BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
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Programme: M.Sc., Biomedical Science

Course Code: BM35C5
Course Title: Molecular Biology

Unit-III

Transcription

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Unit III:

Transcription: Concept of transcription, RNA polymerases, transcriptional factors, regulatory elements, Mechanism of transcription in Prokaryotes and eukaryotes, Distinction between prokaryotic and eukaryotic transcription. Concept and mechanism of post transcriptional modification- 5‟ capping, polyadenylation, splicing of nuclear pre-mRNA, nuclear export of mRNA-mRNA stability

PRESENTATION: 1

TRANSCRIPTION

Concept

- Transcription is the process by which the information encoded in a gene (DNA) is copied into a messenger RNA (mRNA) molecule.
- This is the first step in gene expression, where the genetic code is transferred from the DNA to RNA, which is then used as a template for protein synthesis during translation.
- Transcription is carried out by the enzyme RNA polymerase, which binds to a specific region of the DNA (the promoter) and synthesizes an RNA strand complementary to the DNA template.

RNA POLYMERASES

- **RNA polymerases** are essential enzymes responsible for **synthesizing RNA from a DNA template** during the process of transcription.
- They play a critical role in gene expression by catalyzing the formation of the RNA strand, ensuring the transfer of genetic information from DNA to RNA, which is then used to guide protein synthesis.

Types of RNA Polymerases

- There are different types of RNA polymerases, depending on the organism:

In Prokaryotes (like bacteria):

- **RNA Polymerase:** Prokaryotes have a single type of RNA polymerase that transcribes all types of RNA, including messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). This enzyme is a complex made up of multiple subunits:
  - **Core Enzyme:** Comprised of five subunits (α2, β, β', and ω), which carry out the catalytic activity of RNA synthesis.
  - **Sigma Factor (σ):** This additional subunit helps the core enzyme recognize and bind to specific promoter regions on the DNA, initiating transcription.

2. In Eukaryotes (like plants, animals, and fungi):

- Eukaryotes have three main types of RNA polymerases, each responsible for transcribing different sets of genes:
- **RNA Polymerase I**: Transcribes **most of the rRNA genes** which are components of the ribosome, essential for protein synthesis.
- **RNA Polymerase II**: Transcribes all protein-coding genes into mRNA and also transcribes some small nuclear RNAs (snRNAs) and microRNAs (miRNAs).
- **RNA Polymerase III**: Transcribes **small RNAs,** and other small non-coding RNAs involved in various cellular functions.

Transcriptional Factors

- **Transcription factors** are proteins that regulate the process of transcription, which is the first step in gene expression where RNA is synthesized from a DNA template.
- These factors are essential for controlling which genes are turned on or off in a cell, thereby determining cell function, identity, and response to environmental signals.

REGULATORY ELEMENTS

- **Regulatory elements** are specific sequences of DNA that play a critical role in controlling the expression of genes.
- They act as binding sites for transcription factors and other proteins that regulate the transcription process, determining when, where, and how much a gene is expressed.

TRANSCRIPTION IN PROKARYOTES

1. Initiation:

- **Promoter Recognition:**
    - In prokaryotes, transcription begins when the RNA polymerase holoenzyme (core enzyme and sigma (σ) factor) binds to the promoter region of DNA. The promoter contains conserved sequences at the -10 (TATAAT, known as the Pribnow box) and -35 regions (TTGACA) upstream of the transcription start site.
- **Formation of the Open Complex:**
    - After binding to the promoter, the RNA polymerase unwinds a short stretch of DNA, creating an open complex. This is where the DNA strands separate, exposing the template strand that will be used to synthesize RNA.
- **Transcription Initiation:**
    - Once the open complex is formed, RNA polymerase begins synthesizing RNA.
    - The σ factor helps the polymerase recognize the promoter and initiate transcription.
    - After the first few nucleotides are synthesized, the σ factor is released, and the core enzyme continues transcription.

2. Elongation:

RNA Synthesis:

- During elongation, the core RNA polymerase moves along the DNA template strand in the 3’ to 5’ direction, synthesizing RNA in the 5’ to 3’ direction by adding ribonucleotides complementary to the DNA template.

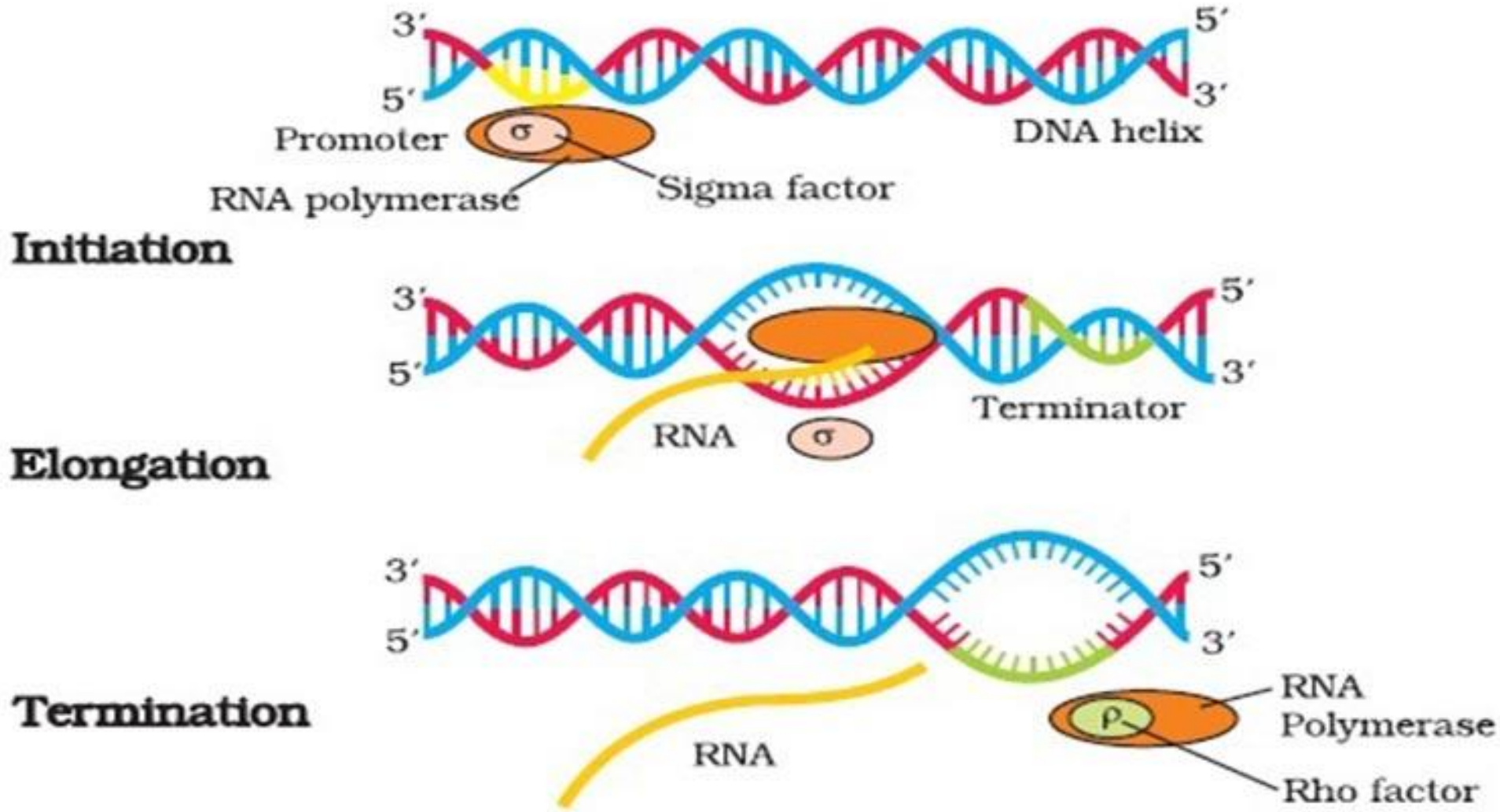
Formation of the Transcription Bubble:

- The RNA polymerase maintains a "transcription bubble," where about 17 base pairs of DNA are unwound at any given time. This allows the RNA strand to grow as the polymerase moves downstream.

3. Termination:

- **Rho-Independent Termination:**
  - In this mechanism, a GC-rich region in the RNA forms a hairpin loop followed by a series of uracil (U) residues. The formation of the hairpin **destabilizes the RNA-DNA hybrid**, causing the **RNA polymerase to release the RNA transcript and detach from the DNA**.
- **Rho-Dependent Termination:**
  - This mechanism requires the **rho (ρ) protein, which binds to a specific site** on the nascent **RNA transcript** (called the rut site). The ρ protein moves along the RNA toward the RNA polymerase, using ATP **to unwind the RNA-DNA** hybrid, causing the RNA polymerase to dissociate from the DNA template and release the transcript.

TRANSCRIPTION IN PROKARYOTES



TRANSCRIPTION IN EUKARYOTES

Transcription in eukaryotes is more complex due to the presence of chromatin, additional regulatory elements, and different types of RNA polymerases.

1. Initiation:

- **Chromatin Remodeling:**
  - Before transcription can begin, the **chromatin structure must be modified to make DNA accessible**. Chromatin remodelers and histone-modifying enzymes, such as **histone acetyltransferases (HATs), modify histones to relax the chromatin, exposing the DNA**.

- **Assembly of the Pre-Initiation Complex (PIC):**
  - **General transcription factors (GTFs) are essential for the initiation of transcription.** These factors, including TFIID, TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH, assemble at the promoter region to form the pre-initiation complex (PIC).
  - **TFIID binds to the TATA box** (or another promoter element) through its TATA-binding protein (TBP) subunit. Other general transcription factors (GTFs) and RNA polymerase II are recruited to form the PIC.
- **Promoter Clearance and Phosphorylation:**
  - **TFIIH** has **helicase activity that unwinds DNA** to form an open complex and kinase activity that phosphorylates the C-terminal domain (CTD) of RNA polymerase II.

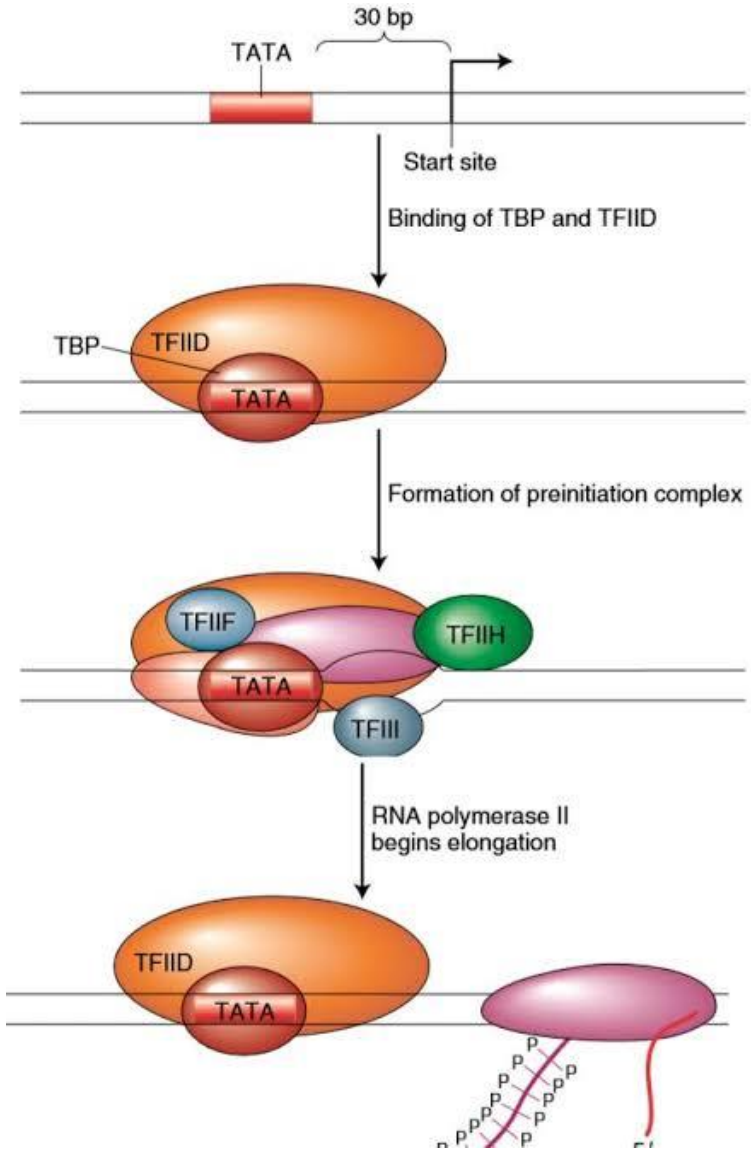
2. Elongation:

- **RNA Synthesis:**
  - **RNA polymerase II synthesizes RNA in the 5’ to 3’ direction using the DNA template.** As it moves downstream, it elongates the RNA transcript by adding ribonucleotides complementary to the DNA template.
- **RNA Processing:** In eukaryotes, the nascent RNA undergoes several co-transcriptional modifications, including:
  - **5' Capping:** A modified guanine nucleotide (**7-methylguanosine) is added to the 5' end of the RNA transcript.** This cap is essential for RNA stability, export from the nucleus, and translation initiation.
  - **Splicing: Introns (non-coding regions) are removed**, and exons (coding regions) are joined together by the spliceosome.
  - **3' Polyadenylation: A poly-A tail (a stretch of adenine nucleotides) is added to the 3' end of the RNA** transcript, which helps protect the RNA from degradation and aids in its export from the nucleus.

3. Termination:

- **Cleavage and Polyadenylation:**
  - Transcription termination in eukaryotes involves the **cleavage of the pre-mRNA downstream of the polyadenylation signal sequence (AAUAAA).** The RNA is cut, and a poly-A tail is added to the 3’ end.
- **Dissociation of RNA Polymerase II:**
  - RNA polymerase II continues to transcribe beyond the cleavage site, but it eventually dissociates from the DNA template due to degradation of the downstream RNA or dephosphorylation of its CTD.

TRANSCRIPTION IN EUKARYOTES



Difference between Prokaryotic and Eukaryotic Transcription

Aspect	Prokaryotes	Eukaryotes
RNA Polymerase	One RNA polymerase transcribes all types of RNA	Three RNA polymerases (I, II, III) specialized for different RNA types
Promoters	Simple promoters with -10 and -35 consensus sequences	Complex promoters with TATA box, initiator (Inr), etc.
Transcription Factors	Sigma factor required for initiation	Multiple general and specific transcription factors required
Chromatin	No chromatin; DNA is more accessible	DNA is packed into chromatin, requiring chromatin remodeling
RNA Processing	Little to no processing; transcription and translation can occur simultaneously	Extensive processing (capping, splicing, polyadenylation)
Termination	Rho-dependent or rho-independent mechanisms	Involves cleavage and polyadenylation of the pre-mRNA
Location	Transcription occurs in the cytoplasm	Transcription occurs in the nucleus, and translation occurs in the cytoplasm

ACKNOWLEDGEMENT

- The presentation is being used for educational and non-commercial purposes.
- Thanks are due to all the original contributors and entities whose pictures were used to create this presentation.