

I. Genetic Material:

1. Oswald Avery, Colin Macleod and Maclyn McCarty repeated Griffith's experiment in an in vitro system.
  - \* R-strain – Rough, non virulent.
  - \* S-strain – smooth, virulent.
  - \* This experiment proved that the biochemical substance which can be the genetic material is DNA.
  - \* The doubt was on protein, or RNA or DNA which had the possibility to be a genetic material.
  - \* Steps involved were:
    - a. Protein part was destroyed and denatured. S-strain (heat killed) was added to R-strain, virulent S-strain appeared.
    - b. RNA was destroyed and S-strain (heat killed) was added to rough strain again. S-Strain appeared.
    - c. DNA was destroyed and S-strain (heat killed) was added to Rough strain no S-Strain appeared, no transformation occurred. So, DNA is the genetic material.
2. Hershey and Chase experiment on T<sub>2</sub> bacteriophage (virus).

Scientist Alfred Hershey and Martha Chase also called as blender experiment.

  - \* There was a doubt that proteins can be the genetic material rather than DNA. This experiment is done to prove that DNA is the genetic material.
  - \* Nucleic acid of DNA contains phosphorus.
  - \* Proteins are made of amino acids and some amino acids like (methionine and cysteine contain sulphur).
  - \* To prove it as DNA they take Phosphorus P<sup>32</sup>.
  - \* To prove it as protein they take Sulphur isotope S<sup>35</sup>.

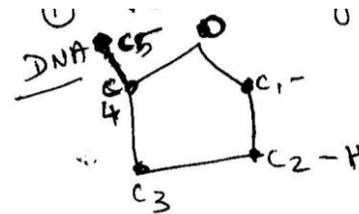
- \* Two different culture medium is taken one medium has bacteria growing in P<sup>32</sup> and Bacteriophage (virus) infecting it. Another medium has bacteria growing in S<sup>35</sup> and Bacteriophage (virus) infecting it.
- \* Bacteriophage (virus) which grew in S<sup>35</sup> has labelled Sulphur and Bacteriophage (virus) which grew in P<sup>32</sup> has labelled Phosphorus.
- \* The two labelled S<sup>35</sup> & P<sup>32</sup> phages are allowed to infect unlabelled bacteria E.coli.
- \* The phages infect the bacteria and they are given a slight agitation before lysis with a blender.
- \* They found that P<sup>32</sup> has entered the Bacterial cell, and S<sup>35</sup> remained in the outside medium proving DNA is the genetic material.

Chemistry of Nucleic acids

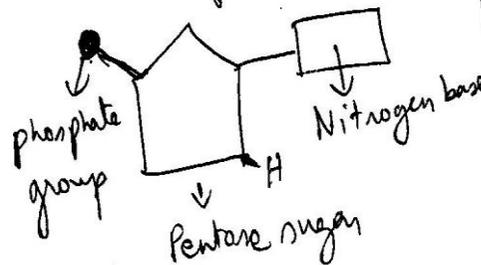
DNA and RNA

DNA is made (i) Pentose sugar (ii) Nitrogen base (iii) Phosphate group

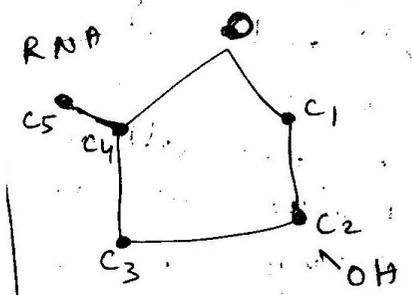
1. Pentose sugar



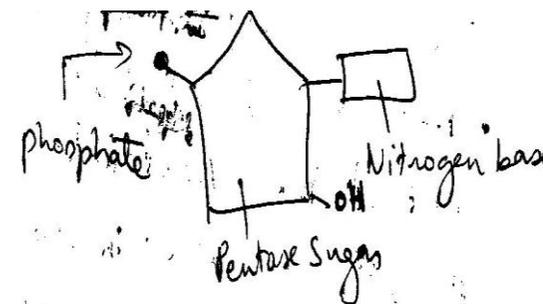
C2-only 'H' in DNA no 'OH' group makes DNA more stable



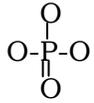
RNA



2. Basic structure of RNA



## DNA and RNA Phosphate group



1. Phosphate group gives negative charge and acidic nature which retains it within cell or nuclear membrane
2. Oxygen molecules give negative charge
3. Release of H<sup>+</sup> ions
  - \*from phosphate group gives acidic nature to nucleic acid

### 3. Nitrogen Base DNA

Accepts H<sup>+</sup> ions gives basic nature

- \* There are two types of nitrogen bases  
Purines and Pyrimidines
- \* Purines are Adenine, Guanine
- \* Pyrimidines are cytosine, Thymine
- \* Thymine gives more stability to DNA  
Purines two nitrogen rings
- \* Pyrimidine one nitrogen ring

### Nitrogen Base RNA:

Accepts H<sup>+</sup> ions gives basic nature

- \* There are two types of nitrogen bases purines and Pyrimidines
- \* Purines are Adenine and Guanine
- Pyrimidines:  
Pyrimidines are cytosine and uracil.
- Uracil makes RNA more unstable and reactive.
- Purine two rings Pyrimidine are nitrogen ring

### 4. DNA – Double standard:

Hydrogen bonds are found between Purines and Pyrimidines  
A=T → two hydrogen bonds  
G≡C → three hydrogen bonds

DNA is double stranded and it gives more stability

RNA single standard only tRNA seems to form Hydrogen bonds by pairing of complementary base pairs forming bonds (Hydrogen) only in certain regions.

### Packaging of DNA helix:

haploid human

genome (23) chromosomes

has 3.2 billion base pairs length of DNA double helix is 2.2m

### Packaging:

Prokaryotes lacks (no) chromatin

Eukaryotes it is complex, chromatin is formed of repeating units called nucleosomes

Nucleosomes → proposed by Kornberg

- \* Four histones - proteins are present
- \* Each type of histone has two molecules
- \* The four histones are H2A, H2B, H3, H4
- \* Each histone with two molecules make a octomere (eight molecules)  
H2A-two, H3-two  
H2B-two, H4-two

\*DNA is wrapped twice around the Nucleosome

\*200bp are packed around a Nucleosome

\*The Nucleosomes are connected by H1

\*DNA is negatively charged and the histones are positively charged.

\* Solenoids are formed of 6-Nucleosomes per turn.

\*DNA is made of 40-folds of Solenoid

\*Chromatin of interphase is 200-300nm (nanometer) in diameter

### DNA replication:

\*Semi conservative replication of DNA was postulated by Watson and Crick

\*Meselson and Stahl proved this semi conservative replication of DNA postulated by Watson and Crick was correct

\* Two culture mediums with two different isotopes was used

\* One set of Bacteria E-coli was grown in culture medium with heavy isotope <sup>15</sup>N and other light isotope <sup>14</sup>N (NH<sub>4</sub>Cl)

\* After many generations each formed DNA with purely Heavy <sup>15</sup>N and <sup>14</sup>N

\* They were distinguished by cesium chloride density gradient centrifugation.

\* Each type settled in two distinct separate bands

\* Heavy culture was transferred to plain NH<sub>4</sub>Cl and after the first replication made to undergo centrifugation and showed the band settling between heavy and light band, (intermediate position) proving it had one heavy and one light band.

\* Again samples were taken after second replication, they found DNA settling at intermediate and light band position proving semi consecutive replication

## Replication

### Replication of DNA in prokaryotes

Enzymes:

DNA, I, II, III polymerase

DNA – I → Kornberg enzyme used for repair

DNA-II polymerase for repair

DNA-III main enzyme for DNA replication

Energy component for replication is → Deoxy nucleotide triphosphate initiation at ori-site

- \* DNA – unwinds
- \* Two strands are formed
- \* One leading strand → 3' → 5' polarity
- \* One lagging strand → 5' → 3' polarity
- \* Leading strand replication is continuous
- \* Lagging strand replication is discontinuous
- \* Leading strand is 3" to 5" because DNA polymerase can add bases from 5' and 3' end
- \* lagging strand replication is discontinuous causing formation of okazali fragments.

### Replication in Eukaryotes:

Enzymes are

- \* Helicase → unwinds the DNA strand
- \* Topoisomerase → keep the strand open till replication is completed
- \* Replication fork is formed
- \* DNA polymerase adds complementary nucleotides
- \* RNA primase – adds primer to help DNA polymerase to start replication
- \* Exonuclease - removes RNA primers
- \* Gaps are filled by DNA polymerase
- \* Ligase seals, the gaps

### Transcription – Synthesis of RNA

- \* Always only template strand with 3' to 5' used, it must have 3' to 5' polarity
- \* The other strand coding strand is not transcribed
- \* Monocistronic structural gene is seen in Eukaryotes (one gene one protein)
- \* Polycistronic structural gene is seen in prokaryotes (cluster of many related genes are presented) having many related genes, related proteins forming 'operon'

Transcription unit of DNA has

- \* Promoter region
- \* Structural gene
- \* Terminator

### Promoter region:

- \* Prokaryotes have pribnow box
- \* Eukaryotes have Gold berg Hongness box or TATA box
- TATA → Thymine, adnine rich region
- \* Structural gene
- Prokaryote – polycistronic (operon)

### Eukaryote – monocistronic

In prokaryotes:

RNA polymerase enzyme helps in transcription has two parts one is core enzyme and other sigma subunit, core enzyme ( $\beta'$ ,  $\beta$ ,  $\alpha$ ,  $\alpha$ ) helps RNA synthesis.

Sigma helps recognition of promoter.

- \* The gene ends in terminator termination is by rho( $\rho$ )
- \* Initiation factor is sigma
- \* And the termination factor is rho
- \* RNA polymerase associates with these factors to start and stop transcription.

Eukaryotes:

- \* There are three RNA polymerases
- \* RNA -I → transcribes rRNA
- \* RNA -II transcribes – mRNA (hnRNA)
- hnRNA has (exon, intron)
- RNA III for T-RNA
- Intron → mobile gene, non coding sequence
- \* Introns are intervening sequence
- \* Exons are expressed sequence
- \* Introns have to be spliced and capping and tailing has to be done to change hn RNA to mRNA which can come out of Nucleus
- \* Capping by methyl gnanosine triphospahte
- \* Tailing by polyadenyl residues
- Capping and tailing protects MRNA from enzymes of cytoplasm.