RNA PROCESSING AND SPLICING

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Molecular Biology

Semester I

Week 7

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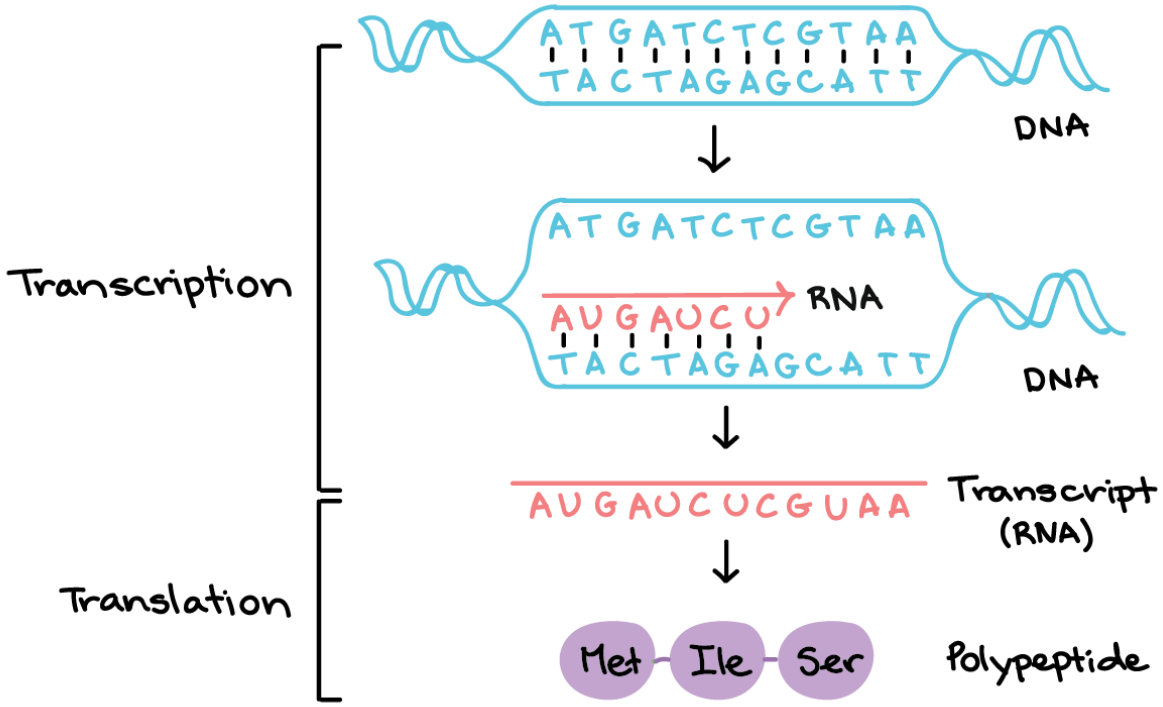
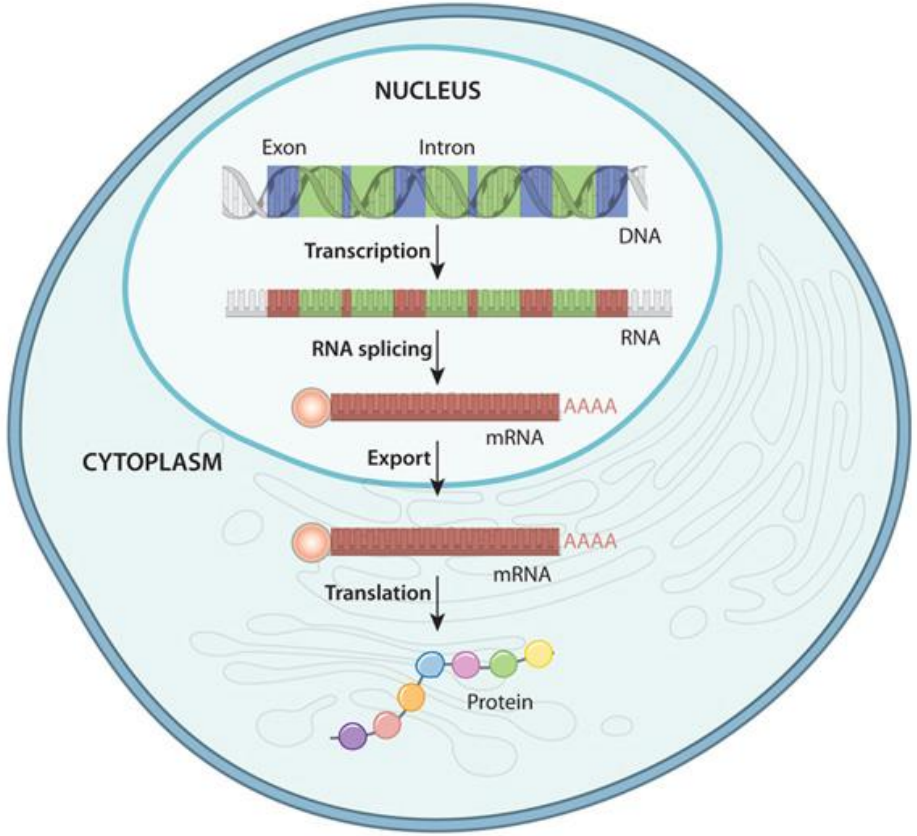
Learning Objectives

- **Outline** the main steps of RNA processing (capping, splicing, polyadenylation).
- **Identify** key splice sites and the components of the spliceosome.
- **Explain** how introns are removed and exons joined.
- **Describe** the importance of alternative splicing.
- **Recognize** diseases and therapies related to splicing defects.

Review


Transcription
Translation
ATGATCTCGTAA
TACTAGAGCATT
DNA
ATGATCTCGTAA
AUGAUCU
RNA
TACTAGAGCATT
DNA
AUGAUCUCGUAA
Transcript (RNA)
Met
Ile
Ser
Polypeptide

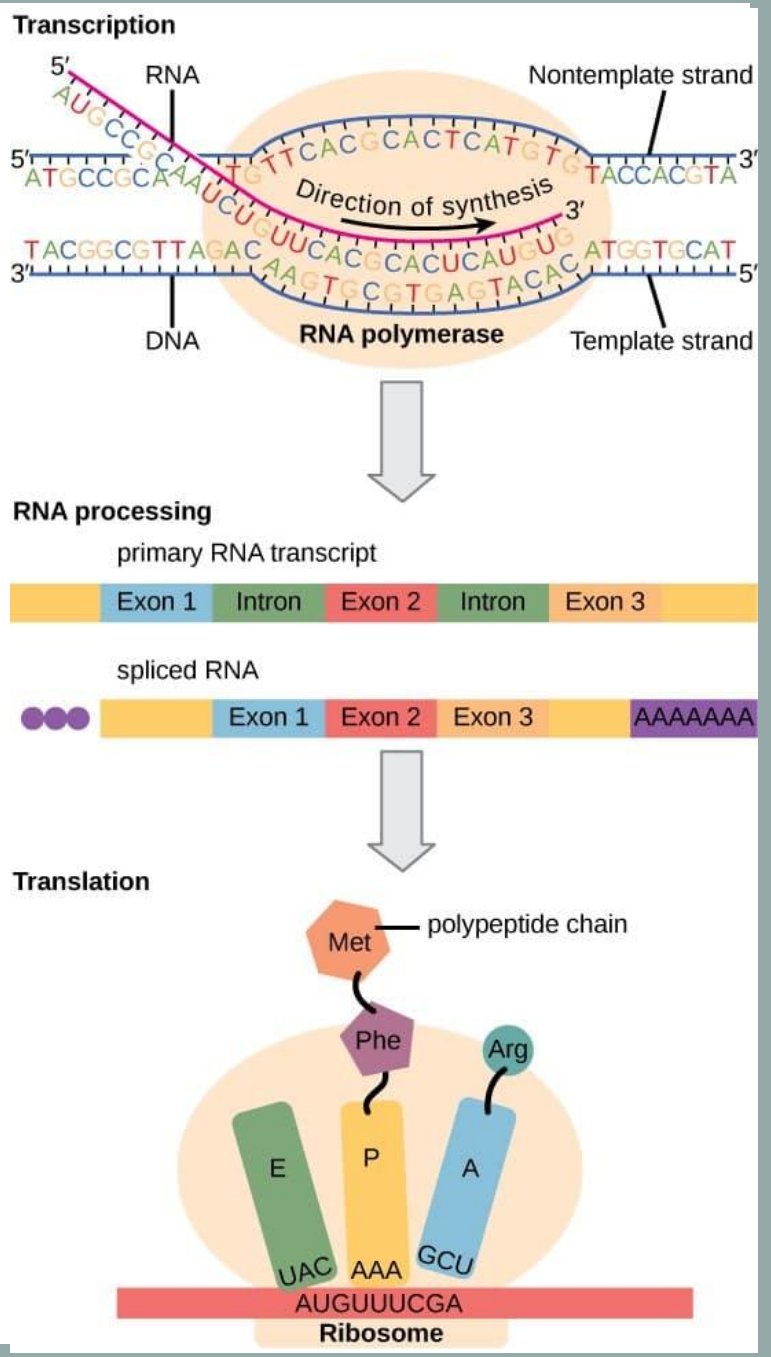

NUCLEUS
Exon
Intron
DNA
Transcription
RNA
RNA splicing
AAAA
mRNA
CYTOPLASM
Export
AAAA
mRNA
Translation
Protein

Overview of RNA Processing

Eukaryotic pre-mRNA undergoes three major modifications before leaving the nucleus:

1. **5′ capping** at the start of the transcript,
2. **splicing** to remove introns and join exons, and
3. **polyadenylation** at the 3′ end.

These events stabilize the mRNA, protect it from exonucleases, and ensure efficient translation. Without these modifications, the mRNA would be quickly degraded or fail to be recognized by the ribosome.



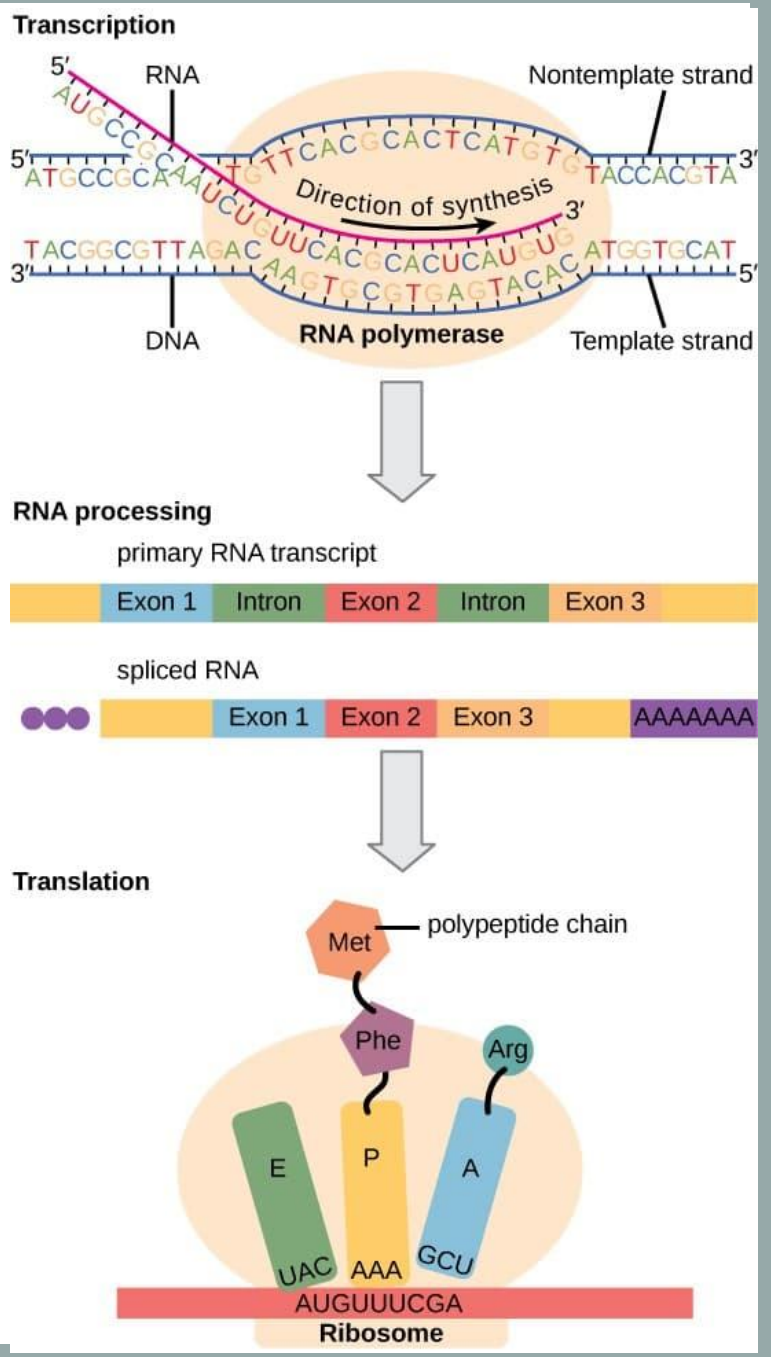
Overview of RNA Processing

Unlike bacteria, eukaryotes have introns and exons. The presence of introns offers evolutionary benefits such as alternative splicing, but it requires quality control.

Processing:

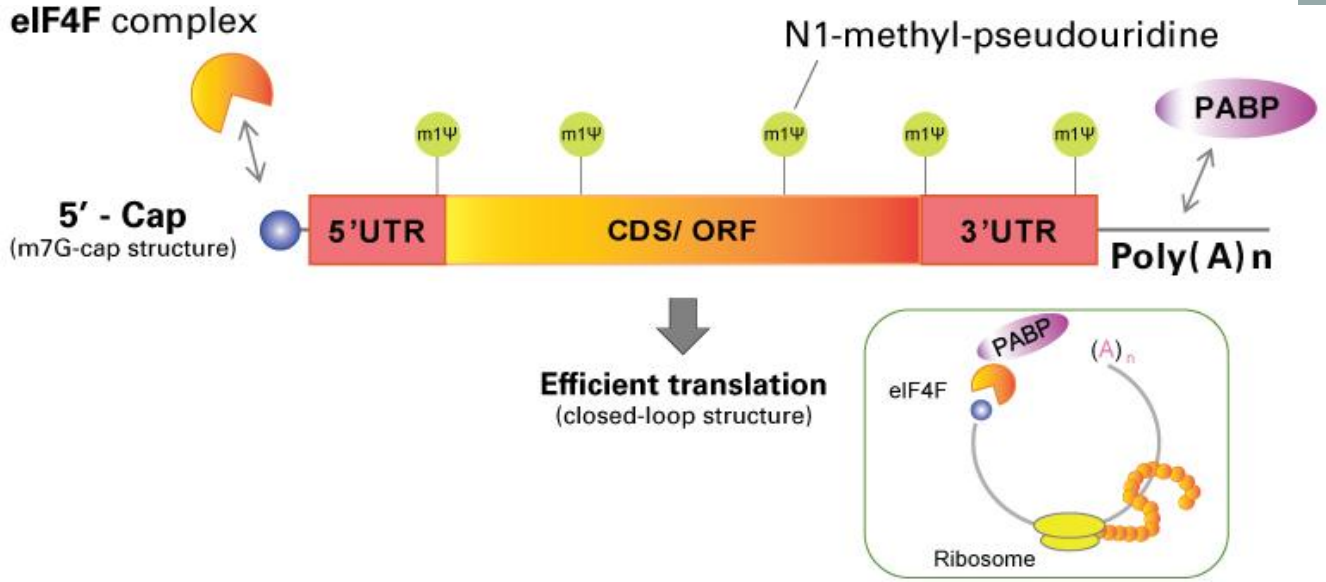
- Protects RNA stability
- Ensures nuclear export through nuclear pore complexes
- Mark mRNA as ‘self’ to differentiate from viral RNA
- Promotes accurate ribosome loading

The nucleus acts like a ‘production facility’ ensuring only fully processed transcripts reach the cytoplasm.



The 5′ Cap Structure

- The 5′ m7G cap is one of the earliest modifications of mRNA. It is added when the transcript is around 20–30 nucleotides long. The cap consists of a 7-methylguanosine nucleotide linked via a unique 5′–5′ triphosphate bridge.
- This special linkage makes the RNA resistant to 5′ exonucleases. It also recruits the translation machinery, particularly eIF4E, which recognizes the cap and directs the ribosome to initiate scanning.



5’ Capping: Mechanism

- **Three enzymes carry out capping:**

  1) RNA triphosphatase removes a phosphate.

  2) Guanylyltransferase adds GMP in reverse orientation.

  3) Methyltransferase methylates the guanine to create m7G.

- This happens co-transcriptionally, meaning the cap helps regulate transcription elongation itself. The cap also interacts with the cap-binding complex (CBC), which accompanies the mRNA through splicing and nuclear export.

Functions of the 5’ Cap

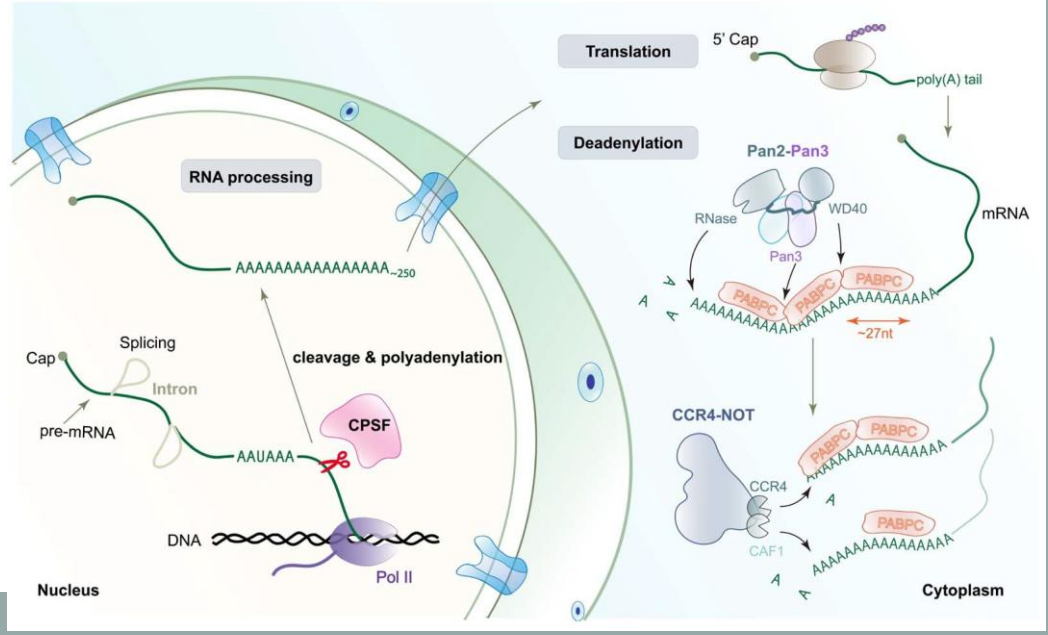
The cap has multiple roles:

- Protects from exonuclease digestion.
- Required for ribosome recognition.
- Promotes efficient translation initiation.
- Helps mRNA circularization by interacting with the poly(A)-binding proteins.
- Facilitates mRNA export out of the nucleus.

Viruses such as influenza steal caps from host mRNAs, a process known as ‘cap snatching’ to stabilize their own RNA.

3′ End Polyadenylation

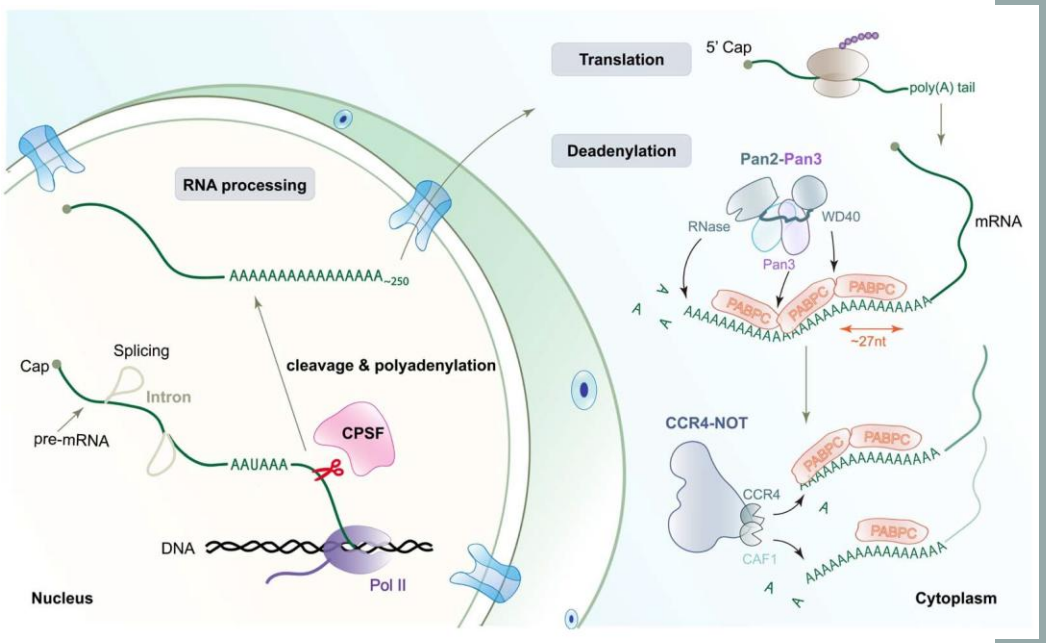
Near the end of transcription, RNA polymerase encounters a polyadenylation signal, usually the sequence AAUAAA. Proteins such as CPSF (cleavage and polyadenylation specificity factor) bind this site and guide the cleavage reaction. Poly(A) polymerase then adds 150–250 adenine residues without requiring a template. The poly(A) tail's length correlates with mRNA stability; the longer the tail, the more stable the mRNA.



Poly(A) Tail Functions

The poly(A) tail plays key roles in mRNA metabolism:

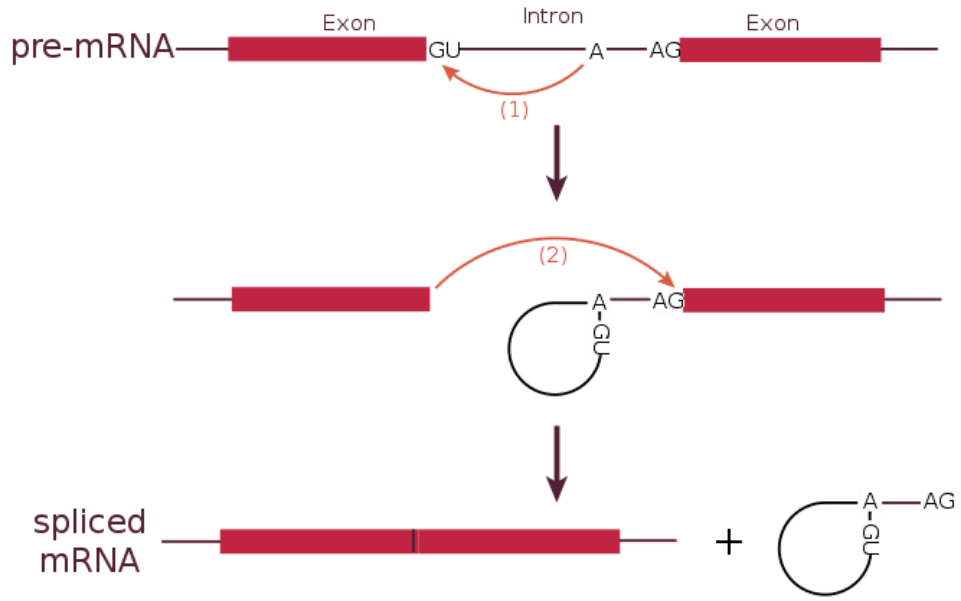
- **Stabilizes** the transcript against degradation.
- Helps **initiate translation** through interaction with PABP (Poly(A)-binding protein).
- Assists in **nuclear export**.
- Promotes **mRNA circularization,** the 5’ cap interacts with the 3’ tail during translation, forming a closed-loop structure that enhances ribosome recycling.



Splicing Introduction

- Splicing removes introns and joins exons to produce a continuous coding sequence.
- Eukaryotic genes contain many introns—some genes like dystrophin have over 70 introns.
- The **spliceosome** must recognize precise GU–AG boundaries and remove introns accurately. Incorrect splicing can lead to dramatic loss of protein function.

A **spliceosome** is a large, complex molecular machine found in eukaryotic cells that is composed of small nuclear RNAs (snRNAs) and proteins. It is responsible for splicing, a process that removes non-coding regions called introns from a pre-messenger RNA (pre-mRNA) transcript and joins the remaining protein-coding regions, called exons. This splicing process is essential for creating mature messenger RNA (mRNA) molecules that can be translated into proteins.



Consensus Splice Sites

Each intron has functional elements required for recognition:

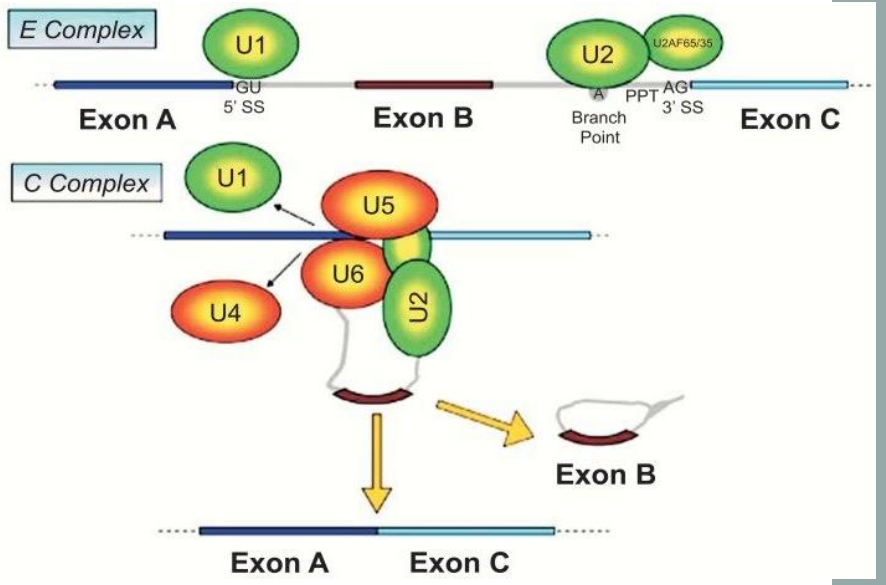
- A **5′ splice site** starting with GU.
- A **branch point adenine (A)** located 18–40 nucleotides upstream of the 3′ splice site.
- A **polypyrimidine tract**, rich in U residues.
- A **3′ splice site** ending with AG.

These cis-elements define where the spliceosome will cut and join the RNA.

The Spliceosome Components

The spliceosome is a dynamic ribonucleoprotein complex containing five snRNP particles: U1, U2, U4, U5, and U6.

- **U1 snRNP** binds the 5′ splice site.
- **U2 snRNP** binds the branch point A.
- **U4/U6.U5 tri-snRNP** joins to form an active catalytic core.
  The snRNAs act as enzymes, forming the catalytic center that carries out the **transesterification** reactions.



Step-by-Step Splicing Mechanism

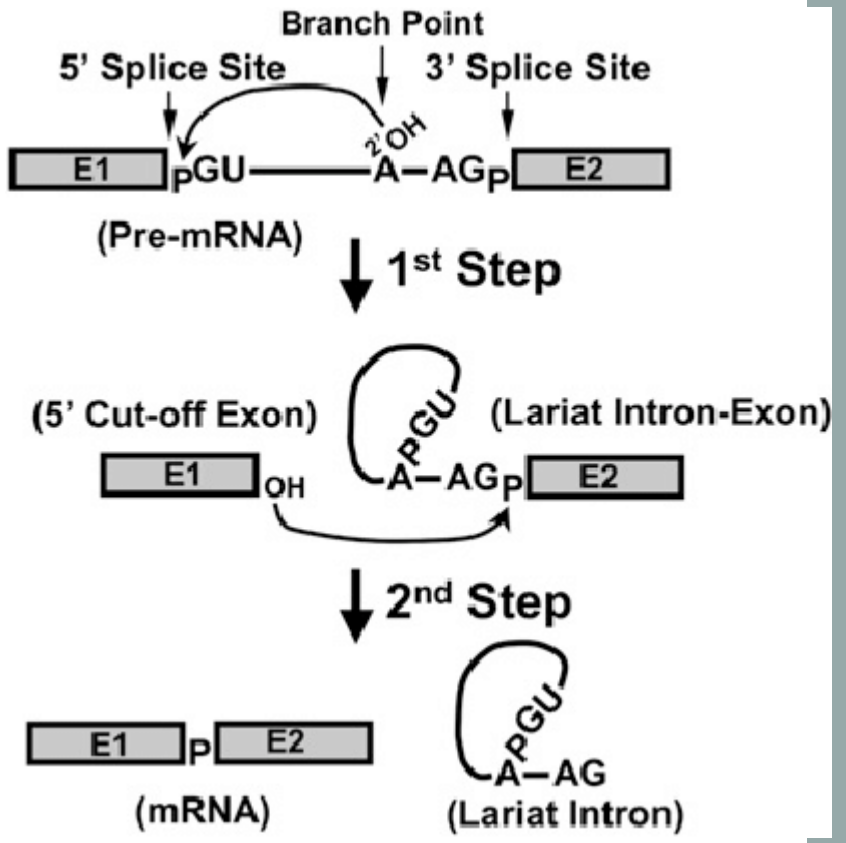
Splicing proceeds via two major transesterification reactions:

Reaction 1: The 2′-OH of branch point A attacks the 5′ splice site, releasing exon 1 and forming a lariat intron.

Reaction 2: The 3′-OH of exon 1 attacks the 3′ splice site, joining exon 1 and exon 2.

The intron lariat is then released and degraded.

Both reactions require no external energy, they rely entirely on RNA catalysis.

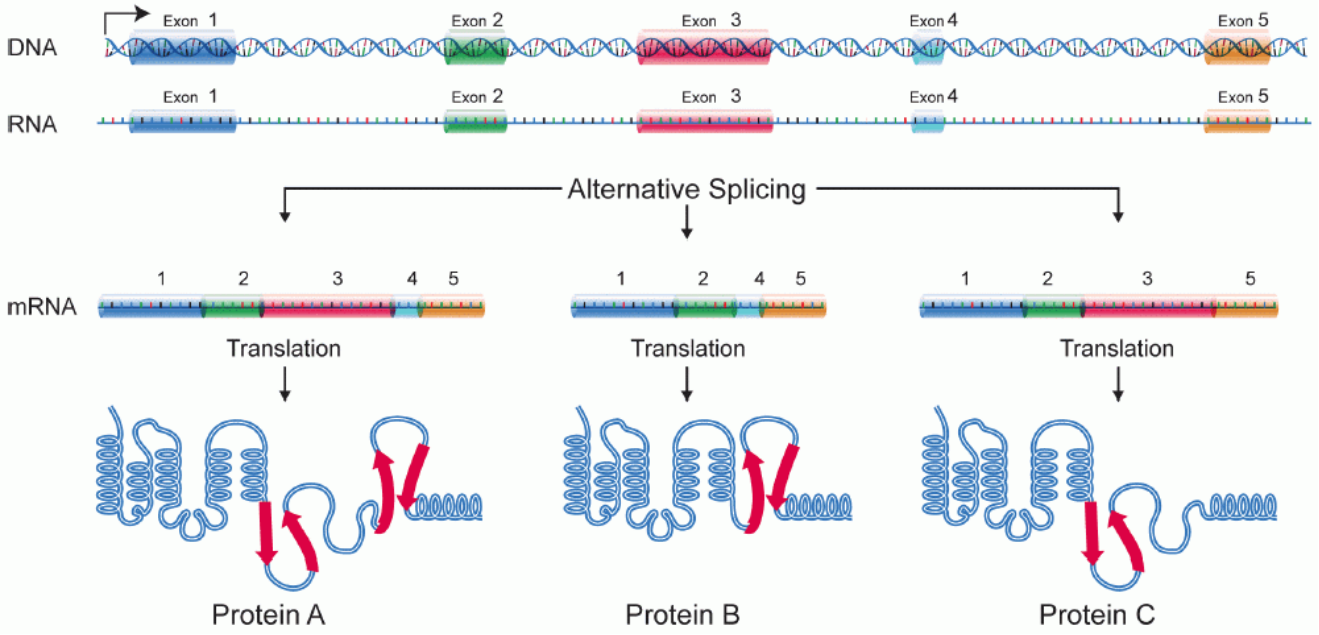


Alternative Splicing

Alternative splicing allows a single gene to produce multiple protein isoforms.

Major types:

- Exon skipping
- Alternative 5′ splice sites
- Alternative 3′ splice sites
- Mutually exclusive exons
- Intron retention



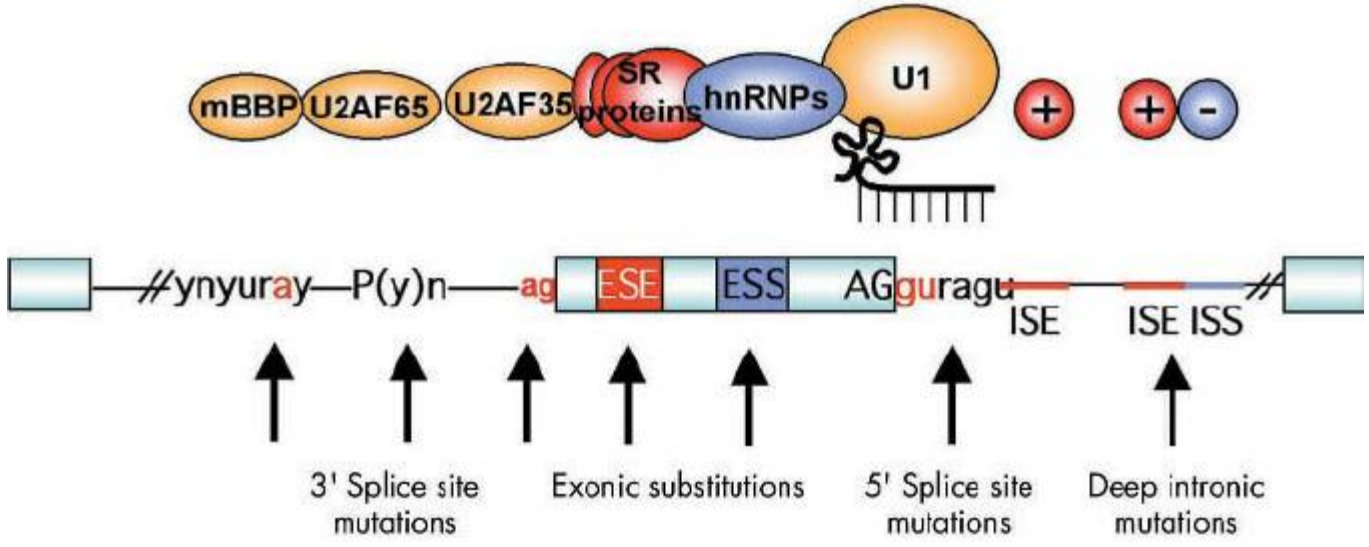
Over 90% of human genes undergo alternative splicing, dramatically expanding proteomic diversity. This is why humans can produce ~100,000 proteins from only ~20,000 genes.

Regulation of Alternative Splicing

Alternative splicing is tightly regulated by RNA-binding proteins:

- **SR proteins** enhance splicing at nearby sites.
- **hnRNP proteins** repress splicing by blocking spliceosome assembly.
  Splicing decisions are influenced by tissue type, developmental stage, stress, signaling pathways, and chromatin structure.
  For example:

• The FGFR2 gene produces epithelial and mesenchymal isoforms depending on the cell type.



Splicing Errors and Human Disease

Many genetic diseases arise from splicing defects:

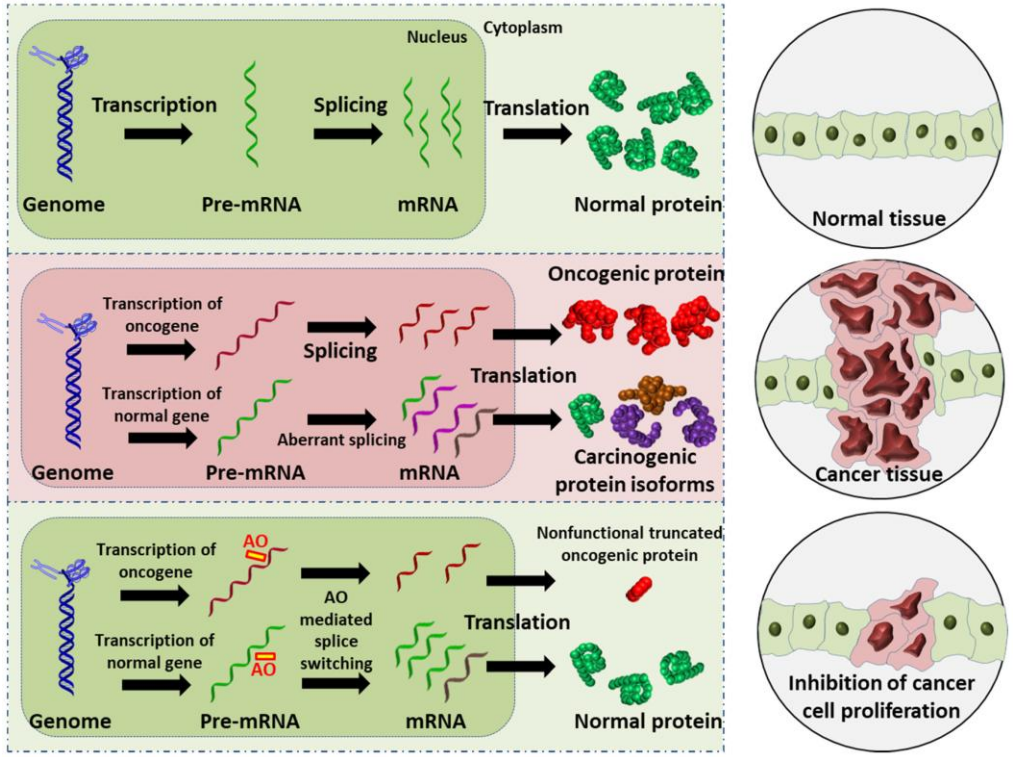
- **β-thalassemia** caused by mutations destroying the 5′ or 3′ splice sites of β-globin.
- **Duchenne muscular dystrophy** due to frameshifting exon skipping.
- **Cystic fibrosis (CFTR gene)** has splice-site mutations.
- Cancers frequently express abnormal splice isoforms that promote survival, invasion, or drug resistance.

  Up to 35% of all disease-causing mutations affect splicing.

Therapeutics Targeting Splicing

Modern therapies directly target splicing:

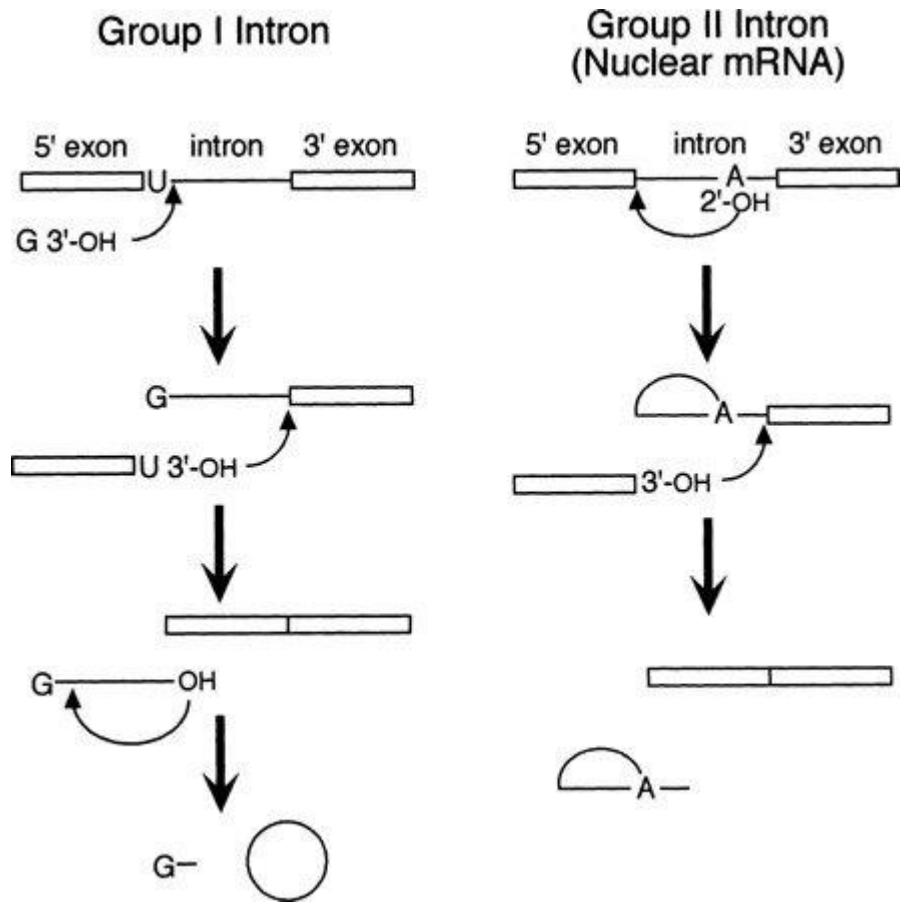
- **Antisense oligonucleotides (ASOs)** redirect splice-site selection.
  - Example: **Nusinersen (Spinraza)** for spinal muscular atrophy.
- **Small molecule splicing modulators** (H3B-8800, E7107) target the spliceosome in leukemia.
- **CRISPR/Cas9 splice editing** allows removal of pathogenic exons without altering coding sequence. These strategies represent a promising area of precision medicine.



Self-Splicing Introns

Not all splicing requires the spliceosome.

- **Group I introns** (Tetrahymena) use a guanosine cofactor.
- **Group II introns** self-splice similarly to spliceosomal introns and are believed to be ancestral precursors to the spliceosome. Their discovery proved RNA can act as a true enzyme — ribozyme — which earned a Nobel Prize.



Summary

- mRNA processing ensures stability and correct expression.
- Splicing is a highly precise but flexible mechanism.
- Alternative splicing is a major source of protein diversity.
- Splicing errors are a major cause of disease.
- Splicing is now a powerful target for modern therapeutics.

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