Lecture Outline: Structure, Replication, and Mutation of DNA

I. **Introduction to DNA and Heritable Traits**

A. Historical Context

1. Knowledge of heritable traits existed for millennia, but the mechanism was unknown.
2. Scientific demonstration and evidence of a genetic material was needed.

B. Griffith's Transformation Experiment

1. Used non-virulent (R strain) and virulent (S strain) pneumonia-causing bacteria with mice.
2. Experimental results:
   a. Injected living S cells: mice died.
   b. Injected living R cells: mice lived.
   c. Injected heat-killed S cells: mice lived.
   d. Injected heat-killed S cells mixed with living R cells: mice died surprisingly.
3. Conclusion: Something from the dead S strain transformed the harmless R strain into the harmful S type, demonstrating the existence of a **genetic material**.
4. Mechanism: Bacteria can take up "naked DNA" (DNA outside a cell) from the environment and use it to gain new traits (bacterial transformation).

II. **Identifying the Genetic Material**

A. The Question: What is the actual genetic material?

1. Biologists focused on the four macromolecules: lipids, polysaccharides, proteins, and nucleic acids.
2. Lipids and polysaccharides were quickly discounted due to their known

functions and relative lack of complexity.

3. Primary candidates were **DNA** (a nucleic acid) and **proteins**.
4. Most biologists at the time favored proteins due to their immense complexity and diverse functions in the cell, believing such a complex role required a complex molecule.
5. The function of DNA was largely unknown.

B. Hershey-Chase Experiment (using bacteriophages)

1. Rationale: Viruses, specifically bacteriophages (phages), are composed solely of **DNA and protein**, making them ideal for testing which component is the genetic material.
2. Viral operation: A virus attaches to a host cell and injects its nucleic acid, which then directs the host cell to reproduce the virus.
3. The key question was: Which of the two components (DNA or protein) gets injected into the host cell?
4. Experimental Design: **Radiolabeling** was used to track DNA and protein.
    a. One batch of phages was grown in a medium with **radioactive sulfur (^{35}S)**, which labels only **proteins** (sulfur is found in some amino acids like cysteine, but not in DNA).
    b. Another batch was grown in a medium with **radioactive phosphorus (^{32}P)**, which labels only **DNA** (phosphorus is abundant in DNA's phosphate groups, but not significantly in proteins).
5. Procedure:
    a. Labeled phages were allowed to infect bacteria.
    b. A blender was used to dislodge the empty viral shells (capsids) from the surface of the bacterial cells without rupturing the cells.
    c. The mixture was centrifuged to separate the heavier bacterial cells (pellet) from the lighter viral shells (supernatant).
6. Results:
    a. In the batch labeled with radioactive sulfur (protein), radioactivity was found primarily in the supernatant (viral shells), not in the bacterial pellet.

b. In the batch labeled with radioactive phosphorus (DNA), radioactivity was found primarily in the pellet (bacterial cells), not in the supernatant.

7. Conclusion: This provided strong evidence that **DNA, not protein, is the genetic material**.

III. **DNA Structure**

A. Importance of Structure: Understanding how DNA works fundamentally depends on knowing its molecular structure.

B. Nucleotides: The Monomers of DNA

1. A nucleotide consists of three parts:
   a. A pentose sugar (deoxyribose in DNA).
   b. A nitrogenous base.
   c. One or more phosphate groups.
2. The information in DNA is stored in the specific sequence of the four different nitrogenous bases: Adenine (A), Guanine (G), Cytosine (C), and Thymine (T).
3. Nucleic acids are called "nucleic" because they were first found in the nucleus of eukaryotic cells, and "acid" due to their acidic phosphate groups. "Polynucleotide" is a more descriptive term.

C. The Polynucleotide Strand (Sugar-Phosphate Backbone)

1. Nucleotides are linked end-to-end in a specific way: the sugar of one nucleotide is covalently bonded to the phosphate of the next, forming the "sugar-phosphate backbone."
2. This backbone is composed of **strong covalent bonds**, which are crucial for maintaining the integrity and sequence of the DNA molecule.
3. Unlike some polysaccharides, polynucleotides are unbranched polymers, similar to polypeptides.
4. The nitrogenous bases are attached to the sugar-phosphate backbone but do not participate in holding the strand together; they "hang off" the backbone.
5. Each strand has a directionality, with a 5' end and a 3' end, which is critical for DNA synthesis.

D. The Double Helix Model

1. Rosalind Franklin's X-ray crystallography work provided critical data suggesting DNA had a **helical structure**.
2. Watson and Crick used this and other chemical data to propose the famous **double helix model** of DNA.
3. A complete DNA molecule consists of **two separate polynucleotide strands** twisted into a double helix.
4. The structure can be visualized as a ladder:
    a. The two vertical "rails" are the sugar-phosphate backbones.
    b. The horizontal "rungs" are formed by pairs of nitrogenous bases.
5. The two strands are held together by **weak hydrogen bonds** between the paired nitrogenous bases.
    a. These weak bonds are important because they allow the two strands to be "unzipped" for processes like replication and transcription.
    b. Despite being individually weak, millions of these bonds collectively provide overall stability to the DNA molecule.

E. Chargaff's Rules and Complementarity

1. Erwin Chargaff observed that in DNA samples from various organisms, the amount of purines always equaled the amount of pyrimidines.
2. Nitrogenous bases are categorized as:
    a. **Purines** (two-ring structures): Adenine (A) and Guanine (G).
    b. **Pyrimidines** (one-ring structures): Cytosine (C) and Thymine (T).
3. The uniform width of the DNA ladder (double helix) is maintained because each rung consists of one purine and one pyrimidine.
4. This leads to **>strict complementarity**: specific base pairing occurs.
    a. **Adenine (A) always pairs with Thymine (T)**, forming two hydrogen bonds.
    b. **Guanine (G) always pairs with Cytosine (C)**, forming three hydrogen bonds.
5. This strict complementarity is vital because it means the sequence of

one strand dictates the sequence of the other, allowing DNA to serve as a **template** for exact self-replication.

IV. **DNA Replication (Semiconservative Model)**

A. Definition: DNA replication is the process by which DNA makes exact copies of itself.

B. Importance of Complementarity: Because of strict complementarity, each strand of the original DNA molecule can serve as a template for synthesizing a new, complementary strand.

C. Models of Replication (Experimentally Tested)

1. **Conservative Model**: Proposed that the original double helix remains entirely intact, and a completely new double helix is synthesized. This was disproven by experiments.
2. **Semiconservative Model**: Proposed that each new double helix consists of one original (old) strand and one newly synthesized (new) strand. This model was **experimentally confirmed**.
3. Dispersive Model: Proposed that each new double helix contains a mixture of old and new DNA fragments within each strand. This was disproven.
4. The Meselson-Stahl experiment used heavy nitrogen (^{15}N) and light nitrogen (^{14}N) in conjunction with centrifugation to distinguish between DNA densities, conclusively proving the semiconservative model.

D. Replication Process in Prokaryotes vs. Eukaryotes

1. Prokaryotes: Have a single, circular chromosome and typically one **origin of replication** where unwinding begins.
2. Eukaryotes: Have multiple, linear chromosomes and **multiple origins of replication** along each chromosome to speed up the replication of their larger genomes.
3. The basic enzymatic processes are similar in both.

E. Key Steps and Enzymes in DNA Replication (at a replication fork)

1. Unwinding the DNA:
    a. **Helicase**: An enzyme that unwinds and separates the two DNA strands, breaking the hydrogen bonds between bases, forming a

replication bubble with two replication forks.

   b. **Single-strand binding proteins**: Proteins that bind to the separated single DNA strands, preventing them from re-pairing and keeping them stable for replication.

   c. **Topoisomerase**: An enzyme that relieves the tension and overwinding that occurs ahead of the replication fork as DNA unwinds, by cutting and rejoining the DNA strands.

2. Initiating New Strand Synthesis:

   a. **Primase**: An enzyme that synthesizes a short RNA segment called an **RNA primer**.

   b. A primer is necessary because DNA polymerase cannot initiate a new DNA strand from scratch; it can only add new nucleotides to an existing 3' end of a growing strand. RNA nucleotides, however, can be laid down initially.

3. Elongating the New Strand:

   a. **DNA Polymerase III**: The primary enzyme responsible for synthesizing new DNA strands by adding DNA nucleotides (as nucleotide triphosphates) to the 3' end of the RNA primer or existing DNA strand, following the rules of complementarity.

   b. The energy for forming the phosphodiester bond comes from the cleavage of two phosphate groups from the incoming nucleotide triphosphate (an anabolic, endergonic process).

   c. **Leading Strand**: This strand is synthesized continuously in the 5' to 3' direction (towards the replication fork) because DNA polymerase III can continuously add nucleotides as the fork opens. It requires only one initial primer.

   d. **Lagging Strand**: This strand is synthesized discontinuously in short segments called **Okazaki fragments**. It is built in the 5' to 3' direction (away from the replication fork), meaning it must restart synthesis as the fork opens further. Each Okazaki fragment requires a new RNA primer.

4. Replacing RNA Primers and Joining Fragments:

   a. **DNA Polymerase I**: An enzyme that removes the RNA nucleotides

of the primers and replaces them with DNA nucleotides, filling in the gaps.

b. **DNA Ligase**: An enzyme that forms the final covalent phosphodiester bond ("seals the nicks") between the newly synthesized DNA fragments (e.g., between Okazaki fragments or after primer replacement), creating a continuous DNA strand.

F. **Proofreading and Repair Mechanisms**

1. DNA replication is highly accurate, but errors can occur.
2. A system of enzymes, including DNA Polymerase I and II, acts as a proofreading mechanism to recognize and repair mistakes.
3. **Excision Repair**: Involves nucleases that cut out damaged or incorrect segments of DNA (e.g., a mismatched base pair or a **thymine dimer** caused by UV radiation).
4. DNA polymerase then fills the gap using the intact complementary strand as a template.
5. DNA ligase then seals the final phosphodiester bond, restoring the DNA to its correct, functional state.

G. **Telomeres and Telomerase (Eukaryotic Specific Problem)**

1. Problem: Due to the linear nature of eukaryotic chromosomes and the inability of DNA polymerase to add nucleotides without an existing 3' end, the very ends of the lagging strand templates cannot be fully replicated after primer removal.
2. This leads to a progressive shortening of eukaryotic chromosomes with each replication cycle if not addressed.
3. Solution: The enzyme **telomerase**.
4. Telomerase adds repetitive, non-coding DNA sequences called **telomeres** to the ends of chromosomes. This provides a buffer, protecting the essential genes from being lost due to successive rounds of replication.

V. **DNA Organization in the Eukaryotic Nucleus**

A. Compaction: An immense length of DNA (over a meter per human cell) is tightly packed into the microscopic nucleus.

B. **Chromatin**: The complex of **DNA and proteins** found in the eukaryotic nucleus.

C. Histones: The primary proteins involved in DNA packaging; they are typically arranged in groups of eight.

D. Levels of Packing:

1. **Nucleosomes**: The basic unit of chromatin, formed by a segment of DNA wrapped around a core of eight histone proteins. This structure resembles "beads on a string."
2. The nucleosomes then coil further into a thicker chromatin fiber.
3. This fiber forms loops, leading to even higher levels of compaction.
4. **Chromosome**: During cell division (M phase), chromatin becomes highly condensed and visible as distinct chromosomes. Each replicated chromosome consists of two identical sister chromatids joined at the centromere.