**DNA = Genetic Material & Mechanism of Replication**

Series of "Classical" Studies in Molecular Biology

- **Avery, MacLeod & McCarty – 1944**
  Griffith's "Transforming Principle" is DNA

- **Hershey & Chase – 1952**
  "Waring Blender" Experiment DNA is Genetic Material

- **Chargaff – 1950**
  "Chargaff's Rule" Total Pyrimidines (C+T) = Total Purines (A+G) in DNA

- **Watson & Crick – 1953**
  Deduce Double Helical Structure of DNA
  from X-ray Crystal Structure Derived by Wilkins & Franklin

- **Meselson & Stahl – 1957**
  DNA Replication is Semi-Conservative

Electron Micrograph of a T2 Bacteriophage Infecting an *E.coli* Cell
Clear zones are plaques (~1 mm dia) where a single phage initially infected a single bacterium. Each plaque contains ~10^5 – 10^6 infectious phage.

If infected cells are grown in the presence of radioactive ^32^P or ^35^S, during infection, T2 DNA or proteins, respectively, will become radioactively labeled.

\[ ^{32}\text{PO}_4 \] 
Incorporated into deoxy-nucleotides
Subsequently incorporated into phage DNA during infection

\[ ^{35}\text{SO}_4 \] 
Incorporated into the amino acids methionine & cysteine
Subsequently incorporated into phage proteins during infection

**EXPERIMENT**

**Question:** Which component of a bacteriophage-DNA or protein is the hereditary material that enters a bacterial cell to direct the assembly of new virus particles?

**Experiment 1**

- ^32^P-containing DNA
- Bacteria
- Hershey-Chase “Waring Blender” Experiment
- Little ^32^P in supernatant
- Pellet

**Experiment 2**

- ^35^S-containing phage coats
- Bacteria
- METHOD
- Most ^35^S in supernatant
- Pellet

**RESULTS**

Conclusion: DNA, not protein, enters bacterial cells and directs the assembly of new virus particles.
Chargaff's Rule \([A+G] = [C+T]\)

\[
\begin{array}{ccc}
A & + & G \\
\hline
T & + & C
\end{array}
\]

Note: Chargaff did not extend his observation to conclude that \([A] = [T] \& [G] = [C]\) or that "A" base pairs with "T" \& "G" base pairs with "C"

Purines = Pyrimidines

### 11.1 Percentages of Bases in the DNA of Some Well-Studied Species

<table>
<thead>
<tr>
<th>DNA Origin</th>
<th>Amount of Base (Percentage of Total DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Human (Homo sapiens)</td>
<td>31.0</td>
</tr>
<tr>
<td>Corn (Zea mays)</td>
<td>25.6</td>
</tr>
<tr>
<td>Fruit fly (Drosophila melanogaster)</td>
<td>27.3</td>
</tr>
<tr>
<td>Bacterium (Escherichia coli)</td>
<td>26.1</td>
</tr>
</tbody>
</table>

Ratio of \([A+T]\) to \([G+C]\) Varies Widely Among Organisms

Main Features of the Double Helical Structure for DNA

- **Antiparallel Strands**

  - 3.4 nm Helical pitch
  - 10 base pairs per helical turn
  - Right-handed helix

- **Main Features**
  - 0.34 nm Bases
  - 2 nm Uniform Diameter
  - 3.4 nm Helical pitch
  - 10 base pairs per helical turn
  - Right-handed helix
  - Minor groove
  - Major groove

Phosphodiester Bonds Comprise Sugar-Phosphate "Backbone"  
Glycosidic Bonds Link Bases to Sugar

2 Hydrogen Bonds form A-T base pair

Complementary base pairing explains Chargaff's Rule
Provides simple copying mechanism. Each strand serves as a template to specify the sequence of its complement.

3 Hydrogen Bonds form G-C base pair

Stability of Structure conferred by: Hydrogen bonds & Hydrophobic "Base-stacking" Interactions between adjacent planar bases

3 Possible Mechanisms for DNA Replication

(a) Semiconservative

Original DNA  After one round of replication

Each product contains 1 old & 1 new strand

(b) Conservative

1 Product contains 2 new strands & 1 Product contains 2 old strands

(c) Dispersive

Both products contain old & new DNA interspersed along each strand
Meselson & Stahl Part I – The Most Elegant Experiment in Molecular Biology

A saturated CsCl solution has a buoyant density = DNA dissolved in ~6M CsCl

After a brief time Cs atoms begin to sediment towards bottom of tube generating a CsCl density gradient

At equilibrium CsCl density gradient is maintained within the tube centrifugation balances diffusion

Can prepare DNA with either "Heavy" or "Light" density by growing cells in either $^{15}$N or $^{14}$N NH$_4$Cl, respectively. N incorporated into purines & pyrimidine bases

Centrifugation "drives" DNA towards bottom of tube. DNA reaches CsCl with same Density & "floats" or "bands" at that position. Centrifugation prevents diffusion to top of tube & "heavier" CsCl prevents DNA from moving to bottom of tube

At equilibrium, the $^{15}$N and $^{14}$N-containing DNAs are separated into 2 distinct fractions based on their differing densities "light" nearer to the top "heavy" nearer to the bottom

Meselson & Stahl Part II – The Most Elegant Experiment in Molecular Biology

**EXPERIMENT**

**Question:** Does DNA replicate semiconservatively, or by some other mechanism?

**METHOD**

Cells grown for many generations in presence of $^{15}$NH$_4$Cl to generate heavy-heavy DNA

DNA extracted at indicated times

Cells transferred to growth media with $^{14}$NH$_4$Cl

**RESULTS**

$^{14}$N/$^{14}$N (light) DNA

$^{15}$N/$^{14}$N (intermediate) DNA

$^{15}$N/$^{15}$N (heavy) DNA

Sample at

Sample after

Sample after

10 minutes

20 minutes

40 minutes

top

bottom

Parental

First generation

Second generation

100% HH

100% HL

50% HH

50% HL

50% LL

**INTERPRETATION**

Note - This means that the 2 parental strands must separate from each other during replication

Contribution: DNA replication is semiconservative.
DNA Synthesis Occurs Only 5’ - 3’ and Requires a Pre-existing Template

Phosphodiester bond formation requires energy provided by hydrolysis of dNTP: dNMP + PP_i and subsequent hydrolysis of PP_i = 2 Pi.

dNTP = dCTP, dATP, dGTP or dTTP

DNA Replication Initiates at Discrete Origins

(a) Circular chromosome

Circular bacterial chromosome has a single Origin Ori
Replication proceeds bidirectionally from the Ori

(b) Linear chromosomes

Linear Chromosomes e.g., human, have multiple origins of DNA Replication
Replication proceeds bidirectionally from these origins
DNA Synthesis Requires an RNA Primer

What displaces this complementary DNA strand so that RNA Primer can be synthesized?