Chapter 9: DNA Structure and Analysis

Honors Genetics
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Fundamental Questions to Answer in this Chapter

• How were we able to determine that DNA, and not some other molecule, serves as the genetic material in bacteria, bacteriophages, and eukaryotes?

• How do we know that the structure of DNA is in the form of a right-handed double helical model?

• How do we know that in DNA, G pairs with C and A pairs with T as complementary strands are formed?

• How do we know that repetitive DNA sequences exist in eukaryotes?
10.1: Four Characteristics of Genetic Material

• Replication
  – Fundamental property of living things
  – Diploid to diploid in somatic cells
  – Diploid to haploid in gametic cells

• Storage of Information
  – Repository of information even if not being used by the cell.

• Expression of Information
  – Central Dogma of Biology

• Variation by mutation
  – Provides the raw material for processes of evolution.
Central Dogma/Information Flow

DNA → Transcription → mRNA, rRNA, tRNA → Ribosome → Translation → Protein
10.2: Observations Favored Protein as the Genetic Material

- Both proteins and nucleic acids were considered likely candidates as the biomolecules of inheritance.
- Proteins were favored from late 1800’s until 1940’s
  - Abundant diversity of proteins
  - More knowledge about protein chemistry
- DNA lacked the chemical diversity believed to be needed to store genetic information.
10.3: Griffith Experiment and Transformation

- DIAGRAMS a. & b.: Griffith used 2 strains (types) of *D. pneumoniae*.
  - Smooth = virulent = dead mouse
  - Rough = avirulent = live mouse

- DIAGRAM c.: **Heat killing** the virulent strain failed to produce disease.

- DIAGRAM d: Mixing heat-killed smooth and living rough DID kill mice.

- Concluded that some “factor” was transferred from dead virulent strain to living avirulent strain and caused disease.
10.3: Avery, McCarty, and MacLeod

**Determining that DNA is the hereditary material**

1. Remove the lipids and carbohydrates from a solution of heat-killed S cells. Proteins, RNA, and DNA remain.

2. Subject the solution to treatments of enzymes to destroy either the proteins, RNA, or DNA.

3. Add a small portion of each sample to a culture containing R cells. Observe whether transformation has occurred by testing for the presence of virulent S cells.

**Concentration:** Transformation cannot occur unless DNA is present. Therefore, DNA must be the hereditary material.
Use of a bacteriophage; a virus that infects a bacteria.

The specific phage infects *E. coli* bacteria.

Phages are labeled with radioactive material.

   - Adhere to the phosphorus of the DNA molecule and the sulfur of the protein coat.

Because the protein coat of the phage remained OUTSIDE of the bacterial cell, the protein was not involved in the production of new phages.
DNA as Hereditary Material

- Griffith, Avery et al, and Hershey-Chase experiments provided convincing evidence that DNA is the molecule responsible for heredity.
10.4: DNA in Eukaryotes

- The results of the transformation experiments provided conclusive evidence that DNA was the biomolecule that transmitted hereditary information in PROKARYOTES.
- Eukaryotic cells could not be experimented on in the same ways.
- Indirect Evidence and Direct Evidence used to prove that DNA was UNIVERSAL in all LIVING THINGS.
INDIRECT EVIDENCE

• DNA is located where genetic functions occur; nucleus, chloroplast, mitochondria.
• DNA content of somatic vs gametes.
• Mutagenesis

DIRECT EVIDENCE

• Recombinant DNA technology has provided conclusive evidence.
  – Splicing DNA from one organism into another and allowing that gene product to be expressed.
10.5: RNA as Genetic Material in Some Viruses

• Directs the production of all components necessary for viral reproduction.
• Retroviruses use RNA as a template for the synthesis of a complementary DNA molecule.
  – HIV is a retrovirus
<table>
<thead>
<tr>
<th></th>
<th>DNA</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
<td>DeoxyriboNucleic Acid</td>
<td>RiboNucleic Acid</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td>In Eukaryotes, restricted to nucleus</td>
<td>Can move from nucleus to cytoplasm</td>
</tr>
<tr>
<td><strong>Sugar</strong></td>
<td>Deoxyribose sugar</td>
<td>Ribose sugar</td>
</tr>
<tr>
<td><strong>Bases</strong></td>
<td>A, T, G, C</td>
<td>A, U, G, C</td>
</tr>
<tr>
<td><strong>Structure</strong></td>
<td>Double helix</td>
<td>Single strand</td>
</tr>
</tbody>
</table>
10.6: Nucleic Acid Chemistry

- Nucleic acids are composed of monomers called **NUCLEOTIDES**
  - 5-carbon sugar (deoxyribose in DNA/ribose in RNA)
  - Phosphate group
  - Nitrogen base
- **DNA nucleotides**
  - Adenine, guanine, cytosine, thymine
- **RNA nucleotides**
  - Adenine, guanine, cytosine, uracil

- [https://www.youtube.com/watch?v=qUaFYzFFbBU](https://www.youtube.com/watch?v=qUaFYzFFbBU)
- [https://www.youtube.com/watch?v=POdWsi7Al](https://www.youtube.com/watch?v=POdWsi7Al)
**PURINES**

- Double ring with 9 members
- Adenine

![Chemical Structure of Adenine]

- Chemical Name: 6-amino purine
- BINDS to SUGAR at #9 Position

![Chemical Structure of Guanine]

- Chemical Name: 2-amino 6-oxy purine
**PYRIMIDINE**

- 6-member ring

- Thymine
- Cytosine
- Uracil

**Chemical Name**

- 2-oxy 4-oxy 5-methyl pyrimidine
- 2-oxy 4-amino pyrimidine
- 2-oxy 4-oxy pyrimidine

**BINDS to SUGAR at #1 Position**
5-Carbon Sugars

Deoxyribose

Ribose
Phosphate Group
DNA Nucleotide

RNA Nucleotide
Polynucleotides

• The creation of long chains of nucleotides to create a strand of DNA or RNA.
• Forms through the creation of phosphodiester bonds between the phosphate group and the 3’ carbon in the 5-carbon sugar ring.
OK, PRACTICE

• In groups of 3
  – 1 member make a PYRIMIDINE
  – 1 member make a DEOXYRIBOSE SUGAR
  – 1 member make a PHOSPHATE

• Join your pieces together to make a NUCLEOTIDE.

• Join your nucleotide to another nucleotide, eventually joining all nucleotides together to create a POLYNUCLEOTIDE CHAIN.

Does the GEOMETRY and CHEMISTRY make sense?
Color Code

• CARBON = Black (4 holes)
• OXYGEN = Red
• NITROGEN = Blue (3 holes)
• HYDROGEN = small White
• PHOSPHORUS = Purple
• Make sure to use double bonds where needed!
10.7: Structure of DNA = Function

- **Chargaff’s Rule**
  - %A = %T and %G = %C
  - %A/G = %C/T or % purine = % pyrimididine
  - The math of it: if %A = 30 then %T = 30
    so G = 20 and C = 20

- **X-Ray Diffraction**
  - Rosalind Franklin created x-ray photograph of geometry showing the structure to be some sort of helix.

- **Watson-Crick Model**
• X-Ray Diffraction

Rosalind Franklin and her X-Ray Diffraction Picture
Watson – Crick Main Features

- Two long polynucleotide chains coiled around a central axis.
- The two chains are ANTIPARALLEL (opposite directions).
- The bases are FLAT structures, stacked .34 nanometers (3.4 Å) apart on INSIDE of the double helix.
- Base pairing of A – T with 2 hydrogen bonds
  Base pairing of G – C with 3 hydrogen bonds
- Each complete turn of the helix is 3.4 nanometers (34 Å).
  or a total of 10 base pairs.
- Alternation of MAJOR and MINOR grooves along the length of the molecule.
- The double helix has a diameter of 2.0 nanometers (20 Å).
The Double Helix
Electrophoresis

- Analysis of nucleic acids
- Separates different-sized fragments of DNA and RNA
- Invaluable molecular genetics technique
- Separates DNA or RNA in a mixture, forcing them to migrate under the influence of an electric current.
- The fragments move through a semi solid porous substance, like gel, to separate into bands.
- These bands have a similar charge-to-mass ratio.
- The bands will settle at different locations along the gel based on their size differences.
By using a MARKER LANE of known fragment lengths, scientists can use gel electrophoresis to compare unknown fragments of DNA based on where they migrate along the length of the gel.
1. DNA fragments

2. Well

3. Cathode (-)
   
4. Molecules move at a rate inversely proportional to their length

5. Anode (+)
   
   Perform autoradiography or incubate with fluorescent dye

   Bands with longer fragments

   Bands with shorter fragments
Analytical Techniques

- Absorption of UV light
- Sedimentation
- Denature/Renature of Nucleic Acids
- Molecular Hybridization
- Electrophoresis
Absorption of UV Light

- Nucleic acids absorb UV light at 254-260 nm
- This is due to the ring formations of purines and pyrimidines
- Critical in isolation following sedimentation.
Sedimentation

- Nucleic acids separate based on size following centrifugation.
- The banding properties are based on concentration gradient and will separate according to size and migrate at different rates.
- Once each sediment is separated out of the tube, UV absorption can then be measured to produce a profile of the sample.
Tubes placed in ultracentrifuge and rotated at high speed; Sample is separated into its two components.
Denature/Renature

- Melting profile will change by increasing the UV absorption causing a hyperchromic shift.
- Heating will breaking the hydrogen bonds but not the covalent bonds that hold the nucleotide together.
- Analysis of the denatured single strand will allow to estimate the base composition of DNA.
- If allowed to cool slowly, the complimentary strands will reassemble into a double-stranded helix.
Molecular Hybridization

• Property of Denaturation/Renaturation is used in this technique.
• Renatured nucleic acid strands do not necessarily come from the same nucleic acid source.
• Double stranded molecular HYBRIDS can be created with complementary strands from two different organisms.
• Cycle of heating and cooling allows these hybrids to be created.
FISH

• Fluorescent In Situ Hybridization
• Uses TARGETS for the hybridization to take place.
• Use of fluorescent probes to monitor the hybridizing process.
• Can identify specific sequences and their locations along the chromosomes, essentially searching for similarities and differences.