UNIT-4 LIPIDS AND NUCLEIC ACID

LIPIDS

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. Lipids are a class of compounds distinguished by their insolubility in water and solubility in nonpolar solvents.

Lipids have the common property of being relatively insoluble in water and soluble in nonpolar solvents such as ether and chloroform. Lipids are important in biological systems because they form the cell membrane, a mechanical barrier that divides a cell from the external environment. Lipids also provide energy for life and several essential vitamins are lipids.

- **Functions of lipids**

1. **They are stored in adipose tissue** (triglycerides) and are one of the major energy sources. Carbohydrates, on average, gives 4 kcal/g, as proteins, lipids provide, on average, 9 kcal/g. Mostly of Nutrition Organizations recommend that lipids must contribute up to 30% (with saturated fatty acids only less than 10%) of the total daily energy intake.
2. Some lipids are **essential nutrients** like fat-soluble vitamins A, (necessary for vision) and D (necessary for calcium metabolism), present in some fats and oils of animal origin, vitamin E (prevention of autoxidation of unsaturated lipids), present in vegetable oils, and vitamin K (normal clotting of blood) present in green leaves, essential fatty acids, in particular linoleic and α-linolenic acids, founders of the family of omega-6 and omega-3 fatty acids respectively.
3. During growth they are utilized as “bricks” for construction of **biological membranes** (phospholipids, cholesterol and glycolipids together with proteins), so contributing to construction of that barrier that separates intracellular environment from extracellular one and, inside cell, circumscribes organelles like mitochondria, Golgi apparatus or nucleus, and whose integrity is the basis of life itself; moreover they are also important for maintenance, physiochemical properties and repairing of cell membranes themselves.
4. **Many hormones are lipids**: steroid hormones, like estrogens, androgens and cortisol, are formed from cholesterol (essential also during

embryogenesis), prostaglandins, prostacyclin, leukotrienes, thromboxanes, and other compounds (all eicosanids) from omega-3 and omega-6 polyunsaturated fatty acids with 20 carbon atoms.

5. On plasmatic cell membranes they can act as **receptors, antigens and membrane anchors** for proteins and can modify the structure, and therefore the functionality, of membrane enzymes.
6. Many lipids, like diacylglycerol, ceramides, sphingosine and platelet-activating factor act as regulators of intracellular processes.
7. There are fat deposits not accessed during a fast, classified as **structural fat**, the function of which is to hold organs and nerves in the right position protecting them against traumatic injuries and shock; fat pads on the palms and buttocks protect the bones from mechanical pressure.
8. A subcutaneous layer of fat is present in humans: it insulates the body **reducing the loss of body heat** and contributing to maintain body temperature.
9. On epidermis they are involved in maintaining water barrier.
10. They are **electrical insulator of axon of neurons** that are covered over and over again by plasmatic membranes of Swann cells, in peripheral nervous system, and of oligodendrocytes in central nervous system; these plasmatic membranes have lipid content greater than that of the other cells. This lipoprotein coating is called myelin sheath.
11. On digestive tract they f**acilitate the digestive process** depressing gastric secretion, slowing gastric emptying and stimulating biliary and pancreatic flow.
12. **Bile salts** (by-products of cholesterol) are natural detergents synthesized in the liver and secreted into bile. They solubilize phospholipids and cholesterol in the bile, permitting the secretion of cholesterol into the intestine (the excretion of both cholesterol and bile salts is the major way by which cholesterol is removed from the body). Bile salts also aid in the digestion and absorption of fat and soluble-fat vitamins in gut.
13. In many animals, some lipids are secreted into external environment and act as **pheromones** that attract or repel other organisms.
14. They affect the **texture and flavour of food** and so its palatability. Food manufacturers use fat for its textural properties, e.g. in baked goods fat increase the tenderness of the product. Chefs know that fat addiction add to the palatability of meal and increase satiety after a meal.

- **CLASSIFICATION OF LIPIDS**

Lipids are classified as follows:

I. Simple lipids

(1) Fats and oils
(2) Waxes

II. Compound lipids

(A) Phospholipids, e.g. lecithins, cephalins, plasmalogens etc.

(B) Glycolipids, e.g. cerebrosides, gangliosides.

(C) Other compound lipids, e.g. Lipoproteins, Sulpholipids, Aminolipids, etc.

III. Derived lipids

e.g. Fatty acids, Glycerols, Sterols, Sex hormones, prostaglandins, sphingolipids, etc.

(I) SIMPLE LIPIDS

Simple lipids are the esters of fatty acids with various alcohols.

(1) FATS AND OILS

Fats are the esters of fatty acids with glycerol, which are found richly in nature. Fats are solid at room temperature whereas oils are liquid.

Functions :

(a) Acts as food reservoir in the human body.
(b) Acts as insulator for the loss of body heat.
(c) Acts as padding material for protecting internal organs.

(2) WAXES

These are the esters of fatty acids with higher alcohols and formed as secretions, which are mostly protective in function, by many animals. They resemble fats and are usually solid.

The common wax present in human body is esters of cholesterol, found in blood. Waxes contain fully reduced hydrocarbon chains; they are insoluble in water and are very resistant to atmospheric oxidation. They have high melting points and because of these properties they find uses in furniture polishing's.

Some important waxes are -

i. Carnuba wax, obtained from leaves of Carnuba palm of Brazil. They are used as an ingredient in the manufacture of polishes; they make an excellent protective coating.
ii. Bees wax, it contains palmitic acid ester of myricyl alcohol.
iii. Lanolin or wool fat - It is a palmitic, oleic or stearic acid ester of cholesterol.' It is obtained from wool and used as base in making ointments.
iv. Spermaceti - It is a liquid wax and it is palmitic acid ester of cetyl alcohol. It has a value as lubricant.

Functions of waxes

i. As a protective agent on the surfaces of animals and plants.
ii. Waxes are found on the surfaces of feathers and hair with the result they remain soft and pliable.

(II) COMPOUND LIPIDS

Compound lipids are the esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

The various compound lipids are as follows

(A) **Phospholipids:**

Phospholipids are the esters of fatty acids with glycerol containing an esterified phospharic acid and a nitrogen base. They are present in nerve tissue, brain, liver, kidney, pancreas and heart.

Functions

i. Phospholipid is one of the important constituent of cell wall membrane, and acts as carrier of inorganic ions across the membranes.
ii. They help in blood coagulation process.
iii. (Hi) They help in absorption of lipids from the GIT, e.g. lecithin.
iv. They actively involve in the structures of myelin sheath, microsomes and mitochondria.
v. They act as prosthetic group to certain enzymes, (vi) They increase the rate of fatty acid oxidation.

Classification of Phospholipids :

These are classified as -

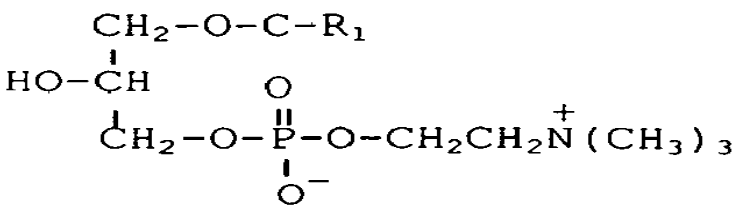
i. Glycerophosphatide -Lecithins, Cephalins, Phosphatidyl serine Plasmalogens, Diphosphatidyl glycerols etc.

ii. Phosphatidyl inositol - Lipositol or phosphoionositides

iii. Phosphosphingosides or spingolipids.

i. Glycerophosphatldes

Lecithins: They are also called as phosphatidyl cholines. Upon hydrolysis they yield glycerol, fatty acids, phosphoric acid and choline. Lecithins generally contain a saturated fatty acid at α-position and an unsaturated fatty acid at β position. They can exist in α or β forms.



Lecithin

The commonly occuring fatty acids in lecithins are palmitic, stearic, oleic, linolenic and archidonic acids.

Lecithins are waxy, white or yellowish grey solids, becomes brown soon when exposed to air. They are soluble in ether and alcohol but not in acetone. They have the property of forming complexes with lipids, proteins, carbohydrates, etc. and this property is concerned in the ionization and function of many macromolecular cellular components. When the hydroxyl group of choline is acetylated, acetyl choline is formed.

Lecithins get broken down by the enzyme lecithinase to lysolecithin. The enzyme lecithinase occurs in venoms of bee and snake cobra. And when injected in blood, lysolecithins bring about rapid haemolysis i of R.B.C.S. Egg yolk is the richest source of lecithins.

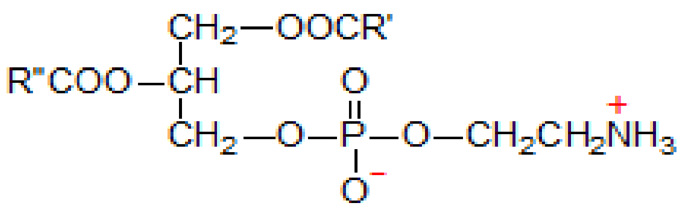
Functions:

i. Lecithins lower the surface tension of water and help in emulsification of lipid-water mixtures, that help in the digestion as well as absorption of lipids from the GIT.
ii. They have very useful function of keeping cholesterol and its. esters in the dissolved state, in plasma.
iii. It also has an important role in fat metabolism in the liver.
iv. They facilitate combination with proteins to form lipoproteins of plasma and cells.
v. Acetyl choline formed from choline is essentially involved in the transmission of nerve impulses.

Cephalitis (Phosphatidyl ethanolamines):

They always occur in tissues in conjunction with lecithins and having similar properties. The only difference is in the nitrogenous base. It contains ethanolamine.

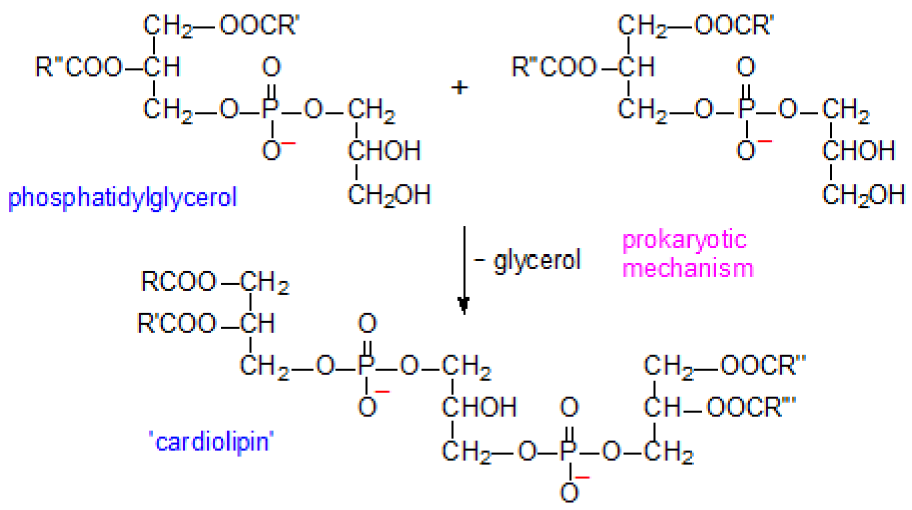
The fatty acid components of cephalins are generally stearic, oleic, linoleic and archidonic acids. They have ethanolamine, colamine and sometimes serihe in place of choline.



phosphatidylethanolamine

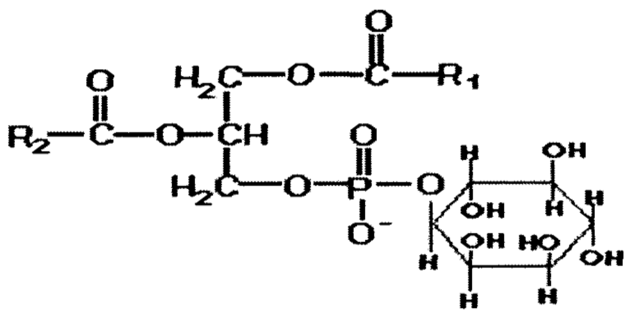
Diphosphotidyl glycerols :

These are two molecules of phosphatidic acid combined by a bridge of glycerol. Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerols and phospholipids. Cardiolipin is formed from phosphatidyl glycerol.



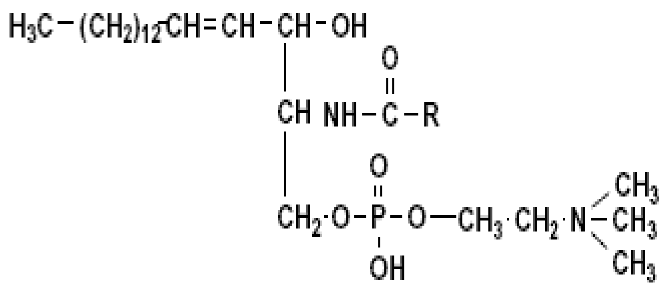
(2) Phosphoinositides or lipositol (Phosphatldyl inositol)

They have hexahydric alcohol 'inositol'. They can be either mono or diphosphoinositides.depending upon either one phosphate or two phosphates, attached in a- position or in the two a positions joined together by the inositol as bridge.



(3) **Phosphosphingosides (Spingolipids) :**

These are the lipids in which the trihydroxy alcohol, glycerol, is replaced by a complex amino alcohol 'spingol.' Fatty acid choline and phosphoric acid are the other constituents.



Sphingomyelin

Spingomylins are present in brain and nervous tissues. The concentration of these phospholipids is increased in Niemann - pick disease in the liver and spleen

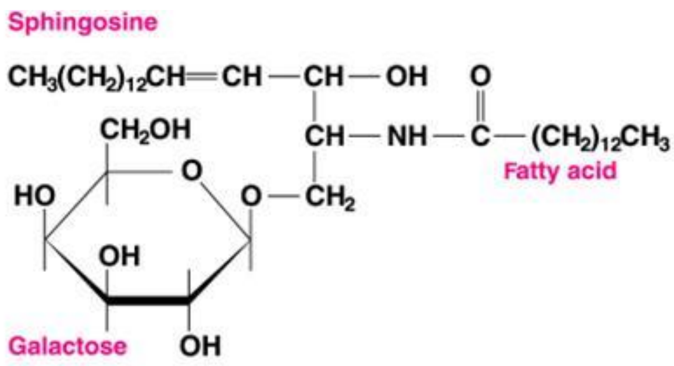
(B) Glycolipids

These are the lipids containing spingol, a carbohydrate-galactose, and fatty acid. The amino alcohol, 'spingol,' with an amide, linkage to fatty acid and glycosidically to a carbohydrate moiety. These are also called as galactolipids. They are present in the white matter of the brain and in the mylein sheaths of nerves. They accumulate in the liver and spleen in Gaucher's disease.

They are further classified as :

(1) Cerebrosides :

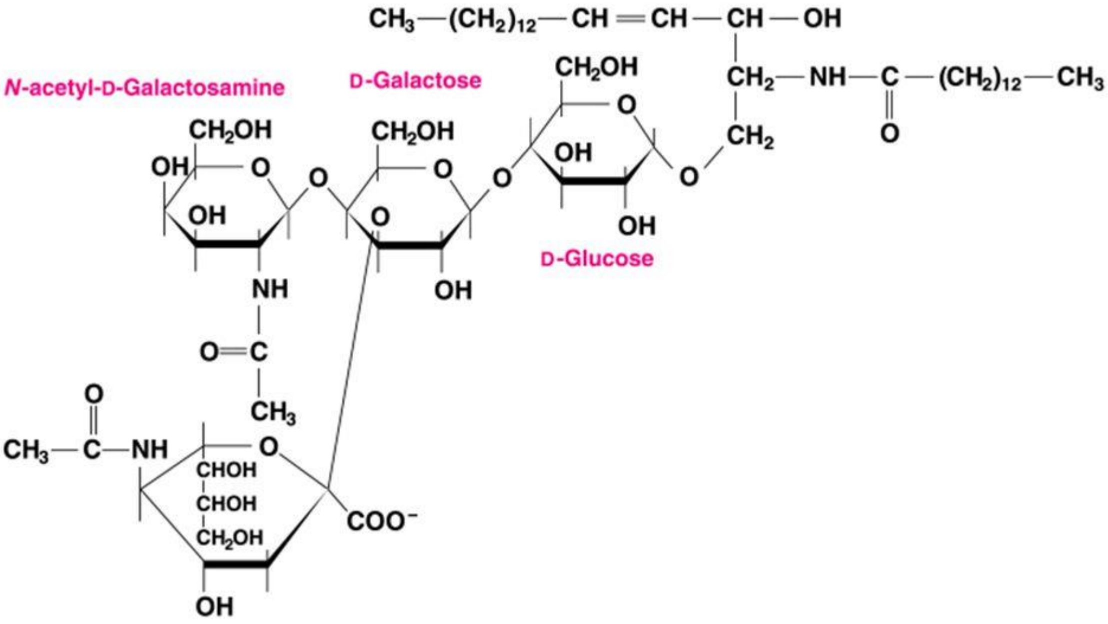
They contain galactose and spingosine and are the chief constituents of myelin sheath.
e.g. Kerasin, Cerebron, Nervon, oxynervon, they may be differentiated by the type of fatty acid in the molecule.



Galactocerebroside, a glycosphingolipid

(2) Gangliosides: They occur in the brain and contain ceramide, i.e. sphingosine + fatty acids, glucose, galactose, N - acetyl -galactosamine and sialic acid.

Ganglioside Structure



(C) **Other compound lipids**

(I) Lipoprotelns: Lipoprotein is a hydrophilic complex containing triacylglycerol, phospholipids, cholesterol and cholesteryl esters, free fatty acids and proteins.

Four major groups of lipoproteins have been identified, which have clinical applications in some metabolic disorders of fat metabolism:

a. **Chylomicrons and VLDL** (Very **low density lipoproteins)**
   The concentrations of these are increased in atherosclerosis and coronary thrombosis etc.
b. **LDL (Low density lipoproteins):** The concentration of which is increased in atherosclerosis and coronary thrombosis, etc.
c. **HDL (High density lipoproteins)** (ii) **Sulpholipids (Sulphatides) :**
   These are sulphate derivatives of the galactosyl residue in cerebrosides, and have been isolated from brain and animal tissues.

(II) DERIVED LIPIDS

These are the lipids derived from simple and compound lipids by hydrolysis.

(1) Fatty Acids:

Fatty acids are the hydrolysis products of fats. They contain C, H and O. Most of the naturally occuring fatty acids are straight chain derivatives and contain an even number of carbon atoms.

They may be 'saturated' (Containing no double bond) or 'unsaturated' (Containing one or more double bonds).

(A) **Saturated / Non-essential Fatty Acids:**

These are the fatty acids containing no double bonds and are represented by the general formula - $C_n H_{2n+1}$ COOH. These fatty acids can be synthesized in the body, hence are not essential to be supplied through diet. Hence, these are also called as **'non-essential' fatty acids.**

The molecular weight of saturated fatty acids contributes the physical properties. Those fatty acids that contain 10 carbon atoms or fewer in their molecules are liquid at room temperature; the remainders are solids whose melting point rises with increasing molecular weight. Fatty acids with 4 carbon atoms or fewer are miscible with water in all proportions. As the length of carbon atom increases beyond this, the solubility rapidly decreases to zero. The terminal carbon atom of saturated fatty acid is having three hydrogen atoms while the other is having a carboxyl group. The example includes -

Acetic acid (2)
Butyric acid (4)
Caproic acid (6)
Caprylic acid (8)
Capric acid (10)
Lauric acid (12)
Myristic acid (14)
Palmitic acid (16)
Stearic.acid (18)
Arachidic acid (20)
Lignoceric acid (24)

(The number in the bracket showing the no. of carbon atom they contain).

(B) Unsaturated / Essential Fatty Acids:

These are the polyunsaturated fatty acids which are not synthesized in the body but are essentially obtained from the diet or natural sources, hence are also called as **'essential fatty acids.'** They are represented by the general formula - $C_n H_{2n-1} COOH$.

These are characterized by the presence of one or more double bonds in the molecule. Because of the presence of double bonds, the unsaturated fatty acids are much more reactive than the saturated fatty acids; the reactivity increases with increasing number of double bonds.

The unsaturated fatty acids are capable of taking one molecule of water, oxygen, hydrogen, bromine or iodine, at each double bond, and the amount of such substance (e.g. iodine) absorbed by the given weight of acid, is used to determine its degree of unsaturation.

The deficiency of essential fatty acids leads to lesions of skin, tissue necrosis, loss of hair, etc.

Functions:

(1) In high cone, along with lipids constitute the structural elements of tissue.
(2) They prolong the blood coagulation period. *
(3) They cure skin lesions.
(4) They are involved in gonads for reproductive functions.
(5) In babies due to deficiency cause eczema.

The examples of unsaturated fatty acids include
Linoleic acid,
Linolenic acid,
Oleic acid,
Palmitoleic acid,
Arachidonic acid

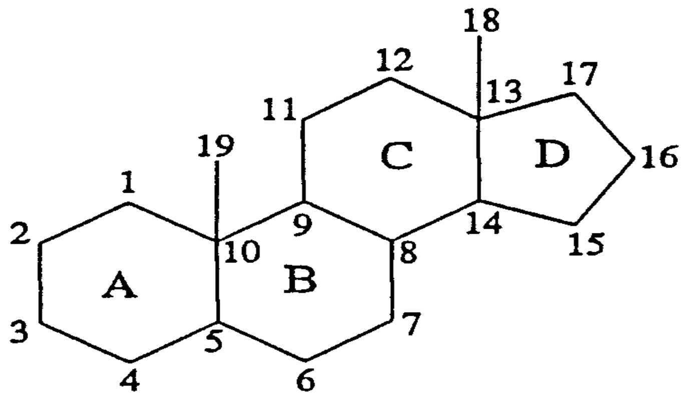
Functions of Fatty Acids

1) Fatty acids are required for the synthesis of phospholipids.
2) Fatty acids are required for the energy production i.e. ATP synthesis, in mitochondria.

3) These are essential for the building of nervous tissues and cell membranes.
4) Fats provide upto 40 % of total building of nervous tissues and cell membranes.
5) These are also required for the synthesis of lipoproteins and glycoproteins.
6) It acts as a carrier for fat-soluble vitamins.
7) Acts as heat insulator.

(2) Sterols

The compound having one or more hydroxyl group and no carbonyl or carboxyl groups in a phenanthrene nucleus are called sterols or steroids.

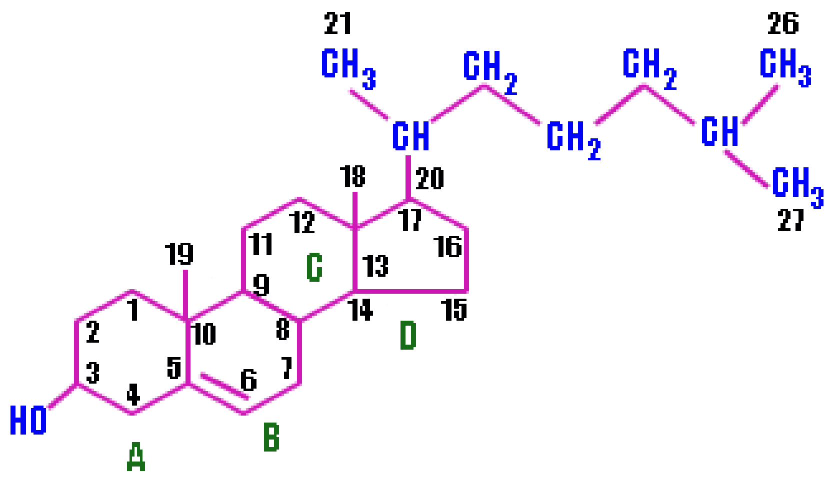


Cyclopentanophenanthrene nucleus

CHOLESTEROL

The most abundant and most important steroid present in the body, is cholesterol. Cholesterol literally means bile-solid-alcohol, derives its name from the fact that, it was first isolated from human gall-stones, of which it is generally the chief component. The amount of cholesterol in animal varies widely. It is particularly abundant in brain, nervous tissues, adrenal glands and egg yolk.

Cholesterol is usually accompanied by dihydrocholesterol and 7-dehydrocholesterol. It has molecular formula C_{27} $H_{45}OH$ and has the 21 structure as shown below



Cholesterol is an important intermediate in the synthesis of steroid hormones. It serves as a membrane component, mostly in the plasma membranes of red blood cells and in the myelinated nerve cells. In the blood it occurs both in free and esterified forms.

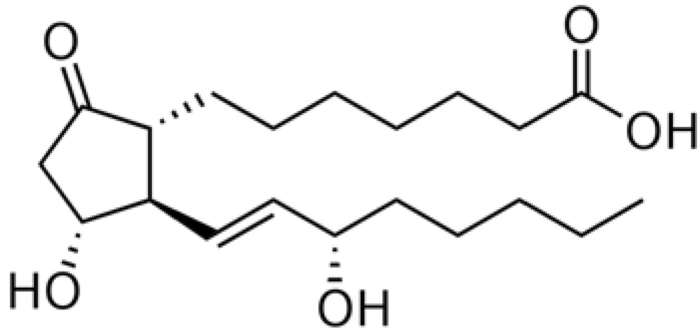
The normal range of cone, of cholesterol in the serum is from 150 -250 mg/dl. The cone, of cholesterol gets elevated in several diseases like atherosclerosis, nephrosis, diabetes mellitus and hypothyrodism. The atherosclerosis disease has got notoriety (bad name) because of its involvement in hardening of arteries and destroys the elasticity of arteries. Moreover, cholesterol is also well known as a constituent of gall stones.

- **Prostaglandins**

The **prostaglandins** (**PG**) are a group of physiologically active lipid compounds having diverse hormone-like effects in animals. Prostaglandins have been found in almost every tissue in humans and other animals. They are derived enzymatically from fatty acids. Every prostaglandin contains 20 carbon atoms, including a 5-carbon ring. They are a subclass of eicosanoids and of the prostanoid class of fatty acid derivatives.

Prostaglandins are found in most tissues and organs. They are produced by almost all nucleated cells. They are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. They are synthesized in the cell from the essential fatty acids (EFAs).

Prostaglandins are produced following the sequential oxidation of arachidonic acid, DGLA or EPA by cyclooxygenases (COX-1 and COX-2) and terminal prostaglandin synthases.



Functions

i. cause constriction or dilation in vascular smooth muscle cells
ii. cause aggregation or disaggregation of platelets
iii. sensitize spinal neurons to pain
iv. induce labor
v. decrease intraocular pressure
vi. regulate inflammation
vii. regulate calcium movement
viii. regulate hormones
ix. control cell growth
x. acts on thermoregulatory center of hypothalamus to produce fever
xi. acts on mesangial cells (specialised smooth muscle cells) in the glomerulus of the kidney to increase glomerular filtration rate
xii. acts on parietal cells in the stomach wall to inhibit acid secretion

Glycolipids

Glycolipids are lipids with a carbohydrate attached by a glycosidic bond.[1] Their role is to maintain stability of the membrane and to facilitate cellular recognition.[2] The carbohydrates are found on the outer surface of all eukaryotic cell membranes. They extend from the phospholipid bilayer into the aqueous environment outside the cell where it acts as a recognition site for specific chemicals as well as helping to maintain the stability of the membrane and attaching cells to one another to form tissues

The basic structure of a glycolipid is the presence of a carbohydrate monosaccharide or oligosaccharide bound to a lipid moiety. The lipid complex is most often composed of either a glycerol or sphingosine backbone, which gives rise to the two main categories of glycolipids, glyceroglycolipids and sphingolipids. Lipids are non-polar molecules, providing them the capability of interacting with the lipid-bilayer of the cell membrane and anchoring the glycolipid to the surface of the cell. Carbohydrates are used as the ligand component of glycolipids and their structure varies depending on the structure of the molecule it binds to. The carbohydrate contains polar groups that enable the molecule to be soluble in the aqueous environment surrounding the cell.[3] The two molecular groups form a glycoconjugate through a covalent bond referred to as a glycosidic bond. The anomeric carbon of the sugar binds to the hydroxyl group present on the lipid.

Saponification

Usually, a process by which triglycerides are reacted with sodium or potassium hydroxide to produce glycerol and a fatty acid salt, called 'soap'. When sodium hydroxide is used, a hard soap is produced. Using potassium hydroxide results in a soft soap.

Lipids that contain fatty acid ester linkages can undergo hydrolysis. This reaction is catalyzed by a strong acid or base. Saponification is the alkaline hydrolysis of the fatty acid esters.

Example: The chemical reaction between any fat and sodium hydroxide is a saponification reaction.

triglyceride + sodium hydroxide (or potassium hydroxide) → glycerol + 3 soap molecules

Vegetable oils and animal fats are the main materials that are saponified. These greasy materials, triesters called triglycerides, are mixtures derived from diverse fatty acids. Triglycerides can be converted to soap in either a one- or a two-step process. In the traditional one-step process, the triglyceride is treated with a strong base (e.g., lye), which accelerates cleavage of the ester bond and releases the fatty acid salt and glycerol. This process is the main industrial method for producing glycerol. If necessary, soaps may be precipitated by salting it out with saturated sodium chloride. The saponification value is the amount of base required to saponify a fat sample. For soap making, the triglycerides are highly purified, but saponification includes other base hydrolysis of unpurified triglycerides.

Significance

High saponification value indicates low molecular weight fatty acids and vice versa.

NUCLEIC ACIDS

Nucleic acids are present in all living organisms, whether plants, animals or viruses. They are generally associated with proteins to form *nucleoproteins*. There are two types of nucleic acids, *deoxyribose nucleic acid* (DNA) and *ribose nucleic acid* (RNA). DNA is found predominantly in the nucleus, while RNA is predominant in the cytoplasm. DNA is the genetic material of most organisms, including many viruses. Some viruses, however, have RNA as their genetic material.

DEOXYRIBOSE NUCLEIC ACID (DNA)

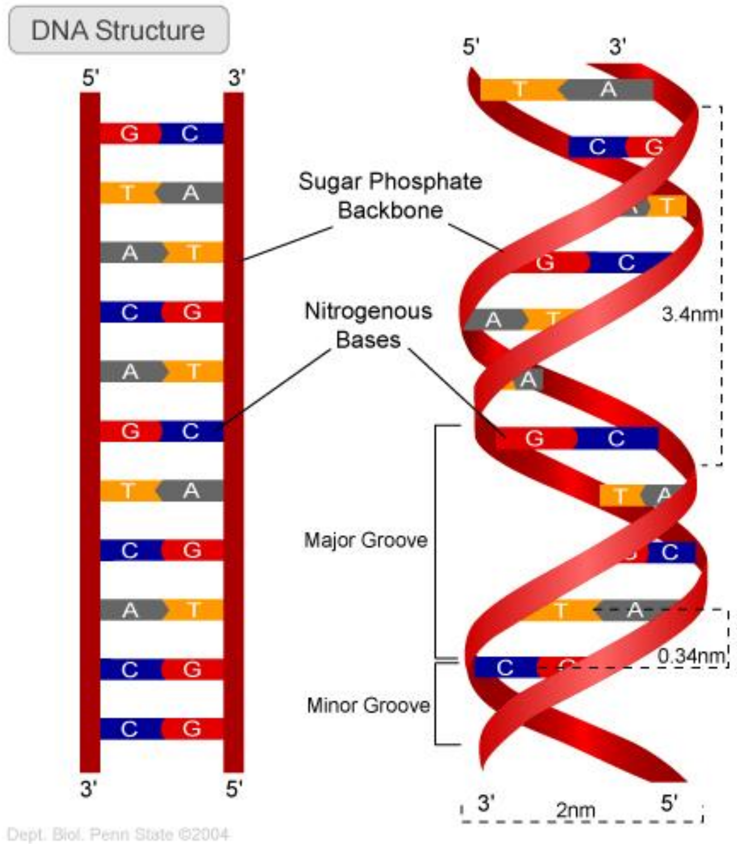
DNA is present in the cells of all plants, animals, prokaryotes and in a number of viruses. In eukaryotes it is combined with proteins to form *nucleoproteins.* In prokaryotes (e.g. *Escherichia coli,* a bacterium) the genetic material consists of a single giant molecule of DNA about 1,000 microns in length, without any associated proteins. DNA is present mainly in the chromosomes. It has also been reported in cytoplasmic organelles like mitochondria and chloroplasts. The DNA of all plants and animals and many viruses (polyoma virus, small-pox virus, bacteriophages T2, T4 and T6) is double stranded. In the bacteriophage Φx 174, however, it is single stranded. In some viruses the genetic material is RNA. In the tobacco mosaic virus (TMV), A tobacco virus, influenza virus poliomyelities virus and the bacterial viruses F2 and R17 the RNA is single stranded. In reovirus and the wound tumour virus, however, RNA is double stranded. In bacteria and in higher plants and animals both DNA and RNA are present. Viruses usually contain either DNA or RNA.

Table: Genetic material of organisms.

Double Stranded DNA. (dsDNA)	Higher animals and plants, Bacteria. Polyoma virus and small-pox virus. The T-even bacteriophages (T2,T4,T6).
Single Stranded DNA. (ssDNA)	The bacteriophage Φx 174, and several bacterial viruses.
Double Stranded RNA. (dsRNA)	Reo group of viruses. Wound tumour virus.
Single Standed RNA. (ssRNA)	Tobacco mosaic virus. A tobacco virus. Influenza virus. Poliomyelitis virus. Bacterial viruses F2 and R17.

STRUCTURE OF DNA

The widely accepted molecular model of DNA is the double helix structure proposed by Watson and Crick (1953). The DNA molecule consists of two helically twisted strands connected, together by 'steps' as shown in figure.



1. Each strand consists of alternating molecules of *deoxyribose* (a pentose sugar) and *phosphate* groups.
2. Each step is made up of a double ring *purine* base and a single ring - *pyrimidine* base.
3. The purine and pyrimidine bases are connected to deoxyribose sugar molecules.
4. The two strands are intertwined in a clockwise direction, i.e. in a right hand helix, and run in opposite directions.
5. The strand completes a turn each 34A. Each nucleotide occupies 3.4A. Thus, there are 10 nucleotides per turn.
6. Each successive nucleotide turns 36 degrees in the horizontal plane.
7. The width of the DNA molecule is 20A.
8. The twisting of the strands results in the formation *of deep* and *shallow spiral grooves.*

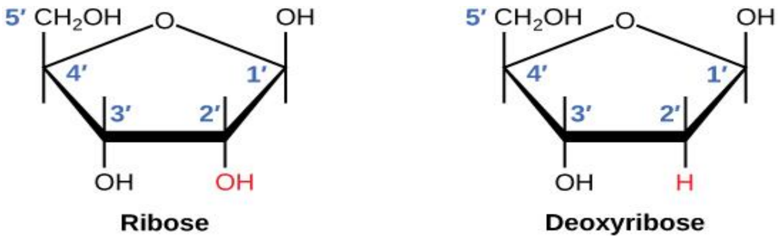
The DNA molecule is a *polymer* consisting of several thousand pairs of *nucleotide monomers.* Each nucleotide consists of the pentose sugar *deoxyribose, & phosphate* group and a *nitrogenous base* which may be either a *purine* or a *pyrimidine.* Deoxyribose and a nitrogenous base together form a *nucleoside.* A nucleoside and a phosphate together form a *nucleotide.*

Nucleoside=Deoxyribose+Nitrogenous base.

Nucleotide=Deoxyribose+Nitrogenous base+Phosphate.

(1) Deoxyribose

It is a pentose sugar with five carbon atoms. Four of the five carbon atoms plus a single atom of oxygen form a five-membered ring.



The fifth carbon atom is outside the ring and forms a part of a $-CH_2$ group. The four atoms of the ring are numbered 1' 2', 3' and 4'. The carbon atom of $-CH_2$ is numbered 5'. There are three-OH groups in positions 1', 3' and 5'. Hydrogen atoms are attached to carbon atoms 1', 2', 3' and 4'.

Ribose, the pentose sugar of RNA, has an identical structure except that there is an -OH group instead of H on carbon atom 2'.

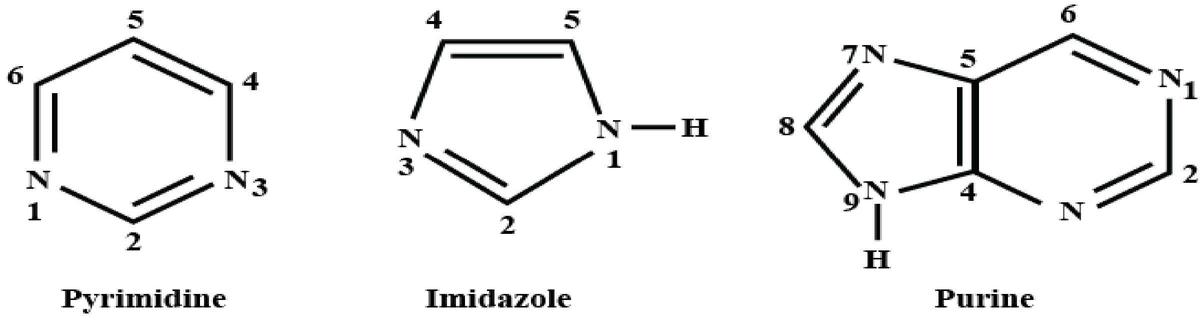
All the sugars in one strand are directed to one end, i.e. the strand has polarity. The sugars of the two strands are directed in opposite directions.

(2) **Nitrogenous bases.**

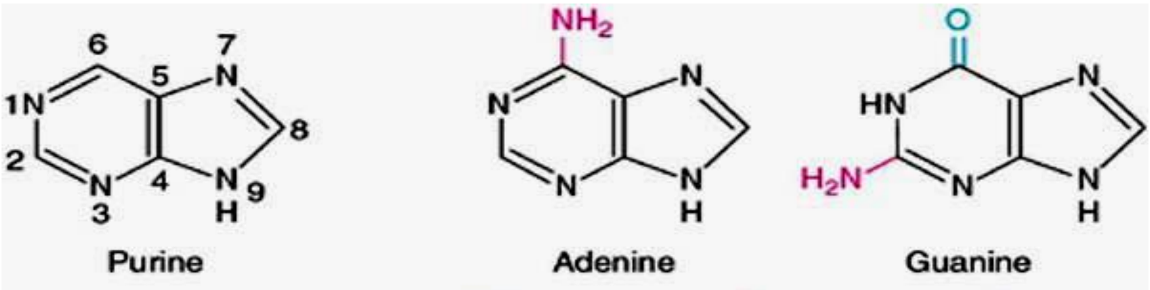
There are two types of nitrogenous bases, *purines* and *pyrimidines.*

Purines

These are double ring compounds. A purine molecule consists of a 5-membered *imidazole* ring joined to a pyrimidine ring at positions 4' and 5'.

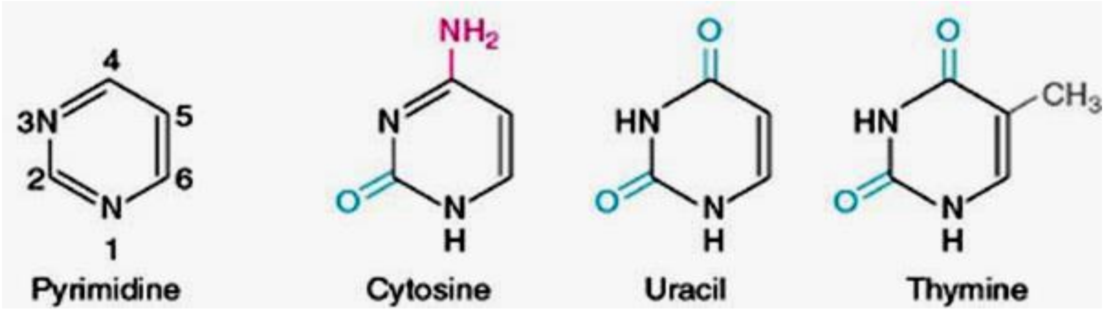


The two most common purines of DNA are *adenine* (A) and *guanine* (G).



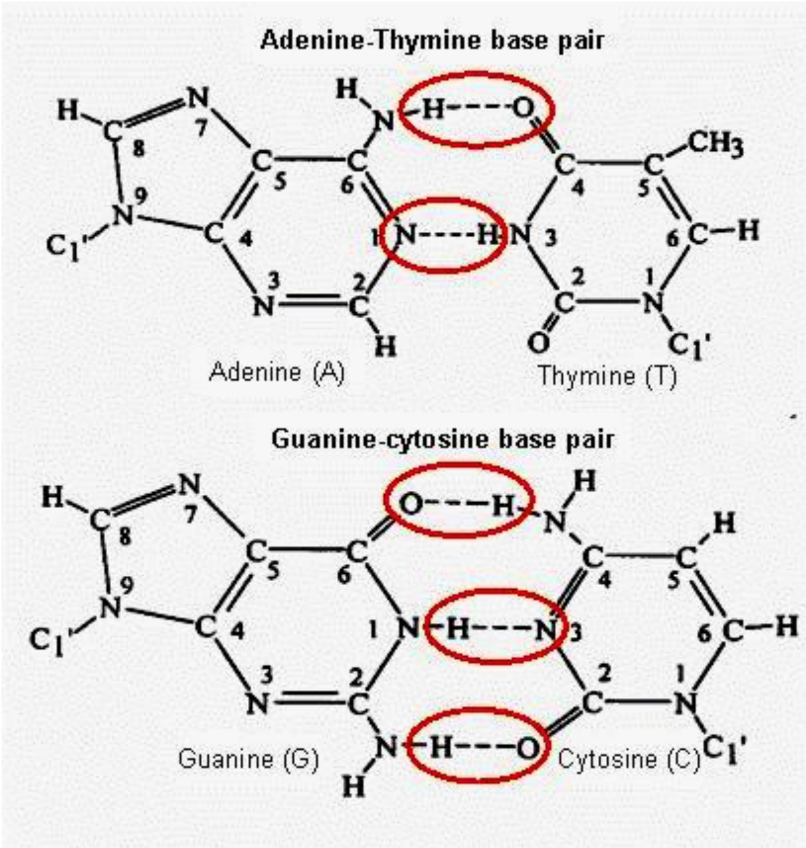
Pyrimidines

These are single ring compounds, with nitrogen in positions 1' and 3' of a 6-membered benzene ring. The two most common pyrimidines of DNA are *cytosine* (C) and *thymine* (T).



Base pairing

Each 'step' of the DNA ladder is made up of a purine and a pyrimidine pair, i.e. of a double ring and a single ring compound.



Two purines would occupy too much space, while two pyrimidines would occupy too little. Because of the purine-pyrimidine pairing the total number of purines in a double-stranded DNA molecule is equal to the total number of pyrimidines. Thus A/T = 1 and G/C = 1 or A+G = C+T. The ratio A+T/G+C, however, rarely equals 1, and varies with different species from 0.4 to 1.9. This ratio is commonly low in micro-organisms and high in higher animals.

The purine and pyrimidine bases pair only in certain combinations. Adenine pairs with thymine (A-T) and guanine with cytosine (G-C). The total width of the pair is 10.7A. Adenine and thymine are joined by two hydrogen bonds through atoms attached to positions 6' and 1'.

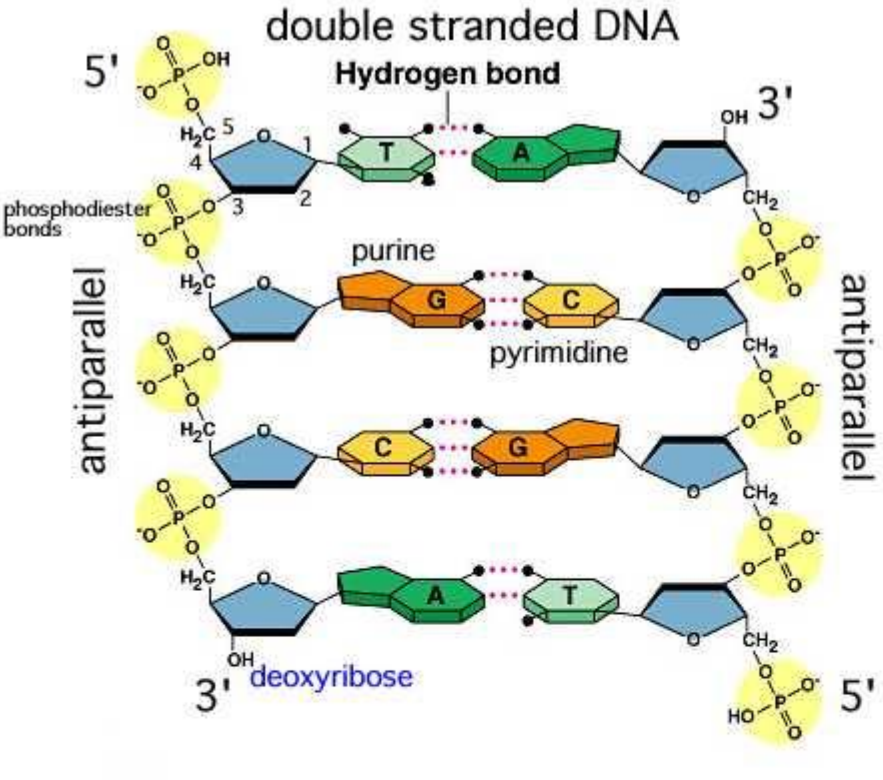
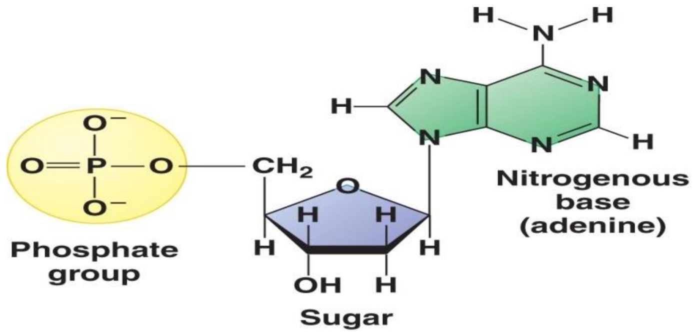


Fig: Segment of DNA molecule showing pairing between two nucleotides.

Cytosine and guanine are joined by *three* hydrogen bonds through positions 6', 1' and 2'. The hydrogen atom with its positive charge is shared between an oxygen atom and a nitrogen atom, both with slight negative charges. Although hydrogen bonds are weak, their more number gives stability to the DNA molecule. The weak hydrogen bonding enables the two strands of the DNA to separate during replication. The pyrimidine and purine bases; are linked to the deoxyribose sugar molecules. The linkage in pyrimidine nucleosides is between position 1' of deoxyribose and 3' of the pyrimidine. In purine nucleosides it is between position 1' of deoxyribose and position 9' of the purine.

(3) **Phosphate.** In the DNA strand the phosphate groups alternate with deoxyribose. Each phosphate group is joined to carbon atom 3' of one deoxyribose and to carbon atom 5' of another. Thus each strand has a 3' end and a 5' end. The two strands are oriented in opposite directions. The 3' end of one

strand corresponds to the 5' end of the other. Consequently the oxygen atoms of deoxyribose point in opposite directions in the two strands.



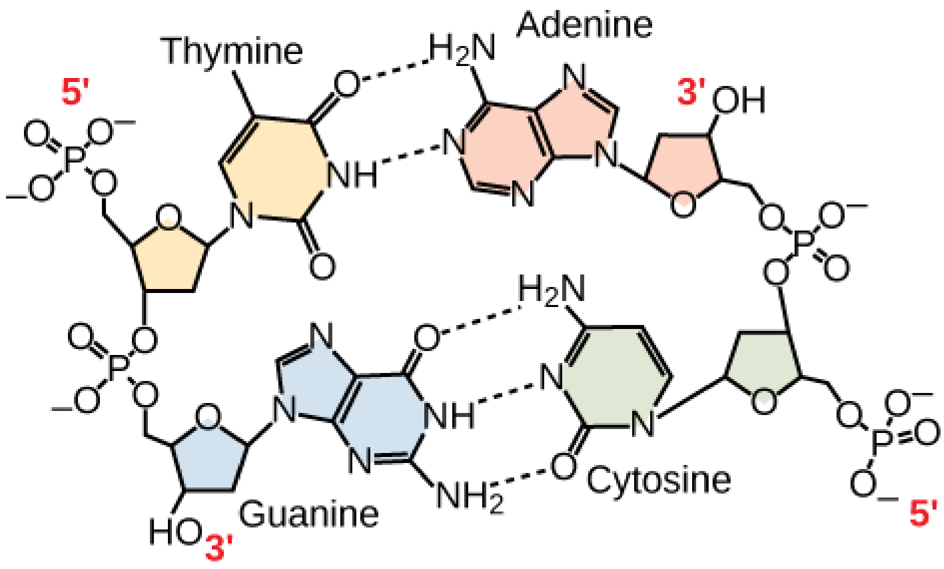
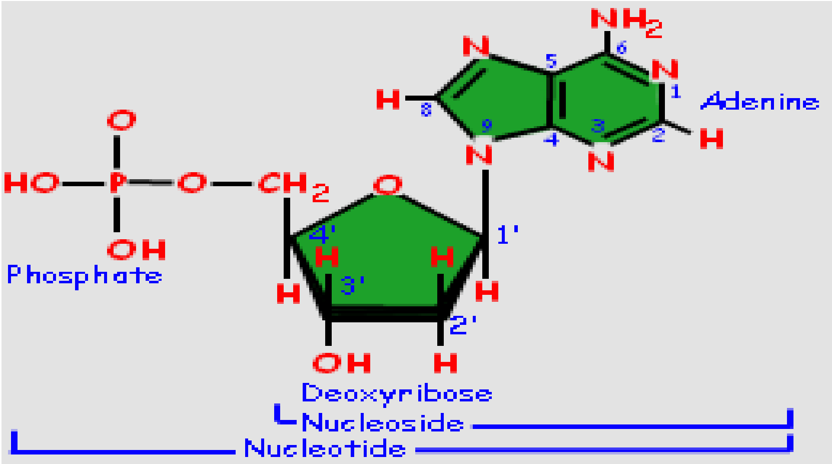
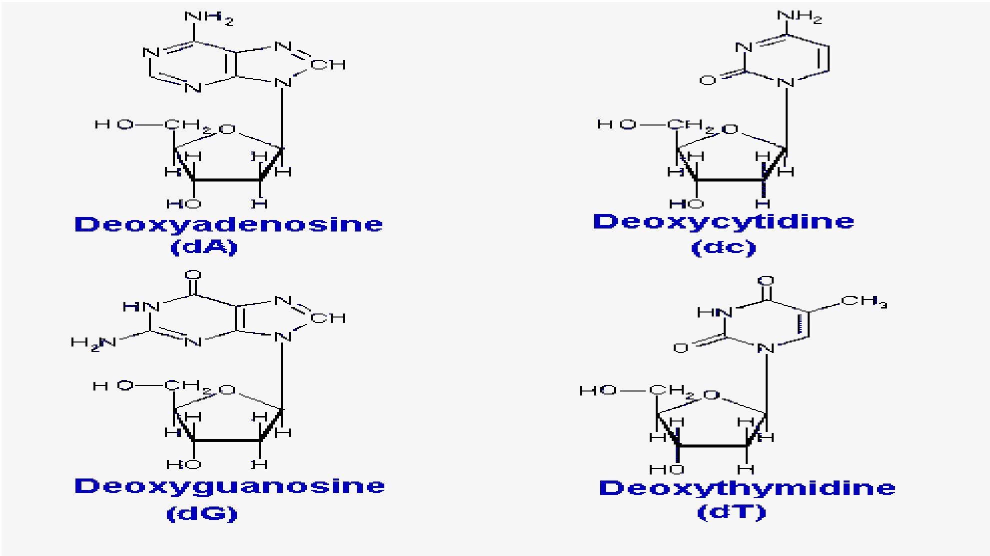


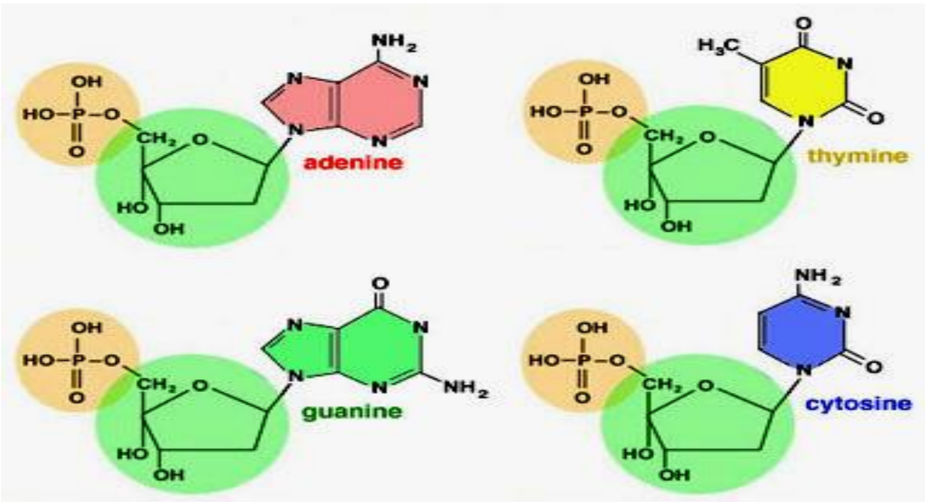
Fig: Two pairs of nucleotides showing bonding between phosphate, deoxyribose and nitrogenous bases.

Nucleosides and nucleotides. A *sugar molecule* and a *nitrogenous base* form a *nucleoside*, and a nucleoside plus a *phosphate* group form a *nucleotide*. In other words a nucleoside is a *base-sugar combination* and a nucleotide is a *nucleoside phosphate*. The nucleotides of RNA are called *ribonucleotides*, and those of DNA *deoxyribonucleotides*. Ribonucleotides contain the sugar ribose and deoxyribonucleotides the sugar *deoxyribose*.





Nucleosides



Nucleotides

- **Types / Forms of DNA**

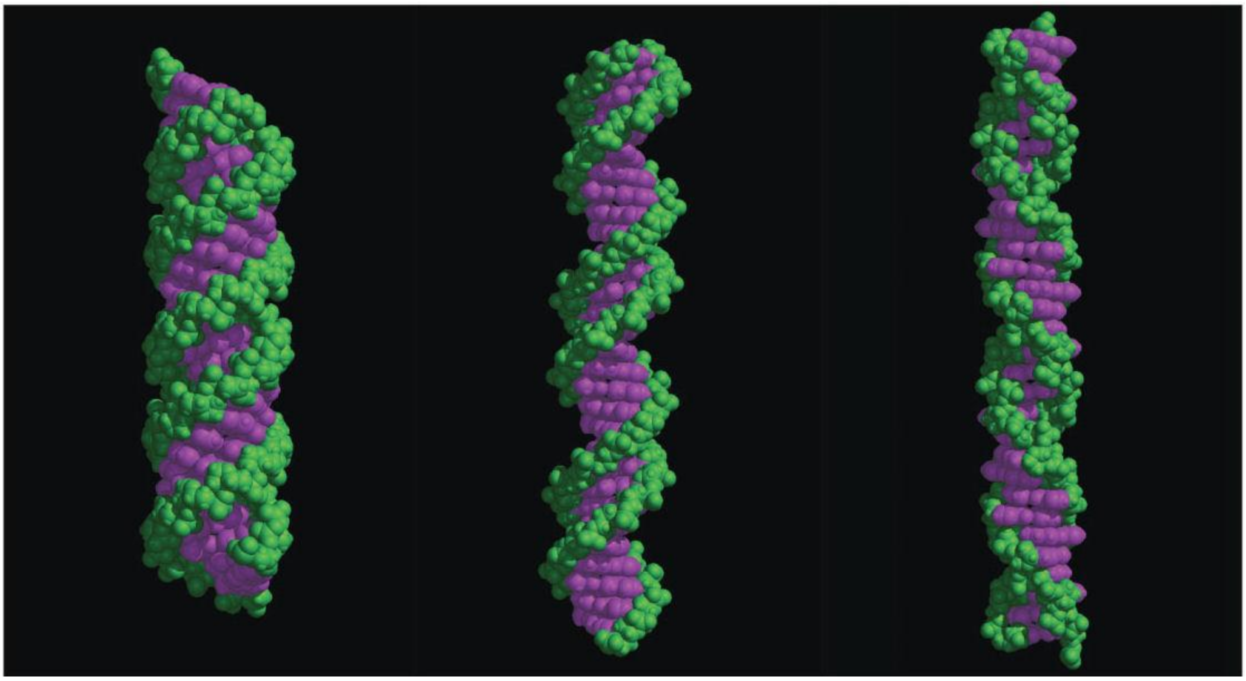
DNA can exist as single, double-stranded, or mixed forms. dsDNA has a linear sequence (primary structure), secondary structure (right handed double helix), and tertiary/quaternary structure (it is folded and packed in the cell).The **primary structure** is the sequence of nucleoside monophosphates (usually written as the sequence of bases they contain).The **secondary structure** refers to the shape a nucleic acid assumes as a result of the **primary structure**. A-DNA, B-DNA and Z-DNA are forms of **secondary structure. Tertiary structure** refers to large-scale folding in a linear polymer that is at a higher order than **secondary structure**. The **tertiary structure** is the specific three dimensional shape into which an entire chain is folded.

A DNA: - The **right-handed 'A' form** occurs in crystalline structures where the water concentration is reduced. The structure is distorted and the bases are no longer co-planar. A-DNA occurs when DNA is dehydrated, but also in DNA/RNA hybrids. It is a right-handed double helix fairly similar to the more common and well-known B-DNA form, but with a shorter more compact helical structure whose base pairs are not perpendicular to the helix-axis as in B-DNA.

The same helical conformation is the most commonly seen one in double stranded RNA's. There is a slight increase in the number of base pairs (bp) per rotation (resulting in a tighter rotation angle), and smaller rise per turn. This results in a deepening of the major groove and a shallowing of the minor. A thicker right-handed duplex with a shorter distance between the base pairs has been described for RNA-DNA duplexes and RNA-RNA duplexes.

B DNA: - The **right-handed 'B' form** is the standard form in biological systems. The double stranded DNA molecule is a right-handed helix as determined by Watson and Crick using Franklin's x-ray diffraction images. This B-form of DNA has approximately 10 nucleotides per turn of the helix and is the most common form of DNA found in nature. The most common form, present in most DNA at neutral pH and physiological salt concentrations, is B-form. That is the classic, right-handed double helical structure. The major difference between A-form and B-form nucleic acid is in the conformation of the deoxyribose sugar ring. It is in the C2' endo-conformation for B-form,whereas it is in the C3' endo-conformation in A-form. In the C2' endo-conformation, the C2' atom is above the plane, whereas the C3' atom is above the plane in the C3' endo-conformation. The latter conformation brings the 5'and3' hydroxyls (both esterified to the phosphates linking to the next nucleotides) closer together than is seen in the C2' endo-confromation. Thus the distance between adjacent nucleotides is reduced by about 1 Angstrom in A-form relative to B-form nucleic acid. In B-form, the base-pairs are almost centred over the helical axis, but in A-form, they are displaced away from the central axis and closer to the major groove. The result is a ribbon-like helix with a more open cylindrical core in A-form.

Z DNA: - The **left-handed Z form** occurs occasionally in the middle of a **B-**form molecule. A small amount of the DNA in a cell exists in the Z form. Z-DNA is a radically different duplex structure, with the two strands coiling in left-handed helices and a pronounced zig-zag (hence the name) pattern in the phosphodiester backbone. Bases are co-planar in both the **B** and **Z** forms. The high salt and GC base-pairs, used to form the DNA crystals because the helix to twist in a left-handed way, creating a structure called Z-DNA. A third form of duplex DNA has a strikingly different, left-handed helical structure. This Z-DNA is formed by stretches of alternating purines and pyrimidines, e.g. GCGCGC, especially in negatively supercoiled DNA. The big difference is at the G nucleotide. It has the sugar in the C3' endo-conformation (like A-form nucleic acid, and in contrast to B-form DNA) and the guanine base is in the syn-conformation. This places the guanine back over the sugar ring, in contrast to the usual anti-conformation seen in A- and B-form nucleic acid. Note that having the base in the anti-conformation places it in the position where it can readily form H-bonds with the complementary base on the opposite strand. The duplex in Z-DNA has to accommodate the distortion of this G nucleotide in the syn-conformation. The cytosine in the adjacent nucleotide of Z-DNA is in the "Normal" C2' endo, anti-conformation.



Feature	A-DNA	B-DNA	Z-DNA
Type of helix	Right-handed	Right-handed	Left-handed
Helical diameter (nm)	2.55	2.37	1.84
Rise per base pair (nm)	0.29	0.34	0.37
Distance per complete turn (pitch) (nm)	3.2	3.4	4.5
Number of base pairs per complete turn	11	10	12
Topology of major groove	Narrow, deep	Wide, deep	Flat
Topology of minor groove	Broad, shallow	Narrow, shallow	Narrow, deep

Functions of DNA:

1. Genetic Information (Genetic Blue Print):

DNA is the genetic material which carries all the hereditary information. The genetic information is coded in the arrangement of its nitrogen bases.

2. Replication:

DNA has unique property of replication or production of carbon copies (Autocatalytic function). This is essential for transfer of genetic information from one cell to its daughters and from one generation to the next.

3. Chromosomes:

DNA occurs inside chromosomes. This is essential for equitable distribution of DNA during cell division.

4. Recombination's:

During meiosis, crossing over gives rise to new combination of genes called recombinations.

5. Mutations:

Changes in sequence of nitrogen bases due to addition, deletion or wrong replication give rise to mutations. Mutations are the fountain head of all variations and evolution.

6. Transcription:

DNA gives rise to RNAs through the process of transcription. It is heterocatalytic activity of DNA.

7. Cellular Metabolism:

It controls the metabolic reactions of the cells through the help of specific RNAs, synthesis of specific proteins, enzymes and hormones.

8. Differentiation:

Due to differential functioning of some specific regions of DNA or genes, different parts of the organisms get differentiated in shape, size and functions.

9. Development:

DNA controls development of an organism through working of an internal genetic clock with or without the help of extrinsic information.

10. DNA Finger Printing:

Hypervariable microsatellite DNA sequences of each individual are distinct. They are used in identification of individuals and deciphering their relationships. The mechanism is called DNA finger printing.

11. Gene Therapy:

Defective heredity can be rectified by incorporating correct genes in place of defective ones.

12. Antisense Therapy:

Excess availability of anti-mRNA or antisense RNAs will not allow the pathogenic genes to express themselves. By this technique failure of angioplasty has been checked. A modification of this technique is RNA interference (RNAi).

- **RNA (Ribose Nucleic Acid)**

- Ribonucleic acid (RNA) is a biopolymer macromolecule as DNA. It consists of small subunits called nucleotides composed of:

  − Purine nucleobases [Adenine−(A), Guanine−(G)]
  − Pyrimidine nucleobases [Cytosine−(C), Uracil−(U)]
  − Ribose pentose sugars [$C_5H_{10}O_5$]
  − Phosphate groups [PO_4^{3-}]

- The nitrogenous base is attached on the Ribose by an N−glycosidic bond
- The ribose is bonded to the phosphate group through ester bonds
- The backbone bonding between RNA nuclotides (i.e. the bonds between the phosphate group and an adjacent ribose sugar) occurs through phosphodiester bonds. A phosphate group is attached to the 3'−carbon position of one ribose and on the 5'−carbon position of the next

Characteristics

- RNA does not self-replicate in order to multiply; instead it is encoded by DNA genes
- RNA is synthesized in order for the translation of DNA
- They contain a continuous sequence of nucleotides encoding a specific polypeptide;

- They are found in the cytoplasm; and
- They are either attached to ribosomes or capable of such an attachment in order to be translated.
- Consistent with the range of molecular masses observed for polypeptides, mRNAs vary in length from a few hundred nucleotides to several thousand nucleotides;
- mRNAs generally are more labile than rRNAs and tRNAs, although the turnover rate for some mRNAs is quite slow.

Types of RNA

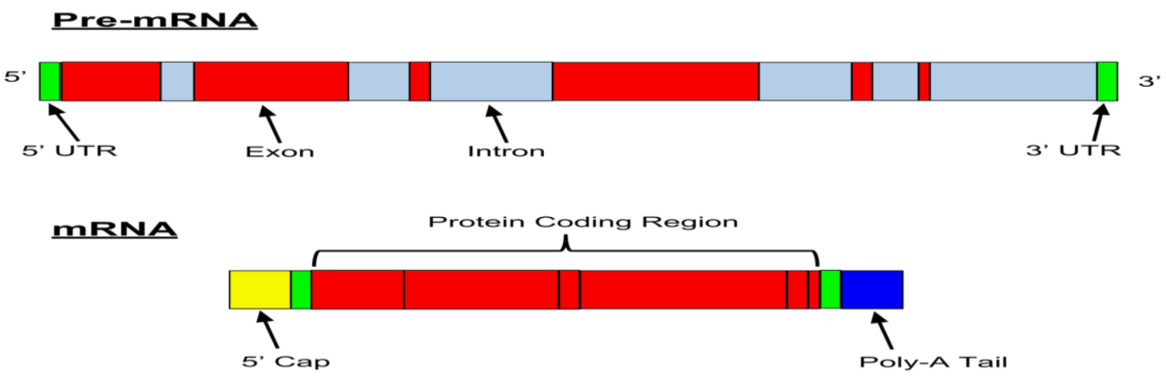
1. messenger RNA (mRNA),
2. ribosomal RNA(rRNA)
3. transfer RNA (tRNA).

1. messenger RNA (mRNA)

Messenger RNA serves as the template for protein synthesis. It constitutes 2 – 5 per cent of the total RNA of the normal cell. It was first detected by Hershey (1956). The name and concept of messenger RNA was first given by F. Jacob and J. Monod (1961). Messenger RNA is a linear molecule transcribed from one strand of DNA. It carries the base sequence complementary to DNA template strand. The base sequence of mRNA is in the form of consecutive triplet codons. Ribosomes translate these triplet codons into amino acid sequence of polypeptide chain. m-RNA is formed from the template strand of the DNA duplex. Messenger RNAs encode the amino acid sequences of proteins. Messenger RNA (mRNA) is the RNA that carries information from DNA to the ribosome, the sites of protein synthesis (translation) in the cell. The coding sequence of the mRNA determines the amino acid sequence in the protein that is produced. The sequence carried on m-RNA is read in the form of codon.

Length of mRNA depends upon the length of polypeptide chain it Codes for. Polypeptide length varies from a chain of a few amino acids to thousands of amino acids. For example, a sequence of 600 nucleotides will code for a polypeptide having a chain of 200 amino acids. The message is read in the groups of three consecutive bases from a fixed starting point.

All mRNAs have two types of regions. The coding region consists of series of codons. But the mRNA is longer than the coding regions. Length of newly synthesized mRNA is much larger than the length of mRNA used for translation. The coding regions are called exons. Between the coding regions lie various non-coding regions called introns. Genes with these intervening sequences are called Split genes or Interrupted genes.



2. ribosomal RNA (rRNA)

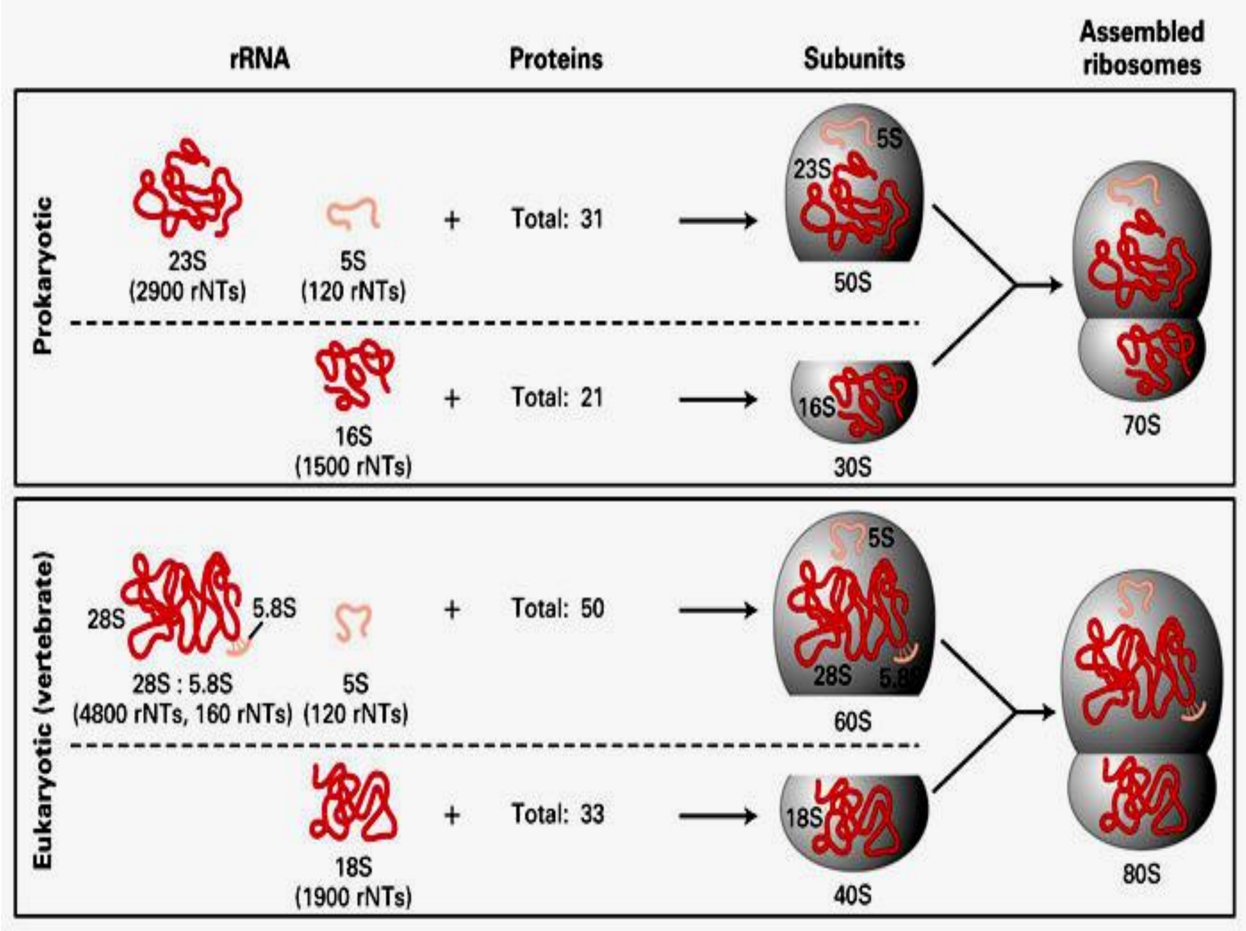
Most of the RNA of the cell is in the form of ribosomal RNA which constitutes about 85% of the total RNA. Ribosomes consist of many types of rRNA. The 70S ribosome of prokaryotes, in its smaller subunit of 30S has 16S rRNA. The 50S larger subunit consists of 23S and 5S rRNA. Similarly 80S ribosome has 18S rRNA in its smaller subunit of 40S. The 60S larger subunit has 28S, 5.8S and 5S rRNA.

The rRNA molecules form secondary structure of double stranded stems and single stranded loops by extensive complementary base pairings. The rRNA plays major role in protein synthesis. They interact with mRNA and tRNA at each step of translation or protein synthesis.

The 3′ terminus of rRNA of 16S rRNA interacts with initiation site on mRNA which is called Shine-Dalgarno sequence and lies just before the start codon AUG.

The 23S rRNA plays an active role in peptidyl transferase activity. Movement of tRNA between A and P site on ribosome is aided by 23 S rRNA.

The rRNA molecules form complexes with specific proteins in ribosomes. The RNA- protein complexes are called ribonucleoproteins (RNP).



3. transfer RNA (tRNA)

It transports amino acids to ribosome and decodes the information of mRNA. Each nucleotide triplet codon on mRNA represents an amino acid. The tRNA plays the role of an adaptor and matches each codon to its particular amino acid in the cytopolasmic pool.

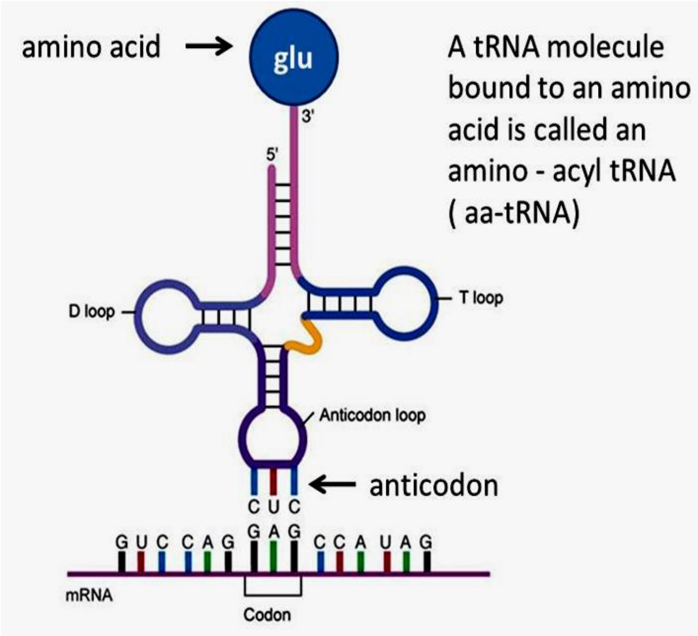
The tRNA has two properties:

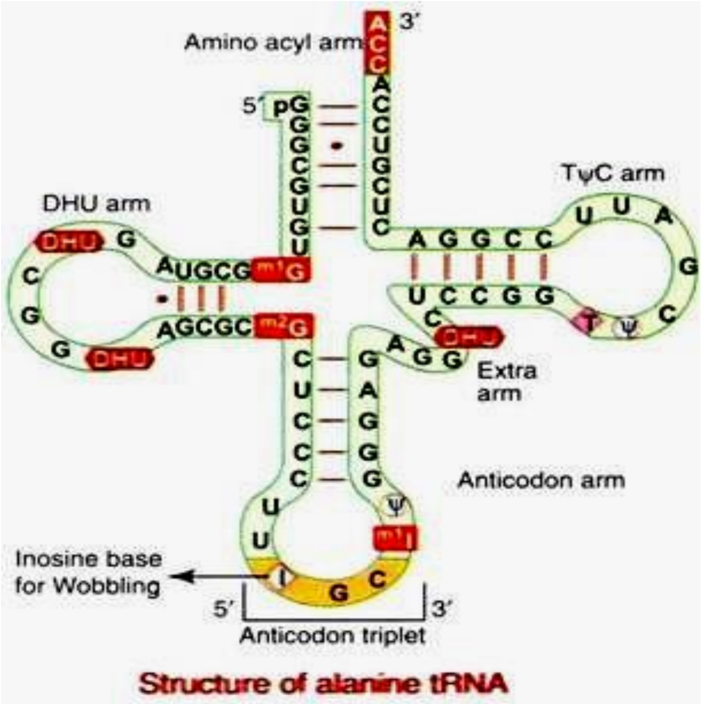
(a) It represents a single amino acid to which it binds covalently.

(b) It has two sites. One is a trinucleotide sequence called anticodon, which is complementary to the codon of mRNA. The codon and anticodon form base pairs with each other. The other is amino acid binding site.

There are many different kinds of tRNA molecules in a cell. Each tRNA is named after the amino acid it carries. For example if tRNA carries amino acid tyrosine it is written as $tRNA^{Tyr}$. Sometimes there are more than one tRNA for an amino acid, then it is denoted as $tRNA_1^{Try}$ and $tRNA_2^{Try}$. A minimum of 32 tRNAs are required to translate all 61 codons.

The tRNA charged with an amino acid is called amino acyl tRNA.





Structure of alanine tRNA

Clover Leaf Structure of tRNA:

The primary structure of all tRNA molecules is small, linear, single stranded nucleic acid ranging in size from 73 to 93 nucleotides. The tRNA due to its property of having stretches of complementary base pairs forms secondary structure, which is in the form of a cloverleaf.

Several regions of the single stranded molecule form double stranded stems or arms and single stranded loops due to folding of various regions of the molecule. These double stranded stems have complementary base pairs. A typical tRNA has bases numbering from 1-76, using the standard numbering convention where position 1 is the 5′ end and 76 is the 3′ end.

The various regions of the clover leaf model of tRNA are as follows:

1. Amino acid arm:

It has a seven base pairs stem formed by base pairing between 5′ and 3′ ends of tRNA. At 3′ end a sequence of 5′-CCA-3′ is added. This is called CCA arm or amino acid acceptor arm. Amino acid binds to this arm during protein synthesis.

2. D-arm:

Going from 5′ to 3′ direction or anticlockwise direction, next arm is D-arm. It has a 3 to 4 base pair stem and a loop called D-loop or DHU-loop. It contains a modified base dihydrouracil.

3. Anticodon arm:

Next is the arm which lies opposite to the acceptor arm. It has a five base pair stem and a loop in which there are three adjacent nucleotides called anticodon which are complementary to the codon of mRNA.

4. An extra arm:

Next lies an extra arm which consists of 3-21 bases. Depending upon the length, extra arms are of two types, small extra arm with 3-5 bases and other a large arm having 13-21 bases.

5. T-arm or TψC arm:

It has a modified base pseudouridine ψ. It has a five base pair stem with a loop.

There are about 50 different types of modified bases in different tRNAs, but four bases are more common. One is ribothymidine which contains thymine which is not found in RNA. Other modified bases are pseudouridine ψ, dihyrouridine and inosine.

Difference Between: **DNA and RNA**

Sr No	DNA	RNA
1.	It usually occurs inside nucleus and some cell organelles. (Mitochondria and Chloroplast in plants)	Very little RNA occurs inside the nucleus. Most of it is found in the cytoplasm.
2.	It is double stranded with exception of some viruses (e.g., Øx174)	It is single stranded with exception of some viruses (*Reovirus*)
3.	DNA contains over a million nucleotides	Depending on the type, RNA contains 70-12,000 nucleotides.
4.	The sugar portion of DNA is 2-deoxyribose	The sugar portion of RNA is ribose
5.	Purine and Pyrimidine bases are in equal in number.	There is no proportionality in between numbers of Purine and Pyrimidine bases.
6.	The base present in DNA are adenine	The base present in RNA are adenine

	(A), guanine (G) ,thymine (T) and cytosine (C)	(A), guanine (G) , uracil (U) and cytosine (C)
7.	Hydrogen bonds are formed between complementary nitrogen bases of the opposite strands (A-T, C-G)	Base pairing through hydrogen bonds, occurs in the coiled parts.
8.	DNA is spirally twisted to produce a regular helix.	The strand may get folded at places to produce a secondary helix or pseudohelix.
9.	It replicates to form new DNA molecules	It cannot normally replicate itself.
10.	It occurs in the form of chromatin or chromosomes.	It occurs in ribosomes or forms association with ribosomes.
11.	The function of DNA is to transfer genetic information from one generation to the next.	The function of RNA is to direct synthesis of proteins in the body.
12.	DNA transcribes genetic information to RNA. Its quantity is fixed for cell.	RNA translates the transcribed message for forming polypeptides. The quantity of RNA of a cell is variable.
13.	Renaturation after melting is slow.	It is quite fast
14.	DNA is only two types: intra nuclear and extra nuclear.	Three different types of RNA: m-RNA, t-RNA and r-RNA.
15.	It is long lived	Some RNAs are very short lived while others have somewhat longer life.