CHAPTER 9

Chemistry of Nucleic Acids

INTRODUCTION

Nucleic acids are macromolecules present in all living cells in combination with proteins to form ***nucleoproteins.*** The protein is usually **protamines** and **histones.** Genetic information is encoded in a nucleic acid molecule.

NUCLEIC ACIDS

- Nucleic acids are polymers of ***nucleotides,*** linked by **phosphodiester** bond, they are therefore called **polynucleotides.**
- The nucleic acids are of two main types:
  - Deoxyribonucleic acid or DNA
  - Ribonucleic acid or RNA.
- DNA is present in nuclei and small amounts are also present in **mitochondria,** whereas 90% of the RNA is present in cell **cytoplasm** and 10% in the **nucleolus.**

NUCLEOTIDE

Each nucleotide consists of three components:

1. A nitrogenous base
2. A pentose sugar
3. A phosphate group.

Nitrogenous Bases of RNA and DNA

Two classes of nitrogenous bases namely **purines** and **pyrimidines** are present in RNA and DNA.

Purine Bases

- Two principal purine bases found in DNAs, as well as RNAs **(Figure 9.1)** are:
  i. Adenine (A)
  ii. Guanine (G).

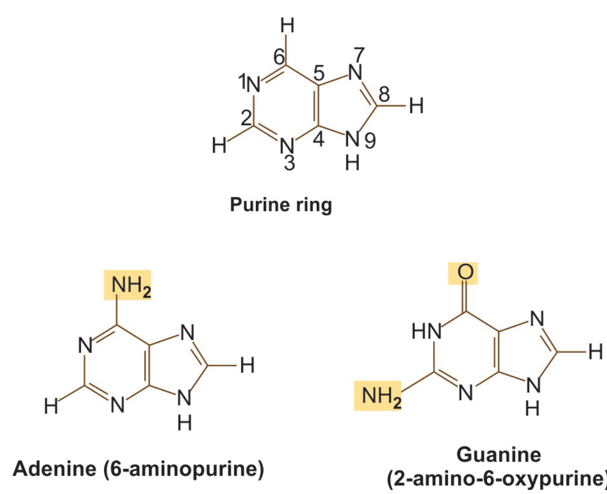

Figure 9.1: Structure of purine ring and purine bases

Pyrimidine Bases

- Three major pyrimidine bases **(Figure 9.2)** are:
  i. Cytosine (C)
  ii. Uracil (U)
  iii. Thymine (T).
- Cytosine and uracil are found in RNAs and cytosine and thymine in DNA.
- Both DNA and RNA contain the pyrimidine cytosine but they differ in their second pyrimidine base. DNA contains thymine whereas RNA contains uracil.

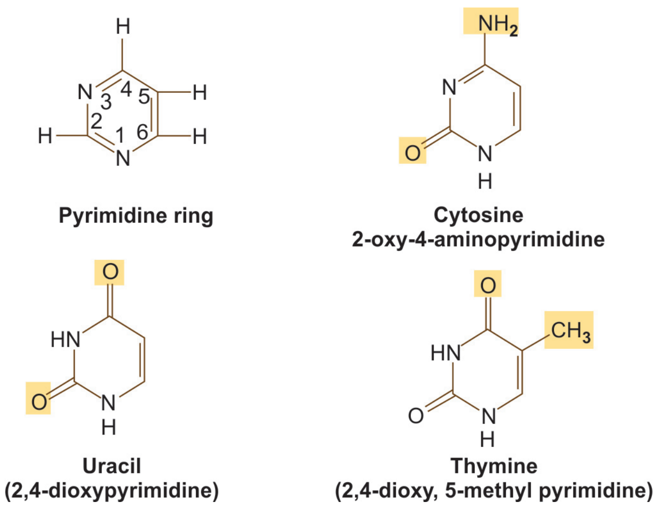

Figure 9.2: Structure of pyrimidine ring and pyrimidine bases

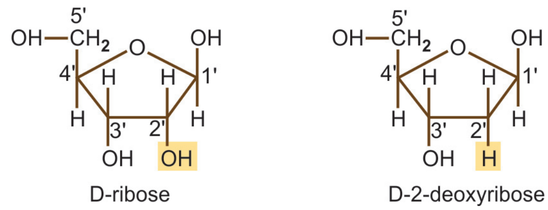

Figure 9.3: Structure of sugars present in nucleic acid

Pentose Sugars Present in RNA and DNA

- The pentose sugar is either ***D-ribose*** or ***D-2-deoxy-ribose*** **(Figure 9.3).** DNA and RNA are distinguished on the basis of the pentose sugar present. DNA contains D-2-deoxyribose and RNA contains D-ribose.
- A pentose sugar (D-ribose or D-2-deoxyribose) is linked to a base (purine or pyrimidine) via covalent **N-glycosidic bond (Figure 9.4).** The term **nucleoside** is used for structures containing only sugar and nitrogen base.
- This linkage joins nitrogen-9 of the purine base or nitrogen 1 of the pyrimidine base with carbon 1 of pentose sugar **(Figure 9.4).**
- The atoms of the base in nucleosides are given ***cardinal*** numbers, whereas the carbon atoms of the sugars are given **primed** numbers as shown in **Figure 9.4** to distinguish sugar atoms from those of the nitrogen base.

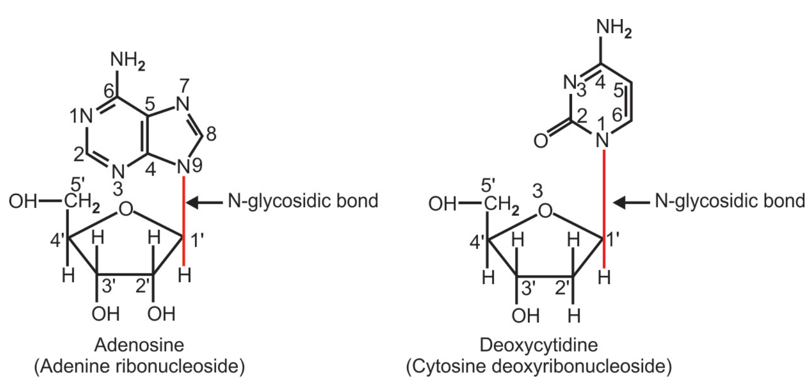

Figure 9.4: Structures of nucleoside

Table 9.1: Different major bases with their corresponding nucleosides and nucleotides

Base	*Ribonucleoside*	*Ribonucleotide*
Adenine (A)	Adenosine	Adenosine monophosphate (AMP)
Guanine (G)	Guanosine	Guanosine monophosphate (GMP)
Uracil (U)	Uridine	Uridine monophosphate (UMP)
Cytosine (C)	Cytidine	Cytidine monophosphate (CMP)
Base	*Deoxyribonucleoside*	*Deoxyribonucleotide*
Adenine	Deoxyadenosine	Deoxyadenosine monophosphate (dAMP)
Guanine	Deoxyguanosine	Deoxyguanosine monophosphate (dGMP)
Uracil	Deoxyuridine	Deoxyuridine monophosphate (dUMP)
Cytosine	Deoxycytidine	Deoxycytidine monophosphate (dCMP)
Thymine	Deoxythymidine	Deoxythymidine monophosphate (dTMP)

- The nucleosides of A, G, C, T and U are named adenosine, guanosine, cytidine, thymidine and uridine respectively.
- If the sugar is ribose, ribonucleoside is produced; if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced. The structures of these two types of nucleoside are illustrated by the examples in the **Figure 9.4. Table 9.1** indicates the bases and their corresponding nucleosides.

Structure of nucleotides

Nucleotides are phosphorylated nucleosides. Nucleosides are nitrogen bases containing pentose sugar. The phosphate group is attached to the nucleoside by an ester linkage to the hydroxyl group of the pentose sugar. The nucleotides are of two types depending on the kind of pentose sugar present.

1. **Deoxyribonucleotides:** These nucleotides contain pentose sugar, **deoxyribose** and are monomeric units of DNA **(Figure 9.5)**.
2. **Ribonucleotides:** These nucleotides contain pentose sugar, D-ribose and are monomeric units of RNA **(Figure 9.5)**.

- Mononuleotides are nucleosides in which single phosphate group is attached to hydroxyl group of the pentose sugar. For example, AMP (adenosine monophosphate) is adenine + ribose + phosphate.
- If an additional phosphate group is attached to the pre-existing phosphate of mononucleotide
  - A nucleoside diphosphate, e.g. ADP
  - A nucleoside triphosphate, e.g. ATP results **(Figure 9.6)**.
- The principle bases, their respective nucleosides and nucleotides found in the structure of nucleic acids are given in **Table 9.1.**

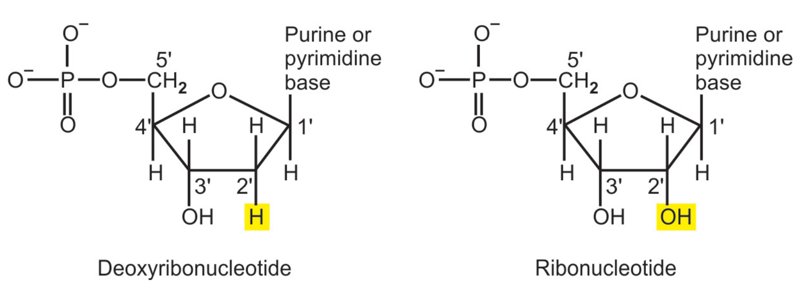

Figure 9.5: Structure of nucleotide

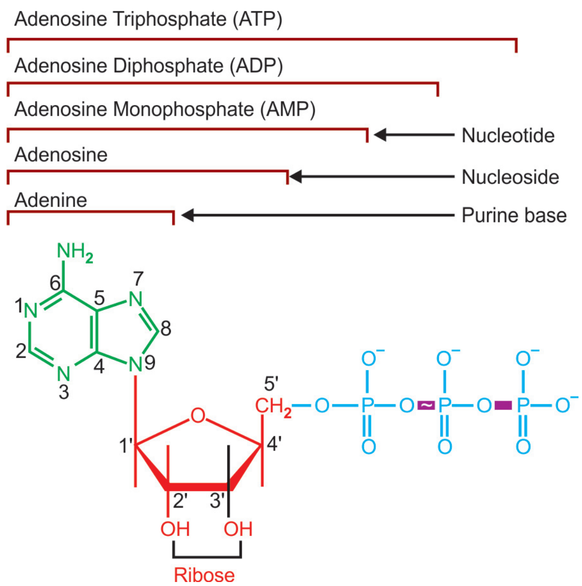

Figure 9.6: Structure of ATP and its components

BIOLOGICALLY IMPORTANT NUCLEOTIDES

Besides being the structural components of nucleic acids, several nucleotides such as ATP, ADP, c-AMP, GTP, GDP, c-GMP, UDP, CTP, CDP, etc. participate in several biochemical and physiological functions. Such nucleotides are called **biologically important nucleotides.** Nucleotides involved in a various biochemical proceses are discussed below.

ATP

ATP serves as the main biological source of energy in the cell. ATP is required as a source of energy in several metabolic pathways, e.g. fatty acid synthesis, glycolysis, cholesterol synthesis, protein synthesis, gluconeogenesis, etc. and in physiologic functions such as muscle contraction, nerve impulse transmission, etc.

AMP

AMP is the component of many coenzymes such as NAD^+, $NADP^+$, FAD, coenzyme A, etc. These coenzymes are essential for the metabolism of carbohydrate, lipid and protein.

C-AMP (Cyclic adenosine 3', 5'-monophosphate)

- c-AMP is formed from ATP by the action of ***adenylate* cyclase.**
- c-AMP acts as a second messenger for many hormones, e.g. epinephrine, glucagon, etc. c-AMP affects a wide range of cellular processes by acting as a second messenger.
- It enhances the degradation of storage fuels like fat and glycogen by stimulating lipolysis, glycogenolysis.
- It inhibits the aggregation of blood platelets.
- c-AMP increases the secretion of acid by the gastric mucosa.

GDP and GTP

- These guanosine nucleotides participate in the conversion of **succinyl-CoA to succinate,** a reaction which is coupled to the substrate level phosphorylation of GDP to GTP in citric acid cycle.
- GTP is required for activation of adenylate cyclase by some hormones.
- GTP serves as an energy source for protein synthesis.

C-GMP (Cyclic guanosine 3', 5'-monophosphate)

- c-GMP is formed from GTP by ***guanylyl cyclase.***
- c-GMP is an intracellular signal or second messenger that can act antagonistically to c-AMP.
- c-GMP is involved in relaxation of smooth muscle and vasodilation.

UDP (Uridine diphosphate)

- UDP participates in glycogenesis.
- UDP-glucose and UDP-galactose take part in galactose metabolism and required for synthesis of lactose and cerebrosides.
- UDP-glucuronic acid is required in detoxification processes and for biosynthesis of mucopolysaccharides such as heparin, hyaluronic acid, etc.

CTP (Cytidine triphosphate) and CDP (Cytidine diphosphate)

- CTP and CDP are required for the biosynthesis of some phospholipids. CDP-choline is involved in the synthesis of sphingomyelin.

SYNTHETIC ANALOGUES OF NUCLEOTIDES OR ANTIMETABOLITES

Chemically, synthesized analogues of purines and pyrimidines, their nucleosides and their nucleotides have therapeutic applications in medicine. An analogue is prepared either by altering the heterocyclic ring or sugar moiety. These are used **chemotherapeutically** (treatment of disease by the use of chemical substances) to control **cancer** or **infections**.

Analogues of purines and pyrimidines used in the treatment of infections or in cancer chemotherapy are:

- The nucleoside ***cytarabine (arabinosyl cytosine; ara-c)*** in which arabinose replaces ribose, is used in the chemotherapy of cancer and viral infections.

- The purine analogue ***allopurinol*** used in treatment of hyperuricemia and gout.
- ***Azidothymidine (AZT)*** is a structural analogue of thymidine used in the treatment of acquired immunodeficiency syndrome (AIDS), a disease caused by the human immunodeficiency virus (HIV).
- ***Azathioprine,*** which is used during organ transplantation to suppress events involved in immunologic rejection.
- ***5-iododeoxyuridine,*** a nucleoside analogue has antiviral activity and is used in the treatment of herpetic keratitis, an infection of the cornea by *Herpes* virus.

DNA STRUCTURE AND FUNCTION

DNA serves as the genetic material for cells both prokaryotes and eukaryotes. In eukaryotes, DNA is located in the nucleus separated from cytoplasm by the nuclear membrane. Because prokaryotes lack internal membrane systems, their DNA is not separated from the rest of the cellular contents. Eukaryotic DNA is bound to proteins, forming a complex known as chromatin.

DNA is also present in mitochondria (less than 0.1% of the total DNA) and in chloroplast of plants. Many viruses also contain DNA as their genetic material.

Structure of DNA

- DNA is a very long, thread like macromolecule made up of a large number of **deoxyribonucleotides.** Deoxyribonucleotide is composed of a ***nitrogenous base, a sugar*** and ***phosphate group.***
- The bases of DNA molecule carry genetic information, whereas their sugar and phosphate groups perform a structural role.
- The sugar in a deoxyribonucleotide is ***deoxyribose.***
- The purine bases in DNA are **adenine (A),** and **guanine (G)**.
- Pyrimidine bases are **thymine (T)** and **cytosine (C).**
- DNA is a polymer of many deoxyribonucleotides linked covalently by **3', 5' phosphodiester bonds**. The 3'-hydroxyl group of the sugar moiety of one deoxyribonucleotide is joined to the 5'-hydroxyl group of the adjacent sugar moiety of deoxyribonucleotide by a phosphodiester linkage **(Figure 9.7).**

The Watson-Crick DNA Double Helical Structure

In 1953, James Watson and Francis Crick deduced the three-dimensional structure of DNA. The important features of their model of DNA are as follows:

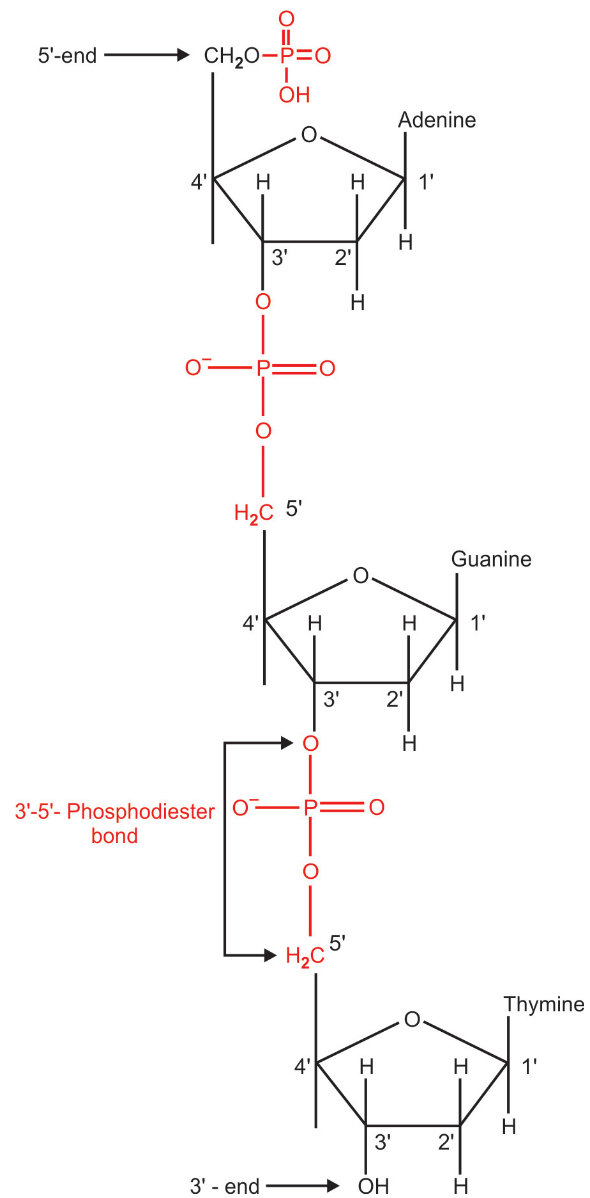

Figure 9.7: Structure of polynucleotide chain of DNA

- Two helical polynucleotide chains are coiled around a common axis. The chains run in opposite direction (anti parallel) **(Figure 9.8)**.
- The purine and pyrimidine bases are on the inside of the helix, whereas the phosphate and deoxyribose units are on the outside.
- The diameter of the helix is 20 Å. Adjacent bases are separated by 3.4 Å along the helix axis and the helical structure repeates after ten residues on each chain, i.e. at intervals of 34 Å **(Figure 9.9)**.

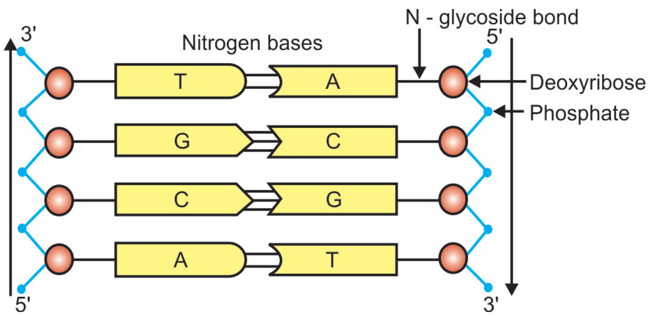

Figure 9.8: Antiparallel strands of DNA

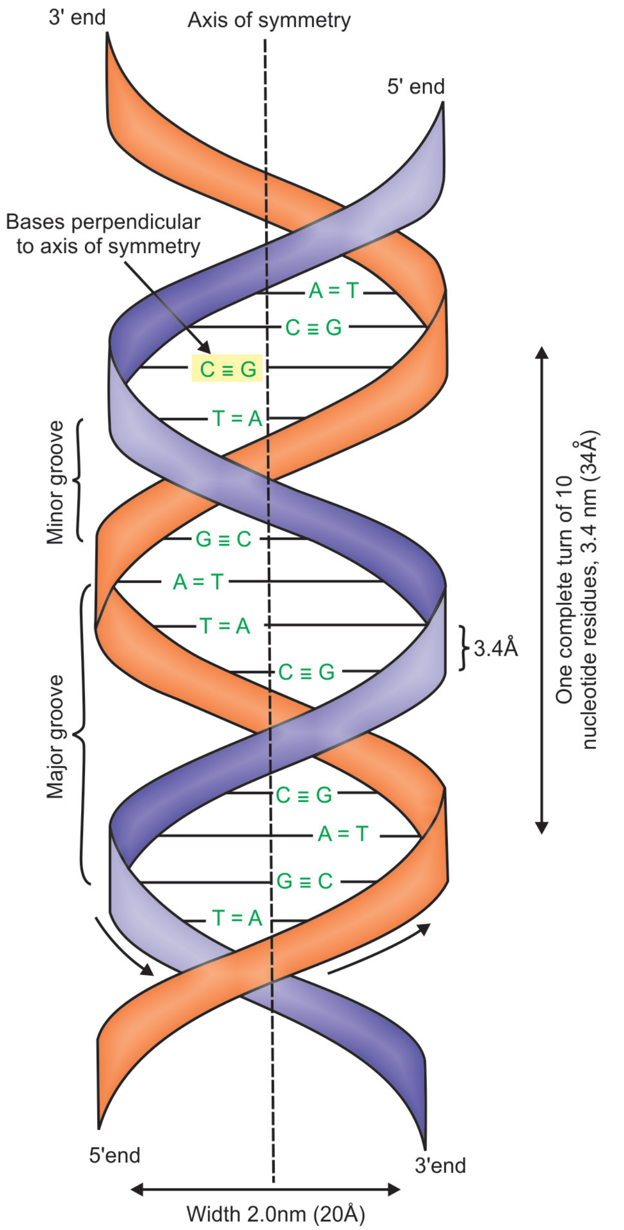

Figure 9.9: A diagrammatic representation of the Watson and Crick model of the double-helical structure of the B form of DNA

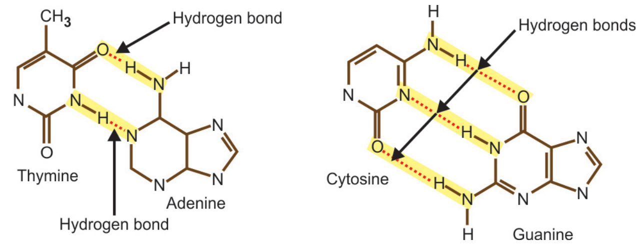

Figure 9.10: Base pairing between adenine and thymine involves the formation of two hydrogen bonds and base pairing between cytosine and guanine involves the formation of three hydrogen bonds

- The two chains are held together by hydrogen bonds between complementary pairs of bases:
  – ***Adenine is always paired with thymine by formation of two hydrogen bonds. Guanine is always paired with cytosine by formation of three hydrogen bonds*** **(Figure 9.10).**
- The two strands are always complementary to each other. In a double stranded DNA molecule, the content of adenine equals to that of thymine and the contents of guanine equals to that of cytosine. The complementary base pairing proves the **Chargaff's rule** (discussed later).
- The model proposed by Watson and Crick **(Figure 9.9)** is a **B form of DNA *(B-DNA)*** which is a right handed helix of 10 base pairs per turn, containing grooves of alternate size, known as major and minor grooves.
- Other forms of DNA may also occur, such as ***A-DNA*** and ***Z-DNA***. Under physiologic conditions, DNA is almost entirely in Watson-Crick B form.

Chargaff's Rule

- Ervin Chargaff discovered that in DNA of all species, quantity of purines is the same as that of pyrimidines (A+G=T+C). He observed that in DNA, content of adenine equals that of thymin (A=T) and content of guanine equals that of cytosine (G=C).
- Watson and Crick deduced that adenine must pair with thymine and guanine with cytosine, because of stearic and hydrogen bonding factors. Adenine cannot pair with cytosine; guanine cannot pair with thymine.
- Thus, one member of a base pair in a DNA must always be a purine and the other a pyrimidine.
- This base pairing restriction explains that in a double stranded DNA molecule, the content of A equals that of T and the content of G equals that of C.

- The ratio of purine to pyrimidine bases in the DNA is always one, i.e. G+A **:** T+C = 1. This is that of ***Chargaff's rule.***

Functions of DNA

DNA is the store of genetic information. The genetic information stored in the DNA serves two functions.

1. It is the source of information for the synthesis of all protein molecules of the cell and
2. It provides the information inherited by daughter cells or offspring.

ORGANIZATION OF DNA

Prokaryotic DNA

A prokaryotic cell generally contains a single chromosome composed of double stranded circular DNA, which contains over 4 x 10^6 base pairs. Because DNA molecules are so large, they require special organization/packaging to enable them to reside within cells. In *E. coli*, the circular DNA is supercoiled and attached to an RNA-protein core.

Eukaryotic DNA

- Eukaryotes contain over 1,000 times the amount of DNA found in prokaryotes. Consequently, their method of organizing or packing DNA is much more complex.
- A typical human cell contains 46 chromosomes, whose total DNA is approximately two meter in length.
- The packing of DNA in a chromosome represents a 10,000 fold shortening of its length from primary B-form DNA.
- In resting nondividing eukaryotic cells, the chromosomal material is called **chromatin**. Chromatin is made up of nucleosomes.

Different Levels of Organization of Eukaryotic DNA

Nucleosomes

- Nucleosomes are primary structural units of chromatin; which consists of **DNA bound to protein histones**.
- There are five types of histones, designated **H1, H2A, H2B, H3** and **H4.** Two molecules of each H2A, H2B, H3 and H4 associate with one another to form a structural core called **histone octomer (Figure 9.11)**.
- Around histone octomer a segment of the DNA double helix is wound nearly twice forming **nucleosome core** particles (a "bead").

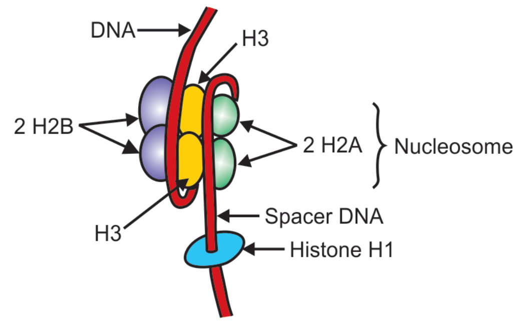

Figure 9.11: Diagrammatic representation of nucleosome

Chromatin fiber

- Chromatin is made up of repeating units of nucleosomes in which one nucleosome core joins to the next to form chromatin fiber.
- Nucleosomes are separated by spacer DNA to which histone H1 is attached to stabilize the complex **(Figure 9.12).** This continuous string of nucleosomes representing beads on a string form of chromatin is termed as **10 nm fiber.**

Solenoid structure

In addition to the net shortening of a DNA strand produced by winding of it around the histones, additional shortening packaging of eukaryotic DNA is brought about by further coiling of 10 nm chromatin fiber to produce 30 nm fiber which has a **solenoid structure** with six nucleosomes per turn. The solenoids are further folded into supercoiled loops to produce 300 nm form **chromosomes.**

A schematic view of the different levels of organization of DNA in the chromosome is shown in **Figure 9.13.**

RNA STRUCTURE AND FUNCTION

Unlike double stranded helical structure of DNA, the RNAs are single stranded. RNA is an unbranched linear polymer of ribonucleotides joined by 3′, 5′ phosphodiester bonds. The phosphodiester bonds join the 3′-OH group of ribose of one nucleotide unit to the 5′-OH group of ribose sugar of the next nucleotide.

Differences Between RNA and DNA

- In RNA, the sugar is **ribose** rather than **2'-deoxyribose** of DNA.
- RNA does not possess **thymine** except in the rare case. Instead of thymine, RNA contains **uracil.**

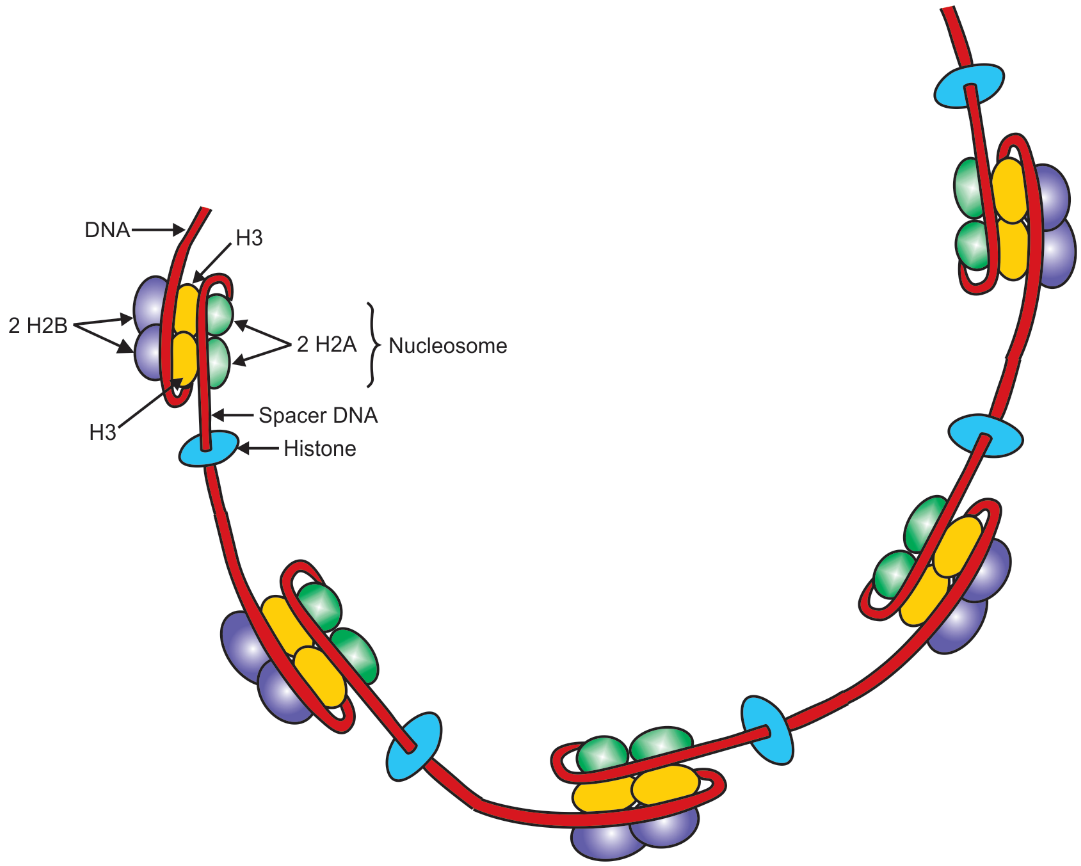

Figure 9.12: Schematic representation of chromatin fiber (Beads on a string structure)

- In RNA, adenine pairs with uracil rather than thymine.
- Unlike DNA, RNA is a single stranded and does not exhibit the equivalence of adenine with uracil and cytosine with guanine.

Types of RNA

Cell contains three major types of RNA:

1. Messenger RNA (mRNA)
2. Transfer RNA (tRNA)
3. Ribosomal RNA (rRNA).

All of these are involved in the process of protein biosynthesis. Each differs from the others by size and function.

Messenger RNA (mRNA)

Structure of mRNA (Figure 9.14)

- The mRNA comprises only about 5-10% of total cellular RNA.
- mRNA is synthesized in the nucleus as heterogenous RNA (hnRNA), which are processed into functional mRNA.
- The mRNA carries the genetic information in the form of codons. Codons are a group of three adjacent nucleotides that code for the amino acids of protein.
- In eukaryotes mRNAs have some unique characteristics, e.g. the 5' end of mRNA is ***"capped"*** by a ***7-methyl-guanosine triphosphate.***
- The cap is involved in the recognition of mRNA in protein biosynthesis and it helps to stabilize the mRNA by preventing attack of 5'-exonucleases.
- A poly (A**)** ***"tail"*** is attached to the other 3'-end of mRNA. This tail consists of series of **adenylate residues**, 20-250 nucleotides in length joined by 3' to 5' phosphodiester bonds.
- The function of poly A tail is not fully understood, but it seems that it helps to stabilize mRNA by preventing the attack of 3'-exonuclease.

Function of mRNA

mRNAs serve as template for protein biosynthesis and transfer genetic information from DNA to protein synthesizing machinery.

If the mRNA codes for only one peptide, the mRNA is **monocistronic.** If it codes for two or more different polypeptides, the mRNA is **polycistronic.** In eukaryotes most mRNA are monocistronic.

Transfer RNA (tRNA)

tRNA molecules vary in length from 74 to 95 nucleotides. In eukaryotic cells, 10-20% of the nucleotides of tRNA may be modified and known as unusual nucleotides **(Figure 9.15)**, e.g.

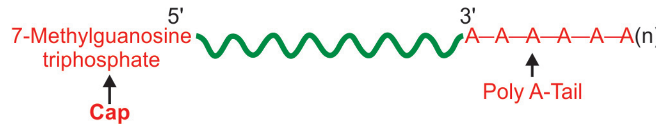

Figure 9.14: Structure of mRNA

- ***Dihydrouridine (D)***, in which one of the double bonds of the base is reduced.
- ***Ribothymidine (T),*** in which methyl group is added to uracil to form thymine.
- ***Pseudouridine (ψ)***, in which uracil is attached to ribose by a carbon-carbon bond rather than a nitrogen bond.

Structure of tRNA

- All single stranded transfer RNA molecules get folded into a structure that appears like a **clover leaf.**

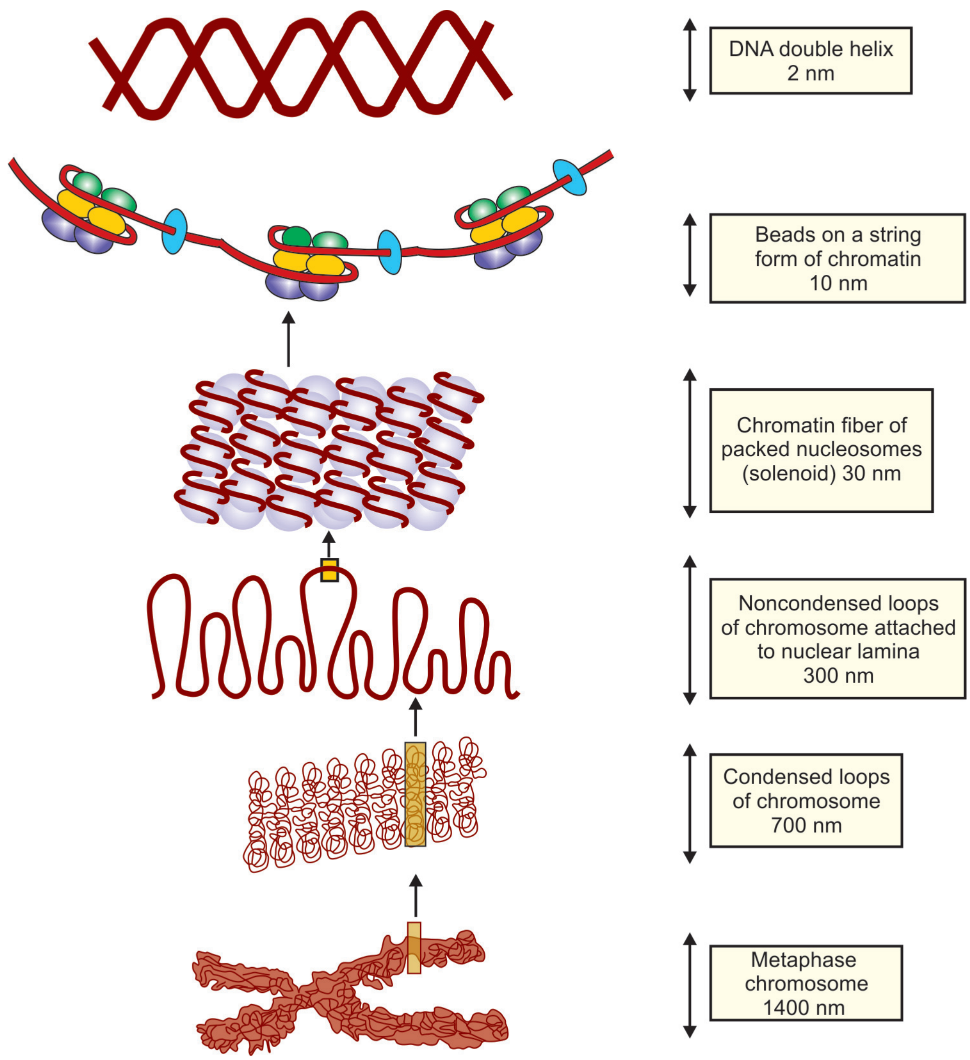

Figure 9.13: Packaging of DNA, arranged in increasing order of organization from top to bottom

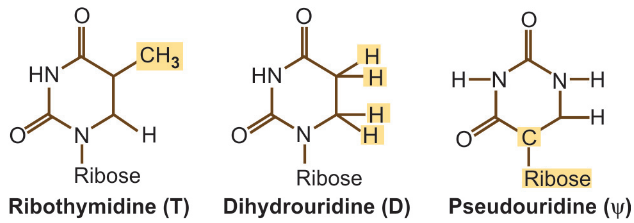

Figure 9.15: Three unusual (modified) nucleosides present in tRNA

All t-RNAs contain four main arms:

1. The acceptor arm
2. The D arm
3. The anticodon arm
4. The TψC arm.

The arms have base paired stems and unpaired loops as shown in **Figure 9.16.** The structure of tRNA molecules is maintained by the base pairing in these arms or stem regions.

- The **acceptor arm** consists of a base paired stem that terminates in the sequence **CCA** at the 3' end. This is the attachment site for the amino acid.
- The **D arm** is named for the presence of the base dihydrouridine (D).
- **The anticodon arm** contains the **anticodon** that base pairs with the codon on mRNA. Anticodon has nucleotide sequence complementary to the codon of mRNA and is responsible for the specificity of the tRNA.
- The TψC arm contains both **ribothymidine (T)** and **pseudouridine (ψ, psi)**

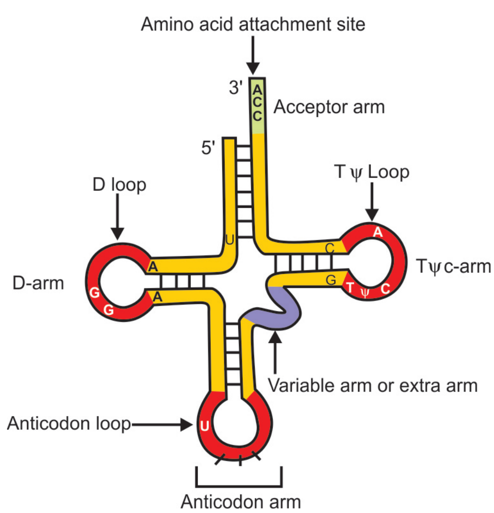

Figure 9.16: Structure of clover leaf transfer RNA

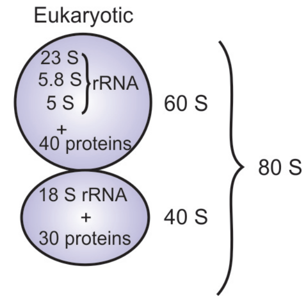

Figure 9.17: The components of eukaryotic ribosomal subunits

- The extra arm is also known as variable arm because it varies in size, is found between the anticodon and TψC arms.

Function of tRNA

tRNA carries amino acids in an activated form to the ribosome for the protein synthesis.

Ribosomal RNA (rRNA)

The RNA of the ribosomes is called the rRNA.

- A ribosome is a cytoplasmic nucleoprotein that acts as a machinery for the synthesis of proteins.
- The ribosome is a spheroidal particle and is composed of a large and a small nucleoprotein subunit.
- The eukaryotic ribosomes are composed of 60S and 40S subunits **(Figure 9.17)**.
- Each subunit is composed of one or more strand of rRNA and numerous protein molecules **(Figure 9.17).**
- The 60S subunit contains 28S rRNA, 5S rRNA and 5.8S rRNA, while the 40S subunit contains 18S rRNA.

Functions of ribosomal RNA

The function of the ribosomal RNA molecules in the ribosomal particle are not fully understood, but they are:

- Necessary to maintain ribosomal structure and also participate in protein synthesis by binding of mRNA to ribosome.
- Recent studies suggest that ribosomal RNAs may also provide some of the catalytic activities and thus is an enzyme "a ***ribozyme*".**

Other Nuclear and Cytoplasmic RNAs

Besides mRNA, tRNA and rRNA, eukaryotes have some other RNAs. These are:

- Heterogenous RNAs (hnRNAs)
- Small cytoplasmic RNAs (scRNAs)
- Small nuclear RNAs (snRNAs).

Table 9.2: Different types of cellular RNAs and their functions

Types of RNA	*Functions*
mRNA (messenger RNA)	Carries the genetic information from DNA to the cytosol, where it is used for protein synthesis
tRNA (transfer RNA)	Serves as an "adaptor" molecule that carries specific amino acid to the site of protein synthesis
rRNA (ribosomal RNA)	In association with protein serves as the sites for protein synthesis. Provides catalytic activities (peptidyltransferase activity)
hnRNA (heterogeneous nuclear RNA)	Serves as precursor for mRNA
scRNA (small nuclear cytoplasmic RNA)	Involved in recognition of signal sequence in protein synthesis on membrane bound ribosomes
snRNA (small nuclear RNA)	Involved in excising introns and splicing exons

Various RNAs and their functions are given in **Table 9.2.**

SUMMARY

- The nucleic acids DNA and RNA are poly-nucleotides.
- In DNA and RNA, nucleotides are linked by 3'-5' phosphodiester bonds.
- Each nucleotide contains a nitrogenous base, a sugar and a phosphate.
- Nucleosides contain, D-ribose or D-2-deoxyribose, linked to N-1 of pyrimidines or N-9 of purines, by a N-glycosidic bond.
- DNA contains the purine bases: adenine (A) and guanine (G) and pyrimidine bases: cytosine (C) and thymine (T).
- RNA contains uracil (U) instead of thymine.
- Several synthetic analogues of purine and pyrimidine bases and their derivatives are used chemotherapeutically as anticancer drugs.
- DNA is organized into two strands by the pairing of bases A to T and G to C, on complementary strands. In contrast to DNA, RNA is single stranded structure.
- The three major types of RNA are mRNA, tRNA and rRNA, all are involved in some aspects of protein synthesis.

EXERCISE

Multiple Choice Questions (MCQs)

1. **Which of the following holds DNA strands together?**
   a) Phosphodiester bond b) Hydrogen bond
   c) Glycosidic bond d) Phosphate ester

2. **Which carbon of the pentose is in ester linkage with the phosphate in a nucleotide structure?**
   a) C_1 b) C_2
   c) C_3 d) C_4

3. **Thymine is present in which of the following:**
   a) Ribosomal RNA
   b) Messenger RNA
   c) Transfer RNA
   d) None of the above

4. **Unusual nucleotide pseudouridylic acid is present in:**
   a) mRNA b) tRNA
   c) rRNA d) hnRNA

5. **A nucleoside can be composed of, *except*:**
   a) Purine base
   b) Pentose sugar
   c) Phosphate group
   d) Pyrimidine base

6. **Nucleotides perform all of the following functions *except:***
   a) Structural units of DNA and RNA
   b) Catalytic in nature
   c) Regulators of metabolic reactions
   d) Components of certain coenzymes

7. **Which of the following is not a feature of Watson-Crick model of DNA?**
   a) Helical
   b) Two strands are held by hydrogen bond
   c) A + T = C+G
   d) Two strands are right handed

8. **The number of hydrogen bonds between guanosine and cytosine in DNA are:**
   a) One b) Two
   c) Three d) Four

9. DNA is present in:
a) Only nucleus
b) Only mitochondria
c) Both nucleus and mitochondria
d) Cytoplasm

10. RNA is present in:
a) Nucleus
b) Only cytoplasm
c) Mitochondria
d) Cytoplasm and nucleolus

Correct answers for MCQs

1-b	2-a	3-c	4-b
5-c	6-b	7-c	8-c
9-c	10-d		