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جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

كلية العلوم

قسم التقنيات الاحيائية الطبية

Molecular Biology

Lec. 4

DNA Transcription

by

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Lecture Three: DNA Transcription

1. Introduction

- DNA transcription is the **process by which a gene's DNA sequence is copied into RNA**.
- It is the **first step in gene expression**, leading to protein synthesis.
- **Central Dogma:** DNA → RNA → Protein
- Importance:
  - Allows cells to produce **proteins as needed**.
  - Enables **cell specialization** in multicellular organisms.
- Historical notes:
  - Discovered in the 1950s–60s with the elucidation of RNA polymerase and mRNA.
  - Early experiments by Jacob and Monod explained gene regulation in prokaryotes.

2. Types of RNA Produced

- **mRNA (messenger RNA):** carries coding information from DNA to ribosome.
- **tRNA (transfer RNA):** brings amino acids to ribosome during translation.
- **rRNA (ribosomal RNA):** forms the structural and catalytic core of ribosomes.
- **Other RNAs:** microRNA, snRNA, lncRNA – regulate gene expression.

3. DNA Template and RNA Synthesis

- **Template strand:** read by RNA polymerase to make complementary RNA.
- **Coding strand:** same sequence as RNA (U instead of T).
- **Directionality:** RNA is synthesized **5' → 3'**, reading DNA **3' → 5'**.
- Diagram suggestion: show DNA double helix with template/coding strands and growing RNA strand.

4. RNA Polymerases

Prokaryotic RNA Polymerase

- Single RNA polymerase with **sigma factor** for promoter recognition.
- Synthesizes all RNA types.
- Subunits: α, β, β', ω, σ.

Eukaryotic RNA Polymerases

Polymerase	RNA Type	Location
RNA Pol I	rRNA	Nucleolus
RNA Pol II	mRNA	Nucleoplasm
RNA Pol III	tRNA, 5S rRNA	Nucleoplasm

- Accessory factors: transcription factors (TFs) are essential for initiation.

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5. Promoters and Regulatory Elements

- **Promoter:** DNA sequence where RNA polymerase binds.
- **Prokaryotes:** -10 (TATAAT) and -35 (TTGACA) regions.
- **Eukaryotes:** TATA box (~25 bp upstream), initiator elements, enhancers, silencers.
- **Enhancers:** increase transcription efficiency; may be far from gene.
- **Silencers:** suppress transcription.
- Diagram suggestion: show promoter, enhancer, and transcription factor binding.

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6. Transcription Initiation

- **Prokaryotic Initiation:**
  - Sigma factor binds promoter, RNA polymerase forms **closed complex** → DNA unwinds → **open complex**.
- **Eukaryotic Initiation:**
  - Multiple **general transcription factors (GTFs)** recruit RNA Pol II.
  - Pre-initiation complex (PIC) forms at promoter.
  - DNA unwinding exposes template strand.
- Energy source: ATP helps in unwinding DNA.

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7. Transcription Elongation

- RNA polymerase moves along DNA, synthesizing RNA.
- Nucleotides added complementary to template strand.
- **Proofreading:** RNA polymerase can correct mistakes.
- **Supercoiling**: DNA ahead of polymerase is positively supercoiled; DNA gyrase/topoisomerase relaxes it.
- Diagram suggestion: RNA polymerase moving along DNA, RNA strand elongating.

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8. Transcription Termination

- **Prokaryotic Termination:**
  - **Rho-independent:** GC-rich hairpin followed by U sequence destabilizes RNA-DNA hybrid.
  - **Rho-dependent:** Rho helicase unwinds RNA from DNA.
- **Eukaryotic Termination:**
  - RNA Pol II continues past coding region until polyadenylation signal (AAUAAA).
  - RNA is cleaved; poly-A tail added for stability.

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9. RNA Processing in Eukaryotes

- **5’ capping:** Protects RNA, aids ribosome recognition.
- **3’ polyadenylation:** Adds poly-A tail; increases stability, export to cytoplasm.
- **Splicing:** Introns removed, exons joined to form mature mRNA.
- **Alternative splicing:** One gene → multiple mRNA variants → protein diversity.
- Diagram suggestion: pre-mRNA → spliced mRNA with cap and tail.

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10. Regulation of Transcription

- **Prokaryotic regulation:** operons (e.g., lac operon) respond to environmental signals.
- **Eukaryotic regulation:**
  - Transcription factors, enhancers, silencers.
  - Epigenetics: DNA methylation, histone acetylation/methylation.
- Examples: heat shock genes, steroid hormone response genes.

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11. Techniques to Study Transcription

- **RT-PCR:** Quantify RNA levels.
- **Northern blot:** Detect specific RNA.
- **RNA sequencing (RNA-seq):** Transcriptome-wide analysis.
- **Chromatin Immunoprecipitation (ChIP):** Identifies protein-DNA interactions.
- **Reporter assays:** Assess promoter activity (e.g., luciferase).

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12. Biological Significance

- Enables **cell-specific protein production**.
- Plays role in **development, differentiation, and stress response**.
- Transcription errors → diseases: cancer, genetic disorders.
- RNA therapeutics (siRNA, mRNA vaccines) rely on understanding transcription.