The Search for Genetic Material :→

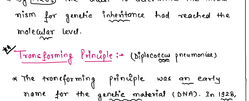
- Mendel proposed the principles of Inheritance in 1866. (factors)
- Meischer discovered nuclein in 1869.
- Sutton & Boveri proposed chromosome theory of Inheritance in 1902 indicating that genetic material is contained in chromosomes.
- In 1933 Morgan confirmed the binding of sutton and Boveri. He proved that genes are located in the chromosomes and reside in the nucleus of most cells.

Question ?? what molecule act as genetic material, has not been answered

* By 1926, the quest to determine the mechanism for genetic inheritance had reached the molecular level.

Transforming Principle :→ (Diplococcus pneumoniae)

* The transforming principle was an early name for the genetic material (DNA). In 1928, Frederick Griffith, in a series of experiment with Streptococcus pneumoniae (bacterium responsible for pneumonia) witnessed a miraculous transformation in the bacteria. During the course of his experiment, a living organism (bacteria) had changed in physical form.



Step-1 S-strain → Inject into mice → Mice die

Step-2 R-strain → Inject into mice → Mice live

"means S-strain is virulent because mice develop pneumonia & die from it." (or Pathogenic)

R-strain → non-virulent, do not cause pneumonia. (or non-pathogenic)

* Griffith was able to kill bacteria by heating them.

Step-3 S-strain (Heat killed) → Inject to mice → Mice live

Step-4 S-strain (Heat-killed) + R-strain (Live) → Inject to mice → Mice die (non-pathogenic)

* He observed that heat killed S-strain bacteria injected into mice did not kill them.
* When he injected a mixture of heat-killed S and live R-bacteria, then mice died. Moreover, he recovered living bacteria from dead mice.

Griffith concluded that the R-strain bacteria had somehow been transformed by the heat-killed S strain bacteria. Some 'transforming Principle', transferred from the heat-killed S strain, had enabled the R strain to synthesise a smooth polysaccharide coat and become virulent. This must be due to transfer of genetic material.

However, the biochemical nature of genetic material was not distinct from this experiment.

Biochemical characterisation of Transforming Principle

* Prior to work of Oswald Avery, Colin MacLeod and Maclyn McCarty (1933-44), the genetic material was thought to be Protein.
* They worked to determine the biochemical nature of 'Transforming Principle' in Griffith's Experiment.
* They purified biochemicals (Protein, DNA, RNA etc) from the heat killed S cells to see which one could transform live R cells into S cells
* They discovered that DNA alone from S bacteria caused R bacteria to become transformed.
* They also discovered that protein-digesting enzymes (Proteases) and RNA-digesting enzymes (RNAases) did not affect transformation, so that transforming substance was not protein or RNA.
* Digestion with DNase did inhibit transformation, suggesting that the DNA caused the transformation.
* They conclude that DNA is the heredity material but not all biologists were convinced.

The Genetic Material is DNA.

* The unequivocal (unmistakable) proof that DNA is the genetic material came from Transduction Experiment (bacteriophage experiments) of Alfred Hershey and Martha Chase (1952).
* They worked with viruses that infected the bacteria called bacteriophages ⇒ "T_2-Phage"
* Hershey and chase worked to discover whether it was protein or DNA from the viruses that entered the bacteria.

Bacteriophages are viruses

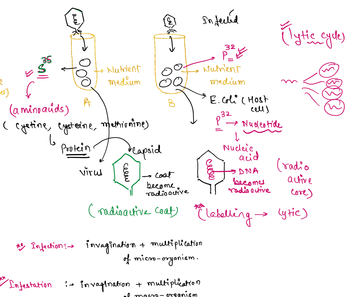
TMV

Bacteriophage (lipid) → Coat (Protein), core (DNA)

"T_2-phage"

Labelling of Protein Coat and DNA of bacteriophages with radioactive Traces

Hershey and chase (1952) grew two cultures of E. coli (host cell) (radioactive isotopes). One culture was supplied with radioactive ^{35}S. The other culture was provided with radioactive phosphorus, ^{32}P. Radioactive sulphur gets incorporated into sulphur containing aminoacids (cysteine and methionine), and therefore, became part of bacterial proteins. Radioactive phosphorus gets incorporated into nucleotides which form nucleic acids, mostly DNA. Therefore, bacteria of both the cultures become labelled. [with the help of lytic cycle]



After Labelling, three steps were followed.

1. Infection :→ Both types of labelled phages were allowed to attach to E. coli. Then as infection proceed DNA was introduced and protein coat was left out.
2. Blinding :→ As the infection proceed, the viral coat were removed from the bacteria by agitating them in a blender.
3. Centrifugation :→ The virus particles were separated from the bacteria by spinning them in a centrifuge.

The heavier bacteria settled down in the form of pellet. The supernatant contain lighter viral coats which do not enter the bacterial cells. (ghost viruses)

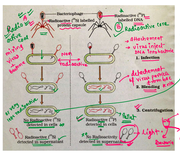
Bacteria which was infected with viruses that had radioactive DNA were radioactive indicating that DNA was the material that passed from virus to bacteria. (radioactive core)

Bacteria that were infected with viruses that had radioactive proteins were not radioactive. This indicates that proteins did not enter the bacteria from the viruses.

DNA is therefore the genetic material that is passed from virus to bacteria → "(unequivocal) proof"

Infection :→ Invagination + multiplication of micro-organism.

Infestation :→ Invagination + multiplication of macro-organism



"The Hershey-Chase Experiment"
(This experiment is also KIA)
Blender Experiment

Properties of Genetic material (DNA Versus RNA)

* Now it is clear that the debate between protein v/s DNA as genetic material was unequivocally resolved from Hershey-chase experiment
* However, it subsequently became clear that in some viruses, RNA is genetic material (for example (Tobacco mosaic viruses, BB bacteriophages.
* Some Questions like (storage information)
  1. Why DNA is predominant genetic material
  2. RNA performs dynamic functions of messenger and adapter.

→ These can be explained by differences between chemical structures of the two nucleic acid molecules.

A molecule that can act as a genetic material must fulfill the following criteria

(1) Replication power → It should be able to replicate (Duplicate)
(2) Stability → It should be stable chemically and structurally.

R-Strain

1. Culture medium + Proteins & Amino sugars = No change.
2. Culture medium + RNA of S-type (R-strain) = No change.
3. Culture medium + DNA of S-Type (R-strain) + DNase = No change (b/c DNase digest DNA)
4. Culture medium + DNA of S-type (R-strain) (without DNase) = Will be change (S-strain)

(3) Mutation :- It should provide the scope for slow changes (mutation) that are required for evolution.

Mutation → variation → Evolution

(genetic) DNA, RNA → Protein (1,2,3,4) (doesn't have power of Replication)

DNA → RNA → Protein (forward process)

DNA stability :→ The genetic material should be stable enough not to change with different stages of life cycle, age or with change in physiology of organisms.

Stability as one of the properties of genetic material was very evident in Griffith's 'transforming principle' itself that heat, which killed the bacteria, at least did not destroy some of the properties of genetic material.

This can be explained by

(1) The two strands of DNA being complementary. If separated by heating come together when appropriate conditions are provided. (dc nature + temp)
(2) 2'-OH group not at every nucleotide in RNA is a reactive group and makes RNA labile and easily degradable. (Ribozyme)
(3) RNA is also known to be catalytic, hence reactive.

∴ DNA is chemically is less reactive and structurally more stable compared to RNA. Therefore, among the two nucleic acids, DNA is a better genetic material.

(4) Presence of thymine at the uracil also confers additional stability to DNA → CH_3
(5) Both DNA and RNA are able to mutate. In fact RNA being unstable mutate at a faster rate. Consequently, viruses having RNA genome having shorter life span mutate and evolve faster
(6) RNA can directly code for synthesis of proteins, hence can easily express the characters.

DNA however, is dependent on RNA for synthesis of protein.

(7) Protein synthesising machinery has evolved around RNA.

The above discussion indicate that DNA and RNA can function as genetic material, but DNA being more stable is preferred for storage of genetic information. For transmission of genetic information RNA is better.

6.3 RNA World :→

* There is a little doubt that early life was RNA centric with every important functions being controlled by it.
* The first genetic material was RNA.
* There is now enough evidences to suggest that essential life processes like metabolism translation, splicing etc evolved around RNA.
* The first biocatalysts were RNAs.
* RNA use to act as genetic material as well as catalyst.
* RNA work well in early unstable condition
* RNAs were replaced in two of its functions.

(1) RNA being a catalyst was reactive and hence unstable. Therefore, DNA has evolved with chemical modification that make it more stable.

DNA being double stranded and having complementary strand further resists chang by evolving a process of repair.

(2) For biocatalysts, RNA was replaced protein enzymes. The latter were more stable, more efficient and more varied.

- The increase metabolic activity paved the way for evolution of more complex organisms.

Splicing → Process of transcription

A = C
G = T