Chapter 13
DNA
The Genetic Material
Replication

Genes are on chromosomes
- T.H. Morgan
  - working with Drosophila (fruit flies)
  - genes are on chromosomes
  - but is it the protein or the DNA of the chromosomes that are the genes?
    - through 1940 proteins were thought to be genetic material... Why?

The “Transforming Factor”
- Frederick Griffith
  - Streptococcus pneumoniae bacteria
    - was working to find cure for pneumonia
  - harmless live bacteria mixed with heat-killed infectious bacteria causes disease in mice
  - substance passed from dead bacteria to live bacteria = “Transforming Factor”

DNA is the “Transforming Factor”
- Avery, McCarty & MacLeod
  - purified DNA, RNA, & proteins from pathogenic Streptococcus pneumoniae bacteria and added them* to a new bacterial culture to see which can transform non-pathogenic bacteria...
  - *digested purified protein
    - bacteria still transformed
  - *digested purified RNA
    - bacteria still transformed
  - *digested purified DNA
    - bacteria DID NOT transform!

The “Transforming Factor”
- 1928

Confirmation of DNA
- 1952 | 1969

Hershey & Chase
- classic “blender” experiment
- worked with bacteriophage
  - viruses that infect bacteria
- grew phage viruses in 2 media, radioactively labeled with either
  - 35S in their proteins
  - 32P in their DNA
- infected bacteria with labeled phages

The "Transforming Factor"
- 1928

Transformation?
something in heat-killed bacteria could still transmit disease-causing properties
**CCHS AP Biology**

**Goldberg**

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**Hershey & Chase**

Protein coat labeled with $^{35}$S  
DNA labeled with $^{32}$P  

T2 bacteriophages are labeled with radioactive isotopes $S$ vs. $P$  

bacteriophages infect bacterial cells  

bacterial cells are agitated to remove viral protein coats and then centrifuged “spun down”  

$^{35}$S radioactivity found in the liquid medium  

$^{32}$P radioactivity found in the bacterial cells  

Hershey & Chase

Martha Chase  
Alfred Hershey

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**“Blender” Experiment**

- Radioactive phage & bacteria in blender  
  - $^{35}$S phage  
    - radioactive proteins stayed in supernatant  
    - therefore protein did NOT enter bacteria  
  - $^{32}$P phage  
    - radioactive DNA stayed in pellet  
    - therefore DNA did enter bacteria  
  - **Confirmed DNA is “transforming factor”**

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**Chargaff**

- DNA composition: “Chargaff’s rules”  
  - varies from species to species  
  - all 4 bases not in equal quantity  
  - bases present in characteristic ratio

- DNA composition table:

<table>
<thead>
<tr>
<th>Organism</th>
<th>A %</th>
<th>T %</th>
<th>G %</th>
<th>C %</th>
<th>A/T</th>
<th>G/C</th>
<th>A+T</th>
<th>G+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>31.3</td>
<td>18.7</td>
<td>31.7</td>
<td>18.3</td>
<td>1.81</td>
<td>1.64</td>
<td>3.45</td>
<td>3.45</td>
</tr>
<tr>
<td>Mouse</td>
<td>26.8</td>
<td>23.3</td>
<td>27.2</td>
<td>13.7</td>
<td>1.13</td>
<td>1.14</td>
<td>2.27</td>
<td>2.27</td>
</tr>
<tr>
<td>Yeast</td>
<td>31.4</td>
<td>27.4</td>
<td>28.4</td>
<td>12.8</td>
<td>1.14</td>
<td>1.16</td>
<td>2.30</td>
<td>2.30</td>
</tr>
<tr>
<td>Chicken</td>
<td>28.0</td>
<td>21.0</td>
<td>28.4</td>
<td>13.6</td>
<td>1.28</td>
<td>1.27</td>
<td>2.55</td>
<td>2.55</td>
</tr>
<tr>
<td>Ostrich</td>
<td>29.3</td>
<td>20.5</td>
<td>20.7</td>
<td>19.1</td>
<td>1.47</td>
<td>1.47</td>
<td>3.06</td>
<td>3.06</td>
</tr>
<tr>
<td>Grasshopper</td>
<td>30.8</td>
<td>17.7</td>
<td>17.3</td>
<td>32.2</td>
<td>1.77</td>
<td>1.77</td>
<td>3.54</td>
<td>3.54</td>
</tr>
<tr>
<td>Sea Urchin</td>
<td>29.8</td>
<td>17.7</td>
<td>17.3</td>
<td>32.2</td>
<td>1.77</td>
<td>1.77</td>
<td>3.54</td>
<td>3.54</td>
</tr>
</tbody>
</table>

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**Structure of DNA**

- Watson & Crick  
  - developed double helix model of DNA  
  - other scientists working on question:  
    - Rosalind Franklin  
    - Maurice Wilkins  
    - Linus Pauling

- 1953 | 1962

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**Watson and Crick**

Watson and Crick

Erwin Chargaff

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Rosalind Franklin (1920-1958)

Double Helix Structure of DNA

- The structure of DNA suggested a mechanism for how DNA is copied by the cell.

Directionality of DNA
- You need to number the carbons!
  - it matters!

\[
\begin{align*}
\text{ribose} & : \text{OH} \quad \text{CH}_2 \quad \text{OH} \\
\text{deoxyribose} & : \text{OH} \quad \text{CH}_2 \quad \text{OH}
\end{align*}
\]

The DNA Backbone
- Putting the DNA backbone together
  - refer to the 3’ and 5’ ends of the DNA
    - the last trailing carbon

Base Pairing in DNA
- Purines
  - adenine (A)
  - guanine (G)
- Pyrimidines
  - thymine (T)
  - cytosine (C)
- Pairing
  - A : T
  - C : G
Anti-parallel Strands
- Phosphate to sugar covalent bond involves carbons in 3’ & 5’ positions
  - DNA molecule has “direction”
  - complementary strand runs in opposite direction

“It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.”
Watson & Crick

Models of DNA Replication
- Alternative models
  - so how is DNA copied?

DNA Replication
- Large team of enzymes coordinates replication

Bonding in DNA
- Hydrogen bonds
- Covalent phosphodiester bonds

Copying DNA
- Replication of DNA
  - base pairing allows each strand to serve as a pattern for a new strand

Replication: 1st step
- Unwind and open up DNA
  - Helicase enzyme
    - unwinds part of DNA helix at ori
    - forms replication forks
    - stabilized by single-stranded binding proteins
**Replication: 1st step**
- Unwind and open up DNA
  - topoisomerase enzyme
    - relieves stress of coiling at front of replication fork due to helicase

**Replication: 2nd step**
- Bring in new nucleotides to match up to template strands

**Energy of Replication**
- Where does the energy for the bonding come from?

**Energy of Replication**
- The nucleotides arrive as nucleosides
  - DNA bases with P–P–P
  - DNA bases arrive with their own energy source for bonding
  - bonded by DNA polymerase III

**Replication**
- Adding bases
  - can only add nucleotides to 3’ end of a growing DNA strand
  - strand grows 5’→3’
  - carried out by DNA polymerase III

**Priming DNA Synthesis**
- DNA polymerase III can only extend an existing DNA molecule
  - cannot start new one
    - cannot place first base
  - short RNA primer is built first by primase
    - starter sequences
  - DNA polymerase III can now add nucleotides to RNA primer
  - Priming DNA Synthesis
    - DNA template
    - DNA polymerase III
    - RNA primer
    - New DNA
Cleaning Up Primers

DNA polymerase I removes sections of RNA primer and replaces with DNA nucleotides

Leading & Lagging Strands

Leading strand - continuous synthesis

Ligase - "spot welder" enzyme

Replication Enzymes

- helicase
- single-stranded binding proteins
- topoisomerase
- DNA polymerase III
- primase
- DNA polymerase I
- ligase

Replication Bubble

Adds 1000 bases/second!

- Which direction does DNA build?
- List the enzymes & their role

DNA Polymerase Review

- DNA polymerase III
  - 1000 bases/second
  - main DNA building enzyme
- DNA polymerase I
  - 20 bases/second
  - editing, repair & primer removal
Editing & Proofreading DNA
- 1000 bases/second = lots of typos!
- DNA polymerase I
  - proofreads & corrects typos
  - repairs mismatched bases
  - excises abnormal bases
  - repairs damage throughout life
  - reduces error rate from ~1 in 10,000 to ~1 in 100 million bases

Fast & Accurate!
- It takes \textit{E. coli} <1 hour to copy 5 million base pairs in its single chromosome
  - divide to form 2 identical daughter cells
- Human cell copies its 6 billion bases & divide into daughter cells in only few hours
  - remarkably accurate
  - only ~1 error per 100 million bases
  - ~30 errors per cell cycle

And in the end...
- Ends of chromosomes are eroded with each replication
  - an issue in aging?
  - ends of chromosomes are protected by telomeres

Telomeres
- Expendable, non-coding sequences at ends of DNA
  - short sequence of bases repeated 1000s times
  - TTAGGG in humans
- Telomerase enzyme in certain cells
  - enzyme extends telomeres
  - prevalent in cancers
  - Why?

Telomeres
- Blackburn, Greider, & Szostak
  - for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase

The “Central Dogma”
- flow of genetic information within a cell

1977 | 2009

![Telomerase enzyme](image)
Polymerase Chain Reaction (PCR)

- What if you want to study a specific segment (gene)?
- What if you have to copy DNA with not a lot to begin with?
  - PCR is a method for making many copies of a specific region of DNA
  - only need 1 molecule of DNA to start

Kary Mullis

- development of PCR technique
  - a copying machine for DNA

PCR Process

- It’s copying DNA in a test tube!
- What do you need?
  - template strand
  - DNA polymerase enzyme
  - nucleotides
  - primer

PCR Primers

- The primers are critical!
  - need to know a bit of sequence to make proper primers
  - primers bracket target sequence
    - start with long piece of DNA & copy a specified shorter segment
    - primers define section of DNA to be ‘cloned’

The Polymerase Problem

- Heat DNA to denature it
  - 90°C destroys DNA polymerase
  - have to add new enzyme every cycle
    - almost impractical!
- Need enzyme that can withstand 90°C...
  - Taq polymerase
    - from hot springs bacteria
      - Thermus aquaticus

PCR Process

- What do you need to do?
  - heat (~90°C) DNA to separate strands (denature)
  - cool (~60°C) to hybridize (anneal)
  - raise temp (~72°C) to build new DNA (extension)