ii. Early evidences and Experiments of DNA

ii. Early evidences and Experiments of DNA as the genetic material, Chemistry of Nucleic acids, Nucleotides, Chargaff's rule

Early Evidence and Experiments Demonstrating DNA as the Genetic Material

1. **Historical Context**
   - At the turn of the 20th century, chromosomes were recognized as carriers of hereditary information. These chromosomes contained both proteins and nucleic acids.
   - Proteins were initially favored as genetic molecules because of their diversity and complexity. Nucleic acids—"nuclein," discovered in 1869 by Friedrich Miescher—were generally considered too simple to encode the vast complexity of life.
2. **Griffith's Transformation Experiment (1928)**
   - **Background**: Frederick Griffith studied *Streptococcus pneumoniae*, which exists in two forms: the smooth (S) strain (virulent) and the rough (R) strain (non-virulent).
   - **Key Finding**: When heat-killed S bacteria were mixed with live R bacteria, the R bacteria somehow "transformed" into virulent S bacteria, causing pneumonia in mice.
   - **Conclusion**: Griffith inferred the presence of a "transforming principle," but he did not identify its exact chemical nature.
3. **Avery–MacLeod–McCarty Experiment (1944)**
   - **Goal**: Determine the chemical identity of Griffith's "transforming principle."
   - **Approach**:
     1. Prepared extracts from heat-killed S-type bacteria.
     2. Treated these extracts with enzymes that destroyed proteins (proteases), RNA (RNases), or DNA (DNases).
   - **Key Observation**: Transformation of R cells into S cells only failed to occur when the extracts were treated with DNases, implicating DNA as the transformative agent.
   - **Impact**: This was a groundbreaking demonstration that **DNA is the substance responsible for hereditary transformations** in bacteria.
4. **Hershey–Chase Experiment (1952)**
   - **System**: Bacteriophage T2 (a virus infecting *E. coli*) composed of a protein coat and DNA core.
   - **Method**:
     1. Radioactively labeled phage proteins with 35S^{35}\mathrm{S}35S (sulfur is in proteins, not in DNA).
     2. Radioactively labeled phage DNA with 32P^{32}\mathrm{P}32P (phosphorus is in DNA, less commonly in proteins).
     3. Allowed labeled phages to infect *E. coli*, then separated the phage "ghosts" (coats) from the bacterial cells using a blender.
   - **Result**: 32P^{32}\mathrm{P}32P-labeled DNA was detected inside the bacterial cells, whereas 35S^{35}\mathrm{S}35S-labeled protein remained mostly in the supernatant.
   - **Conclusion**: DNA, not protein, was injected by the phage into the bacteria to direct viral replication, confirming DNA's role as the genetic material.

Collectively, these experiments **shifted the paradigm** from protein to DNA as the primary molecule of inheritance, setting the stage for the discovery of DNA's structure and replication mechanisms.

Chemistry of Nucleic Acids

1. **Definition**
   - Nucleic acids are polymers (polynucleotides) composed of nucleotide monomers.
   - Two main classes: **Deoxyribonucleic Acid (DNA)** and **Ribonucleic Acid (RNA)**.
2. **Basic Composition**

- **Nitrogenous bases**: Purines (adenine AAA, guanine GGG) and pyrimidines (cytosine CCC, thymine TTT in DNA, uracil UUU in RNA).
- **Pentose sugar**: Deoxyribose in DNA (lacking a 2′-OH group) and ribose in RNA (with a 2′-OH group).
- **Phosphate group**: Typically one phosphate per nucleotide in nucleic acids, but nucleotides can exist in mono-, di-, or triphosphate forms when free (e.g., ATP, GTP).

3. **Sugar–Phosphate Backbone**
    - Nucleotides link via **phosphodiester bonds** between the 3′-hydroxyl of one sugar and the 5′-phosphate of the next sugar.
    - This linkage forms a repeating sugar–phosphate backbone with protruding nitrogenous bases.
4. **Key Structural Features**
    - **Polarity/Directionality**: A polynucleotide strand has a 5′ end (with a free phosphate) and a 3′ end (with a free hydroxyl).
    - **DNA Forms**: Under physiological conditions, DNA most commonly adopts the right-handed **B-form** double helix. Alternative forms (A-DNA, Z-DNA) exist under special conditions.
    - **RNA Structures**: Usually single-stranded but can form complex secondary and tertiary folds (hairpins, stem-loops, ribozymes).
5. **Chemical Stability**
    - DNA is more stable than RNA, as the 2′-OH group in RNA can facilitate hydrolysis of the phosphodiester bond.
    - This stability is evolutionarily advantageous for storing genetic information long-term.

Nucleotides: Structure and Functions

1. **Components**
    - **Nitrogenous Base**: A, G, C, T (DNA), or A, G, C, U (RNA).
    - **Pentose Sugar**: Ribose or deoxyribose.
    - **Phosphate Group**: Usually one in nucleic acid polymers, but may be two or three in free nucleotides (e.g., ATP, GTP).
2. **Functions Beyond Genetic Coding**
    - **Energy Currency**: ATP (adenosine triphosphate) is the universal energy currency in cells; GTP also plays roles in protein synthesis and signaling.
    - **Coenzymes**: Nucleotides are part of coenzymes like NAD^+, FAD, and Coenzyme A.
    - **Signal Transduction**: Nucleotides such as cAMP (cyclic AMP) and cGMP (cyclic GMP) act as second messengers in hormone and neurotransmitter pathways.
3. **Polymerization into Nucleic Acids**
    - Nucleotides form polynucleotides (DNA or RNA) through **dehydration (condensation) reactions**, creating phosphodiester linkages.
    - The **sequence** of bases in a strand encodes genetic information, specifying amino acid sequences (via the genetic code) and regulatory signals.

Chargaff's Rule

1. **Erwin Chargaff's Observations (Early 1950s)**
    - By analyzing the base composition of DNA from various species, Chargaff observed two critical patterns:
        1. **[A] ≈ [T] and [G] ≈ [C]** in DNA from any given organism.
        2. Different species exhibit different overall proportions of A+T vs. G+C, indicating **species-specific** base composition.
2. **Implications for DNA Structure**
    - These compositional rules greatly influenced Watson and Crick's **double-helix** model (1953), where A pairs with T via two hydrogen bonds and G pairs with C via three hydrogen bonds.
    - The complementarity implied by Chargaff's ratios explained how DNA could be copied (one strand serving as a template for the other) and how genetic information remains consistent across generations.
3. **Legacy and Further Insights**
    - Chargaff's findings underscored the **informational specificity** of DNA.
    - Variation in G+C content among species helped pave the way for evolutionary and phylogenetic studies at

the molecular level, demonstrating that even subtle changes in DNA composition correlate with lineage divergence.

Concluding Remarks

From **Griffith’s groundbreaking transformation studies** to **Hershey and Chase’s definitive bacteriophage work**, scientists uncovered the central role of DNA in heredity, overturning the once-dominant protein-centric view. The **chemical structure** of DNA (a polymer of nucleotides linked by phosphodiester bonds) and **Chargaff’s rule** provided the final clues that led to the Watson–Crick double helix model—revealing a simple, elegant mechanism for replication and molecular continuity across generations.

Today, these discoveries form the bedrock of modern biology, underpinning everything from **genetics** and **molecular medicine** to **biotechnology** and **genomics**. Understanding the structure and function of nucleic acids continues to shape scientific inquiry, leading to innovations such as **PCR**, **DNA sequencing**, **CRISPR-based gene editing**, and beyond.