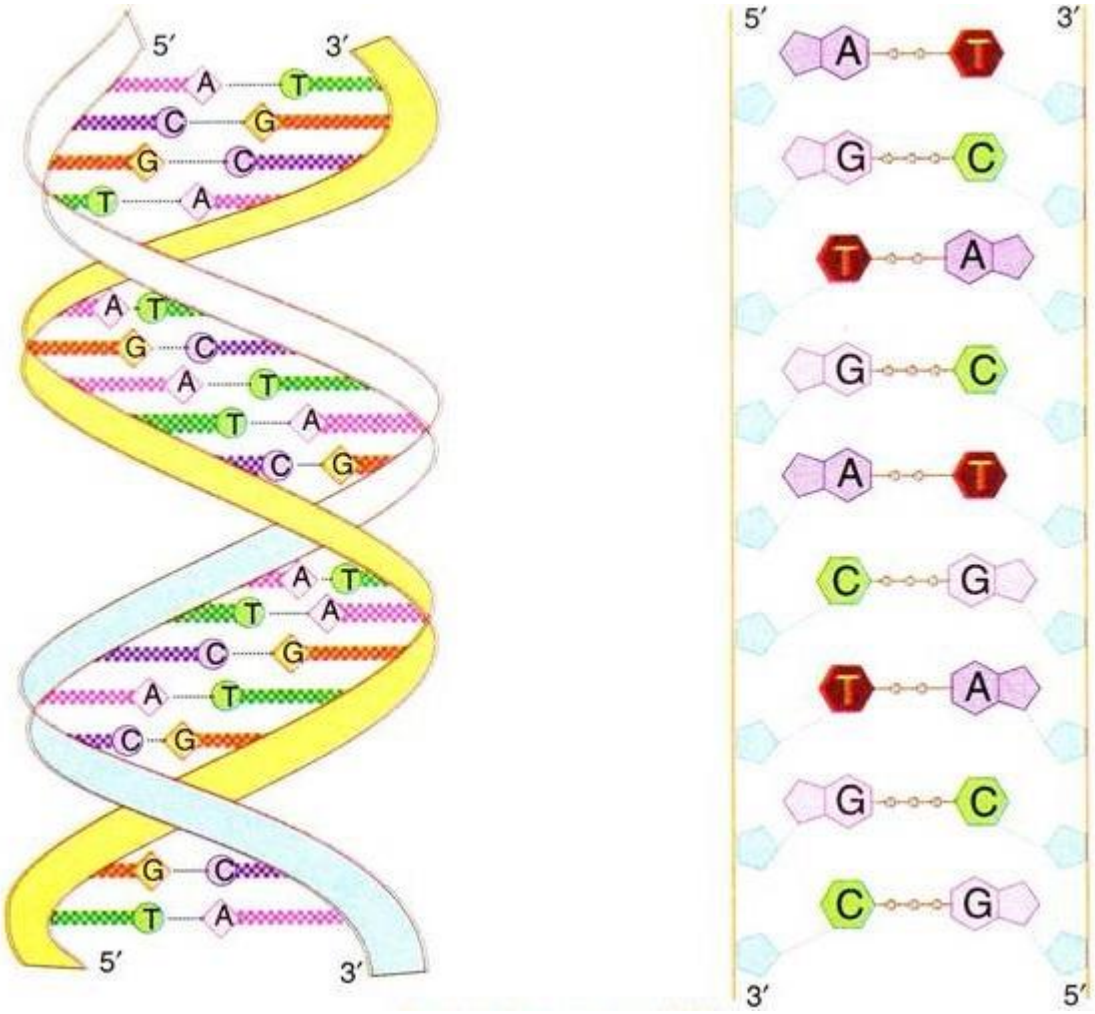
Deoxyribonucleic Acid (DNA):

Deoxyribonucleic acid, also abbreviated as DNA, is the principal informational macromolecule of the cell, which stores, translates and transfers the genetic information. In the prokaryotes, the DNA is found mostly in the nuclear zone. In eukaryotes it is found in the nucleus, mitochondria and chloroplast. The present understanding of the storage and utilization of the cell's genetic information is based upon the discovery of the structure of DNA by Watson and Crick in 1953.

Structure of DNA:

DNA is made of two helical chains coiled around the same axis, to form a right-handed double helix.

2. The two chains in the helix are anti-parallel to each other, i.e., the 5′-end of one polynucleotide chain and the 3′-end of the other polynucleotide chain is on the same side and close together.



The distance between each turn is 3.6 nm (formerly 3.4 nm).

4. There are 10.5 nucleotides per turn (formerly 10 nucleotides).

5. The spatial relationship between the two strands creates major and minor grooves between the two strands. In these grooves some proteins interact.

6. The hydrophilic backbones of alternating deoxyribose and negatively charged phosphate groups are on the outside of the double helix.

7. The hydrophobic pyrimidine and purine bases are inside the double helix, which stabilizes the double helix of the DNA.

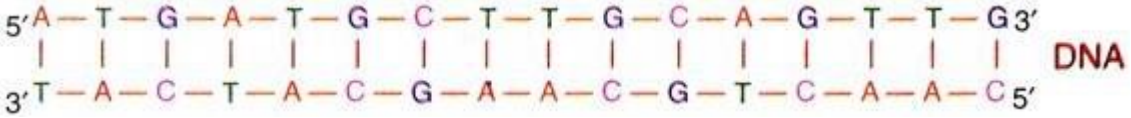
8. The double helix is also stabilized by inter-chain hydrogen bond formed between a purine and pyrimidine base.

9. A particular purine base, pairs by hydrogen bonds, only with a particular pyrimidine base, i.e., Adenine (A) pairs with Thymine (T) and Guanine (G) pairs with Cytosine (C) only.

10. Two hydrogen bonds pairs Adenine and Thymine (A = T), whereas three hydrogen bonds pairs Guanine and Cytosine (G ≡ C).

11. The base pairs A = T and G ≡ C are known as complementary base pairs.

Due to the presence of complementary base pairing, the two chains of the DNA double helix are complementary to each other.



Hence the number of A' bases are equal to the number of T' bases (or 'G' is equal to 'C) in a given double stranded DNA.

13. One of the strands in the double helix is known as sense strand, i.e., which codes for RNA/proteins and the other strand is known as antisense strand.

The DNA molecules exist in four different structural forms or organizations under different physiological conditions or in different cells or at different points in the same DNA.

Comparison between different structural forms of DNA

	A	B	Z	H
1.	Shorter and wider	Normal reference strand– Watson and Crick model	Longer and thinner	It is a long stretch (part) of DNA with alternating T and C or polypurine/ polypyrimidine
2.	Right-handed double helix	Right-handed double helix	Left-handed double helix	Triple helix
3.	Distance between each turn is 2.3 nm	Distance between each turn is 3.6 nm	Distance between each turn is 3.8 nm	—
4.	11 base pairs per turn	10.5 base pairs per turn	12 base pairs per turn	—
5.	Stable in solutions devoid of water	Most stable under physiological conditions	Doubtful existence in physiological state	Helps in gene regulation

Functions of DNA:

The base sequence of the DNA constitutes the informational signal called the genetic material. This nucleotide base sequence enables the DNA to function, store, express and transfer the genetic information. Hence it programs and controls all the activities of an organism directly or indirectly throughout its life cycle.

(a) DNA stores the complete genetic information required to specify (form) the structure of all the proteins and RNA's of each organism.

(b) DNA is the source of information for the synthesis of all cellular body proteins. Some of the proteins are structural proteins and some are enzymes. These enzymes arrange micro-molecules to form macromolecules. These macromolecules are arranged to form supra-molecular complexes or cell organelles which associate to form cells. These cells group to form tissues which in turn form different organs of a body, specifically peculiar to that organism during foetal development, growth and repair. Hence DNA programs in time and space the orderly biosynthesis of cells and tissue components.

(c) It determines the activities of an organism throughout its life cycle, i.e., the period of gestation, birth, maturity, senescence and death.

(d) It determines the individuality and identity of a given organism.

(e) It duplicates (replicates to form two daughter DNA) itself and transfers one of the copy to the daughter cell during cell division, thus maintaining the genetic material from generation to generation.

CHEMICAL AND PHYSICAL PROPERTIES OF DNA

I. ABSORPTION

- A. The bases in DNA absorb ultraviolet light at the wavelength of 260 nm
  1. This absorption can be monitored using a spectrophotometer
  2. This is one method used to figure the concentration of DNA in solution
- B. The less ordered the bases the more ultraviolet light is absorbed
  1. Free bases absorb 1.60 units at 260 nm
  2. Single stranded DNA absorb 1.37 units at 260 nm
  3. Double stranded DNA absorb 1.00 units at 260 nm

II. DENSITY

- A. Density can be measured by CsCl-density ultracentrifugation
  1. CsCl, upon ultracentrifugation, will form a density gradient, with the most dense solution at the bottom
  2. Macromolecules, such as DNA, will concentrate in the area of CsCl that has the same density as themselves
     a. Hence, more dense DNA will migrate downward and less dense DNA upwards forming bands
- B. Density can be used to estimate G+C content
  1. GC base pairs are more dense than AT base pairs
  2. Therefore, DNA with more GC base pairs will form bands lower down than an equal number of base pairs with high AT content
- C. Density studies show the existence of satellite DNA
  1. If chromosomal DNA is cut into about equal size pieces and subjected to CsCl-density ultracentrifugation two bands are formed
     a. One band contains most of the DNA from the genome
     b. The second band (the satellite) contains about 5% of the DNA from the genome and has a highly repetitive sequence
     c.

III. DENATURATION

A. Definition

1. DNA is considered denatured when the double stranded DNA molecule is converted into two single stranded molecules
2. This can be monitored by noting the increase in absorption of ultraviolet light

B. Temperature

1. As thermal energy increases, the frequency of hydrogen bonds breaking between the molecules increases
   a. As temperature increases, the two molecules will separate into single-stranded molecules
2. The *Tm* (melting temperature) of a DNA molecule is the temperature in which half the DNA molecules are denatures
   a. The *Tm* is used to estimate the G+C content of a DNA molecule
      1. G-C base pairs are held together by three hydrogen bonds (A-Ts by two) and it therefore takes more energy (higher temperatures) to separate molecules with high GC contents

C. Hydrophobicity of solvent

1. Substances that are hydrophobic tend to decrease the *Tm* of DNA molecules
   a. Hydrophobic substances will allow the bases in DNA to dissolve into the solvent
   b. Hence, the bases are not constricted to being stacked upon one another
      1. This will make it easier to disrupt the hydrogen bonding between DNA molecules
2. Substances that are hydrophilic tend to increase the *Tm* of DNA molecules
   a. These will keep the bases of DNA stacked upon one another in the orientation that most favors hydrogen bonding between DNA strands

D. pH

1. Acids
   a. pHs lower then one result in the breakage of phosphodiester bonds between nucleotides and breakage of the N-glycosidic bond between the sugar and purine bases

b. pH of around 4 results in the selective breakage of N-glycosidic bonds between the sugar and purines
    1. DNA treated this way is referred to a apurinic acid, since the purines have been removed

2. Alkali
    a. Base tends to change the polarity of groups involved in hydrogen bonds
        1. Above pH 11.3, all hydrogen bonds are disrupted and the DNA is totally denatured
    b. DNA is resistant to hydrolysis to about pH 13
        1. Unless it is apurinic, then it is hydrolyzed
    c. RNA is hydrolyzed into ribonucleotides around pH 11

E. Ionic strength
1. The phosphates of the DNA sugar-phosphate backbones are negatively charged
    a. Like charges repel each other
    b. DNA in distilled water will spontaneously denature into single stranded DNA
2. Salts that dissociate into ions will neutralize the charges of the phosphate groups
    a. Salts will stabilize the DNA double helix resulting in a higher *Tm*

F. G+C content
1. Variation
    a. Most higher organisms have a G+C content of about 0.5 (0.49 - 0.51 for primates)
    b. Lower organisms range widely from 0.27 to 0.76 for some bacteria
2. Estimating G+C content
    a. G+C content of a DNA molecule can be estimated from its thermal melting temperature (*Tm*)

IV. SOLUBILITY

A. RNA is more soluble in aqueous solutions then DNA
1. Ribose has a 2'-OH group where deoxyribose contains a 2'-H
    a. Hydroxyl groups are polar and dissolve in water better
    b. C-H is a non-polar bond and is therefore hydrophobic

B. RNA is less stable then DNA
   1. The hydroxyl group on the 2' carbon of ribose is more reactive the hydrogen found in deoxyribose

V. SIZE

A. Electrophoresis
   1. DNA has a negative charge that is proportional to its size
      a. This is due to the negatively charged phosphates in the sugar-phosphate backbone
      b. If DNA is placed in an electrical field it will migrate towards the positive electrode (the cathode)
   2. If DNA is electrophoresed through a gel, smaller pieces will migrate faster than larger pieces
      a. Larger pieces have trouble squeezing through the gel matrix and are hence retarded while smaller pieces migrate easier
      b. Type of gels
         1. Agarose is used to separate fairly large DNA molecules
            a. 5 million to a few thousands base pairs
         2. Polyacrylamide is used to separate small pieces of DNA
            a. 2 to several hundred base pairs
   3. The size of DNA is estimated by comparing its migration through the gel to DNA molecules of known size

B. Velocity sedimentation
   1. Sedimentation velocity is dependent upon two variables: density and shape
      a. The more dense the DNA the quicker it will sediment upon centrifugation
      b. Globular (more compact) molecules will sediment faster than linear molecules

C. Electron microscopy
   1. The size of DNA molecules can be determined by electron microscopy
      a. The DNA is visualized on a grid of known size so that the size of the DNA molecule can be estimated

VI. DNA CONCENTRATION

A. Absorption
   1. DNA absorbs ultraviolet light at 260 nm

2. The more DNA present, the higher the absorption
    a. DNA concentrations can be estimated by comparing its absorption to known concentrations of DNA
    b. DNA most be fairly pure, since many contaminating substances (e.g., proteins) also absorb around this wavelength

VII. RENATURATION STUDIES

A. DNA that has been denatured will often come back together when condition are met
    1. This is referred to as renaturation
    2. Renaturation occurs because hydrogen bonds of complimentary base pairs reform
        a. Slowly lowering the temperature or adding ions to solution may lead to renaturation
B. Renaturation rates are dependent on DNA concentration
    1. The rate limiting step in renaturation is the collision of complimentary DNA molecules
        a. The more molecules of complimentary DNA molecules present, the faster they can find each other and renature
        b. DNA molecules in low concentration in solution will take awhile to find a complimentary partner, and will therefore renature slower
C. In eukaryotes, three major drops in absorbance occur in renaturation studies
    1. The first drop in absorption is when the highly repetitive DNA sequence renatures
        a. Since these are repeated so often, they are in the highest concentration
    2. The second drop in absorption occurs when the moderately repetitive DNA renatures
    3. Unique DNA sequences are the last to renature
        a. These are in the lowest concentration and take the longest time to find each other

Temperature of Melting (Tm)

The **Temperature** of **Melting** (Tm) is defined as the **temperature** at which 50% of double stranded **DNA** is changed to single-standard **DNA**. The higher the **melting temperature** the greater the guanine-cytosine (GC) content of the **DNA**.

The **temperature** at which the **DNA** strands are half denatured, meaning half double-stranded, half single-stranded, is called the **melting temperature** (Tm). The amount of strand separation, or **melting**, is measured by the absorbance of the **DNA** solution at 260nm.