NUCLEIC ACIDS

Structure of nucleic acid

Nucleic acid consists of many nucleotides. Each nucleotide consists of a nitrogenous base, a five carbon (pentose) sugar and a phosphate group.

There are two types of **nitrogenous bases**. They are purines and pyrimidines. **Purines** have two rings in the structure, a five membered ring and a six membered ring. **Adenine** and **Guanine** are the important purines. **Pyrimidines** have only a six membered ring in their structure. **Cytosine**, **Thymine** and **Uracil** are the important pyrimidines.

Pentose sugar in the nucleic acid is the **deoxyribose** sugar in DNA and **ribose** sugar in RNA. A **phosphate group** is present in each of the nucleotides.

The first carbon atom of the pentose sugar is linked with the nitrogenous base by N-glycosidic bond to form **nucleoside**. (Nucleoside = Pentose sugar + Nitrogenous base). The phosphate sugar is linked with the C-3 of the sugar of nucleoside to form **nucleotide** (Nucleotide = Nucleoside + Phosphate group).

Structure of DNA

James Watson and Francis Crick proposed a model for the structure of DNA called **Watson and Crick model** of DNA. According to this model, the DNA molecule has two polynucleotide chains coiled around a common axis to form a right handed **Double helix**. The chains run in opposite directions. At one end of DNA molecule, one polynucletide chain will have the free phosphate group called 5' end while the other chain will have the free –OH group called the 3' end. The chains are said to be **antiparallel** to each other.

Each double helix has a **minor groove** of $12A^0$ width and a **major groove** of $22A^0$ width.

Each polynucleotide is made up of many **deoxyribonucleotides**. Each deoxyribonucleotide consists of a **deoxyribose sugar**, a **nitrogenous base** and a **phosphate group**.

The bases present in DNA are the purines, **adenine** and **guanine** and the pyrimidines, **cytosine** and **thymine**. They are present inside the helix while the phosphate group and the sugar on the outer side of the helix. The bases are perpendicular to the helix axis. The diameter of the helix is $20A^0$. Two nucleotides are separated by a distance of 3.4 A^0. The DNA helix makes a turn for every 10.5 nucleotides and the distance of the turn is $36A^0$.

The two chains are held together by hydrogen bonds between the base pairs. A purine always pairs with a pyrimidine. Adenine always pairs with thymine by two hydrogen bonds (**A=T**) while guanine always pairs with cytosine by three hydrogen bonds (**G ≡ C**). These base pairs are commonly referred to as **complementary bases** and the polynucleotide chains are **complementary** to each other. The number of A bases is equal to T and the number of G bases is equal to C in a given double stranded DNA.

Replication of DNA

The DNA molecule synthesises an exact replica of itself and the process is called replication. The replication of DNA takes place during the interphase between two mitotic cycles and in the interphase before meiosis.

The **semi-conservative** mode of DNA replication was proposed by Watson and Crick. In this method, one strand of DNA molecule (parent strand) serves as the template for the synthesis of a new complementary strand. Thus from a single double helical parent DNA two double helical daughter DNA are formed. Each daughter DNA has one parental old polynucleotide chain and the other newly synthesised strand.

DNA replication requires more than 20 enzymes and factors, which are collectively called DNA replication system or **Replisome**.

Replication of DNA starts at a particular point called **initiation point** or **ori (**origin of replication). The ori is recognised by specific **initiator proteins**. The replication starts with a nick (break) in one of the two parental strands made by an **incision enzyme**. The hydrogen bonds between the complementary nucleotides break from the initiator point. This process is helped by the **deoxyribonuclease enzyme**. The unwinding of the DNA helix is done by the **helicase** enzyme. Some **DNA binding proteins** (DBP or SSP) bind to the strands keeping

them apart. The region where the proteins bind forms a Y-shaped **replication fork.** The enzyme **DNA Gyrase or Topoisomerase** puts a knot a little away from the replication fork to prevent the complete unwinding of the duplex DNA.

The initiation of replication requires a short length of **RNA as primer**, because the enzyme DNA polymerase III can not initiate synthesis of a new strand but can only elongate an existing strand. The complementary RNA is synthesised in the 5' →3' direction by the enzyme **primase** present in the complex called **primosome.** To the 3' end of this RNA primer, deoxyribonucleotides complementary to the template DNA, are added by the enzyme **DNA polymerase III.**

The enzyme DNA polymerase III can add nucleotides only to the free 3' end of the chain (i.e. in 5' →3' direction). Hence, only one of the two starnds can be continuously elongated and is called the **leading strand**. The other starnd is made in snall pieces known as **Okazaki fragments** and is called **lagging strand**. The short Okazaki fragments are then joined together by the enzyme **DNA ligase.**

The RNA primer is removed and replaced by complementary deoxyribonucleotides by the enzyme **DNA polymerase I**.

When both the strands are replicated the DNA binding proteins are removed and each of the daughter molecules rewind separately. Thus each daughter DNA molecule has a new strand and a parental strand.

The replication may proceed in one (**unidirectional**) or both (**boidirectional)** directions.

Evidences to prove semi-conservative mode of DNA replication

1. **Meselson and Stahl's Experiment**

Meselson and Stahl proved experimentally the semi-conservative mode of DNA replication. They cultured ***E.coli*** for 14 generations in a medium containing N^{15} isotope. The N^{15} was incorporated and the DNA of *E.coli* became heavier. This DNA was called **heavier**

DNA. After 14 generations the *E.coli* was washed and transferred to a culture medium with normal nitrogen (N^{14}) which was lighter.

After the first division, the DNA was extracted and all of it was found to be a hybrid (N^{14}+ N^{15}) with intermediate density. After the second division two types of DNA were found, half of normal N^{14} DNA and the other half hybrid (N^{14}+ N^{15}) DNA.

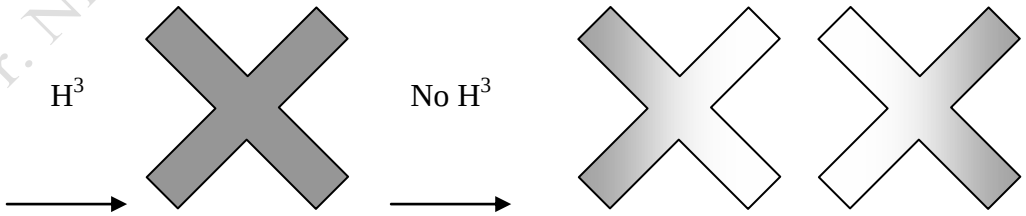
After each generation, the normal DNA showed greater accumulation. This proved that the heavier strand was the parental strand and the other strand was the newly synthesised daughter strand. This clearly proved semi-conservative method of DNA replication.

2. Taylor's Experiment

Taylor and his co-workers used autoradiographic technique to prove semi- conservative method of DNA replication.

The roots of *Vicia faba* bean were grown in a medium containing radioactive thymidine (tritiated thymidine). After the incorporation of the tritiated thymidine in the chromosome, the root tips were transferred to the unlabelled medium containing colchicines which prevents cell division without affecting chromosome duplication and the cells were studied for radioactivity.

It was found that after one cycle of duplication, both the chromatids of the chromosome were labelled indicating that the original strand of DNA helix was labelled with radioactivity and in each chromatid only one of the two strands was labelled. In the second cycle of duplication, in each chromosome, one of the two chromatids was found to be labelled.



Evidences of DNA as genetic material

Several experiments have been done to prove DNA is the genetic material

1. **Bacterial Transformation (Griffith's Experiment)**

Griffith revealed that DNA was the genetic material. He was working on *Pneumococcus pneumoniae* (the bactria causing pneumonia). He found that this bacteria exists in two strains, Virulent and Avirulent. The **virulent strain (SIII)** secretes a polysaccharide capsule around it so that it forms smooth colony. They cause pneumonia. The **Avirulent strain (SII)** lacks the polysaccharide capsule and forms rough colony. They do not cause pneumonia.

In his experiments, Griffith injected virulent SIII strains into the body of mice. They developed pneumonia and died. When avirulent SII strains were injected to the mice, they remained healthy. The virulent strain were heated to 65^0C and killed. The heat killed SII strains were injected to mice, they remained healthy.

When the mice were injected with a mixture of living avirulent and heat killed virulent strain, the mice developed pneumonia and died. Bacteria recovered from the dead mice were found to contain both virulent and avirulent strains. Griffith concluded that some factor from the heat killed virulent strain caused a transformation in the live avirulent strains, changing them into virulent form.

Later Avery, McCarty and Macleod identified the transforming factor as a DNA. They extracted DNA from the virulent strain and were added to the culture medium of avirulent strain. After a few hours, the culture contained both virulent and avirulent strains. Thus they concluded that DNA is the genetic material.

2. **Hershey and Chase's Experiment**

Bacteriophage is a virus that infects bacteria. The virus consists of a head and a tail. The head consists of a

protein coat which encloses the DNA. The tail consists of protein coat alone. The phage attaches to the bacterial cell with the help of tail fibres. After the attachment the DNA present

in the head is transferred into the bacteria while the head and protein coat remains outside. Inside the bacteria the phage DNA replicates and daughter phages are produced.

Hershey and Chase allowed the phage particles to multiply in a medium containing radioactive phosphorus P^{32} and radioactive sulphur S^{35}.

Structure of RNA

Ribonucleic acid (RNA) is formed of many ribonucleotides. Each ribonucleotide consists of a ribose sugar, a nitrogenous base and a phosphorus group. The nitrogenous bases are the purines, adenine and guanine and the pyrimidines, uracil and cytosine. RNA has a linear structure formed of many ribonucleotides linked by phosphodiester bonds.

There are 3 major classes of RNA – messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA). The **messenger RNA (mRNA)** is present in the nucleus and cytoplasm. It is synthesised by the DNA. The mRNA is complementary to the DNA from which it is synthesized. They carry the genetic message from the DNA to the cytoplasm. The message in mRNA is translated to form the aminoacid sequence of proteins. This genetic message is in the form of triplet nucleotides and is called the genetic code or codon.

Transfer RNA (tRNA) serves as adaptors during protein synthesis. It brings the appropriate aminoacid from the aminoacid pool to the site of protein synthesis. Each tRNA has three nucleotides complementary to a particular genetic code and is called the **anticodon**.

Ribosomal RNA (rRNA) are found in larger quantities in a cell and are of different types like 5S, 5.8S, 16S, 18S, 23S and 28S rRNAs. They are found in the ribosomes and give definite shape to the ribosomes. They are the structural and functional units of ribosomes. They allow only the appropriate tRNA to bind to the ribosome and identify the starting point of mRNA.

Secondary (3-D) structure of t-RNA

All the tRNAs have the same general secondary structure. This structure is known as the **clover leaf structure.** It has four major arms and an extra arm. The four arms are:

1. **Aminoacid arm** (acceptor arm) – it has a base paired stem which ends in an unpaired sequence. The terminal nucleotide bears the aminoacid at the second and third –OH groups. This arm has 7 base pairs.

2. **TΨC arm** – it has a base paired stem and an unpaired loop. The stem has 5 base pairs. The modified bases **ribosyl thymidine** (T) and **psuedouridine** (Ψ) and **cytosine** (C) are present.

3. **Anticodon arm –** it has the anticodon triplet in the loop. The stem is formed of 5 base pairs. The anticodon interacts with the codon of mRNA during protein synthesis.

4. **DHU arm** – it has the modified base dihydrouridine in the loop. The stem is formed of 4 base pairs.

5. **Extra arm** – between the TΨC arm and the anticodon arm, there is an arm called extra arm which may be small or large.