Genetic Material: Protein or DNA?

Until the early 1950’s no one knew for sure, but it was generally thought that protein was the genetic material. Why?

- protein is made of 20 different amino acids
- DNA is made of only 4 different nucleotides
- protein could theoretically store more info
  - a “20 letter alphabet” vs a “4 letter alphabet”
- it was assumed that life was so complex, therefore a “bigger alphabet” was necessary to somehow encode it!

Some classic experiments would prove otherwise…
Transformation of Bacteria

**EXPERIMENT**
- Living S cells (control)
- Living R cells (control)
- Heat-killed S cells (control)
- Heat-killed S cells and living R cells

**RESULTS**
- Mouse dies
- Mouse healthy
- Mouse healthy
- Mouse dies

*(Frederick Griffith, 1928)*

Demonstrated the transfer of a genetic trait between different bacteria. The nature of that genetic material was still unknown.

What is the Genetic Material of Bacteriophages?

Bacteriophages are viruses that infect bacteria.
- consist of a protein capsid which contains DNA

What enters the bacterial host cell, viral protein, DNA, or both?
- whatever enters the host cell should be the genetic material

Bacteriophage Genetic Material is DNA

**EXPERIMENT**
- Phage
- Bacterial cell
- Batch 1: Radioactive sulfur (³⁵S)
- Batch 2: Radioactive phosphorus (³²P)

**RESULTS**
- Phage DNA
- Empty protein shell
- Radiolabeled phage (³²P) in liquid
- Radioactivity (³²P) in pellet
- Pellet (bacterial cells and contents)

*(Alfred Hershey & Martha Chase, 1952)*
The Discovery of DNA Structure

Using the technique of x-ray crystallography, Rosalind Franklin, James Watson & Francis Crick figured out the structure of DNA.

• Watson & Crick used the X-ray diffraction data of Rosalind Franklin to deduce the structure of DNA.

DNA: a Polymer of 4 Nucleotides

<table>
<thead>
<tr>
<th>pyrimidines</th>
<th>purines</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
</tr>
</tbody>
</table>

DNA: A single DNA polymer or "strand" consists of a sugar-phosphate backbone with the bases project out.

The ends of a DNA strand are different, with one end having a free 5' phosphate, and the other having a free 3' hydroxyl group.
**Structure of Double-stranded DNA**
- the 2 strands are anti-parallel and interact via base pairs

![Diagram of DNA structure](image)

**DNA “Base-Pairing”**
Base pairs are held together by hydrogen bonds.

Why only A:T and C:G?
- the position of chemical groups involved in H-Bonds
- the size of the bases (purine & pyrimidine)

Sugar
Sugar
Sugar
Sugar

Adenine (A)  Thymine (T)
Guanine (G)  Cytosine (C)

Purine + purine: too wide
Pyrimidine + pyrimidine: too narrow
Purine + pyrimidine: width consistent with X-ray data

**The DNA “Sequence”**
The DNA sequence is the linear order of nucleotides in a DNA strand:
- each DNA strand in the double helix has its own sequence
- the sequences in each strand are considered as complementary to each other
  - they differ, but “fit just right” with each other
  - ea strand will “fit” with only 1 complementary strand

**DNA sequence examples**
- 5’ A–C–A–C–A–C–A–C–A–C–A 3’
2. DNA Replication

Chapter Reading – pp. 318-330

How is DNA Replicated?

Every time a cell reproduces (i.e., divides) it must replicate its chromosomes (DNA) during S phase.

The process of DNA replication was originally proposed to depend on the rules of base pairing:

• A:T & T:A, C:G & G:C
• the sequence of one strand dictates the sequence of the other
• each strand of the double helix could serve as a template to make a complementary strand

Model for DNA Replication

(a) Parent molecule (b) Separation of strands (c) "Daughter" DNA molecules, each consisting of one parental strand and one new strand

The semiconservative model of DNA replication proposed that each original strand serves as a template to produce a new complementary strand.

• note that each original strand ends up in a different molecule
(a) Conservative model

CONSERVATIVE
The original DNA strands stay together

(b) Semiconservative model

SEMICONSERVATIVE
The original DNA strands remain intact in separate molecules

(c) Dispersive model

DISPERSE
The original DNA strands are dispersed among the all daughter strands

Testing the Models

In this experiment, bacteria with DNA containing the “heavy” isotope $^{15}$N were allowed to reproduce in medium containing lighter $^{14}$N.

Density-gradient centrifugation revealed that DNA replication is semiconservative.

Matthew Meselson & Franklin Stahl, 1958

DNA Replication in Bacteria

(a) Origin of replication in an *E. coli* cell

Initiation of DNA replication requires an origin of replication
(b) Origins of replication in a eukaryotic cell

Origin of replication

Double-stranded DNA molecule

Parental (template) strand

Daughter (new) strand

Bubble

Replication fork

Two daughter DNA molecules

Eukaryotic DNA replication requires multiple origins of replication

Overview of DNA Replication

Leading strand

Lagging strand

Origin of replication

Overall directions of replication

Overview of DNA Replication proceeds 5' to 3'

DNA polymerase can add only to the 3' end
Enzymes involved in DNA Replication

**DNA Polymerase** – synthesizes new DNA

**Helicase** – unwinds DNA double helix

**Topoisomerase** – relieves tension due to DNA unwinding

**Primase** – makes short RNA primers

**DNA Ligase** – connects DNA fragments

Leading Strand DNA Synthesis

Proceeds toward unwinding replication fork:

- DNA polymerase can only synthesize DNA in a 5' to 3' direction.
- DNA polymerase requires an RNA primer which it can extend in a continuous manner toward the unwinding replication fork.

Lagging Strand DNA Synthesis

Proceeds away from the unwinding replication fork:

- DNA synthesis is discontinuous on the lagging strand.
- DNA polymerase synthesizes DNA in Okazaki fragments.
- Each fragment requires an RNA primer.
- Another DNA polymerase will replace the RNA with DNA.
- DNA ligase will link the fragments together.

Overall direction of replication: 3' → 5'
Summary of DNA Replication

DNA replication proceeds in this manner in ALL living organisms.

Current Model of DNA Replication

DNA Repair

When DNA is damaged it is essential that the DNA is repaired so it can be replicated and expressed properly.

• special enzymes recognize and remove the damaged portion of DNA
• a DNA polymerase will fill in the gap
• DNA ligase will then connect the newly made DNA to the adjacent strand
The Problem with Telomeres

The ends of linear chromosomes, the telomeres, cannot be completely copied on the lagging strand. This results in progressive shortening of the chromosome every time it is replicated.

Telomerase will solve this problem in certain cell types

...more on Chromatin

Chromatin refers to the complex of DNA and histone proteins in eukaryotic nuclei:

- chromosomal DNA wraps around histone proteins to form structures called nucleosomes that look like “beads on a string”
- different parts of a chromosome can be in various states of “packing”

EUCHROMATIN – loosely packed DNA
HETEROCHROMATIN – tightly packed DNA
**Key Terms for Chapter 16**

- bacterial transformation, bacteriophage
- X-ray crystallography
- pyrimidine, purine, base-pair, complementary
- double helix, anti-parallel, sugar-phosphate backbone
- DNA replication, template
- conservative, semiconservative, dispersive
- DNA polymerase, helicase, topoisomerase, primase, DNA ligase
- leading, lagging strand; continuous, discontinuous
- Okazaki fragment, telomere, telomerase
- chromatin, nucleosome, euchromatin, heterochromatin

---

**Relevant Chapter Questions**

1-7, 9