Biology

MOLECULAR BASIS OF INHERITANCE

CHAPTER 6

EDUCATION SOURCE

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Class: - 12th

EDUCATION SOURCE

MOLECULAR BASIS OF INHERITANCE
CHAPTER 6th

I. Introduction: -

The Structure and Functions of DNA

1. Introduction

- Inheritance patterns and genetic basis were unclear during Mendel's time.
- Investigative efforts over a century revealed DNA as the primary genetic material for most organisms.
- Nucleic acids, specifically DNA and RNA, are polymers of nucleotides.

2. Types of Nucleic Acids

- DNA serves as the genetic material in the majority of organisms.
- RNA, acting as a genetic material in some viruses, primarily functions as a messenger and has additional roles as an adapter, structural, and sometimes catalytic molecule.

3. Nucleotide Structures and Polymer Formation

- Nucleotides are the monomer units of nucleic acids.
- DNA and RNA structures were covered in Class XI.
- This chapter delves into the detailed structure of DNA and its significance.

4. Structure of DNA

- DNA is a double-stranded helix with complementary base pairing (adenine with thymine, cytosine with guanine).
- The specific arrangement of nucleotides encodes genetic information.

5. DNA Replication

- DNA undergoes replication, ensuring faithful transmission of genetic information during cell division.
- Enzymes and specific processes are involved in the accurate duplication of DNA.

6. Transcription: DNA to RNA

- The process of transcription involves the synthesis of RNA from DNA.
- RNA acts as a messenger, carrying genetic information from the DNA to the cellular machinery.

7. Genetic Code and Protein Synthesis

- The genetic code determines the sequence of amino acids in proteins.
- Translation, the process of protein synthesis, involves decoding the genetic information carried by mRNA into a specific sequence of amino acids.

8. Regulation of Gene Expression

- Elementary basis of gene regulation is discussed.
- Processes that control when and how genes are expressed are crucial for cellular function.

9. Genomics and Human Genome Sequencing

- The complete nucleotide sequence of the human genome was determined in the last decade.
- This achievement ushered in the era of genomics, providing profound insights into human biology.

10. Conclusion

- Understanding the structure and functions of DNA is foundational to comprehending genetic processes.
- Human genome sequencing has opened up new avenues for research and applications in genomics.

These notes serve as an overview of the chapter, covering key topics such as DNA structure, replication, transcription, translation, and the significance of genomics in the modern era.

II. THE DNA

The Structure of DNA: A Detailed Overview

1. **Polymer Composition:**
    - DNA is composed of deoxyribonucleotides, which are monomers made up of a deoxyribose sugar, a phosphate group, and one of four nitrogenous bases: adenine (A), thymine (T), cytosine (C), or guanine (G).
    - The sequence of these bases along the DNA chain carries genetic information.

2. **Length and Measurement:**
    - DNA length is typically measured by the number of nucleotides or base pairs it contains.

- For instance, the bacteriophage ϕ ×174 has 5386 nucleotides, while Bacteriophage lambda has 48502 base pairs. Escherichia coli has a genome of 4.6 × 10^6 base pairs, and the haploid content of the human DNA is 3.3 × 10^9 base pairs.

3. **Characterization of Organisms:**
   - The length of DNA is a defining characteristic of an organism.
   - Variations in DNA length contribute to the diversity observed in different species.
4. **Double-Stranded Helical Structure:**
   - DNA has a double-stranded helical structure, resembling a twisted ladder or a spiral staircase.
   - The two strands run antiparallel to each other, meaning they run in opposite directions.

5. **Complementary Base Pairing:**
   - Adenine (A) pairs with thymine (T) through two hydrogen bonds.
   - Cytosine (C) pairs with guanine (G) through three hydrogen bonds.
   - This complementary base pairing ensures the specificity and stability of the DNA structure.

6. **Major and Minor Grooves:**
   - The helical structure results in major and minor grooves along the DNA molecule.
   - These grooves provide accessibility to enzymes and other molecules involved in various cellular processes.

7. **Functions of DNA:**
   - DNA serves as the repository of genetic information, encoding instructions for the synthesis of proteins and the regulation of cellular activities.
   - The precise sequence of nucleotides determines the genetic code.

8. **Replication:**
   - DNA undergoes replication, ensuring the faithful transmission of genetic information during cell division.
   - Enzymes, such as DNA polymerase, catalyze the synthesis of a new DNA strand complementary to the original template.

9. **Significance in Genetics:**
   - Understanding the structure of DNA is fundamental to comprehending the principles of inheritance and genetic variation.

- Genetic information is passed from one generation to the next through the faithful replication of DNA.

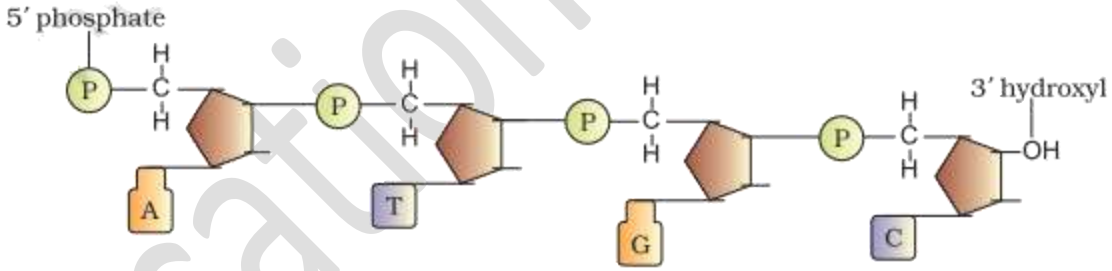
In the subsequent sections of this chapter, we will delve into the intricacies of DNA replication, transcription (the synthesis of RNA from DNA), the genetic code, and the process of protein synthesis. The structure of DNA is the key to unraveling the mysteries of heredity and the fundamental processes that govern life.

(a) Structure of Polynucleotide Chain

Recapitulation of Polynucleotide Chain Structure and DNA Discovery

1. **Nucleotide Composition:**
   - Nucleotides consist of a nitrogenous base (purines: Adenine, Guanine; pyrimidines: Cytosine, Thymine in DNA, Uracil in RNA), a pentose sugar (ribose or deoxyribose), and a phosphate group.

2. **Nucleosides and Nucleotides:**
   - Nucleosides form when a nitrogenous base links to the 1' C of the pentose sugar.
   - Nucleotides are created when a phosphate group attaches to the 5' C of a nucleoside, forming a phosphoester linkage.

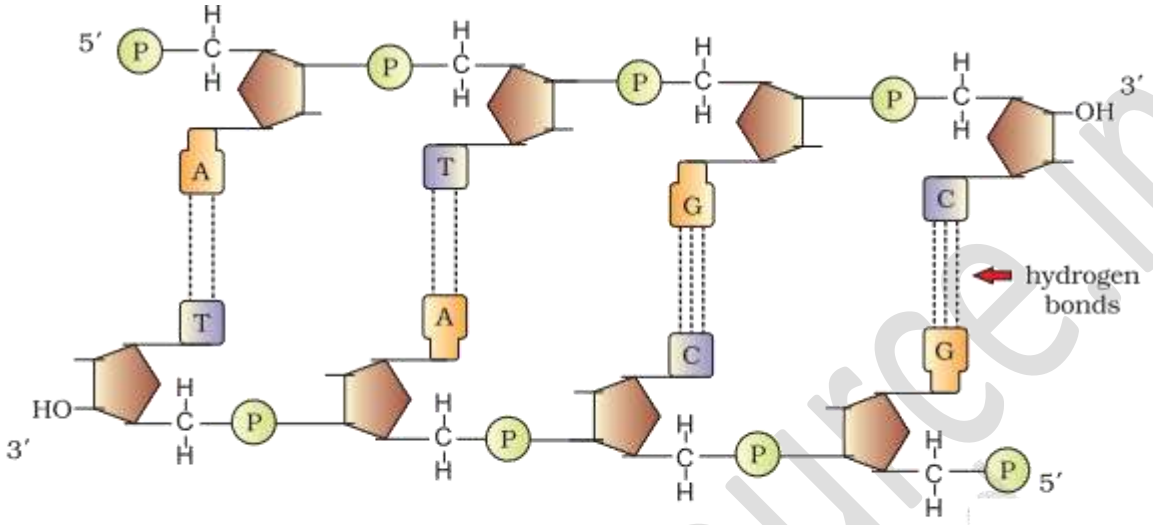


A Polynucleotide chain

3. **Dinucleotides and Polynucleotide Chains:**
   - Two nucleotides connect through 3'-5' phosphodiester linkage to form a dinucleotide.
   - Polynucleotide chains result from the linkage of multiple nucleotides.

4. **Chain Ends and Backbone:**
   - Polynucleotide chains have a free phosphate at the 5'-end and a free OH group at the 3'-end.
   - The backbone consists of alternating sugar and phosphate units.

5. **RNA Distinctions:**
   - RNA has an additional -OH group at the 2'-position of ribose.
   - Uracil replaces thymine in RNA.



Double stranded polynucleotide chain

6. **DNA Discovery and Early Identification:**
   - Friedrich Meischer identified DNA in 1869, naming it 'Nuclein.'
   - Technical limitations hindered intact DNA isolation for a long time.

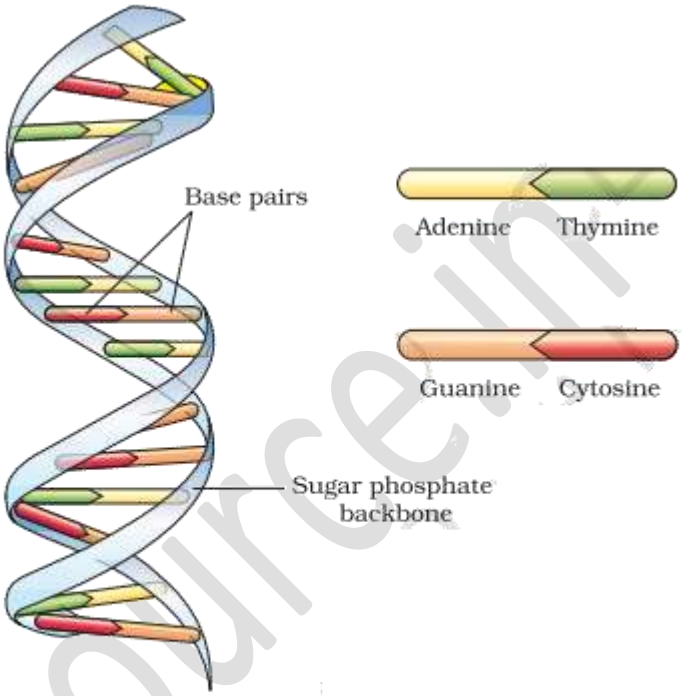
7. **Double Helix Model:**
   - James Watson and Francis Crick proposed the Double Helix model in 1953 based on X-ray diffraction data from Maurice Wilkins and Rosalind Franklin.
   - Base pairing and Chargaff's observations were critical to their proposition.

8. **Key Features of Double-Helix Structure:**
   - Two anti-parallel polynucleotide chains form the backbone.
   - Bases pair through hydrogen bonds: Adenine with Thymine (two H-bonds) and Guanine with Cytosine (three H-bonds).
   - The helix is right-handed, with a pitch of 3.4 nm and approximately 10 base pairs per turn.
   - Stacking of base pairs and hydrogen bonds confer stability.

9. **Genetic Implications:**
   - Complementary base pairing allows the prediction of one strand's sequence if the other is known.
   - DNA replication results in identical daughter DNA molecules.

10.Central Dogma of Molecular Biology:

- Proposed by Francis Crick, the central dogma states that genetic information flows from DNA to RNA to protein.
- Some viruses exhibit reverse information flow (from RNA to DNA).

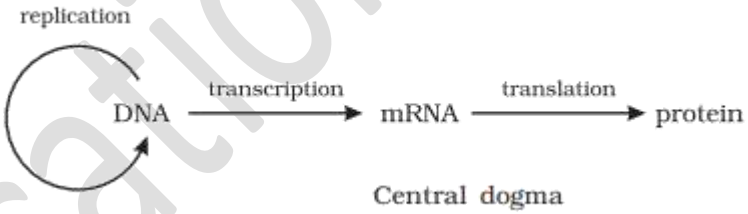


DNA double helix

11.Question for Consideration:

- Propose a simple name for the process when information flows from RNA to DNA in viruses.

Understanding the structure of DNA and its implications laid the foundation for molecular biology, providing insights into genetic information transfer and inheritance mechanisms. The discovery of the double helix structure remains one of the most significant milestones in the history of science.



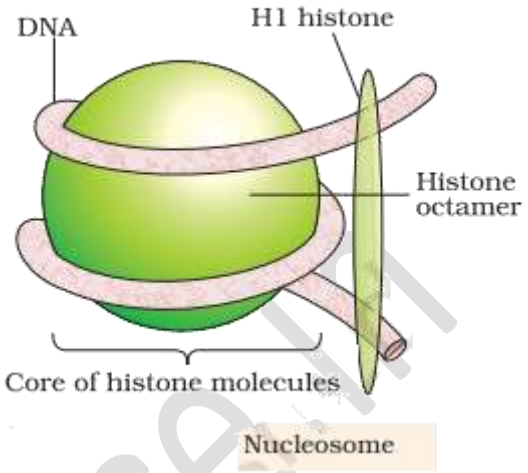
Central dogma

(b) <u>Packaging of DNA Helix</u>

Packaging of DNA in the Cell: From Nucleoid to Chromosomes

1. **DNA Length Calculation:**
   - The length of DNA in a mammalian cell is calculated by multiplying the total number of base pairs (bp) with the distance between two consecutive base pairs (0.34 nm/bp).
   - For example, 6.6 × 10^9 bp × 0.34 × 10^(-9) m/bp results in approximately 2.2 meters, exceeding the dimensions of a typical nucleus.

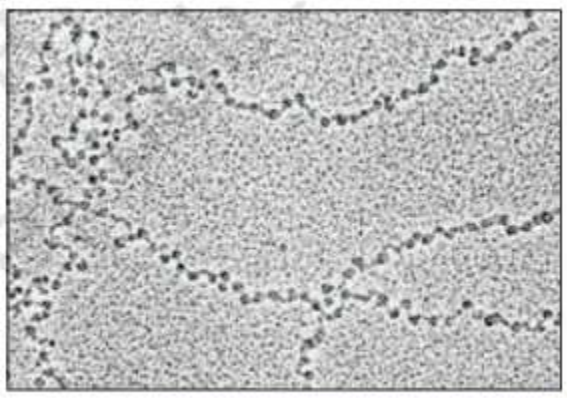
2. **E. coli DNA Length and Base Pairs:**
    - Given the length of E. coli DNA (1.36 mm), you can calculate the number of base pairs by dividing the length by the distance between two consecutive base pairs (0.34 nm/bp).



3. **DNA Organization in Prokaryotes (E. coli):**
    - In prokaryotes like E. coli, DNA is held in a region termed 'nucleoid,' organized into large loops held by proteins.
    - The negative charge of DNA is balanced by positively charged proteins.

4. **Eukaryotic DNA Organization - Nucleosomes:**
    - Eukaryotes use histones, basic proteins rich in lysine and arginine, to organize DNA.
    - DNA wraps around a histone octamer to form a nucleosome, the repeating unit of chromatin.
    - A nucleosome typically contains 200 bp of DNA helix.

5. **Chromatin Structure:**
    - Chromatin, consisting of nucleosomes, appears as a 'beads-on-string' structure under an electron microscope.
    - Theoretical estimation: The number of nucleosomes in a mammalian cell can be imagined by dividing the total length of DNA by the length of a nucleosome.



EM picture - 'Beads-on-String'

6. **Chromatin Packaging into Chromosomes:**
    - Chromatin fibers are further coiled and condensed during the metaphase stage of cell division to form chromosomes.
    - Non-histone chromosomal (NHC) proteins aid in this higher-level packaging.

7. **Euchromatin and Heterochromatin:**
    - Euchromatin is loosely packed and stains lightly, indicating transcriptional activity.
    - Heterochromatin is densely packed and stains dark, suggesting inactivity.

Understanding how DNA is packaged is essential for grasping the dynamic organization of genetic material in cells. The transition from nucleoid or nucleosome structure to the highly condensed chromosomes during cell division reflects the complexity of DNA organization in both prokaryotic and eukaryotic cells.

III. THE SEARCH FOR GENETIC MATERIAL

The journey to unravel the mystery of the genetic material was indeed a long and intricate process. Here's a breakdown of the key events leading up to the confirmation of DNA as the genetic material:

1. **Friedrich Miescher (1869):**
   - Discovered nuclein (DNA) while working with white blood cells.
   - Identified a substance in the cell nucleus that contained both phosphorus and nitrogen.

2. **Gregor Mendel (1865):**
   - Established principles of inheritance through his work with pea plants.
   - The concept of discrete hereditary factors (genes) was introduced.

3. **Walter Sutton and Thomas Hunt Morgan (Early 20th Century):**
   - Proposed the chromosome theory of inheritance.
   - Observed that genes were located on chromosomes during the study of fruit flies.

4. **Chromosome as the Carrier of Genetic Information:**
   - The work of Mendel, Sutton, and Morgan focused attention on chromosomes as potential carriers of genetic information.
   - Chromosomes were identified as entities involved in the transmission of hereditary traits.

5. **Frederick Griffith's Transformation Experiment (1928):**
   - Conducted experiments with Streptococcus pneumoniae, revealing a transforming principle.
   - Heat-killed S strain bacteria could transform live R strain bacteria, suggesting a transfer of genetic material.
   - The nature of the transforming substance remained unknown.

6. **1926 - The Molecular Level Quest:**
    - The scientific community aimed to understand the molecular basis of genetic inheritance.
    - The search was narrowed to the chromosomes, but the specific molecule carrying genetic information remained elusive.

7. **Avery, MacLeod, and McCarty (1933-44):**
    - Conducted groundbreaking experiments to identify the transforming principle in Griffith's work.
    - Determined that DNA alone, not proteins or RNA, was responsible for the transformation.
    - Concluded that DNA is the hereditary material.

8. **Watson and Crick (1953):**
    - Proposed the double helix structure of DNA based on X-ray diffraction data from Rosalind Franklin and Maurice Wilkins.
    - Provided a physical model for the structure of DNA.

In summary, the convergence of research efforts over several decades, from the discoveries of Miescher and Mendel to the experiments of Griffith and the biochemical investigations of Avery, MacLeod, and McCarty, led to the identification of DNA as the genetic material. Watson and Crick's elucidation of the DNA structure in 1953 solidified this understanding and paved the way for the modern era of molecular genetics. The journey showcased the collaborative efforts of scientists across various disciplines in unraveling the complexities of genetic inheritance.

Transforming Principle

Frederick Griffith's transformation experiment in 1928 was a pivotal moment in the search for the genetic material. Here's a summary of the key findings and conclusions from his groundbreaking work:

Experiment Overview:

1. **Bacterial Strains:**
    - Streptococcus pneumoniae (pneumococcus) bacteria were used.
    - Two distinct strains:
        - **S strain (smooth colonies):** Virulent, causing pneumonia in mice.
        - **R strain (rough colonies):** Nonvirulent, harmless to mice.

S strain ⟶ Inject into mice ⟶ Mice die

R strain ⟶ Inject into mice ⟶ Mice live

2. **Polysaccharide Coat:**
   - S strain bacteria had a mucous (polysaccharide) coat that made them virulent.
   - R strain bacteria lacked this coat and were nonvirulent.

S strain (heat-killed) ⟶ Inject into mice ⟶ Mice live

S strain (heat-killed) + R strain (live) ⟶ Inject into mice ⟶ Mice die

3. **Heat-Killing Bacteria:**
   - Griffith observed that heating S strain bacteria killed them while retaining their structural components.
4. **Transformation Observation:**
   - Mice injected with heat-killed S strain bacteria alone survived, as expected.
   - Mice injected with live R strain bacteria alone also survived.
   - Surprisingly, when a mixture of heat-killed S strain and live R strain bacteria was injected, the mice died.
5. **Recovery of Living S Bacteria:**
   - Griffith recovered living S strain bacteria from the deceased mice.
   - This indicated that something from the heat-killed S strain had transformed the live R strain into a virulent form.
6. **Conclusion:**
   - Griffith proposed the existence of a "transforming principle" transferred from the heat-killed S strain to the live R strain.
   - The transforming principle allowed the R strain to synthesize a smooth polysaccharide coat, making it virulent.
   - This implied the transfer of genetic material, but the biochemical nature of this material remained undefined.

Significance and Impact:

- Griffith's experiment laid the foundation for the concept of transformation in bacteria, where external genetic material could alter the phenotype of living bacteria.
- The term "transforming principle" marked the beginning of the search for the actual genetic material responsible for inheritance.
- The biochemical identity of the transforming principle was later determined to be DNA by Avery, MacLeod, and McCarty in the 1940s, marking a crucial milestone in the understanding of genetics.

Biochemical Characterisation of Transforming Principle

Biochemical Characterization of the Transforming Principle: Avery, MacLeod, and McCarty (1933-44)

1. **Objective:**
    - Oswald Avery, Colin MacLeod, and Maclyn McCarty aimed to identify the biochemical nature of the transforming principle in Griffith's experiment.
2. **Experimental Approach:**
    - They purified various biochemicals (proteins, DNA, RNA, etc.) from the heat-killed S cells.
    - Tested each purified component to determine which one could transform live R cells into S cells.
3. **Key Findings:**
    - **DNA Alone Caused Transformation:**
        - When DNA alone from S bacteria was introduced to live R bacteria, it caused transformation into the S phenotype.
        - This suggested that DNA was the active component responsible for the transformation.
    - **Proteases and RNases Did Not Affect Transformation:**
        - Protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNases) did not hinder the transformation.
        - Indicated that the transforming substance was neither a protein nor RNA.
    - **DNase Inhibited Transformation:**
        - Digestion with DNase, an enzyme that degrades DNA, inhibited the transformation.
        - Suggested that the presence of DNA was crucial for the observed changes.

4. **Conclusion:**
    - Avery, MacLeod, and McCarty concluded that DNA is the hereditary material responsible for the transformation observed in Griffith's experiment.
    - This landmark discovery challenged the prevailing view that proteins were the carriers of genetic information.
5. **Skepticism and Acceptance:**
    - Despite the compelling evidence, not all biologists were initially convinced of DNA's role as the genetic material.
    - The shift from proteins to DNA as the primary carrier of genetic information took time to gain widespread acceptance.

Difference Between DNAs and DNase:

- **DNA (Deoxyribonucleic Acid):**
    - DNA is a complex macromolecule that carries genetic information.
    - It consists of a sequence of nucleotides, each containing a phosphate group, a deoxyribose sugar molecule, and one of four nitrogenous bases (adenine, thymine, cytosine, or guanine).
    - DNA is the hereditary material responsible for passing genetic instructions from one generation to the next.
- **DNase (Deoxyribonuclease):**
    - DNase is an enzyme that specifically cleaves phosphodiester bonds in DNA.
    - It functions by breaking down DNA into smaller fragments.
    - DNase digestion can be used in experiments to degrade DNA selectively.

In summary, the distinction between DNA and DNase lies in their roles and structures. DNA is the genetic material, whereas DNase is an enzyme that acts on DNA by cleaving its bonds, ultimately leading to its degradation. The experiments by Avery, MacLeod, and McCarty provided crucial evidence supporting DNA as the hereditary material.

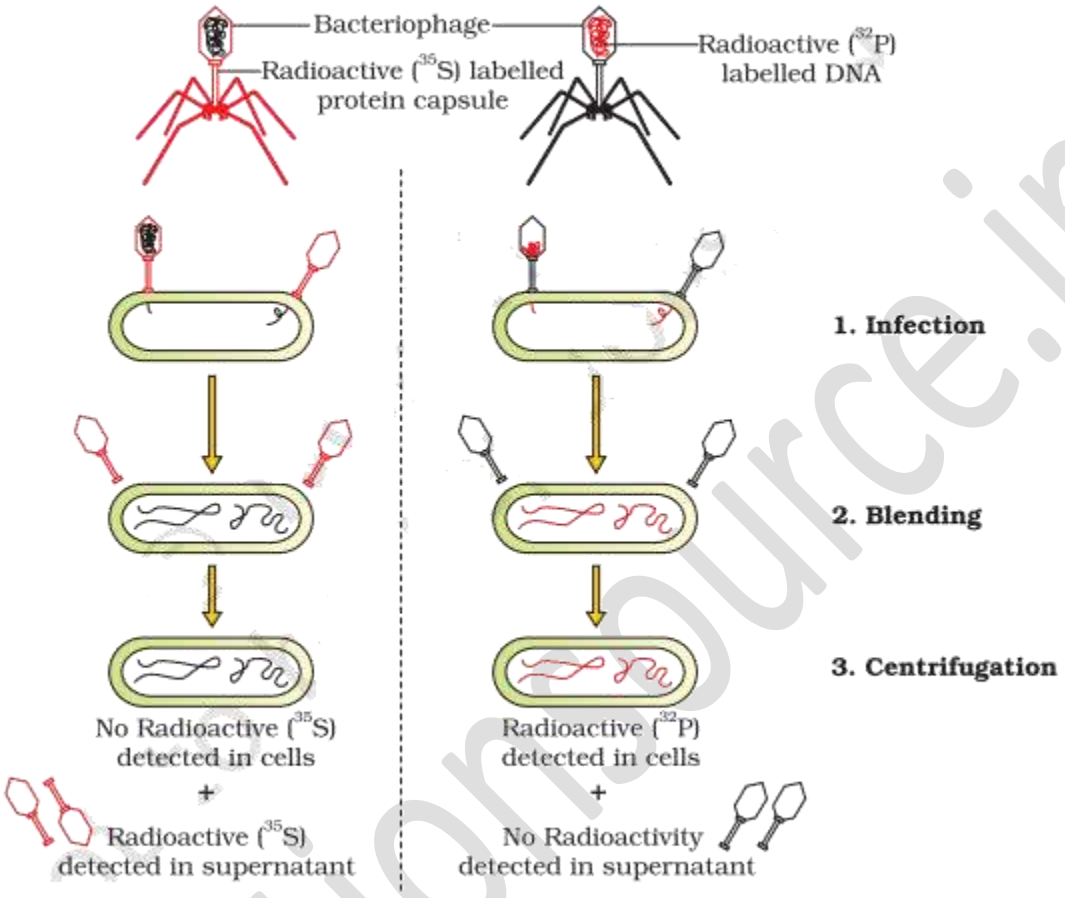
(a) <u>The Genetic Material is DNA</u>

Hershey and Chase's Experiment: Confirming DNA as the Genetic Material (1952)

Alfred Hershey and Martha Chase conducted an elegant experiment using bacteriophages (viruses that infect bacteria) to provide conclusive evidence that DNA, and not protein, is the genetic material. Here's an overview of their groundbreaking experiment:

1. **Background:**
    - Bacteriophages consist of a protein coat and genetic material (either DNA or RNA).
    - The question was whether it was the viral protein or DNA that played a crucial role in the infection process.
2. **Experimental Design:**
    - Grew two batches of bacteriophages: one in a medium containing radioactive phosphorus (32P) and the other in a medium containing radioactive sulfur (35S).
    - Phosphorus is present in DNA but not in proteins, while sulfur is present in proteins but not in DNA.
3. **Virus Attachment and Infection:**
    - Allowed the radioactive phages to infect Escherichia coli (E. coli) bacteria.
    - The radioactive phosphorus-labeled phages had radioactive DNA, and the sulfur-labeled phages had radioactive protein.
4. **Blending and Centrifugation:**
    - Used a blender to separate the viral protein coats from the bacteria after infection.
    - Spun the mixture in a centrifuge to separate heavier bacterial cells from lighter viral protein coats.
5. **Results:**
    - Bacteria infected with the phosphorus-labeled phages (containing radioactive DNA) were found to be radioactive.
    - Bacteria infected with the sulfur-labeled phages (containing radioactive protein) were not radioactive.
6. **Conclusion:**
    - The radioactive DNA from the viral phages was the genetic material that entered the bacterial cells.
    - The absence of radioactivity in the bacterial cells infected with protein-labeled phages ruled out proteins as the genetic material.
7. **Significance:**
    - The experiment provided definitive evidence that DNA, not protein, carries genetic information.
    - This confirmed and extended earlier work, establishing DNA as the universal genetic material.

Alfred Hershey and Martha Chase's experiment played a crucial role in solidifying the understanding that DNA is the hereditary material responsible for transmitting genetic information. This pivotal experiment laid the groundwork for the later discoveries in molecular biology, including the structure of DNA and the mechanisms of DNA replication and protein synthesis.



The Hershey-Chase experiment

(b) Properties of Genetic Material (DNA versus RNA)

Chemical Differences Between DNA and RNA:

1. **Sugar Type:**
   - **DNA:** Contains deoxyribose sugar.
   - **RNA:** Contains ribose sugar.
2. **Nitrogenous Bases:**
   - **DNA:** Adenine (A), Thymine (T), Cytosine (C), Guanine (G).
   - **RNA:** Adenine (A), Uracil (U), Cytosine (C), Guanine (G).

Properties of Genetic Material:

1. **Replication:**
   - ***DNA:*** Can replicate itself through the complementary base pairing mechanism.
   - ***RNA:*** Can also replicate, but the process is more complex and less accurate compared to DNA replication.
2. **Chemical and Structural Stability:**
   - ***DNA:*** More stable chemically and structurally. The presence of thymine instead of uracil contributes to stability.
   - ***RNA:*** Less stable due to the presence of the reactive 2'-OH group and susceptibility to degradation.
3. **Mutation and Evolution:**
   - ***DNA:*** Mutations can occur, but the slower mutation rate contributes to greater stability.
   - ***RNA:*** Prone to mutations at a faster rate, contributing to the evolution of viruses with RNA genomes.
4. **Expression of Mendelian Characters:**
   - ***DNA:*** Dependent on RNA for protein synthesis. DNA indirectly expresses genetic information.
   - ***RNA:*** Can directly code for protein synthesis, allowing for the direct expression of genetic traits.

Conclusion:

- DNA and RNA can both function as genetic material.
- DNA is favored for storage due to its greater stability, less reactivity, and slower mutation rate.
- RNA is more dynamic, suitable for rapid mutations and direct protein synthesis.
- DNA's stability and ability to replicate accurately make it well-suited for long-term storage and transmission of genetic information.

In summary, the chemical differences between DNA and RNA contribute to their distinct roles in the cell. DNA's stability makes it suitable for long-term storage, while RNA's dynamic nature allows for rapid changes and immediate protein synthesis. The interplay between DNA and RNA ensures the efficient functioning of genetic processes in living organisms.

IV. RNA WORLD

RNA World Hypothesis: The Early Genetic Material

The RNA World hypothesis proposes that RNA (ribonucleic acid) was the first genetic material and played a central role in the early stages of life on Earth. Here are key points related to the RNA World hypothesis:

1. **RNA as the First Genetic Material:**
    - The RNA World hypothesis suggests that in the early stages of life, RNA served as both the genetic material and a functional molecule capable of catalyzing biochemical reactions.
    - RNA possesses both information-carrying capabilities (like DNA) and catalytic properties (like proteins).

2. **Essential Life Processes Involving RNA:**
    - Metabolism, translation (protein synthesis), splicing, and other crucial life processes are believed to have evolved around RNA molecules.
    - RNA acted as a versatile molecule, carrying genetic information and catalyzing biochemical reactions necessary for cellular functions.

3. **RNA Catalysts (Ribozymes):**
    - Some biochemical reactions in living systems are catalyzed by RNA molecules known as ribozymes, rather than by protein enzymes.
    - Ribozymes demonstrate the catalytic capabilities of RNA, supporting the idea that RNA could have played a dual role in the early stages of life.

4. **RNA's Reactivity and Instability:**
    - RNA, while versatile, is more reactive and less stable than DNA.
    - The presence of a 2'-OH group in RNA makes it more prone to chemical reactions and degradation.

5. **Evolution to DNA:**
    - DNA is thought to have evolved from RNA through chemical modifications that enhanced stability.
    - DNA is double-stranded, providing a backup copy of genetic information, and its complementary strands allow for error correction through repair mechanisms.

6. **DNA's Stability and Repair Mechanisms:**
    - DNA's double-stranded structure and the presence of complementary strands make it more stable than RNA.
    - The evolution of repair mechanisms in DNA contributes to maintaining genetic information over generations.

Conclusion:

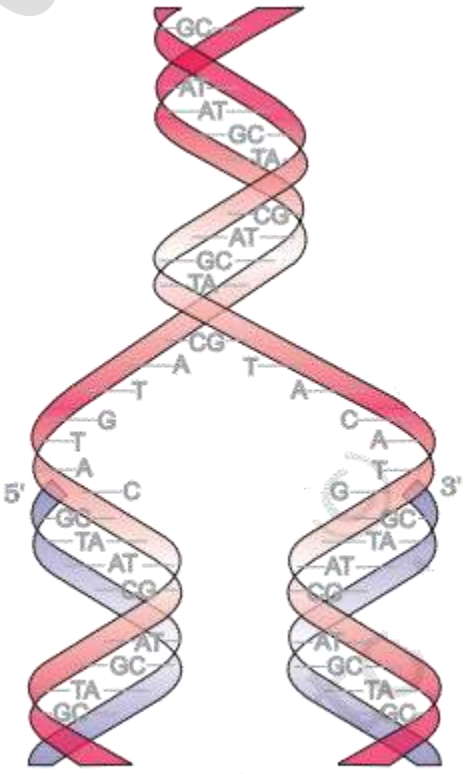
- The RNA World hypothesis provides a framework for understanding the transition from non-living to living entities, suggesting that RNA played a crucial role as the first genetic material.
- Over time, DNA evolved as a more stable repository of genetic information, allowing for the complexity and diversity observed in contemporary life.

The RNA World hypothesis offers insights into the early stages of life's evolution, emphasizing the central role of RNA in the emergence of cellular processes and genetic information storage. This theory aligns with the dynamic nature of RNA and its ability to serve dual roles in genetic and catalytic functions.

V. REPLICATION

Semiconservative DNA Replication: The Watson-Crick Proposal

Watson and Crick's Original Proposal (1953): Watson and Crick, in their seminal paper proposing the double helical structure of DNA, also put forth a model for the replication of DNA. The key idea was that the specific base pairing observed in the double helix immediately suggested a mechanism for copying genetic material.



Watson-Crick model for semiconservative DNA replication

Semiconservative DNA Replication:

1. **Separation of Strands:**
   - The two strands of the DNA double helix would separate or "unzip," exposing the nitrogenous bases.

2. **Template for Synthesis:**
   - Each separated strand would then act as a template for the synthesis of a new complementary strand.
   - The specificity of base pairing (A with T, G with C) would guide the addition of appropriate nucleotides to form a new strand.

3. **Synthesis of Complementary Strands:**
    - Enzymes, specifically DNA polymerases, would catalyze the addition of nucleotides to the exposed templates, creating two new complementary strands.
    - Adenine (A) on one strand pairs with thymine (T) on the other, and guanine (G) on one pair with cytosine (C) on the other.

4. **Formation of Replicated DNA Molecules:**
    - After replication, each resulting DNA molecule would consist of one parental (original) strand and one newly synthesized strand.
    - This process ensures that the genetic information is preserved in the newly synthesized DNA.

Significance of Semiconservative Replication:

- The term "semiconservative" indicates that each replicated DNA molecule contains one strand from the original DNA molecule and one newly synthesized strand.
- This model provides a mechanism for the accurate transmission of genetic information from one generation of cells to the next.

Experimental Confirmation:

- The semiconservative model was experimentally confirmed by Matthew Meselson and Franklin Stahl in 1958 using isotopic labeling and density gradient centrifugation.
- Their experiments provided strong support for the semiconservative nature of DNA replication.

Conclusion:

- Watson and Crick's proposal of semiconservative DNA replication laid the foundation for our understanding of how genetic information is faithfully passed on during cell division.
- The semiconservative model has been a fundamental concept in molecular biology and is a key process in the maintenance of genetic continuity.

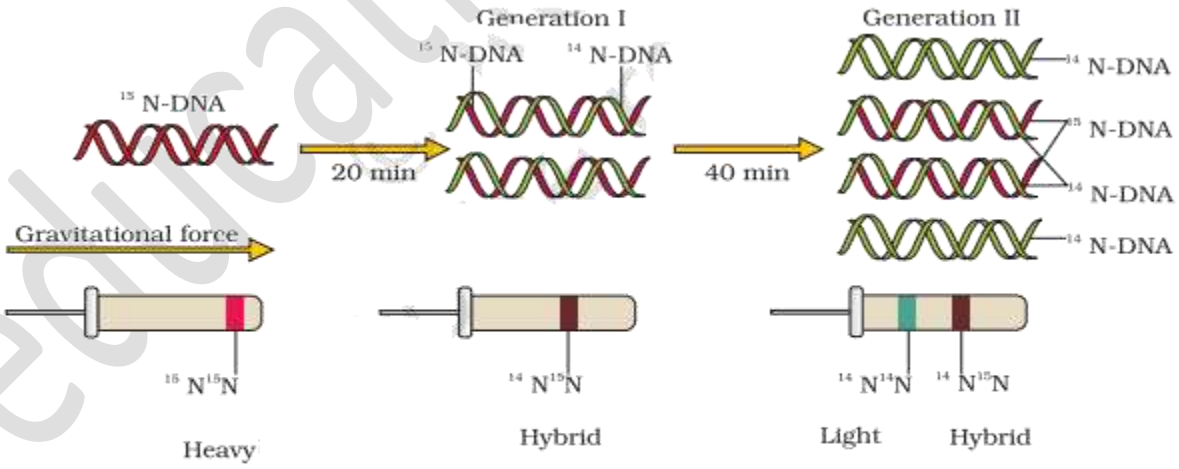
(a) <u>The Experimental Proof</u>

Experimental Proof of Semiconservative DNA Replication: Meselson and Stahl Experiment

Objective of the Experiment: Matthew Meselson and Franklin Stahl designed an experiment in 1958 to investigate the mode of DNA replication—whether it was conservative, semiconservative, or dispersive.

Experimental Design:

1. **Isotopic Labeling:**
   - E. coli bacteria were grown in a medium containing heavy nitrogen isotope, 15N (15NH4Cl), as the sole nitrogen source.
   - This resulted in the incorporation of 15N into the newly synthesized DNA, making it distinguishable from the normal DNA containing the lighter isotope, 14N.
2. **Transfer to 14N Medium:**
   - The bacteria were then transferred to a medium containing normal nitrogen isotope, 14N (14NH4Cl), and allowed to multiply.
   - Samples were taken at various time intervals as the cells replicated.
3. **Density Gradient Centrifugation:**
   - The extracted DNA samples were subjected to density gradient centrifugation in cesium chloride (CsCl).
   - Centrifugation separates molecules based on their density. Heavier molecules sediment faster under centrifugal force.
4. **Interpretation of Results:**
   - After one generation (20 minutes), the DNA extracted had an intermediate density, reflecting the incorporation of both 15N and 14N.
   - After the second generation (40 minutes), the DNA had a hybrid density, consisting of equal amounts of the original 15N-labeled DNA and newly synthesized 14N-containing DNA.
   - Subsequent generations showed a progressive increase in the proportion of 14N-containing DNA.



(Separation of DNA by Centrifugation)

Meselson and Stahl's Experiment

Interpretation of Results:

- The experiment provided clear evidence for semiconservative DNA replication. After one generation, a hybrid DNA was observed, consistent with the semiconservative model proposed by Watson and Crick.
- The presence of equal amounts of 15N and 14N in the second generation supported the idea that each strand of the original DNA served as a template for the synthesis of a new complementary strand.

Centrifugal Force and Sedimentation:

- Centrifugal force involves spinning samples at high speeds.
- Molecules with higher mass or density sediment faster and move farther down the gradient during centrifugation.

Conclusion:

- The Meselson and Stahl experiment provided conclusive evidence for semiconservative DNA replication in E. coli.
- The results supported Watson and Crick's model, and the experiment is considered a classic in molecular biology.
- Similar experiments in other organisms, such as Vicia faba, confirmed the semiconservative nature of DNA replication in eukaryotes as well.

(b) <u>The Machinery and the Enzymes</u>

DNA Replication: Machinery and Enzymes

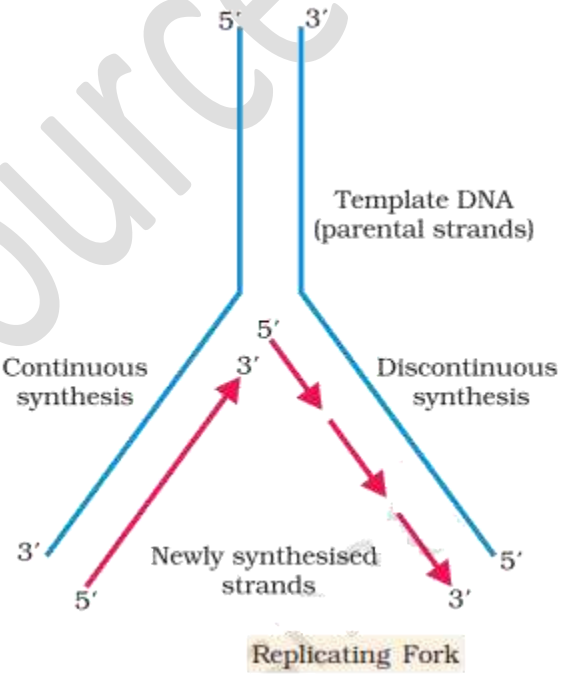
1. **DNA Polymerase:**
    - **Function:** DNA replication is catalyzed by enzymes called DNA polymerases.
    - **Specificity:** DNA-dependent DNA polymerase uses a DNA template to synthesize a new strand of DNA by catalyzing the polymerization of deoxynucleotides.
    - **Efficiency:** These enzymes are highly efficient to cope with the requirement of synthesizing a large number of nucleotides in a short time. E. coli, with 4.6 × 10^6 base pairs, completes replication within 18 minutes.

2. **Polymerization Rate:**
    - **Rate:** The average rate of polymerization during E. coli replication is approximately 2000 base pairs per second.
    - **Accuracy:** DNA polymerases must ensure a high degree of accuracy to prevent mutations.
3. **Energy Source:**
    - **Deoxyribonucleoside Triphosphates (dNTPs):** Serve as substrates for DNA polymerases and provide energy for the polymerization reaction. The two terminal phosphates in dNTPs are high-energy phosphates.

4. **Replication Fork:**
    - **Definition:** The replication fork is the region where the DNA double helix is unwound, and new strands are synthesized.
    - **Directionality:** DNA polymerases catalyze polymerization only in the 5' to 3' direction.

    - **Continuous and Discontinuous Synthesis:** On the template strand with polarity 3' to 5', replication is continuous. On the template strand with polarity 5' to 3', replication is discontinuous, leading to the formation of Okazaki fragments.
    - **Fragment Joining:** Discontinuous fragments are joined by DNA ligase.

5. **Initiation of Replication:**
    - **Origin of Replication:** Replication does not initiate randomly; there is a specific region in DNA termed the origin of replication.
    - **Vectors in Recombinant DNA:** Origin of replication is essential for DNA propagation. Vectors used in recombinant DNA procedures provide the necessary origin of replication.



6. **Coordination with Cell Cycle:**
    - **Eukaryotic Replication:** In eukaryotes, DNA replication occurs during the S-phase of the cell cycle.
    - **Coordination:** Replication and the cell division cycle are highly coordinated to avoid anomalies like polyploidy, which can result from a failure in cell division after DNA replication.

Conclusion:

- DNA replication involves a complex interplay of enzymes and molecular machinery.
- The process is highly regulated, efficient, and accurate to ensure faithful transmission of genetic information during cell division.
- Coordination with the cell cycle is crucial for maintaining genomic stability.

VI. TRANSCRIPTION

Transcription: Copying Genetic Information

Overview:

- **Definition:** Transcription is the process of copying genetic information from one strand of DNA into RNA.
- **Principle of Complementarity:** Similar to replication, transcription follows the principle of complementarity. Adenosine in DNA pairs with uracil in RNA instead of thymine.

Key Points:

1. **Complementarity in Transcription:**
    - Adenosine in DNA pairs with uracil in RNA during transcription.
    - The process is governed by the principle of complementarity, ensuring accurate copying of genetic information.
2. **Selective Transcription:**
    - Only a segment of DNA and one of the strands are copied during transcription.
    - Demarcating Boundaries: Specific regions and strands of DNA are defined for transcription.
    - Why Only One Strand:
        - Avoids coding for RNA molecules with different sequences from both strands.
        - Prevents the simultaneous production of complementary RNA molecules, which would form double-stranded RNA, hindering protein translation.
3. **Rationale for Single-Strand Transcription:**
    - **Avoiding Sequence Ambiguity:** Transcription from both strands would lead to RNA molecules with different sequences.
    - **Preventing Double-Stranded RNA Formation:** Simultaneous transcription from both strands would produce complementary RNA molecules, forming double-stranded RNA and hindering translation.
4. **Genetic Information Complexity:**
    - Single-strand transcription simplifies the genetic information transfer machinery.
    - Ensures that one DNA segment codes for a specific protein without ambiguity.

Conclusion: Transcription is a precise process that selectively copies genetic information from one strand of DNA into RNA. The decision to transcribe only one strand avoids complications in genetic information transfer and maintains the integrity of the protein-coding process.

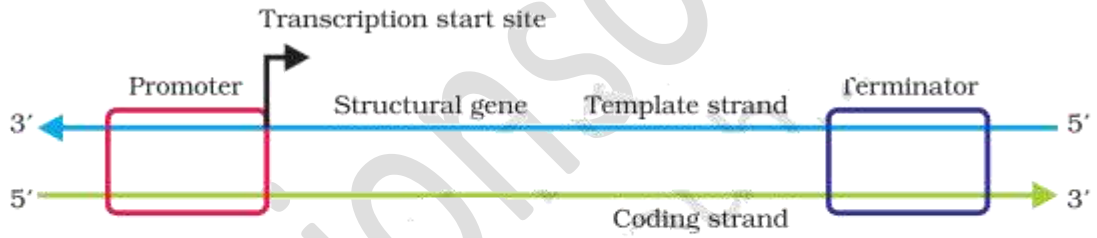
(a) Transcription Unit

Transcription Unit: Definition and Components
Definition: A transcription unit in DNA is defined by three key regions:

1. **Promoter**
2. **Structural Gene**
3. **Terminator**

Key Points:

1. **Template Strand and Coding Strand:**
   - **Template Strand:** The strand with the polarity 3'→5', which acts as a template during transcription.
   - **Coding Strand:** The strand with the polarity 5'→3', having the same sequence as RNA (except thymine replaced by uracil).
   - The DNA-dependent RNA polymerase catalyzes polymerization only in the 5'→3' direction on the template strand.



Schematic structure of a transcription unit

2. **Sequence Example:**
   - **Template Strand:** 3' -ATGCATGCATGCATGCATGCATGC-5'
   - **Coding Strand:** 5' -TACGTACGTACGTACGTACGTACG-3'
   - **RNA Sequence Transcribed:** 5' -AUGCAUGCAUGCAUGCAUGCAUGC-3'
3. **Promoter and Terminator:**
   - **Promoter Location:** Toward the 5' -end (upstream) of the structural gene.
   - **Promoter Function:** Provides the binding site for RNA polymerase, defining the template and coding strands.
   - **Terminator Location:** Toward the 3' -end (downstream) of the coding strand.
   - **Terminator Function:** Defines the end of the transcription process.
4. **Regulatory Sequences:**

- Additional regulatory sequences may exist upstream or downstream of the promoter.
- These sequences play a role in the regulation of gene expression.

Conclusion: A transcription unit is a functional segment of DNA defined by a promoter, structural gene, and terminator. The template and coding strands are determined by the orientation of the promoter. The terminator marks the end of the transcription process, and additional regulatory sequences contribute to the regulation of gene expression.

(b) Transcription Unit and the Gene

Transcription Unit, Gene, and Genetic Definitions

1. Gene as a Functional Unit:

- A gene is considered the functional unit of inheritance.
- Genes are located on DNA, and they play a crucial role in determining traits and characteristics.

2. Definition Challenges:

- Defining a gene in terms of DNA sequence can be challenging.
- Genes can code for various molecules, including tRNA or rRNA.
- A cistron, defined as a DNA segment coding for a polypeptide, can be monocistronic (mostly in eukaryotes) or polycistronic (mostly in bacteria or prokaryotes).

3. Eukaryotic Structural Genes:

- Monocistronic structural genes in eukaryotes often have interrupted coding sequences.
- Genes in eukaryotes are split, with coding sequences called exons and non-coding sequences called introns.
- **Exons:** Sequences present in mature or processed RNA.
- **Introns:** Intervening sequences that do not appear in mature RNA.

4. Split-Gene Arrangement:

- The split-gene arrangement complicates the definition of a gene in terms of a continuous DNA segment.

5. Inheritance and Regulatory Sequences:

- Inheritance of a character is influenced not only by coding sequences but also by promoter and regulatory sequences.
- Regulatory sequences, although not coding for RNA or protein, play a crucial role in gene expression and function.

- Regulatory sequences are sometimes loosely defined as regulatory genes.

Conclusion: The definition of a gene is complex, especially considering the diversity of molecules it can code for and the split-gene arrangement in eukaryotes. Inheritance is influenced by coding sequences, as well as promoter and regulatory sequences. Regulatory sequences, although not directly involved in coding for molecules, play a significant role in gene expression and function.

(c) Types of RNA and the process of Transcription

1. Types of RNA in Bacteria:

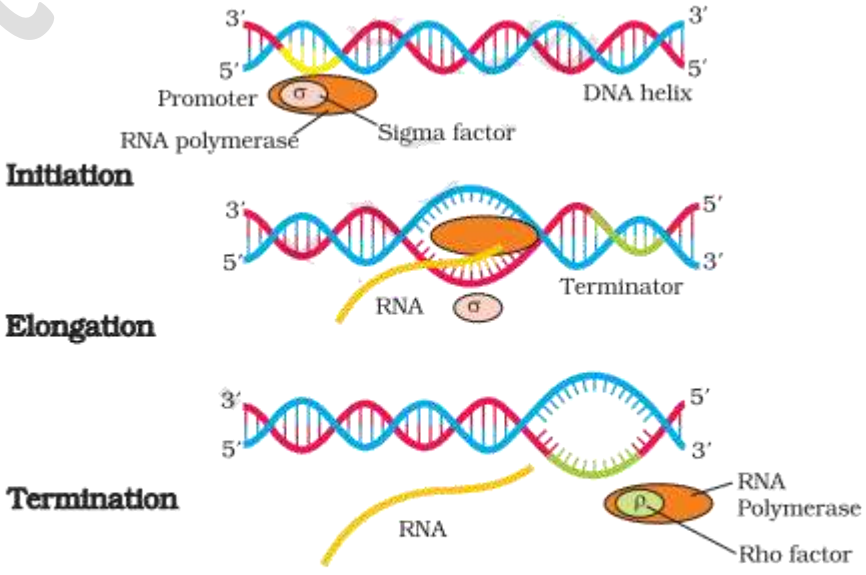
- **mRNA (Messenger RNA):** Provides the template for protein synthesis.
- **tRNA (Transfer RNA):** Transfers amino acids and reads the genetic code.
- **rRNA (Ribosomal RNA):** Plays structural and catalytic roles during translation.

2. Transcription in Bacteria:

- Single DNA-dependent RNA polymerase catalyzes transcription of all RNA types.
- **Initiation:** RNA polymerase binds to the promoter and initiates transcription.
- **Elongation:** Polymerase uses nucleoside triphosphates to polymerize in a template-dependent fashion.
- **Termination:** Once the polymerase reaches the terminator region, nascent RNA falls off, resulting in transcription termination.
- RNA polymerase associates with initiation-factor (σ) and termination-factor (ρ) for initiation and termination, respectively.

3. Coupled Transcription and Translation in Bacteria:

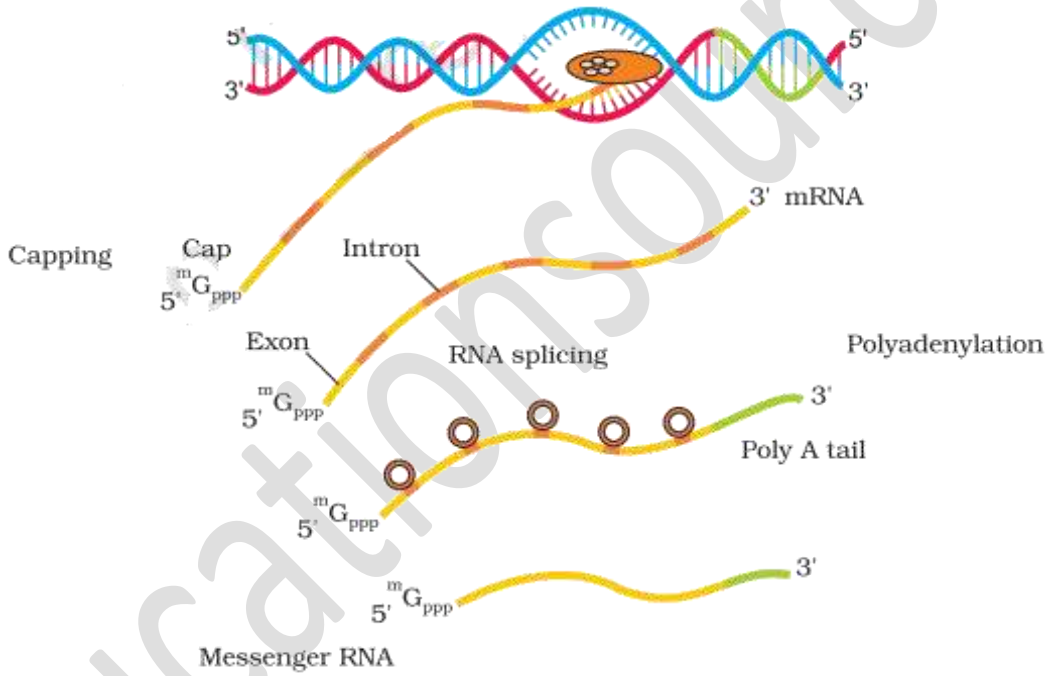
- In bacteria, translation can begin before mRNA is fully transcribed.
- Transcription and translation are coupled because there is no separation of cytosol and nucleus in bacteria.



Process of Transcription in Bacteria

4. Complexities in Eukaryotic Transcription:

- **Multiple RNA Polymerases:** Eukaryotes have at least three RNA polymerases in the nucleus.
  - **RNA polymerase I:** Transcribes rRNAs (28S, 18S, 5.8S).
  - **RNA polymerase II:** Transcribes precursor mRNA (hnRNA).
  - **RNA polymerase III:** Transcribes tRNA, 5srRNA, snRNAs.
- **Processing of Primary Transcripts:**
  - Primary transcripts in eukaryotes contain both exons and introns and are non-functional.
  - Splicing removes introns and joins exons in a defined order.
  - hnRNA undergoes capping and tailing before being called mRNA.
  - mRNA is transported out of the nucleus for translation.



Process of Transcription in Eukaryotes

5. Significance of Complexities:

- Split-gene arrangements and the presence of introns represent ancient features of the genome.
- Splicing reflects the dominance of an RNA-world.
- Understanding RNA and RNA-dependent processes is gaining importance in recent times.

Conclusion: The transcription process, types of RNA, and the associated complexities vary between bacteria and eukaryotes. Bacteria exhibit coupled transcription and translation, while eukaryotes have multiple RNA polymerases and involve additional processing steps like splicing, capping, and tailing for functional mRNA production. Understanding these processes is crucial for deciphering the intricacies of genetic information flow in living systems.

VII. GENETIC CODE

Genetic Code: Deciphering the Language of Life

The process of translation, where genetic information is transferred from nucleic acids to proteins, necessitated the existence of a genetic code. Unlike replication and transcription, which are based on complementarity between nucleotides, translation involves the synthesis of amino acids, with no theoretical complementarity between nucleotides and amino acids.

George Gamow's Proposition:

- George Gamow, a physicist, proposed that the genetic code should consist of combinations of three nucleotides (triplets).
- Despite the potential generation of 64 codons (43), Gamow argued that the code should code for 20 amino acids, leading to some redundancy.

Deciphering the Code:

- Har Gobind Khorana's chemical method and Marshall Nirenberg's cell-free system for protein synthesis played crucial roles.
- Severo Ochoa's enzyme facilitated the enzymatic synthesis of RNA with defined sequences.
- A checkerboard for the genetic code was prepared.

Salient Features of Genetic Code:

1. **Triplet Codon:** The code is a triplet, with 61 codons coding for amino acids, and 3 codons functioning as stop codons.
2. **Degeneracy:** Some amino acids are coded by more than one codon, making the code degenerate.
3. **Contiguous Reading:** The codon is read in mRNA in a contiguous fashion without punctuations.
4. **Universality:** The code is nearly universal, but exceptions exist in mitochondrial codons and some protozoans.

5. **Dual Function of AUG:** AUG codes for Methionine (Met) and serves as the initiator codon.
6. **Stop Codons:** UAA, UAG, UGA function as stop terminator codons.

The Codons for the Various Amino Acids

First position	Second position U	Second position C	Second position A	Second position G	Third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Practical Application:

- Given the mRNA sequence - AUG UUU UUC UUC UUU UUU UUC, predicting the amino acid sequence using the checkerboard.
- Given the amino acid sequence - Met-Phe-Phe-Phe-Phe-Phe-Phe, predicting the RNA sequence.

Correlation of Properties:

- The properties learned include the triplet nature of the codon, its degeneracy, contiguous reading, universality, the dual function of AUG, and the presence of stop codons (UAA, UAG, UGA).

Understanding the genetic code was a monumental task that involved contributions from various scientific disciplines, leading to a profound comprehension of the language that dictates the synthesis of proteins in living organisms.

(a) Mutations and Genetic Code

Mutations and the Genetic Code: Understanding Point Mutations

The relationship between genes and DNA is best illuminated through mutation studies, which provide insights into the effects of alterations in genetic sequences. While the effects of large deletions and rearrangements in DNA segments are relatively straightforward, the impact of point mutations, particularly frameshift mutations, will be elucidated.

Point Mutation and Sickle Cell Anemia:

- A classical example of a point mutation is observed in the gene for the beta globin chain, leading to the substitution of a single base pair. This change results in the replacement of the amino acid residue glutamate with valine, causing sickle cell anemia.

Analogy with Word Sequence:

- Consider a statement composed of three-letter words, akin to the genetic code: "RAM HAS RED CAP."
- Using an analogy, the insertion or deletion of letters in this statement demonstrates the concept of frameshift mutations.

Frameshift Mutations:

1. **Insertion of a Letter (e.g., B):**
    - RAM HAS BRE DCA P
2. **Insertion of Two Letters (e.g., BI):**
    - RAM HAS BIR EDC AP
3. **Insertion of Three Letters (e.g., BIG):**
    - RAM HAS BIG RED CAP
4. **Deletion of Letters (e.g., R, E, D):**
    - RAM HAS EDC AP
    - RAM HAS DCA P
    - RAM HAS CAP

Observations:

- Insertion or deletion of one or two bases alters the reading frame from the point of insertion or deletion.
- Frameshift mutations refer to these alterations, disrupting the normal reading frame.

Effect of Three or Its Multiple Bases:

- Insertion or deletion of three or its multiples does not change the reading frame, as it aligns with the triplet nature of the genetic code.
- For example, the insertion or deletion of three bases would affect one codon, preserving the reading frame.

Understanding frameshift mutations is crucial in comprehending how changes at the genetic level, even at the single base pair level, can lead to significant alterations in the resulting protein sequence, potentially causing diseases or other functional consequences.

(b) <u>tRNA– the Adapter Molecule</u>

From the outset of the genetic code proposition, Francis Crick recognized the need for a mechanism to interpret and link the code to amino acids. A crucial element in this process is the adapter molecule, which both reads the genetic code and binds specifically to amino acids. This adapter molecule, known as tRNA (transfer RNA), was

initially referred to as sRNA (soluble RNA) and was recognized before the formulation of the genetic code. However, its role as an adapter molecule became apparent later.

Key Features of tRNA:

1. **Anticodon Loop:**
   - tRNA possesses an anticodon loop with bases that are complementary to the genetic code. This loop allows tRNA to recognize and bind to the corresponding codons in mRNA.

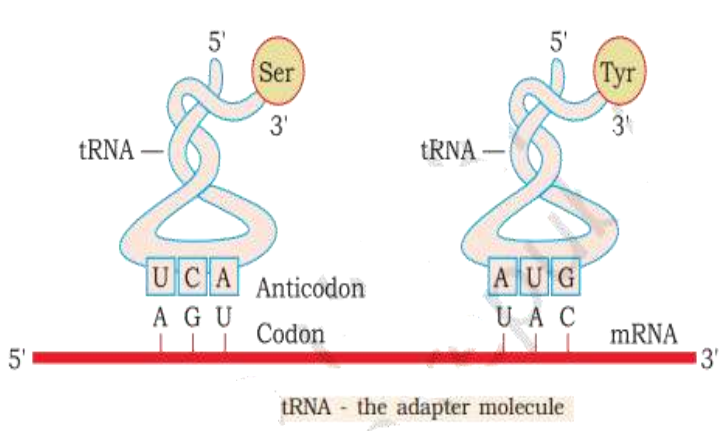
2. **Amino Acid Acceptor End:**
   - tRNA also has an amino acid acceptor end where it binds to specific amino acids. This ensures the accurate pairing of the genetic code with the corresponding amino acids.

3. **Specificity for Amino Acids:**
   - Each tRNA molecule is specific for a particular amino acid. This specificity ensures that the correct amino acid is attached to the tRNA with the corresponding anticodon.

4. **Initiator tRNA:**
   - For initiation of translation, a specific tRNA known as initiator tRNA is involved. This tRNA initiates the translation process and plays a crucial role in the formation of the initiation complex.

5. **Secondary Structure:**
   - The secondary structure of tRNA is often represented as a clover-leaf shape, as shown in Figure. However, the actual structure is more compact and resembles an inverted L.

Understanding the structure and function of tRNA is essential for the accurate translation of the genetic code into proteins. tRNA acts as a critical intermediary, ensuring the faithful conversion of the nucleotide language of mRNA into the amino acid language of proteins during the process of translation.



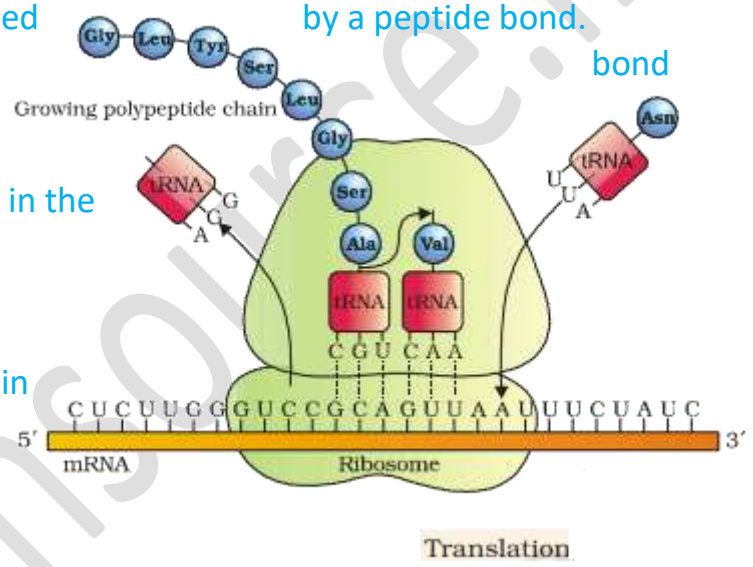
tRNA - the adapter molecule

VIII. TRANSLATION: -

Translation Process:

1. **Polymerization of Amino Acids:**
   - **Process:** Polymerization of amino acids forms a polypeptide (Figure 5.13).
   - **Sequence Determination:** Amino acid sequence is defined by mRNA base sequence.

2. **Peptide Bond Formation:**
   - **Bond Type:** Amino acids are joined by a peptide bond.
   - **Energy Requirement:** Peptide bond formation requires energy.
   - **Activation:** Amino acids are activated in the presence of ATP in the charging of tRNA.



Translation

3. **Ribosome as Cellular Factory:**
   - **Structure:** Ribosome consists of structural RNAs and 80 proteins in two subunits (large and small).
   - **Activation:** Ribosome becomes active during translation.

4. **Translation Initiation:**
   - **Start Codon:** Ribosome binds to mRNA at the start codon (AUG).
   - **Initiator tRNA:** Recognized by initiator tRNA.

5. **Elongation Phase:**
   - **Codon Recognition:** Amino acid-tRNA complexes bind to mRNA codons.
   - **Movement:** Ribosome moves from codon to codon.
   - **Translation:** Amino acids are added sequentially, translating into polypeptide sequences.

6. **Ribosome as Catalyst:**
   - **Catalyst Function:** Ribosome acts as a catalyst (23S rRNA in bacteria) for peptide bond formation.

7. **Translation Termination:**
   - **Stop Codon:** Release factor binds to the stop codon.
   - **Termination:** Translation concludes, and the complete polypeptide is released from the ribosome.

8. **mRNA Structure:**
    - **Translational Unit:** Sequence between start (AUG) and stop codon codes for a polypeptide.
    - **Untranslated Regions (UTR):** Non-translated sequences at 5' and 3' ends for efficient translation.

9. **Catalyst Enhancement:**
    - **Catalyst Effect:** Catalyst enhances the rate of peptide bond formation.

10. **tRNA Charging:**
    - **Activation:** Amino acids are linked to their cognate tRNA in the presence of ATP.
11. **Codon-Anticodon Interaction:**
    - **Interaction:** Complementary base pairing between tRNA anticodon and mRNA codon.
12. **Role of Release Factor:**
    - **Binding:** Release factor binds to the stop codon.
    - **Termination:** Marks the end of translation.

Note: Translation involves the synthesis of proteins with the ribosome acting as a key cellular factory. The process includes initiation, elongation, and termination phases, with specific sequences on mRNA directing the synthesis of polypeptides. The role of tRNA charging, codon-anticodon interaction, and the catalytic function of ribosomes are crucial in this biological process. Untranslated regions in mRNA play a role in the efficiency of translation.

IX. REGULATION OF GENE EXPRESSION

Regulation of Gene Expression:

1. **Definition:**
    - **Broad Term:** Involves control at various levels.
    - **Levels:** Transcriptional, processing, transport of mRNA, translational.
2. **Eukaryotic Regulation:**
    - **(i) Transcriptional Level:** Controls primary transcript formation.
    - **(ii) Processing Level:** Regulates splicing.
    - **(iii) mRNA Transport:** From nucleus to cytoplasm.
    - **(iv) Translational Level:** Controls translation into a polypeptide.
3. **Functional Gene Expression:**
    - **Purpose:** Genes expressed for specific functions.
    - **Example:** E. coli synthesizes beta-galactosidase for lactose hydrolysis.

4. **Regulation Basis:**
    - **Conditions:** Metabolic, physiological, environmental factors.
    - **Example:** Expression based on the availability of lactose as an energy source.
5. **Development and Differentiation:**
    - **Embryo to Adult:** Coordinated gene expression regulates development.
    - **Result:** Expression of gene sets determines the developmental outcome.
6. **Prokaryotic Regulation:**
    - **Transcriptional Initiation Control:** Predominant in prokaryotes.
    - **RNA Polymerase Regulation:** Interaction with accessory proteins.
    - **Accessory Proteins:** Act as activators (positive) or repressors (negative).
7. **Operator Interaction:**
    - **Operator Sequences:** Regulate promoter accessibility in prokaryotic DNA.
    - **Operon Regulation:** Proteins interact with operators.
    - **Repressor Binding:** Operator binds a specific repressor protein.
8. **Specificity in Operons:**
    - **Lac Operon Example:** lac operator interacts specifically with lac repressor.
    - **Unique Regulation:** Each operon has a specific operator and repressor.

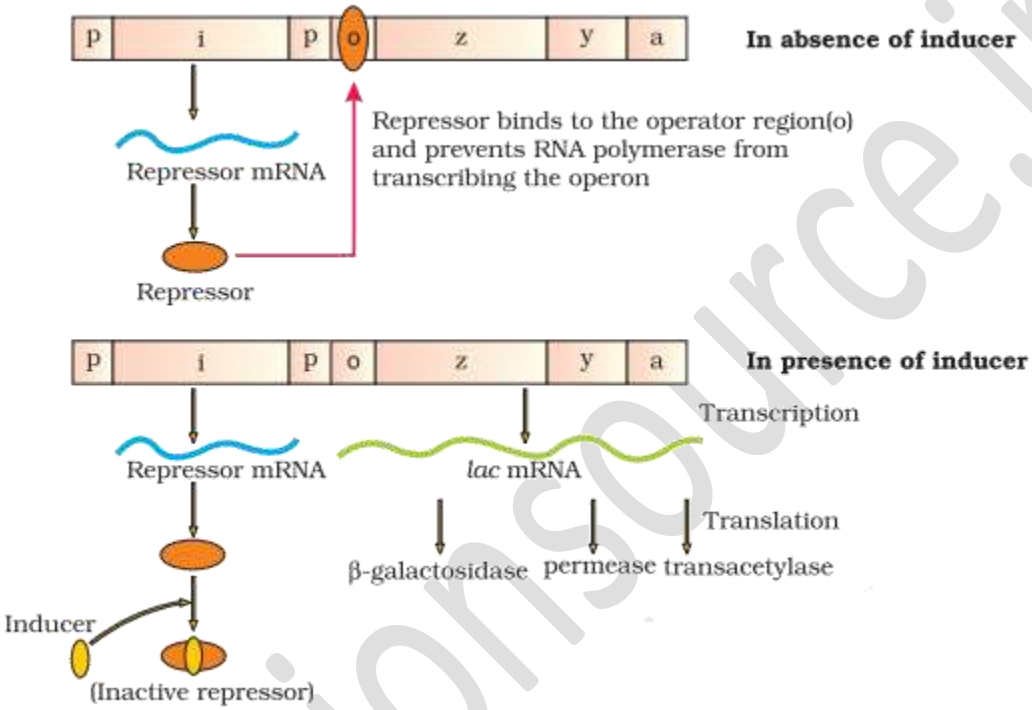
Note: Regulation of gene expression is a complex process occurring at multiple levels. In eukaryotes, control occurs during transcription, processing, mRNA transport, and translation. The regulation is often influenced by metabolic, physiological, or environmental conditions. In prokaryotes, transcriptional initiation control is predominant, with RNA polymerase activity regulated by accessory proteins. The interaction of proteins with operator sequences in operons plays a crucial role, with specificity in binding ensuring precise regulation of gene expression.

- **<u>The Lac operon: -</u>**

1. **Discovery and Collaborators:**
    - **Geneticist and Biochemist:** Francois Jacob and Jacque Monod.
    - **First Transcriptionally Regulated System:** Lac operon elucidation.
2. **Operon Concept:**
    - **Definition:** Polycistronic structural gene with a common promoter and regulatory genes.
    - **Examples:** lac operon, trp operon, ara operon, his operon, val operon, etc.
3. **Components of Lac Operon:**
    - **Regulatory Gene (i Gene):** Codes for the repressor of the lac operon.
    - **Structural Genes (z, y, a):** z codes for beta-galactosidase, y for permease, a for transacetylase.

- **Requirement:** All three genes needed for lactose metabolism.

4. **Function of Gene Products:**
   - **Beta-Galactosidase (z):** Hydrolysis of lactose into galactose and glucose.
   - **Permease (y):** Increases cell permeability to beta-galactosides.
   - **Transacetylase (a):** Encodes a transacetylase.



5. **Lactose as Inducer:**
   - **Inducer Definition:** Substance regulating the operon on/off state.
   - **Lactose Role:** Serves as an inducer for the lac operon.
   - **Substrate for Beta-Galactosidase:** Lactose is the substrate.
6. **Operon Activation:**
   - **Permease Action:** Lactose enters cells with the help of permease.
   - **Repressor Inactivation:** In the presence of lactose or allolactose, repressor is inactivated.
   - **RNA Polymerase Access:** Repressor removal allows RNA polymerase access to the promoter.
7. **Negative Regulation:**
   - **Repressor Function:** Binds to the operator, preventing transcription.
   - **Inducer Inactivation:** Inducer inactivates the repressor, allowing transcription.
8. **Duration of Expression:**
   - **Continuous Expression:** Low-level expression required for lactose entry.

- **Induction Duration:** Operon expression continues as long as lactose is present.

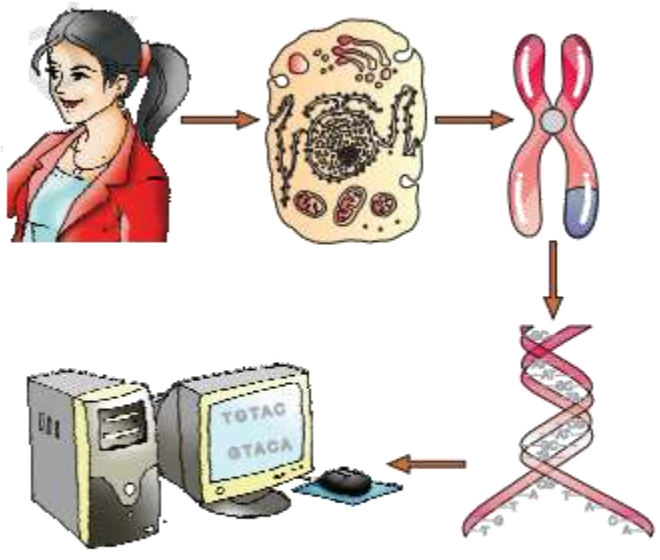
9. **Inducer Specificity:**
    - **Non-Inducers:** Glucose or galactose cannot act as inducers for lac operon.
    - **Specificity:** Lactose or allolactose required for induction.
10. **Positive Regulation:**
    - **Beyond Discussion Scope:** Lac operon is under positive regulation, but not discussed at this level.

Note: The Lac operon, a classic example of gene regulation, involves the coordination of regulatory and structural genes for lactose metabolism. Lactose serves as an inducer, enabling the expression of the operon. The interplay between repressor, inducer, and RNA polymerase controls the on/off state of the operon. This regulation is termed negative regulation. The operon is expressed as long as lactose is present in the environment, emphasizing the role of substrate-induced gene expression.

X. HUMAN GENOME PROJECT: -

Human Genome Project (HGP):

1. **Background:**
    - **DNA Sequence Importance:** Genetic information lies in DNA sequences.
    - **Individual Differences:** DNA differences reflect individual variations.
2. **Initiation (1990):**
    - **Genetic Engineering:** Techniques allowed DNA isolation and cloning.
    - **Ambitious Project:** Launched in 1990 to sequence the entire human genome.
3. **Magnitude of the Project:**
    - **Genome Size:** Human genome approximately 3 x 10^9 base pairs.
    - **Estimated Cost:** $3 per base pair, totaling around 9 billion US dollars.
    - **Information Storage:** If typed, 3300 books with 1000 pages each would be needed for a single human cell's DNA.



A representative diagram of human genome project

4. **Computational Demands:**
    - **Bioinformatics:** Emergence due to data storage, retrieval, and analysis needs.
    - **High-Speed Devices:** Required for handling vast amounts of data.

5. **Project Goals:**
    - **Gene Identification:** Identify 20,000-25,000 human genes.
    - **DNA Sequencing:** Determine sequences of 3 billion base pairs in human DNA.
    - **Database Storage:** Store information in databases.
    - **Analytical Tools:** Improve tools for data analysis.
    - **Technology Transfer:** Apply related technologies in other sectors.
    - **Ethical, Legal, and Social Issues (ELSI):** Address arising ethical, legal, and social concerns.
6. **Duration and Coordination:**
    - **13-Year Project:** Coordinated by the U.S. Department of Energy and the National Institute of Health.
    - **Partnerships:** Wellcome Trust (U.K.), Japan, France, Germany, China, and others contributed.

7. **Completion (2003):**
    - **Successful Completion:** The Human Genome Project concluded in 2003.
8. **Applications:**
    - **Medical Advancements:** DNA variation knowledge aids in diagnosis, treatment, and prevention of disorders.
    - **Beyond Human Biology:** Non-human organism DNA sequencing has applications in health care, agriculture, energy, and environmental remediation.

9. **Methodologies:**
    - **Two Approaches:** Expressed Sequence Tags (ESTs) for gene identification and Whole Genome Sequencing for coding and non-coding sequences.
    - **Fragmentation and Cloning:** DNA isolated, fragmented, cloned in bacteria or yeast using BAC and YAC vectors.
    - **Sequencing:** Automated DNA sequencers based on Frederick Sanger's method.
    - **Overlapping Sequences:** Sequences aligned based on overlapping regions using specialized computer programs.
    - **Chromosome Sequencing:** Completed in May 2006 (Chromosome 1 was the last).
10. **Mapping Challenges:**
    - **Genetic and Physical Maps:** Polymorphism and microsatellites used for mapping.
    - **Completion Challenge:** Assignment of maps to the genome.

Note: The Human Genome Project, initiated in 1990 and completed in 2003, aimed to sequence the entire human genome, providing valuable insights into genetic variations. This ambitious project involved advanced genetic engineering and computational techniques, with significant contributions from various countries. The generated knowledge has applications in medicine, biology, and diverse fields. The project's success marked a milestone in understanding human genetics and paved the way for further advancements in genomics.

(a) Salient Features of Human Genome:

1. **Genome Size:**
   - Total Bases: Human genome contains 3164.7 million base pairs.
2. **Gene Characteristics:**
   - **Average Gene Size:** Approximately 3000 bases.
   - **Gene Size Variation:** Sizes vary significantly; the largest known human gene is dystrophin at 2.4 million bases.
   - **Estimated Total Genes:** Approximately 30,000, significantly lower than previous estimates (80,000 to 140,000).
3. **Genetic Uniformity:**
   - **Nucleotide Consistency:** Almost all (99.9%) nucleotide bases are identical in all individuals.
4. **Functional Knowledge:**
   - **Unknown Functions:** Over 50% of discovered genes have unknown functions.
5. **Protein-Coding Portion:**
   - **Protein-Coding Region:** Less than 2% of the genome codes for proteins.
6. **Repeated Sequences:**
   - **Large Portion:** Repeated sequences make up a significant part of the human genome.
   - **Function:** Thought to have no direct coding functions but provide insights into chromosome structure, dynamics, and evolution.
7. **Chromosome Distribution:**
   - **Gene Distribution:** Chromosome 1 has the most genes (2968), while the Y chromosome has the fewest (231).
8. **Repetitive Sequences:**
   - **Definition:** Stretches of DNA sequences repeated many times (hundreds to thousands).
   - **Function:** Shed light on chromosome structure, dynamics, and evolution.

9. **Single Nucleotide Polymorphisms (SNPs):**
    - **Identified Locations:** About 1.4 million locations with single-base DNA differences (SNPs) in humans.
    - **Significance:** Revolutionizes the search for disease-associated sequences and traces human history.

Note: Insights from the Human Genome Project reveal the vast complexity of the human genome. While the genome is large, the actual protein-coding portion is relatively small. The discovery of repeated sequences and the identification of SNPs offer valuable information for understanding chromosome structure, genetic variation, and evolutionary processes. The project has significantly refined our knowledge of the human genome, paving the way for advancements in genetics, medicine, and evolutionary studies.

(b) Applications of Human Genome Sequencing:

1. **Biological Understanding:**
    - **Research Advancements:** DNA sequences contribute to our understanding of biological systems.
    - **Decades of Research:** Ongoing research using DNA sequences will shape biological studies in the coming decades.
2. **Interdisciplinary Collaboration:**
    - **Expertise and Creativity:** Requires the collaboration of tens of thousands of scientists from diverse disciplines.
    - **Public and Private Sectors:** Involvement from both public and private sectors globally.
3. **Radical Approach to Research:**
    - **Impact of HG Sequence:** Human Genome (HG) sequence enables a radical shift in biological research approaches.
    - **Broad-Scale Studies:** Whole-genome sequences and high-throughput technologies allow systematic, comprehensive studies.
4. **Systematic Study of Genes:**
    - **From One to Many Genes:** Previous research focused on one or a few genes; now, researchers can study entire genomes.
    - **Tissue and Organ Studies:** Systematic examination of all genes, transcripts, or proteins in specific tissues, organs, or tumors.
5. **Network Studies:**
    - **Gene and Protein Networks:** Exploration of how tens of thousands of genes and proteins work together in interconnected networks.

- **Chemistry of Life:** Understanding the orchestration of life's chemistry through comprehensive studies.

Future Challenges:

1. **Data Management:**
   - **Enormous Data:** Handling and managing vast amounts of genomic data.
   - **Storage and Retrieval:** Efficient methods for storage, retrieval, and analysis of genomic information.
2. **Interpretation Complexity:**
   - **Functional Annotation:** Assigning functions to genes with unknown roles.
   - **Integration of Data:** Coordinating information from various studies for comprehensive understanding.
3. **Ethical, Legal, and Social Implications (ELSI):**
   - **Addressing Concerns:** Continuing to address ethical, legal, and social issues arising from genomic research.
   - **Privacy Considerations:** Balancing the benefits of genomic information with individual privacy concerns.
4. **Technological Advancements:**
   - **Continuous Innovation:** Staying abreast of technological advancements in sequencing and analysis.
   - **Cost-Effectiveness:** Ensuring accessibility and cost-effectiveness of evolving technologies.
5. **Translation to Clinical Applications:**
   - **Medical Implications:** Translating genomic knowledge into practical applications for disease diagnosis, treatment, and prevention.
   - **Clinical Integration:** Integrating genomics into routine clinical practices for personalized medicine.
6. **Global Collaboration:**
   - **Collaborative Research:** Fostering international collaboration for a global understanding of genomic diversity.
   - **Shared Resources:** Sharing data and resources for collective advancements.
7. **Educational Initiatives:**
   - **Public Awareness:** Increasing public awareness and understanding of genomic research.
   - **Educational Programs:** Promoting education and training in genomics for scientists, healthcare professionals, and the public.

Note: The applications of human genome sequencing are vast, offering insights into biological systems and enabling a paradigm shift in research approaches. However, future challenges include managing enormous data, interpreting functional complexities, addressing ethical considerations, keeping pace with technological advancements, translating genomic knowledge into clinical applications, fostering global collaboration, and promoting education and awareness. Overcoming these challenges will further unlock the potential of genomics for scientific and medical advancements.

XI. DNA Fingerprinting:

Introduction:

- **Genetic Similarity:** 99.9% of the human genome is identical; differences in DNA sequence contribute to individual uniqueness.
- **Comparing DNA Sequences:** Sequencing the entire DNA for comparison is impractical and expensive.
- **DNA Fingerprinting:** Quick method to compare DNA sequences of individuals.

Principles of DNA Fingerprinting:

- **Repetitive DNA Regions:** Identification of differences in specific repetitive DNA regions.
- **Separation by Centrifugation:** Repetitive DNA separated as satellite DNA peaks during density gradient centrifugation.
- **Satellite DNA Classification:** Based on base composition, length, and number of repetitive units; includes micro-satellites, mini-satellites, etc.
- **High Polymorphism:** These sequences, while not coding for proteins, exhibit high polymorphism, forming the basis of DNA fingerprinting.

Applications:

- **Forensic Identification:** DNA fingerprinting is useful in forensic applications for identifying individuals from various tissues.
- **Paternity Testing:** Inheritable polymorphisms aid in paternity testing, resolving disputes.

Polymorphism and DNA Fingerprinting:

- **Genetic Mapping:** Polymorphism is crucial for genetic mapping and DNA fingerprinting.
- **Mutation Basis:** Polymorphism arises from mutations, which can occur in somatic or germ cells.
- **Allelic Variation:** Allelic sequence variation is considered polymorphism if the frequency is greater than 0.01.
- **Mutation Frequency:** If a mutation is inheritable and occurs at a high frequency in a population, it's termed DNA polymorphism.

Types of Polymorphisms:

- **Range of Polymorphisms:** Varied types, from single nucleotide changes to large-scale changes.
- **Evolutionary Role:** Important for evolution and speciation, contributing to genetic diversity.

DNA Fingerprinting Technique:

- **Developed by Alec Jeffreys:** Technique based on Variable Number of Tandem Repeats (VNTR) in satellite DNA.
- **Southern Blot Hybridization:** Initial technique involves radiolabeled VNTR probe and Southern blot hybridization.

- **Procedure:**
  1. Isolation of DNA.
  2. Digestion by restriction endonucleases.
  3. Electrophoresis for DNA fragment separation.
  4. Blotting of DNA fragments to synthetic membranes.
  5. Hybridization using labeled VNTR probe.
  6. Detection of hybridized fragments by autoradiography.

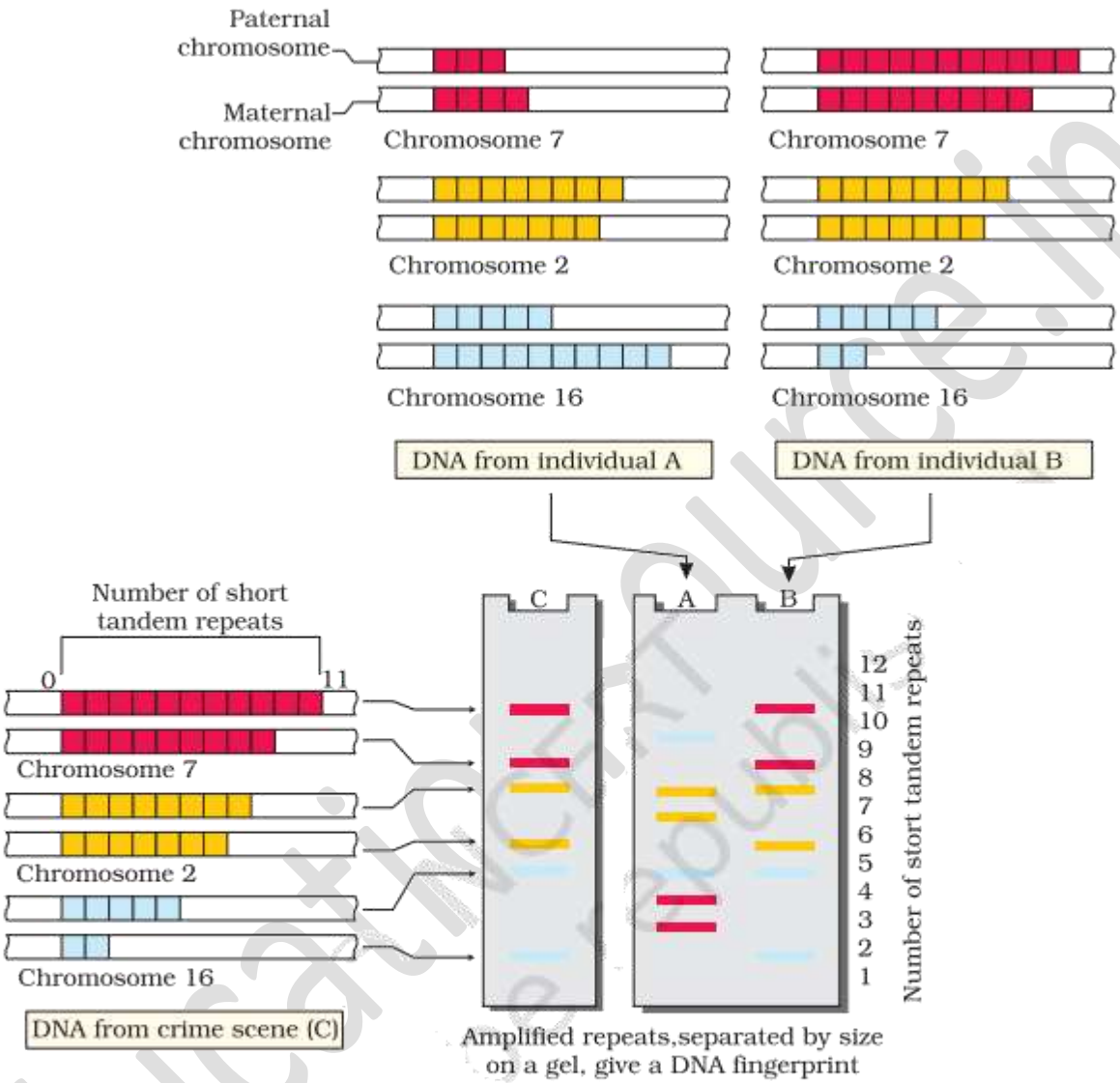
- **VNTR Characteristics:** Belongs to the mini-satellite class; tandem arrangement with varying copy numbers.
- **Polymorphism Display:** VNTR size varies (0.1 to 20 kb), resulting in bands of differing sizes on autoradiogram.
- **Individual Patterns:** Unique characteristic patterns for individuals, except monozygotic twins.
- **PCR Enhancement:** Sensitivity increased with Polymerase Chain Reaction (PCR); DNA from a single cell sufficient for analysis.

Wider Applications:

- **Forensic Science:** Widely used in forensic investigations.
- **Genetic Diversity:** Used in determining population and genetic diversities.
- **PCR Advancements:** Multiple probes used for diverse DNA fingerprinting applications.

Note: DNA fingerprinting, pioneered by Alec Jeffreys, utilizes repetitive DNA regions to compare genetic differences among individuals. The technique, initially involving VNTR probes and Southern blot hybridization, has evolved with the Polymerase Chain Reaction (PCR), making it more sensitive. It finds extensive use in forensic science, paternity testing, and assessing genetic diversities in populations. DNA

fingerprinting contributes to our understanding of genetic polymorphism, mutation, and the intricate variations that shape the human genome.



Schematic representation of DNA fingerprinting: Few representative chromosomes have been shown to contain different copy number of VNTR. For the sake of understanding different colour schemes have been used to trace the origin of each band in the gel. The two alleles (paternal and maternal) of a chromosome also contain different copy numbers of VNTR. It is clear that the banding pattern of DNA from crime scene matches with individual B, and not with A.