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Department of Vety. Physiology & Biochemistry

7.Biochemistry of Nucleic Acids

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Introduction

Nucleotides have a variety of roles in cellular metabolism and they are the constituents of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), the molecular repositories of genetic information. The ability to store and transmit genetic information from one generation to the next is a fundamental condition for life.

DNA was discovered in 1869 by Johann Friedrich Miescher, a Swiss researcher. The demonstration that DNA contained genetic information was first made in 1944, by Avery, Macleod and MacCary.

A segment of a DNA molecule that contains the information required for the synthesis of a functional biological product, whether protein or RNA, is referred to as a **gene.** A cell typically has many thousands of genes, and DNA molecules, not surprisingly, tend to be very large. The storage and transmission of biological information are the only known functions of DNA.

RNAs have a broader range of functions, and several classes are found in cells. **Ribosomal RNAs (rRNAs)** are components of ribosomes, the complexes that carry out the synthesis of proteins. **Messenger RNAs (mRNAs)** are intermediaries, carrying genetic information from one or a few genes to a ribosome, where the corresponding proteins can be synthesized. **Transfer RNAs (tRNAs)** are adapter molecules that faithfully translate the information in mRNA into a specific sequence of amino acids.

Components of nucleic acids

Nucleic acids are the polymers of nucleotides (polynucleotides) held by 3' and 5' phosphate bridges.

Nucleotides have three characteristic components:

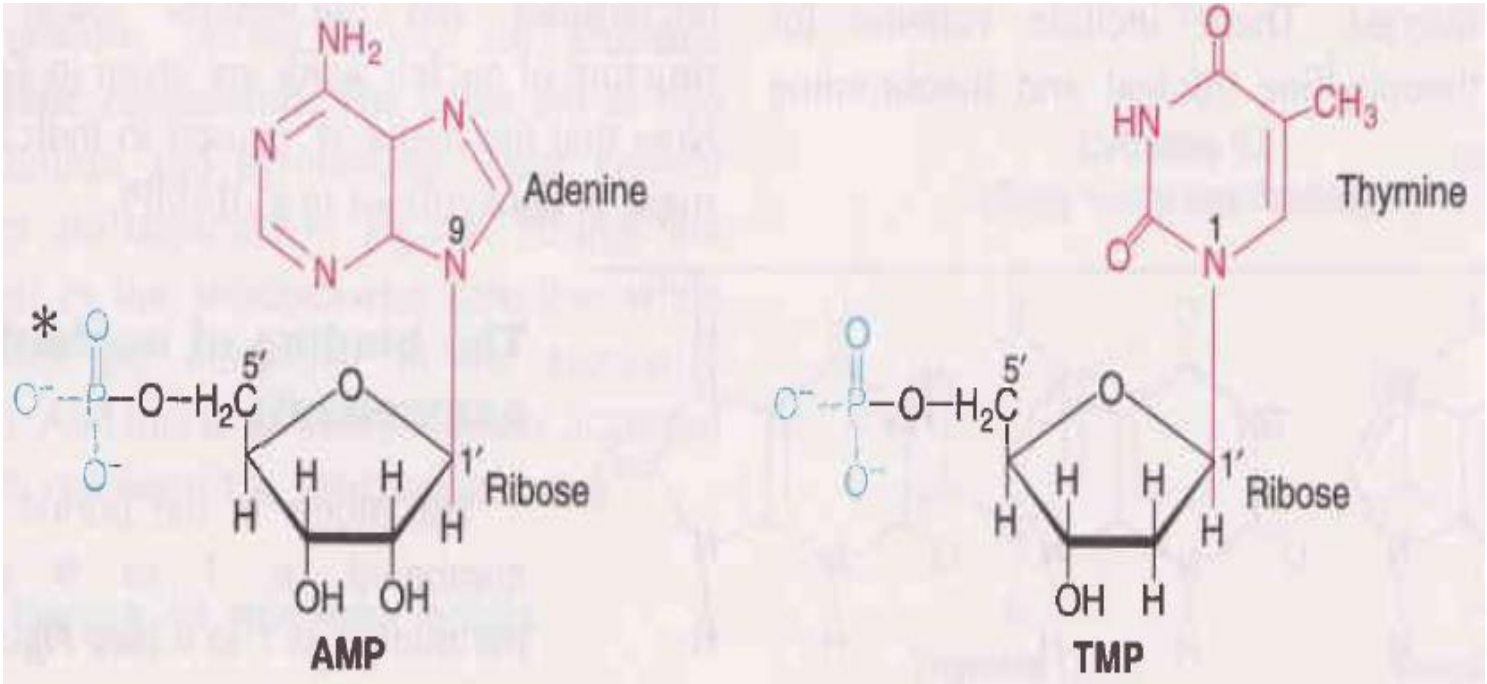
(1) a nitrogenous (nitrogen-containing) base

(2) a pentose, and

(3) a phosphate

The molecule without the phosphate group is called a nucleoside. The nitrogenous bases are derivatives of two parent compounds, **pyrimidine** and **purine.**

The base of a nucleotide is joined covalently (at N-1 of pyrimidines and N-9 of purines) in an *N*-β glycosyl bond to the 1 carbon of the pentose, and the phosphate is esterified to the 5 carbon.

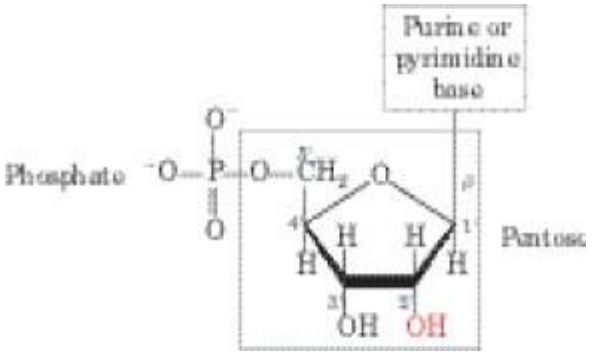


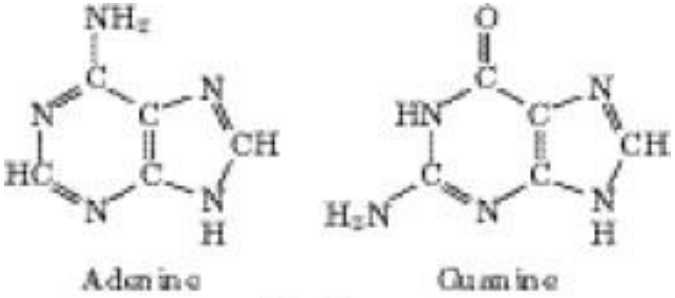
Both DNA and RNA contain two major purine bases, **adenine** (A) and **guanine** (G), and two major pyrimidines.

In both DNA and RNA one of the pyrimidines is **cytosine** (C), but the second major pyrimidine is not the same in both: it is **thymine** (T) in DNA and **uracil** (U) in RNA. Only rarely does thymine occur in RNA or uracil in DNA.

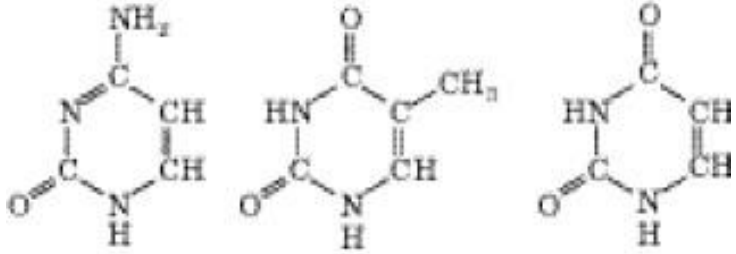
Table 5.1 Principal bases, nucleosides and nucleotides

Base	*Ribonucleoside*	*Ribonucleotide (5′-monophosphate)*	*Abbreviation*
Adenine (A)	Adenosine	Adenosine 5′-monophosphate or adenylate	AMP
Guanine (G)	Guanosine	Guanosine 5′-monophosphate or guanylate	GMP
Cytosine (C)	Cytidine	Cytidine 5′-monophosphate or cytidylate	CMP
Uracil (U)	Uridine	Uridine 5′-monophosphate or uridylate	UMP
Base	***Deoxyribonucleoside***	***Deoxyribonucleotide (5′-monophosphate)***	***Abbreviation***
Adenine (A)	Deoxyadenosine	Deoxyadenosine 5′-monophosphate or deoxyadenylate	dAMP
Guanine (G)	Deoxyguanosine	Deoxyguanosine 5′-monophosphate or deoxyguanylate	dGMP
Cytosine (C)	Deoxycytidine	Deoxycytidine 5′-monophosphate or deoxycytidylate	dCMP
Thymine (T)	Deoxythymidine	Deoxythymidine 5′-monophosphate or deoxythymidylate	dTMP

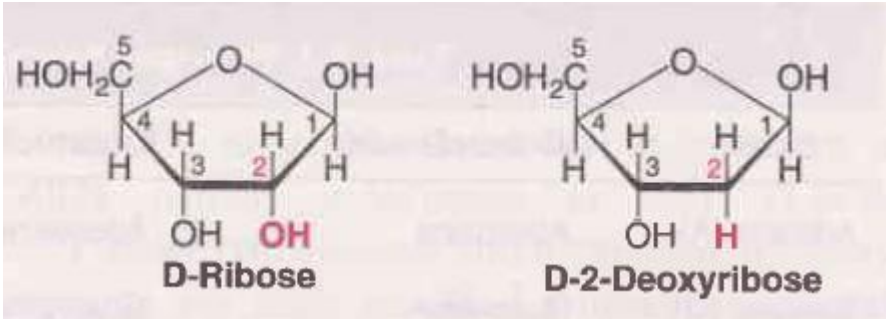




Purines



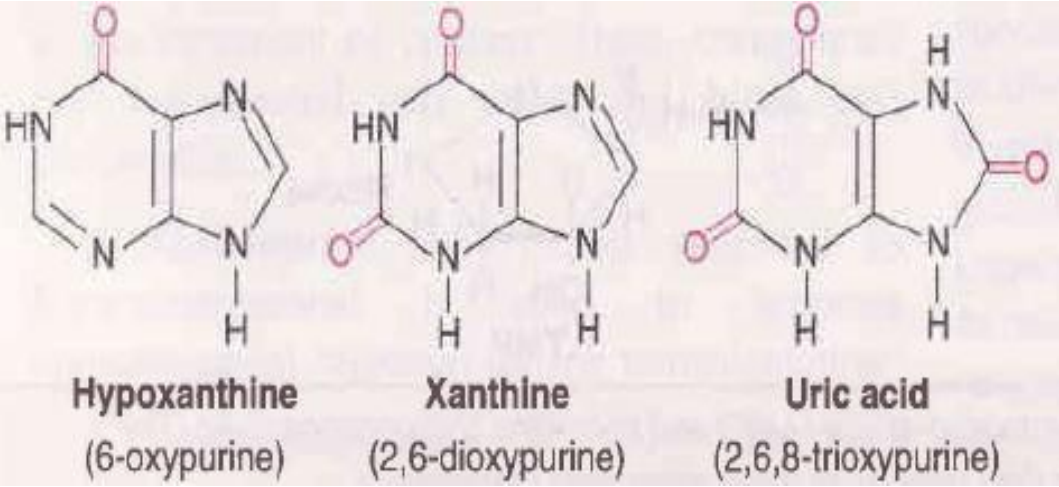
Pyrimidines



Biologically important purines

The bases such as hypoxanthine, xanthine and uric acid are present in the free state in the cells and biogically important purines. The first two are the intermediates in purine synthesis while uric acid is the end product of purine degradation.

Purine bases of plants:- Caffeine (of coffee), theophylline (of tea) and theobromine (of cocoa).



PURINE, PYRIMIDINE AND NUCLEOTIDE ANALOGS

1. Allopurinol - is used in the treatment of hyperuricemia and gout.

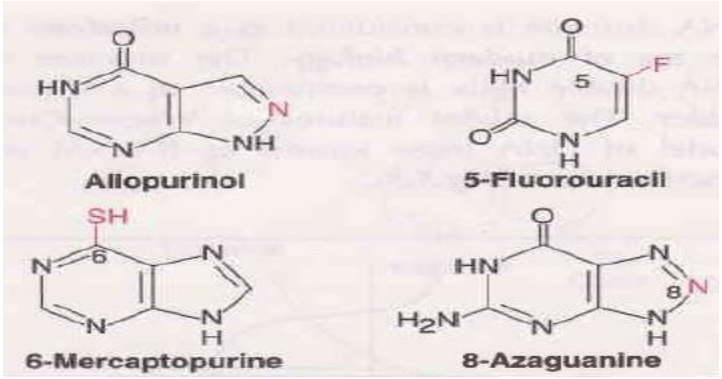
2.5-Fluorouracil, 6-mercaptopurine, 8-azaguanine, 3-deoxyuridine, 5- or 6-azauridine, 5- or 6-azacytidine and 5-idouracil are employed in the treatment of cancers.

3.Azathioprine (which gets degraded to 6-mercaptopurine) is used to suppress immunological rejection during transplantation.

4. Arabinosyladenine - is used for the treatment of neurological disease, viral encephalitis.

5. Arabinosylcytosine – is used in cancer therapy.

6. Zidovudine or AZT (3-azido 2',3' -dideoxythymidine) and didanosine (di-deoxyinosine) are sugar modified synthetic nucleotide analogs.



Structure of DNA

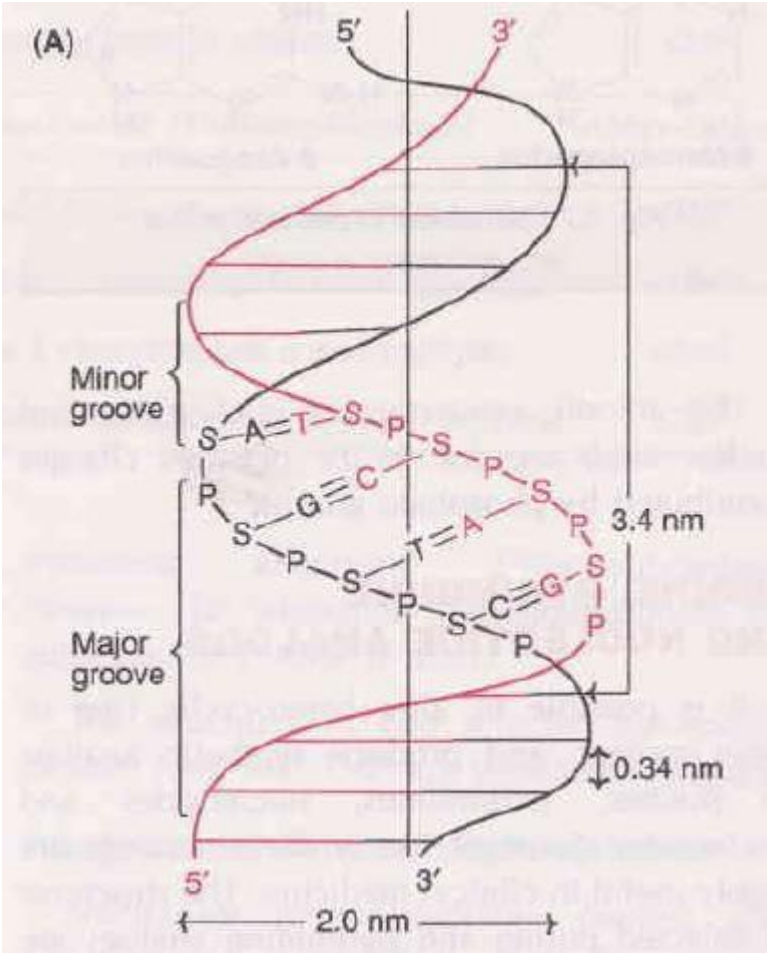
DNA is a polymer of deoxyribonucleotides (or simply deoxynucleotides). It is composed of monomeric units namely deoxyadenylate (dAMP), deoxyguanylate (dGMP), deoxycytidylate (dCMP) and deoxythymidylate (dTMP).

Chargaff's rule of DNA composition- He observed that in all the species he studied, DNA had equal numbers of adenine and thymine residues (A = T) and equal numbers of guanine and cytosine residues (G = C). This is known as Chargaff's rule of molar equivalence between the purines and pyrimidines in DNA structure.

Single-stranded DNA, and RNAs which are usually single-stranded, do not obey Chargaff's rule.

DNA DOUBLE HELIX:-

The double helical structure of DNA was proposed by lames Watson and Francis Crick in 1953 (Nobel Prize, 1962). The structure of DNA double helix is comparable to a twisted ladder.



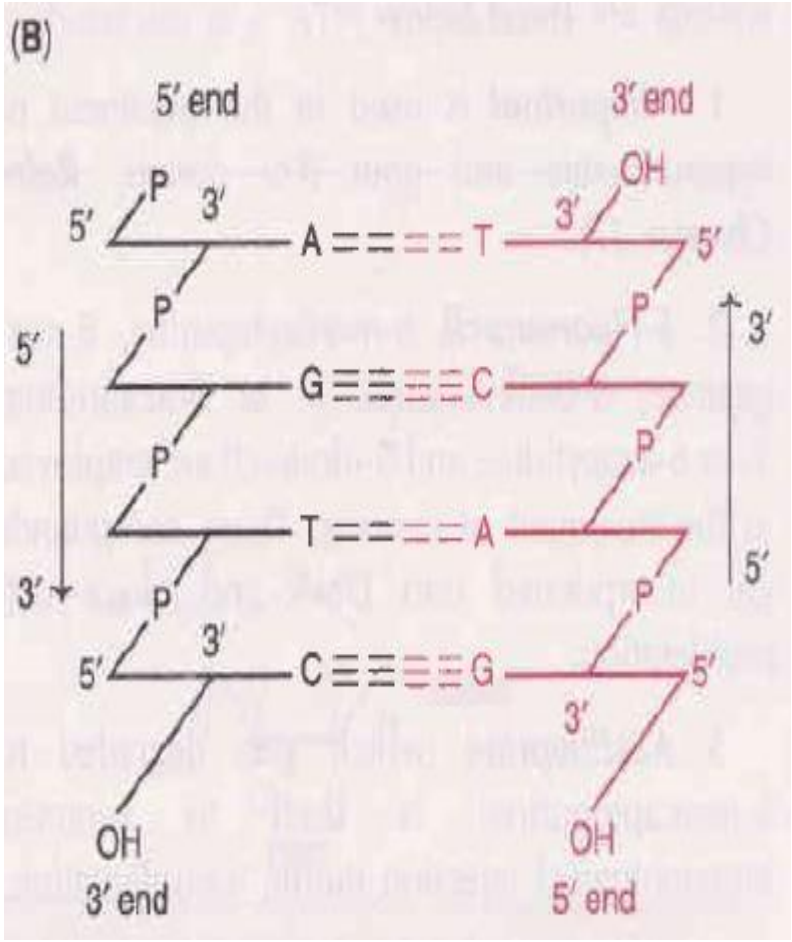


Fig.- (A) Watson-Crick model of DNA helix
(B) Complementary base pairing in DNA helix.

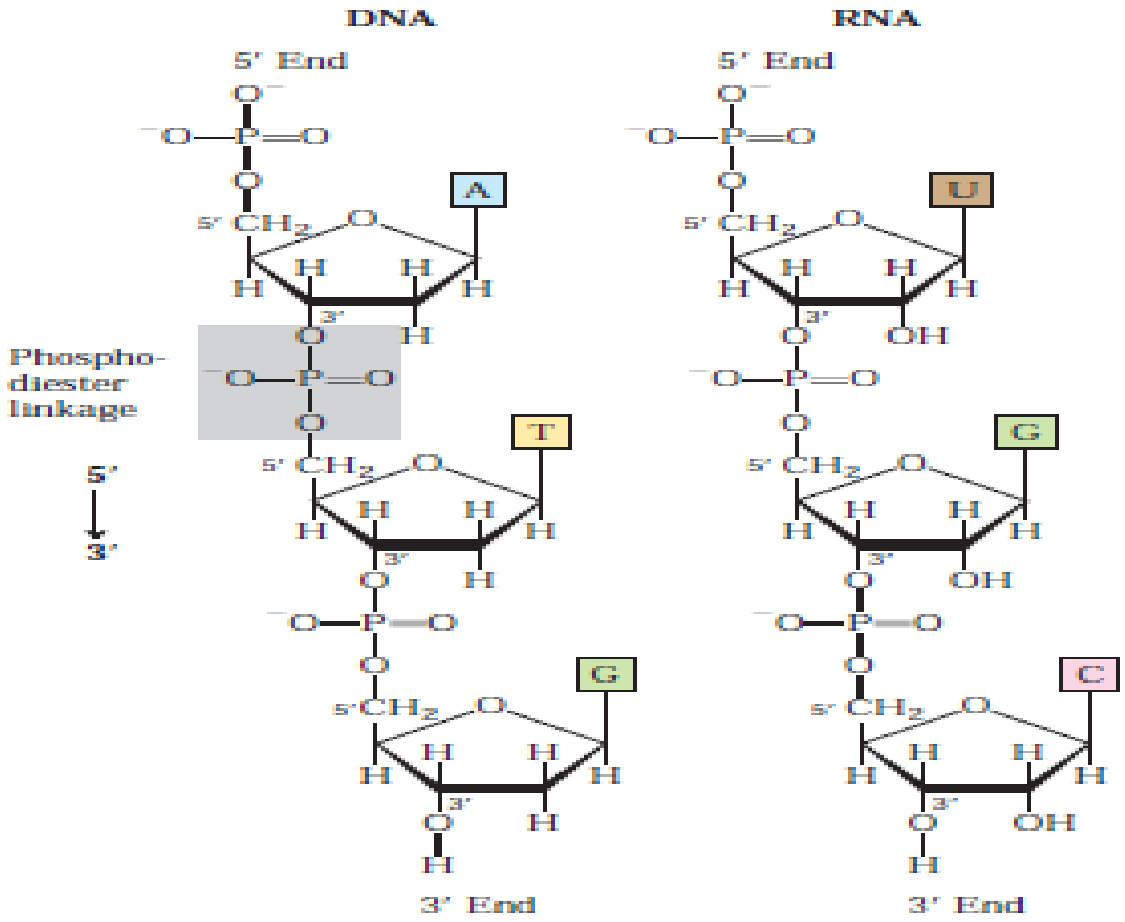


Fig.- Phosphodiester linkages in the covalent backbone of DNA and RNA.

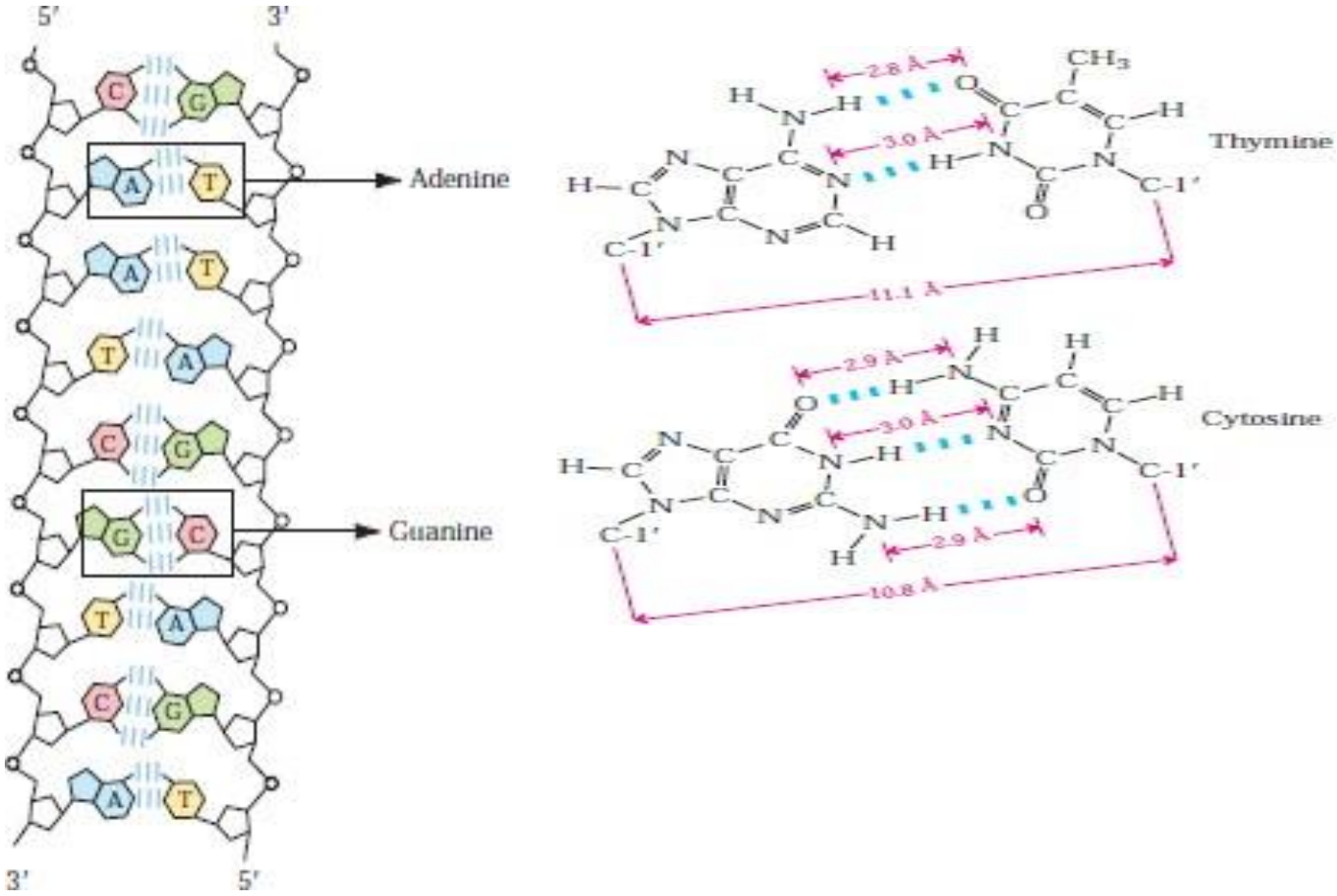


Fig.- Hydrogen-bonding patterns in the base pairs defined by Watson and Crick

The salient features of Watson-Crick model of DNA

1.The DNA is a right handed double helix. It consists of two polydeoxyribonucleotide chains (strands) twisted around each other on a common axis.

2.The two strands are antiparallel, i.e., one strand runs in the 5' to 3' direction while the other in 3' to 5'direction. This is comparable to two parallel adjacent roads carrying traffic in opposite direction.

3. The width (or diameter) of a double helix is 20 A° (2 nm).

4. Each turn (pitch) of the helix is 34 A° (3.4 nm) with 10 pairs of nucleotides, each pair placed at a distance of about 3.4 A°.

5.Each strand of DNA has a hydrophilic deoxyribose phosphate backbone (3'-5' phosphodiester bonds) on the outside (periphery) of the molecule while the hydrophobic bases are stacked inside (core).

6.The two polynucleotide chains are not identical but complementary to each other due to base pairing.

7.The two strands are held together by hydrogen bonds formed by complementary base pairs. The A-T pair has 2 hydrogen bonds while G-C pair has 3 hydrogen bonds. The G = C is stronger by about 50% than A=T.

8.The hydrogen bonds are formed between a purine and a pyrimidine only. lf two purines face each other, they would not fit into the allowable space. And two pyrimidines would be too far to form hydrogen bonds. The only base arrangement possible in DNA structure, from spatial considerations is A-T, T-A, G-C and C-G.

9. The complementary base pairing in DNA helix proves Chargaffs rule. The content of adenine equals to that of thymine (A = T) and guanine equals to that of cytosine (G = C).

10. The genetic information resides on one of the two strands known as template strand or sense strand. The opposite strand is antisense strand.

The double helix has (wide) major grooves and (narrow) minor grooves along the phosphodiester backbone.

Different Conformations of DNA Double Helix

Characteristics	A-DNA	B-DNA	C-DNA	Z-DNA
Conditions	75 % relative humidity; Na^+, K^+, Cs^+ions	92 % relative humidity; Low ion strength	60 % relative humidity; Li^+ions	Very high salt concentration
Shape	Broadest	Intermediate	Narrow	Narrowest
Helix sense	Right -handed	Right -handed	Right -handed	Left-handed
Helix diameter	25.5 A°	23.7 A°	19.0 A°	18.4 A°
Rise per base pair (A°)	2.3 A°	3.4 A°	3.32 A°	3.8 A°
Base pairs per turn of helix ('n')	11	10.4	9.33	12 (=6 dimers)
Helix pitch	25.30 A°	35.36 A°	30.97 A°	45.60 A°
Rotation per base pair	+32.72 A°	+34.61 A°	+38.58 A°	-60° (per dimer)
Base pair tilt	19°	1°	7.8°	9°
Major groove	Narrow and very deep	Wide and quite deep	-	Flat
Minor groove	Very broad and shallow	Narrow and quite deep	-	Very narrow and deep

OTHER TYPES OF DNA STRUCTURE

Bent DNA:-

Bending in DNA structure has been reported due to photochemical damage or mispairing of bases.

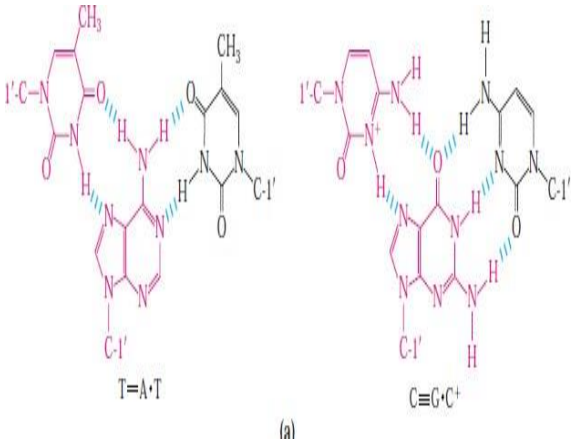
Certain antitumor drugs (e.g. cisplatin) produce bent structure in DNA. Such changed structure can take up proteins that damage the DNA.

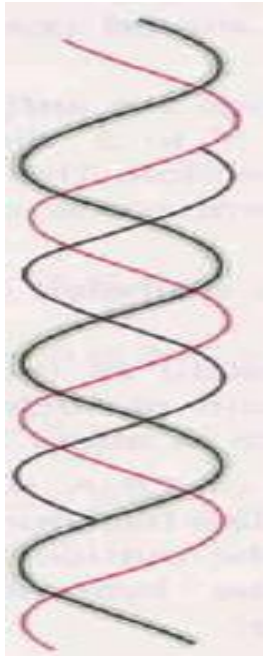
Triple-stranded DNA:-

Triple-stranded DNA formation may occur due to additional hydrogen bonds between the bases. Thus, a thymine can selectively form two **Hoogsteen hydrogen bonds (non-Watson-Crick pairing)** to the adenine of A-T pair to form T-A-T and cytosine can also form two hydrogen bonds with guanine of G-C pairs that results in C-G-C.

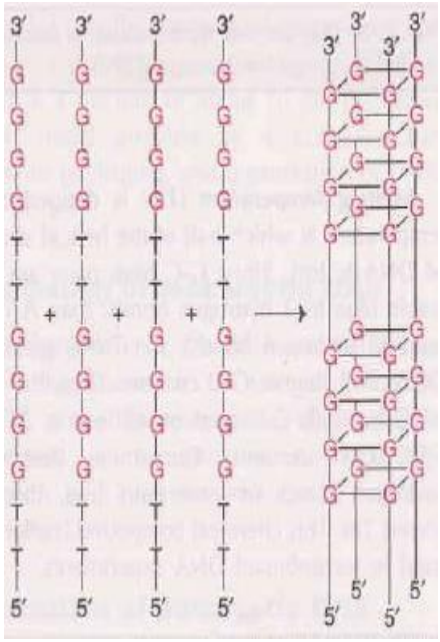
Four-stranded DNA:-

Polynucleotides with very high contents of guanine can form a novel tetrameric structure called G-quartets. These structures are planar and are connected by Hoogsteen hydrogen bonds.

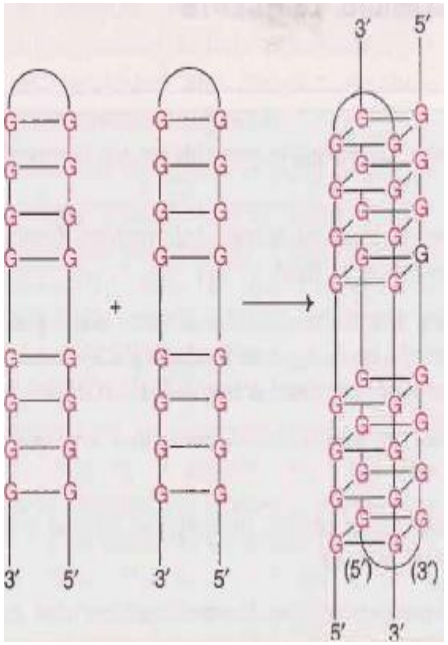




(b)



C (i)



C (ii)

(a) Base-pairing patterns in one well-characterized form of triplex DNA. The Hoogsteen pair in each case is shown.

(b) An outline of Hoogsteen triple helical structure of DNA.

(C) Four-stranded DNAs tructure (i) Parallel G-tetraplex (ii) Antiparallel G-tetraplex

Denaturation of DNA Strands

The two strands of DNA helix are held together by hydrogen bonds. Disruption of hydrogen bonds (by changes in pH or increase in temperature) results in the separation of polynucleotide strands. This phenomenon of loss of helical structure of DNA is known as denaturation.

Renaturation (or Reannealing) is the process in which the separated complementary DNA strands can form a double helix.

The phosphodiester bonds are not broken by denaturation. Loss of helical structure can be measured by increase in absorbance at 260 nm (in a spectrophotometer).

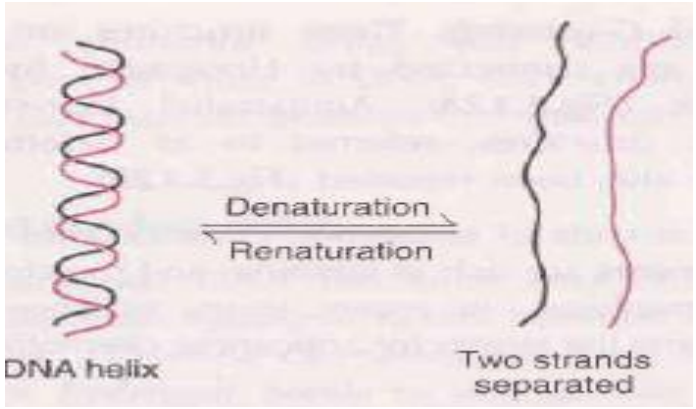


Fig.- Diagrammatic representation of denaturation and renaturation of DNA.

Melting temperature (Tm) is defined as the temperature at which half of the helical structure of DNA is lost.

Since G-C base pairs are more stable (due to 3 hydrogen bonds) than A-T base pairs (2 hydrogen bonds), the Tm is greater for DNAs with higher G-C content.

Thus, the Tm is 65°C for 35 % G-C content while it is 70°C for 50 % G-C content.

Oganization of DNA in the Cell

The double-stranded DNA helix in each chromosome has a length that is thousands times the diameter of the nucleus. In humans, a 2-meter long DNA is packed in a nucleus of about 10 µm diameter.

This is made possible by a compact and marvellous packaging, and organization of DNA inside in cell.

In prokaryotic cells, the bacterial chromosomes are packed in the form of **nucleoids**, by interaction with proteins and certain cations (polyamines).

In eukaryotic cells, the DNA is associated with various proteins to form chromatin which then gets organized info compact structures namely **chromosome.**

The DNA double helix is wrapped around the core proteins namely **histones** which are basic in nature. The core is composed of two molecules of histones (H2A, H2B, H3 and H4). Each core with two turns of DNA wrapped round it is termed as a **nucleosome, the basic unit of chromatin.**

RNA vs. DNA

RNA is a polymer of ribonucleotides held together by 3',5'-phosphodiester bridges.

RNAs have specific differences from DNA-

1.Pentose:- The sugar in RNA is ribose in contrast to deoxyribose in DNA.

2.Pyrimidine:- RNA contains the pyrimidine uracil in place of thymine (in DNA).

3. Single strand:- RNA is usually a single stranded polynucleotide.

4. Chargaff's rule-not obeyed:-Due to the single-stranded nature, there is no specific relation between purine and pyrimidine contents.

5. Susceptibility to alkali hydrolysis:- DNA cannot be subjected to alkali hydrolysis due to lack of hydroxyl group.

6.Orcinol colour reaction:- RNAs can be histologically identified by orcinol colour reaction due to the presence of ribose.

FUNCTIONS OF NUCLEOTIDES

ATP is the principal form of chemical energy, available to cells. It is used as a phosphorylating agent and is also involved in muscle contraction, active transport and maintenance of ionic gradients.

Nucleotides are the monomeric units of nucleic acids (DNA & RNA).

Nucleotides are used in the synthesis of second messengers like cAMP and cGMP for the hormonal functions.

Many of the regulated steps of metabolic pathways are controlled by intracellular concentrations of nucleotides.

They serve as a carrier of high-energy intermediates in the biosynthesis of carbohydrates, lipids and proteins. e.g.

- GTP is involved in the synthesis of glucose (gluconeogenesis).
- GDP is involved in the oxidation of α - Ketoglutaric acid to succinyl CoA to form GTP.
- Uracil derivatives UTP is involved in the synthesis of glycogen and also in the epimerization of galactose and glucose (lactose biosynthesis).
- Cytosine derivatives are involved in the biosynthesis of phosphoglycerides in animal tissues.

Nucleotides are also structural components of several coenzymes of B complex vitamins. e.g. NAD, FAD, and pantothenic acid in Co- enzyme A.

Biologically important nucleoside, S- adenosylmethionine (adenosyl derivative) is involved in several transmethylation processes.

Types of RNA & their functions

The three major types of RNAs-

1. Messenger RNA (mRNA): 5-10 %
2. Transfer RNA (tRNA): 10-20 %
3. Ribosomal RNA (rRNA): 50-80 %

Type of RNA	*Abbreviation*	*Function(s)*
Messenger RNA	mRNA	Transfers genetic information from genes to ribosomes to synthesize proteins.
Heterogeneous nuclear RNA	hnRNA	Serves as precursor for mRNA and other RNAs.
Transfer RNA	tRNA	Transfers amino acid to mRNA for protein biosynthesis.
Ribosomal RNA	rRNA	Provides structural framework for ribosomes.
Small nuclear RNA	snRNA	Involved in mRNA processing.
Small nucleolar RNA	snoRNA	Plays a key role in the processing of rRNA molecules.
Small cytoplasmic RNA	scRNA	Involved in the selection of proteins for export.
Transfer–messenger RNA	tmRNA	Mostly present in bacteria. Adds short peptide tags to proteins to facilitate the degradation of incorrectly synthesized proteins.

Messenger RNA (mRNA)-

The eukaryotic mRNA is capped at the 5'-terminal end by 7-methylguanosine triphosphate and this cap helps to prevent the hydrolysis of mRNA by 5'-exonucleases and involved in the recognition of mRNA for protein synthesis.

The 3'-terminal end of mRNA contains a polymer of adenylate residues (20-250 nucleotides) which is known as poly (A) tail. This tail may provide stability to mRNA and preventing it from the attack of 3'-exonucleases.

Transfer RNA (tRNA)-

Transfer RNA (soluhle RNA) molecule contains 71-80 nucleotides (mostly 75) with a molecular weight of about 25,000. The structure of tRNA (for alanine) was first elucidated by Holley.

The structure of tRNA (Clover leaf modal)-

tRNA contains mainly four arms, each arm with a base paired stem.

1.The acceptor arm:-This arm is capped with a sequence CCA (5' to 3‘). The amino acid is attached to the acceptor arm.

2.The anticodon arm:- This arm, with the three specific nucleotide bases (anticodon), is responsible for the recognition of triplet codon of mRNA.

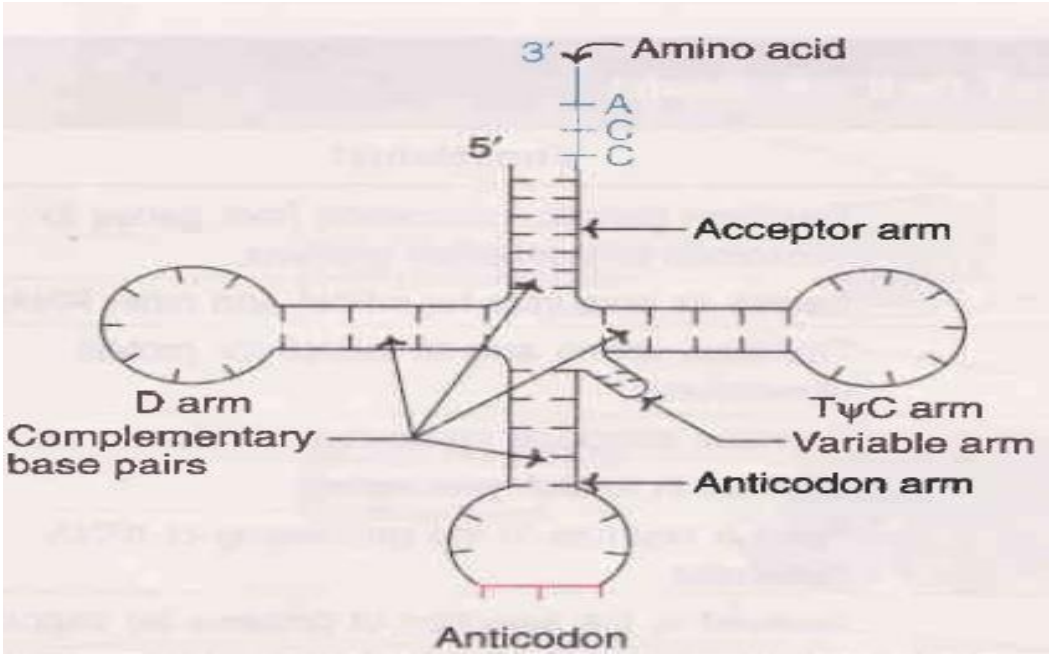
3.The D arm:- lt is so named due to the presence of dihydrouridine.

4.The TYC arm:- This arm contains a sequence of T, pseudouridine (represented by psi, Y) and C.

5. The variable arm:- This arm is the most variable in tRNA. Based on this variability, tRNAs are classified into 2 categories:

(a)Class I tRNAs : The most predominant (about 75 %) form with 3-5 base pairs length.

(b) Class ll tRNAs : They contain 13-20 base pair long arm.



Ribosomal RNA (rRNA)-

The ribosomes are the factories of protein synthesis. The eukaryotic ribosomes are composed of two major nucleoprotein complexes-60S subunit and 40S subunit. The 60S subunit contains 28S rRNA, 5S rRNA and 5.8S rRNA while the 40S subunit contains 18S rRNA. They play a significant role in the binding of mRNA to ribosomes and protein synthesis.

CATALYTIC RNAs-RIBOZYMES-

The RNA component of a ribonucleoprotein (RNA in association with protein) is catalytically active.

Ribonuclease P (RNase P) is a ribozyme containing protein and RNA component.

It is believed that ribozymes (RNAs) were functioning as catalysts before the occurrence of protein enzymes, during the course of evolution.

THANKS