DNA

- DNA is a part of nucleic acid and stands for deoxyribonucleic acid.
- DNA stores information for the synthesis of protein.
- DNA contains all the information of the cell to reproduce and all these information are transferred from parents to their children therefore DNA is called as a genetic or heredity material of the cell.
- The main role of DNA in the cell is the long-term storage of information.
- Approximately **3 billon** base pairs of DNA arranged into 46 chromosomes.

Structure of DNA:

- DNA is made up of molecules called **nucleotides.**
- Each nucleotide contains:

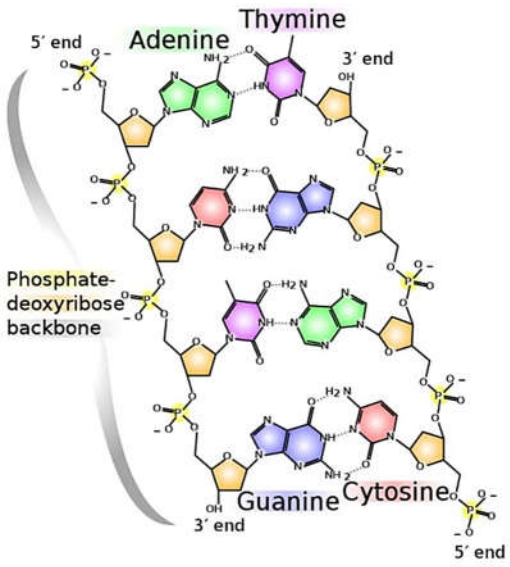
1. A phosphate group

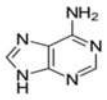
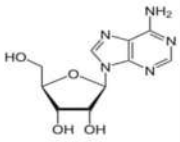
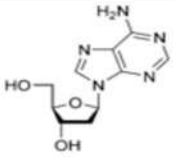
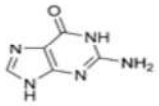
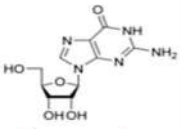
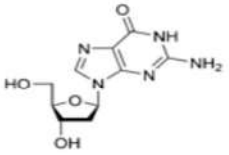
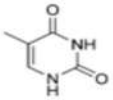
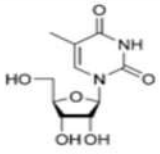
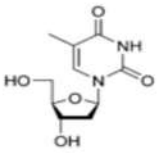
2. A sugar group (deoxyribose sugar)

3. A nitrogen base. The four types of nitrogen bases are adenine (A), thymine (T), guanine (G) and cytosine (C).

- The structure of DNA is a helical, double-stranded macromolecule with bases present inside of the molecule of DNA. These two strands are always complementary in sequence. One strand serves as a template for the formation of the other during DNA replication, a major source of inheritance. This unique feature of DNA provides a mechanism for the existence of life.
- The structure of DNA was found by Rosalind Franklin when she used x-ray crystallography to study the genetic material of the cell. The x-ray photo she obtained revealed the physical structure of DNA as a helix.
- DNA has a double helix structure. The outer edges are formed by alternating deoxyribose sugar molecules and phosphate groups, which make up the sugar-phosphate backbone.
- The two strands run in opposite directions, one moved in a **3' to 5'** direction and the other moved in a **5' to 3'** direction.
- The nitrogenous bases are present inside the helix structure like "rungs on a ladder," due to the hydrophobic effect, and stabilized by hydrogen bonding.

- The two strands run in opposite directions to form the double helix. The strands are held together by **hydrogen bonds and hydrophobic interactions.**
- The H-bonds are formed between the base pairs of the anti-parallel strands. The base in the first strand forms an H-bond only with a specific base in the second strand.
- Those two bases form a base-pair (H-bond interaction that keeps strands together and form double helical structure). The base–pairs in DNA are adenine-thymine (A-T) and cytosine-guanine (C-G). Such interactions provide us an understanding that nitrogen-containing bases are located inside of the DNA double helical structure, while sugars and phosphates are located outside of the double helical structure.
- The component consisting of the base and the sugar is known as the **nucleoside.** DNA contains deoxyadenosine (deoxyribose sugar bonded to adenine), deoxyguanosine (deoxyribose sugar bonded to guanine), deoxycytidine (deoxyribose sugar bonded to cytosine), and deoxythymidine (deoxyribose sugar bonded to thymine).
- The linkage of the bonds between the bases to the sugar is known as the beta-N-Glycosidic linkage. In **purines**, this occurs between the **N-9 and C-1' and** in **pyrimidines** this occurs between the **N-1 and C-1'**.
- A nucleoside and a phosphate group make up a **nucleotide**. The bond between the deoxyribose sugar of the nucleoside and the phosphate group is a **3'-5' phosphodiester linkage**.
- The bases, located inside the double helix, are stacked. Stacking bases interact with each other through the Van der Waals forces. Although the energy associated with a Van der Waals interaction is relatively small, in a helical structure.
- The distance between two neighboring bases that are perpendicular to the main axis is **3.4 Å.**
- The DNA structure is repetitive. There are ten bases per turn that is the structure repeats after **34 Å,** so every base has a **36°** angle of rotation. The radius of the double helix is approximately **10 Å**.
- An easy way to differentiate between **Nucleosides and Deoxynucleosides** is the atoms bonded to C-2 on the sugar unit. If the structure is a deoxynucleoside, then C-2 bears two hydrogens. If it is a nucleoside, then C-2 bears one hydrogen.



Nitrogenous base	Nucleoside	Deoxynucleoside
 Adenine	 Adenosine A	 Deoxyadenosine dA
 Guanine	 Guanosine G	 Deoxyguanosine dG
 Thymine	 5-Methyluridine m^5U	 Deoxythymidine dT

Early foundation for DNA structure:

- One of most important parts of determining the structure of DNA comes from the work **of Erwin Chargaff and his colleagues in the late 1940s**. They found that the four nucleotide

bases of DNA of different organisms and that the amounts of certain bases are closely related. They concluded the following about the structure of DNA:

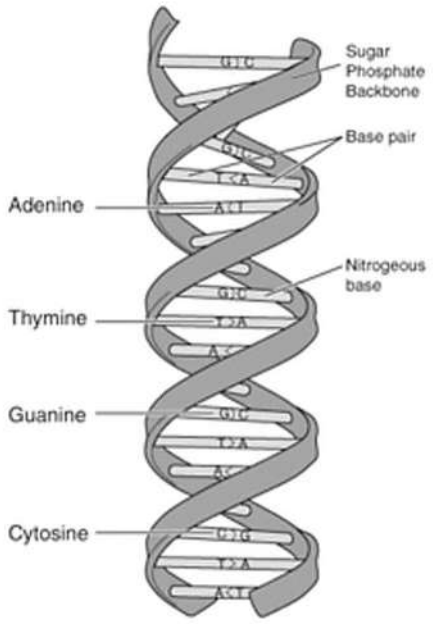
DNA general structure and its bases:

1. The base composition of DNA generally varies from one species to another.

2. DNA specimens isolated from different tissues of the same species have the same base composition.

3. The base composition of DNA in a given species does not change over time, nutritional states, or environment.

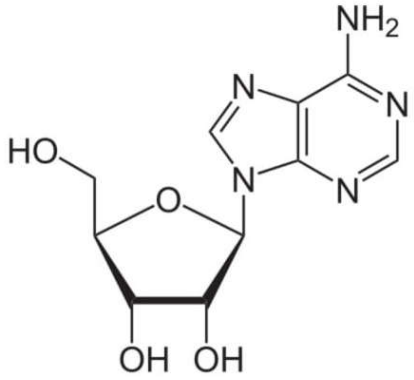
4. In all cellular DNA, regardless of the species, the number of adenines is equal to the number of thymine (A=T) and the number of guanines is equal to the number of cytosine (G=C).



DNA general structure and its bases

- Later in **1953, Rosalind Franklin and Maurice Wilkins** used a powerful X-ray diffraction technique called X-ray crystallography to give the DNA structure. Franklin and Wilkins found that DNA molecules are helical with two cycles along their long axis, a primary one of **3.4A** and a secondary one of **34A.**
- **Watson and Crick** later based their model of DNA upon the data they were able to extract from Wilkins and Franklin's X-ray diffraction photo.

- DNA structure given by Watson and Crick is still accepted today. In this structure, they proposed that two helical DNA chains of opposite direction wound around the same axis to form a right handed double helix. The purine and pyrimidine bases of both strands are stacked inside the double helix and stabilized by Van Der Waals interactions.
- The double-helix has a diameter of **10 Å.** Each adjacent base on one strand of the double-helix is **3.4 Å** apart. Every 10 base-pairs constitutes a is **3.4 Å** apart. Every 10 base-pairs constitute a **360°** turn in the helix, and the length of the helix is determined by **34 Å per 10 base-pairs.**



Nucleoside (adenosin) with beta glycosidic bond

Forces involved in DNA:

- The DNA double helix is held together by two main forces:
  1. Hydrogen bonds between complementary base pairs inside the helix
  2. Van der Waals base-stacking interaction

Hydrogen bonds:

- Watson and Crick found that the hydrogen bonded base pairs, G with C, A with T, are those that best fit within the DNA structure.
- It is important to note that three hydrogen bonds can form between G and C, but only two bonds can be found in A and T pairs. This conclusion was made possible by a known fact that in each species the G content is equal to that of C content and the T content is equal to that of A content.

Denaturating or Annealing of DNA:

- Ultraviolet (UV) light can detect whether bases are stacked or unstacked. Stacked bases within the DNA structure facilitate shielding from light; therefore the absorbance of UV light of double helical DNA is much less than single stranded DNA. This characteristic is known as the **hypochromic effect**, in which less color is emitted from the double helix of DNA molecules.
- **The melting temperature (Tm)** is the temperature in which DNA is half way of the DNA is double stranded and half is single stranded. The Tm depends greatly on base composition. Since G-C base pairs are stronger due to more Hydrogen bonds, DNA with high G-C content will have a higher Tm than that of DNA with greater A-Tcontent.
- When heat is applied to a double-stranded DNA, each individual strand will eventually separate **(denature)** because hydrogen bonds are disrupted between base pairs. Upon separation, the separated strands spontaneously reassociate to form the double helix again. This process is known as **annealing**.
- In biological systems, both denaturing and annealing can occur. Helicases use chemical energy (from ATP) to disrupt the structure of double-stranded nucleic acid molecules. The study of the ability of DNA to reanneal within the laboratory is important in discovering gene structure and expression.

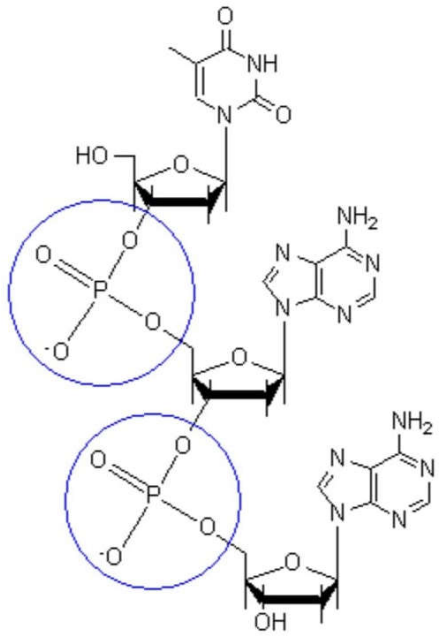
Base stacking interactions:

- The two strands of double-stranded DNA are held together by a number of weak interactions such as hydrogen bonds, stacking interactions, and hydrophobic effects. Of these, the stacking interactions between base pairs are the most significant. The strength of base stacking interactions depends on the bases. It is strongest for stacks of G-C base pairs and weakest for stacks of A-T base pairs.
- The hydrophobic effect stacks the bases on top of one another. The stacked base pairs attract one another through **Van der Waals forces**, typically from **2 to 4 kJ/ mol-1.** In addition, base stacking in DNA is favored by the conformations of the somewhat rigid five membered rings of the backbone phosphate-sugars. The base-stacking interactions, which are largely nonspecific with respect to the identity of the stacked base, make the major contribution to the stability of the double helix.

Phosphodiester bond:

"A phosphodiester bond is the linkage formed between the 3' carbon atom and the 5' carbon of the sugar deoxyribose in DNA"

- The phosphate groups in a phosphodiester bond are negatively-charged. The pKa of phosphate groups are near to 0, therefore they are negatively-charged at neutral pH (pH=7). This charge-charge repulsion forces the phosphates groups to take opposite positions of the DNA strands and is neutralized by proteins (histones), metal ions such as magnesium, and polyamines.
- The tri-phosphate or di-phosphate forms of the nucleotide building are blocks, first have to be broken apart to release the energy require to drive an enzyme-catalyzed reaction for a phosphodiester bond to form and for the nucleotide to join. Once a single phosphate or two phosphates (pyrophosphates) break apart and participate in a catalytic reaction, the phosphodiester bond is formed.
- An important role in repairing DNA sequences is due to the hydrolysis of phosphodiester bonds being catalyzed by phoshodiesterases, an enzyme that facilitates the repairs.
- One reason that made DNA more stable than RNA is absence of the 2'-OH group in DNA. The presence of OH group on 2'C makes RNA more susceptible for reactions.

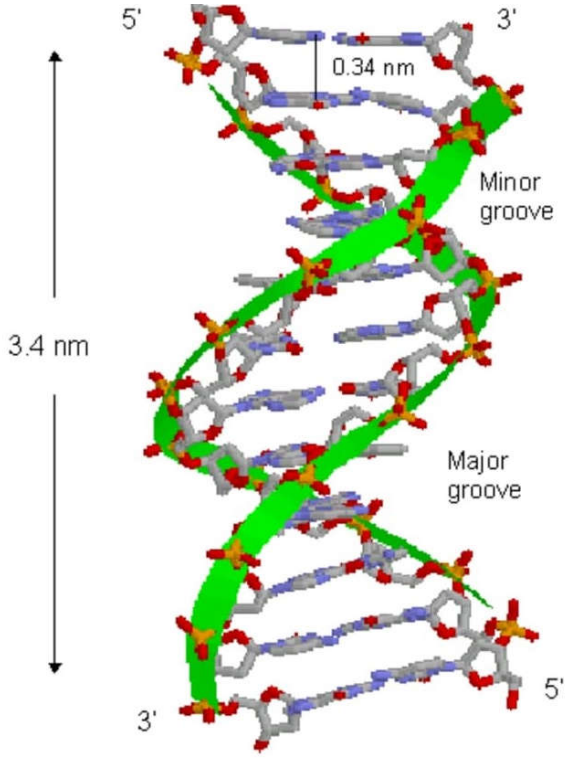


Phosphodiester Bond between nucleotides

Secondary structure of DNA:

Base pairing of complementary nucleotides make up the secondary structure of DNA.

- A single-stranded DNA may participate in intramolecular base pairing between complementary base pairs and therefore make up secondary structure as well.
- The molecule has two asymmetric grooves. One groove is smaller than the other is larger. This asymmetry is a result of the geometrical configuration of the bonds between the phosphate, sugar, and base groups that forces the base groups to attach at **120 degree angles instead of 180 degree**. The larger groove is called the **major groove**, occurs when the backbones are far apart; while the smaller one is called the **minor groove**, and occurs when they are close together.
- Since the major and minor grooves expose the edges of the bases, the grooves can be used to tell the base sequence of a specific DNA molecule. The possibility for such recognition is critical, since proteins must be able to recognize specific DNA sequences on which to bind in order for the proper functions of the body and cell to be carried out. As you might expect, the major groove is more information rich than the minor groove, allowing the DNA proteins to interact with the bases. This fact makes the minor groove less ideal for protein binding.



Visual Representation of Major and Minor Grooves in DNA Structure

<u>Forms of DNA in secondary structure:</u>

- There are three forms of DNA in secondary structure:

  1. A form

2. B form

3. Z form

A form:

- These following features represented different characteristics of A-form DNA structure:

1. Most RNA and RNA-DNA duplex in this form.

2. Shorter, wider helix than B.

3· Deep, narrow major groove not easily accessible to proteins

content than major groove.

5· Favored conformation at low water concentrations.

6· Base pairs tilted to helix axis and displaced from axis.

7· Sugar pucker C3'-endo (in RNA 2'-OH inhibits C2'-endo conformation)

8· Right handed.

9· Size is about 26 angstroms.

10· Needs 11 base pairs per helical turn.

11· Anti conformation in Glycosyl bond.

B form:

1. The double helical structure of normal DNA takes a right-handed form called the B-helix.

2. It is about 20 angstroms with a C-2' endo sugar pucker conformation.

3. The helix makes one complete turn approximately every 10 base pairs (= 34 A per repeat/3.4 A per base).

4. B-DNA has two principal grooves, a wide major groove and a narrow minor groove.

5. Many proteins interact in the space of the major groove, where they make sequence-specific contacts with the bases. In addition, a few proteins are known to make contacts via the minor groove.

Z form:

1. DNA sequences can turn to from a B form to a Z form and vice versa.

2. Z form of DNA is a more radical departure from the B structure.

3. The most obvious distinction is the left-handed helical rotation.

4. The Z form is about 18 angstroms and there are 12 base pairs per helical turn, and the structure appears more slender and elongated.

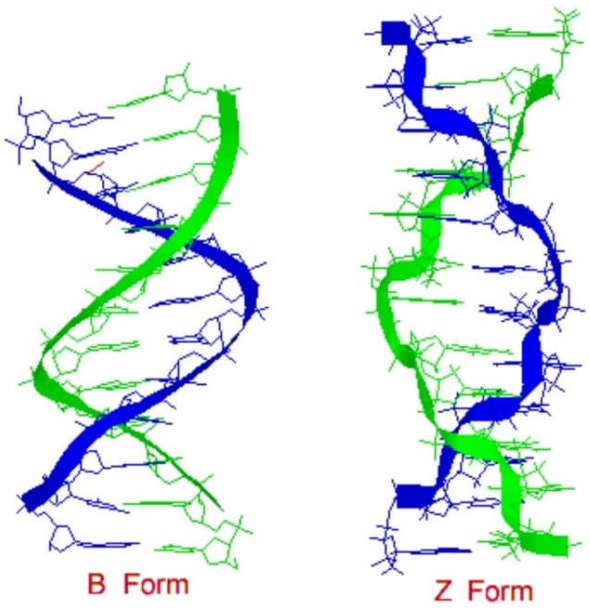
5. The DNA backbone takes on a zigzag appearance. Certain nucleotide sequences fold into left-handed Z helices much more readily than others. Prominent examples are sequences in which pyrimidines alternate with purines, especially alternating C and G or 5-methyl-C and G residues.

6. To form the left-handed helix in Z-DNA, the purine residues turn to the syn conformation alternating with pyrimidines in the anti-conformation.

7. The major groove is barely apparent in Z-DNA, and the minor groove is narrow and deep.

8. For pyrimidines, the sugar pucker conformation is C-2' endo and for purines, it is a C-3' endo.

9. Z-DNA formation occurs during transcription of genes, at transcription start sites near promoters of actively transcribed genes. During transcription, the movement of RNA polymerase induces negative supercoiling upstream and positive supercoiling downstream the site of transcription. The negative supercoiling upstream favors Z-DNA formation; a Z-DNA function would be to absorb negative supercoiling. At the end of transcription, topoisomerase relaxes DNA back to B conformation.



B and Z form DNA

	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	26 A	20 A	18 A
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 A	3.4 A	3.7A
Base tilt normal to the helix axis	20^0	6^0	7^0
Sugar pucker conformation	C-3' endo	C-2' endo	C-2' endo for pyrimidines and C-3'endo for purines

Tertiary structure of DNA:

The tertiary structure of DNA molecule is made up of the two strands of DNA wind around each other.

- DNA double helix can be arranged in space, in a tertiary arrangement of strands.
- Linking Number (Lk) in a covalently closed circular DNA, where the two strands cannot be separated will result in a constant number of turns in a given molecule. Lk of DNA is an integral composed of two components:

  1. Twist (Tw): number of helical turns of DNA strand

  2. Writhe (Wr): number of supercoiled turns in DNA strand
- Normally, DNA has Lk of about **25,** meaning it is underwound. However, DNA can also be supercoiled with two "underwindings" which is made up of negative supercoils. This is much like the two "turns- worth" of a single stranded DNA and no supercoils.
- This kind of interconversion of helical and superhelical turns is important in gene transcription and regulation.

Quaternary structure of DNA:

The binding of DNA to histones protein to form nucleosomes is referring the DNA quaternary structure.

- The quaternary structure affects the DNA sequences in transcription process for the expression of genes.
- DNA is connected with histones and non-histone proteins to form the chromatin. The negative charge due to the phosphate group in DNA makes it relatively acidic. This negative charge binds to the basic histone groups.

Histone modifications:

- Recent studies provide that actively transcribed regions are characterized by specific modification pattern of histone.
- The experiments carried on by the dynamics of histone modification shows that there is a significant kinetic distinction between methylation, phosphorylation, and acetylation. This suggests that the role of these modifications has different roles in gene expression patterns.
- Histones are proteins which DNA wraps around and forms a chromatin.

- The basic unit of a chromatin is a nucleosome which is formed by histone octomer of 2 molecules of H2A, H2B, H3, and H4 along with **147 base pairs** of DNA wrapped in a superhelix.
- The accessibility of DNA is regulated by higher-order chromatin structures that of which can be obtained by the packing of nucleosomes.
- It is believed that the N-Termini tails of the histone molecules contributes to the chromatin function in that it mediates inter-nucleosomal interactions and are involved in the induct of non-histone proteins to the chromatin.
- The N-termini tail directs interactions to the chromatin binders which is thought to be the driving force of balance chromatin structure. However, there are other ways modifications can occur such as that observed by the unfolding or assembly of nucleosome and it is also involved in gene regulation.

Structural variations in DNA:

- The structural variations in DNA is occur mostly due to following:

1. Difference in deoxyribose conformations (4 total conformations)
2. Rotation around the contiguous bonds in the phosphodeoxyribose backbone (between the C1-C3 and C5-C6)
3. Free rotation around C1-N-glycosyl bond.