier, the attachment of phosphoric acid to the pentose of the nucleoside may be at 3' and 5' in the deoxyribose molecule and it may be at 2', 3', or 5' in the ribose molecules. 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.

Ribose sugar of RNA has hydroxyl group at 2' position. As a consequence, in addition to 3' and 5' phosphates it can also form 2' phosphate.

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.

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 | [] |

SAQ 3

Draw the structure for each of the following:

- UDP
- GMP
- TTP
- Cytidine-5'-phosphate

After describing the nucleotides, let us now explain how polynucleotides are formed.

11.4 FORMATION AND REPRESENTATION OF POLYNUCLEOTIDES

Polynucleotides (**Nucleic acids**) are composed of long strands of nucleotide subunits linked by phosphodiester bonds. These linkages join the group of one nucleotide to the group of the next nucleotide. Such linkages are shown in Fig. 12.1. In the formation of nucleic acids nucleotide triphosphates are the starting materials. DNA is synthesized by enzymes called **DNA polymerases**, and RNA is synthesized by enzymes called **RNA polymerases**.

The nucleotide strand is formed as a result of nucleophilic attack by a 3' OH group of one nucleotide triphosphate on the phosphorus group of another nucleotide triphosphate, breaking a phosphoanhydride bond and eliminating pyrophosphate. The product formed in this step undergoes another nucleophilic substitution reaction with second molecule of nucleotide triphosphate and finally after multiple steps a polynucleotide is formed. A formation of polynucleotide is illustrated in Fig. 12.2.

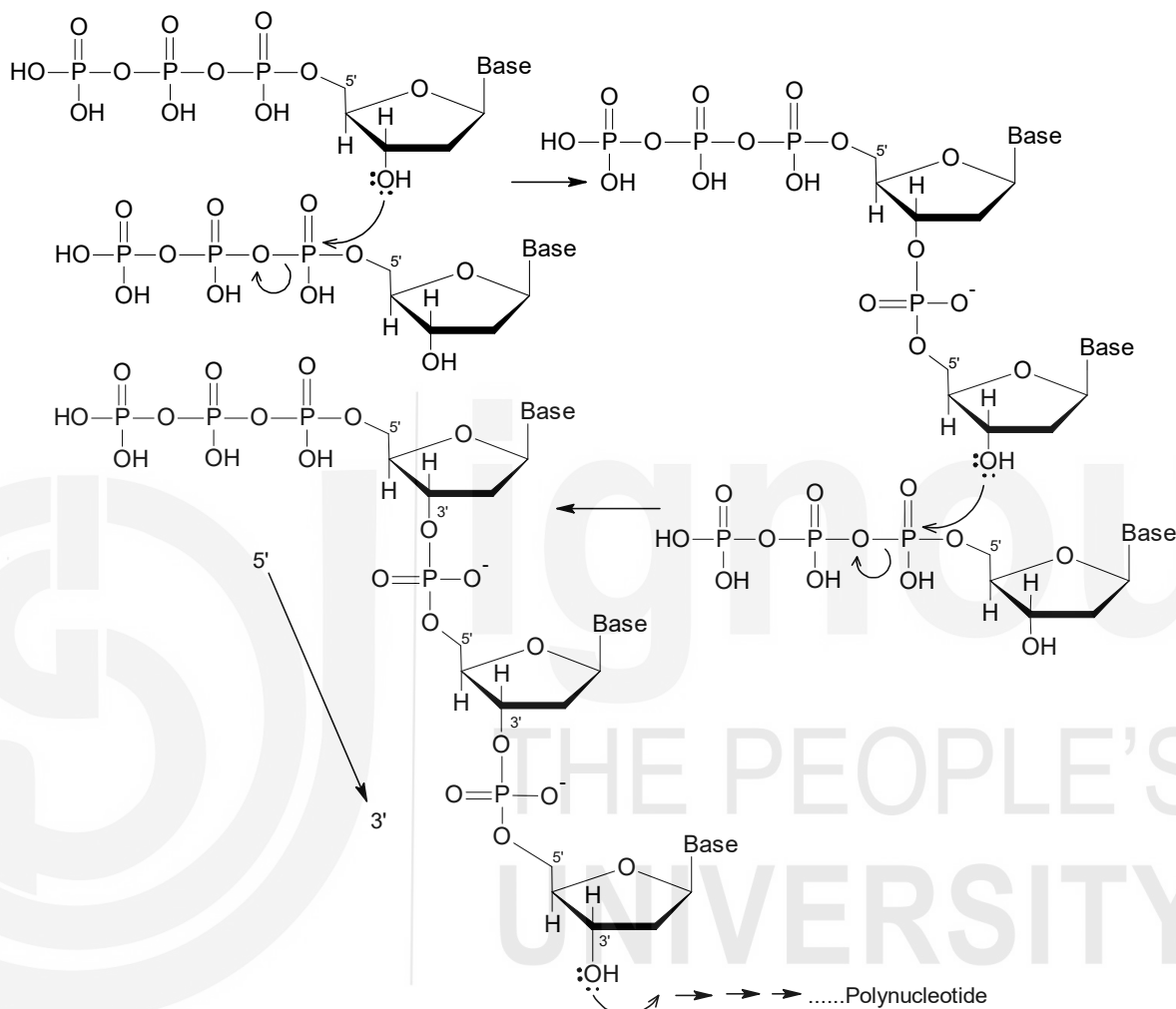


Fig. 12.2: Formation of polynucleotide

As illustrate earlier in Fig. 12.1 and also in Fig. 12.2, the growing polymers are synthesized in the 5'→3' direction. RNA strands are also formed in the same way, using ribonucleotides instead of deoxyribonucleotides. The **primary structure** of a nucleic acid is the sequence of bases in the strand. After describing the nucleotides and formation of polynucleotides in the preceding section, let us now explain in detail about the types of nucleic acids, namely DNA and RNA.

12.5 DEOXYRIBONUCLEIC ACIDS (DNA)

As mentioned earlier, DNA is the polymer of deoxyribonucleotides, with a structure similar to that of RNA, except that the pentose unit is 2'-deoxyribose (2'-deoxyfuranoribose) as compared to the ribose (furanoribose) in RNAs, and the four nitrogenous bases are adenine (A),

thymine (T), guanine (G) and cytosine (C). 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 **Watson & Crick 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. 12.3b).

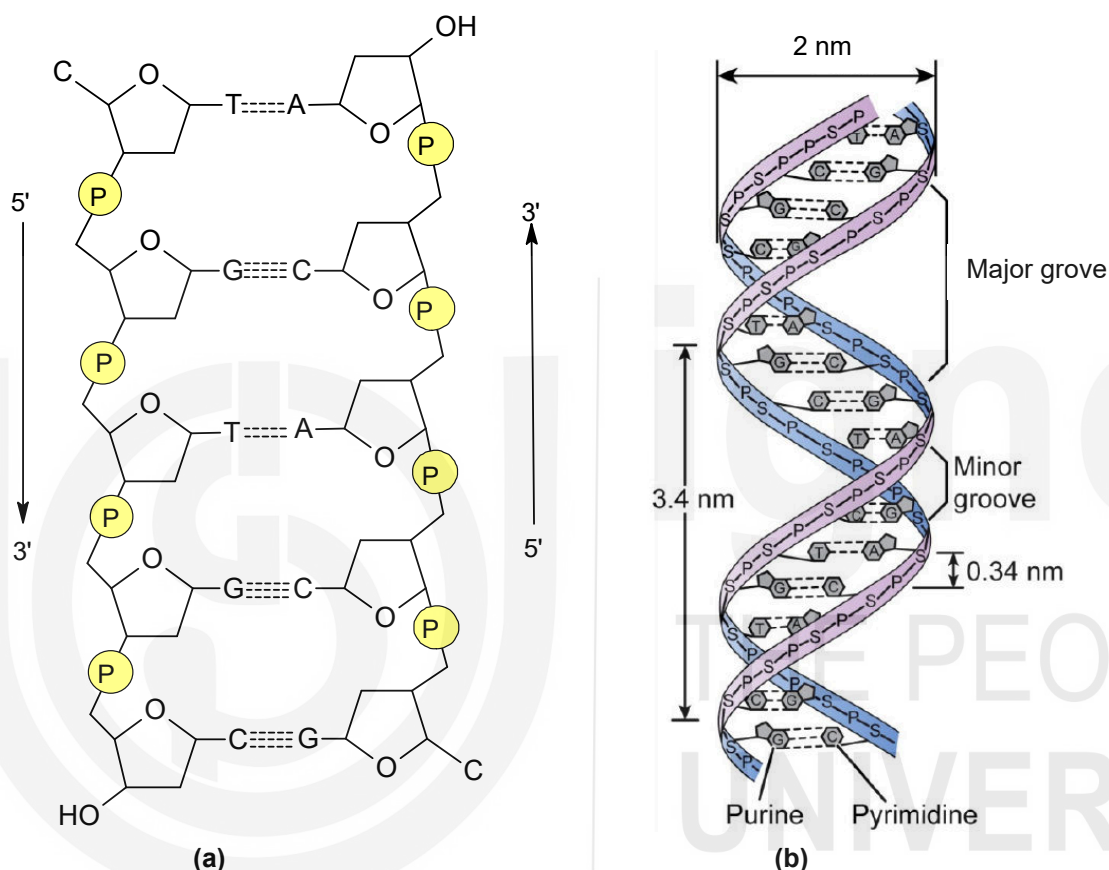


Fig. 12.3: (a) Complementary base pairing in DNA double helix. Adenine always pairs with thymine; guanine always pairs with cytosine; (b) A schematic representation of the DNA double helix. The two spiralling strands coiled around a common axis are held together by hydrogen bonds between bases on the opposite strands. Here P represent phosphate and S represent sugar

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 centred around the long axis. The idea of complementary 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. 12.4a and 12.4b):

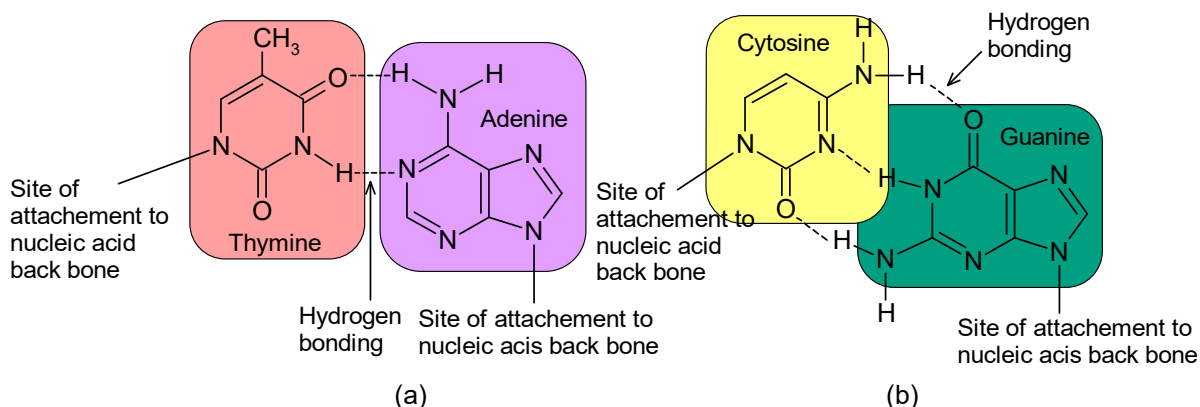


Fig. 12.4: Hydrogen bonding between base pairs. (a) Thymine (T) and adenine (A) form one base pair with two hydrogen bonds; (b) Cytosine (C) and guanine (G) form another base pair with three hydrogen bonds. Adenine can also pair to uracil (U) in a similar manner as to thymine in RNA

The diameter of the helix is 1.8 nm (or 18 Å). Adjacent bases are separated by 0.34 nm (3.4 Å) along the helix and rotated by 36° . Thus the helical structure is repeated after ten nucleotide residues on each chain, or every 3.4 nm (34 Å). 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 predicated 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.

Table 12.1: 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 base 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 humidity (92%) and solutions of low ionic strength.

C-form contains less number of bases 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 per turn, 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 number of base pairs per turn. It possess 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. 12.5).

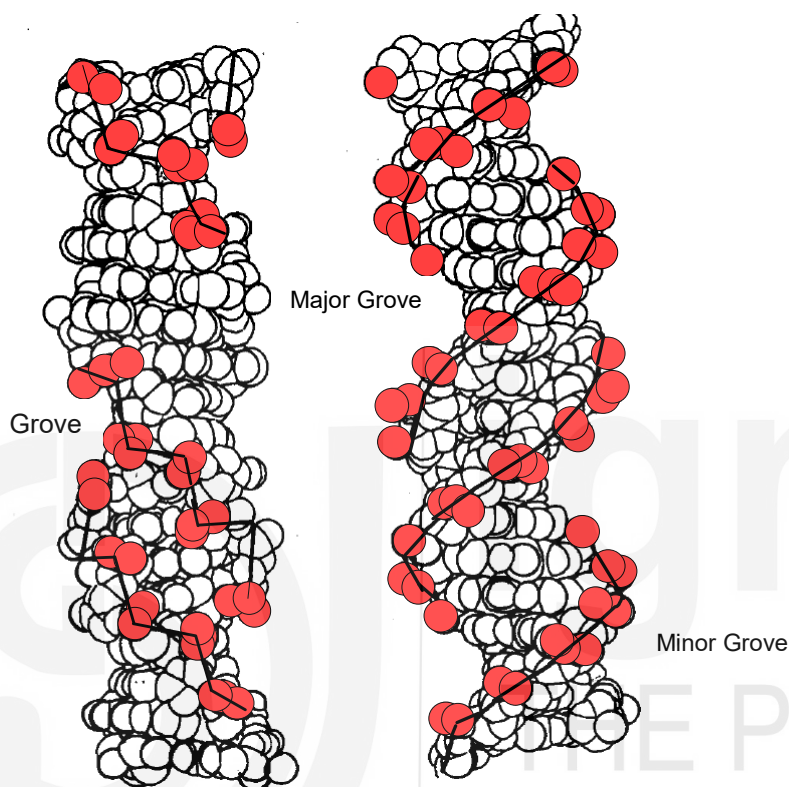


Fig. 12.5: B-DNA and Z-DNA present different double helical structures for DNA.

The double helix has two grooves, one major and one minor (Fig. 12.3) and 12.5). 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 sequence, 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 super helices 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 1 m end to end.

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

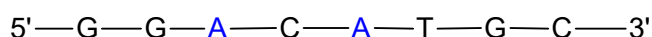
SAQ 4

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

SAQ 5

What is the sequence of bases in the complementary strand if in a DNA strand has the following sequence of bases running in the 5'→3' direction?



12.5.1 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 sequence 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 11) and for imparting unique structures to proteins (Unit 6). Therefore, it is more common to refer to them as “stacking interaction”.

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.

12.5.2 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. 12.6. The mid-point of the temperature range over which the change takes place is called the **melting temperature**, denoted by T_m .

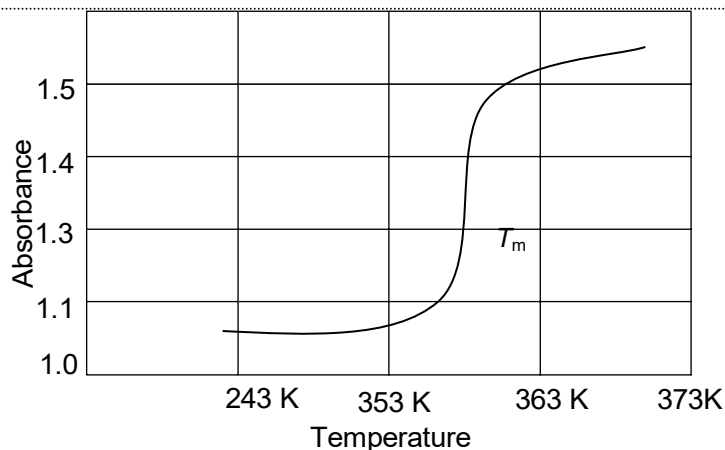


Fig. 12.6 : The denaturation of DNA, which is followed by an increase in optical density, is described by T_m

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. 12.7). Very high pH also sometimes causes denaturation.

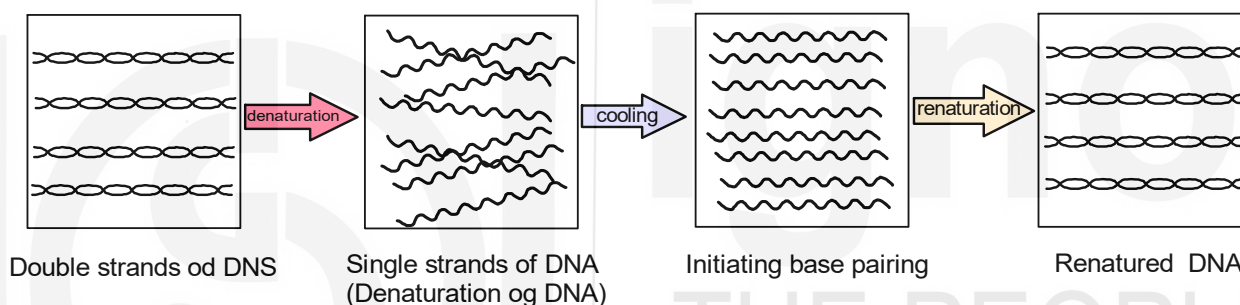


Fig. 12.7: Denatured single strands of DNA renature to the stranded form.

12.5.3 DNA Polymorphism

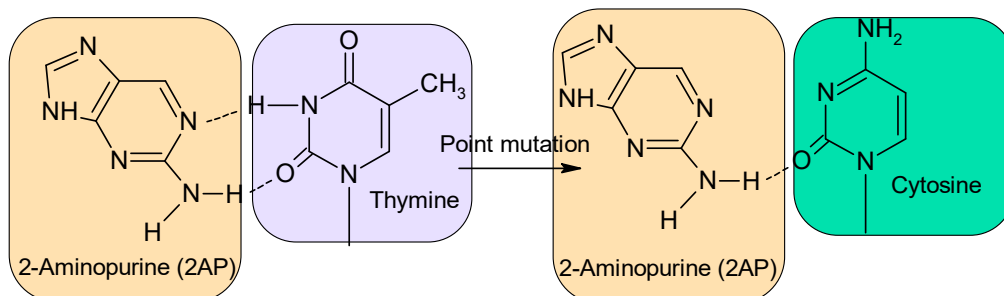
Most of the time, DNA accurately passes the same information from one generation to the next. However, sequences of nucleotides in DNA can also change. Changes in sequences of nucleotide in DNA are called DNA variants. Mutation and polymorphism are two terms used to describe DNA variants. DNA variants can occur due to errors in DNA replication or external factors such as UV and chemicals. A mutation refers to a DNA variant in a particular individual whereas polymorphism refers to DNA variants within a population.

The main difference between mutation and polymorphism is that mutation is a change of a nucleotide sequence of a DNA strand of a particular organism whereas polymorphism is a mutation that occurs in a population with a frequency of 1% or higher. Mutations can be either hereditary or acquired. Let us discuss both these terms in some more detail.

Mutation

Mutations usually occur from unrepaired DNA damage, replication errors, or mobile genetic elements. There are several major classes of DNA mutations. A point mutation occurs when a single nucleotide is added, deleted or substituted (see the example of base pairing by adenine analog 2-aminopurine). Along with point mutations, the whole structure of a

chromosome can be altered, with chromosomal regions being flipped, deleted, duplicated, or translocated such mutations are called as chromosomal mutations. Mutations can be inherited from parents (germline mutations) or acquired over the life of an individual (somatic mutations), the latter being due to arise by environmental factors such as UV and chemicals. Germline mutations occur in the gametes. Since the offspring is initially derived from the fusion of an egg and a sperm, germline mutations of parents may also be found in each nucleated cell of their progeny.

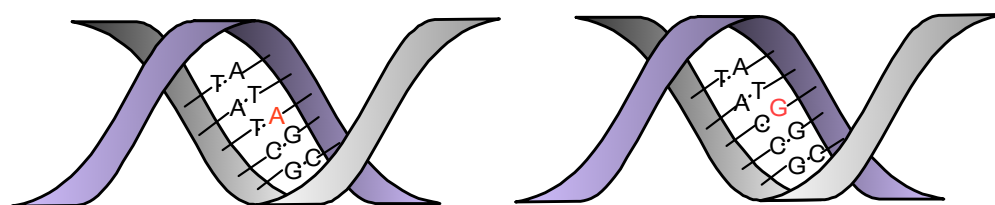


Base pairing by the adenine analog 2-aminopurine: Thymine base is replaced by cytosine base

Many common human diseases are due to mutations. Cancer is the most common human genetic disease; it is caused by mutations occurring in a number of growth-controlling genes. Sometimes cancer-causing genes can exist at birth, increasing a person's chance of getting cancer.

Polymorphism

A variation in the DNA sequence that occurs in a population with a frequency of 1 % or higher is termed a polymorphism. The higher incidence in the population suggests that a polymorphism is naturally occurring, with either a neutral or beneficial effect. Polymorphisms can also be of one or more nucleotide changes, just like mutations. In Fig. 12.8 we have shown an example of a Single Nucleotide Polymorphisms (SNP). In this case single nucleotide is substituted by a different nucleotide. Polymorphisms are common enough to be considered a normal variation in the DNA. Polymorphisms are responsible for many of the normal differences between people such as eye colour, hair colour, and blood type. Although many polymorphisms have no negative effects on a person's health, some of these variations may influence the risk of developing certain disorders.



SNP variation A, G

Fig. 12.8: SNP in DNA strand.

DNA polymorphisms particularly SNPs have become a powerful tool in reconstructing human origins, evolution and prehistoric migrations. Earlier we used to depend on archaeological and paleontological evidences to reconstruct human evolution. In the absence of these traditional evidences DNA analysis became an alternative tool to reconstruct human evolution.

Mutations and polymorphisms are two types of DNA variants. The main difference between mutation and polymorphism is the frequency of occurrence of each type of variants within the population. This knowledge of DNA variants can be exploited for human benefit. 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 directly or indirectly caused 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 genetic disease 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 was initiated in 1990. It has continued to provide a wealth of information which is useful in planning gene therapy and treating the genetic disorders.

It has now been established that the genetic information is carried in the form of specific sequences of bases (**codon**) in DNA. Different segments of DNA function as genes, i.e. code for specific character.

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 genetically 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.

Thus, the 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.

SAQ 6

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) Formation of DNA is catalysed by DNA polymerase [True/False]
 - d) G:C and A:T ratios are unity in DNA [True/False]
-

Let us now describe the RNA molecule in detail.

12.6 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. 12.9).

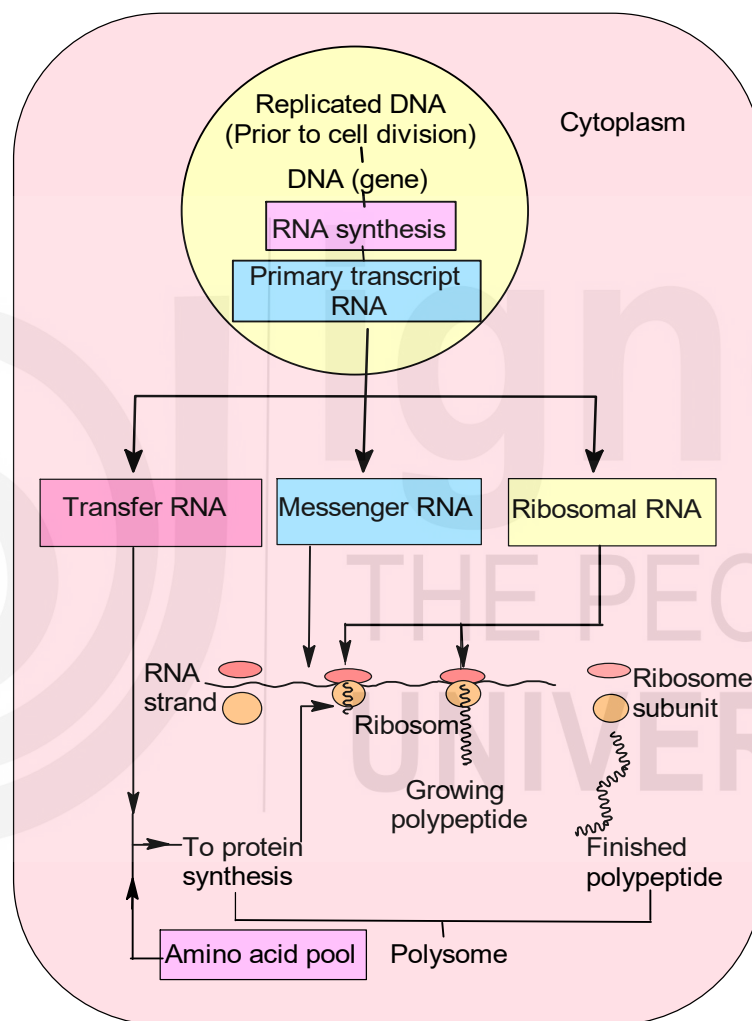


Fig. 12.9: Diagrammatic representation of the relationship of nuclear DNA to different RNAs, and to the synthesis of polypeptides

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 60% 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 strands. 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 tRNA also). The collective result of the hydrogen bonding and the single stranded nature of rRNA give it a peculiar/irregular three-dimensional shape. 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. In spite 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 known, they are sites of polypeptides 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 attribute 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 discuss this in Unit 13.

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



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

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. There are two important regions with complementary base pairing. All tRNAs have a CCA sequence at the 3' end. The three bases at the bottom of the loop are called an **anticodon** (Fig. 12.10). During protein synthesis, tRNA can carry amino acids bound as an ester to its terminal 3' OH group. Each tRNA can carry only one particular amino acid. A tRNA that carries alanine is designated as tRNA^{Ala}.

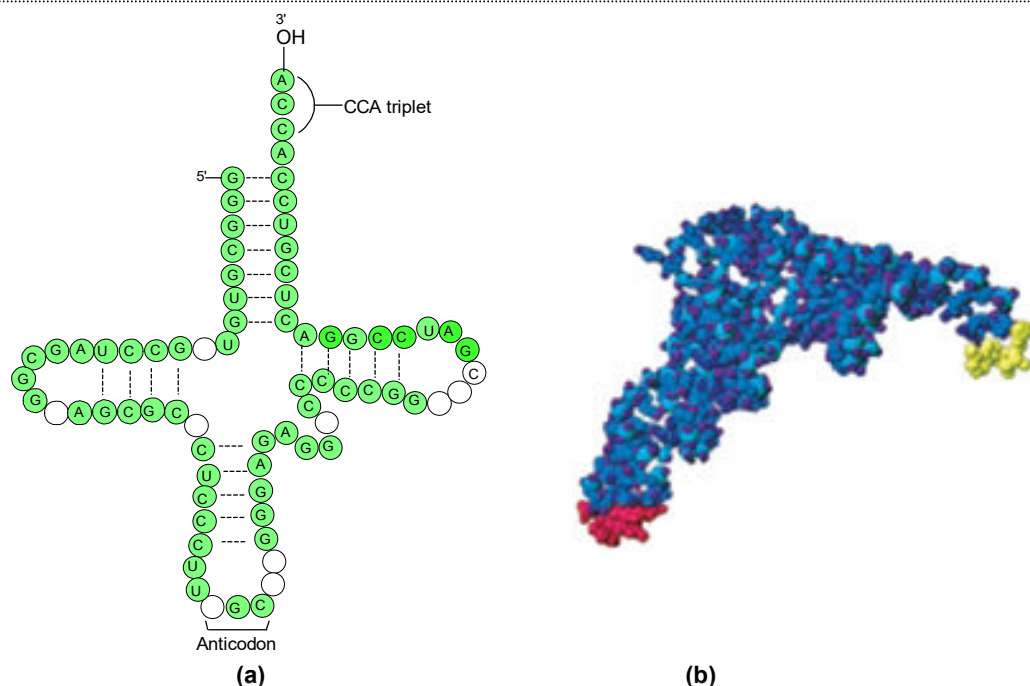
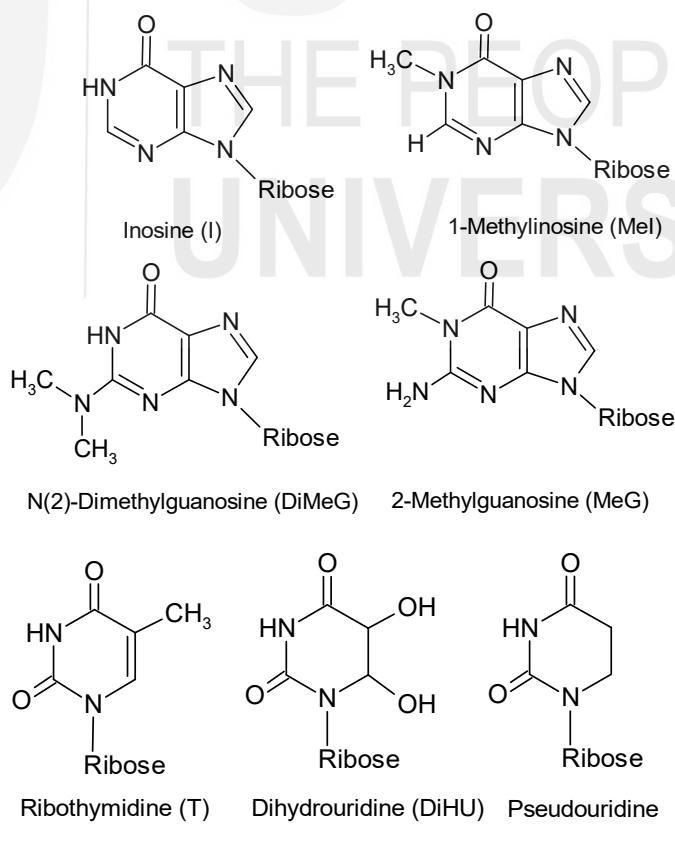


Fig. 12.10 : Transfer RNA (tRNA) for alanine (a) This is a highly schematic representation to highlight the occurrence of double-stranded regions due to extensive base pairing, unusual bases are shown as empty circles; (b) The model of t-RNA: the anticodon is shown in red; the amino acid binding site is shown in yellow.

It was found that the basic three dimensional arrangements are the same for all tRNA molecules. One of the most striking feature of tRNA base sequence is the occurrence of unusual modified bases, i.e. bases other than A,G,C or U. These unusual modified bases are shown below:



Modified bases in tRNA: Structures of some of the modified bases found in tRNA

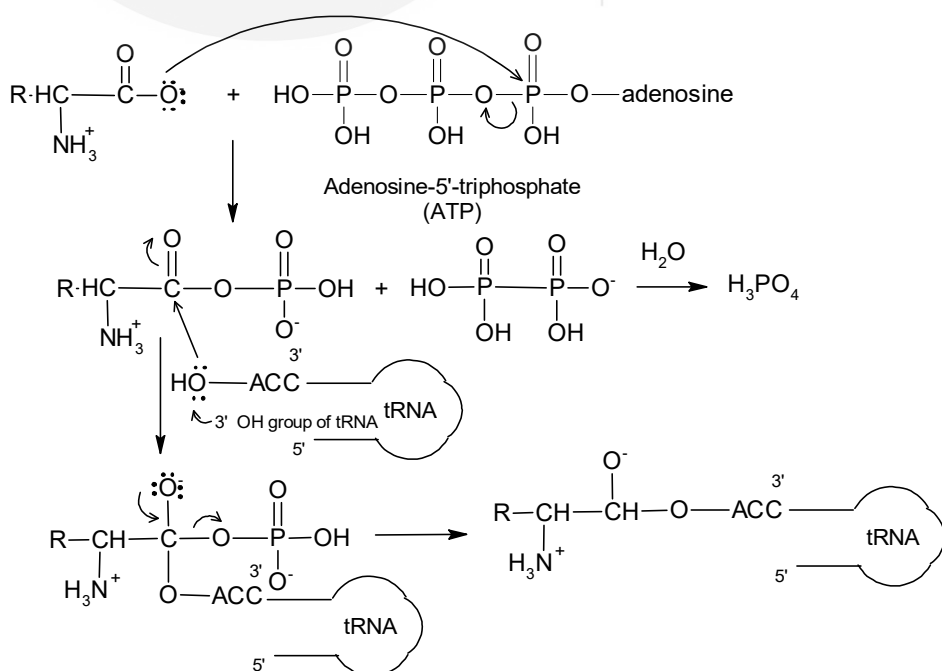
These unusual bases arise from the enzymatic modifications of the existing polynucleotides. The presence of modified nucleosides is important in

stabilising the tRNA structure. 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 interpreter, like the one needed in a talk between two persons, who do not speak the same language. The interpreter 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. 12.10). 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 described 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 understand how amino acids are attached with tRNA.

Mechanism of amino acid attachment to tRNA

Similar to DNA formation process, ATP makes the first move for the attachment of the amino acid to tRNA. This process is catalysed by an enzyme called aminoacyl-tRNA synthetase. In the first step, the carboxyl group of the amino acid attacks the ATP and activates the carboxyl group by forming an acyl adenylate. In the second step, the carbonyl group of the acyladenylate undergoes nucleophilic attack with the 3' OH group of tRNA, forming a tetrahedral intermediate. Finally, aminoacyl tRNA is formed when AMP is expelled from the tetrahedral intermediate. All the steps take place at the active site of the enzyme. Each amino acid has its own aminoacyl tRNA synthetase. Each synthetase has two specific binding sites, one for the amino acid and one for the tRNA that carries that amino acid. The proposed mechanism of this whole process is shown below:



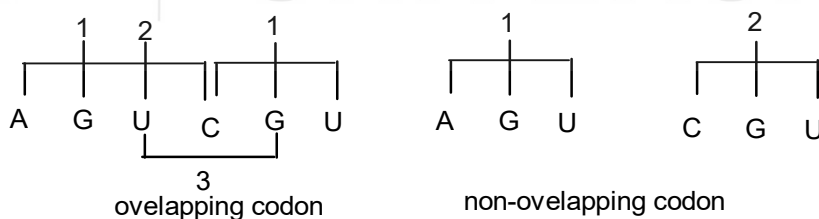
12.7 GENETIC CODE

We have been using the terms codons, anticodons, and codes in this unit and in next unit which is on replication, transcription and translation. It is now appropriate to define these terms more precisely. The genetic material is the DNA molecule (except in a few RNA viruses, where RNA is the genetic material). It has now been established that the genetic information is carried in the form of specific sequence of bases (**codon**) in DNA. Different segments of DNA function as genes, i.e. code for specific proteins by specifying the sequence of amino acids. The relationship between the sequence of bases in DNA, or its RNA-transcript (i.e. messenger RNA), and the sequence of amino acids in proteins specified by them is called the **genetic code**.

As you know already, proteins contain 20 different amino acids which are to be coded for by the four bases present in a DNA molecule. It is obvious that if a single base is to code for one amino acid, then only four amino acids can be coded for. Simple arithmetic shows that if a combination of two bases coded for one amino acid, the maximum number of such pairs is only sixteen (i.e. 4^2). This is not adequate explanation for the twenty amino acids found in the proteins. Therefore, it was realised that minimum code size would be triplet of bases. Number of different triplets made from four bases is sixty four (i.e. 4^3), which are sufficient to code all the amino acids. Later on by genetic experiments, it was confirmed that indeed the **genetic code is triplet of bases: three adjacent nucleotides specify an amino acid**. The first codon to be identified was UUU which codes for phenylalanine.

Each amino acid is usually represented by a three-letter abbreviation
Phenylalanine = Phe
Leucine = Leu
Methionine = Met:
etc.

The genetic codes possess certain characteristics such as non-overlapping and degeneracy. By **non-overlapping** we meant that any base can be part of one triplet (codon) and is not used in another codon. Thus, each base is “read” only once.



Degeneracy refers to one amino acid being coded for by more than one triplet. Only methionine and tryptophan are coded for by a single triplet each which are, AUG and UGG, respectively. For all other amino acids, there are more than one triplet codons. It may be stressed, however, that any triplet codes for a single amino acid only.

Furthermore, a given triplet codes for the same amino acid in all the species. The only exceptions are in the protein biosynthesis in mitochondria where small differences are observed. Thus, the code is not universal but applicable to almost all species. It is referred to as the “standard” genetic code and is shown in Table 12.3.

Table 12.3: The “Standard” Genetic Code; Codon Assignments

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

CT * indicates a signal codon for chain-termination.

AUG functions as a signal for chain initiation or starting point for transition.

Note that the codes are given for messenger RNA. In DNA U will be replaced by T.

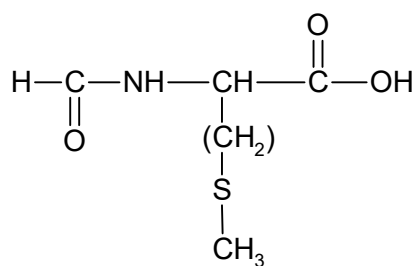
Today we know all codes for all amino acids (Table 12.3). Out of the ($4^3 = 6^4$) possible triplets from our bases, only 61 triplets have been known to code for specific amino acids. The other three, namely, UAA, UAG and UGA, do not code for any amino acids. These function as chain terminator or stop signals. The triplet AUG (coding for methionine) also serves as the initiator codon, i.e. for starting the synthesis of a new polypeptide. Let us discuss these codons in some details.

Messenger RNA Contain Start and Stop Signal for Protein Synthesis

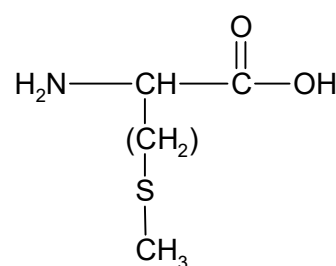
Initiation Codons

mRNA is generally polycistronic, i.e. it carries message for the synthesis of several different protein. It is, therefore, necessary that it should have “start” and “stop” signals, so that message or information for each protein is demarcated and separated from that for another proteins. As shown in

Table 12.3, such signals do exist in the genetic code. The triplet AUG functions both as the codon for methionine, when it occurs in the interior of the message, and also for chain initiation when it occurs in the beginning of the message. In the latter case, it codes for N-formylmethionine and not for free methionine.



N-Formylmethionine (FMet)



Methionine (Met)

There are two types of tRNAs for methionine, namely tRNA^{FMet} and tRNA^{Met}. They have the same anticodon but are specific for formyl-methionine and methionine, respectively. They combine with mRNA at AUG codon at the start and in the interior of the message respectively. Because of this, each nascent polypeptide chain has a methionine residue at the N-terminal. In some cases, the triplet GUG functions as the start signal. In eukaryotes, the initiating methionine is not formylated.

Termination Codons

Three triplets do not code for any amino acid. These are UAA, UAG and UGA. They are called **termination** or **release** or **nonsense codons**. Three codons are not read by any aminoacyl-tRNA (aa-tRNA) but instead are recognized at the appropriate stage (termination) in translation by specific proteins called release factors, consequently a completed polypeptide is released. It has been observed that in some cases, the release codon works singly whereas in others two successive stop codons do the job of chain termination (Fig. 12.11).

Human haemoglobin chain mRNA 5' AAAUACCGU**UAA**AGCU....

E. Coli lactose repressors mRNA 5'GAAAGCGGCGAG**UGA**CCGCAAGGCCAAUUA..

MS2 "A" protein mRNA 5'CGGCUCUCUAGAU**UAG**AGCCCUCAA....

MS2 Coat protein mRNA UCCGGCAUCUAC**UAAUAG**ACGCCG...

Fig. 12.11: The bold triplet indicates the termination codons.

SAQ 7

Which of the following is not true of the genetic code?

- a) There are three nucleotides per codon
- b) Each codon codes for only one kind of amino acid
- c) The codons are carried on mRNA
- d) There is just one codon for each type of amino acid.

In next unit you will learn how 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 process called **transcription** which is similar to DNA replication, this will also be covered in that unit. Protein synthesis in detail has also been dealt in Unit 13.

12.8 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 queries 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'→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 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 molecules 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 DNA 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.
- 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' →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 codons from DNA in the cell nucleus to the site of polypeptides synthesis. The tRNA 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 combat these disease.
- The genetic code is inscribed on the mRNA in form of triplet of bases. Each code is unique and codes for one amino acid only. The genetic code is degenerate and non-overlapping. There is a codon which initiate the polypeptide synthesis process (AUG). Other than the initiation codon, there are three other codons UAA, UAG and UGA. They are often referred to as nonsense or release or termination codons.
- Amino acid does not have affinity towards the RNA and are required to be activated. The activation is achieved by the amino acid with the specific tRNA. The process is catalysed by a set of enzymes, very specific and remarkably efficient, called aminoacyl synthetase. The energy for the process of activation is provided by ATP.

12.9 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.
6. What is the significance of genetic code?
7. Explain briefly the terms codons and anticodons.
8. Illustrate the importance of initiation and termination codons.

4.10 ANSWERS

Self-Assessment Questions

1. d
2. d
3. Draw the structures as we have shown in Subsec. 12.3.1.

4. five, uracil.
5. Sequence of bases in the complementary strand are in second row.

$$\begin{array}{cccccccc}
 5' & -G- & G- & A- & C- & A- & T- & G- & C- & 3' \\
 3' & -C- & C- & T- & G- & T- & A- & C- & G- & 5'
 \end{array}$$
6. (a) True; (b) True; (c) True; (d) True
7. d) One amino acid may be coded by more than one triplet of bases.

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, in turn, joined by 5' 3' phosphodiester links into a polynucleotide chain. The nitrogenous base 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 present at 3'/5' positions.
3. The pentose sugar in RNA is ribose, whereas in DNA it is deoxyribose. Besides, RNA do not have thymine and DNAs do not have uracil. Further DNA consists of 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 Watson & Crick, 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. rRNA, which are integral parts of ribosomes, combine with mRNA to form the sites of polypeptide synthesis. tRNAs, which happen to be smallest RNA

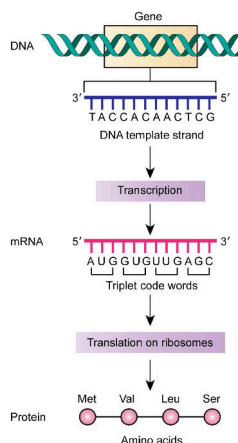
molecule capable of easy movement within the cell, carry amino acid molecules to the ribosome for protein synthesis.

6. See Sec. 12.7
7. Codons: The genetic code is a dictionary that gives the correspondence between a sequence of bases (nucleotide bases) and a sequence of amino acids. The individual words in the code are each composed of three nucleotide bases. These genetic words are called codons.

Anticodons: The selection of a particular amino acid from the pool of cellular amino acids is detected by set of three bases in the tRNA which are called anticodons and are complementary to the codons on the mRNA.

8. Refer to Sec. 12.7.





REPLICATION AND TRANSCRIPTION

Structure

13.1	Introduction	13.6	Protein Synthesis: Translation
	Objectives	13.7	Structure and Role of Ribosomes
13.2	DNA-The Genetic Material	13.8	Mechanism of Protein Synthesis
	Experiments of Griffith (1928) and Avery and co-workers (1944)		Activation of Amino Acids
	Hershey –Chase experiment (1952)		Elongation of the Polypeptide Chain
	DNA content of eukaryotic cells		Termination of Polypeptide Chain
13.3	DNA - Replication	13.9	Summary
13.4	RNA Synthesis: Transcription	13.10	Terminal Questions
13.5	Similarities between DNA-Replication and RNA Transcription		

13.1 INTRODUCTION

The structure and chemistry of some bio-molecules described in earlier units, with an introduction of their physiological role. Carbohydrates (other than 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, structural, 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

Information flows from DNA to RNA to protein i.e. DNA directs the synthesis of RNA, which in turn directs the synthesis of proteins. This flow of information is known as **central dogma** of molecular biology (Fig. 13.1).

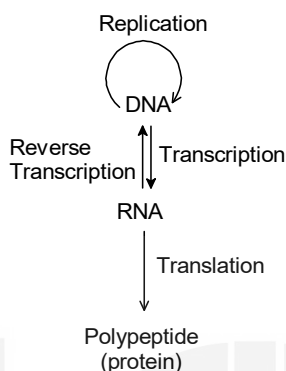


Fig. 13.1: Central dogma of life

transfer of a particular characteristic from parent to the daughter cells, say ability to synthesis a particular protein like insulin. It has since been shown that deoxyribonucleic acid (DNA) and, in a limited number of cases, ribonucleic acid (RNA)] performs the functions attributed to genes. In this unit, we will study the evidence in support of this statement. We have already studied structure of DNA and RNA along with genetic code in Unit 12. In this unit we will learn how DNA (i.e., a collection of genes) is duplicated. This process is being called "Replication". In the living cells the genetic information contained in DNA molecule is first "copied" or "transcribed" in the form of specific RNAs. It is then being translated into the synthesis of various proteins. The process of transcription is very similar to that of replication and, therefore, this will also be described in this unit.

RNAs play very important role in protein synthesis. In Unit 12, we have briefly described how the sequence of nucleotides (codon) in the mRNA is translated into sequence of amino acids in the resulting protein molecules. We also mentioned the role of tRNA and its anticodon in the protein synthesis. The process of protein synthesis is called translation. In this unit we will take up protein synthesis in more detail. Last section of this unit is devoted on protein biosynthesis. You are advised to go through Unit 12 again before preceding on with this unit this will help you to understand concepts discussed in this unit.

Expected Learning Outcomes

After studying this unit and having the experiments performed, 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);
- ❖ describe the synthesis of RNA where DNA is employed as template;
- ❖ explain role of RNAs and ribosomes in protein synthesis; and
- ❖ describe biosynthesis of protein.

13.2 DNA-THE GENETIC MATERIAL

Now the question which generally comes to our minds is what qualifies DNA as the genetic material? Why not protein or lipid or some other biomolecules? DNA is the genetic blueprint of life. It functions as the molecule that carries on the genetic information from one generation to another, let us understand how. Nitrogenous bases linked to sugar phosphate backbone in a sequential manner stores and transfer the genetic information. Bases have a property to form pairs with the help of hydrogen bonds. Because of this **base pairing** nucleic acids tend to form a double stranded helical structure, and also base pairing provides a mechanism for copying this genetic information.

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 seventy five years elapsed between the discovery and isolation of DNA from nuclei of the leucocytes by Friedrich Miescher (1860) 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 Griffith (1928) and Avery and Coworkers (1944)

In the first half of the 20th century, scientists believed that the proteins in chromosomes formed the basis of the genetic information that was passed from generation to generation. For them, DNA was too simple molecule to be able to carry complex information. However, later through experiments scientists discovered that in fact it was DNA, not protein, which carries the genetic information.

Experiment of Frederick Griffith; was the first experiment suggesting that bacteria are capable of transferring genetic information through a process known as transformation. Later Avery and co-worker found that DNA is the material that communicated this genetic information.

In his experiments, Griffith used two strains that are distinguishable by the appearance of their colonies when grown in laboratory cultures. The virulent form (ability to cause disease or death) 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. Griffith's other strain, a mutant nonvirulent type that grows in mice but is not lethal. In this case the polysaccharide coat is absent, giving colonies a rough appearance. 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 animal. On the other hand, injecting heat-killed S' cells and R cells do not produce disease or death. Griffith injected a mixture of live R cells and heat killed S' cells into mice and found that the mice became sick and died (Fig. 13.1).

Moreover, blood of these 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.

Later Avery and coworkers (1944) 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.

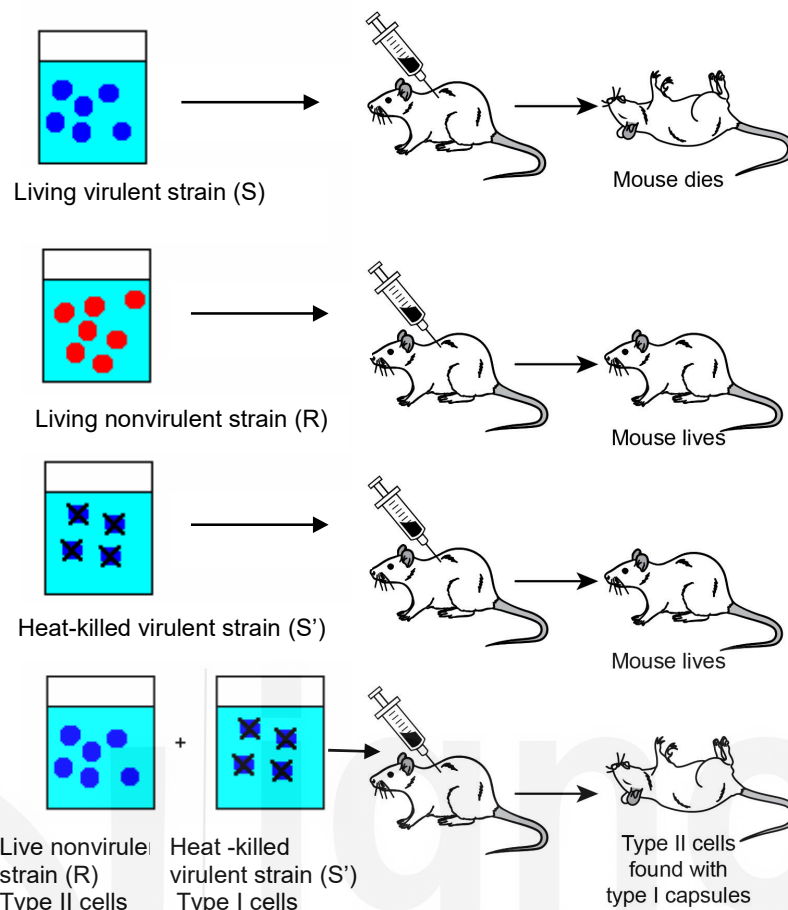


Fig. 13.1: Griffith's experiment demonstrating the effect of rough, smooth, heat-killed (smooth) and mixture of rough and heat-killed smooth strains of *Pneumococci*.

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 heat killed S' cells

13.2.2 Hershey-Chase Experiment (1952)

Alfred Hershey and Martha Chase further supported the findings from the work of Avery and coworkers. Hershey and Chase used a virus called T2 to know if the genetic information was passed on via DNA or proteins. T2 virus can attack bacterial cells to make more copies of itself. Virus which attack bacteria are called bacteria-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 fibers

(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 grew phage particles on *E. Coli* in a medium containing nutrients with radio-active isotopes. ^{32}P and ^{35}S . Note that only nucleic acids contain phosphorus atoms and only proteins contain sulphur-containing amino acids (methionine and cysteine,). 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 progeny. Therefore, DNA must be the genetic material.



Fig. 13.2 : Bacterial viruses or bacteriophages of the type called 'T2 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 fibers which attach to the cell wall of the bacterium.

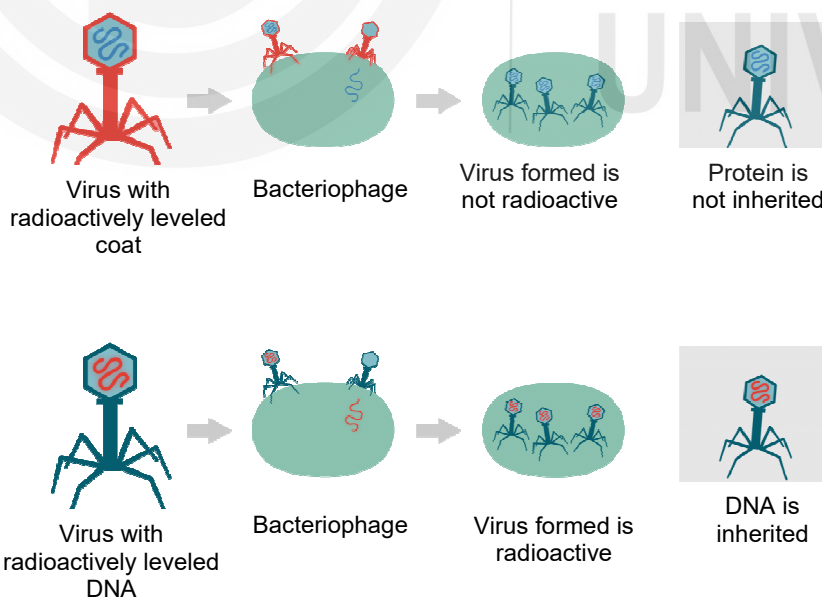


Fig. 13.3: A Schematic representation of the Hershey and Chase experiment.

SAQ 2

What are bacteriophages?

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 makeup, and therefore the hereditary characteristics of each of spring.

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 it may be equally divided between the two daughter cells. A major characteristic of the genetic material is that it must be able to duplicate by the cell machinery. In the next section, we will see how the unique double helical structure of DNA is well suited for duplication or "replication", i.e., producing or causing to produce another replica of itself.

SAQ 3

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

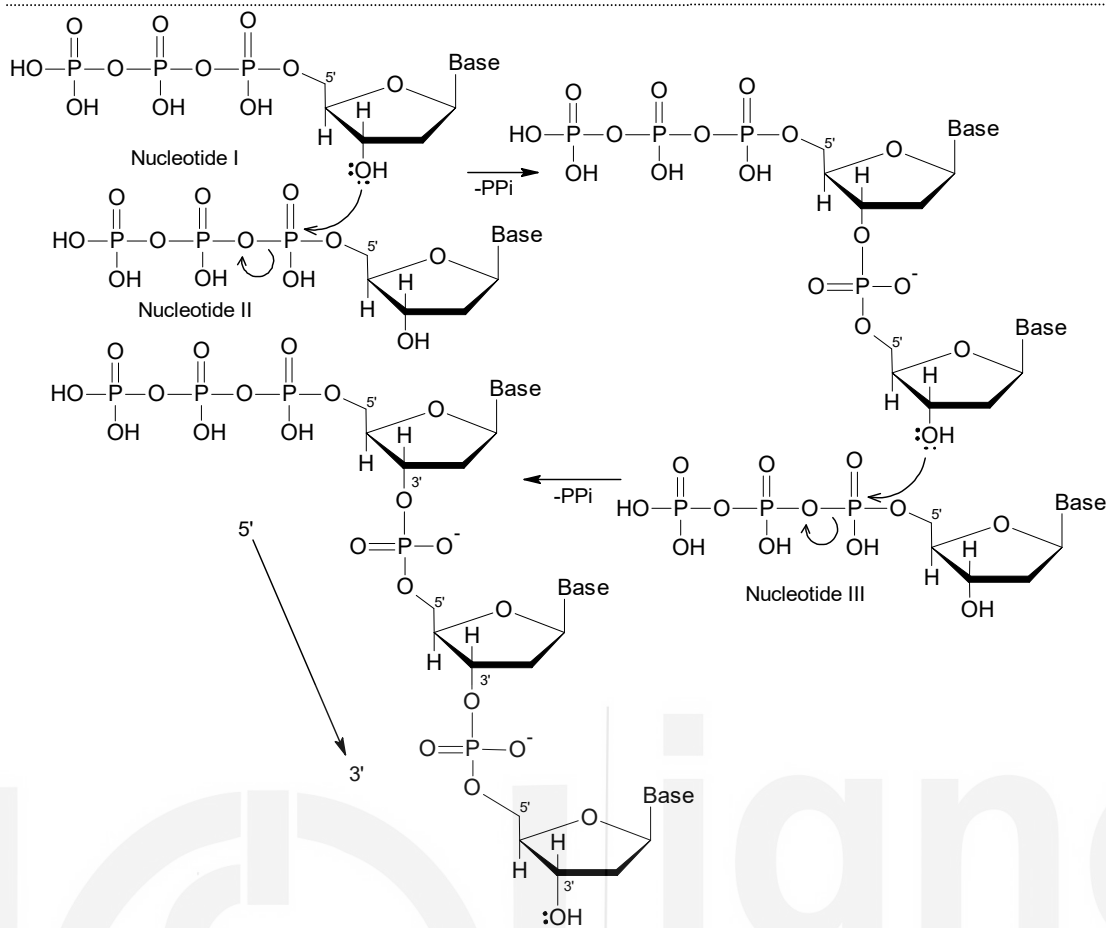
- (a) Somatic cells of eukaryotes has equal amount of DNA as compared to corresponding germ cell. []
- (b) Two germ cells, one from each parent, combine to form a single cell. []
- (c) The characteristics of the genetic material is not to duplicate. []

13.3 DNA REPLICATION

Before going to explore the DNA replication, let us explore the basic principle operating behind its base pairing, which is popularly known as Chargaff's Rule. The two basic rules of base pairing (or nucleotide pairing) are:

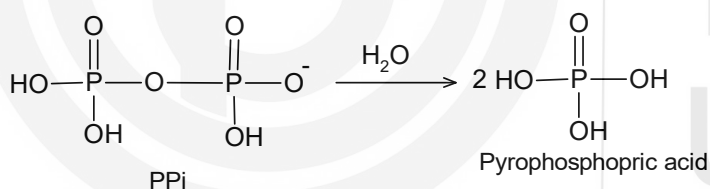
- i. adenine (**A**) with thymine (**T**): the purine adenine always pairs with the pyrimidine thymine through hydrogen bonds
- ii. cytosine (**C**) with guanine (**G**): the pyrimidine cytosine always pairs with the purine guanine through three hydrogen bonds

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

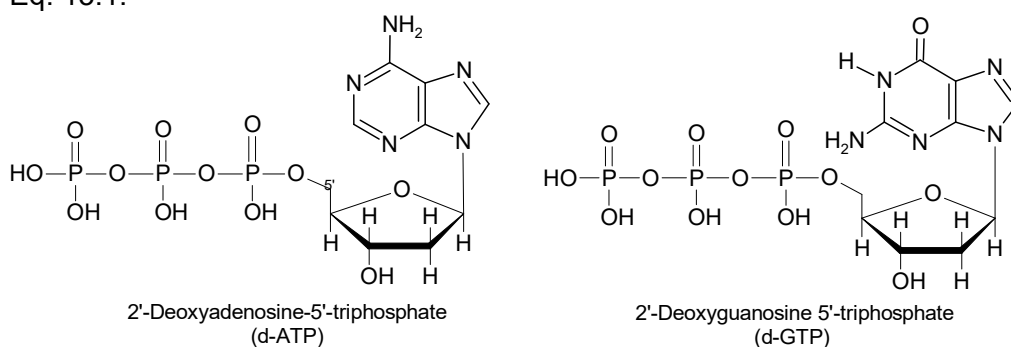


Eq.13.1

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



Since this hydrolysis is practically irreversible, it helps to drive the reaction of Eq. 13.1 to completion. Note that a poly-deoxy nucleotide built by repetition of this reaction will have two distinct ends, namely a 5' end which will be towards the top in the structure depicted in Eq. 13.1 and 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.



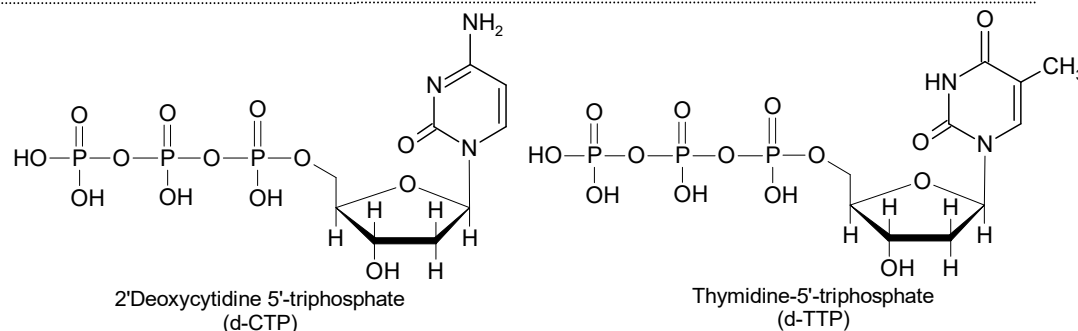


Fig. 13.4: Structures of deoxynucleotide triphosphate precursors of DNA.

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 fidelity 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 12, 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 adenine of one strands lie alongside thymine of the other strand (A-T pair) and guanine and cytosine of different strand lie side by side (G-C pair). Specificity and stability of each bases pair is due to base-base hydrogen bonding (see Unit 12, Section 12.5). Such a structure provides the basis for replication of DNA. Each strand can function as a template for the synthesis of a new strand 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 dotted vertical lines in this figure represent the hydrogen bonds (two in A-----T and three in G-----C pair). If we imaging that the two strand 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 color. The next logical step will be polymerisation of the precursors by a succession of reactions as shown in Eq.13.1, except that nucleotide '1' 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 earlier. 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 polynucleotide chain. This will give rise to two double strands (Fig.13.5(d)) which are identical to each other and similar to the starting parent DNA molecule. Note that this type of replication is "semi-conservative" where each new, daughter, 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.

The selection of the particular precursor is based on the specificity of base pairing as described above. 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 right for the bottom pair of Fig. 13.5(d).

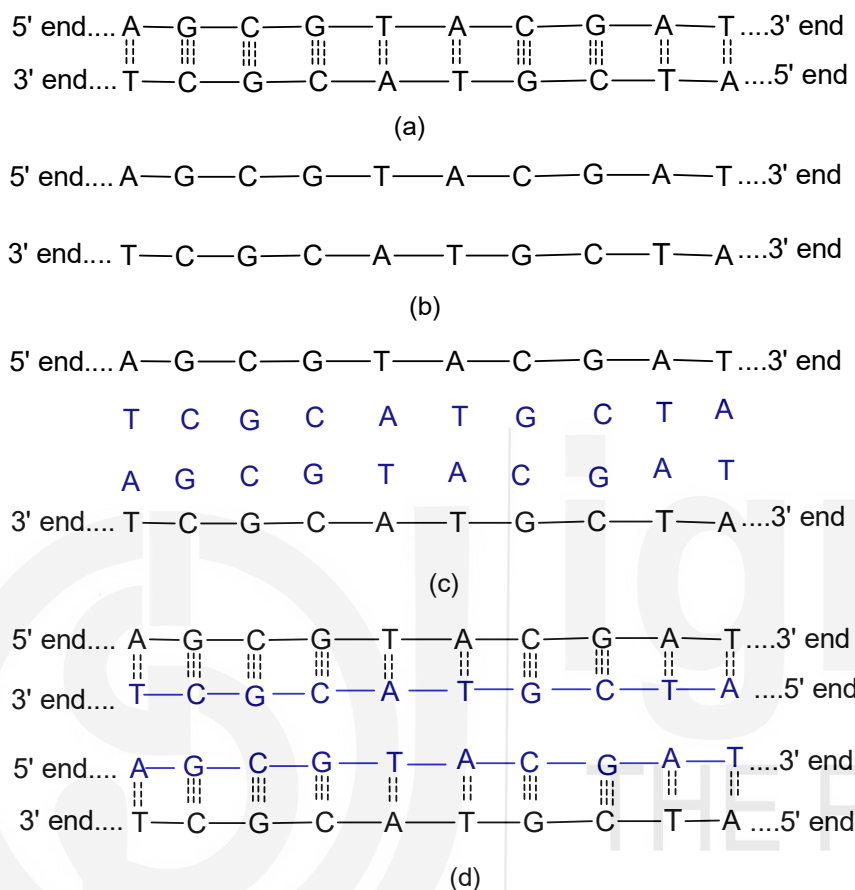


Fig. 13.5: Diagrammatic representation of the replication of DNA (a) Double stranded parent DNA. The vertical dotted lines represent the hydrogen bonds. (b) Separate strands of the parents DNA (c) Alignment of the bases of the deoxy-nucleoside triphosphate precursors (blue coloured) along the two strands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

Up to now you have learnt about the basic process of DNA replication. Now let us explore at the enzymes involved in DNA replication.

DNA replication employs a large number of structural proteins and enzymes, each of which plays a critical role during the process. An important enzyme involved in DNA replication 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 nucleotide 'I' being substituted by 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., nucleotide '1' 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. The RNA primer is synthesized by an enzyme called primase, which is a type of RNA polymerase, before DNA replication can occur.

In the parent DNA there are specific nucleotide sequences called *origins of replication* where replication begins. The origin of replication is recognized by certain proteins that bind to this site. An enzyme called *helicase* unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. As the DNA opens up, Y-shaped structures called *replication forks* are formed (see Fig. 13.6). Two replication forks are formed at the origin of replication and these get extended bi-directionally as replication proceeds. Single-strand binding proteins coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix.

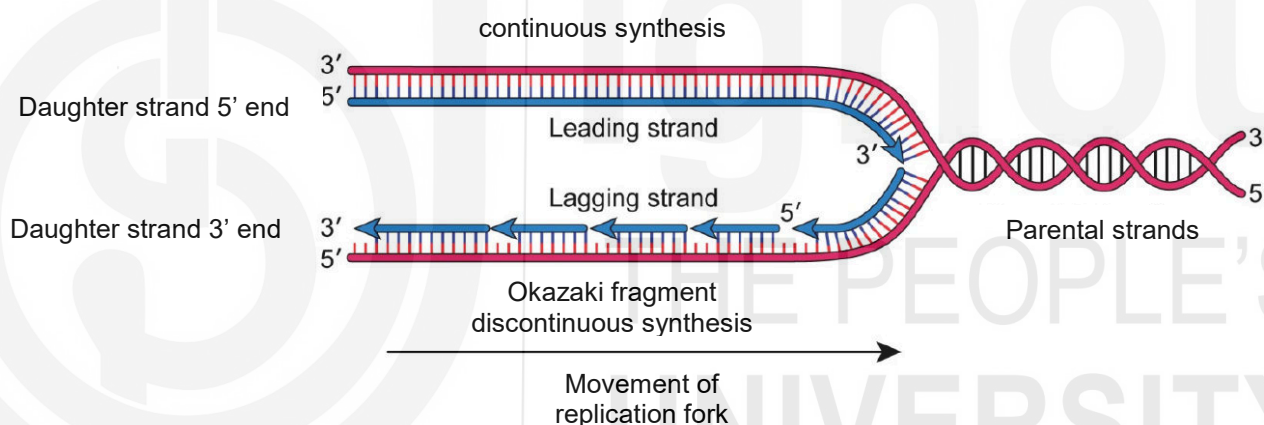


Fig. 13.6 : Replication fork, Replication of both the strands proceeds from 5' to the 3' end. In one of these the replication proceeds continuously (leading strand). In the other, short polynucleotide chains (100-200 bases) are synthesised in a discontinuous manner (lagging strand). The structure on the left hand side of the vertical border line is shown as two sets of parallel lines. Actually these represent two parent stands.

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 synthesized strands must proceed in opposite directions. Thus only one new DNA strand that is complementary to the 3' to 5' parental DNA strand, can be synthesized continuously towards the replication fork in a single piece (because it is synthesized in the 5' to the 3' direction). This continuously synthesised strand is known as the leading strand. It has been shown that when replication of one strand has progressed for some length (the leading strand), replication of the second strand which is complementary to the 5' to 3' parent DNA commences in opposite direction (Fig.13.6). This daughter strand needs to grow in the 5' to the 3' direction, so it is synthesized discontinuously in small pieces. This strand is replicated away from the replication fork. A new fragment is initiated every time when the

replication of the first strand (continuously replicated) has progressed somewhat further. The fragments in the discontinuous strand are 100-200 nucleotides in length and are called Okazaki fragments. Subsequently these fragments are covalently linked to each other by the action of another enzyme called DNA-ligase. The parent DNA is continuously being unwound as the replication progresses.

It must be clear that specific base pairing is responsible for the exceedingly high precision of DNA replication. The complementarity between base sequences of the newly synthesised DNA strand and its template was first demonstrated by hybridization 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 Okazaki fragment?

13.4 RNA SYNTHESIS: TRANSCRIPTION

There are three types of RNAs in the cell, namely the ribosomal RNAs (rRNA), transfer RNAs (tRNA) and messenger RNAs (mRNA). They have similar chemical structure, i.e., they are all polynucleotides in which the individual nucleotides are linked by phosphodiester linkages. However, they differ with respect to the size, secondary structure and function (see Units 12). 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-5'-triphosphate (ATP), guanosine-5'-triphosphate (GTP), cytidine-5'-triphosphate (CTP) and uridine-5'-triphosphate (UTP) (Fig. 13.7).

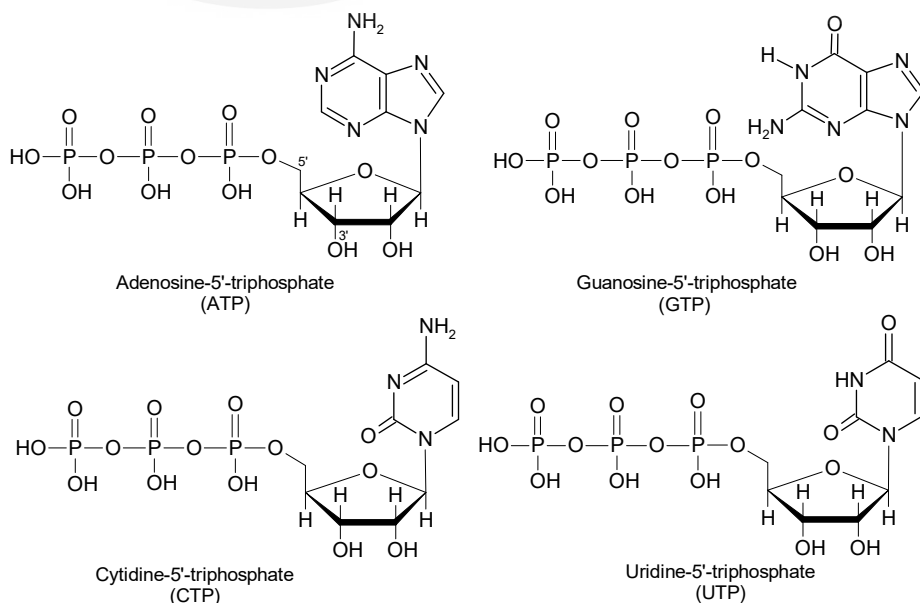


Fig. 13.7: Structures ribonucleotide triphosphates, the precursors in RNA synthesis.

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.

DNA contains sequences of bases known as promoter sites. The promoter sites mark the beginning of transcription. An enzyme identifies a promoter site and binds to it, initiating RNA synthesis. The DNA at a promoter site unwinds to give two single strands, exposing the bases. One of the strands is called the sense strand or coding strand and second one is called the template strand. The template strand is read in the $3' \rightarrow 5'$ direction; therefore, this strand can be used for RNA transcription (as RNA transcription is taken place only in the $5' \rightarrow 3'$ direction (see Fig. 13.8. This is in contrast to DNA synthesis where both the strands are replicated. Further RNA polymerase does not require any primer. RNA polymerase binds at promoter site on DNA 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 next section, because they are closely related to the regulation of protein biosynthesis. Further, RNA polymerase does not possess any exonuclease activity.

Transcription is a continuous process in which RNA is transcribed in $5' \rightarrow 3'$ 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.

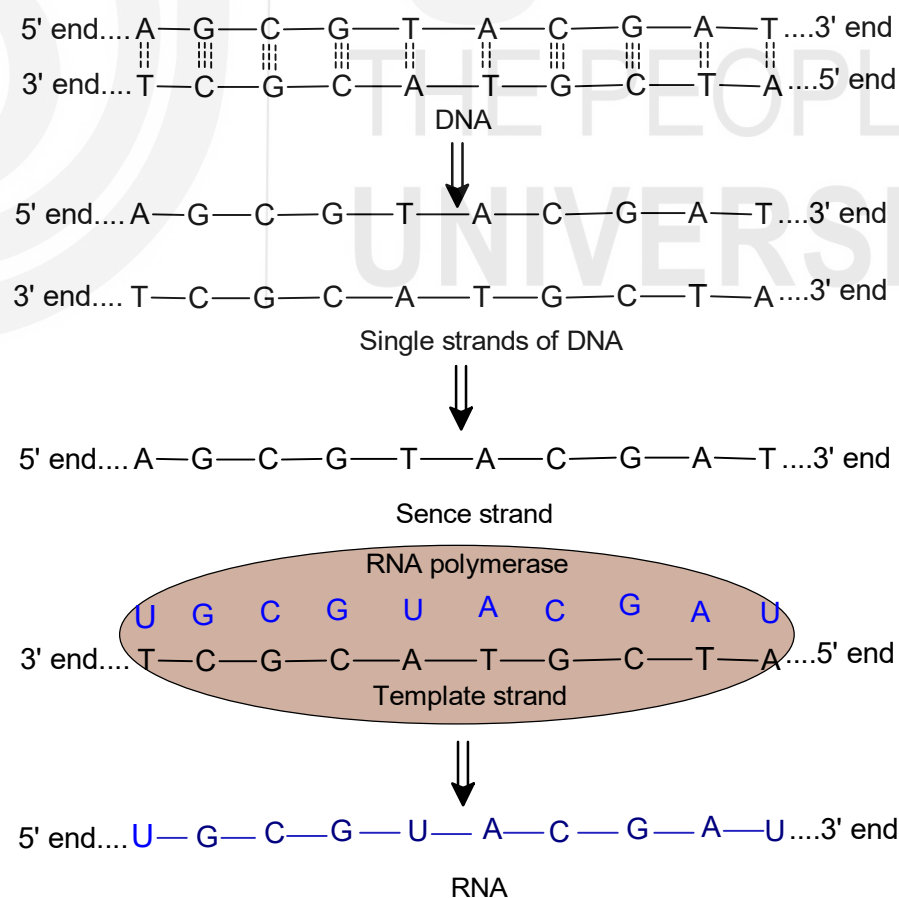


Fig. 13.8: Graphical illustration of the process of RNA synthesizes using the RNA polymerase

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 group of the following chain on the 5' -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 specificity of base pairing with DNA template.

13.6 PROTEIN SYNTHESIS: TRANSLATION

As discussed earlier translation is the process by which the genetic message in DNA that has been passed to mRNA is decoded and used to build proteins. Hundreds of proteins in the human body can be synthesized from single mRNA as per the cell need. Before going in detail of protein biosynthesis let us understand different steps involved in this process.

Protein synthesis occurs on ribosomes. Each ribosome particle consists of RNA molecules and about 52 different protein molecules and carries the enzymes system needed to form peptide bonds between amino acids. Ribosomes provide a site for binding the mRNA and sites for bringing in and aligning the amino acids in preparation for their assembly into the finished polypeptide chain. Amino acids themselves are unable to interact with the ribosome and cannot recognise bases in the mRNA molecule. There exists a collection of "carrier molecules" described in the Unit 12, namely, the tRNA. These molecules contain a site for amino acid attachment and a region called the anticodon that recognises the appropriate base sequence (the codon) in the mRNA (Fig. 12.10 Unit 12). Proper selection of the amino acids for assembly is determined by the positioning of the tRNA molecules, which in turn is determined by hydrogen-bonding or base pairing between the anticodon of each tRNA molecule and the corresponding codon of the mRNA. When an amino acid is attached to a tRNA molecule, the tRNA is said to be aminoacylated or charged. This linking of an amino acid to its corresponding tRNA is catalysed by the enzyme aminoacyl-tRNA synthetase and requires ATP. For each amino acid, there is at least one kind of tRNA and aminoacyl-tRNA synthetase.

Each amino acid is usually represented by a three-letter abbreviation
Phenylalanine = Phe; Methionine = Met etc.

Actual protein synthesis takes place in three stages: initiation, chain elongation, and termination. Before we study the protein biosynthesis in detail, let us learn about the structure and role of ribosome.

13.7 STRUCTURE AND ROLE OF RIBOSOME

A ribosome is composed of two irregularly shaped subunits, which fit together snugly. The two subunits do move apart slightly during translation.

The dalton is a unit of mass nearly equivalent to the mass of a single hydrogen atom, the terms dalton and molecular mass are use interchangeable.

The loci of protein biosynthesis are ribosomes. These are large complexes of protein and ribosomal RNA (rRNA) (Fig.13.9). They consist of two subunits - one "large" and one "small", whose relative sizes are generally given in terms of their sedimentation coefficients, or S values. The *E. Coli* ribosomes have a particle mass of approximately 2.5×10^6 daltons and sedimentation coefficient equal to 70 S and their subunits are 50 S and 30 S. Subunits can be further split into their constituent proteins and RNAs. The 30 S subunit contains 21 different proteins and a 16 S RNA molecule. The 50 S subunits contain 32 different proteins and two RNA molecules, 5 S RNA and 23 S RNA.

Eukaryotic ribosomes are larger, namely 80 S. These have subunits of 40 S and 60 S. Small subunits have one 18 S RNA molecule and 33 different protein, The larger subunit contains three RNA molecules (28 S, 5.8 S and 5 S) and 45 different proteins. There is no functional difference between the prokaryotic ribosome and eukaryotic ribosome.

Specific ribosomal proteins are directly involved in binding mRNA and tRNA. rRNA apparently does not participate directly in these binding sites but serves as a structural polymer holding the multiplier particles in a compact configuration.

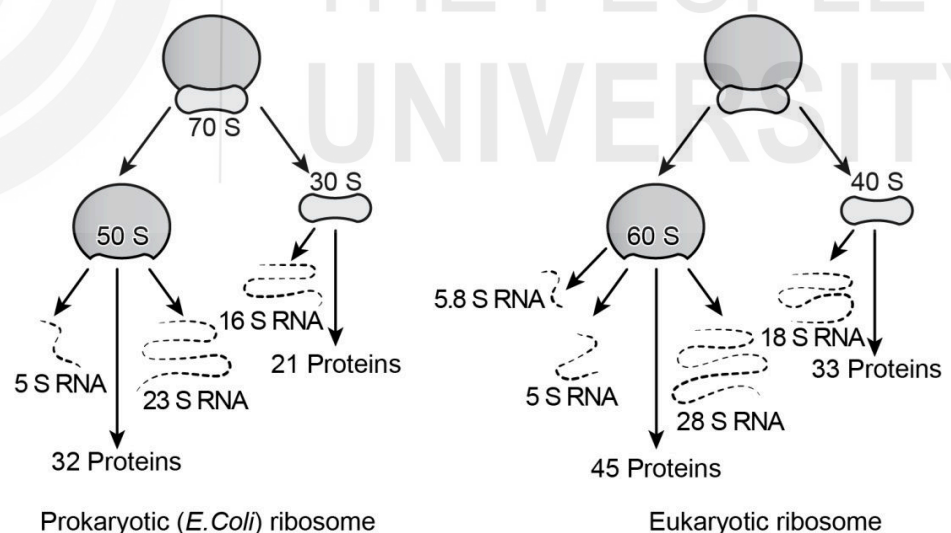


Fig. 13.9: Ribosomal composition.

The ribosome has two binding sites for tRNA molecules, the A (aminoacyl) and P (peptidyl) sites; each extends over both subunits (Fig.13.10). Together, they cover two neighbouring codons of mRNA. During translation, the A site on the ribosome binds an incoming aminoacyl-tRNA as specified by the codon currently occupying this site. This codon specifies the next amino acid to be added to the growing peptide chain.

The P site codon is occupied by the growing chain peptidyl-tRNA. This tRNA carries the chain of amino acids that has already been synthesised. See Fig. 13.11 for an illustration of the role of the A and P site in translation.

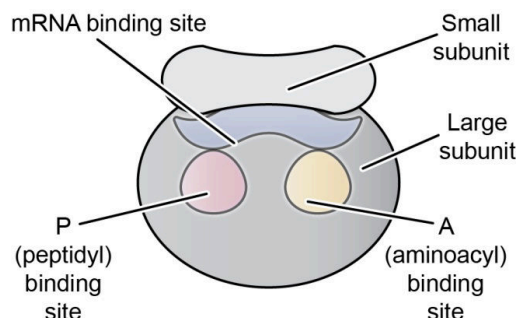


Fig. 13.10: The binding sites of a ribosome are located mainly on the large subunit.

13.8 MECHANISM OF PROTEIN SYNTHESIS

We shall now describe the complex sequence of reactions in which tRNA, mRNA, ribosomes, enzymes and proteins are involved. There are four broad steps in the synthesis of a protein

- activation of amino acid,
- initiation of polypeptide chain formation
- elongation of the polypeptide chain
- termination of polypeptide chain formation

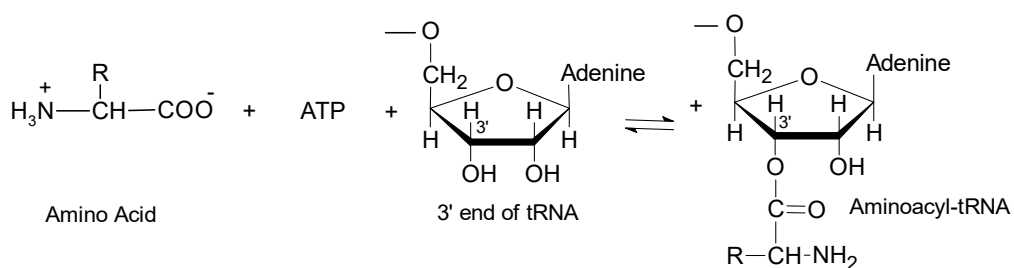
The process is summarised in Fig. 13.11

13.8.1 Activation of Amino Acids

Thermodynamically, peptides are less stable than free amino acids. Therefore, the latter must be "activated" by expenditure of energy so that the peptide synthesis becomes thermodynamically feasible. Secondly, the amino acids have no specificity for the bases of mRNA codons therefore, they do not recognise them. In protein biosynthesis, both these "problems" are solved by attaching amino acids to their specific tRNAs. This reaction requires ATP and is referred to as activation of amino acids. The resultant amino aminoacyl-tRNA (aa-tRNA) then participates in the protein biosynthesis.

The triplet base sequence at anticodon is directly related to the amino acid carried by that tRNA molecule.

In *E. coli*, initiation begins by the attachment of FMet-tRNA to the same alter subunit of ribosome (30 S), which has the initiator proteins and mRNA molecules already bonded.



Formation of aa-tRNA is catalysed by specific enzymes called aminoacyl-tRNA synthetases. This helps in establishing an ester linkage between 3'-OH

group of the terminal ribose of tRNA and amino acid. The overall reaction requires ATP and may be represented as:

The detailed mechanism how amino acid attached to tRNA has been described in Unit 12, Sec 12.6.

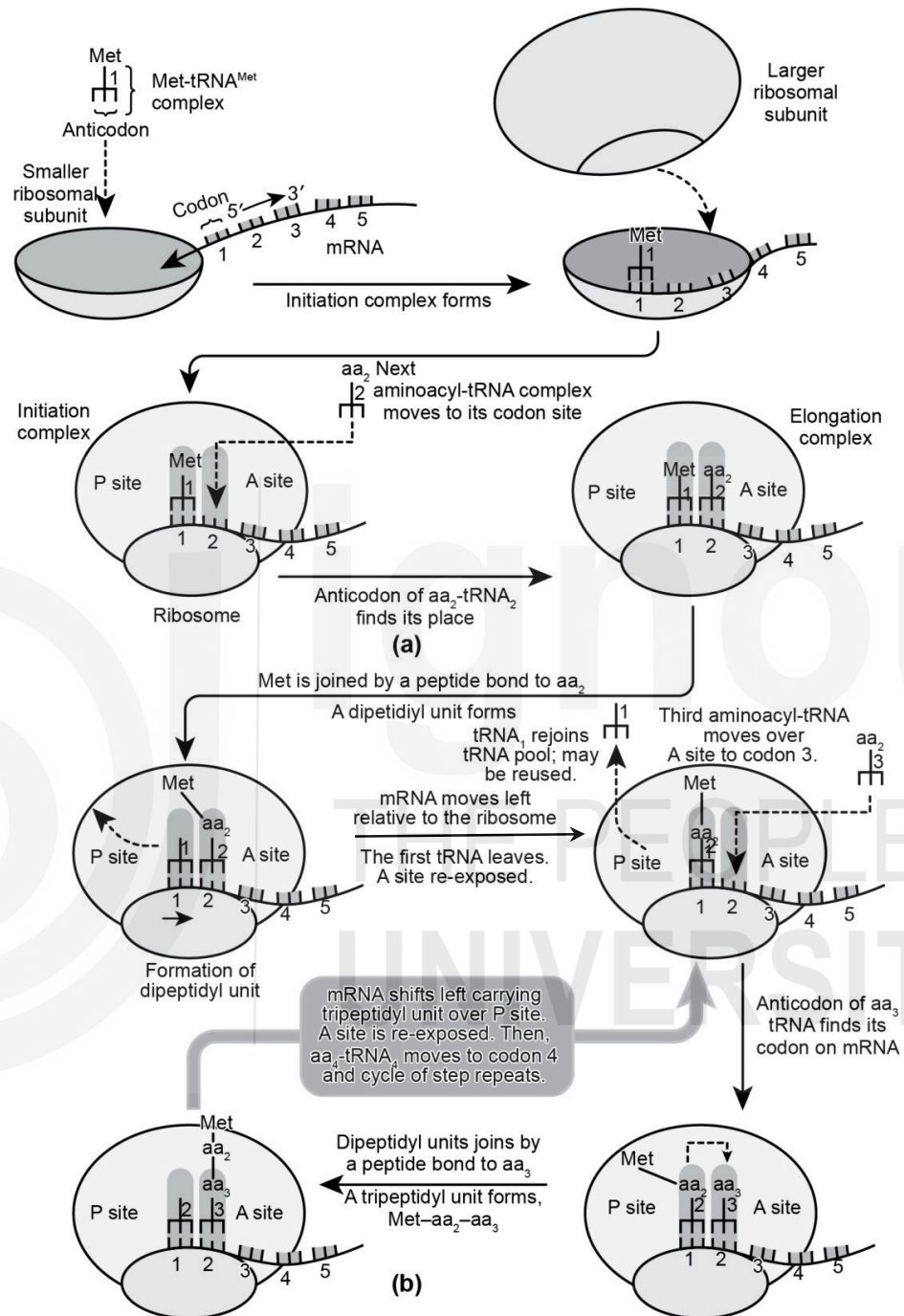
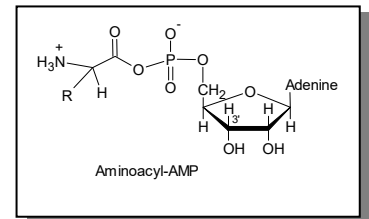
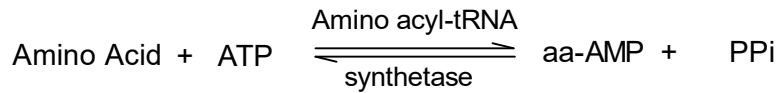


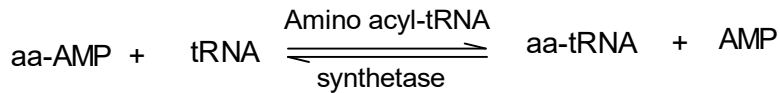
Fig. 13.11: (a) Formation of the elongation complex at the beginning of the synthesis of a polypeptide; (b) The elongation steps in the synthesis of a polypeptide. The dipeptidyl unit, Met-aa₂ formed by the process of (a) now has a third amino acid residue, aa₃ added to it.

The reaction is driven to completion by hydrolytic removal of pyrophosphate ion (PPi) catalysed by inorganic pyrophosphatase. Thus, the activation of amino acids for protein biosynthesis resembles that of fatty acids in the biosynthesis of fats. As in the latter, the activation of amino acids is also a two-step process with enzyme-bound aminoacyl-AMP as an intermediate.

1. Activation of the amino acid by the enzyme (aminoacyl tRNA synthetase)



2. The transfer of the amino acid from aa-AMP to the specific tRNA



An aa-tRNA is also referred to as a "charged" tRNA.

The aa-tRNA synthetases are remarkably specific, they recognize not only the correct amino acid from the cellular amino acid pool containing amino acids with very similar structures (e.g. Ile, Val, Phe, Tyr, etc.), but also the appropriate tRNA molecule from the whole range of tRNAs. The process is strikingly error-free.

13.8.2 Initiation of Polypeptide Chain Formation

The activated amino acid, i.e., aminoacyl-tRNAs diffuse to the ribosomes, which are the sites of polypeptide formation. The polypeptide synthesis never takes place in the solution but instead always requires ribosomes, that orient the aa-tRNA and the mRNA for accurate alignment in accordance with the genetic code. Methionine is invariably the peptide initiating residue in eukaryotes, and as N-formylmethionine (FMet) in prokaryotes.

The formyl group is introduced and removed enzymatically. In *E.coli*, initiation begins by the attachment of FMet-tRNA to the smaller subunit of ribosome (30 S), which has the initiator proteins and mRNA molecule already bonded, see Fig. 13.11a. The mRNA molecule bonds to the P site, of the ribosome. At the P site, all of the necessary enzymes for translation are present. The anticodon of the FMet-tRNA (CAU) binds to the codon (AUG) on the mRNA (Fig 13.12).

The initiating Met-tRNA^{Met} and F-Met-tRNA^{fmet} are only aminoacyl-tRNAs which go directly to the P site. All other aminoacyl tRNA enter through the A site of the ribosome.

Initiation complex- an aggregate composed of mRNA, an intact ribosome, and the initial aminoacyl-tRNA adduct.

The sequence of bases in mRNA determines the sequence of amino acids in a protein.

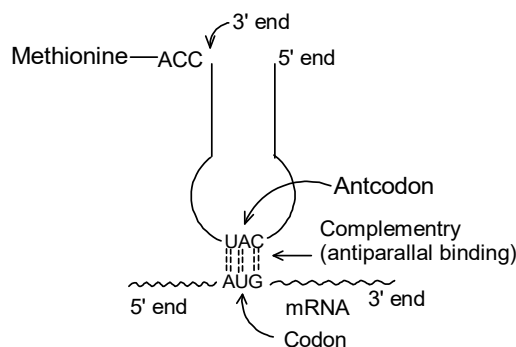
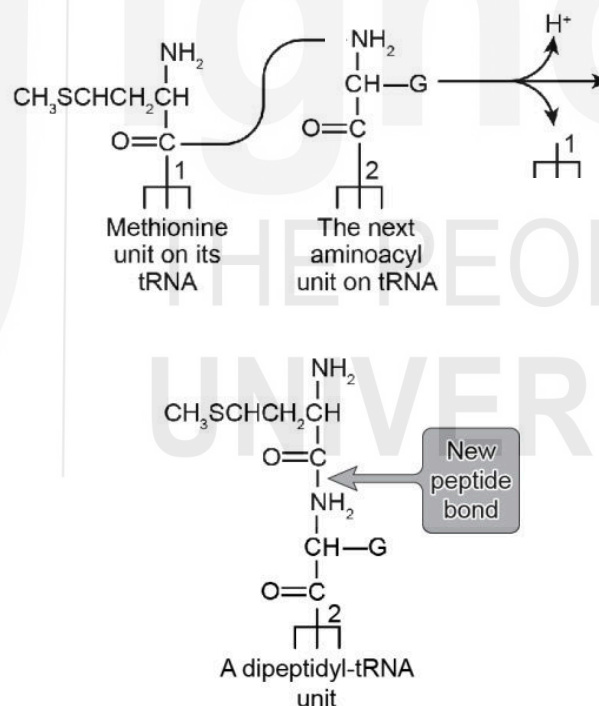


Fig. 13.12: Complementary binding of the Aminoacyl-tRNA anticodon of methionine-tRNA (CAU) to mRNA codon for methionine (AUG). Amino acid attachment site is also shown

To complete the initiation stage, the larger ribosomal unit binds to the complex and produces the initiation complex. Now the second aa-tRNA₁ unit, tRNA having aminoacyl group aa₂ binds to the matching codon at site A, i.e. aminoacyl binding site. Consequently, the aminoacyl units lie side by side and peptide bond formation (chain elongation) starts.

13.8.3 Elongation or the Polypeptide Chain

The first charged tRNA, i.e. tRNA₁ (tRNA^{Met}) transfers its amino acid (Met) to the aa₂ amino acid of the second charged tRNA (tRNA₂), forming an amide (peptide) bond. The process is catalysed by certain enzymes, called **peptidyl transferases**. The freed tRNA (tRNA₁) on the P site leaves the mRNA and mRNA moves one codon over. The third charged tRNA (tRNA₃) finds its anticodon matching on the third codon over the A-site vacated by leftward movement of one codon. The elongation takes place by transferring the dipeptide Met-aa₂ to the aa₃ amino acid and mRNA shifts one codon leftward from A-site to P-site as the free tRNA (tRNA₂) dissociates. The process is repeated over and over again till the required chain length is reached. The sequence of bases in mRNA determines the sequence of amino acids in a protein.



It is very important to realise at this point that the peptide chain grows stepwise with addition of a single amino acid starting with the amino terminal and ending with the carboxyl terminal. The codons of mRNA are "read" from 5'- to the 3'-end.

13.8.4 Termination or Polypeptide Chain Formation

Once the ribosome has moved down to one of the chain terminating codons (nonsense codons or stop codon), the polypeptide is released and the ribosome can be reused for synthesis of another protein or polypeptide chain.

Several details have been left out in this simplified description of protein biosynthesis. For example, the role of various "initiation factors" in the formation of initial Met-tRNA^{Met}-mRNA-ribosome complex has not been described. Similarly details of GTP-requiring movement of ribosome along mRNA and release factors which bring about release of completed polypeptide chain have been omitted. Interested students may consult any advanced text book of biochemistry for these details.

SAQ 6

The elongation of polypeptide chain takes place at:

- a) A-site
 - b) P-site
 - c) between A and P site
 - d) both at A and P sites
-

13.9 SUMMARY

- DNA, a poly-deoxynucleotide, is the genetic material for all cellular life and for most viruses. It has been shown first experimentally by Frederick Griffith (1928) that bacteria are capable of transferring genetic information through a process known as transformation. Later Avery and co-worker (1944) found that DNA is the material that communicated this genetic information. Experiments with bacteriophages by Hershey-Chase (1952) also showed that the phage DNA can enter in the bacterial cell. The infected cell had all the information 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 precursors 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 which drives the reaction to completion.
- Existing DNA is unwound by helicases and other proteins to produce short regions of single strand DNA 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 strands 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 produced which are identical to each other and to the

starting 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 DNA as a template. Nucleoside 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 precursors get selected by virtue of specific base pairing as in DNA replication. In RNA synthesis, only one strand of DNA is transcribed.
- In protein synthesis, the ribosomes play the vital role. They provide site for binding the mRNA. The tRNA having a clover leaf like structure carries the anticodons complementary to the codons on the mRNA. It brings the amino acid to the site of polypeptide synthesis. The process is remarkably error free and amino acids are attached to the processing polypeptide chain in the sequence coded by the codons on the mRNA. The terminating codons cause the chain termination and the completed polypeptide is released. It has been established that smaller polypeptides are synthesised in translation and these then unite to give the resultant proteins.

13.10 TERMINAL QUESTIONS

1. In Hershey and Chase experiment why did the radioactivity pass into the bacterial cell only from DNA labeled bacteriophages?
2. What is the direction of RNA synthesis?
3. Is exonuclease activity is present in RNA polymerase?
4. How are the bases paired in 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?
8. Give a brief account of the steps involved in protein biosynthesis

13.11 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 strand are 100-200 nucleotides in length and are called Okazaki.

5. Three types: Transfer RNA, Ribosomal RNAs and Messenger RNAs.
6. b)

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. Protein part Bacteriophages remain outside the bacterial cell.
2. It is from 5' to 3'
3. No, exonuclease activity is not present in RNA polymerase.
4. Adenine of one strand lies alongside thymine of the other strand. (A-T pair) and guanine and cytosine to different strands lie side by (G-C pair).
5. The thymine is replaced by Uracil in RNA.
6. DNA polymerase.
7. Please see section 13.5.
8.
 - i) Charged tRNAs i.e. aminoacyl-tRNAs diffuse to the ribosome.
 - ii) mRNA attaches to a ribosome.
 - iii) Charged tRNA brings the first amino acid (FMet) and binds to the first start codon mRNA on the ribosome surface at P site.
 - iv) Another charged tRNA brings the second amino acid (aa₂) and binds to the second codon of mRNA at A site of ribosome.
 - v) The first amino acid is joined to the second amino acid on the second tRNA.
 - vi) The empty first tRNA leaves and the mRNA and second tRNA move on the ribosome, bringing the third codone into A site on the ribosome.
 - vii) The charged tRNA bearing the amino acid specified by the third codon binds to the A site.
 - viii) Steps 5 to 7 are repeated with each amino acid until a "Stop" codon on the mRNA is reached.

