I MSC ZOOLOGY

CELL AND MOLECULAR BIOLOGY – UNIT IV – DR.S.ARULJOTHISELVI

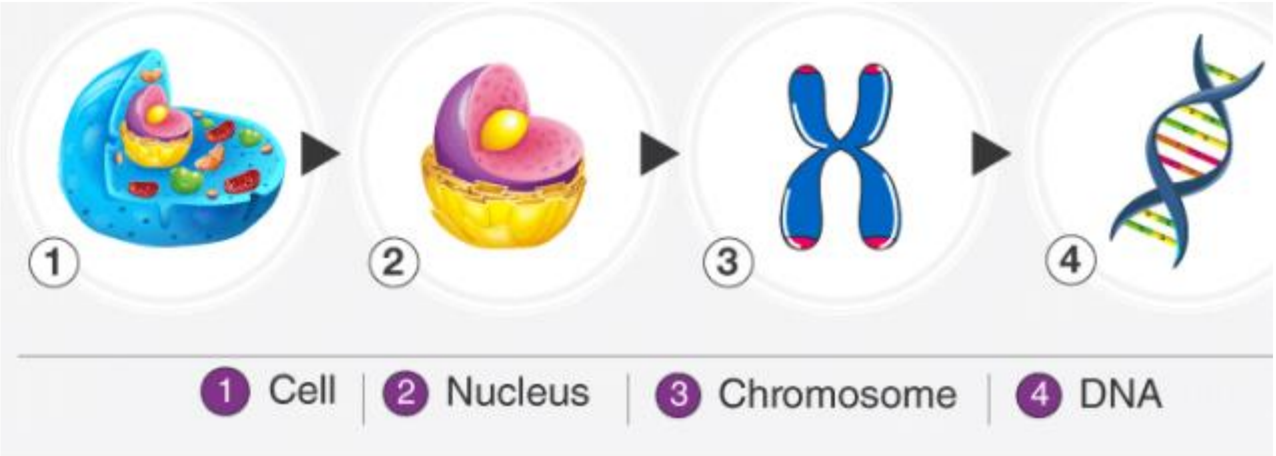
07.11.2020

UNIT-IV: CHEMISTRY OF NUCLEIC ACIDS

Chemistry of DNA - DNA replication – Experimental proof of semiconservative replication – enzymes in replication.

Chemistry of RNA - Different types of RNA and their functions.

CHEMISTRY OF DNA



INTRODUCTION

Nucleic acids

- Nucleic acids are the polymers in which nucleotides are monomers. These are biomolecules present in nuclei of all living cells in the form of nucleoproteins .They are also called as polynucleotides .

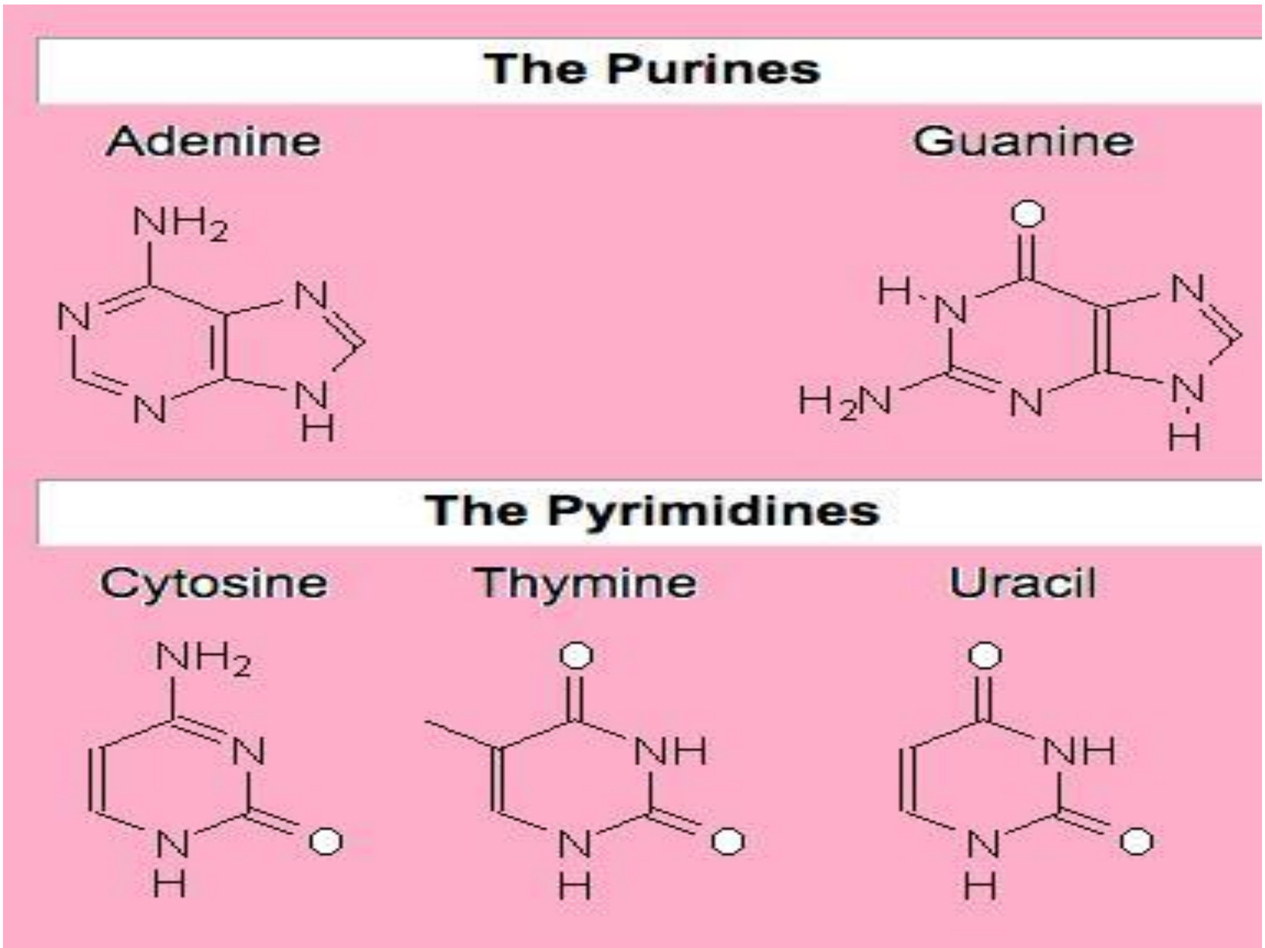
They help in the role of transmission of hereditary characters and synthesis of proteins.

Each nucleotide consists of 3 parts:

- A pentose sugar
- A nitrogenous base
- A phosphate group
- The nitrogenous base and a pentose sugar are called as nucleoside.

Nitrogenous bases are of two types: Purines and Pyrimidines

- Purines: adenine and guanine
- Pyrimidines: cytosine , thiamine and uracil



Purines and Pyrimidines are linked together by hydrogen bonds

- Adenine always bond with thiamine by double bond or vice versa.
- Cytosine always pairs with guanine by triple bond or vice versa.

Types of nucleic acids

- Deoxyribonucleic acid (DNA)
- Ribonucleic acid (RNA)

DNA

- It occurs in nucleus of cell. It has double stranded helical structure

DNA contains:

- Deoxyribose sugar
- Nitrogenous bases :
- Purines (adenine and guanine), Pyrimidines (thiamine and cytosine)
- A phosphate group
- It can undergo replication
- It helps in transfer of genetic information from parents to offspring

RNA

- It occurs in cytoplasm of cell

It consist of:

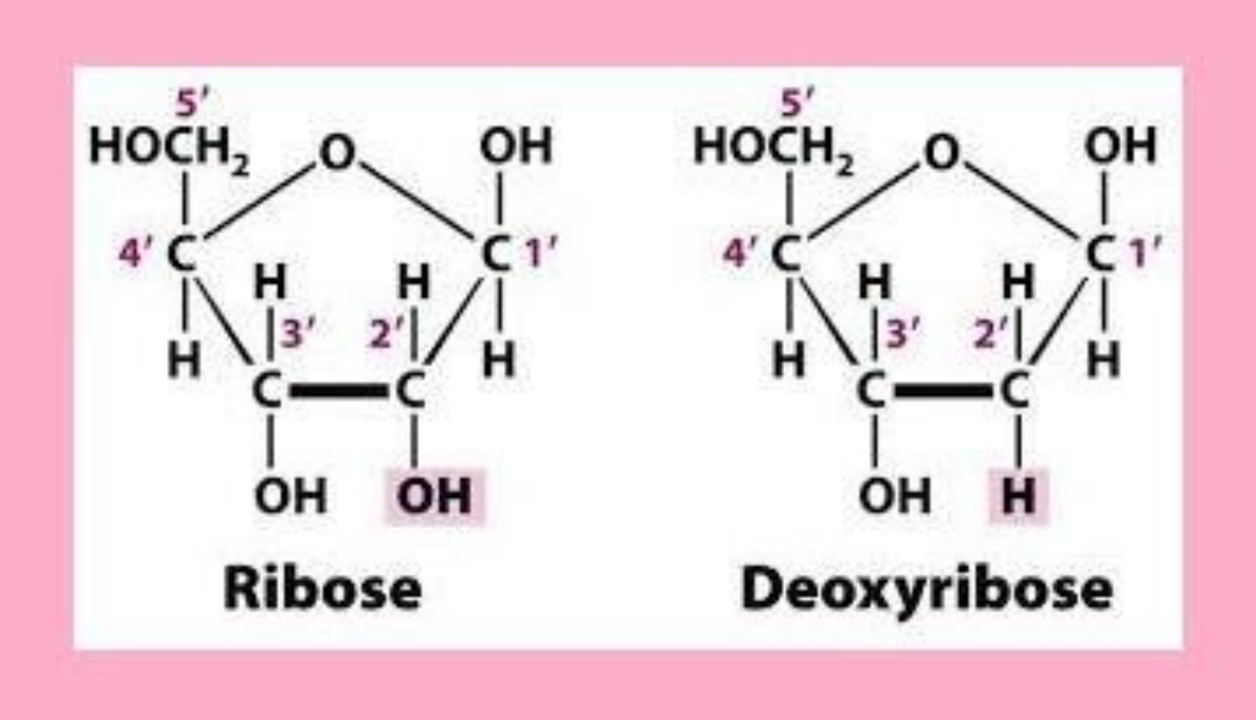
- Ribose sugar

- Nitrogenous base
- Purines: adenine and guanine
- Pyrimidines: cytosine and uracil
- A phosphate group
- It has a single strand helical structure
- It doesn’t undergo replication
- It controls synthesis of proteins

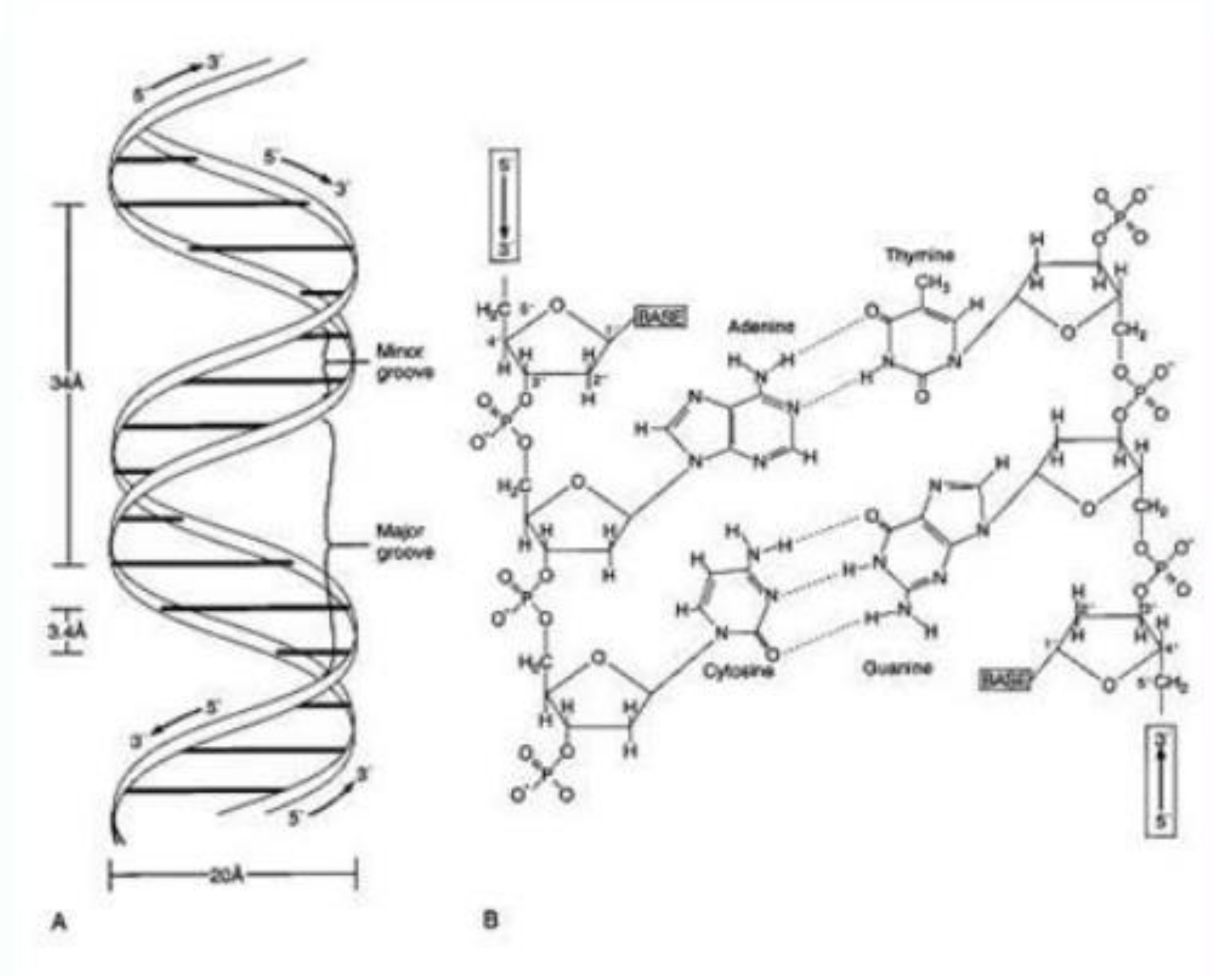
The structure of deoxyribose and ribose sugar is given:

Structure of nucleic acids

1. **Primary structure**
   - The nucleic acids are formed by the condensation of thousands of molecules of nucleotides.
   - On hydrolysis the nucleotides produces phosphoric acid and nucleoside .it means nucleosides on hydrolyses form Purine and Pyrimidines base and sugar moiety.
   - A nucleic acid-- ^{NH_3}ànucleotides –$^{aq\ NH3}$à nucleosides + phosphoric acid –$^{dilute\ HCL}$--àPurines + Pyrimidines + sugar.
   - Nucleotides are building blocks of nucleic acids.
   - These nucleotides are linked together with one another in a particular sequence, phosphate groups forming bridges between C-5 of the sugar residue of the one nucleoside and C-3 of the sugar residue of the other nucleoside.
   - The manner in which the sugar, phosphate and bases are linked with one another in nucleic acids is known as primary structure of nucleic acids.



2. **Secondary structure:**
   - Watson and Crick explained the double helix structure of DNA. The nucleotides in each strand are connected by phosphate ester bond and bases of one strand by hydrogen bonds.

- Adenine pairs with thiamine through two double hydrogen bonds whereas cytosine pairs with guanine by triple hydrogen bonds.
- The two strands of DNA are complementary to each other that is if one side there is Purine then on other side at same position Pyrimidine is present. For example if base sequence on strand is ACTCGCCA, then on the other strand the sequence will be complementary that is: TGAGCGGT
- **The primary and secondary structure is shown below:**
- 

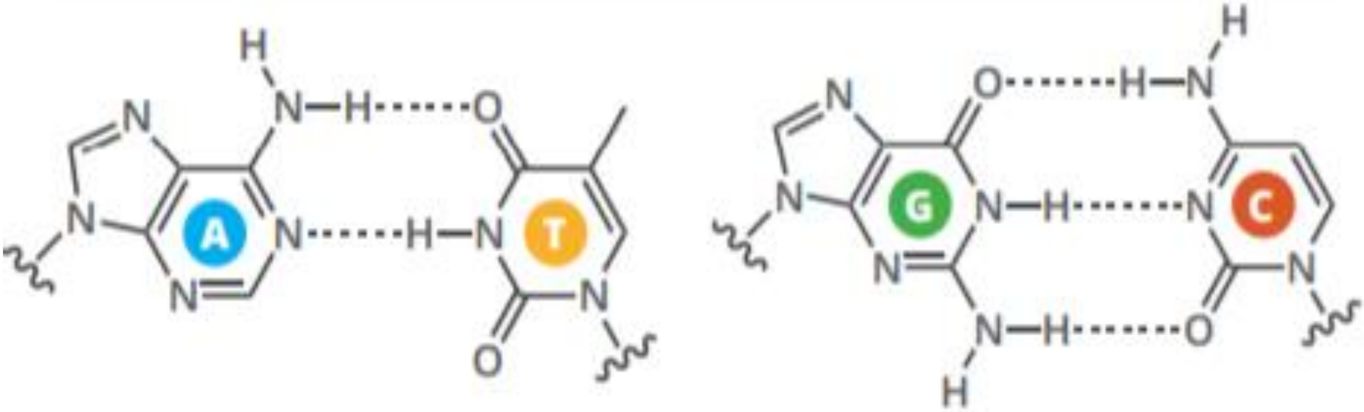
Watson and Crick model of DNA
Functions of nucleic acids

- **Replication**: The genetic information of cell is contained in the sequence of bases A, T, C and G in DNA molecule .In the division of cell, DNA molecules replicate and makes exact copies of themselves so that each daughter cell will have DNA identical to that of the parent cell.
- **Protein synthesis: The** specific information coded on DNA has to be translated and expressed in the form of synthesis of specific proteins which performs various functions in the cell. This synthesis is done in two steps:
- Transcription and translation.
- **Gene and genetic code**: Each segment of DNA molecule that codes for specific protein or a polypeptide is known as The relationship between nucleotides triplets and the amino acids are called the genetic code .This is gene and genetic code.
- **Mutation**: It is a chemical change in DNA molecule, which leads to the synthesis of proteins with a changed amino acid sequence.
    - These changes are caused by radiation, viruses or chemical agents.

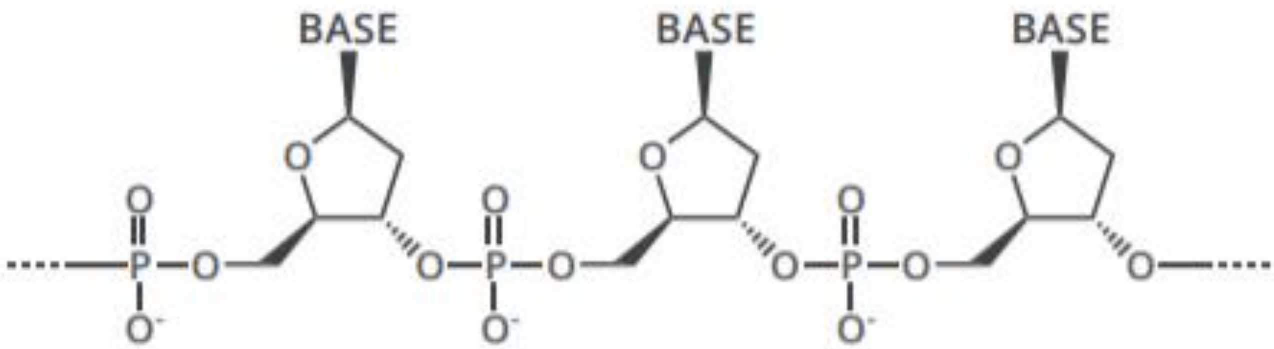
- The majority of changes in DNA are replicated by special enzymes in the cell, but if there is failure to repair by the enzymes then it can cause mutation.

WHAT HOLDS DNA STRANDS TOGETHER?

DNA strands are held together by hydrogen bonds between bases on adjacent strands. Adenine (A) always pairs with thymine (T), while guanine (G) always pairs with cytosine (C). Adenine pairs with uracil (U) in RNA.



THE SUGAR PHOSPHATE 'BACKBONE'



DNA is a polymer made up of units called nucleotides. The nucleotides are made of three different components: a sugar group, a phosphate group, and a base. There are four different bases: adenine, thymine, guanine and cytosine.

FROM DNA TO PROTEINS

The bases on a single strand of DNA act as a code. The letters form three letter codons, which code for amino acids - the building blocks of proteins.

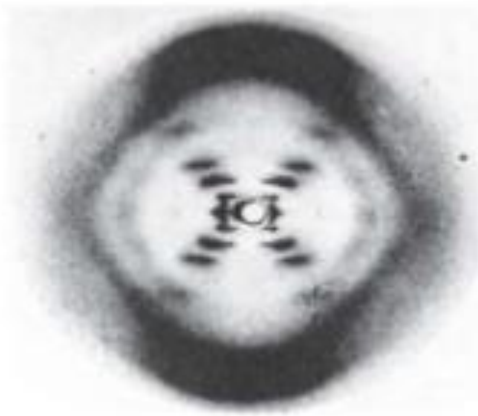
An enzyme, RNA polymerase, transcribes DNA into mRNA (messenger ribonucleic acid). It splits apart the two strands that form the double helix, then reads a strand and copies the sequence of nucleotides. The only difference between the RNA and the original DNA is that in the place of thymine (T), another base with a similar structure is used: uracil (U).

In multicellular organisms, the mRNA carries genetic code out of the cell nucleus, to the cytoplasm. Here, protein synthesis takes place. 'Translation' is the process of turning the mRNA's 'code' into proteins. Molecules called ribosomes carry out this process, building up proteins from the amino acids coded for.

HISTORY

- In 1869, **Miescher discovered "nuclein" (DNA) in the** cells from pus & later he separated it into a protein and an acid molecule. It came to known as nucleic acid after 1874.
- 1926 , **Levene proposed "Tetra nucleotide theory"** which states that Nucleic acid consists of only 4 nitridesas it gives 4 different nucleotides on hydrolysis.

Rosalind Franklin used X-ray crystallography to help visualize the structure of DNA

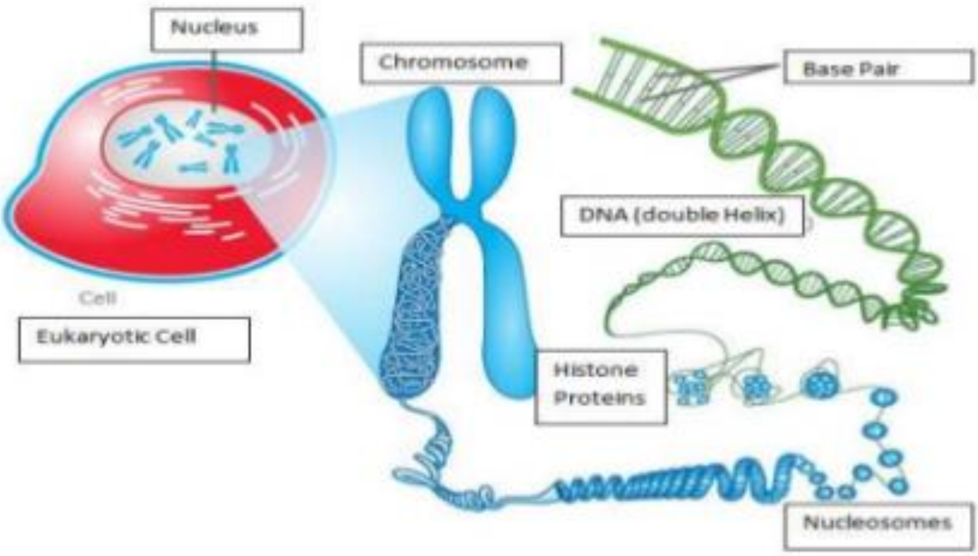


(b) Franklin's X-ray diffraction photograph of DNA

James D. Watson and Francis Crick, co-originators of the doublehelix model.

BRIEFING ON DNA...

- DNA is found in the cells of all living things.
- DNA contains all of the genetic information that makes you who you are and every
- individual organism has unique DNA like a finger print.

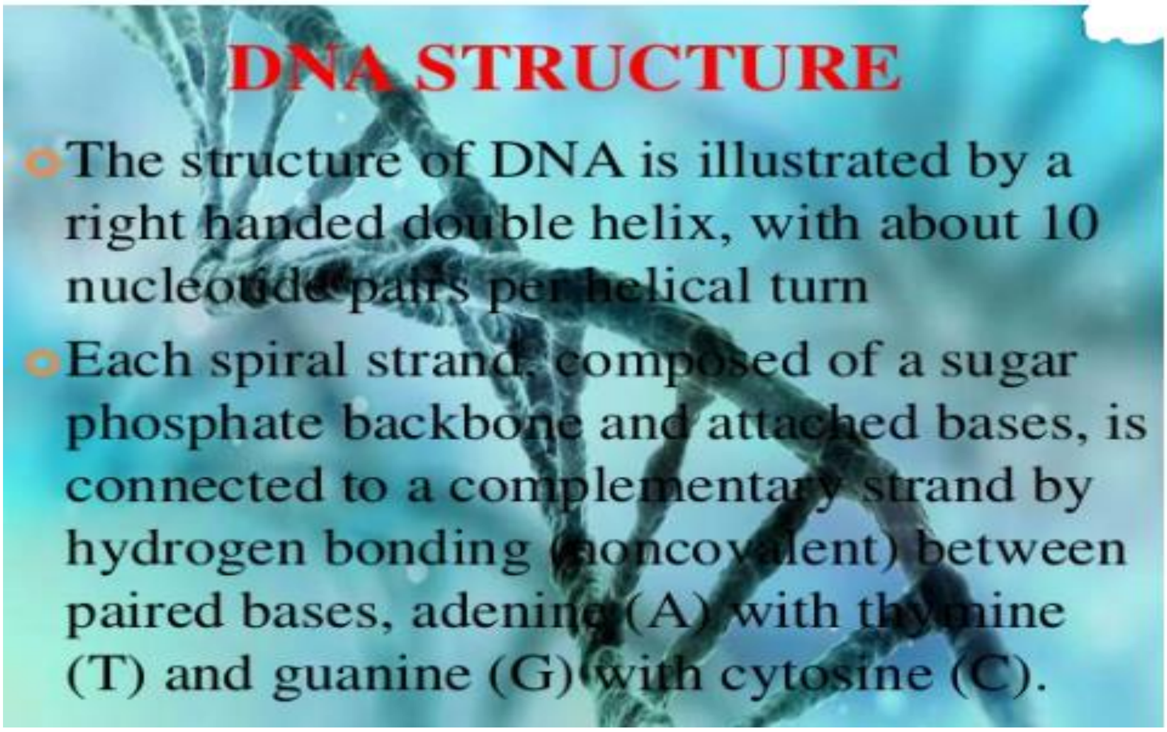


- It contained phosphorus in the form of phosphate
- Deoxyribo nucleic acid
- It is a molecule that encodes the genetic instructions.
- Most DNA molecules are double-stranded helices.
- Each molecule consists of two long biopolymers made of simpler units called nucleotides—each nucleotide is composed of a nucleobase recorded using the letters G, A, T, and C.
- DNA is well-suited for biological information storage.

- DNA Stands for “DeoxyriboNcleic Acid”.
- Term DNA was given by Zaccharis
- DNA is biopolymer consist of nucleotide as monomeric unit.
- DNA is double helical structure in eukaryote and prokaryote, but in virus it may be double stranded or single stranded and presented as monopartite or multipartite.
- In eukaryotes, DNA is presented in nucleus surrounded by nuclear membrane
- In prokaryotes, DNA is presented in nucleoid region of Cytoplasm without nuclear membrane
- In virus, DNA is presented in the core of virus surrounded by Protein layer (called as capsid).

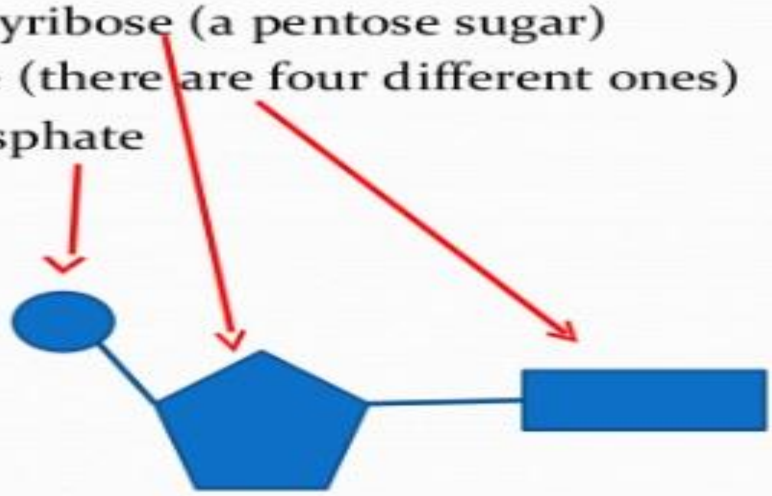
What is DNA?

- DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person's body has the same DNA.
- Where is it located?
- Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA).

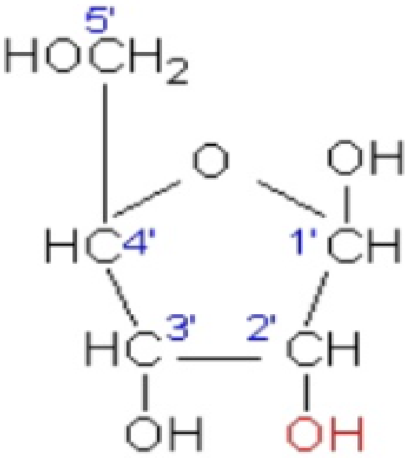


DNA Structure

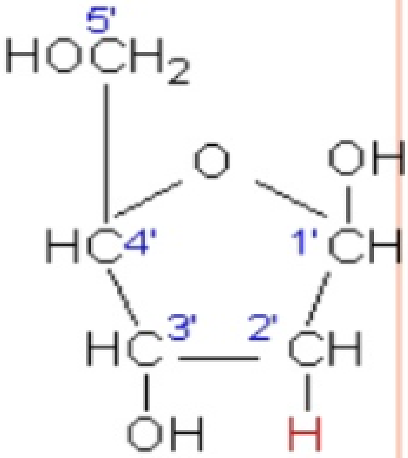
- DNA has three main components
  - 1. deoxyribose (a pentose sugar)
  - 2. base (there are four different ones)
  - 3. phosphate



THE SUGARS



Ribose
(in RNA)



2'-Deoxyribose
(in DNA)

The Bases

- They are divided into two groups
  - Pyrimidines and purines
- Pyrimidines (made of one 6 member ring)
  - Thymine
  - Cytosine

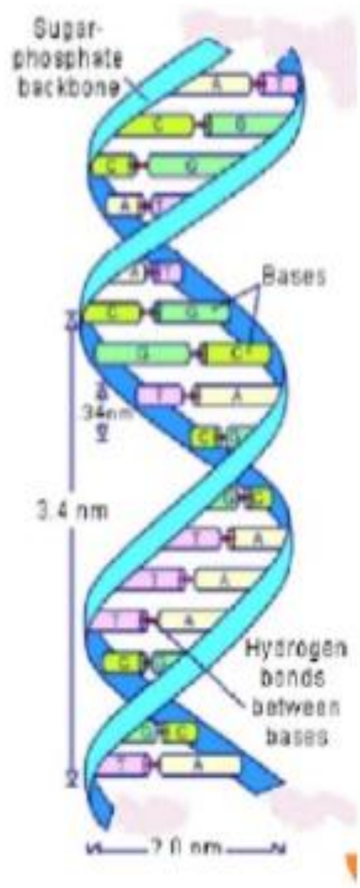
- Purines (made of a 6 member ring, fused to a 5 member ring)
  - Adenine
  - Guanine

- The rings are not only made of carbon (specific formulas and structures are not required for IB)

DNA STRUCTURE

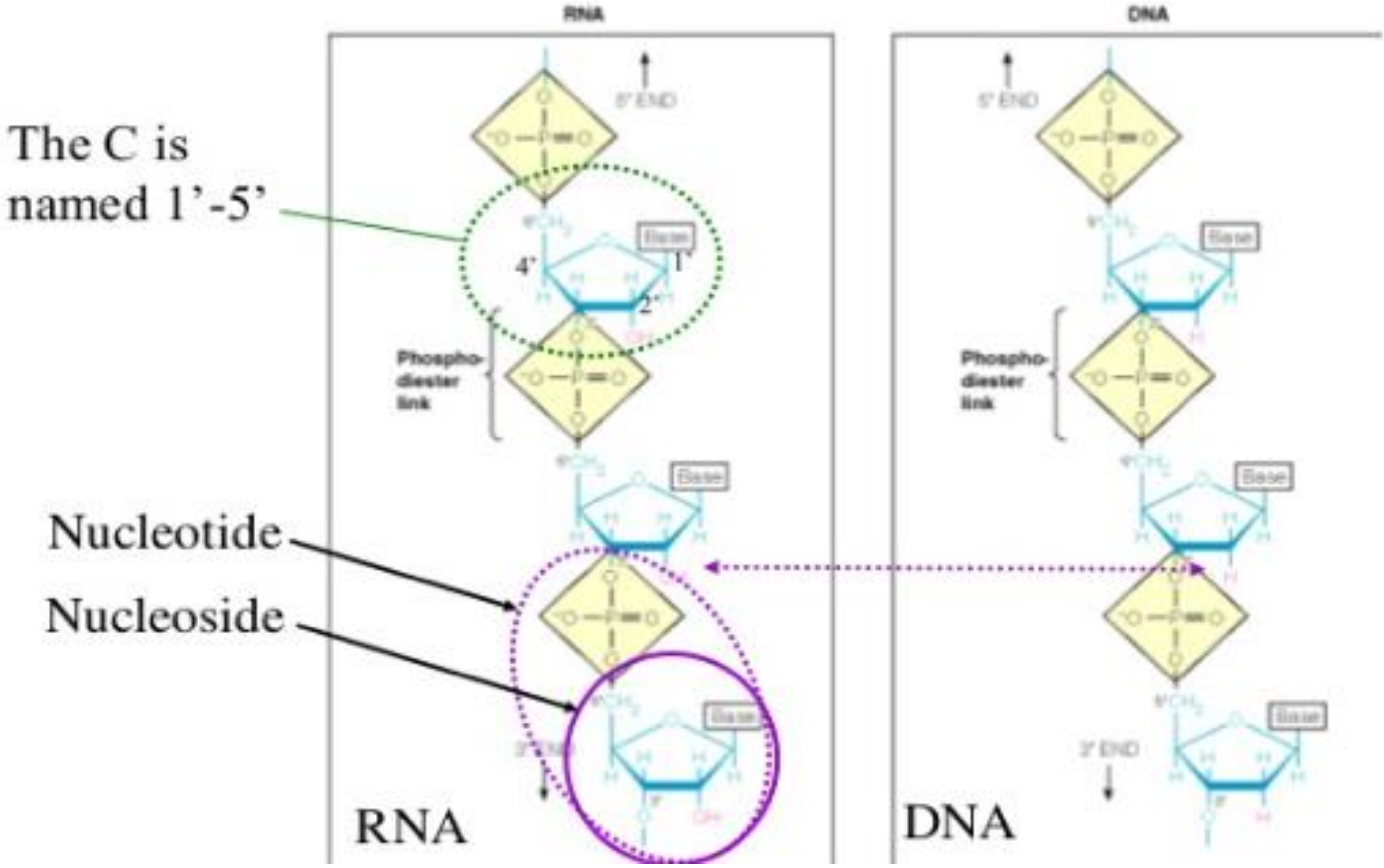
In 1953, Watson and Crick postulated a three dimensional model of DNA structure that accounted for both the X-ray data and the characteristic base pairing in DNA.

- It consist of two helical polynucleotide chains.
- Two polynucleotide chains coil around the same axis to form a right –handed double helix.
- In the helix, the two chains or strands are anti parallel i.e. have an opposite polarity.
- Backbone of each chain which consist of alternate sugar-phosphate residues, (hydrophilic) are on the out side of the double helix, facing the surrounding.

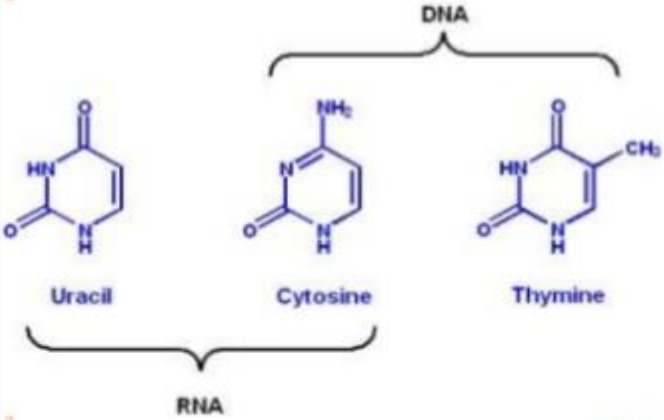


- The purine and pyrimidine bases of each strand face inward towards each other.
- The bases are stacked perpendicular to the long axis of the double helix.
- The base pair are 0.34 nm apart in DNA helix. A complete turn of helix takes 3.4 nm, therefore in each helical turn, 10 bases are present. The external diameter of helix is 2 nm.
- The helix has two external grooves, the narrow groove is called as **minor groove** while the wide groove is called as **major groove** . The major groove is the site for DNA binding proteins. The minor grooves often are the site for binding small molecules.

CHEMICAL STRUCTURE OF DNA AND RNA

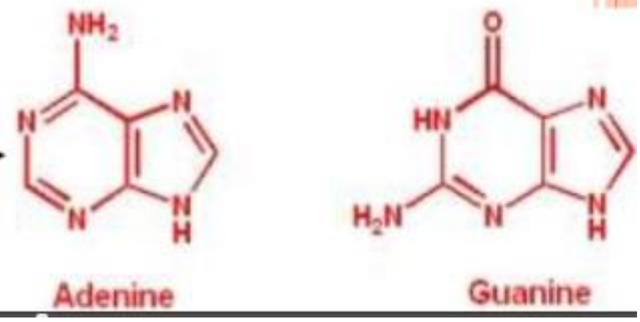


THE BASES

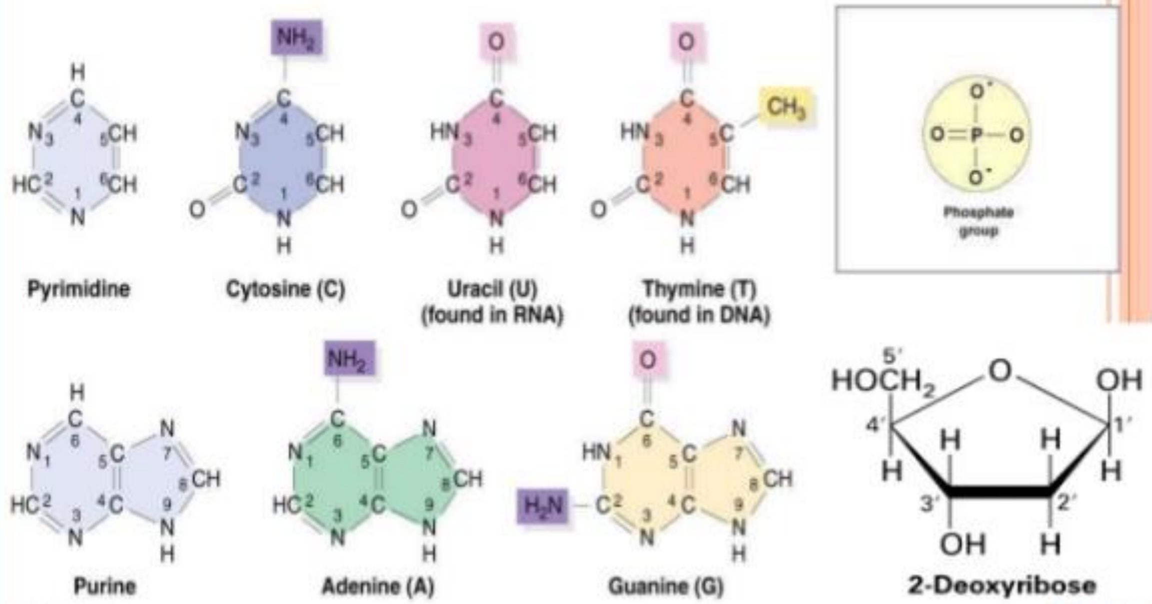


← PYRIMIDINES

PURINES →



NITROGENOUS BASES

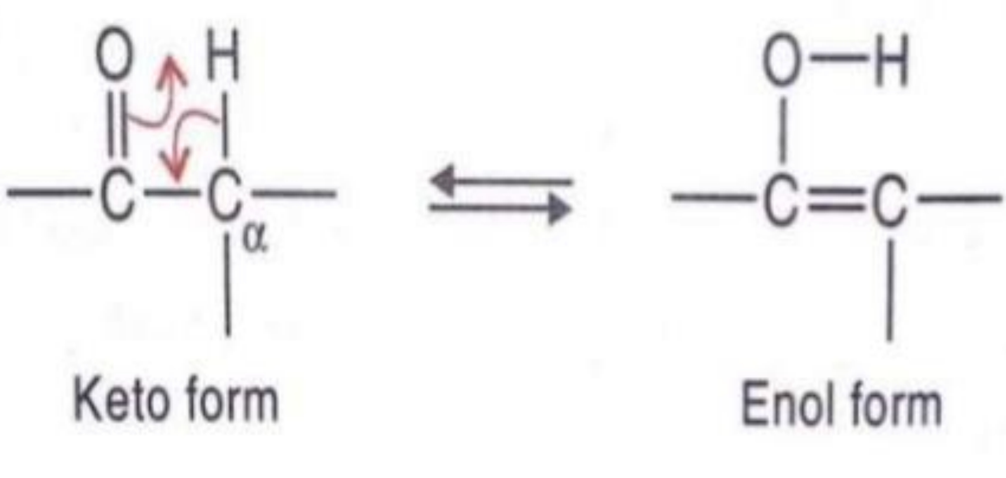


TAUTOMERISM

- **Tautomers** are isomers of a compound which differ only in the position of the protons and electrons. ... A reaction which involves simple proton transfer in an intramolecular fashion is called a **tautomerism**. Keto-enol **tautomerism** is a very common process, and is acid or base catalysed.

KETO-ENOL TAUTOMERISM

Aldehydes, ketones and some other compounds undergo this special type of tautomerism. It involves the migration of a proton from ά-carbon to the carbonyl oxygen.



- The pairs of bases are always between a purine and pyrimidine, specifically the pairs A-T and G-C, which are the base pairs found by Chargarff.
- There is hydrogen bonding between the bases. 2H-bonds between A & T and 3H-bonds between G & C
- Two chains do not have the same base composition (not identical), but two chains are **complementary** to each other. Such chains are called as **complementary** chains.

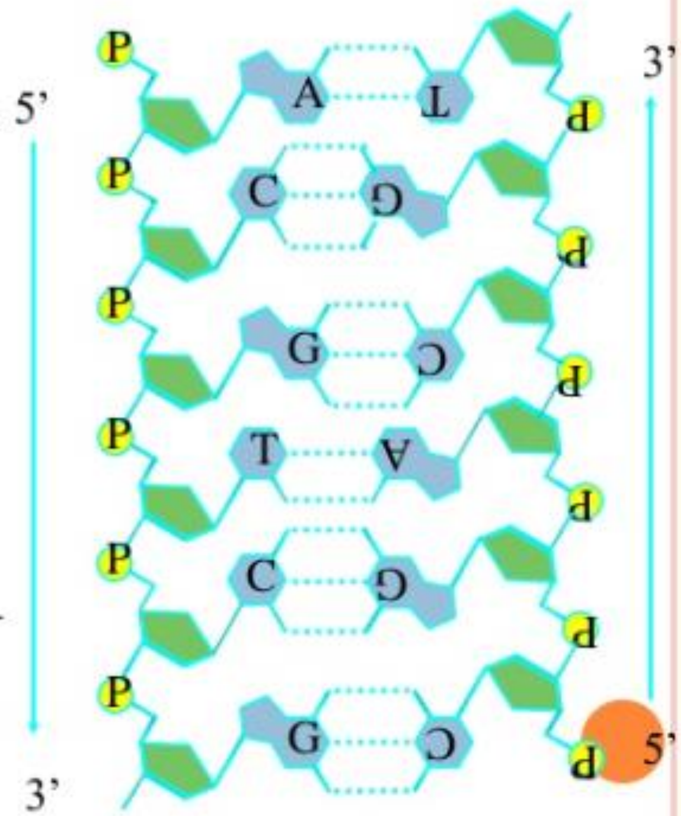
  1st chain ---- ATACGCAC---3A, 1T, 3C, 1G
  2nd chain ---- TATGCGTG---1A, 3T, 1C, 3G

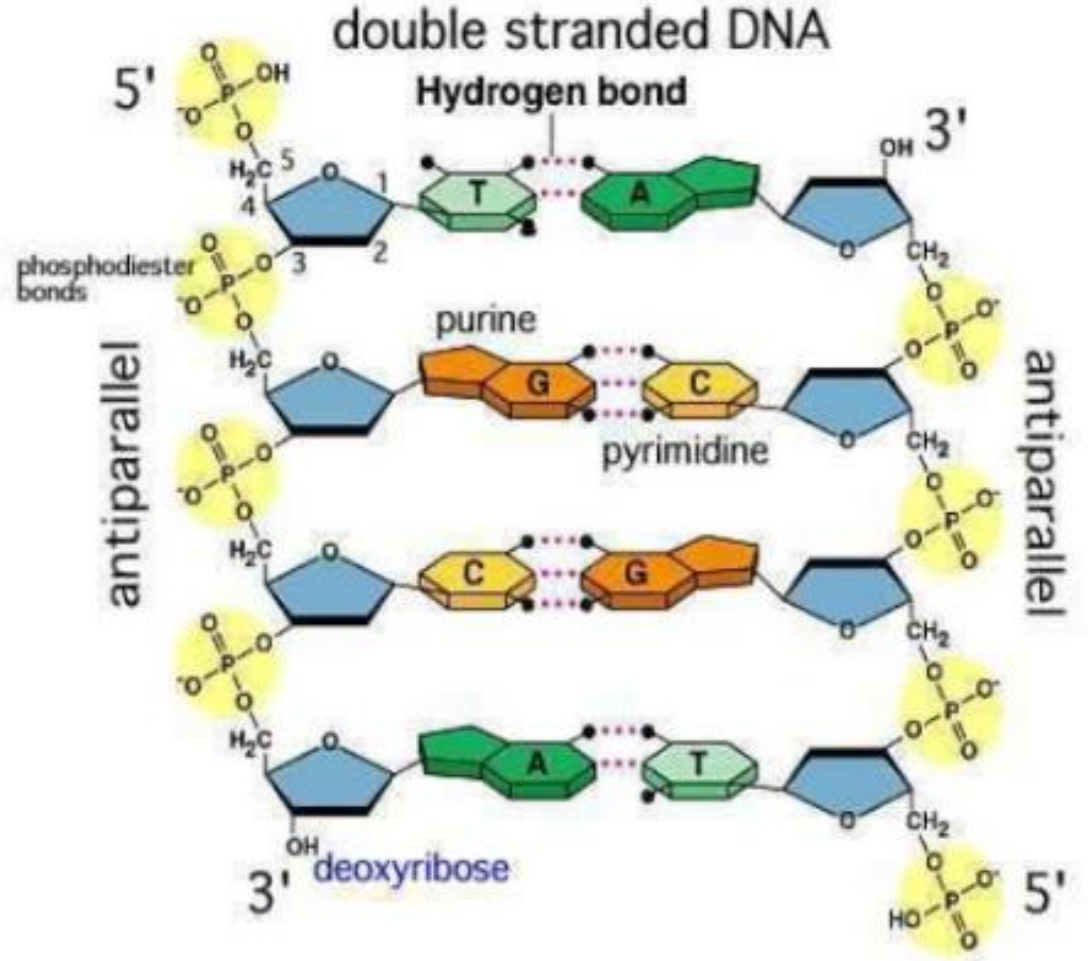
DNA IS A DOUBLE HELIX

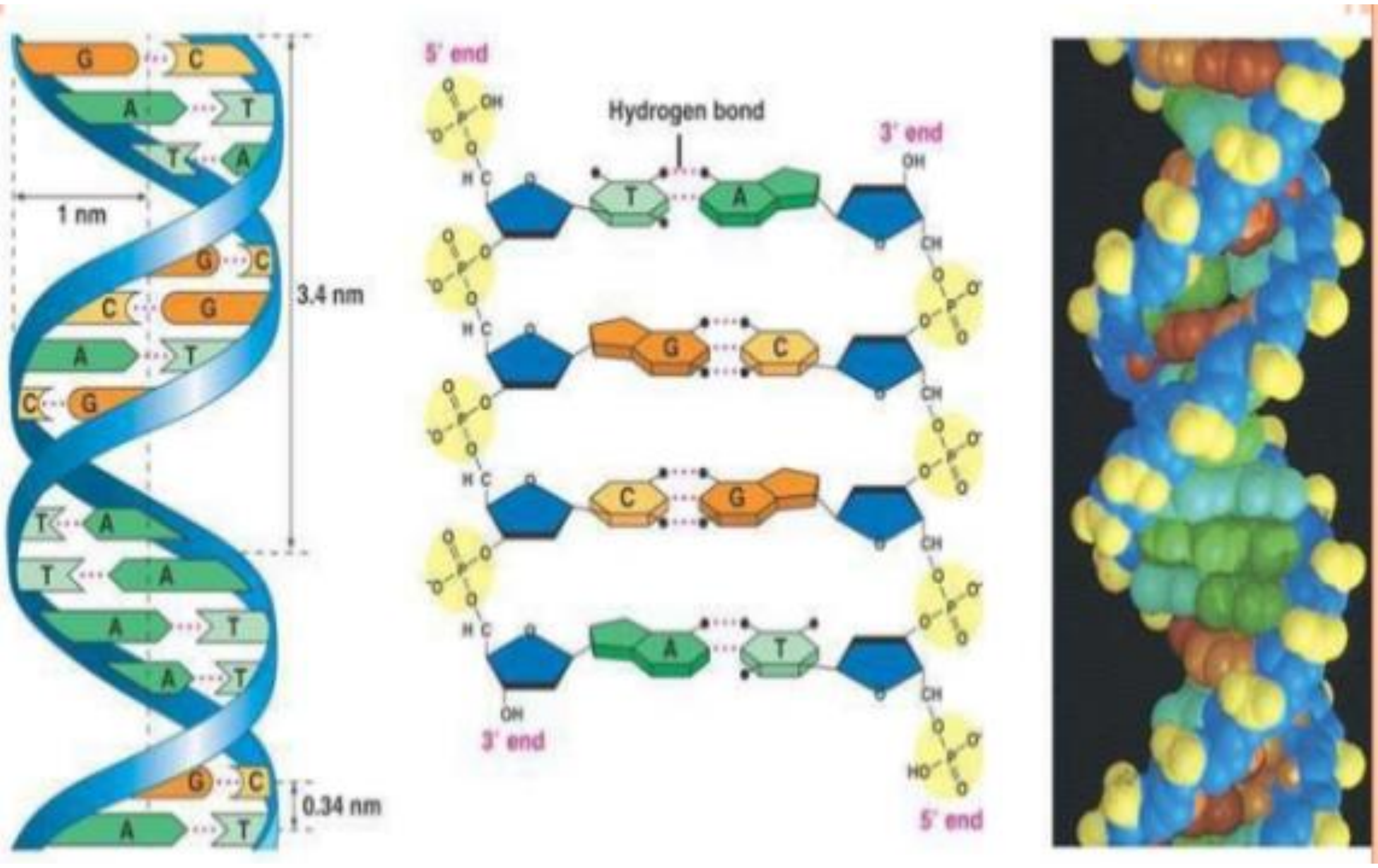
A sugar and phosphate "backbone" connects nucleotides in a chain.

Hydrogen bonds between paired bases hold the two DNA strands together.

DNA strands are antiparallel.




double stranded DNA
5'
Hydrogen bond
3'
phosphodiester bonds
purine
pyrimidine
antiparallel
antiparallel
T
A
G
C
C
G
A
T
3'
deoxyribose
5'


1 nm
3.4 nm
0.34 nm
5' end
Hydrogen bond
3' end
3' end
5' end

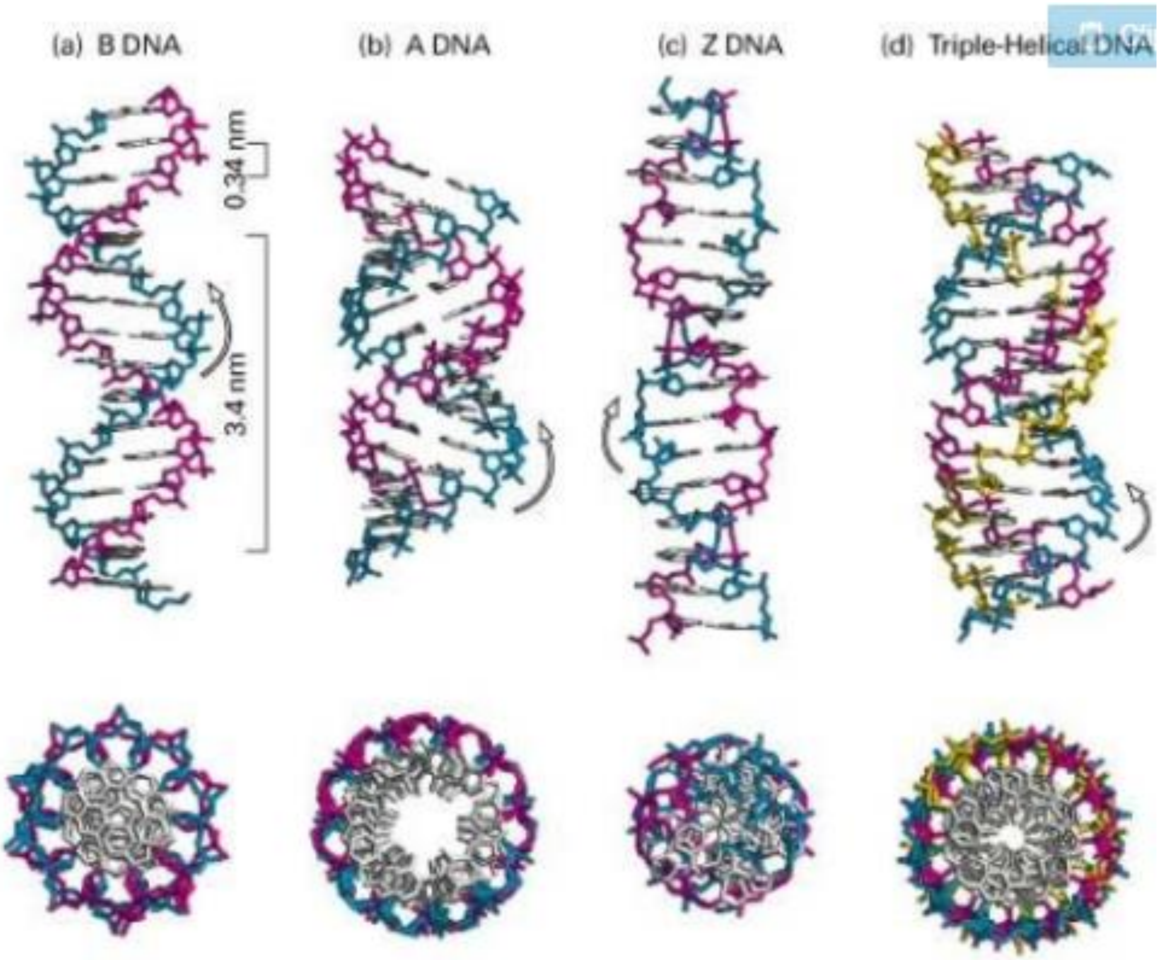
A-DNA

- **A-DNA is one of the many possible double** helical structures of DNA.
- It is most active along with other forms.
- Helix has left-handed sense, shorter more compact helical structure.
- It occurs only in dehydrated samples of DNA, such as those used in crystallograph experiments.

A-DNA

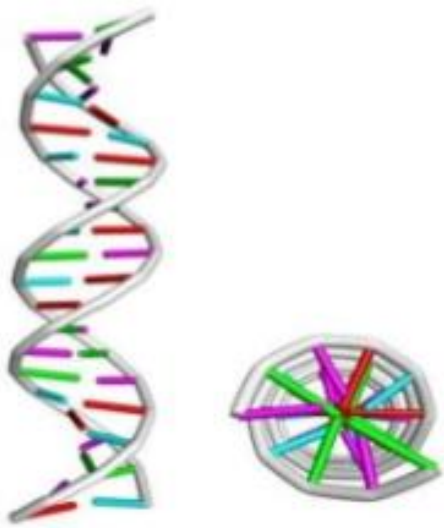
- **Structure**
- A-DNA is fairly similar to B-DNA.
- Slight increase in the number of bp/ rotation (resulting in a tighter rotation angle), and smaller rise/turn.
- deep major groove and a shallow minor groove.
- Favoured conformation at low water concentrations.
- In a solution with higher salt concentrations or with alcohol

added, the DNA structure may change to an **A form, which is** still right-handed, but every 2.3 nm makes a turn and there are 11 base pairs per turn.



B-DNA

- Most common DNA conformation in vivo.
- Favoured conformation at high water concentrations.
- Also known as Watson & Crick model of DNA.
- First identified in fibre at 92% relative humidity.

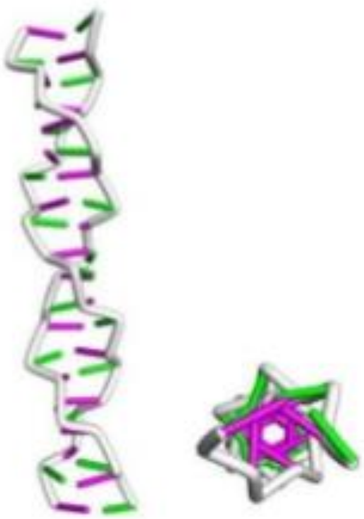


B-DNA

- Structure
- Narrower, more elongated helix than A.
- Wide major groove easily accessible to proteins & Narrow minor groove.
- Base pairs nearly perpendicular to helix axis One spiral is 3.4nm or 34Å.
- Distance between two H-bonds is 0.34nm or 3.4Å.

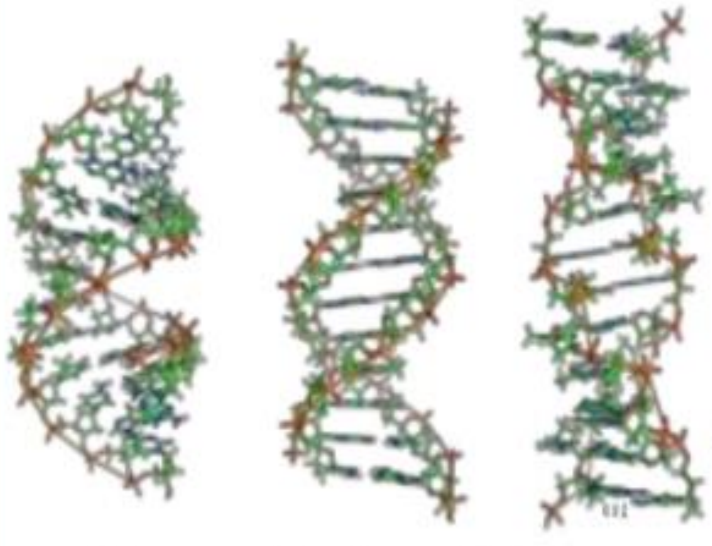
Z-DNA

- **Z-DNA is one of the many possible** double helical structures of DNA.
- Helix has left-handed sense.
- It is most active double helical structure.
- Can be formed in vivo, given proper sequence and super helical tension, but function remains obscure.

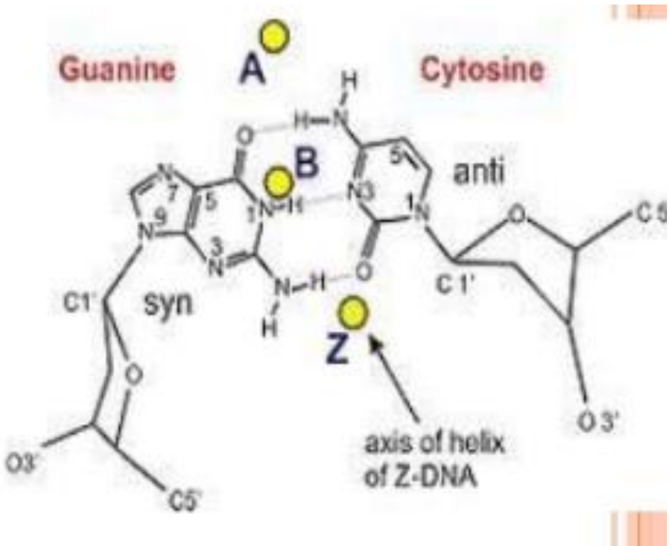


Z-DNA

	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrmidines;

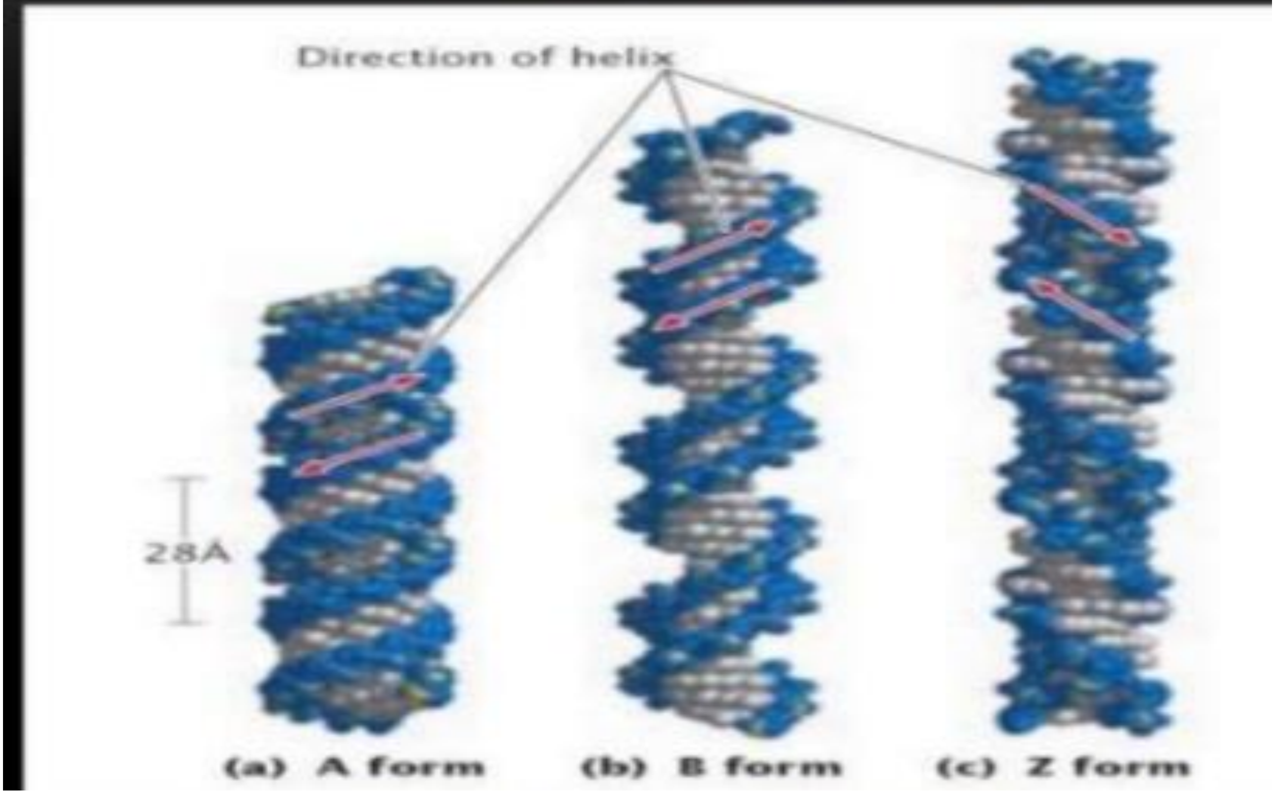


Side view of A-, B-, and Z-DNA.



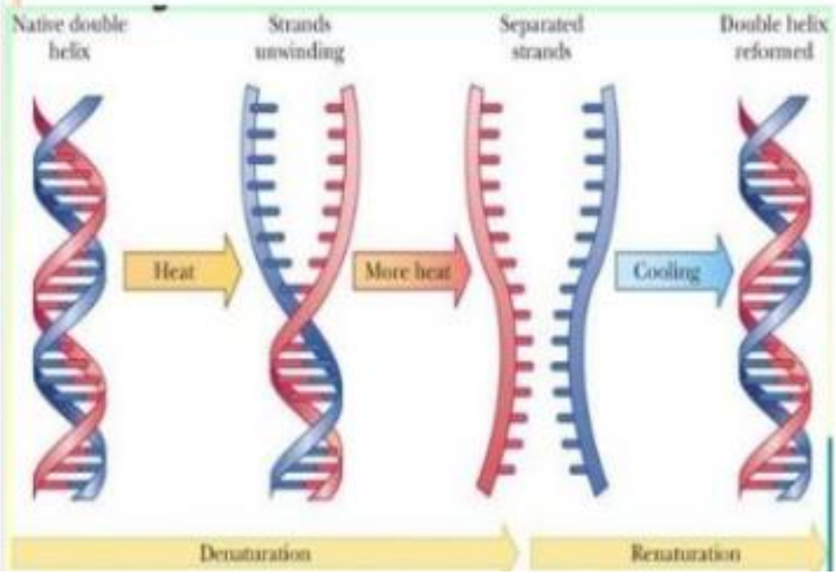
The helix axis of A-, B-, and Z-DNA.

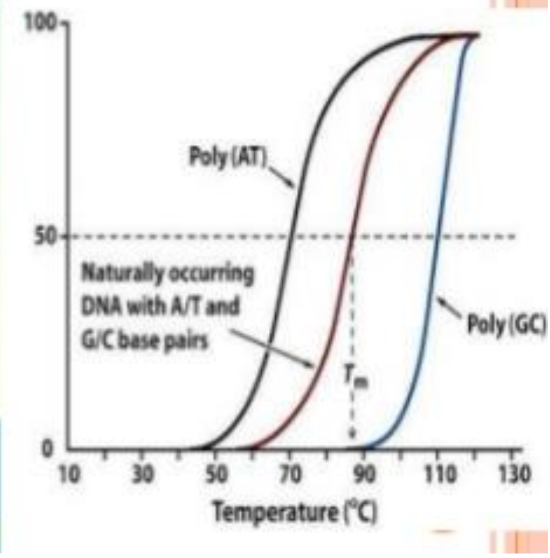
Direction of Helix



."DNA Denaturation"

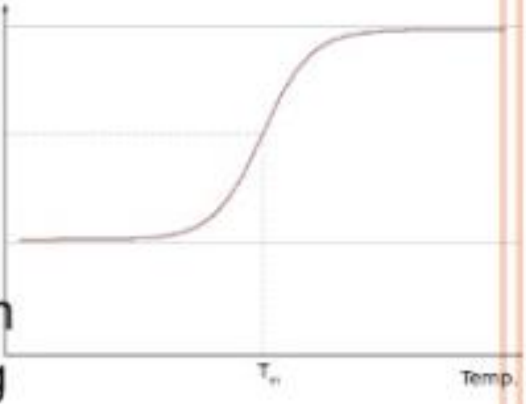
- DNA Denaturation is the separation of a double strand into two single strands, which occurs when the **hydrogen bonds** between the strands are broken.





HYPERCHROMIC EFFECT

- is the increas of absorbance (*optical density*) of a material. The most famous example is the hyperchromicity of DNAthat occurs when the DNA duplex is denatured. The UV absorption is increased when the two single DNA strands are being separated, either by heat or by addition of denaturant or by increasing the pH level. The opposite, a decrease of absorbance is called **hypochromicity**.



CONCLUSION

- The secondary structure of DNA is important in many events in cellular life. Replication, transcription and regulation of expression of many genes depends on local differences or changes in DNA structure. Recombination which leads to rearrangement of genes takes advantage of the ability to form an unusual structure called a Holliday's structure. Also different kinds of mutations occur as a result of specific DNA structure.

IMPORTANT NOTES

What is the structure of DNA?

DNA is a double helical structure composed of nucleotides. The two helices are joined together by hydrogen bonds. The DNA also bears a sugar-phosphate backbone.

What are the three different types of DNA?

The three different types of DNA include:

- A-DNA
- B-DNA
- Z-DNA

How is Z-DNA different from other forms of DNA?

Z-DNA is a left-handed double helix. The helix winds to the left in a zig-zag manner. On the contrary, A and B-DNA are right-handed DNA.

What are the functions of DNA?

The functions of DNA include:

- Replication
- Gene expression
- Mutation
- Transcription
- Translation

What type of DNA is found in humans?

B-DNA is found in humans. It is a right-handed double-helical structure.

What is DNA?	• DNA = **deoxyribonucleic acid** • Holds all our cell's **information** • Located in the cell's **nucleus**
What we already know about DNA	• Codes for proteins essential to life • A nucleic acid macromolecule • Monomer of a nucleic acid is a nucleotide • The three parts of a nucleotide: – 1. Phosphate group – 2. Sugar (deoxyribose) – 3. Nitrogen base
Nitrogen bases	• The nitrogen base can either be a **purine** or a **pyrimidine**. • How many carbon rings does each have? – Purines have 2 – Pyrimidines have 1 • DNA has 4 nitrogen bases: – Thymine (T) – Adenine (A) – Cytosine (C) – Guanine (G) • Adenine and Guanine are purines Cytosine and Thymine are pyrimidines.

<table>
<tr><td>A collaborative effort!</td><td>In the early 1900s, it was known that information had to be passed from cell to cell. However, it was not known what was responsible for carrying this information.
Some scientists thought that it must be protein, others that it was the nucleic acid.
Three major experiments helped show that it was a nucleic acid:
Griffith
Avery-MacLeod-McCarty
Hershey-Chase</td></tr>
<tr><td>Frederick Griffith got lucky?</td><td>Griffith studied pneumonia bacteria
In 1928, he isolated two strains of bacteria, and injected them into mice
Live R strain was harmless (mice lived)
Live S strain caused pneumonia (mice died)
When he injected the S Strain that was heat-killed, the mice lived
BUT.... When he mixed the live R strain with the heat-killed S strain and injected into mice, the mice died.</td></tr>
<tr><td>Griffith's Conclusions</td><td>When the heat-killed bacteria mixed with the live harmless bacteria, something was exchanged between them, making the live harmless bacteria deadly
Transformation = process in which one strain of bacteria changes the gene(s) of another bacteria</td></tr>
<tr><td>Avery-MacLeod-McCarty</td><td>Following Griffith (1943), scientists heat killed the virulent S strain and then selectively destroyed parts of the bacteria before combining with R strain
Destroyed proteins, lipids, carbs = mice died => something different was transforming bacteria
Destroyed nucleic acids = mice lived! => DNA was transforming bacteria
Demonstrated that DNA was the transforming agent</td></tr>
<tr><td>Hershey and Chase</td><td>Experimented (1950) with bacteriophages to see if information is carried on proteins or DNA
Used radioactive elements to "mark" DNA and protein
Only the radioactive DNA was found in bacteria cells (not proteins)
Further supported Avery's experiment that genetic material is DNA</td></tr>
<tr><td>Discovery of the structure of DNA</td><td>Many scientists contributed to determining the structure of DNA
Erwin Chargaff
Rosalind Franklin
James Watson & Francis Crick</td></tr>
</table>

Erwin Chargaff	• Worked with DNA nitrogen bases, discovered (1950):
	• In any sample of DNA,
	  – # adenines (A) = # thymines (T)
	  – # cytosines (C) = # guanines (G)
	• Therefore, in DNA, the bases are always paired: **A** with **T**, and **C** with **G**.
	• This is Chargaff's Rule!

Rosalind Franklin	• Worked with x-ray photography to try to find DNA structure • Her "Photo 51" revealed DNA's structure (1952) • Died of cancer in 1958
Watson and Crick	• Credited with finding the structure of DNA (1953) • Watson got a sneak peak at Franklin's x-ray photos and used them with other evidence • They described DNA as a double helix, with the strands held together by weak hydrogen bonds formed between the bases A-T and C-G.
DNA structure	• Looks like a twisted ladder made of nucleotides – The nucleotide: (hosphate group, sugar, nitrogen base • Sugars and phosphates make the sides of the ladder, nitrogen bases are the rungs • The atoms within the two strands are held together by strong covalent bonds • The two strands are held together by weak hydrogen bonds between the nitrogenous bases.

What bonds with what?	• A bond between two purines would be too wide.
	• A bond between two pyrimidines would be too narrow.
	• THUS, a purine always bonds with a pyrimidine.
	  – A bonds with T
	  – G bonds with C