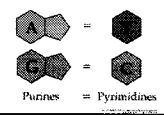


Enzyme: DNA polymerase II

The Molecular Basis of Inheritance

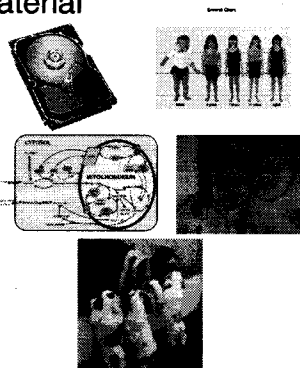


I. Searching for the Genetic Material

A. Genetic material must be able to

1. store information
2. control development of cells.
3. control the metabolic activities of cells.
4. be stable so it can be replicated accurately.
5. undergo mutations that provide the genetic variability required for evolution.

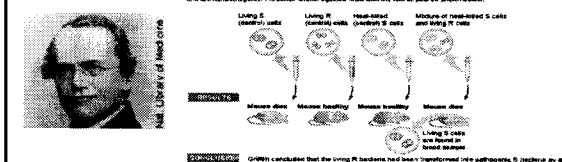
B. In early 1900's debate over hereditary material: Protein or DNA?



I. Searching for the Genetic Material

C. Frederick Griffith (1928)

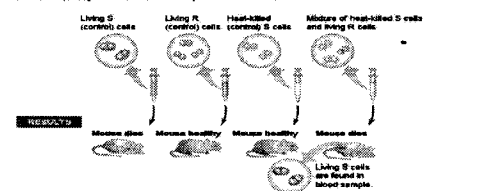
1. DNA Can Transform Bacteria
2. Conducted experiments with *Streptococcus pneumoniae*
 - a) Injected mice with two strains: (S) strain and a (R) strain.
 - b) The S strain is virulent (mice died); it has a mucous capsule



I. Searching for the Genetic Material

- c) The R strain is not virulent (mice lived); it has no capsule.
- d) He injected mice with heat-killed S strain bacteria; the mice lived.

EXPERIMENT Bacteria of the "S" (smooth) strain of *Streptococcus pneumoniae* are pathogenic because they have a capsule that protects them from an animal's defense system. Bacteria of the "R" (rough) strain lack a capsule and are nonpathogenic. Frederick Griffith injected mice with the two strains as shown below.

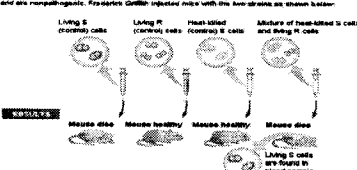


CONCLUSION Griffith concluded that the living R bacteria had been transformed into pathogenic S bacteria by an unknown substance from the dead S cells.

I. Searching for the Genetic Material

- d) He injected mice with a mixture of heat-killed S strain and live R strain bacteria.
- e) The mice died and living S strain pneumococcus were recovered from their bodies.
- f) Griffith concluded, some substance transformed the R strain.

EXPERIMENT Bacteria of the "S" (smooth) strain of *Streptococcus pneumoniae* are pathogenic because they have a capsule that protects them from an animal's defense system. Bacteria of the "R" (rough) strain lack a capsule and are nonpathogenic. Frederick Griffith injected mice with the two strains as shown below.



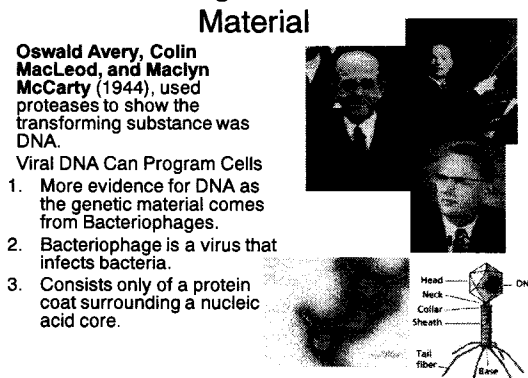
CONCLUSION Griffith concluded that the living R bacteria had been transformed into pathogenic S bacteria by an unknown substance from the dead S cells.

I. Searching for the Genetic Material

D. Oswald Avery, Colin MacLeod, and Maclyn McCarty (1944), used proteases to show the transforming substance was DNA.

E. Viral DNA Can Program Cells

1. More evidence for DNA as the genetic material comes from Bacteriophages.
2. Bacteriophage is a virus that infects bacteria.
3. Consists only of a protein coat surrounding a nucleic acid core.



I. Searching for the Genetic Material

F. Alfred Hershey and Martha Chase (1952)

- Used bacteriophage T2 in their experiments.
- See if protein coat or DNA directed reproduction of virus.
- In two separate experiments, they labeled the protein coat with radioactive ^{35}S and the DNA with radioactive ^{32}P .

The diagram illustrates the experimental setup. In the first experiment, bacteriophages are labeled with ^{35}S in their protein coats. After infecting a bacterial cell and centrifugation, most of the ^{35}S is found in the supernatant, indicating that the protein coats remain outside the cell. In the second experiment, bacteriophages are labeled with ^{32}P in their DNA. After infection and centrifugation, most of the ^{32}P is found in the bacterial pellet, indicating that the DNA enters the cell and directs the production of new phages.

I. Searching for the Genetic Material

- Viral coats are sheared away from bacterial cells and are separated by centrifugation.
- Results: radioactive ^{32}P alone is taken up by bacterial host and incorporated in virus reproduction.
- Their results reinforced the notion that DNA is the genetic material.

The diagram shows the results of the experiment. For the ^{32}P experiment, after centrifugation, there is little ^{32}P in the supernatant, and ^{32}P -labeled DNA is found in the progeny phage. For the ^{35}S experiment, most ^{35}S is in the supernatant, and no ^{35}S -labeled phage coats are found in the progeny phage.

I. Searching for the Genetic Material

G. Additional Evidence for DNA

- Circumstantial Evidence
 - A eukaryotic cell doubles its DNA prior to mitosis.
 - During mitosis, the doubled DNA is equally divided.
 - On organism's diploid cells have twice the DNA as its haploid gametes.

The diagram shows a cell during mitosis with DNA being divided. Below it are micrographs of a diploid cell (Chromosomes 2 + 4) and a haploid cell (Chromosomes 2 + 4).

I. Searching for the Genetic Material

2. Erwin Chargaff (1947)

- Performed detailed analysis of base content of DNA.
- It was known that DNA contained four different types of nucleotides:
 - two with purine bases
 - double-ring structure
 - adenine (A) and guanine (G).
 - two with pyrimidine bases
 - single-ring structure
 - Thymine (T) and cytosine (C)

The diagram shows the chemical structures of the four nucleotides: Cytosine, Thymine, Uracil (in RNA), Adenine, and Guanine.

I. Searching for the Genetic Material

e) Chargaff discovered that within each species, DNA has the constancy required of the genetic material.

f) This constancy is exemplified in Chargaff's Rules:

- The amount of A, T, G, and C in DNA varies from species to species.
- In each species, the amount of A=T and G=C.

The diagram shows Chargaff's Rules: A=T and G=C, and Purines = Pyrimidines.

Organism	Thymine	Adenine	Uracil	Guanine	Cytosine
Sheep sperm	28.1	21.7	24.9	25.2	20.1
Human sperm	28.9	21.6	26.5	18.0	19.9
Human liver	14.1	17.4	24.9	24.4	20.4
Yeast	21.2	20.9	18.7	18.1	17.1

II. Finding the Structure of DNA

A. Rosalind Franklin and Maurice Wilkins


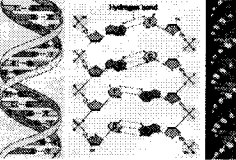
- Produced X-ray diffraction photograph of DNA.
- Franklin's work provided evidence that DNA had the following features:
 - DNA is a helix.
 - One part of the helix is repeated.

The diagram shows photographs of Rosalind Franklin and Maurice Wilkins, and an X-ray diffraction pattern of DNA.

II. Finding the Structure of DNA

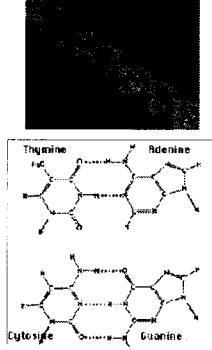
B. James Watson and Francis Crick (1953)

- Used information generated by Franklin
- Built a model of DNA as double helix
- Sugar-phosphate molecules on outside
- Paired bases on inside.
- Sugar-phosphate backbones are antiparallel.

II. Finding the Structure of DNA

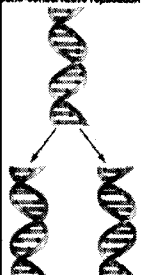
- Using information generated by Chargaff.
 - Width is 2 nm.
 - Width of DNA due to purines paired to pyrimidines.
 - Chargaff's rules are consistent with; A hydrogen-bonded to T; G hydrogen-bonded to C.
- Watson and Crick received the Nobel Prize in 1954 for their model of DNA.



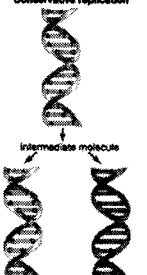
III. How is DNA Replicated

A. Is Replication Conservative, Semi-conservative, or Dispersive?


Hypothesis 1:
Semi-conservative replication



Hypothesis 2:
Conservative replication



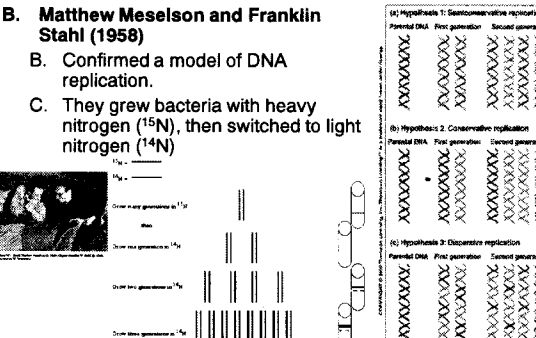
Hypothesis 3:
Dispersive replication



III. How is DNA Replicated

B. Matthew Meselson and Franklin Stahl (1958)

- Confirmed a model of DNA replication.
- They grew bacteria with heavy nitrogen (^{15}N), then switched to light nitrogen (^{14}N)




III. How is DNA Replicated


- After one division, only hybrid DNA molecules were in the cells.
- After two divisions, half the DNA molecules were light and half were hybrid.
- These were exactly the results to be expected if DNA replication is semi-conservative.

DNA extracted and centrifuged to equilibrium in CsCl density gradient.


(a) Heavy DNA (^{15}N)




Original parent molecule




(b) Hybrid DNA ($^{15}\text{N}/^{14}\text{N}$)




First generation daughter molecules



(c) Light DNA (^{14}N)



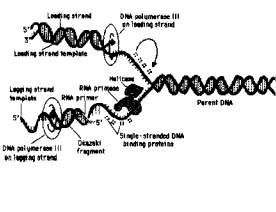
Second-generation daughter molecules



IV. Steps in DNA Replication

- Begins at a specific sequence of nucleotides (origins of replication)
- Unwinding
 - Hydrogen bonds between paired bases are broken.
 - Parent DNA strand is unwound.
 - Both catalyzed by the enzyme **helicase**.
 - Single-strand binding proteins** keep the strands apart.

Collaboration of Proteins at the Replication Fork



IV. Steps in DNA Replication

C. Priming DNA Synthesis

- Short segments of RNA (10 nucleotides long) formed by **primase**.
- RNA primer must be bound to DNA segment to begin replication.

D. Complementary base pairing

- Free nucleotides (Nucleoside triphosphates) bind with complementary bases on unzipped portions of DNA.
- New strands grow in the 5' → 3' direction.

IV. Steps in DNA Replication

- 3' end contains hydroxyl.
- 5' end contains phosphate.
- Process is catalyzed by **DNA polymerase**.
- Polymerase can only elongate at 3' end.
 - Continuous synthesis creates a **leading strand**.
 - Discontinuous synthesis of the complementary strand creates a **lagging strand**.
 - Lagging strand produces **Okazaki fragments**.
 - Fragments are linked by **DNA ligase**.

V. Replication Errors

- Ability to mutate is requirement for genetic material
- Base changes during replication are one way mutations occur.
- A mismatched nucleotide may occur once per 100,000 base pairs.
- Errors minimized because DNA polymerase performs a proofreading function.

V. Replication Errors

- Mismatch repair** corrects mistakes when DNA is synthesized.
- Excision repair** corrects accidental changes that occur in existing DNA.
- Incorrect base pairs that survive the proofreading process contribute to gene mutations

VI. Prokaryotic Versus Eukaryotic Replication

A. DNA Replication in Prokaryotes

- Bacteria have a single loop of DNA that must replicate before the cell divides.
- Replication in prokaryotes may be bidirectional from one point of origin.
- Replication may proceed in one direction only, from 5' to 3'.
- The two single loops separate as the cell enlarges and binary fission occurs.
- Bacterial cells are able to complete DNA replication in about 40 minutes.

VI. Prokaryotic Versus Eukaryotic Replication

B. DNA Replication in Eukaryotes

- Replication in eukaryotes is also bidirectional.
- There are many points of origin (replication bubbles).
- Replication forks are the V-shape ends of the replication bubbles.
- Eukaryotes take hours to complete DNA replication.