The Search for Genetic Material

By 1926 quest to determine the mechanism of genetic material had reached molecular level.

Transforming Principle

In 1928 Fredrick Griffith did Series of experiment with bacteria Streptococcus pneumonia.
Bacteria grown on cultural plate produce two types of colonies

1) **S -strain** (Virulent/ Toxic) – Smooth and Shiny colonies that have a mucus (polysaccharides) coat.
2) **R-strain** (Non- Toxic) **–** Rough colonies that do not have mucus coat.

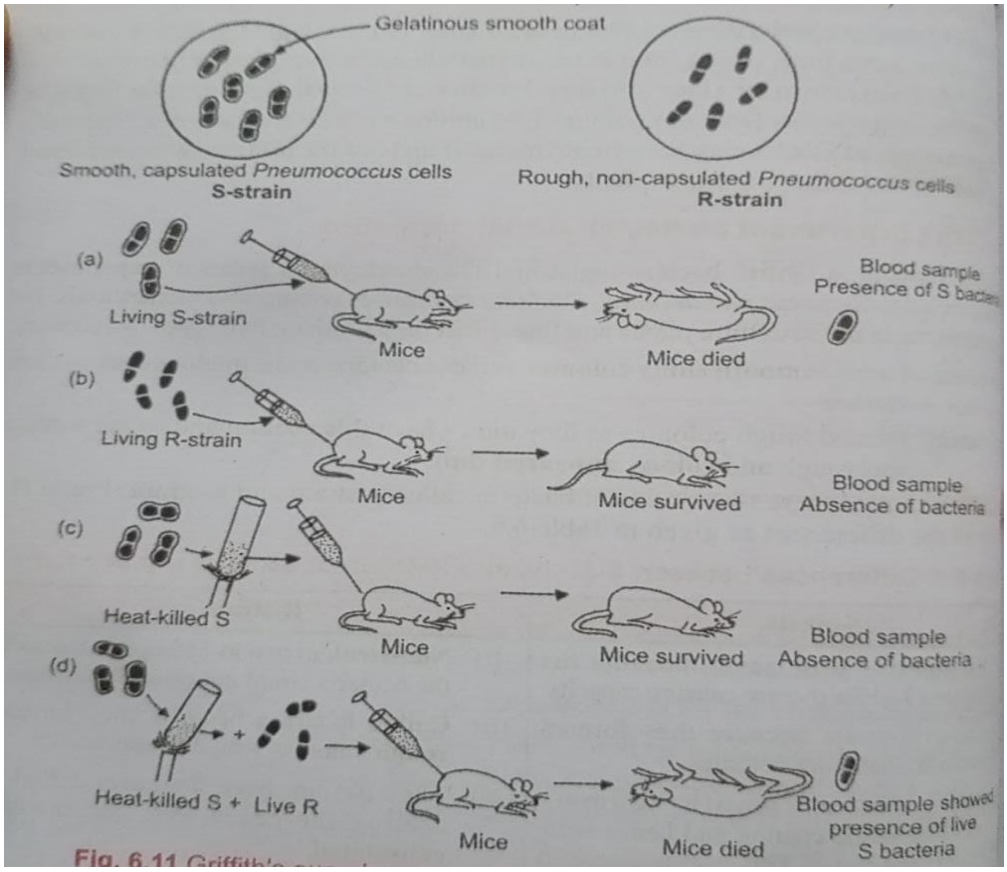


Fig. 6.11 Griffith's

Conclusion: Some

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 the transfer of the genetic material.
Biochemical nature of genetic material was not defined from his experiments.

Biochemical Characterisation of Transforming Principle

Oswald Avery, Colin MacLeod & Maclyn McCarty (1933-44) worked on Griffith's Experiment.
Prior to their work protein was consider as genetic material.
They purified Protein, DNA & RNA from Heat Killed Strain Bacteria and conducted expetiment.

1. Heat Killed S-Strain + Live R Strain + Protease (Protein digesting enzyme) → Mice Died (Transformation Occur)
2. Heat Killed S-Strain + Live R Strain + RNase (Protein digesting enzyme) → Mice Died (Transformation Occur)
3. Heat Killed S-Strain + Live R Strain + DNase (Protein digesting enzyme) → Mice Survived (Transformation Inhibited)

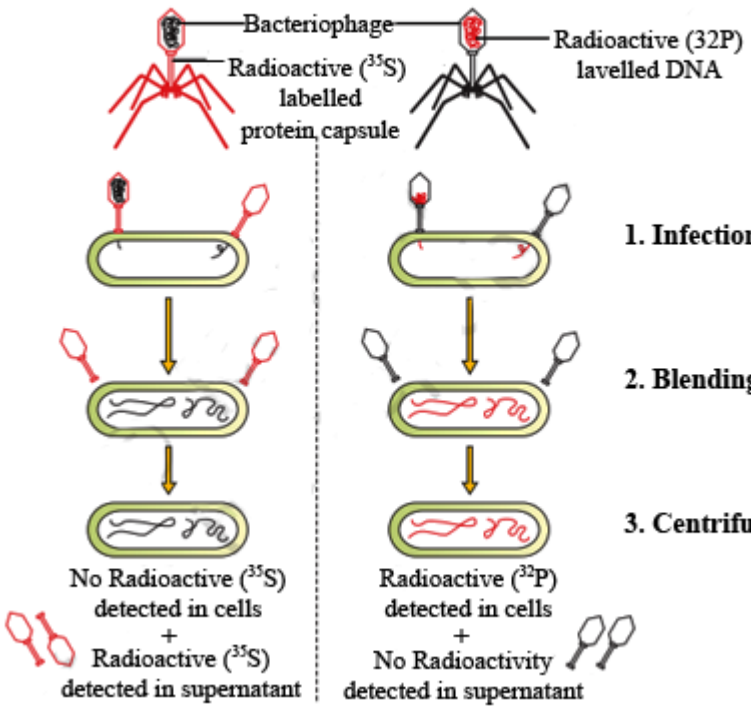
Conclusion: DNA alone from S bacteria causes R bacteria to become transformed.
Hence DNA was genetic material.

But not all scientist were convinced.

The Genetic Material is DNA

- Alfred Hershey & Martha Chase (1952) worked with bacteriophage (bacteria infecting virus).
- Virus transfer its genetic material and infects bacteria
- Bacterial cell treats the viral genetic material as if it was its own manufactures more virus particles.
- Bacteriophage grown on medium containing radioactive Phosphorus contain Radioactive DNA and other grown on medium containing radioactive Sulphur contained radioactive protein.

Hershey and Chase experiment



Radioactive phages were allowed to attach to E. coli bacteria.

Viral coats were removed from the bacteria by agitating them in a blender.

Virus particles were separated from the bacteria by spinning them in a centrifuge.

Conclusion: Bacteria (E-coli) infected with viruses (bacteriophage) with radioactive DNA were radioactive & that infected with radioactive proteins were not radioactive. DNA is therefore the genetic material.

Property of Genetic Material (DNA vs RNA)

- In some viruses like Tobacco Mosaic Virus, QB bacteriophage etc RNA is genetic material but DNA is predominant in most of the organisms.

Features of Good Genetic Material

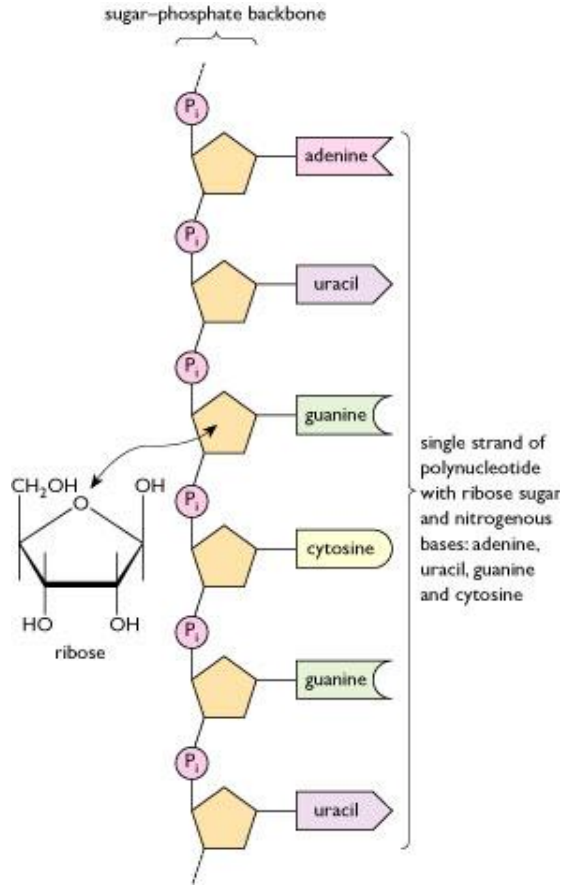
1. It should be Chemically & Structurally **Stable.**
2. Undergo **Slow Mutation** (Fast Changing – Less Stable, Very Slow- No Variations)
3. Undergo **Replication**- Needs to pass in next generation
4. Able to Express itself in form of **Mendelian Characters**.

Since Protein being Unstable (at high Temp & low pH), unable to replicate, or pass on to next generation & was clear from Hershey & Chase Experiment that protein were out of the race of Genetic Material.

	Properties	DNA	RNA	Conclusion
1	Chemical Stability	Thymine (5'methyl Uracil) Stable	Uracil Reactive.	Chemically DNA is More Stable
		2' H makes DNA less reactive	2' OH makes RNA labile & degradable.	
		Never act as Catalyst	RNA act as catalyst is reactive- Ribozyme .	
		Thermally more stable (Proved in Griffith Experiment) If Complementary strand separated by heating than comes together when condition becomes favourable.	Thermally less stable.	
2	Structural Stability	Double Stranded- Complementary strands joined with H-bonds increase stability	Single Stranded	Structurally DNA is More Stable
3	Replication	Can duplicate	Can duplicate	Both can replicate
4	Slow Mutation	Slow Mutation- repair possible due to complementary strand.	Fast Mutation- repair not possible due to single strand. Evolve faster, short life span	DNA Mutates Slowly- better in storing information.
5	Express Mendelian Character	DNA → RNA → Protein → Trait		RNA is better to express
		Express through RNA	Express directly	
Final Conclusion		**Hence DNA is better Genetic Material.**		

RNA World

- RNA was first genetic material.
- Essential life processes (metabolism, translation, splicing etc) evolved around RNA
- RNA being catalyst (Some important biochemical reactions performed by RNA Catalyst-Ribozyme not by protein) was reactive hence unstable.
- Hence DNA evolved (Being more stable- Double Stranded, Complimentary Strand resist changes by evolving repair process) from RNA



Replication

- Watson & Crick -1953 predicted DNA Replication.
- **Semiconservative DNA**- Two Strand Separate & act as Template (Parental) strand for synthesis of new complementary strand. After Replication each DNA have one Template & one New Strand.

Experimental Proof- Semi Conservative DNA

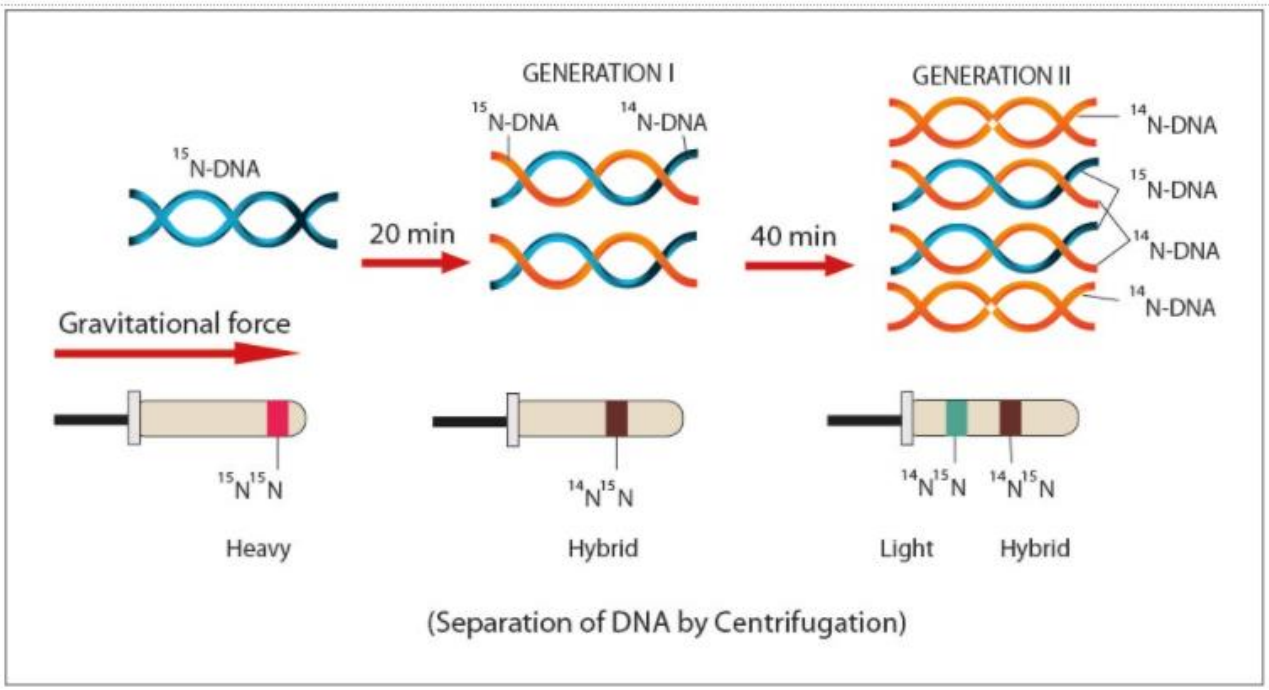
1. Matthew Meselson & Franklin Stahl- on E coli in 1958
2. Taylor& his colleagues – using Radioactive Thymidine on Vicia Faba (Faba Bean) in 1958
3. On Human (Animals)

Meselson & Stahal's Experiment

i. E coli grown on medium containing $^{15}NH_4Cl$ (N^{15}- Heavy Isotope non-radioactive, N^{14}- Light – can be separated based on density). N15 found in DNA.
This heavy isotope could be distinguished by normal by DNA Centrifugation in Cesium Chloride (CsCl) density gradient.

ii. Then Cell transferred into a medium with normal $^{14}NH_4Cl$ & samples were separated independently using CsCl density gradient at different interval of time.

iii. DNA extracted one generation after other (E coli divides in 20 mins) providing Normal ($^{14}NH_4Cl$) Source.

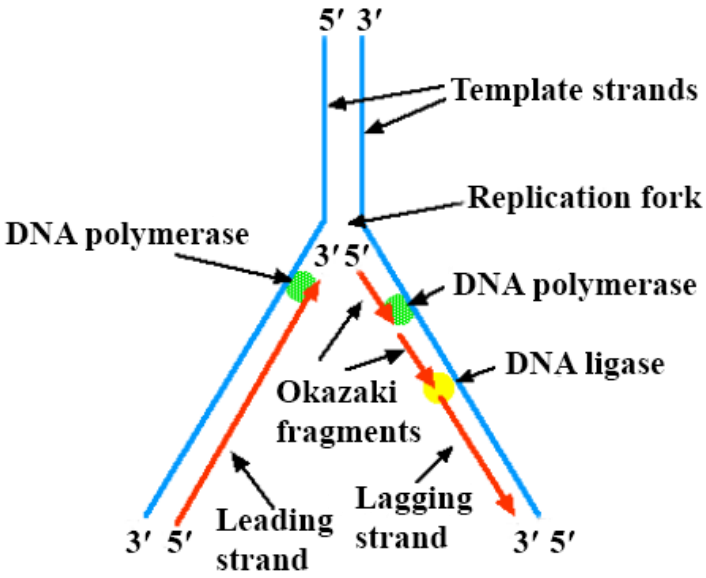


a) **First Generation** (After 20 mins) – Hybrid / Intermediate Density (containing ^{14}N & ^{15}N) – 1 Heavy DNA & 1 Light DNA in each of the two Hybrid strand.
b) **Second Generation** (After 40 mins) - Two Hybrid & Two Normal Strands
c) **Third Generation** (After 60 mins) – Two Hybrid & Six Normal Strands
d) **Fourth Generation** (After 80 mins)- Two Hybrid & Fourteen Normal Strands

The Machinery & the Enzyme

Process Requires set of Catalyst (enzymes:

- DNA Dependent **DNA polymerase**- Main enzyme that uses a DNA template (parental) strand to polymerise deoxynucleotides.
  - **Polymerisation** of nucleotides is **very fast** (2000 BS/sec). Ex: 4.6×10^6 BP of E coli replicates in 18 mins**.**
  - Catalyses reaction with **High degree of accuracy**. (Any mistake results in mutation)
- **Energetically very expensive process** (hence whole length of DNA cannot separate at once)
- **Deoxyribonucleoside triphosphate –** Serve dual purpose-
  a) Act as substrate or building block of DNA
  b) Provide energy for polymerisation- Two terminal Phosphate (highly energised) released energy for polymerisation.
- Replication occur within small opening of helix called **Replication Folk.**
- Catalyses in one direction i.e. 5′ to 3′ (forms continuous or leading strand) and with polarity 3′ to 5′ (forms discontinuous or lagging strand)
- **Origin of Replication-** Definite region where replication starts as replication cannot initiates on its own.
  There is only one origin of replication in prokaryotic circular DNA.
- Replication starts in prokaryotes before fission in cytoplasm.
- In Eukaryotes Replication occurs at S phase in Nucleus.
- Failure of cell division after replication results in polyploidy (chromosomal abnormality).
- **Helicase-** Unwinding enzyme break weak h-Bond and contribute to replication.
- **DNA Ligase-** Enzyme that joins discontinuous DNA fragments (Okazaki fragments).
- **Repair enzyme-** Cut off wrong base pair and replace with correct one.



- **Topoisomerase-** Break & reseal one strand & decrease negative supercoiling.
- **Primase-** Helps in forming primer/ vector-RNA fragment that helps in initiation of DNA replication.