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Programme: M.Sc., Biomedical Science

Course Code: BM35C5
Course Title: Molecular Biology

Unit-I

Structure and Properties of Nucleic Acids

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Unit I:

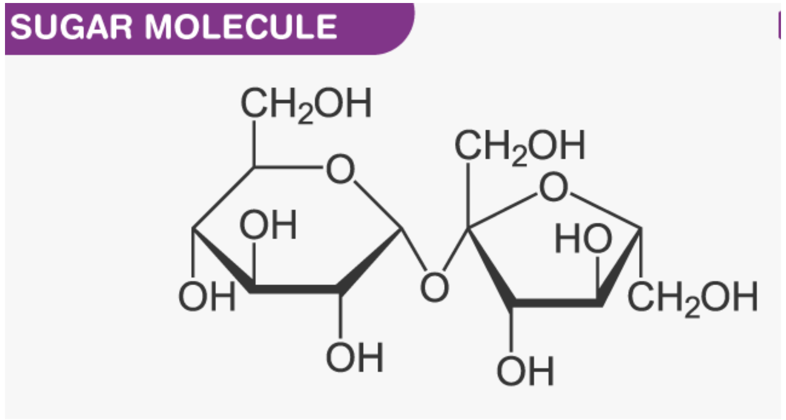
Structure and Properties of Nucleic acids : Nucleic acid as the genetic material (Griffith's experiment, Avery, MacLeod and McCarty‟s experiment, Hershey-Chase experiment)- Components of DNA and RNA -Watson and Crick model of DNA structure, Various forms of DNA- Physical properties of nucleic acids; Denaturation and renaturation, Cot curves – Structure and function of mRNA, rRNA, tRNA- Chromatin structure-Euchromatin, Heterochromatin- Central Dogma of Molecular Biology.

PRESENTATION: 1

Overview of Nucleic Acids

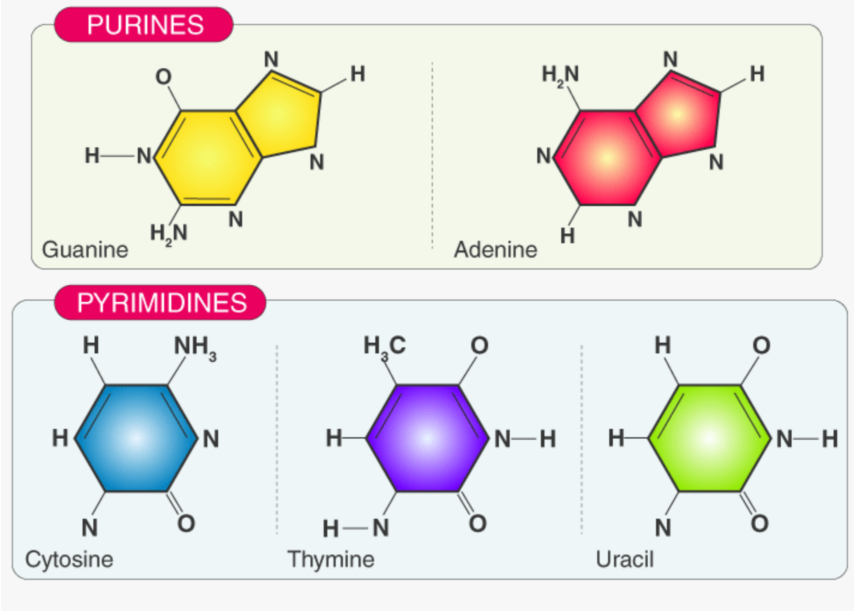
- A nucleic acid is a linear polymer of nucleotides which form an integral part of the information transfer system in cells.
- Examples of nucleic acids are DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid).
- In order to study the structure of nucleic acid, it is essential to study the structure of its monomer.
- Nucleotides are made up of a sugar molecule, a nitrogenous base, and a phosphate group.

Sugar molecule

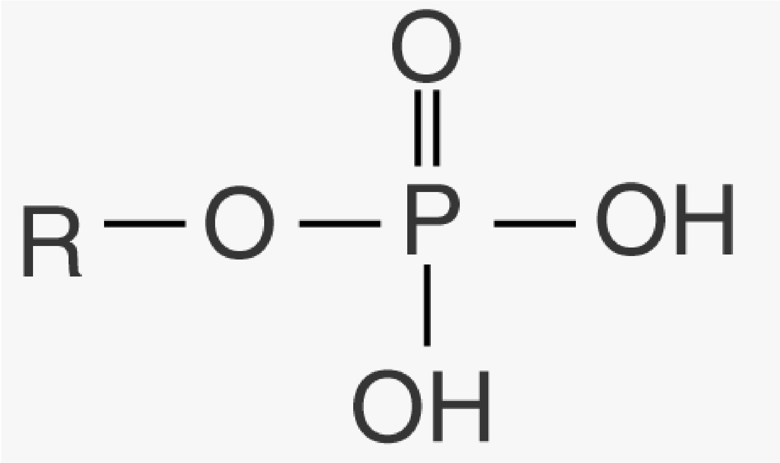


The sugar molecule in nucleotides could be either ribose ($C_5H_{10}O_5$) or deoxyribose ($C_5H_{10}O_4$) and both the sugars are in their Pentagon or furanose state.

Nitrogenous Base

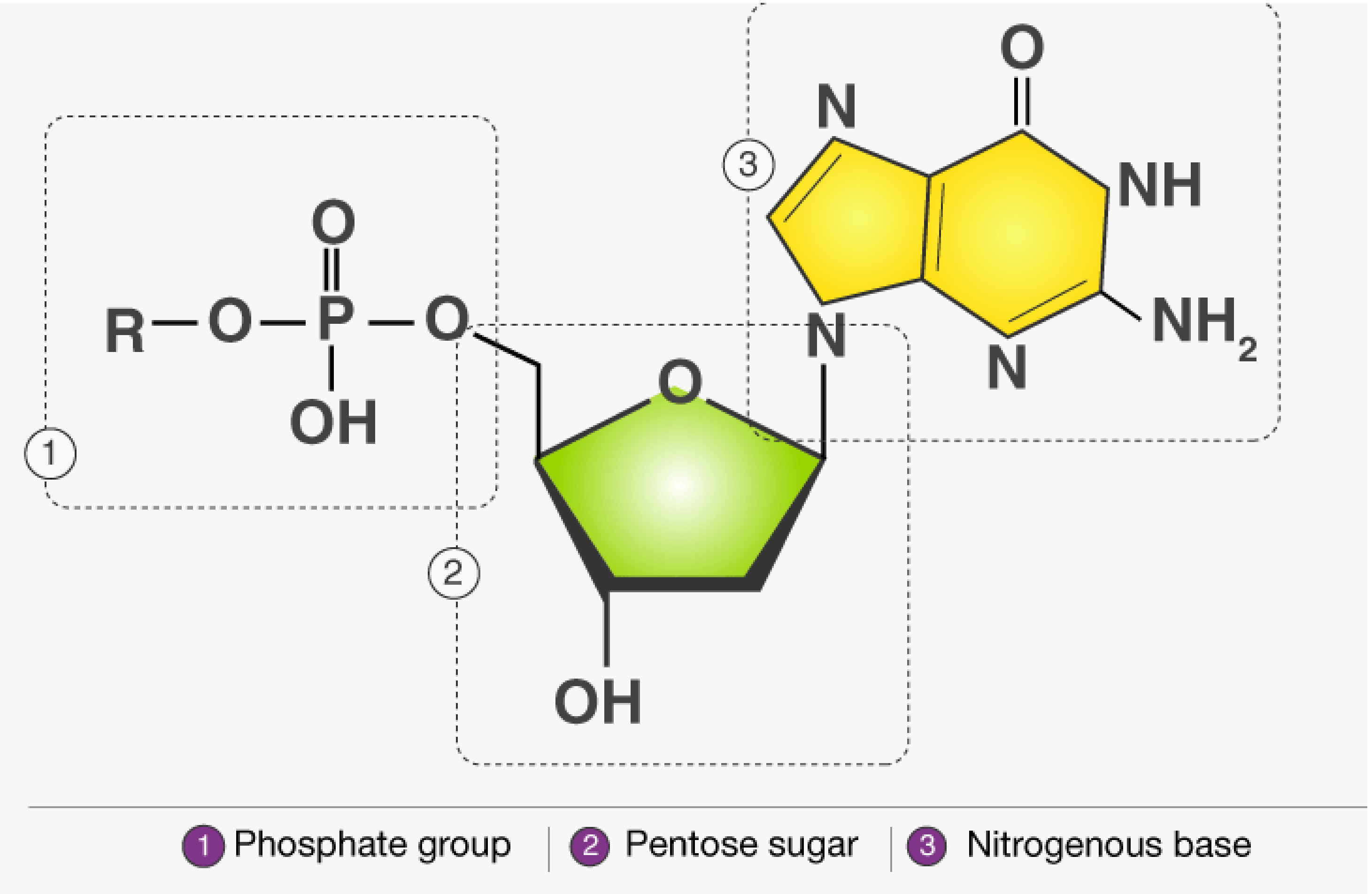


Phosphate group



When a phosphate group is added to a nucleoside (one sugar molecule plus one nitrogenous base), it forms a nucleotide.

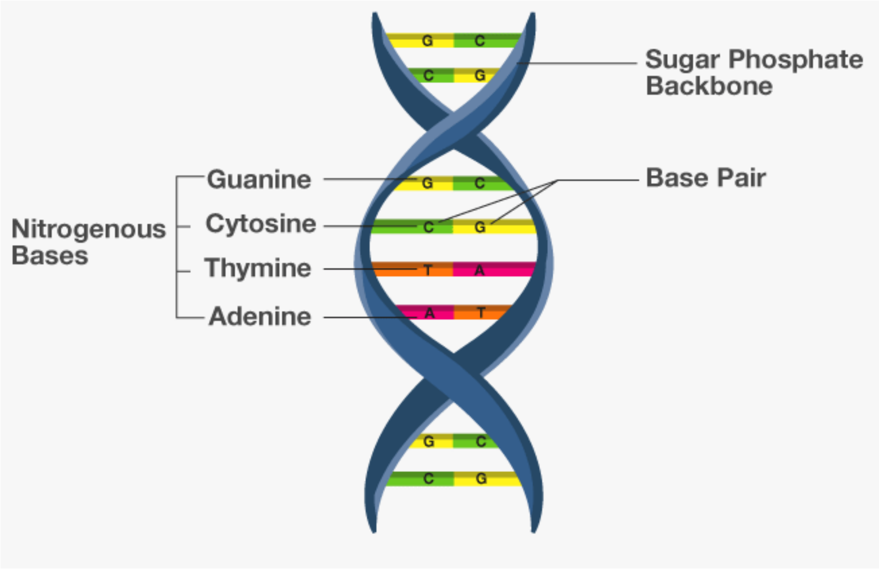
Nucleotide



O
R–O–P–O
OH
O
N
OH
3
N
O
NH
N
NH2
N
1
2
1 Phosphate group
2 Pentose sugar
3 Nitrogenous base

NUCLEIC ACIDS AS GENETIC MATERIAL

DNA

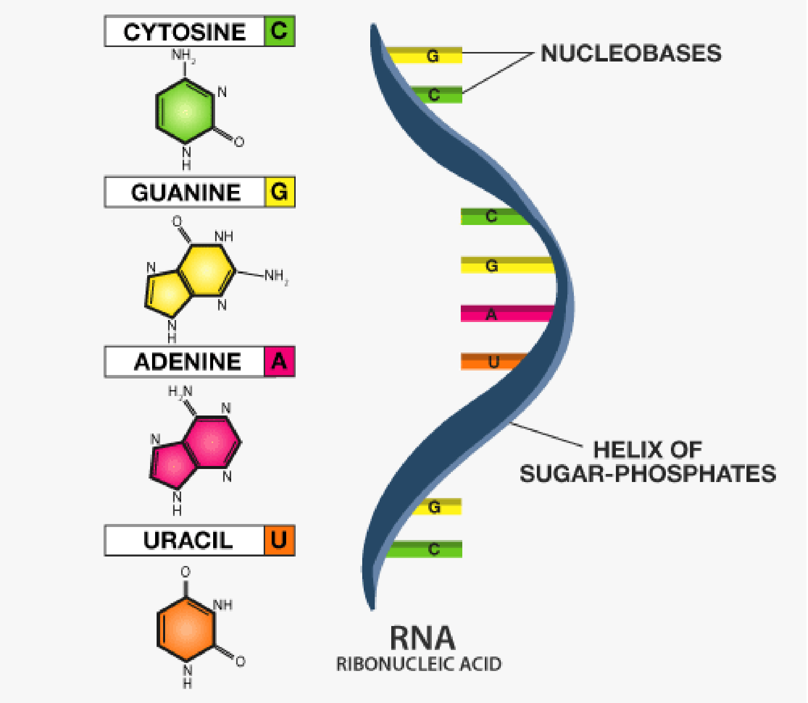


- Genes carry genetic information from one generation to the other.
- The structure of nucleic acids such as DNA consists of a long polymer of nucleotides connected by phosphodiester bonds.
- In the case of DNA, the nucleotides are deoxynucleotides.
- The most widely accepted structure of DNA (right-handed double-helix) was proposed by Watson and Crick in 1953.
- The nitrogenous bases present in DNA are Adenine, Guanine, Thymine, and Cytosine.

Ribonucleic Acid (RNA)

- Ribonucleic acid is an example of the nucleic acid formed by the polymerization of ribonucleotides.
- It is a single-stranded chain formed by 7-12000 ribonucleotides.
- This structure of nucleic acid contains a ribose sugar, a phosphate group, and a nitrogen base.
- The nitrogenous bases in RNA are Adenine, Guanine, Cytosine, and Uracil (U).
- RNA is formed from DNA by the process of ‘transcription’ and is generally involved in protein synthesis.

- Types of RNA include – Ribosomal RNA (rRNA), Transfer RNA (tRNA) and Messenger RNA (mRNA).

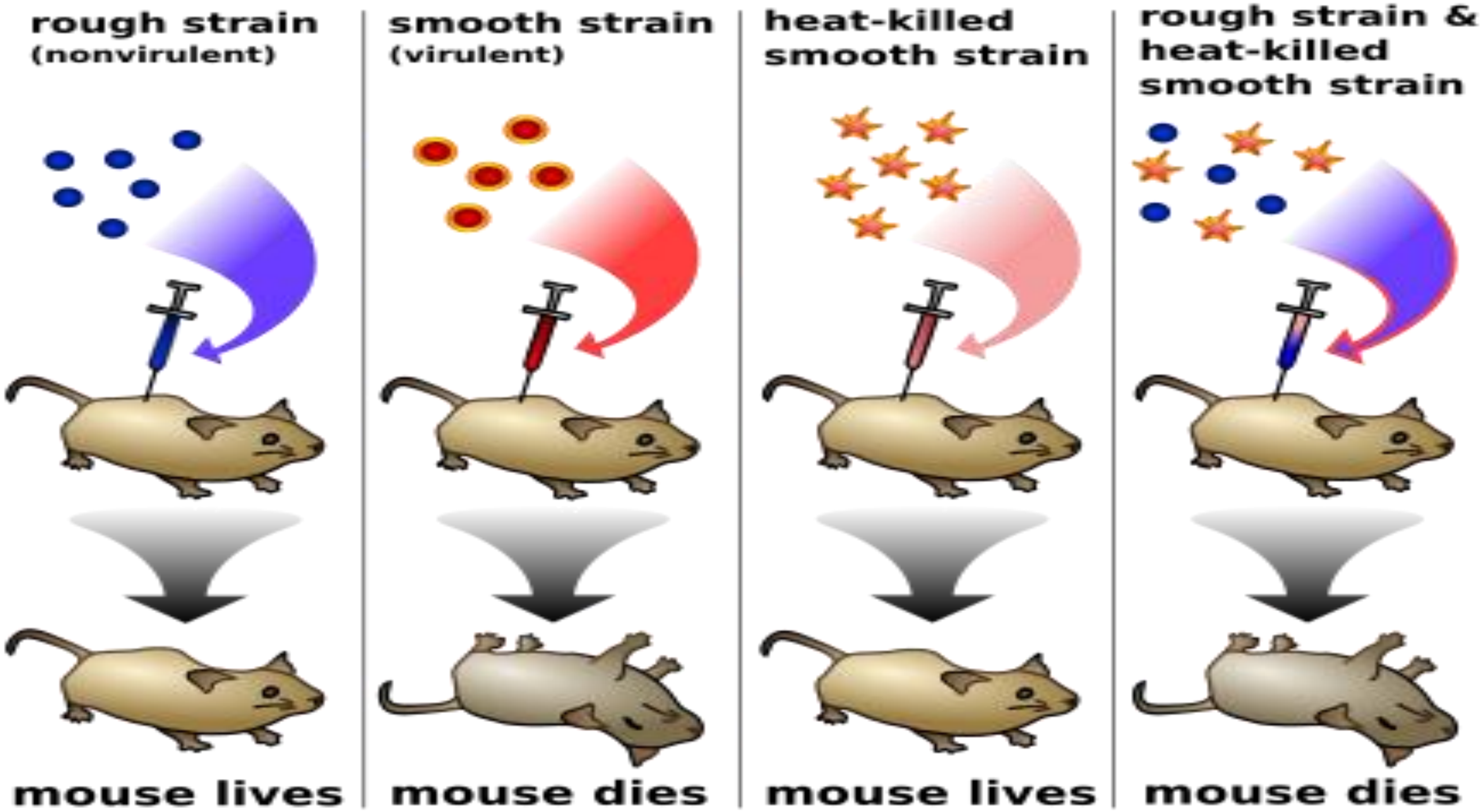


Griffith's experiment

- **Griffith's experiment-** performed by Frederick Griffith (British Bacteriologist) and reported in 1928.
- It was the first experiment suggesting that bacteria are capable of transferring genetic information through a process known as transformation.
- Pneumonia was a serious cause of death in the wake of the post-WWI Spanish Griffith was studying the possibility of creating a vaccine.
- Griffith was investigating two strains of the bacterium Streptococcus pneumoniae: **the virulent S strain**, which has a smooth appearance due to a polysaccharide capsule and causes pneumonia, and the **non-virulent R strain**, which lacks this capsule and has a rough appearance.

The Experiment

1. **Injection of S Strain:**
    1. Mice injected with live S strain bacteria died of pneumonia.
2. **Injection of R Strain:**
    1. Mice injected with live R strain bacteria remained healthy.
3. **Injection of Heat-Killed S Strain:**
    1. Mice injected with heat-killed S strain bacteria remained healthy, as the bacteria were no longer virulent.
4. **Injection of Heat-Killed S Strain and Live R Strain:**
    1. Mice injected with a mixture of heat-killed S strain and live R strain bacteria died of pneumonia.



Griffith's experiment discovering the "transforming principle" in *Streptococcus pneumoniae*

Conclusion:

•Griffith found that the live R strain bacteria had somehow been transformed into the virulent S strain.

•This transformation implied that some "transforming principle" from the dead S strain bacteria was taken up by the live R strain bacteria, enabling them to produce the polysaccharide capsule and become virulent.

Significance:

•This experiment showed the phenomenon of transformation, where genetic material could be transferred from one bacterium to another.

•Griffith's findings laid the groundwork for future research to identify the nature of the transforming principle.

•This led to the further discovery by Avery, MacLeod, and McCarty in 1944 that DNA is the substance responsible for transformation and thus the hereditary material.

Avery, MacLeod, and McCarty's experiment

Avery, MacLeod, and McCarty aimed to identify the transforming principle by isolating and purifying different macromolecules from the heat-killed S strain bacteria and testing their ability to transform the R strain bacteria.

1.Preparation of Extracts:

1. They prepared an extract from heat-killed S strain bacteria containing various macromolecules, including proteins, RNA, and DNA.

2.Fractionation and Treatment:

1. The extract was treated with enzymes that specifically degrade proteins (proteases), RNA (RNase), or DNA (DNase).
2. The purpose of these treatments was to determine which component was responsible for transformation.

3. Transformation Assay:

1. After each treatment, the extracts were mixed with live R strain bacteria and injected into mice or grown in culture to observe the transformation.
2. Extracts treated with proteases or RNase still transformed R strain bacteria into virulent S strain bacteria.
3. Extracts treated with DNase did not transform the R strain bacteria, indicating that DNA was necessary for transformation.

Conclusion:

•Avery, MacLeod, and McCarty jointly concluded that DNA is the substance responsible for transforming the R strain bacteria into the virulent S strain. This served as a strong evidence that DNA is the genetic material.

Significance:

•This experiment was one of the first to suggest that DNA is the hereditary material, whereas it was earlier considered that proteins were the carriers of genetic information.

•Further research was carried out by Hershey and Chase in1952, which provided additional confirmation that DNA is the genetic material in viruses.

Hershey-Chase experiment

Experiment

1. Labeling the Phage Components:

- Hershey and Chase used two sets of bacteriophages:
- One set had its DNA labelled with radioactive phosphorus-32 (^32P), since **DNA contains phosphorus but proteins do not**.
- - The other set had its protein coat labelled with radioactive sulfur-35 (^35S), since **proteins contain sulfur but DNA does not**.
- **2. Infection of Bacteria:**
- The radioactive phages were allowed to infect *Escherichia coli* bacteria.
- During infection, the phages attach to the bacterial surface and inject their genetic material into the bacteria.

3. **Blending and Centrifugation:**

- After a period of time, Hershey and Chase used a blender to separate the phage protein coats from the bacterial cells.
- They then used centrifugation to separate the heavier bacterial cells from the lighter phage protein coats.

4. **Analysis:**

- The bacterial cells, which formed a pellet at the bottom of the centrifuge tube, were examined for radioactivity.
- The liquid supernatant, containing the phage protein coats, was also examined for radioactivity.

Results

- Bacteria infected with phages labelled with ^32P (DNA) were found to have radioactive phosphorus **inside the cells**.
- Bacteria infected with phages labeled with ^35S (protein) had radioactive sulfur in the **supernatant, not inside the cells**.

- It reinforced the findings of Avery, MacLeod, and McCarty and paved the way for the discovery of the structure of DNA by James Watson and Francis Crick in 1953.

Conclusion

- The experiment demonstrated that it was the DNA, not the protein, of the phages that entered the bacterial cells and directed the production of new phages.
- This provided strong evidence that DNA is the genetic material responsible for heredity.

Significance

- The Hershey-Chase experiment confirmed that **DNA, not protein, is the genetic material in all living organisms.**

DNA structure

DNA—Watson-Crick Model

- DNA is the largest macromolecule that represents the genetic material of the cell.
- DNA is a double helix of two antiparallel polynucleotide chains.
- In 1953, J. D. Watson (an American biologist) and F.H.C. Crick (a British Physicist) proposed the three-dimensional model of physiological DNA (i.e. B-DNA) on the basis of X-ray diffraction data of DNA obtained by Franklin and Wilkins.
- For this epoch- making discovery, Watson, Crick and Wilkins (first crystalline symmetrical patterns of DNA) got Nobel Prize in medicine in 1962.

Features of Watson-Crick model or double helix model of DNA

1. The DNA molecule consists of two polynucleotide chains or strands that spirally twisted around each other and coiled around a common axis to form a right-handed double-helix.

2. The two strands are antiparallel i.e. they run in opposite directions so that the 3′ end of one chain facing the 5′ end of the other.

3. The sugar-phosphate backbones remain on the outside, while the core of the helix contains the purine and pyrimidine bases.

4. The two strands are held together by hydrogen bonds between the purine and pyrimidine bases of the opposite strands.

5. Adenine (A) always pairs with thymine (T) by two hydrogen bonds and guanine (G) always pairs with cytosine (C) by three hydrogen bonds. This complementarity is known as the base pairing rule. Thus, the two stands are complementary to one another.

6. The base sequence along a polynucleotide chain is variable and a specific sequence of bases carry the genetic information.

7. The base compositions of DNA obey Chargaff's rules (E.E. Chargaff, 1950).

- The quantity of A equals to the quantity of T, and the quantity of G equals to the quantity of C.
- DNA from any cell of all organisms should have a 1:1 ratio (base pair rule) of purine and pyrimidine bases.

8. The diameter of DNA is 2.0nm or 20 Å . Adjacent bases are separated 0.34 nm or by 3.4 Å along the axis. The length of a complete turn of helix is 3.4 nm or 34 Å i.e. there are 10bp per turn.

9. The DNA helix has a shallow groove called minor groove (˜1.2nm) and a deep groove called major groove (~2.2nm) across.

Fig. 6.7 X-ray diffraction photograph of a DNA fiber (B form) as taken by Rosalind Franklin (1953). The central X-shaped pattern indicated a helix, whereas the dark black arcs on the top and bottom arise from base pairing with a distance of 3.4 Å apart.

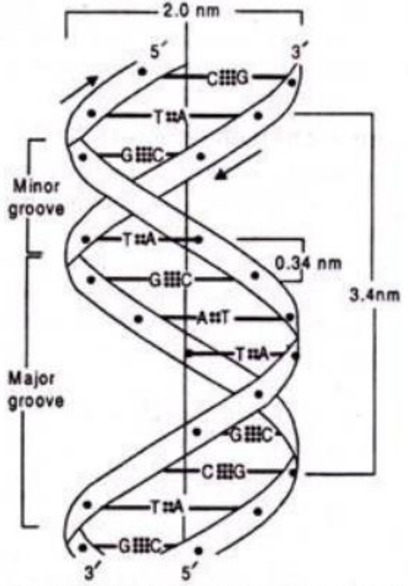


Fig. 6.8 The structure of the Watson and Crick model of double-helical structure of the B-form of DNA (diagrammatic).

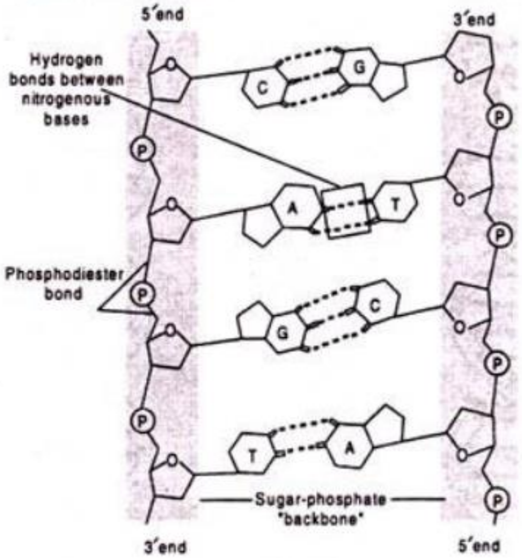


Fig. 6.9 DNA. A hypothetically untwisted DNA lie flat to show the ladder-like appearance. The two sides of the ladder are called the DNA's "backbone". The steps inside the ladder represent "base pairs".

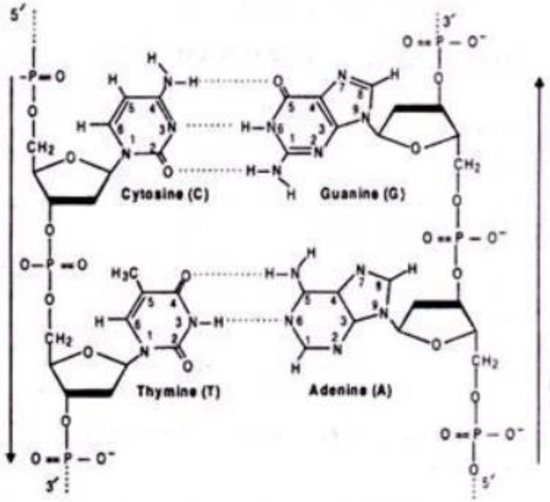


Fig. 6.10 A part of DNA showing complementary base pairing. Three hydrogen bonds link cytosine & guanine, whereas two hydrogen bonds form between thymine and adenine.