

ZOOLOGY

Molecular Biology, Bioinstrumentation & Biotechniques

for

B.Sc. Sem. III

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UNIT 1

Protein Synthesis I: Process of Transcription

FINE STRUCTURE OF GENE

The fine structure of a gene refers to the detailed organization and functional components that make up a gene at the molecular level. It is a concept that was first introduced by Seymour Benzer in the 1950s when he studied the genetic fine structure of bacteriophages. The fine structure focuses on the way the gene is organized in terms of its sequence, coding regions, regulatory elements, and the various molecular mechanisms involved in gene expression.

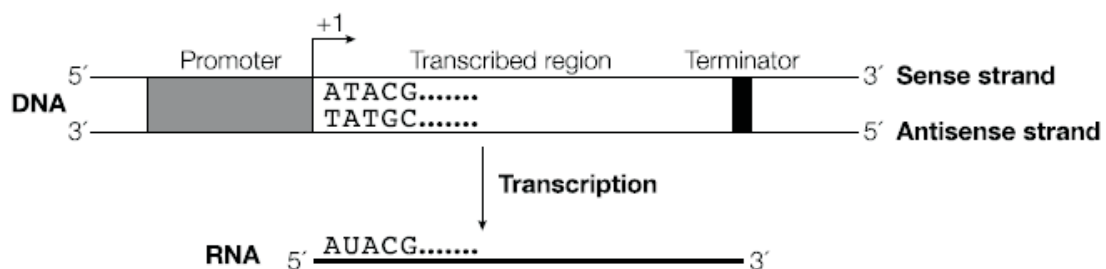
Gene

Definition: A gene is a functional unit of heredity that encodes the information required to produce functional products, primarily proteins or RNA molecules. Genes are located on chromosomes, which are made up of DNA.

Basic Components of a Gene

A typical gene consists of several key regions:

- **Promoter Region:** This is the sequence where RNA polymerase binds to initiate transcription. It is located upstream (toward the 5' end) of the coding region.
- **Transcription Start Site:** The site where transcription begins. In eukaryotic genes, this is typically referred to as the +1 position.
- **Exons:** These are the coding sequences that are transcribed into mRNA and are translated into proteins.
- **Introns:** Non-coding sequences that are transcribed into precursor mRNA but are removed during RNA splicing to form the final mRNA.
- **Exon-Intron Boundaries:** Regions at the borders between exons and introns where splicing occurs.



Structure of a typical transcription unit showing promoter and terminator sequences, and the RNA product.

- **Terminator Region:** This region signals the end of transcription. It includes sequences that cause RNA polymerase to stop transcription and release the RNA transcript.

DNA Sequence Organization

- **Coding Region:** The portion of the gene that is transcribed into mRNA and then translated into a polypeptide chain. It consists of the exons and in protein-coding genes, this is referred to as the open reading frame (ORF).
- **Non-Coding Regions:** These include introns and regulatory sequences that do not code for protein but are crucial for gene regulation.

Regulatory Sequences

Regulatory regions are non-coding DNA sequences that play a crucial role in controlling the transcription of the gene. Some key types of regulatory sequences include:

- **Enhancers:** DNA sequences that enhance the transcription of a gene. They can be located far from the gene they regulate and can work in a tissue-specific manner.
- **Silencers:** These regions suppress the transcription of a gene.
- **Promoters and Proximal Control Elements:** The promoter is the region where RNA polymerase binds, but additional control elements (like TATA boxes and CAAT boxes) help modulate gene expression by binding transcription factors.

Splicing and Post-transcriptional Modifications

- **RNA Splicing:** In eukaryotic cells, the primary RNA transcript (pre-mRNA)

contains both exons and introns. Introns are spliced out, and exons are joined together to form the mature mRNA.

- **5' Cap and 3' Poly-A Tail:** Eukaryotic mRNA transcripts are modified by the addition of a 5' cap and a 3' poly-A tail. The 5' cap protects the mRNA from degradation and helps in its translation, while the poly-A tail aids in stability and nuclear export.

Regulation of Gene Expression

Gene expression is tightly regulated to ensure that proteins are produced at the right time and in the right amount. Regulatory elements include:

- **Transcription factors:** Proteins that bind to specific DNA sequences (such as promoters, enhancers, or silencers) to regulate transcription.
- **Chromatin modifications:** The structure of chromatin can affect gene expression by making the DNA more or less accessible for transcription.
- **RNA interference:** Small RNA molecules like miRNAs or siRNAs can regulate gene expression at the post-transcriptional level by binding to mRNA and preventing its translation or promoting its degradation.

Operons in Prokaryotes

In prokaryotes (such as bacteria), genes that are related in function are often organized into operons, a group of genes that are transcribed together as a single mRNA molecule. Operons include:

- **Promoter:** Where RNA polymerase binds to begin transcription.
- **Operator:** A region where repressor proteins can bind to inhibit transcription.
- **Structural Genes:** Genes that code for proteins involved in a specific metabolic pathway or function.

RNA POLYMERASES

RNA Polymerase is an enzyme that catalyzes the synthesis of RNA from a DNA template during transcription. It plays a central role in gene expression, enabling the conversion of genetic information from DNA into RNA, which is subsequently translated into proteins in the case of mRNA. RNA polymerase is highly conserved across organisms, but its structure and function can vary between prokaryotes and eukaryotes.

Basic Function of RNA Polymerase

RNA polymerase synthesizes RNA by adding nucleotides to the 3' end of a growing RNA chain. The RNA strand is complementary to the DNA template strand, and RNA polymerase reads the DNA from the 3' to 5' direction, synthesizing RNA in the 5' to 3' direction.

Structure of RNA Polymerase

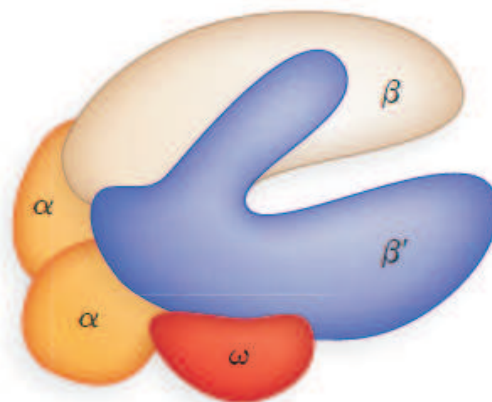
RNA polymerase is a complex enzyme with several subunits. Its structure varies between prokaryotes and eukaryotes.

Prokaryotic RNA Polymerase

In prokaryotes, a single RNA polymerase transcribes all types of RNA (mRNA, rRNA, tRNA) and the transcription and translation processes are coupled.

In prokaryotes (like bacteria), RNA polymerase is a multi-subunit enzyme with a core enzyme and an additional sigma (σ) factor:

- **Core enzyme:** The core enzyme is made up of five subunits:
 - Two α (alpha) subunits
 - One β (beta) subunit
 - One β' (beta prime) subunit
 - One ω (omega) subunit
 These subunits are responsible for the catalytic activity of RNA



Structure of the RNA polymerase holoenzyme

polymerization and can initiate transcription once bound to a promoter.

- **Sigma factor (σ):** The sigma factor is a regulatory protein that associates with the core enzyme to form the holoenzyme, which is capable of recognizing specific promoter sequences on DNA and initiating transcription. Once RNA polymerase starts synthesizing RNA, the sigma factor dissociates, and the core enzyme continues the elongation process.

Eukaryotic RNA Polymerase

In Eukaryotic cells transcription occurs in nucleus and translation in cytoplasm. Eukaryotic cells have three distinct RNA polymerases, each responsible for transcribing different classes of RNA:

- **RNA Polymerase I:** This enzyme synthesizes most rRNA (ribosomal RNA), except for 5S rRNA. It operates in the nucleolus.
- **RNA Polymerase II:** This enzyme is responsible for synthesizing mRNA (messenger RNA) and some small RNAs

(such as snRNA, involved in splicing). It operates in the nucleoplasm.

- **RNA Polymerase III:** This enzyme synthesizes tRNA (transfer RNA), 5S rRNA, and some small RNA molecules (such as U6 snRNA).

Each of these RNA polymerases has distinct subunits and functions:

RNA Polymerase II is the most studied and consists of 12 subunits in total, including several conserved subunits and others involved in transcription regulation.

Core promoter elements for RNA Polymerase II include the TATA box, initiator (Inr) and other regulatory sequences that bind transcription factors to regulate gene expression.

MECHANISM OF TRANSCRIPTION

The process of transcription involves three main stages: initiation, elongation, and termination.

Initiation

Binding to the Promoter:

In prokaryotes, the sigma factor helps RNA polymerase recognize specific promoter sequences (like the -10 and -35 regions) on the DNA.

In eukaryotes, RNA polymerase II requires a variety of transcription factors (TFs) to recognize the promoter region. The TATA-binding protein (TBP), part of the TFIID

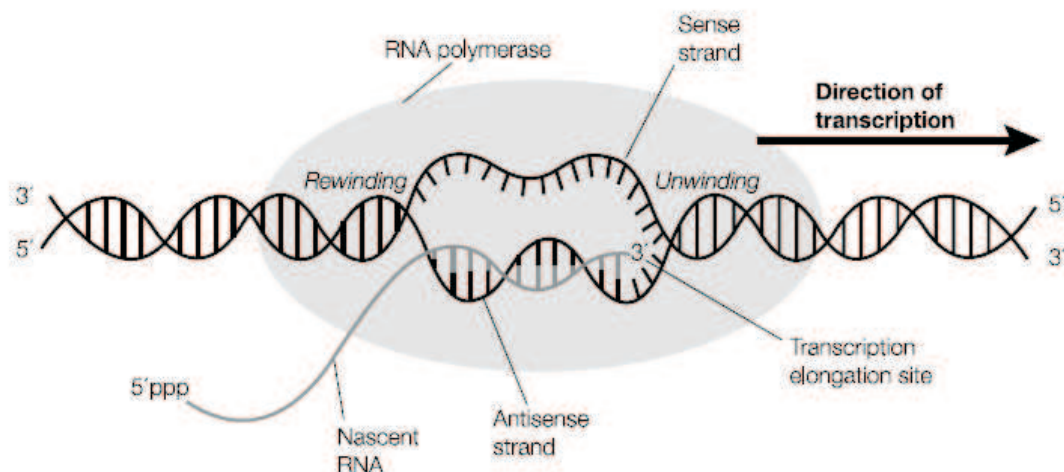
complex, binds to the TATA box, which is crucial for initiating transcription.

Formation of the Transcription Bubble: RNA polymerase unwinds the DNA double helix to form a "transcription bubble" that exposes the template strand, which will be used for RNA synthesis.

Elongation

After initiation, RNA polymerase moves along the DNA template strand, synthesizing RNA in the 5' to 3' direction.

The core enzyme is responsible for the polymerization of RNA by adding



Schematic structure of the transcription bubble during elongation.

ribonucleotides that are complementary to the DNA template.

The RNA polymerase also has a proofreading ability to correct any errors during RNA synthesis.

In eukaryotes, the elongation process is highly regulated, with several elongation factors (like TFIIF and others) interacting with RNA polymerase to modify its activity, stabilize the transcription complex and assist in the progression of transcription.

Termination

👉 Prokaryotic Termination:

Termination can occur in two primary ways:

- **Rho-independent termination:** Involves the formation of a hairpin loop in the RNA transcript, which causes the RNA polymerase to detach from the DNA.
- **Rho-dependent termination:** Requires the Rho protein, which binds to the RNA and moves toward the RNA polymerase. Upon contact, Rho causes the polymerase to dissociate from the DNA.

👉 Eukaryotic Termination:

In eukaryotes, RNA Polymerase II terminates transcription at specific sequences in the DNA, which is often coupled with the cleavage of the RNA transcript by the CPSF (cleavage and polyadenylation specificity factor) complex. This is followed by the addition of a poly-A tail to the mRNA.

Regulation of RNA Polymerase:

RNA polymerase activity is regulated at multiple levels, allowing cells to control gene expression in response to various signals.

- **Promoter and Enhancer Regions:** Regulatory sequences like promoters, enhancers, and silencers control when

and where RNA polymerase binds to DNA and transcribes genes. These elements are bound by various transcription factors.

- **Transcription Factors:** In both prokaryotes and eukaryotes, transcription factors (activators or repressors) influence RNA polymerase's ability to initiate transcription. In prokaryotes, for example, the sigma factor determines which genes are transcribed in response to environmental conditions.
- **Chromatin Remodeling:** In eukaryotes, the accessibility of the DNA is regulated by chromatin structure. Histone modifications and the recruitment of chromatin-remodeling complexes can make the DNA more or less accessible to RNA polymerase.
- **RNA Polymerase Phosphorylation:** In eukaryotic cells, RNA polymerase II is heavily phosphorylated at the C-terminal domain (CTD). Phosphorylation of specific serine residues in the CTD controls the transition from transcription initiation to elongation and also facilitates the recruitment of RNA processing machinery.

Errors and Proofreading

Although RNA polymerase is efficient in transcription, errors can occur during RNA synthesis. Unlike DNA polymerase, RNA polymerase does not have a proofreading exonuclease activity. However, RNA polymerase can backtrack and correct some errors during elongation, but this is less efficient than DNA proofreading.

TRANSCRIPTION FACTORS AND MACHINERY



Transcription is the process of synthesizing RNA from a DNA template, and it involves a complex interplay of enzymes, proteins, and regulatory factors. Transcription factors (TFs) and the associated transcriptional machinery are critical for ensuring that transcription occurs accurately, efficiently and in response to cellular needs.

Transcription Factors (TFs)

Definition: Transcription factors are proteins that bind to specific DNA sequences near genes to regulate the process of transcription. They play a vital role in determining when, where and how genes are expressed.

☞ Types of Transcription Factors:

General Transcription Factors (GTFs):

These are essential for the initiation of transcription in all genes transcribed by RNA polymerase. GTFs assemble at the core promoter to form the pre-initiation complex (PIC) with RNA polymerase. Examples include:

- **TFIID:** Contains the TATA-binding protein (TBP) and other subunits that recognize the promoter region.
- **TFIIB:** Helps position RNA polymerase II at the transcription start site.
- **TFIIF:** Stabilizes the RNA polymerase-DNA interaction.
- **TFIIE:** Recruits TFIIH and has roles in promoter melting.
- **TFIIH:** Has helicase and kinase activities to unwind DNA and phosphorylate RNA polymerase II's CTD (C-terminal domain).

Specific Transcription Factors:

These TFs are gene-specific and regulate transcription in a context-dependent manner (e.g., tissue-specific or signal-responsive).

- **Activators:** Bind to enhancers or promoters to increase transcription.
- **Repressors:** Bind to silencers or other regulatory sequences to inhibit transcription.
- **Coactivators and Corepressors:** Act as intermediaries between TFs and the transcriptional machinery but do not bind DNA directly.

Regulatory Transcription Factors:

- Respond to external signals (hormones, stress, etc.) and modulate transcription accordingly.
- Examples:
 - Steroid hormone receptors (e.g., estrogen receptor).
 - Signal transducers like NF-kB and STAT.

☞ Structural Features of Transcription Factors:

- **DNA-Binding Domains (DBDs):** Allow TFs to recognize and bind specific DNA sequences. Examples:
 - Helix-turn-helix (HTH)
 - Zinc finger domains
 - Leucine zippers
 - Helix-loop-helix (HLH)
- **Transactivation Domains (TADs):** Interact with the transcriptional machinery or coactivators to enhance transcription.
- **Dimerization Domains:** Facilitate the formation of homo- or heterodimers of transcription factors.

☞ Core Promoter and Regulatory Elements:

The core promoter is the DNA region where the general transcription machinery assembles to initiate transcription. Regulatory elements enhance or suppress transcription by interacting with specific transcription factors.

Core Promoter Elements:

- **TATA Box:** A conserved sequence ~25-30 bp upstream of the transcription start site. Recognized by the TATA-binding protein (TBP) within TFIID.
- **Initiator (Inr):** A sequence overlapping the transcription start site.
- **BRE (TFIIB Recognition Element):** Located upstream or downstream of the TATA box, helps TFIIB binding.
- **DPE (Downstream Promoter Element):** Located downstream of the start site.

Regulatory Elements:

- **Enhancers:** Distant sequences that increase transcription by interacting with activators. Can function irrespective of orientation or distance.
- **Silencers:** Sequences that bind repressors to reduce transcription.
- **Insulators:** Prevent inappropriate interactions between enhancers and promoters.
- **Promoter-Proximal Elements:** Located near the promoter, they are binding sites for specific transcription factors.

Transcription Machinery**RNA Polymerase II and Associated Factors:**

RNA Polymerase II: Responsible for synthesizing mRNA and some small nuclear RNAs. Contains multiple subunits, including the CTD, which is heavily phosphorylated during transcription to regulate the process.

Mediator Complex:

- A multi-protein complex that bridges transcription factors and RNA polymerase II.
- Acts as a coactivator to integrate signals from TFs and relay them to the transcriptional machinery.

Chromatin-Remodeling Complexes:

Modify chromatin structure to make DNA accessible to the transcription machinery. Examples: SWI/SNF complex, ISWI family.

Histone Modifications and Epigenetic Regulation:

- **Histone Acetylation:** Performed by histone acetyltransferases (HATs), which loosen chromatin and promote transcription.
- **Histone Methylation:** Performed by histone methyltransferases (HMTs), which can either activate or repress transcription depending on the specific histone marks.
- **Histone Deacetylation and Demethylation:** Performed by HDACs and histone demethylases, respectively, to repress transcription.

Cofactors:

- **Coactivators:** Enhance transcription by bridging TFs and RNA polymerase II (e.g., CBP/p300).
- **Corepressors:** Inhibit transcription by interacting with repressors or modifying chromatin.

Steps in Transcription with Transcription Factors**Pre-Initiation Complex (PIC) Formation:**

- TFIID binds the TATA box through TBP.
- TFIIA and TFIIB stabilize the DNA-TBP complex.
- TFIIF recruits RNA polymerase II to the promoter.
- TFIIIE and TFIIH bind to complete the PIC. TFIIH unwinds DNA and phosphorylates RNA polymerase II.

Initiation:

- RNA polymerase II synthesizes the first few nucleotides of RNA.

- The CTD of RNA polymerase II is phosphorylated, allowing the transition to elongation.

👉 Elongation:

- Elongation factors (e.g., P-TEFb) enhance RNA polymerase II processivity.
- Chromatin remodelers maintain an open DNA template.

👉 Termination:

- Termination factors signal the release of RNA polymerase II from the DNA template.
- RNA is cleaved, and a poly-A tail is added.

👉 Regulation of Transcription Factors:

Transcription factors are regulated by:

- **Post-Translational Modifications:** Phosphorylation, acetylation, ubiquitination, etc.

- **Nuclear Localization:** Some TFs are activated by translocation to the nucleus (e.g., NF-κB).
- **Ligand Binding:** Steroid hormone receptors bind hormones to become active.
- **Protein-Protein Interactions:** TFs often interact with coactivators, corepressors, or other TFs to regulate activity.

👉 Diseases Linked to Transcription Factor Dysfunction:

- **Cancer:** Overexpression or mutations in TFs like MYC, p⁵³, or STAT can lead to uncontrolled cell proliferation.
- **Developmental Disorders:** Mutations in TFs like Hox genes can disrupt normal development.
- **Autoimmune Diseases:** Dysregulation of NF-κB or STAT pathways can result in chronic inflammation.

FORMATION OF INITIATION COMPLEX

The formation of the initiation complex is a crucial step in the transcription process. It occurs at the core promoter region of a gene and involves a series of sequential interactions between the DNA, RNA polymerase, and general transcription factors (GTFs). Below is a detailed explanation of this process:

Key Components Involved

- **RNA Polymerase II:** The enzyme responsible for synthesizing mRNA and some small nuclear RNAs (snRNAs).
- **Core Promoter Elements:** DNA sequences such as the TATA box, Initiator (Inr), BRE, and DPE that provide binding sites for transcription factors.
- **General Transcription Factors (GTFs):** Protein factors required for the recruitment

of RNA polymerase II and initiation of transcription:

- **TFIID:** Recognizes and binds the core promoter.
- **TFIIA:** Stabilizes the TFIID-promoter complex.
- **TFIIB:** Helps RNA polymerase II position at the transcription start site.
- **TFIIF:** Escorts RNA polymerase II to the promoter and stabilizes the complex.
- **TFIIE:** Recruits TFIIH and has roles in DNA melting.
- **TFIIH:** Unwinds DNA at the promoter and phosphorylates RNA polymerase II's C-terminal domain (CTD).

Step-by-Step Formation of the Initiation Complex

👉 Assembly of the Pre-Initiation Complex (PIC)

Binding of TFIID to the Core Promoter:

- The process begins with TFIID recognizing and binding to the TATA box in the promoter.
- TFIID contains the TATA-binding protein (TBP) and TBP-associated factors (TAFs).
- TBP binds directly to the TATA box, causing a local distortion in the DNA helix, making the DNA accessible for other transcription factors.
- If the TATA box is absent, TAFs within TFIID interact with other promoter elements (e.g., Inr or DPE).

Recruitment of TFIIA:

- TFIIA binds to the DNA-TFIID complex, stabilizing TBP's interaction with the promoter and enhancing the recruitment of other factors.

Binding of TFIIB:

- TFIIB binds to the BRE element upstream or downstream of the TATA box.
- TFIIB acts as a bridge between TFIID and RNA polymerase II, helping to position the polymerase at the transcription start site.

Recruitment of RNA Polymerase II and TFIIF:

- TFIIF, which is already associated with RNA polymerase II, binds to the complex.
- TFIIF stabilizes the interaction of RNA polymerase II with TFIIB and the promoter, ensuring proper orientation of RNA polymerase II for transcription.

Recruitment of TFIIH and TFIID:

- TFIIH binds to the complex, creating a docking site for TFIID.

- TFIIH joins the assembly. It has two important activities:

- **Helicase activity:** Unwinds DNA at the transcription start site, creating a single-stranded template for RNA synthesis.
- **Kinase activity:** Phosphorylates the C-terminal domain (CTD) of RNA polymerase II, which is essential for the transition to the elongation phase.

👉 Promoter Melting

- TFIIH uses its helicase activity to unwind the DNA helix at the transcription start site. This step is called promoter melting, which exposes the template strand for base pairing with incoming ribonucleotides.
- ATP hydrolysis provides the energy required for DNA unwinding.

👉 Formation of the Open Complex

- Once the DNA is unwound, RNA polymerase II and the associated factors form the open complex.
- In this state, the template strand is positioned in the active site of RNA polymerase II, ready for RNA synthesis.

👉 Abortive Initiation

- RNA polymerase II synthesizes short RNA chains (2-9 nucleotides) during this phase. This is referred to as abortive initiation, as the short RNA chains are often released before elongation begins.

👉 Transition to the Elongation Phase

Phosphorylation of the CTD:

- TFIIH phosphorylates serine residues in the CTD of RNA polymerase II. This phosphorylation is a key signal for the polymerase to leave the promoter and transition to elongation.
- Phosphorylation also recruits RNA processing factors for capping, splicing and polyadenylation.

Disassembly of GTFs:

- Most GTFs dissociate from the promoter, except for TFIID, which may remain bound to recruit another RNA polymerase II for subsequent rounds of transcription.

Release of the RNA Polymerase II Core Enzyme:

- RNA polymerase II begins the elongation of the RNA transcript, moving along the DNA template.

INITIATION, ELONGATION AND TERMINATION OF TRANSCRIPTION IN PROKARYOTES AND EUKARYOTES

Transcription is the process by which RNA is synthesized from a DNA template. While the basic mechanism of transcription is conserved across prokaryotes and eukaryotes, there are important differences in how the processes occur in each. Here's a detailed comparison of transcription initiation, elongation, and termination in prokaryotes and eukaryotes:

Initiation of Transcription

Prokaryotes:

Promoter Recognition:

- The promoter in prokaryotes typically has two key elements: the -35 box (TTGACA) and the -10 box (TATAAT), located approximately 35 and 10 base pairs upstream of the transcription start site, respectively.
- Sigma Factor (σ) is a subunit of RNA polymerase that recognizes and binds to the promoter elements. The sigma factor helps position the RNA polymerase at the correct start site.

RNA Polymerase Binding:

- The core RNA polymerase (composed of 5 subunits: $2\alpha, \beta, \beta', \omega$) binds the promoter region with the help of the sigma factor to form the holoenzyme (core enzyme + sigma factor).
- The holoenzyme binds to the promoter, causing the DNA to unwind, forming a closed complex.

Formation of the Open Complex:

- RNA polymerase unwinds the DNA around the -10 region, resulting in the formation of the open complex. The transcription bubble is now formed, with the DNA strands separated.

Initiation of RNA Synthesis:

- RNA polymerase starts synthesizing RNA from the DNA template. Initially, it synthesizes short RNA fragments (usually 2-9 nucleotides) in a process known as abortive initiation.
- After several abortive cycles, the sigma factor is released, and the RNA polymerase transitions into the elongation phase.

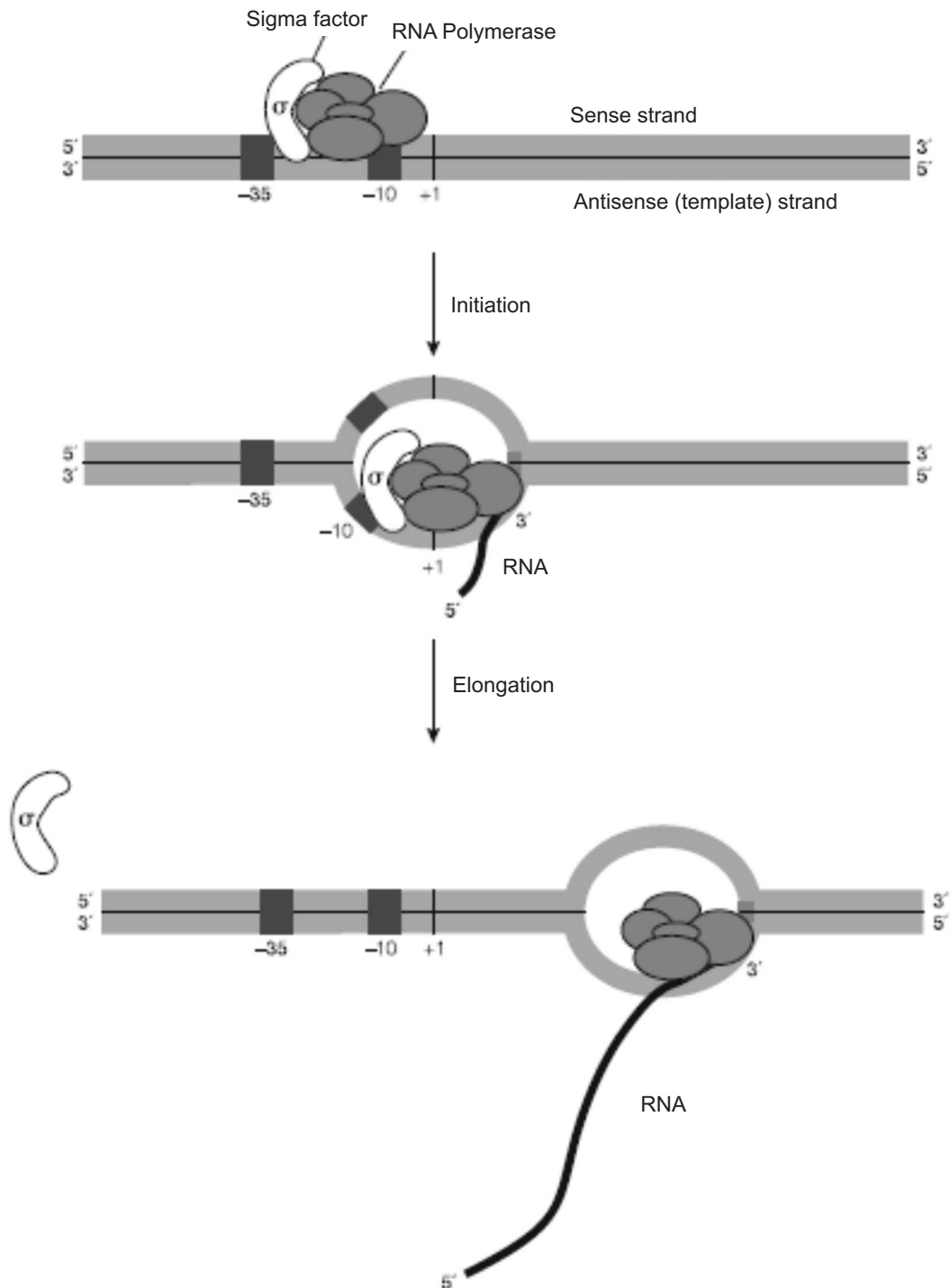
Eukaryotes:

Promoter Recognition:

- The core promoter in eukaryotes often contains a TATA box (located about 25-30 base pairs upstream of the transcription start site) and other regulatory elements like Inr (Initiator) and DPE (Downstream Promoter Element).
- Eukaryotic transcription involves a more complex set of general transcription factors (GTFs) compared to prokaryotes.

Assembly of the Pre-Initiation Complex (PIC):

- TFIID, which contains the TATA-binding protein (TBP), binds to the TATA box.
- Other general transcription factors (e.g., TFIIA, TFIIB, TFIID, TFIIIE, TFIIF) and



Formation of the transcription complex: initiation and elongation.

RNA polymerase II then assemble at the promoter to form the pre-initiation complex (PIC).

- TFIIF has helicase activity that unwinds the DNA to form the open complex and kinase activity that phosphorylates the C-terminal domain (CTD) of RNA polymerase II, enabling the transition to elongation.

Initiation of RNA Synthesis:

- Once the open complex is formed, RNA polymerase II begins RNA synthesis. Like in prokaryotes, initial RNA synthesis involves abortive initiation (short RNA transcripts).
- After several abortive cycles, RNA polymerase II fully transitions into elongation.

Elongation of Transcription

📖 Prokaryotes:

Elongation Process:

- Once initiation is complete and the sigma factor is released, the RNA polymerase proceeds down the DNA template strand.
- RNA polymerase synthesizes the RNA in a 5' to 3' direction, using the DNA template strand as a guide. The RNA polymerase moves along the template strand, unwinding the DNA ahead and rewinding the DNA behind.

RNA Synthesis:

- RNA polymerase adds ribonucleotides (A, U, C, G) complementary to the DNA template strand.
- Elongation continues as RNA polymerase moves along the DNA until a termination signal is reached.

Coupling with Translation:

- In prokaryotes, transcription and translation are coupled. While RNA is

being transcribed, ribosomes begin translating the mRNA immediately.

📖 Eukaryotes:

Elongation Process:

- RNA polymerase II begins elongation after initiating RNA synthesis.
- The CTD (C-terminal domain) of RNA polymerase II is crucial in this phase. It is phosphorylated at different positions to recruit factors necessary for RNA processing, including capping, splicing, and polyadenylation.

RNA Synthesis:

- Like in prokaryotes, RNA polymerase II synthesizes the RNA strand in the 5' to 3' direction. The transcription bubble is maintained, with RNA polymerase moving along the DNA template.

Chromatin Remodeling:

- As RNA polymerase II moves along the DNA, chromatin remodeling complexes (such as SWI/SNF) may be involved in making the DNA template accessible for transcription.
- Histone modifications (e.g., acetylation) play a role in maintaining the chromatin structure conducive to transcription.

Termination of Transcription

📖 Prokaryotes:

Rho-Dependent Termination:

- The Rho protein binds to the nascent RNA and moves toward the RNA polymerase. When it catches up to RNA polymerase, it causes the polymerase to dissociate from the DNA, ending transcription.
- This termination requires the presence of a rut site (Rho utilization site) on the RNA.

Rho-Independent Termination:

- This mechanism involves the formation of a GC-rich hairpin loop in the RNA

transcript, followed by a string of uracils. The hairpin loop causes RNA polymerase to pause, and the weak U-A bonds between the RNA and DNA cause the RNA polymerase to release the RNA transcript.

- No additional protein factors are required for this termination process.

👉 Eukaryotes:

RNA Polymerase I (for rRNA):

- Termination is mediated by specific termination factors that interact with RNA polymerase I and cause it to dissociate from the DNA.

RNA Polymerase II (for mRNA):

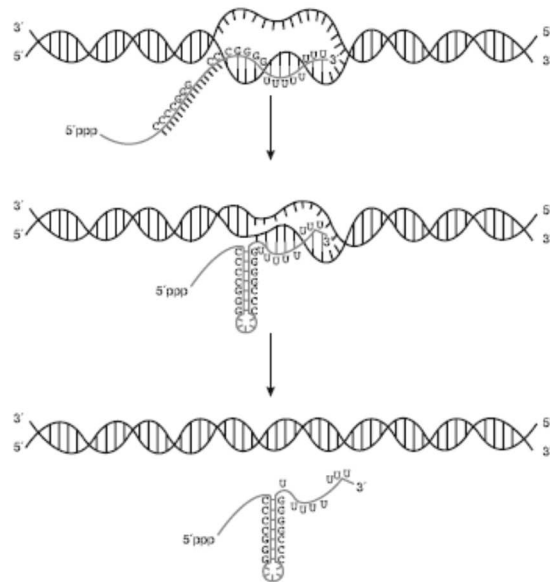
- The termination process is more complex. After the RNA transcript reaches a signal, it is cleaved by cleavage and polyadenylation factors.
- The polyadenylation signal (AAUAAA) is recognized by cleavage factors, and the transcript is cleaved. The 3' end of the RNA is then polyadenylated.
- After cleavage, RNA polymerase II continues to transcribe for a short distance (called torpedo model), and the transcript is eventually released as RNA degradation occurs by XRN2, a 5' to 3' exonuclease.

RNA Polymerase III (for tRNA and small RNAs):

- Termination is also mediated by specific termination factors that bind to sequences within the transcribed RNA and cause the polymerase to release the RNA.

Differences Between Prokaryotes and Eukaryotes

Process	Prokaryotes	Eukaryotes
Promoter Elements	-10 box, -35 box	TATA box, Inr, DPE, BRE



Schematic diagram of rho-independent transcription termination.

RNA Polymerase	Single RNA polymerase (core + sigma factor)	Three types of RNA polymerases (I, II, III)
General Factors	Sigma factor and core RNA polymerase	Multiple general transcription factors (TFIID, TFIIH, etc.)
Initiation	Sigma factor binds to promoter, forms holoenzyme	GTFs assemble at the promoter to form the PIC
Elongation	RNA polymerase synthesizes RNA; no CTD phosphorylation	RNA polymerase II; CTD phosphorylation necessary for elongation
Termination	Rho-dependent or Rho-independent mechanisms	Cleavage, polyadenylation, and termination factors

QUESTIONS



Section A: Very Short Answer Type Questions

1. What is a cistron? (CCSU 2023)

Ans. A functional unit of a gene that codes for a single polypeptide.

2. Define split genes. (CCSU 2022)

Ans. Genes with coding (exons) and non-coding (introns) sequences (e.g., eukaryotic genes).

3. What are promoter regions? (CCSU 2021)

Ans. DNA sequences where RNA polymerase binds to initiate transcription.

4. Which RNA polymerase synthesizes mRNA in eukaryotes? (CCSU 2023)

Ans. RNA polymerase II.

5. Name the RNA polymerase found in prokaryotes. (CCSU 2022)

Ans. A single RNA polymerase (holoenzyme with σ factor).

6. What is the role of σ (sigma) factor? (CCSU 2020)

Ans. Helps prokaryotic RNA polymerase recognize promoter sequences.

7. List two transcription factors in eukaryotes. (CCSU 2023)

Ans. TFIID (binds TATA box) and TFIIH (helicase activity).

8. What is the function of TATA box? (CCSU 2021)

Ans. A eukaryotic promoter sequence guiding RNA polymerase II binding.

9. Why is a primer not needed in transcription? (CCSU 2020)

Ans. RNA polymerase can initiate RNA synthesis de novo (unlike DNA polymerase).

10. What comprises the pre-initiation complex (PIC) in eukaryotes? (CCSU 2022)

Ans. RNA polymerase II + General transcription factors (GTFs) + Mediator.

11. How does prokaryotic transcription initiation differ from eukaryotic? (CCSU 2021)

Ans. Prokaryotes use σ factor, while eukaryotes require multiple GTFs.

12. What signals transcription initiation in prokaryotes? (CCSU 2023)

Ans. σ factor binding to -10 (Pribnow box) and -35 promoter regions.

13. Name the first nucleotide added in eukaryotic transcription. (CCSU 2022)

Ans. Usually a purine (A or G).

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14. What is the role of NusA in elongation? (CCSU 2021)

Ans. A prokaryotic protein preventing transcription termination.

15. How does eukaryotic RNA processing occur during elongation? (CCSU 2020)

Ans. 5' capping and splicing occur co-transcriptionally.

16. What is rho (ρ)-dependent termination? (CCSU 2023)

Ans. ρ protein binds RNA and displaces RNA polymerase at terminator sequences.

17. How does polyadenylation signal termination in eukaryotes? (CCSU 2022)

Ans. AAUAAA sequence triggers cleavage and poly-A tail addition.

Section B: Short Answer Type Questions

18. Define a gene in terms of its fine structure. (CCSU 2022)

Ans. A gene, in terms of its fine structure, is defined as a segment of DNA that contains the necessary information to produce a functional product, usually a protein or RNA molecule. The fine structure of a gene includes:

- **Coding sequences (exons):** Regions that code for amino acids in proteins.
- **Non-coding sequences (introns):** Intervening sequences that are removed during RNA processing in eukaryotes.
- **Regulatory sequences:** Including promoters, enhancers, silencers that control the expression of the gene.
- **Transcription start site:** Where RNA polymerase begins transcription.
- **Termination site:** Where transcription ends.

Thus, a gene is not just a continuous stretch of DNA coding for protein but a complex unit including all elements required for its precise expression and regulation

19. Name the RNA polymerase responsible for transcription in E. coli. (CCSU 2021)

Ans. In *Escherichia coli* (*E. coli*), a single RNA polymerase enzyme is responsible for the transcription of all types of RNA (mRNA, rRNA, tRNA).

- It is called DNA-dependent RNA polymerase or simply E. coli RNA polymerase.

20. What are transcription factors? Give one example. (CCSU 2020)

Ans. Transcription factors (TFs) are proteins that help regulate gene expression by binding to specific DNA sequences near genes to either promote or inhibit transcription. They assist RNA polymerase in initiating transcription and can respond to cellular signals to control when and how much a gene is expressed.

Example: TATA-binding protein (TBP) - a component of the transcription factor complex that binds to the TATA box in eukaryotic promoters, helping to position RNA polymerase II for transcription initiation

21. Differentiate between promoter and terminator in transcription.

(CCSU 2019)

Feature	Promoter	Terminator
Definition	DNA sequence where RNA polymerase binds to initiate transcription	DNA sequence signaling the end of transcription
Function	Initiates transcription by recruiting RNA polymerase and transcription factors	Stops transcription and releases the RNA transcript
Location	Upstream (5' end) of the gene	Downstream (3' end) of the gene
Examples	-35 and -10 sequences in prokaryotes (Pribnow box)	Rho-dependent or rho-independent terminators in prokaryotes
Role	Controls when and where transcription begins	Ensures proper termination and release of the transcript

22. Which sigma factor is used in prokaryotic transcription initiation?

(CCSU 2018)

Ans. The sigma factor $\sigma 70$ (sigma 70) is the primary sigma factor used in *E. coli* for the initiation of transcription of most housekeeping genes during normal growth.

- It helps the RNA polymerase holoenzyme recognize the -35 and -10 promoter regions to start transcription.

Section C: Long Answer Type Questions

23. Explain the process of transcription in prokaryotes, including initiation, elongation, and termination.

(CCSU 2022)

Ans. Transcription is the process by which genetic information from DNA is copied into messenger RNA (mRNA) in prokaryotic cells. It occurs in three main stages:

a) Initiation:

- Transcription begins when the RNA polymerase holoenzyme (core enzyme + sigma factor, usually $\sigma 70$) binds to the promoter region of DNA, located upstream of the gene.
- The promoter contains conserved sequences at the -35 and -10 regions (Pribnow box).
- The sigma factor helps the RNA polymerase recognize and bind specifically to the promoter.
- Upon binding, the DNA strands locally unwind, forming an open complex, allowing RNA polymerase to access the template strand.
- The RNA polymerase starts RNA synthesis by adding ribonucleotides complementary to the DNA template, usually starting with a purine (A or G).

b) Elongation:

- After synthesizing the first few nucleotides, the sigma factor dissociates, and the core RNA polymerase continues RNA synthesis.

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- RNA polymerase moves along the DNA template strand in the 3' to 5' direction, synthesizing RNA in the 5' to 3' direction.
- The DNA ahead of the enzyme unwinds, and behind it rewinds, forming a transcription bubble.
- The RNA transcript grows as nucleotides are added.

c) Termination:

- Transcription stops when RNA polymerase encounters a terminator sequence on DNA.
- Two types of termination occur:

Rho-independent (intrinsic) termination: The RNA forms a GC-rich hairpin followed by a string of U residues, causing the polymerase to pause and dissociate.

Rho-dependent termination: The rho protein binds to the RNA and moves towards RNA polymerase, causing it to dissociate when it catches up.

- The RNA transcript is released, and RNA polymerase detaches from the DNA.

24. Compare and contrast transcription in prokaryotes and eukaryotes. (CCSU 2021)

Ans.

Feature	Prokaryotes	Eukaryotes
Location	Cytoplasm	Nucleus
RNA Polymerases	Single RNA polymerase	Three main RNA polymerases (I, II, III)
Initiation	Requires sigma factor for promoter binding	Requires multiple general transcription factors and formation of initiation complex
Promoters	Simple promoters (-35 and -10 sequences)	Complex promoters (TATA box, enhancers, silencers)
RNA Processing	mRNA often ready for translation immediately (no introns)	Pre-mRNA undergoes capping, polyadenylation, splicing to become mature mRNA
Termination	Rho-dependent and rho-independent	Complex termination mechanisms involving cleavage and polyadenylation signals
Coupling with translation	Transcription and translation occur simultaneously	Transcription and translation are spatially and temporally separated
Chromatin Structure	DNA not packaged in chromatin	DNA packaged in nucleosomes; chromatin remodeling required

25. Describe the role of RNA polymerase II in eukaryotic transcription. (CCSU 2020)

Ans. RNA polymerase II is a key enzyme responsible for synthesizing pre-messenger RNA (pre-mRNA) and some small nuclear RNAs (snRNAs) in eukaryotic cells.

- It binds to promoters with the help of transcription factors and catalyzes the synthesis of RNA from the DNA template.

- RNA polymerase II transcribes protein-coding genes and produces primary transcripts that undergo extensive processing (capping, splicing, polyadenylation).
- It has a unique C-terminal domain (CTD) with repeats of a heptapeptide sequence that is phosphorylated during transcription, coordinating RNA processing events.
- It plays a central role in regulating gene expression, interacting with activators, repressors and chromatin modifiers.

26. How does the transcription initiation complex form in eukaryotes? Discuss the role of TATA box and transcription factors. (CCSU 2019)

Ans. The transcription initiation complex in eukaryotes assembles at the promoter region of a gene, especially at the TATA box, which is a conserved DNA sequence about 25-30 base pairs upstream of the transcription start site.

- The process begins with the binding of the TATA-binding protein (TBP), a subunit of TFIID, to the TATA box.
- This binding causes a bend in DNA and facilitates the recruitment of other general transcription factors (GTFs) like TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH.
- These factors help recruit RNA polymerase II to the promoter, forming the pre-initiation complex (PIC).
- TFIIH has helicase activity that unwinds DNA at the transcription start site and kinase activity that phosphorylates the CTD of RNA polymerase II, triggering the transition from initiation to elongation.
- Other transcription factors and co-activators may bind enhancers or silencers to regulate the efficiency of initiation.

28. Differentiate between rho-dependent and rho-independent termination in prokaryotes? (CCSU 2018)

Ans.

Feature	Rho-dependent termination	Rho-independent termination
Requirement	Requires rho protein	Does not require rho protein
Mechanism	Rho protein binds to rut site on RNA, moves along transcript, and causes RNA polymerase to dissociate	Formation of a GC-rich hairpin loop followed by a series of U residues in RNA causes polymerase to pause and dissociate
Energy source	ATP-dependent helicase activity of rho	No ATP required
RNA sequence signals	Presence of rut (rho utilization) site	Inverted repeats followed by poly-U tract
Outcome	Releases RNA transcript by unwinding RNA-DNA hybrid	Transcription stops due to destabilization of RNA-DNA hybrid

29. Why do eukaryotes have three RNA polymerases while prokaryotes have only one?

Ans. Eukaryotes have three distinct RNA polymerases (I, II, III) because their cellular complexity requires specialized enzymes to transcribe different types of RNA. Each polymerase has unique promoter recognition, transcription factors, and regulatory mechanisms suited to its specific function:

- **RNA Polymerase I:** Synthesizes most ribosomal RNA (rRNA) except 5S rRNA. This is crucial because eukaryotic ribosomes are large and complex, requiring abundant rRNA.
- **RNA Polymerase II:** Transcribes protein-coding genes into pre-mRNA and some small nuclear RNAs (snRNAs). It requires sophisticated regulation because gene expression must be tightly controlled in multicellular organisms.
- **RNA Polymerase III:** Synthesizes transfer RNA (tRNA), 5S rRNA, and other small RNAs essential for translation and RNA processing.

Prokaryotes, being less complex, use a single RNA polymerase that transcribes all types of RNA (mRNA, rRNA, and tRNA). Their simpler genome organization and regulatory needs do not require enzyme specialization.

30. How does alternative splicing affect gene expression?

Ans. Alternative splicing is a process where different combinations of exons are joined together or excluded from the pre-mRNA transcript during RNA processing in eukaryotes. This allows a single gene to produce multiple distinct mRNA isoforms, which can be translated into different protein variants.

Effects on gene expression:

- **Increases protein diversity:** Alternative splicing enables one gene to encode proteins with different structures and functions, expanding the proteome without increasing genome size.
- **Tissue specificity:** Different splicing patterns can occur in different tissues or developmental stages, allowing proteins to be tailored to specific cellular needs.
- **Regulation of function:** Some splice variants can alter protein localization, stability, or activity, influencing cellular pathways.
- **Disease association:** Aberrant alternative splicing can cause diseases by producing dysfunctional proteins or by disrupting gene regulation.

31. What would happen if the sigma factor is mutated in E. coli?

Ans. The sigma factor in E. coli is essential for guiding RNA polymerase to specific promoter sequences to initiate transcription. A mutation in the sigma factor could have several consequences:

- **Reduced or abolished promoter recognition:** The mutated sigma factor may fail to bind promoter regions efficiently, preventing the initiation of transcription for many genes.
- **Impaired gene expression:** Essential genes may not be transcribed, leading to defective cellular functions and possibly cell death.
- **Altered specificity:** If the mutation changes the sigma factor's binding preference, RNA polymerase might bind incorrect promoters, causing misregulation of genes.

- **Overall growth defects:** Since the sigma factor controls the transcription of housekeeping genes, a mutation could impair bacterial growth, stress response, and adaptability.
- In summary, sigma factor mutations can severely compromise the ability of *E. coli* to transcribe genes and survive.

32. Compare the roles of snRNPs in splicing vs. ribozymes in self-splicing introns.

Ans.

Aspect	snRNPs in Splicing	Ribozymes in Self-Splicing Introns
Definition	Small nuclear ribonucleoproteins (snRNPs) are complexes of RNA and protein involved in the removal of introns from pre-mRNA in spliceosomes.	Ribozymes are RNA molecules with catalytic activity that can self-excite introns without protein enzymes.
Mechanism	snRNPs assemble into the spliceosome that recognizes splice sites and catalyzes two transesterification reactions to remove introns.	Ribozymes catalyze their own excision through RNA folding and catalytic activity, carrying out the splicing independently.
Location	Found in eukaryotic nuclei, operating on nuclear pre-mRNA.	Found in some prokaryotic and eukaryotic organellar RNAs (e.g., mitochondrial or chloroplast RNAs).
Protein involvement	Require numerous proteins along with snRNAs for splicing.	Mostly protein-independent, relying on RNA catalytic function.
Splicing complexity	Capable of splicing a wide variety of introns, especially complex splice sites in nuclear genes.	Typically involved in group I and group II introns with simpler and self-contained sequences.
Evolutionary significance	Represents an evolutionarily advanced mechanism involving RNA-protein complexes.	Suggests an ancient RNA world where RNA molecules had catalytic roles before proteins evolved