AL-FARABI KAZAKH NATIONAL UNIVERSITY
FACULTY OF BIOLOGY AND BIOTECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

Lecture 9

DNA Organization, Replication, & Repair. RNA Synthesis, Processing, & Modification

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Lecture Plan

1.Introduction: The Central Dogma of Molecular Biology
2. DNA Organization: From Nucleotides to Chromosomes
3. Chromatin Structure: Histones and Nucleosomes
4. DNA Replication: General Principles and Semiconservative Model
5. Enzymes and Proteins of DNA Replication
6. The Replication Fork and Okazaki Fragments
7. DNA Repair Mechanisms: Types and Importance
8. RNA Synthesis (Transcription): General Principles
9. RNA Polymerases in Prokaryotes and Eukaryotes
10. Transcription in Prokaryotes: Initiation, Elongation, Termination
11. RNA Processing and Modification: Capping, Tailing, Splicing

CONTENTS

Learning objectives

By the end of this lecture, students should be able to:

1. Describe how DNA is organized and packaged into chromatin and chromosomes.
2. Explain the semiconservative model of DNA replication.
3. Identify the key enzymes involved in DNA replication and their functions.
4. Describe the process of DNA replication at the replication fork, including leading and lagging strand synthesis.
5. Explain the major types of DNA repair mechanisms and their importance in maintaining genomic integrity.
6. Describe the process of RNA synthesis (transcription) in prokaryotes and eukaryotes.
7. Explain the three major RNA processing events in eukaryotes: 5' capping, 3' polyadenylation, and splicing.
8. Relate defects in DNA repair and RNA processing to human diseases.

Introduction: The Central Dogma of Molecular Biology

The flow of genetic information in cells is described by the Central Dogma of Molecular Biology:
DNA → RNA → Protein

· DNA Replication: DNA makes a copy of itself before cell division.

· Transcription (RNA Synthesis): DNA is used as a template to synthesize RNA.

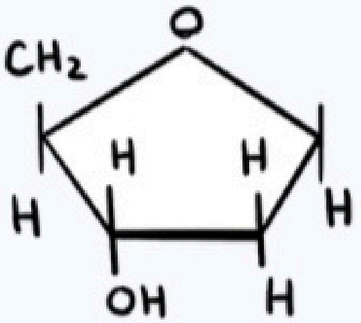
· Translation: RNA (specifically mRNA) is used as a template to synthesize proteins.

DNA vs. RNA

DEOXYRIBONUCLEIC ACID

DOUBLE-STRANDED
SUGAR* PHOSPHATE

* DEOXYRIBOSE



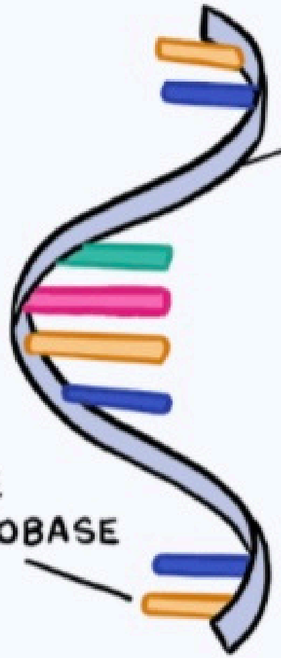


BASE PAIR

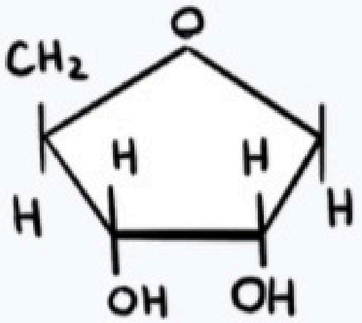
RIBONUCLEIC ACID

USUALLY SINGLE-STRANDED
SUGAR* PHOSPHATE

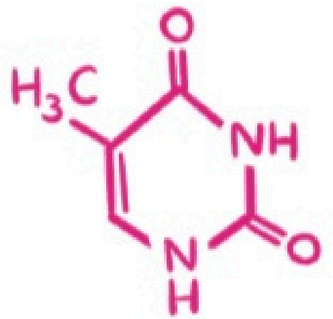
★ RIBOSE



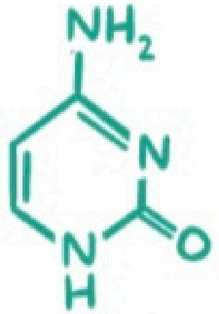
SINGLE
NUCLEOBASE



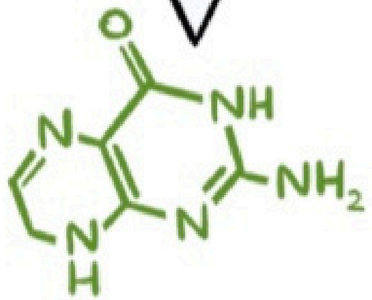
NUCLEOBASES



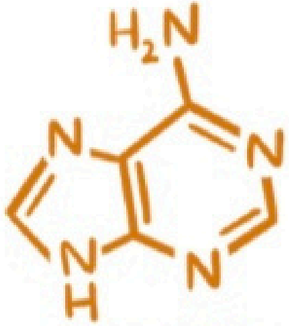
THYMINE



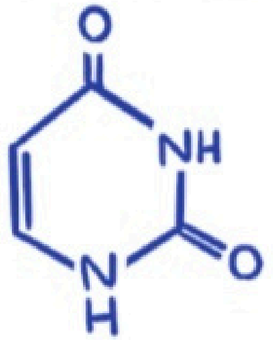
CYTOSINE



GUANINE



ADENINE



URACIL

ThoughtCo.

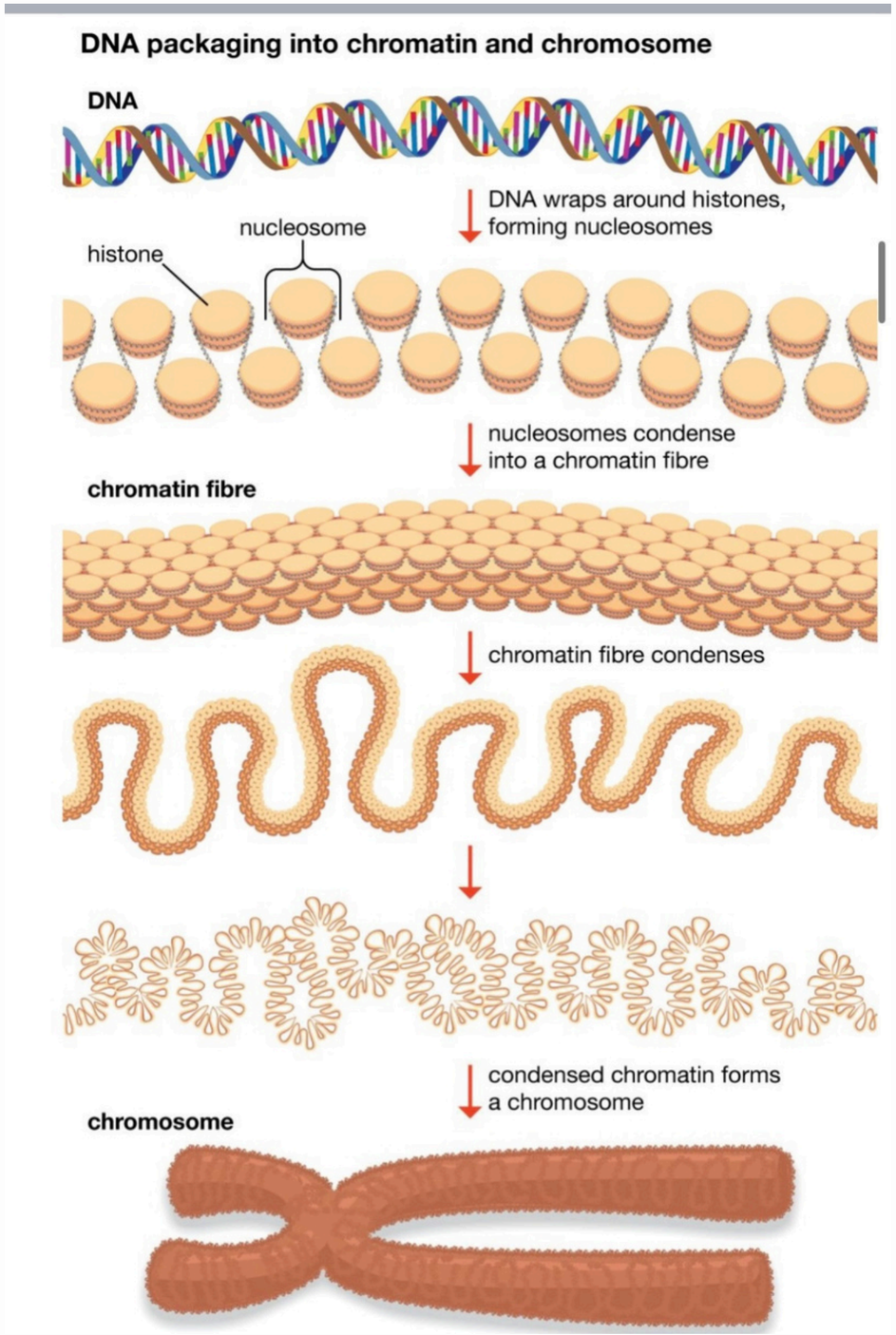
DNA Organization: From Nucleotides to Chromosomes

DNA is a very long molecule that must be compacted to fit inside the nucleus.Levels of DNA organization:

1. Nucleotides: The building blocks of DNA (adenine, guanine, cytosine, thymine).
2. Double Helix: Two antiparallel strands held together by hydrogen bonds (A-T, G-C). The backbone consists of sugar-phosphate linkages.
3. Nucleosomes: DNA wrapped around histone proteins.
4. Chromatin: The complex of DNA and proteins.
5. Chromosomes: Highly condensed chromatin visible during cell division.

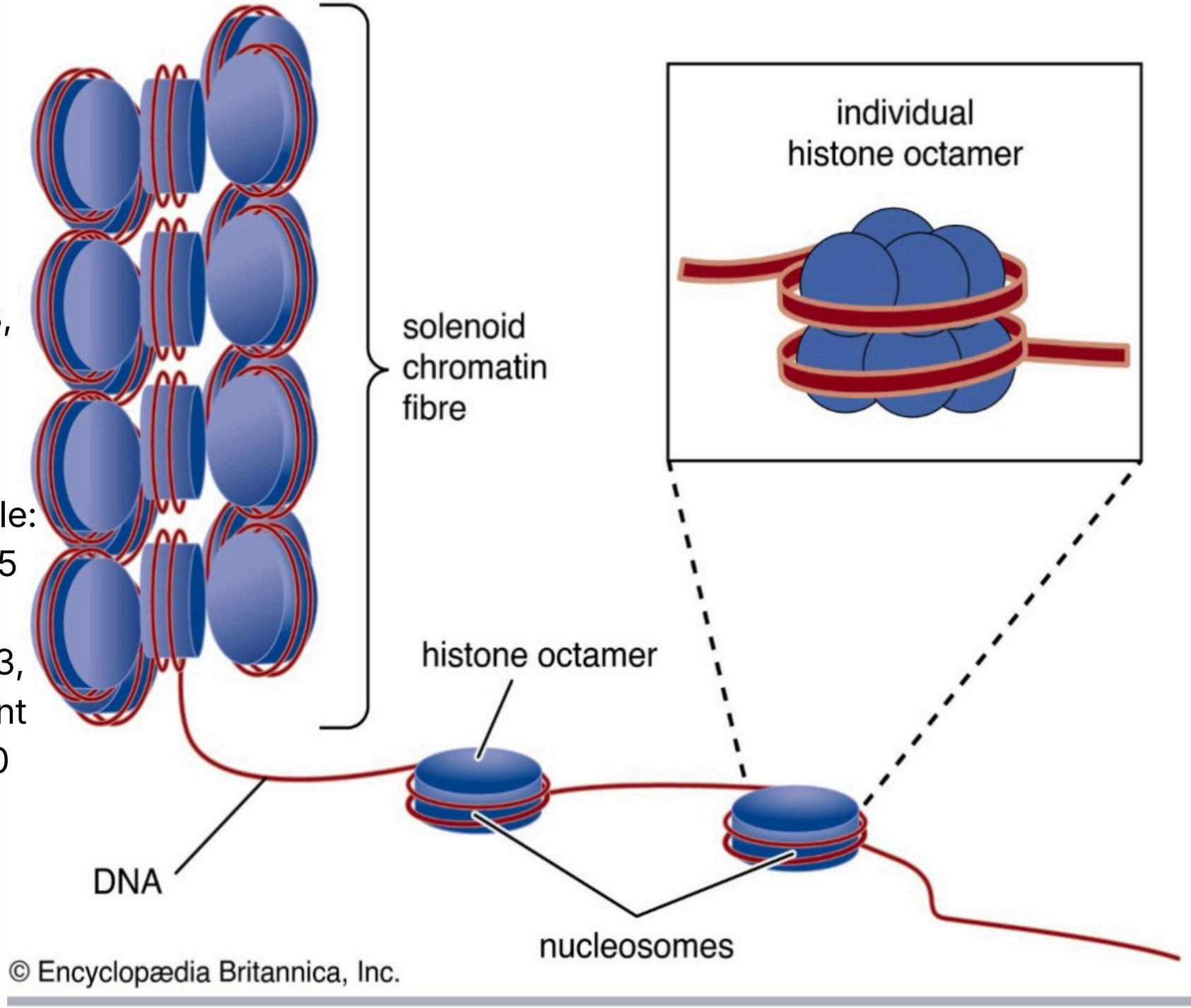
Human genome facts:· The human genome contains approximately 3 billion base pairs of DNA.· If stretched out, the DNA from one human cell would be about 2 meters long.· It must fit inside a nucleus that is only about 6 micrometers in diameter.

Chromatin Structure: Histones and Nucleosomes



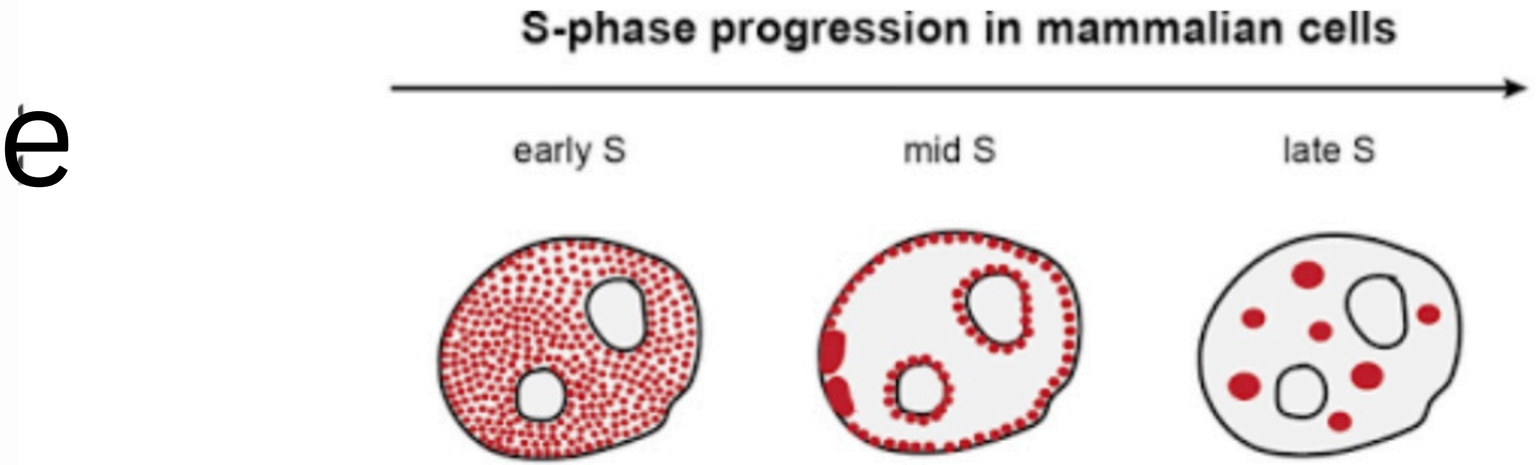
Histones are small, positively charged proteins that bind to negatively charged DNA.Thefive major types of histones:· H1: Linker histone, involved in higher-order chromatin structure.· H2A, H2B, H3, H4: Core histones that form the nucleosome core.

Nucleosome structure:· Core particle: 147 base pairs of DNA wrapped 1.65 turns around an octamer of core histones (two each of H2A, H2B, H3, H4).· Linker DNA: Connects adjacent nucleosomes (approximately 20-80 base pairs).· "Beads on a string" appearance under the electron microscope



Chromatin type

- Euchromatin: Loosely packed, transcriptionally active.
- Heterochromatin: Tightly packed, transcriptionally inactive.



Epigenetic marks	Euchromatin	facultative Heterochromatin	constitutive Heterochromatin
H3K9ac	↑	↓	↓
H3K27me3	↓	↑	↓
H3K9me2	↓	↑	↓
H3K9me3	↓	↓	↑
H4K20me3	↓	↓	↑

DNA Replication: General Principles

DNA replication is the process by which a cell duplicates its DNA before cell division.
Key principles:1. Semiconservative replication: Each new DNA molecule consists of one original (parental) strand and one newly synthesized strand.2. Bidirectional replication: Replication proceeds in both directions from the origin of replication.3. Origin of replication (ori): Specific DNA sequences where replication begins. Prokaryotes have a single origin (oriC); eukaryotes have multiple origins.4. Replication fork: The Y-shaped region where DNA unwinds and new strands are synthesized.5. Direction of synthesis: DNA polymerases synthesize DNA only in the 5' → 3' direction.6. RNA primers: DNA polymerases cannot initiate synthesis de novo; they require a short RNA primer.
Meselson-Stahl experiment (1958): Provided experimental proof for semiconservative replication using isotope labeling of E. coli DNA.

Enzymes and Proteins of DNA Replication

A large team of enzymes and proteins coordinates DNA replication.

Enzyme	Function
DNA polymerase III	The 'replicase' enzyme
DNA polymerase I	A repair enzyme involved in removing errors and filling in gaps in the sequence
DNA ligase	Seals 'nicks' in the sugar-phosphate backbone
RNA polymerase or RNA primase	Make RNA primers to get the DNA polymerase III started
RNase H	Removes the RNA primers once they have completed their function
DNA helicase and DNA gyrase	Unwind the DNA duplex
Helix-destabilizing protein	Prevents the unwound DNA from immediately rewinding

The Replication Fork and Okazaki Fragments

Because DNA strands are antiparallel and DNA polymerase only synthesizes in the 5' → 3' direction, the two strands are synthesized differently:

1. Leading Strand:· Synthesized continuously in the same direction as the replication fork movement.· Requires only one RNA primer.
2. Lagging Strand:· Synthesized discontinuously in short segments called Okazaki fragments (100-200 nucleotides in eukaryotes).· Requires multiple RNA primers.· Each fragment is synthesized 5' → 3' away from the fork.

The Replication Fork and Okazaki Fragments

Steps of lagging strand synthesis:

1. Primase synthesizes an RNA primer.
2. DNA polymerase III extends the primer to form an Okazaki fragment.
3. DNA polymerase I removes the RNA primer and replaces it with DNA.
4. DNA ligase seals the nick between adjacent Okazaki fragments.

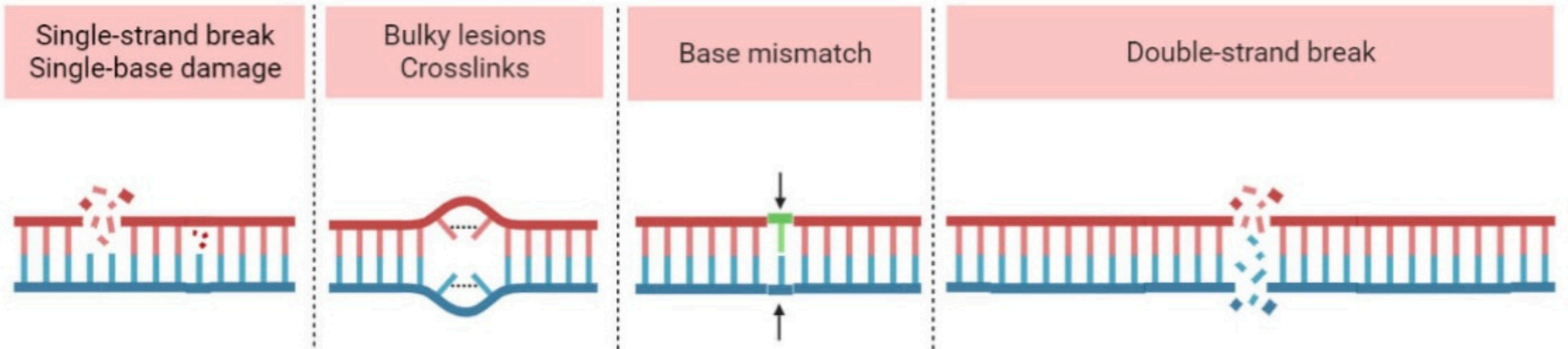
DNA Repair Mechanisms

DNA is constantly damaged by environmental agents and normal cellular processes. Cells have evolved multiple repair mechanisms.

Types of DNA damage:

Mismatches: Incorrect base pairing during replication.· Chemical modifications: Deamination, alkylation, oxidation.· UV radiation: Formation of thymine dimers.· Double-strand breaks: Caused by radiation or oxidative stress.

DNA Repair Mechanisms



Single-strand break Single-base damage	Bulky lesions Crosslinks	Base mismatch	Double-strand break	
Base Excision Repair (BER)	Nucleotide Excision Repair (NER)	Mismatch Mediated Repair (MMR)	Homologous Recombination (HR)	Non-Homologous End-Joining (NHEJ)
OGG1 PARP1 PARP2 XRCC1 Pol β PCNA FEN1 **Ligase III**	GC-NER TC-NER XPD, XPC DDB1/XPE RNA pol β CSA, CSB ERCC1/XPF PCNA Pol δ Pol ε **Ligase I**	MSH2/MSH6 MLH1/PMS2 EXO1/PCNA/RCF Pol δ **Ligase I** **Ligase IV**	ATM MRN complex RPA BRCA2/FANCD RAD51, FANCF Pol δ Pol ε **Ligase I**	KU70, KU80 DNA PKs Artemis XRCC4-XLF Pol μ **Ligase IV**

Clinical relevance:

Defects in NER cause Xeroderma Pigmentosum (extreme sensitivity to UV light and high skin cancer risk).

Defects in MMR are associated with Lynch syndrome (hereditary nonpolyposis colorectal cancer).

RNA Synthesis (Transcription): General Principles

Transcription is the process of synthesizing RNA from a DNA template.Key principles:

1. Only one strand of DNA is used as a template (the template strand or antisense strand). The other strand is the coding strand (sense strand).
2. RNA synthesis proceeds in the 5' → 3' direction.
3. RNA polymerase catalyzes the reaction; it does not require a primer.
4. Uracil (U) replaces thymine (T) in RNA.
5. Transcription is selective: Only specific genes are transcribed in a given cell at a given time.

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TYPES OF RNA

RNA stands for Ribonucleic Acid – a vital biomolecule involved in various cellular functions, especially protein synthesis.

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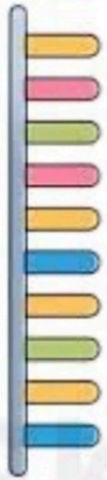
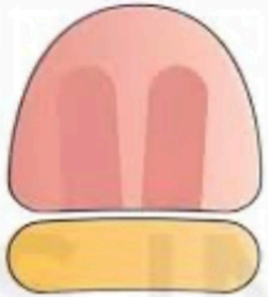
Functions of RNA:

- Protein synthesis (via mRNA, tRNA, and rRNA)
- Gene expression regulation
- Acts as a messenger between DNA and ribosomes
- Some viruses use RNA as genetic material (e.g., coronavirus)







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mRNA
MESSENGER RNA

mRNA – Messenger RNA

- **Function:** Carries genetic code from DNA to ribosomes.
- **Role:** Template for protein synthesis.
- **Fact:** Synthesized during transcription in the nucleus.
- **Mnemonic:** "mRNA = message carrier"

tRNA
TRANSFER RNA

tRNA – Transfer RNA

- **Function:** Transfers specific amino acids to the ribosome.
- **Role:** Matches mRNA codon with the correct amino acid using its anticodon.
- **Structure:** Cloverleaf shape; one end carries an amino acid, the other has an anticodon.
- **Mnemonic:** "tRNA = taxi RNA (delivers amino acids)"

rRNA
RIBOSOMAL RNA

rRNA – Ribosomal RNA

- **Function:** Structural and enzymatic component of ribosomes.
- **Role:** Helps assemble proteins by catalyzing peptide bond formation.
- **Fact:** Combines with proteins to form ribosomes.
- **Mnemonic:** "rRNA = ribosome builder"

Quick Summary

RNA Type	Function	Role
mRNA	Messenger	Carries code from DNA
tRNA	Transporter	Delivers amino acids
rRNA	Ribosomal	Builds ribosomes

RNA Polymerases

In prokaryotes (e.g., E. coli):

· A single RNA polymerase enzyme synthesizes all types of RNA.· Core enzyme: $\alpha_2\beta\beta'\omega$ subunits.· Holoenzyme: Core enzyme + σ (sigma) factor. The sigma factor recognizes the promoter.

In eukaryotes:

There are three distinct RNA polymerases, each responsible for synthesizing different types of RNA

RNA POLYMERASE 1 VS 2 VS 3

RNA POLYMERASE 1	RNA POLYMERASE 2	RNA POLYMERASE 3
RNA polymerase that only transcribes ribosomal RNA (but not 5S rRNA)	Central enzyme that catalyses DNA-directed mRNA synthesis during the transcription of protein-coding genes	RNA polymerase that transcribes small untranslated RNAs, such as tRNAs
Transcribes rRNA genes	Transcribes mRNA, miRNA, snRNA, and snoRNA genes	Transcribes tRNA and 5S rRNA genes
Resides in the nucleolus	Resides in the nucleoplasm	Resides in the nucleoplasm
Transcribes pre-rRNA at ~200 copies	Transcribe ~25,000 genes	Transcribes 30-50 genes at variable copy numbers
Contain the same two non-identical α-like subunits	Has two copies of a different α-like subunit	Contain the same two non-identical α-like subunits
Insensitive to α-amanitin	Sensitive to 1 µg/mL α-amanitin	Sensitive to 10 µg/mL α-amanitin

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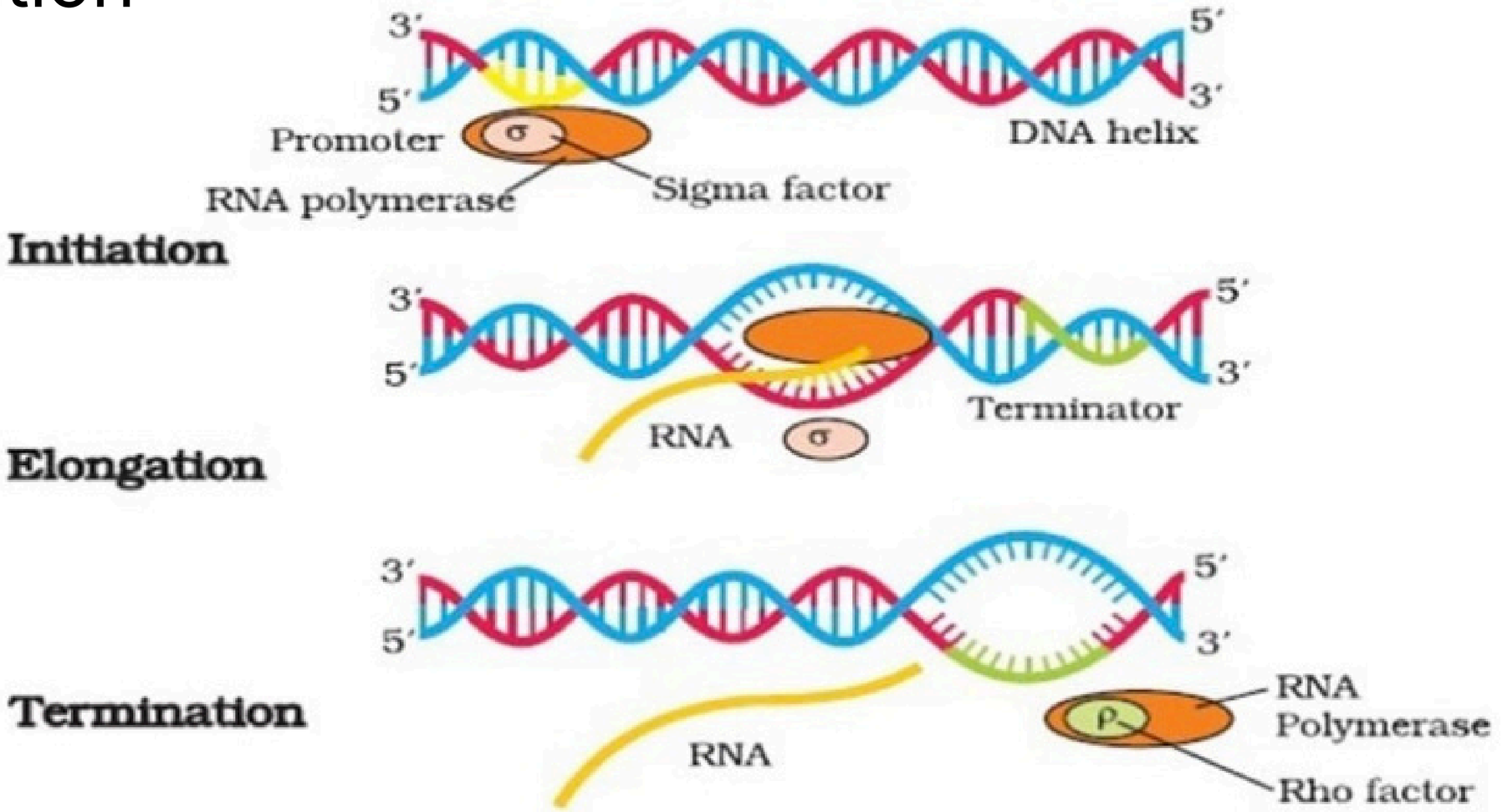
Entry Point 2 & 3: Acetyl-CoA and Acetoacetyl-CoA-Generating Amino Acids

These are the strictly ketogenic amino acids (and some dual ones).
Examples:· Leucine: Degraded into Acetyl-CoA and Acetoacetate (strictly ketogenic).· Lysine: Degraded into Acetyl-CoA and Acetoacetyl-CoA (strictly ketogenic).· Isoleucine: Degraded into both Acetyl-CoA (ketogenic) and Propionyl-CoA (glucogenic).· Phenylalanine & Tyrosine: Degraded into Fumarate (glucogenic) and Acetoacetate (ketogenic).· Tryptophan: Degraded into Alanine (glucogenic) and Acetyl-CoA (ketogenic).
Significance: Because animals cannot convert Acetyl-CoA or Acetoacetyl-CoA into glucose, these amino acids cannot maintain blood glucose levels during fasting. They are instead used for ketone body production or lipid synthesis.

Transcription in Prokaryotes: Initiation, Elongation, Termination



Initiation

RNA polymerase holoenzyme (with sigma factor) binds to the promoter region.· Prokaryotic promoters have two conserved sequences: the -10 sequence (TATAAT, Pribnow box) and the -35 sequence (TTGACA).· The sigma factor recognizes these sequences.· DNA unwinds, forming the transcription bubble.

Elongation:

Sigma factor is released.· RNA polymerase moves along the template strand, adding ribonucleotides complementary to the DNA template.· The RNA transcript grows in the 5' → 3' direction.

Termination:

Rho-dependent termination: The Rho protein recognizes a specific sequence and unwinds the RNA-DNA hybrid.· Rho-independent termination: A GC-rich inverted repeat sequence forms a hairpin loop in the RNA, followed by a run of U residues, causing RNA polymerase to dissociate

PROKARYOTIC VERSUS EUKARYOTIC TRANSCRIPTION

Prokaryotic	Eukaryotic
Prokaryotic transcription occurs in the cell cytoplasm.	Eukaryotic transcription occurs in the cell nucleus.
Here, transcription and translation happen simultaneously.	Transcription and translation differ in space and time.
mRNA is transcribed directly from template DNA molecule.	Initially a pre-mRNA molecule is formed and then processed to yield a mature mRNA.
RNA polymerase consists of five subunits.	RNA polymerase consists of 10-17 subunits.
Holoenzyme (RNA polymerase + sigma factor) recognizes and binds directly to the promoter.	Promoter recognition cannot be carried out by RNA polymerase alone.

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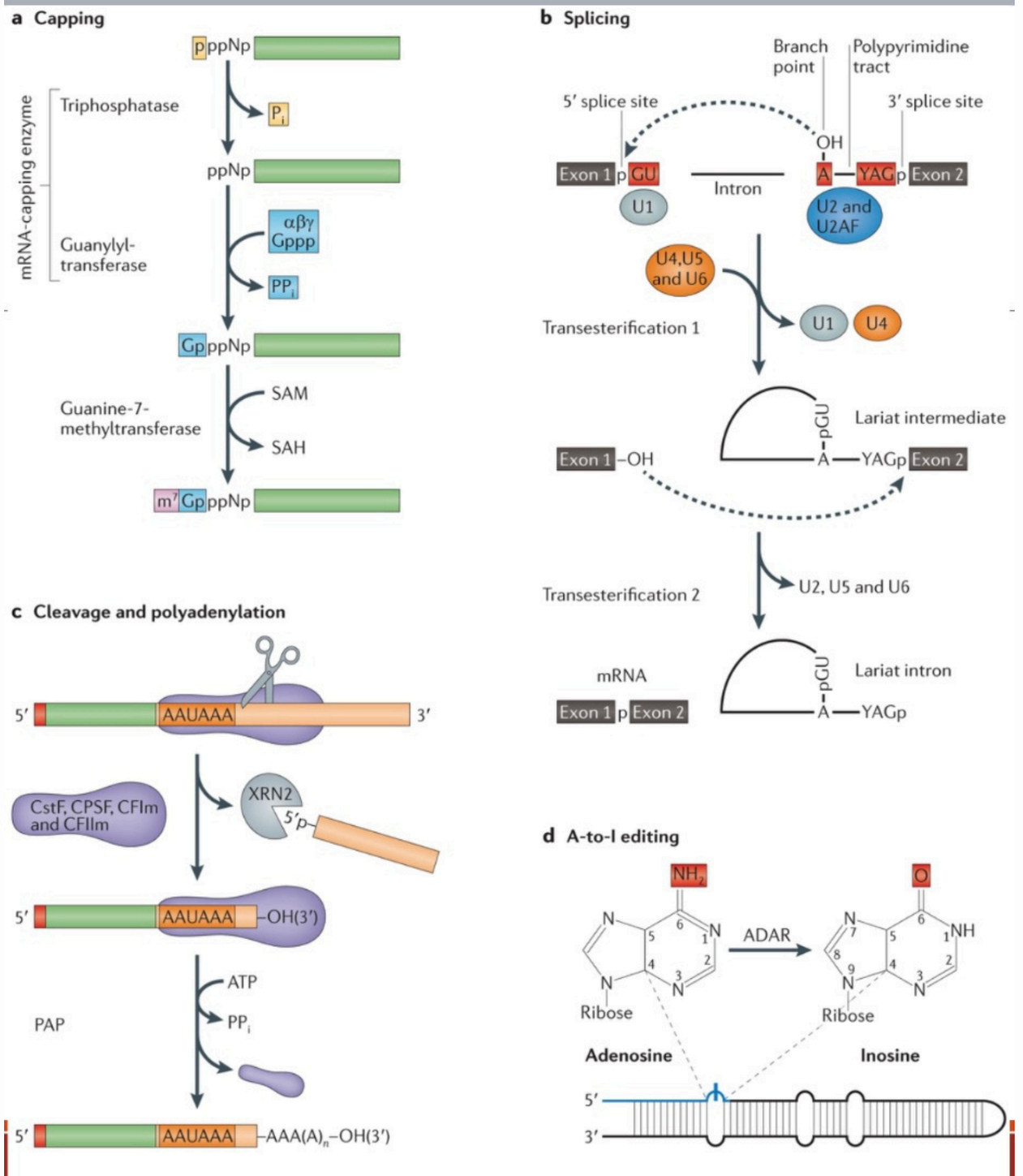

Summary: The Complete Picture

· DNA is organized into chromatin and chromosomes for compact storage.
· DNA replication is semiconservative and requires a coordinated team of enzymes.
· The leading strand is synthesized continuously; the lagging strand is synthesized discontinuously as Okazaki fragments.
· DNA repair mechanisms (MMR, BER, NER, double-strand break repair) maintain genomic integrity.
· Transcription is the synthesis of RNA from a DNA template.
· Prokaryotes have a single RNA polymerase; eukaryotes have three.
· Eukaryotic pre-mRNA undergoes 5' capping, 3' polyadenylation, and splicing before translation.

Clinical Relevance: Metabolic Disorders

· Xeroderma Pigmentosum: Defect in NER; UV sensitivity and skin cancer.
· Lynch Syndrome: Defect in MMR; increased risk of colorectal cancer.
· Spinal Muscular Atrophy (SMA): Caused by mutations in the SMN1 gene and defects in RNA splicing.
· Myotonic Dystrophy: Caused by abnormal RNA processing and toxic RNA gain-of-function.

Student task

Task 1 - Check your understanding:

1.. What is the semiconservative model of DNA replication?

2. Why is DNA replication described as discontinuous on the lagging strand?

3. What are the roles of DNA helicase, primase, DNA polymerase III, DNA polymerase I, and DNA ligase?

4. What are the three major types of DNA repair?

5. What is the difference between the template strand and the coding strand?

6. Which RNA polymerase synthesizes mRNA in eukaryotes?

7. What are the three major RNA processing events in eukaryotes?

Task 2 - Complete the pathway:

Complete the replication of the lagging strand:
Primase → (1) __ → DNA Polymerase III → (2) __ → DNA Polymerase I → (3) __ → DNA Ligase
Then explain:1. Why is an RNA primer required?
2. What would happen if DNA ligase were defective?

Conclusion

DNA organization and packaging allow the long DNA molecule to fit inside the nucleus.

· DNA replication is a highly coordinated process that ensures accurate duplication of the genome.

· DNA repair mechanisms are essential for maintaining genomic integrity and preventing mutations.

· Transcription is the first step in gene expression and is tightly regulated.

· RNA processing and modification are essential for the production of mature, functional RNA molecules.

· Defects in these processes lead to a variety of human diseases, highlighting their importance in cellular function.

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