

BOTANY

Molecular Biology
and
Bioinformatics
for

B.Sc. Sem. V
(Paper 2)

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UNIT 1

Genetic Material

HISTORICAL PERSPECTIVE (FROM MIESCHER TO WATSON & CRICK)

🔑 Friedrich Miescher (1869)

- First isolated "nuclein" from white blood cell nuclei
- **Key findings:**
 - Acidic, phosphorus-rich substance
 - Present in cell nuclei (hence "nuclein")
 - Later identified as DNA

🔑 Griffith's Transformation Experiment (1928)

- Demonstrated bacterial transformation using *Streptococcus pneumoniae*
- Experimental strains:
 - Smooth (S) strain:** Virulent, encapsulated
 - Rough (R) strain:** Non-virulent, non-encapsulated
- **Critical observation:**
 - Heat-killed S strain + live R strain
 - Virulent S strain recovered
- **Conclusion:** Existence of a "transforming principle"

🔑 Avery, MacLeod & McCarty (1944)

- Identified the transforming principle as DNA
- Experimental approach:
 - Systematically destroyed different components:
- Protease treatment (proteins)
 - Transformation still occurred

- RNase treatment (RNA)
 - Transformation still occurred
- DNase treatment (DNA)
 - No transformation
- **Conclusion:** DNA is the genetic material

🔑 Hershey-Chase Experiment (1952)

- Used T2 bacteriophage to confirm DNA as genetic material
- **Experimental design:**
 - Labeled protein coat with ^{35}S
 - Labeled DNA core with ^{32}P
- **Key results:**
 - Only ^{32}P (DNA) entered bacterial cells during infection
 - New phage particles contained ^{32}P -labeled DNA
- Final confirmation that DNA carries genetic information

🔑 Chargaff's Rules (1950)

- Discovered base pairing ratios:
 - $\text{A} = \text{T}$
 - $\text{G} = \text{C}$
 - Total purines = total pyrimidines
- Provided critical evidence for DNA structure

🔑 Rosalind Franklin's X-ray Crystallography (1952)

- Produced Photo 51 - the clearest X-ray diffraction image of DNA

- Key measurements:
 - 3.4 Å between nucleotides
 - 34 Å per complete turn
 - 20 Å diameter
- Revealed:
 - Helical structure
 - Two periodicities
 - Antiparallel strands

👉 **Watson & Crick's Double Helix Model (1953)**

Combined evidence to propose DNA structure:

Two antiparallel polynucleotide chains
 Sugar-phosphate backbone
 Complementary base pairing (A-T, G-C)

Right-handed helix
 Major and minor grooves

Published in Nature (April 1953)

Key Developments Timeline

Year	Scientist(s)	Contribution
1869	Miescher	Isolated nuclein (DNA)
1928	Griffith	Bacterial transformation
1944	Avery et al.	DNA as transforming principle
1950	Chargaff	Base pairing rules
1952	Franklin	X-ray diffraction images
1952	Hershey-Chase	DNA as genetic material
1953	Watson & Crick	Double helix model

«—————» GRIFFITH'S TRANSFORMATION EXPERIMENT (1928) »—————»

Objective: Identify the factor causing bacterial transformation.

Experimental Setup:

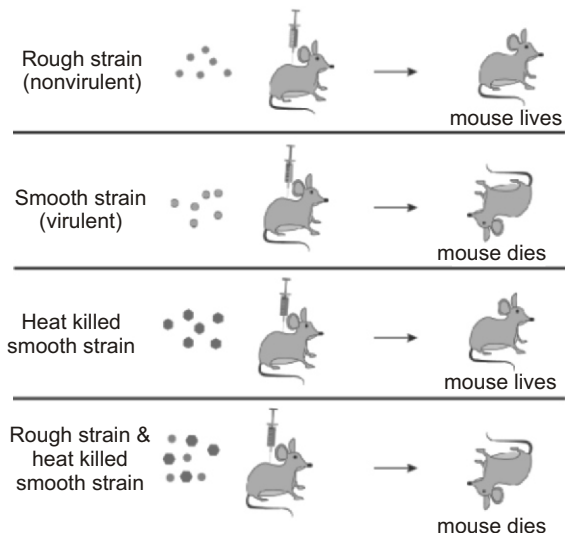
Used *Streptococcus pneumoniae* (pneumococcus) strains:

- **S-strain (Smooth):**
 - Virulent (causes pneumonia)
 - Has a polysaccharide capsule (appears smooth)
- **R-strain (Rough):**
 - Non-virulent (harmless)
 - Lacks capsule (appears rough)

Key Experiments & Observations:

Experiment	Observation	Conclusion
Live S-strain injected into mice	Mice died (S-strain recovered)	S-strain is pathogenic
Live R-strain injected into mice	Mice survived (no infection)	R-strain is non-pathogenic
Heat-killed S-strain injected	Mice survived (bacteria dead)	Heat kills S-strain

Heat-killed S + Live R injected	Mice died (live S-strain recovered!)	R-strain transformed into S-strain
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Griffith's Transformation Experiment

AVERY, MACLEOD & MCCARTY'S EXPERIMENT (1944)

Objective: Identify the chemical nature of Griffith's "transforming principle."

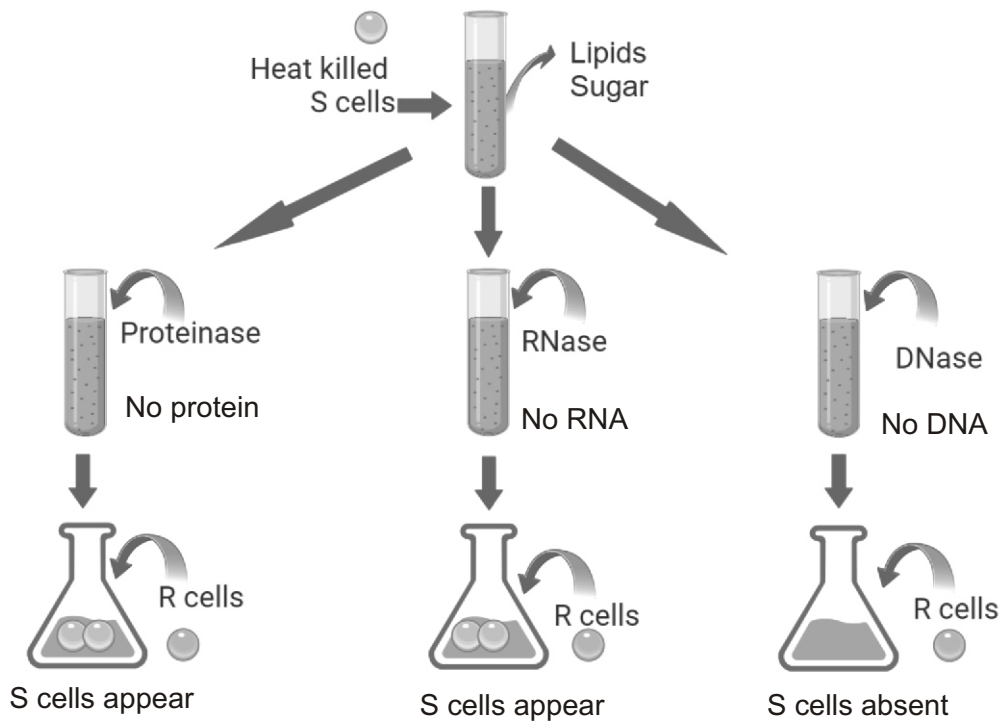
Experimental Approach:

1. Extracted components from heat-killed S-strain:
DNA, RNA, proteins, polysaccharides.
2. Selectively destroyed each component and tested for transformation:

Treatment	Component Destroyed	Transformation Result	Conclusion
Protease	Proteins	Transformation occurred	Proteins not responsible
RNase	RNA	Transformation occurred	RNA not responsible
DNase	DNA	No transformation	DNA is essential
Control (no enzyme)	-	Transformation occurred	Confirms experimental setup

Key Findings:

- Only DNase treatment prevented transformation, proving DNA was the active factor.
- First direct evidence that DNA (not protein) carries genetic information.



Avery, MacLeod & McCarty's Experiment

THE HERSHEY-CHASE EXPERIMENT (1952)

The Blender Experiment" - Confirming DNA as Genetic Material

Background & Objective

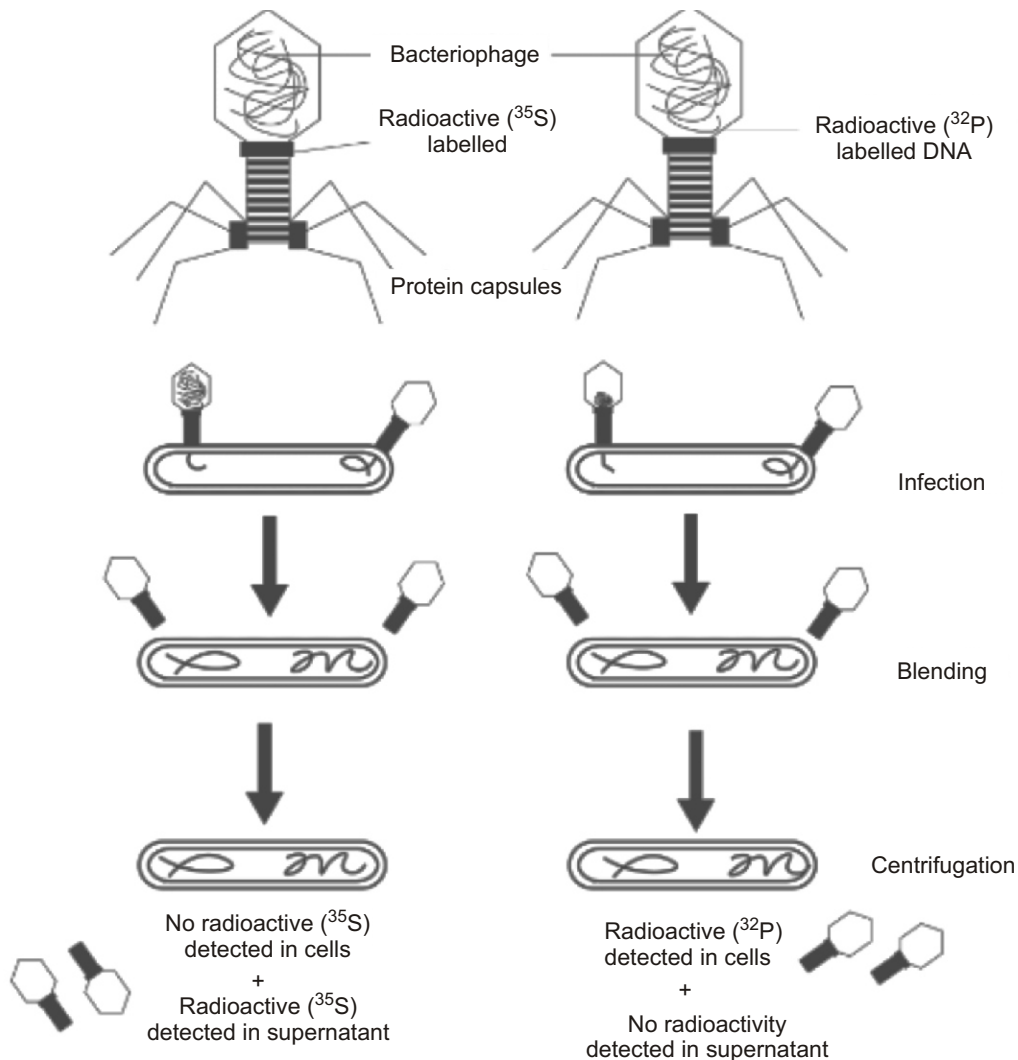
- **Scientists:** Alfred Hershey & Martha Chase
- **Goal:** Determine whether DNA or protein carries genetic information in viruses

- **Organism:** T2 bacteriophage (virus infecting E. coli bacteria)

Experimental Design

Key Principle:

- Viruses consist of only DNA + protein coat
- If DNA is genetic material, it must enter bacteria to make new viruses



The Hershey-Chase Experiment

Radioactive Labeling Strategy:

Component	Tagged Element	Purpose
Protein coat	^{35}S (Sulfur)	Sulfur is present in proteins (methionine, cysteine) but not in DNA
DNA core	^{32}P (Phosphorus)	Phosphorus is in DNA backbone but absent in most proteins

Step-by-Step Procedure**1. Grew two separate batches of T2 phage:**

- **Batch 1:** Grown in ^{35}S medium
Labeled protein coats
- **Batch 2:** Grown in ^{32}P medium
Labeled DNA

2. Infected *E. coli* with each batch:

- Allowed phages to attach and inject genetic material

3. Blended & Centrifuged:

- Used a Waring blender to shear off empty phage coats

- **Centrifuged to separate:**

Pellet (heavy): Bacteria with injected material

Supernatant (light): Empty phage coats

4. Measured radioactivity:

- Where did the labels end up?

Key Results

Label	Location of Radioactivity	Conclusion
^{35}S (Protein)	Supernatant (empty coats)	Protein did NOT enter bacteria
^{32}P (DNA)	Pellet (inside bacteria)	DNA entered bacteria and directed new phage production

Final Proof

- Only ^{32}P -labeled DNA was found in new phage progeny
- Confirmed DNA (not protein) is the genetic material

GENETIC MATERIAL



Genetic material is the substance that stores, transmits, and expresses hereditary information. In living organisms and viruses, the genetic material can be of the following types:

DNA (Deoxyribonucleic Acid)

Found in: Most living organisms (animals, plants, fungi, bacteria)

Types of DNA as Genetic Material:

- **Double-stranded DNA (dsDNA)** - Common in eukaryotes, bacteria, and DNA viruses (e.g., Herpesvirus)

- **Single-stranded DNA (ssDNA)** - Found in some viruses (e.g., Parvovirus)
- **Nuclear DNA** - Linear chromosomes in the nucleus (eukaryotes)
- **Mitochondrial DNA (mtDNA)** - Circular DNA in mitochondria
- **Chloroplast DNA (cpDNA)** - Circular DNA in plant chloroplasts
- **Plasmid DNA** - Small circular DNA in bacteria; can carry genes like antibiotic resistance

RNA (Ribonucleic Acid)

Found as genetic material in: Many viruses (especially RNA viruses)

Types of RNA as Genetic Material:

- *Single-stranded RNA (ssRNA)* - Most RNA viruses (e.g., Influenza, HIV)
 - Positive-sense ssRNA (+ssRNA)* - Can directly act as mRNA (e.g., Poliovirus, SARS-CoV-2)
 - Negative-sense ssRNA (– ssRNA)* - Must be converted to mRNA first (e.g., Rabies virus)
- *Double-stranded RNA (dsRNA)* - Found in some viruses (e.g., Rotavirus)

Note:

In cellular organisms, RNA functions as a messenger or functional molecule (mRNA, tRNA, rRNA), but not usually as genetic material.

Special or Synthetic Types (Used in Biotechnology)

These are lab-made or engineered forms of genetic material:

- **cDNA (complementary DNA)** - Made from mRNA via reverse transcriptase
- **Recombinant DNA** - Artificially joined DNA from different sources
- **Synthetic DNA/RNA** - Used in gene therapy, vaccines (e.g., mRNA COVID-19 vaccines)

Viruses (Based on Genetic Material)

Viruses can be classified based on the type of genetic material:

Type	Example
dsDNA	Herpesvirus, Adenovirus
ssDNA	Parvovirus
dsRNA	Rotavirus
+ssRNA	Poliovirus, Coronavirus
-ssRNA	Rabies, Influenza

RNA with reverse transcriptase	HIV (Retrovirus)
DNA with reverse transcriptase	Hepatitis B virus

DNA Structure: The Double Helix

(As Proposed by Watson & Crick, 1953)

1. Primary Structure

Polymer of nucleotides linked by phosphodiester bonds.

Each nucleotide consists of:

- Phosphate group (attached to 5' carbon of sugar)
- Deoxyribose sugar (5-carbon pentose)
- Nitrogenous base (A, T, G, C)

Key Feature:

- **Directionality:**
 - 5' end (phosphate group)
 - 3' end (free -OH group on sugar)

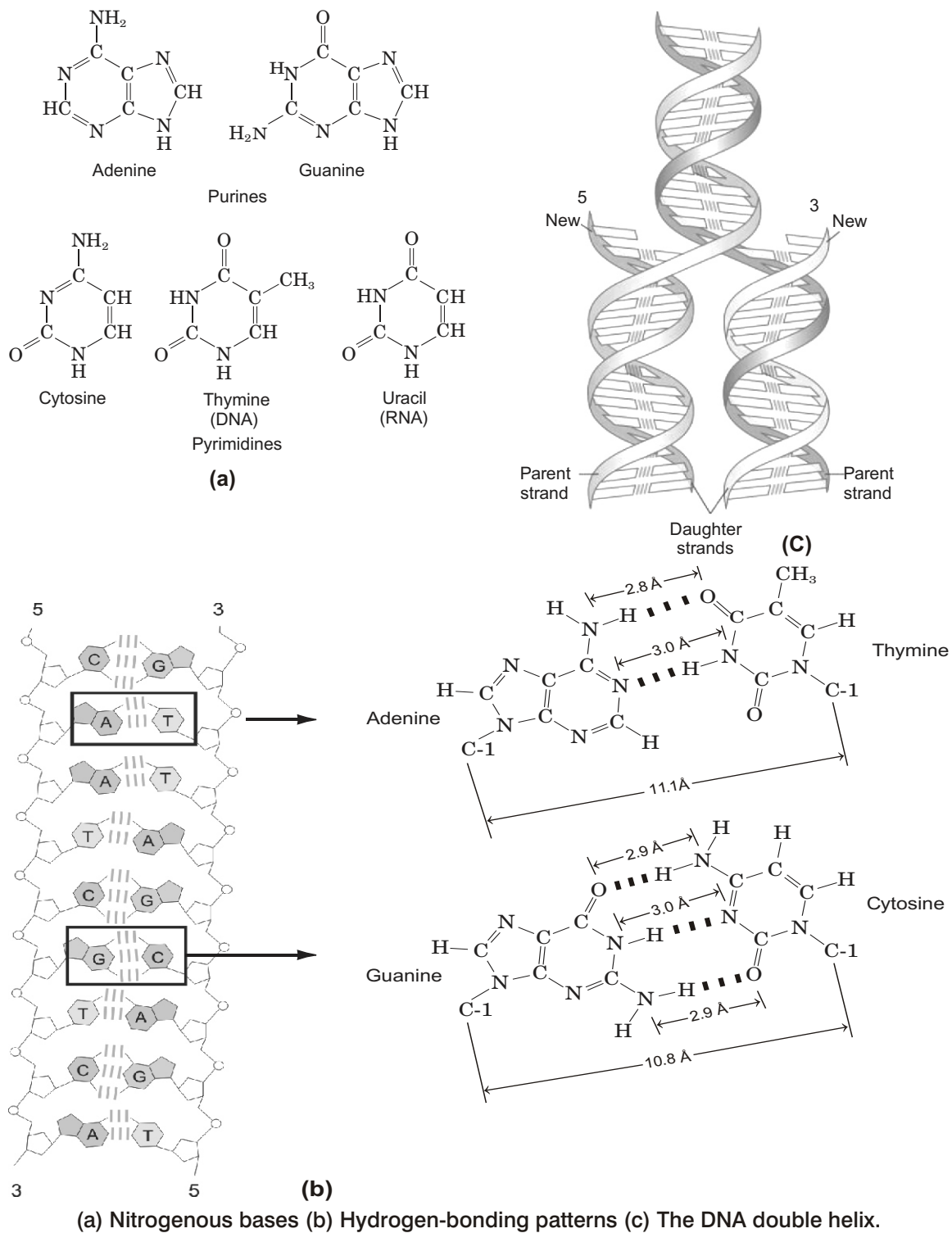
2. Secondary Structure (Double Helix)

Key Features:

- Two antiparallel strands**
One strand runs 5' → 3', the other 3' → 5'.
- Complementary base pairing (Chargaff's rules):**
A = T (2 hydrogen bonds)
G = C (3 hydrogen bonds)
- Sugar-phosphate backbone**
Forms the outer "rails" of the helix.
- Major and minor grooves**
Sites for protein binding (e.g., transcription factors).

Helical Parameters (B-DNA):

Property	Value
Helix diameter	2 nm
Base pairs per turn	10.5
Helical rise per bp	3.4 Å
Rotation per bp	34.3°



Types of DNA Conformations

Type	Features	Occurrence
B-DNA	- Right-handed helix - Most common form - 10.5 bp/turn	Physiological conditions
A-DNA	- Right-handed but shorter/wider - 11 bp/turn	Dehydrated conditions (e.g., viral DNA)
Z-DNA	- Left-handed zig-zag helix - 12 bp/turn	High salt or GC-rich regions

Tertiary Structure (Supercoiling)

- **Negative supercoiling:**
Underwound DNA (facilitates replication/transcription).
- **Positive supercoiling:**
Overwound DNA (rare, induced by certain drugs).
- **Enzymes involved:**
Topoisomerases (relieve supercoiling).

Key Structural Discoveries

1. **Chargaff's Rules (1950):**
A = T, G = C (base pairing ratios).
2. **Franklin's X-ray Diffraction (1952):**
Revealed helical structure
3. **Watson & Crick Model (1953):**
Proposed double helix with antiparallel strands.

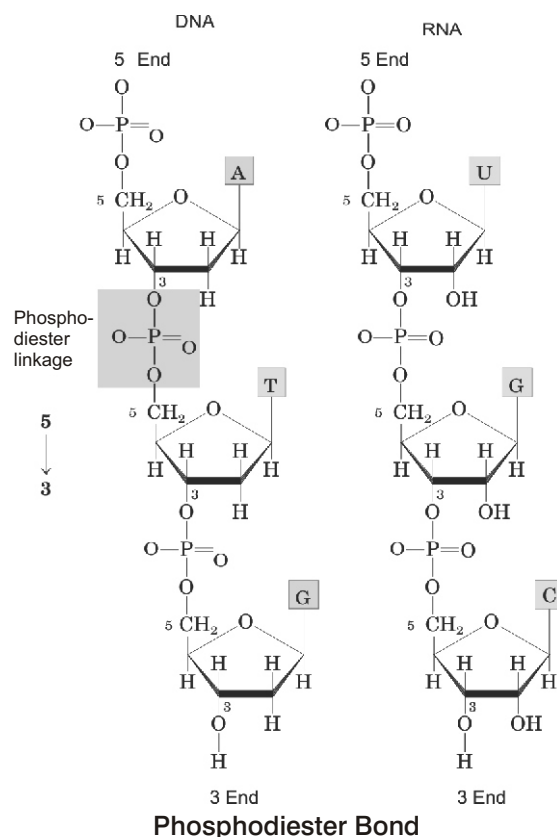
Biological Significance

Stability: H-bonds (base pairing) + hydrophobic stacking.

Replication: Strand separation allows semiconservative copying.

Information storage: Sequence of bases encodes genetic info.

DNA Structure: A Comprehensive Guide



Phosphodiester Bond Formation:

- Covalent linkage between:
5' phosphate of one nucleotide
3' hydroxyl of the next nucleotide
- Forms the sugar-phosphate backbone
- Creates inherent directionality (5' → 3')

Hydrogen Bonding:

- A=T: 2 hydrogen bonds (N6-H...O4 and N1-H...N3)
- G=C: 3 hydrogen bonds (O6-H...N4, N1-H...N3, N2-H...O2)
- Bond strength: G=C > A=T (affects melting temperature)

🔗 Base Stacking Interactions:

- Van der Waals forces between adjacent bases
- Contributes significantly to helix stability
- Stacking energy: ~5-10 kcal/mol per base pair

🔗 Supercoiling

- Negative supercoiling (underwinding):
Facilitates strand separation
Essential for replication/transcription
- Positive supercoiling (overwinding):
Occurs behind replication forks
Resolved by topoisomerases

Topoisomerase Mechanisms:

- Type I: Single-strand breaks (relax supercoils)
- Type II: Double-strand breaks (e.g., DNA gyrase)

🔗 DNA Packaging:

- Nucleosome formation (146 bp around histone octamer)
- 30 nm fiber formation
- Higher-order chromatin structures

Comparative Analysis of DNA Conformations

Parameter	B-DNA	A-DNA	Z-DNA
Helix sense	Right	Right	Left
Base pairs/turn	10.5	11	12
Helix rise/bp	3.4 Å	2.9 Å	3.7 Å
Diameter	20 Å	23 Å	18 Å
Glycosyl bond	anti	anti	anti (pyrimidines), syn (purines)
Conditions	Physiological	Low hydration	High salt/ GC-rich

Special Structures:

- Cruciform DNA (palindromic sequences)
- Triple helices (H-DNA)
- G-quadruplexes (guanine-rich regions)

🔗 Structural Determinants

Forces Maintaining Structure:

1. Hydrogen bonds (base pairing)
2. Hydrophobic interactions (base stacking)
3. Electrostatic repulsion (phosphate groups)
4. Van der Waals forces
5. Cation mediation (Mg^{2+} , polyamines)

Thermodynamic Properties:

- Melting temperature (T°) depends on:
GC content (higher GC = more stable)
Ionic strength
pH
- Denaturation/renaturation kinetics

Biological Implications

Functional Significance:

- Replication: Strand separation at origins
- Transcription: Template accessibility
- Repair: Lesion recognition
- Recombination: Homology search

Protein-DNA Interactions:

- Major groove recognition (sequence-specific)
- Minor groove interactions (shape recognition)
- DNA-binding motifs:
Helix-turn-helix
Zinc fingers
Leucine zippers

Experimental Techniques

Structure Determination Methods:

1. X-ray crystallography (atomic resolution)
2. NMR spectroscopy (solution structure)
3. Cryo-EM (large complexes)
4. AFM (surface topography)

Biophysical Analyses:

- UV melting curves
- Circular dichroism
- Gel electrophoresis (supercoiling analysis)
- Single-molecule studies (optical/magnetic tweezers)

Types of DNA

DNA (Deoxyribonucleic Acid) exists in various structural and functional types depending on its form, source, and role in the cell. Here's a categorized list:

I. Based on Structure (Forms of DNA Helices)**1. A-DNA**

- Right-handed helix
- Short and broad
- Found in dehydrated samples and some DNA-RNA hybrids
- 11 base pairs per turn

2. B-DNA (Most common form)

- Right-handed helix
- Found in most living cells under normal physiological conditions
- 10.5 base pairs per turn

3. Z-DNA

- Left-handed helix
- Zigzag backbone (hence "Z")
- Found transiently in regions with alternating purines and pyrimidines (e.g., GC repeats)
- May play a role in gene regulation

II. Based on Origin and Function**1. Genomic DNA**

- Total DNA present in a cell
- Includes both nuclear and organelle DNA

2. Nuclear DNA

- Located in the nucleus
- Linear chromosomes in eukaryotes

3. Mitochondrial DNA (mtDNA)

- Circular DNA in mitochondria
- Inherited maternally

4. Chloroplast DNA (cpDNA)

- Circular DNA in chloroplasts (plants and algae)

III. Based on Strandedness**1. Double-stranded DNA (dsDNA)**

- Most DNA is double-stranded (B-form)

2. Single-stranded DNA (ssDNA)

- Occurs in some viruses (e.g., Parvovirus)

IV. Based on Function**1. Coding DNA (Exons)**

- Contains genes that code for proteins

2. Non-coding DNA (Introns, Intergenic regions)

- Regulatory, structural, or unknown functions
- Includes promoters, enhancers, repetitive sequences

3. Repetitive DNA

- Tandem repeats, microsatellites, minisatellites

V. Based on Source/Use in Lab/Genetic Engineering**1. cDNA (Complementary DNA)**

- Synthesized from mRNA using reverse transcriptase
- Represents only expressed genes (no introns)

2. Recombinant DNA

- Artificial DNA formed by joining DNA from different organisms

3. Plasmid DNA

- Small, circular, extrachromosomal DNA in bacteria
- Used as cloning vectors

VI. Special Types (in Research and Evolution)**1. Satellite DNA**

- Highly repetitive DNA
- Often found in centromeres

2. Junk DNA

- Older term for non-coding DNA, some of which now have known functions

3. Telomeric DNA

- Repetitive sequences at chromosome ends
- DNA Replication (Prokaryotes & Eukaryotes)

DNA Replication (Prokaryotes and Eukaryotes)

☞ Semi-Conservative Replication

- First demonstrated by Meselson and Stahl using *E. coli* (1958).
- Each daughter DNA molecule consists of one parental strand and one newly synthesized strand.
- Occurs in both prokaryotes and eukaryotes.

☞ Bidirectional Replication

- Replication proceeds in both directions from the origin of replication (OriC).
- Two replication forks are formed moving in opposite directions.
- Found in:

Prokaryotes: Single origin (e.g., *E. coli* at OriC)

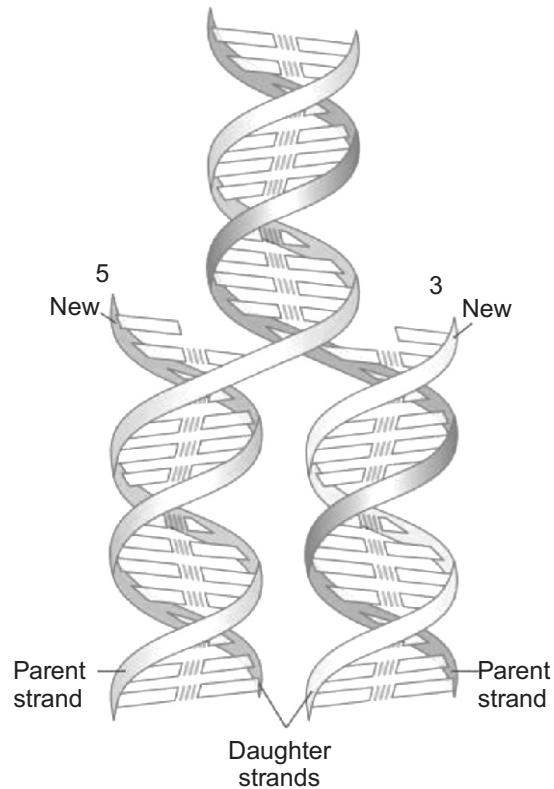
Eukaryotes: Multiple origins per chromosome (to speed up replication)

☞ Semi-Discontinuous Replication

- Leading strand is synthesized continuously (5' → 3' direction).
- Lagging strand is synthesized discontinuously in short fragments called Okazaki fragments.
- Requires multiple RNA primers.
- Occurs in both prokaryotes and eukaryotes.

☞ RNA Priming

- DNA polymerases cannot initiate synthesis on their own.
- Primase enzyme synthesizes short RNA primers (~10 nucleotides).



Semi-conservative replication of DNA at the replication fork.

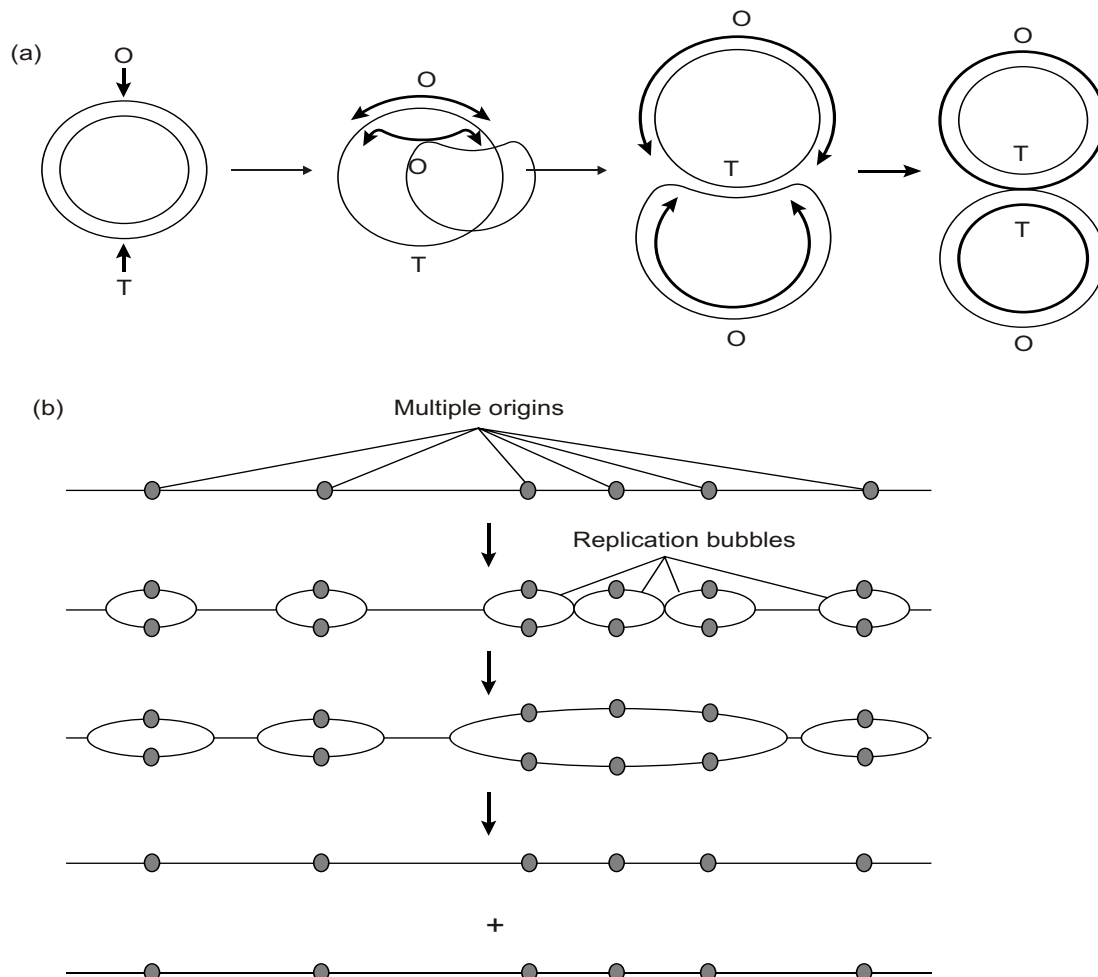
- Primers are later removed and replaced with DNA (by DNA Pol I in prokaryotes; RNase H and DNA Pol β / γ in eukaryotes).

☞ Theta (θ) Mode of Replication (Prokaryotes)

Occurs in circular DNA (e.g., *E. coli*).

The replication bubble resembles the Greek letter θ .

- Starts at OriC, proceeds bidirectionally until the forks meet at the termination site (Ter).
- Results in two identical circular daughter molecules.



Bidirectional replication of a circular bacterial replicon. Two replication forks proceed away from the origin (O) towards the terminus (T). Daughter DNA is shown as a thick line.

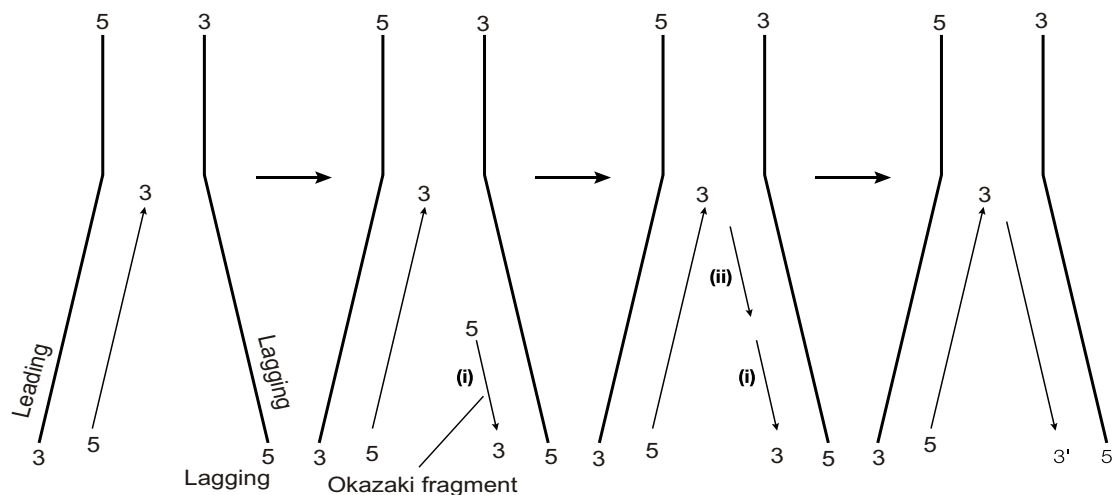
(b) Multiple eukaryotic replicons. Each origin is marked ().

🔍 Replication of Linear dsDNA (Eukaryotes)

- Linear chromosomes replicate using multiple origins to ensure rapid replication.
- DNA polymerases synthesize DNA only in 5' → 3' direction.

🔍 Replicating the 5' End of Linear Chromosomes: The End Replication Problem

- **Problem:** DNA polymerase cannot fully replicate the 5' end of the lagging strand.
- **Solution:** Eukaryotes use an enzyme called telomerase:
A ribonucleoprotein enzyme.



Semi-discontinuous replication. The first (i) and second (ii) Okazaki, or nascent, fragments to be made on the lagging strand are indicated. A single replication fork is shown.

Extends the 3' overhang using its own RNA template.

Prevents loss of coding DNA during replication.

- Absent in most somatic cells, active in germ cells, stem cells, and cancer cells.

Replication Enzymes (Prokaryotes vs Eukaryotes)

Function	Prokaryotes	Eukaryotes
Unwinding DNA	DNA helicase	MCM (Mini Chromosome Maintenance complex)
Stabilizing unwound DNA	SSB (Single-strand binding proteins)	RPA (Replication Protein A)
RNA primer synthesis	Primase	Primase (part of DNA Pol complex)
DNA synthesis	DNA Pol III (main)	DNA Pol (lagging) & DNA Pol (leading)
Primer removal	DNA Pol I (5' → 3' exonuclease)	RNase H & DNA Pol
Ligation	DNA ligase	DNA ligase I
Supercoil removal	DNA gyrase (Topoisomerase II)	Topoisomerase I and II
Telomere extension	Not required (circular DNA)	Telomerase

DNA Replication: Prokaryotes vs Eukaryotes

Feature	Prokaryotes	Eukaryotes
Cell type	Unicellular (e.g., <i>E. coli</i>)	Multicellular and unicellular (plants, animals, fungi, protists)
DNA structure	Circular, double-stranded	Linear, double-stranded
Number of origins of replication	Single origin (<i>oriC</i>)	Multiple origins per chromosome

Mode of replication	(Theta) model	Multiple replicons (replication bubbles)
Replication direction	Bidirectional	Bidirectional
Replication timing	Continuous	S-phase of the cell cycle
Replication speed	~1000 nucleotides/sec	~50–100 nucleotides/sec
Initiator proteins	DnaA, DnaB (helicase), DnaC	ORC (Origin Recognition Complex), MCM helicase
Primer synthesis	Primase (DnaG)	DNA polymerase + primase complex
Main DNA polymerase	DNA Pol III (replication)	DNA Pol (leading), DNA Pol (lagging)
Primer removal	DNA Pol I (has 5' → 3' exonuclease)	RNase H and DNA Pol
Joining of Okazaki fragments	DNA ligase	DNA ligase I
Strand synthesis	Leading: continuous Lagging: discontinuous (Okazaki fragments ~1000-2000 nt)	Leading: continuous Lagging: discontinuous (Okazaki fragments ~100–200 nt)
Proofreading	DNA Pol III (3' → 5' exonuclease)	DNA Pol and (3' → 5' exonuclease)
Handling supercoiling	DNA gyrase (topoisomerase II)	Topoisomerase I and II
Telomere problem	Not present (circular DNA)	Present (solved by telomerase in germ/stem/cancer cells)
Histones	Absent	Present; new histones added post-replication
Replication origin identification	Defined by specific sequence (e.g., OriC in <i>E. coli</i>)	Determined by chromatin structure and epigenetic markers

QUESTIONS



Section A: Very Short Answer Type Questions

1. Who discovered the transforming principle?

Ans. Frederick Griffith in 1928.

2. What is the shape of bacteriophage DNA?

Ans. Linear double-stranded DNA (in most bacteriophages like T2).

3. Name the enzyme that unwinds DNA during replication.

Ans. DNA helicase.

4. Which model organism was used in Hershey-Chase experiment?

Ans. Bacteriophage T2 and *Escherichia coli* (*E. coli*).

5. What is (theta) mode replication?

Ans. A type of circular DNA replication in prokaryotes where the structure resembles the Greek letter θ .

6. Give one difference between prokaryotic and eukaryotic DNA.

Ans. Prokaryotic DNA is circular and lacks histones; eukaryotic DNA is linear and associated with histones.

7. What are Okazaki fragments?

Ans. Short DNA segments synthesized on the lagging strand during replication.

8. Which enzyme removes RNA primers in eukaryotes?

Ans. RNase H (aided by DNA polymerase δ and FEN1).

9. Name the scientist who identified nuclein.

Ans. Friedrich Miescher in 1869.

10. What is the role of single-strand binding proteins (SSBs)?

Ans. They stabilize unwound DNA strands and prevent re-annealing during replication.

Section B: Short Answer Type Questions

11. What did Griffith's transformation experiment prove about genetic material?

Ans. Griffith's experiment demonstrated the phenomenon of transformation, showing that a substance from heat-killed virulent bacteria could genetically transform live non-virulent bacteria. This suggested that genetic information could be transferred between organisms, pointing toward the existence of a "transforming principle" - later identified as DNA.

12. How did Hershey-Chase confirm DNA as the genetic material?

Ans. Hershey and Chase used radioactive labeling of DNA (with phosphorus-32) and protein (with sulfur-35) in bacteriophages. They found that only the DNA entered the bacterial cells and directed viral replication, proving that DNA is the genetic material, not protein.

13. Name the types of DNA based on helix structure.

Ans. The three main types of DNA based on helix structure are:

- A-DNA
- B-DNA
- Z-DNA

14. Differentiate between conservative and semi-conservative DNA replication.

Ans. Conservative replication: The parental DNA remains intact, and an entirely new copy is made.

- **Semi-conservative replication:** Each new DNA molecule consists of one original (parental) strand and one newly synthesized strand. This is the actual mode of DNA replication in cells.

15. What is the role of RNA primer in DNA replication?

Ans. RNA primer provides a free 3'-OH end for DNA polymerase to begin DNA synthesis, as DNA polymerase cannot initiate synthesis on its own.

16. Define (theta) mode of replication with an example.

Ans. The θ mode of replication occurs in circular DNA molecules, such as bacterial chromosomes. It starts at a single origin and forms a theta (θ)-shaped intermediate, resembling the Greek letter θ .

Example: *E. coli* DNA replication.

17. List two enzymes involved in eukaryotic DNA replication.

Ans. DNA polymerase - initiates DNA synthesis by extending the RNA primer.

- **DNA polymerase** - synthesizes the lagging strand and participates in DNA repair.

18. How are telomeres replicated in linear chromosomes?

Ans. Telomeres are replicated by the enzyme telomerase, which adds repetitive nucleotide sequences to the 3' ends of chromosomes, preventing the loss of genetic material during replication.

Section C: Long Answer Type Questions

19. Trace the historical experiments (Griffith, Avery, Hershey-Chase) that established DNA as genetic material. (CCSU 2019, 2022)

Ans. The identification of DNA as the genetic material was a progressive discovery involving several landmark experiments:

- (i) **Frederick Griffith's Transformation Experiment (1928):**

- Griffith worked with *Streptococcus pneumoniae*, which has two forms:
 - S (Smooth):** Virulent with a polysaccharide capsule
 - R (Rough):** Non-virulent, lacking the capsule
- When mice were injected with heat-killed S-strain and live R-strain, the mice died, and live S-strain bacteria were recovered.
- **Conclusion:** A "transforming principle" from the dead S-strain had converted the R-strain into virulent S-strain, suggesting transfer of genetic information.

(ii) **Avery, MacLeod, and McCarty (1944):**

- They repeated Griffith's experiment in vitro and treated the heat-killed S-strain with enzymes:
 - Protease (destroys proteins)
 - RNase (destroys RNA)
 - DNase (destroys DNA)
- Transformation occurred in all cases except when DNA was destroyed.
- **Conclusion:** DNA is the transforming principle, hence the genetic material.

(iii) **Hershey-Chase Experiment (1952):**

- Used bacteriophage T2 (a virus infecting bacteria) and labeled:
 - DNA with ^{32}P (Phosphorus)
 - Protein coat with ^{35}S (Sulfur)
- After infection of *E. coli*, only ^{32}P entered the bacterial cells.
- ^{35}S remained outside with the phage coats.
- **Conclusion:** DNA, not protein, enters the host and carries genetic information.

Collectively, these experiments proved that DNA is the molecule responsible for inheritance.

20. Describe Watson and Crick's DNA double helix model. How does it explain genetic information storage? (CCSU 2020, 2023)

Ans. Watson and Crick's Model (1953): James Watson and Francis Crick proposed the double helix model of DNA based on X-ray diffraction data from Rosalind Franklin and chemical findings from Chargaff.

Key Features of the Model:

1. **Double Helix:** DNA consists of two antiparallel strands coiled into a right-handed helix.
2. **Sugar-Phosphate Backbone:** Each strand has a backbone of deoxyribose sugar and phosphate groups.
3. **Nitrogenous Bases:** The strands are joined by complementary base pairing:
 - Adenine (A) pairs with Thymine (T) via 2 hydrogen bonds.
 - Cytosine (C) pairs with Guanine (G) via 3 hydrogen bonds.
4. **Uniform Diameter:** Because of specific base pairing (purine with pyrimidine), the helix maintains a consistent width (~20 Å).
5. **Helical Structure:** The helix completes one turn every ~10 base pairs (34 Å).

Explanation of Genetic Information Storage:

- **Sequence of Bases:** The linear sequence of nitrogenous bases encodes genetic instructions (like letters in a language).
- **Complementarity:** Enables accurate DNA replication, ensuring genetic fidelity.
- **Stability:** The double helix protects genetic material while allowing it to be unzipped for replication or transcription.
- **Redundancy:** Complementary strands allow repair mechanisms using the undamaged strand as a template.

Conclusion: The model elegantly explained how DNA stores, replicates, and transmits genetic information.

21. Explain semi-conservative and bidirectional DNA replication in prokaryotes. Include Meselson-Stahl experiment. (CCSU 2018, 2021)

Ans. (i) Semi-Conservative Replication:

- In semi-conservative replication, each daughter DNA molecule consists of one parental strand and one newly synthesized strand.

Meselson-Stahl Experiment (1958):

- Proved the semi-conservative nature of replication using *E. coli* and isotopes of nitrogen:
Grown in ^{15}N medium (heavy DNA) shifted to ^{14}N medium
Samples collected after each generation and centrifuged using CsCl density gradient.

Observations:

1. After 1st generation DNA had intermediate density (^{15}N - ^{14}N)
2. After 2nd generation Half intermediate, half light (^{14}N - ^{14}N)

Conclusion: DNA replicates semi-conservatively.

(ii) Bidirectional Replication in Prokaryotes:

- Starts at a single origin of replication (OriC).
- Two replication forks move in opposite directions around the circular chromosome.
- Results in a θ (theta) structure, seen in electron micrographs.

Mechanism of Prokaryotic Replication:

- **Initiation:**
Begins at OriC
Initiator proteins and helicase unwind DNA
RNA primers laid by primase
- **Elongation:**
DNA polymerase III synthesizes new DNA
Leading strand: continuous; Lagging strand: Okazaki fragments
- **Termination:**
Forks meet at terminus; DNA ligase seals nicks

Conclusion: In prokaryotes, DNA replication is both semi-conservative and bidirectional, ensuring quick and accurate copying of genetic material.

22. Compare prokaryotic and eukaryotic DNA replication mechanisms. (CCSU 2020, 2022)

Ans.

Feature	Prokaryotic Replication	Eukaryotic Replication
Origin of replication	Single (OriC)	Multiple origins per chromosome
Replication rate	Fast (~1000 nucleotides/sec)	Slower (~50-100 nucleotides/sec)
Genome structure	Circular DNA	Linear chromosomes
Replication timing	Continuous; starts when needed	Regulated during the S-phase of the cell cycle
DNA polymerases involved	DNA Pol I, II, III	DNA Pol α , β , γ
Okazaki fragment size	Longer (~1000-2000 nucleotides)	Shorter (~100-200 nucleotides)
Telomere problem	Not present (circular DNA)	Present; solved by telomerase
Initiation complex	Simple (few proteins)	Complex (involves ORC, MCM, helicase loaders, etc.)
Proofreading	DNA Pol III has 3' $\rightarrow$ 5' exonuclease activity	DNA Pol α and β have proofreading ability

Conclusion: While the basic mechanism of DNA replication is conserved, eukaryotes have more complex regulation and machinery due to their larger and linear genomes.

23. Discuss the mode of replication in circular DNA. How does it differ from rolling circle replication? (CCSU 2021, 2023)

Ans. (Theta) Mode of Replication:

- Occurs in: Circular DNA of prokaryotes (e.g., E. coli) and some plasmids.
- **Initiation:** Begins at a single origin (OriC).
- **Mechanism:** DNA unwinds at the origin, forming a replication bubble. Two replication forks move in opposite directions (bidirectional).
- **Structure:** As replication proceeds, the molecule resembles the Greek letter θ .
- **Products:** Two identical circular DNA molecules.

Rolling Circle Replication:

- **Occurs in:** Some viruses (e.g., ϕ 174, lambda phage), plasmids, and conjugative DNA transfer.
- **Initiation:** A nick is made on one strand of circular DNA.
- **Mechanism:** The 3' end is extended by DNA polymerase, displacing the 5' end as a single-stranded tail, which can be circularized later.
- Unidirectional replication.
- **Products:** One full circular dsDNA and one ssDNA (which can be made double-stranded later).

Key Differences:

Feature	Replication	Rolling Circle Replication
DNA structure	Bubble-like intermediate	Tail-like structure
Direction	Bidirectional	Unidirectional
Strand displacement	No	Yes (one strand is displaced)
Usage	Chromosomal replication	Viral DNA, plasmids
Final products	Two circular dsDNA	One circular dsDNA, one ssDNA

24. Explain the challenges in replicating 5 ends of linear chromosomes and the role of telomerase.**Ans. Challenge in Replicating 5 Ends:**

(CCSU 2019, 2022)

- During replication, the lagging strand is synthesized in fragments using RNA primers.
- After removal of the terminal RNA primer at the 5 end, there is no upstream 3—OH group available for DNA polymerase to fill the gap.
- **Result:** Each round of replication would shorten the chromosome, leading to loss of genetic material.

Solution: Telomerase Enzyme

- Telomerase is a ribonucleoprotein enzyme with:
 - Reverse transcriptase activity
 - An internal RNA template
- **Function:**
 - Binds to the 3 overhang at the telomere.
 - Extends the 3 end by adding repetitive DNA sequences (e.g., TTAGGG in humans).
 - Allows conventional DNA polymerase to fill in the complementary strand.
- Active in: Germ cells, stem cells, and cancer cells.
- Not active in: Most somatic cells (leading to gradual aging).

Importance:

- Maintains chromosome stability
- Prevents loss of essential genes
- Plays a role in cellular aging and cancer