

Shree H.N.Shukla College of Science
S.Y.B.Sc. Sem-III [BC: 301 Biomolecules]
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UNIT-4 Nucleic Acids and Nucleotides

- ✓ There are two types of nucleic acids, namely deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).
- ✓ Nucleic acids serve as transmitters of genetic information.

❖ **Brief history**

- ✓ 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 McCarty.

❖ **Functions of nucleic acids**

- ✓ DNA is the chemical basis of heredity and may be regarded as the reserve bank of genetic information.
- ✓ DNA is exclusively responsible for maintaining the identity of different species of organisms over millions of years.
- ✓ Every aspect of cellular function is under the control of DNA.
- ✓ The DNA is organized into genes, the fundamental units of genetic information.
- ✓ The genes control the protein synthesis through the mediation of RNA.

DNA → RNA → PROTEIN

The interrelationship of these three classes of biomolecules constitutes the **central dogma of molecular biology** or more commonly **the central dogma of life**.

- ✓ Components of nucleic acids
- ✓ Nucleic acids are the polymers of nucleotides held by 3' and 5' phosphate bridges.
- ✓ In other words, nucleic acids are built up by the monomeric units nucleotides (it may be recalled that protein is polymer of amino acids)

❖ **Nucleotides**

- ✓ Nucleotides are composed of a nitrogenous base, a pentose sugar and a phosphate.

- ✓ Nucleotides perform a wide variety of functions in the living cell, besides being the building blocks or monomeric units in the nucleic acid (DNA and RNA) structure.
- ✓ These include their role as structural components of some coenzymes of B-complex vitamins (e.g. FAD, NAD⁺), in the energy reactions of cells (ATP is the energy currency), and in the control of metabolic reactions.

❖ **STRUCTURE OF NUCLEOTIDES**

- ✓ As already stated, the nucleotides essentially consist of nucleobases, sugar and phosphate.
- ✓ The term nucleoside refers to base + sugar. Thus, nucleotide is nucleoside + phosphate.

➤ **Purines and pyrimidines**

- ✓ The nitrogenous bases found in nucleotides (and, therefore, nucleic acids) are aromatic heterocyclic compounds.
- ✓ The bases are of two types - purines and pyrimidines. Their general structures
- ✓ are depicted in Fig.
- ✓ Purines are numbered in the anticlockwise direction while pyrimidines are numbered in the clockwise direction.
- ✓ And this is an internationally accepted system to represent the structure of bases.

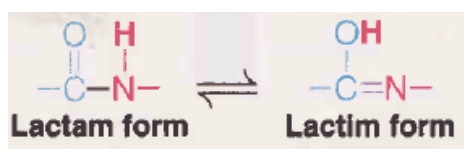
➤ **Major bases in nucleic acids**

- ✓ The structures of major purines and pyrimidines.
- ✓ Found in nucleic acids are shown in Fig.
- ✓ DNA and RNA contain the same purines namely adenine (A) and guanine (G)
- ✓ The pyrimidine cytosine (C) is found in both DNA and RNA. However, the nucleic acids differ with respect to the second pyrimidine base.
- ✓ DNA contains thymine (T) whereas RNA contains uracil (U).
- ✓ Thymine and uracil differ in structure by the presence (in T) or absence (in U) of a methyl group.

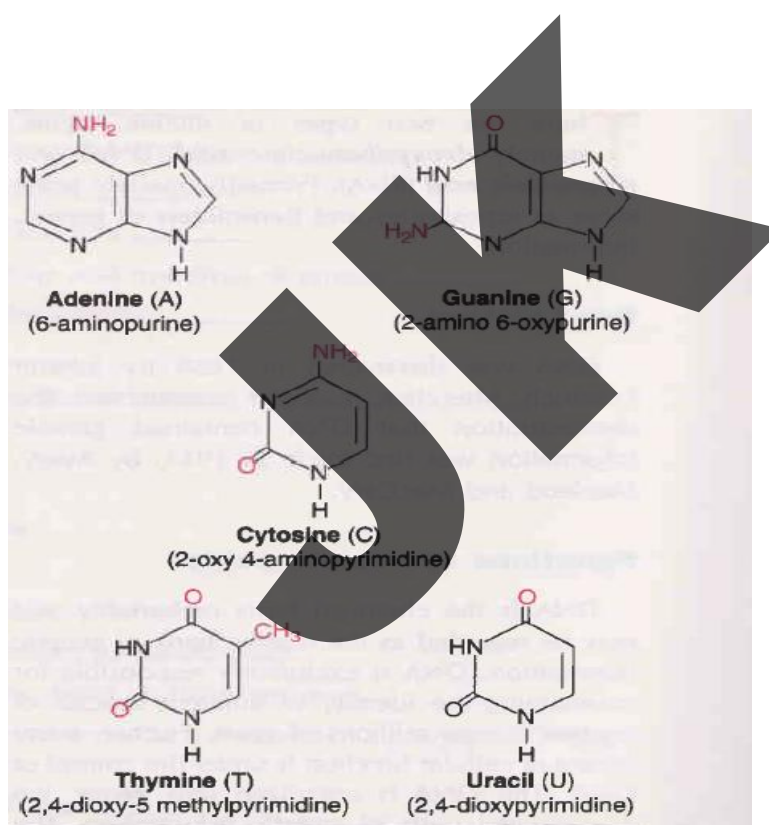
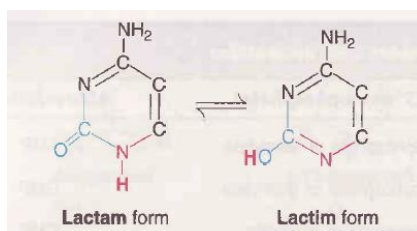
➤ **Tautomeric forms of purines and pyrimidines**

- ✓ The existence of a molecule in a keto (lactam) and enol (lactim) form is known as

- ✓ Tautomerism. The heterocyclic rings of purines and pyrimidines with oxo functional groups exhibit tautomerism as simplified below.

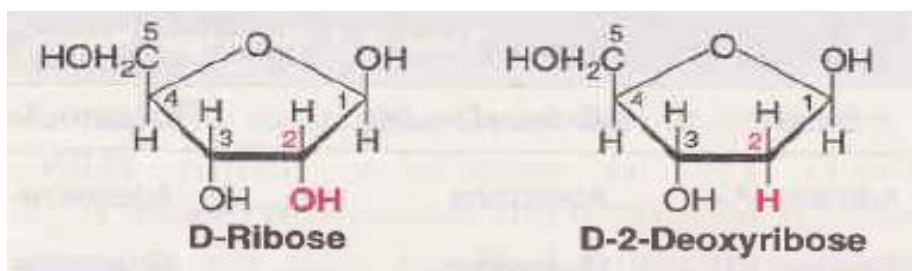


- ✓ The purine-guanine and pyrimidines cytosine, thymine and uracil exhibit
- ✓ Tautomerism. The lactam and lactim forms of cytosine are
- ✓ represented in fig



- **Minor bases found in nucleic acids** : Besides the bases described above, several minor and unusual bases are often found in DNA and RNA.
- ✓ These include 5-methylcytosine, N⁴-acetylcytosine, N⁶-methyladenine, N⁶,dimethyladenine, pseudouracil etc.
- ✓ It is believed that the unusual bases in nucleic acids will help in the
- ✓ recognition of specific enzymes.
- **Other biologically important bases** : The bases such as hypoxanthine, xanthine and uric acid are present in the free state in the cells.

- ✓ The former two are the intermediates in purine synthesis while uric acid is the end
- ✓ product of purine degradation.
- **Sugars of nucleic acids**
- ✓ The five carbon monosaccharides(pentoses) are found in the nucleic acid structure. RNA contains D-ribose while DNA contains D-deoxyribose. Ribose and deoxyribose differ in structure at C2.
- ✓ Deoxyribose has one oxygen less at C2 compared to ribose.



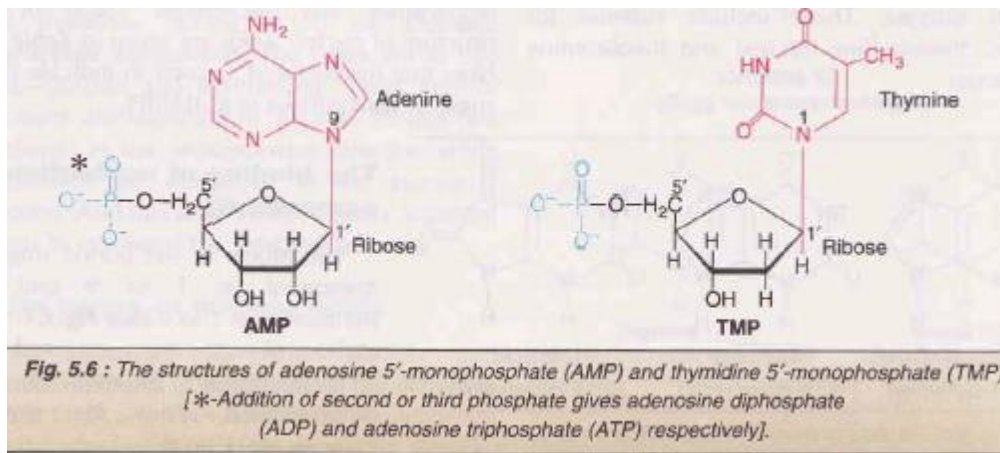
❖ Nomenclature of nucleotides

- ✓ The addition of a pentose sugar to base produces a nucleoside. If the sugar is ribose, ribonucleosides are formed.
Adenosine, guanosine, cytidine and uridine are the ribonucleosides of A, G, C and U respectively.
- ✓ if the sugar is a deoxyribose, deoxyribonucleosides are produced.
- ✓ The term mononucleotide is used when a single phosphate moiety is added to a nucleoside. Thus adenosine monophosphate(AMP) contains adenine + ribose + phosphate.
- ✓ The principal bases, their respective nucleosides and nucleotides found in the structure of nucleic acids are given in table.
- ✓ Note that the prefix 'd' is used to indicate if the sugar is deoxyribose (e.g. dAMP).
- **The binding of nucleotide components**
- ✓ The atoms in the purine ring are numbered as 1 to 9 and for pyrimidine as 1 to 6 The carbons of sugars are represented with an associated prime for differentiation. Thus the pentose carbons are 1' to 5'.
- ✓ The pentoses are bound to nitrogenous bases by β -N-glycosidic bonds.

- ✓ The N⁹ of a purine ring binds with C₁ (1') of a pentose sugar to form a covalent bond in the purine nucleoside. In case of pyrimidine nucleosides the glycosidic linkage is between N₁ of a pyrimidine and C'1 of a pentose.
- ✓ The hydroxyl groups of adenosine are esterified with phosphates to produce 5'- or 3'-monophosphates. 5'-Hydroxyl is the most commonly esterified hence 5' is usually omitted while writing nucleotide names.

Base	Ribonucleoside	Ribonucleotide (5'-monophosphate)	Abbreviation
Adenine (A)	Adenosine	Adenosine 5'-monophosphate or adenylyate	AMP
Guanine (G)	Guanosine	Guanosine 5'-monophosphate or guanylyate	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 deoxyguanylyate	dGMP
Cytosine (C)	Deoxycytidine	Deoxycytidine 5'-monophosphate or deoxycytidylate	dCMP
Thymine (T)	Deoxythymidine	Deoxythymidine 5'-monophosphate or deoxythymidylate	dTMP

- ✓ Thus AMP represents adenosine 5'-monophosphate. However, for adenosine 3'-monophosphate, the abbreviation 3'-AMP is used.
- ✓ The structures of two selected nucleotides namely AMP and TMP are depicted in Fig.
- **Nucleoside di- and tri phosphates**
- ✓ Nucleoside monophosphates possess only one phosphate moiety (AMP, TMP). The addition of second or third phosphate to the nucleoside results in nucleoside diphosphate (e.g. ADP) or triphosphate (e.g. ATP), respectively.
- ✓ The anionic properties of nucleotides and nucleic acids are due to the negative charges contributed by phosphate groups.

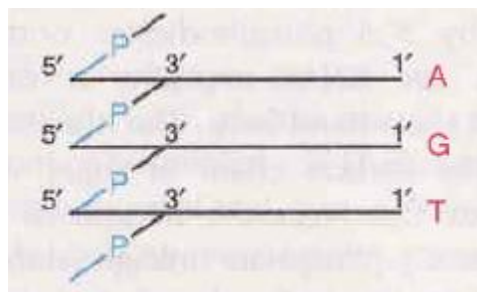


❖ **Structure of DNA**

- ✓ 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) (It may be noted here that some authors prefer to use TMP for deoxythymidylates, since it is found only in DNA).
- ✓ The details of the nucleotide structure are given above.

➤ **Schematic representation of polynucleotides**

- ✓ The monomeric deoxynucleotides in DNA are held together by 3',5'-phosphodiester bridges. DNA (or RNA) structure is often represented in a short-hand form.
- ✓ The horizontal line indicates the carbon chain of sugar with base attached to C₁. Near the middle of the horizontal line is C₃, phosphate linkage while at
- ✓ the other end of the line is C₅, phosphate linkage.

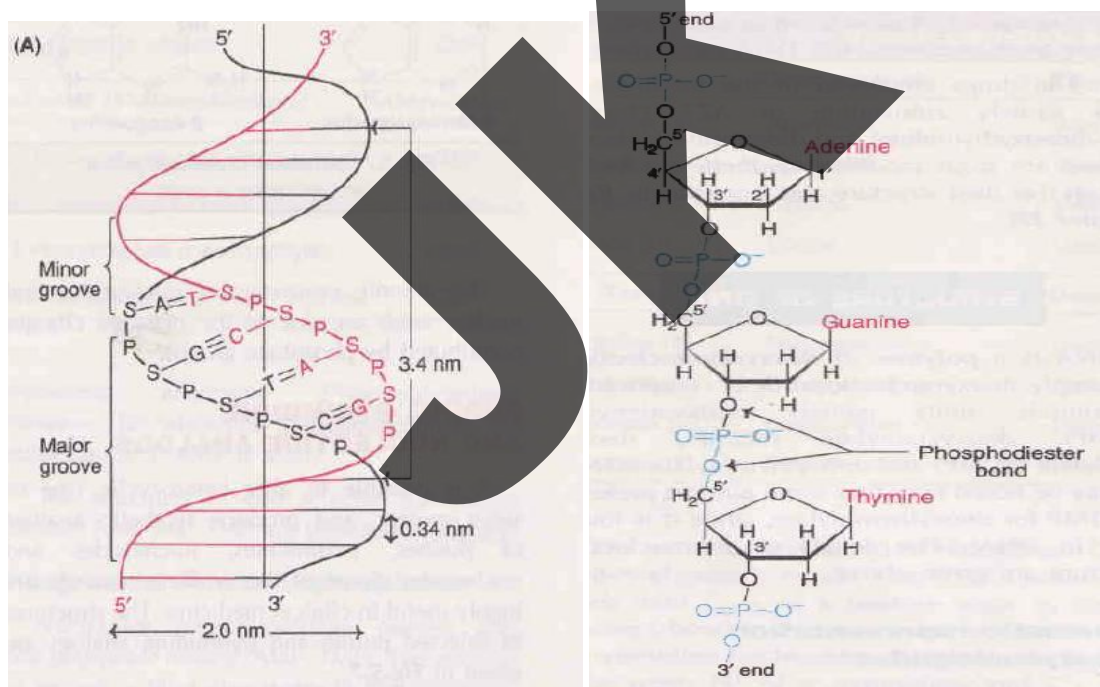


➤ **Chargaff's rule of DNA composition**

- ✓ Erwin Chargaff in late 1940s quantitatively analysed the DNA hydrolysates from different species.
- ✓ 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.
- ✓ The significance of Chargaff's rule was not immediately realised. The double helical structure of DNA derives its strength from Chargaff's rule.
- ✓ Single-stranded DNA, and RNAs which are usually single-stranded do not obey Chargaff's rule. However, double-stranded RNA which is the genetic material in certain viruses satisfies Chargaff's rule.

❖ DNA DOUBLE HELIX

- ✓ The double helical structure of DNA was proposed by James Watson and Francis Crick in 1953 (Nobel Prize, 1962). The elucidation of



- ✓ DNA structure is considered as a milestone in the era of modern biology. The structure of DNA double helix is comparable to a twisted ladder.
- ✓ The salient features of Watson-Crick model of DNA (now known as B-DNA) are described in fig.

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 Å (2 nm).
4. Each turn (pitch) of the helix is 34 Å (3.4 nm) with 10 pairs of nucleotides each pair placed at a distance of about 3.4 Å.
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. If 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 Chargaff's 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. Proteins interact with DNA at these grooves, without disrupting the base pairs and double helix.

❖ **Conformations Of DNA double helix**

- ✓ Variation in the conformation of the nucleotides of DNA is associated with conformational variants of DNA. The double helical structure of DNA exists in at least 6 different forms-A to E and Z.
- ✓ Among these, B, A and Z forms are important. The B-form of DNA double helix, described by Watson and Crick (discussed above), is the most predominant form under physiological conditions.
- ✓ Each turn of the B-form has 10 base pairs spanning a distance of 3.4 nm. The width of the double helix is 2 nm.
- ✓ The A-form is also a right-handed helix. It contains 11 base pairs per turn. There is a tilting of the base pairs by 20° away from the central axis.
- ✓ The Z-form (Z-DNA) is a left-handed helix and contains 12 base pairs per turn. The polynucleotide strands of DNA move in a somewhat 'zig zag' fashion, hence the name Z-DNA.
- ✓ It is believed that transition between different helical forms of DNA plays a significant role in regulating gene expression.

❖ **THE SIZE OF DNA MOLECULE-UNITS OF LENGTH**

- ✓ DNA molecules are huge in size. On an average, a pair of B-DNA with a thickness of 0.34 nm has a molecular weight of 660 daltons.
- ✓ For the measurement of lengths, DNA doublestranded structure is considered, and expressed in the form of base pairs (bp). A kilobase pair (kb) is 10³ bp, and a megabase pair (Mb) is 10⁶ bp and a gigabase pair (Gb) is 10⁹ bp. The kb, Mb and Gb relations may be summarized as follows :

$$\mathbf{1\ kb = 1000\ bp}$$

$$\mathbf{1\ Mb = 1000\ kb = 1,000,000\ bp}$$

$$\mathbf{1\ Gb = 1000\ Mb = 1,000,000,000\ bp}$$

- ✓ It may be noted here that the lengths of RNA molecules (like DNA molecules) cannot be expressed in bp, since most of the RNAs are single-stranded.
- ✓ The length of DNA varies from species to species and is usually expressed in terms of base pair composition and contour length.

- ✓ Contour length represent the total length of the genomic DNA in a cell . Some examples of organisms with bp and contour lengths are listed.

phage virus- 4.8×10^4 bp-contour length 16.5 mm.

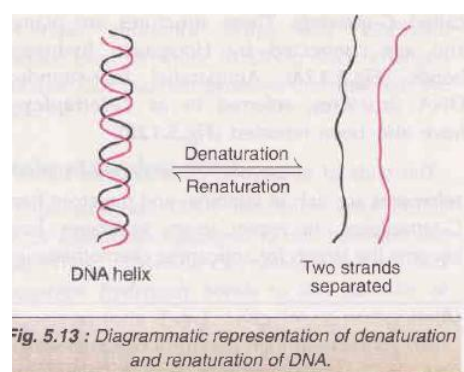
E. coli - 4.6×10^6 bp - contour length 1.5 mm.

Diploid human cell (46 chromosomes-) 6.0×10^9 bp contour length 2 meters.

- ✓ It may be noted that the genomic DNA size is usually much larger the size of the cell or nucleus containing it. For instance in humans, a 2-meter long DNA is packed compactly in a nucleus of about $10\mu\text{m}$ diameter.
- ✓ The genomic DNA may exist in linear or circular forms. Most DNAs in bacteria exist
- ✓ as closed circles. This includes the DNA of bacterial chromosomes and the extra chromosomal DNA of plasmids.
- ✓ Mitochondria and chloroplasts of eukaryotic cells also contain circular DNA.
- ✓ Chromosomal DNAs in higher organisms are mostly linear. human chromosomes contain a single DNA molecule with variable sizes compactly packed. Thus the smallest chromosome contains 34 Mb while the largest one has 263 Mb.

➤ **Denaturation of DNA strands**

- ✓ The two strands of DNA helix are held together by hydrogen bonds. Disruption of hydrogen bonds(by change in pH or increase in temperature) results in the separation of polynucleotide strands.
- ✓ **The loss of helical structure of DNA is known as denaturation.** phosphodiester bonds are not broken by denaturation Loss of helical structure can be measured by increase in absorbance at 260 nm in spectrophotometer.

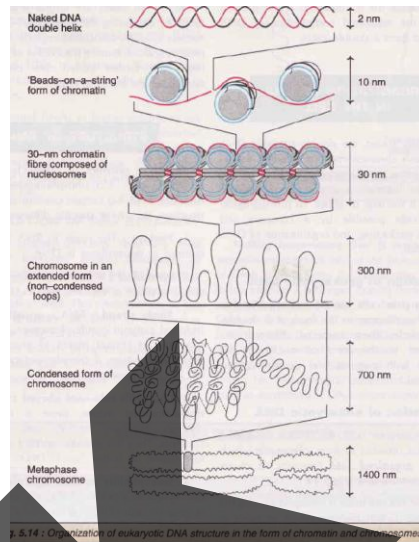


- ✓ **Melting temperature (T_m) is defined as the temperature at which half of the helical structure of DNA is lost.**
- ✓ Since G-C base pairs are more stable (due to 3 hydrogen bonds) than A-T base pairs (2 hydrogen bonds), the T_m is greater for DNAs with higher G-C content. Thus, the T_m is 65°C for 35% G-C content while it is 70°C for 50% G-C content.
- ✓ **Renaturation is the process in which the separated complementary DNA strands can form a double helix.**

❖ **Organization of DNA in the cell**

- ✓ As already stated, the double-stranded DNA helix in each chromosome has a length that is thousands times the diameter of the nucleus. For instance, in humans, 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.
- **Organization of prokaryotic DNA**
- ✓ In prokaryotic cells, the DNA is organized as a single chromosome in the form of a doublestranded circle.
- ✓ These bacterial chromosomes are packed in the form of nucleoids, by interaction with Proteins and certain cations (polyamines).
- **Organization of eukaryotic DNA**
- ✓ In the eukaryotic cells, the DNA is associated with various proteins to form chromatin which then gets organized into compact structures namely chromosomes
- ✓ 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 (approximately with 150 bp) is termed as a nucleosome, the basic unit of chromatin.
- ✓ Nucleosomes are separated by spacer DNA to which histone H1 is attached. This continuous string of nucleosomes, representing beads-on-a string form of chromatin is termed as 10 nm fiber.

- ✓ The length of the DNA is considerably reduced by the formation of 10 nm fiber. This 10-nm fiber is further coiled to produce 30-nm fiber which has a **solenoid** structure with six nucleosomes in every turn.
- ✓ These 30-nm fibers are further organized into 300nm regions namely scaffold-associated regions, protein scaffold.
- ✓ During the course of mitosis, the loops are further coiled, the chromosomes condense and become visible.



✓ **Structure of RNA**

- ❖ RNA is a polymer of ribonucleotides held together by 3',5'-phosphodiester bridges.
- ❖ Although RNA has certain similarities with DNA structure, they have specific differences

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 singlestranded polynucleotide. However, this strand

may fold at certain places to give a doublestranded structure, if complementary base pairs are in close proximity.

4. Chargaff's rule-not obeyed : Due to the single-stranded nature, there is no specific

relation between purine and pyrimidine contents. Thus the guanine content is not equal to cytosine (as is the case in DNA).

5. Susceptibility to alkali hydrolysis : Alkali can hydrolyse RNA to 2',3'-cyclic diesters.

This is possible due to the presence of a hydroxyl group at 2' position.

DNA cannot be subjected to alkali hydrolysis due to lack of this group.

6. Orcinol colour reaction : RNAs can be histologically identified by orcinol colour

reaction due to the presence of ribose.

✓ **TYPES OF RNA**

❖ The three major types of RNAs with their respective cellular composition are given below

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.

❖ Besides the three RNAs referred above, other RNAs are also present in the cells. These include heterogeneous nuclear RNA (hnRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and small cytoplasmic RNA (scRNA). The major functions of these RNAs are given in Table 5.3.

❖ The RNAs are synthesized from DNA, and are primarily involved in the process of protein biosynthesis. The RNAs vary in their structure and function. A brief description on the major RNAs is given.

❖ **Messenger RNA (mRNA)**

- ✓ The mRNA is synthesized in the nucleus (in eukaryotes) as heterogeneous nuclear RNA (hnRNA). hnRNA, on processing, liberates the functional mRNA which enters the cytoplasm to participate in protein synthesis.
- ✓ mRNA has high molecular weight with a short half-life. The eukaryotic mRNA is capped at the 5'-terminal end by 7-methylguanosine triphosphate. It is believed that this cap helps to prevent the hydrolysis of mRNA by 5'-exonucleases.
- ✓ Further, the cap may be also involved in the recognition of mRNA for protein synthesis.
- ✓ The 3'-terminal end of mRNA contains a polymer of adenylate residues (20-25 nucleotides) which is known as poly (A) tail.
- ✓ This tail may provide stability to mRNA, besides preventing it from the attack of 3'-exonucleases.
- ✓ mRNA molecules often contain certain modified bases such as 6-methyladenylates in the internal structure.

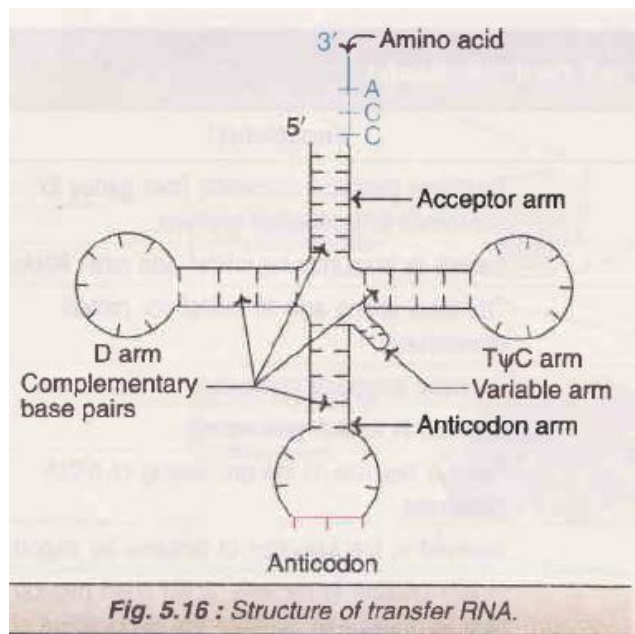
❖ **Transfer RNA (tRNA)**

- ✓ Transfer RNA (soluble RNA) molecule contains 71-80 nucleotides (mostly 75) with a molecular weight of about 25,000. There are at least 20 species of tRNAs, corresponding to 20 amino acids present in protein structure.
The structure of tRNA (for alanine) was first elucidated by Holley.
- ✓ The structure of tRNA, depicted in Fig., resembles that of a clover leaf. 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. The codon and anticodon are complementary to each other.



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

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

5. The variable arm : This arm is the most variable in tRNA. Based on this variability,

tRNAs are classified into 2 categories :

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

Class II tRNAs : They contain 13-20 base pair long arm.

- ✓ **Base pairs in tRNA :** The structure of tRNA is maintained due to the complementary base pairing in the arms.
- ✓ The four arms with their respective base pairs are given below

The acceptor arm - 7 bp

The TΨC arm - 5 bp

The anticodon arm - 5 bp

The D arm - 4bp

❖ **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

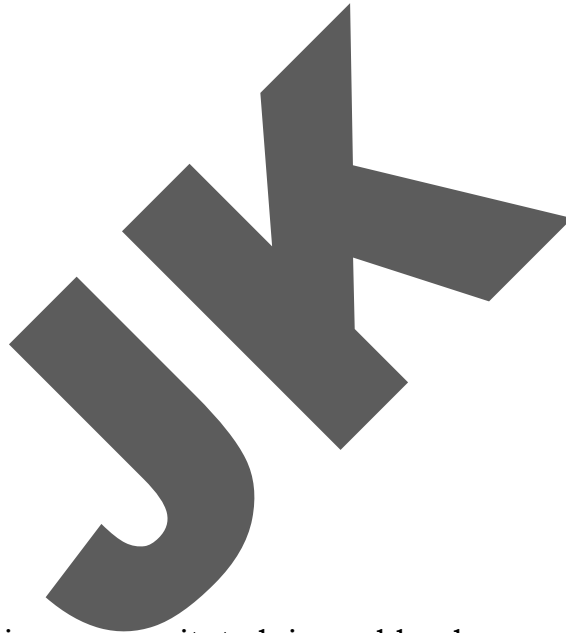
- ✓ 60s subunit contains 28S rRNA, 5s rRNA and 5.8S rRNA while the 40S subunit contains 18S rRNA.
- ✓ The function of rRNAs in ribosomes is not clearly known. It is believed that they play a significant role in the binding of mRNA to ribosomes and protein synthesis.
- ✓ Other RNAs The various other RNAs and their functions are summarised in Table

❖ **DNA is the Genetic material Experiment of Avery et al.**

- In 1944 Oswald Avery, McCleod and McCarty showed that the transforming substance was DNA.
- When the cultures were treated with nuclease, they did not show transformation and R strain was not converted in S strain. Mice lived.
- But when the cultures were treated with protease, they showed transformation and R strain was converted in S strain. Mice died.
- This proved that the DNA, and not the protein was responsible for transformation in original experiment of Griffith.
- The experiment of Avery et al. proved that the prokaryotic genetic material is DNA
- The next step was to demonstrate that DNA provides the genetic material in other living systems also.
- Here is the diagram explaining the work of Avery et al.

❖ **DNA is the Hereditary Material Experiment of Hershey and Chase**

- In 1952, James Hershey and Martha Chases infected bacteria(E.coli) with T₂ phages that had been radioactively labeled either in their DNA component (³²P) or in their proteein component (³⁵S).
- Following diagram illustrates the result of this experiment.



- The infected bacteria were agitated in a blender ,and two fraction were separated by centrifugation.
- One contained the empty phage coats that were released from the surface of the becteria. this consisted of proteins and therefore carried the ³⁵S radioactive label.
- The other fraction consisted of the infected bacteria.
- Most of the ³²p label was present in the infected bacteria.
- The progeny phage particles produced by the infection contained approximately 30% of the original ³²p label.
- the progeny received very little (less than 1%) of the proteins contained in the original phage population .

- This experiment, therefore showed directly that the DNA of parent enters the bacteria and thus become part of the progeny phages.
- This showed the exact pattern of inheritance of genetic material.
- The phage possesses genetic material whose behavior is analogous to that of cellular genomes.
- The case of T₂ , thus concludes that the genetic material is DNA ,whether part of the genome of a cell or virus.
- Bacteria and phages clearly clearly have DNA as their genetic material

❖ **Experiment of meselson & stahl(1958)**

- They performed an experiment to establish the semi conservative nature of DNA replication.
- They used the E.coil strain which having the ¹⁵N first in their DNA.
- Further they allow to grow such organism in the media having light ¹⁴N containing compound & they allow them to divide.
- Then they extracted the DNA & they found the density of helix intermediary of the density of DNA completely made up of ¹⁴N & DNA made up of completely ¹⁵N.
- In this experiment they used the the c_scl₃ gradient & centrifugation process.
- So, they proved that the DNA replication is semiconservative mode of replication.