AQC-321

Post Transcriptional Modification

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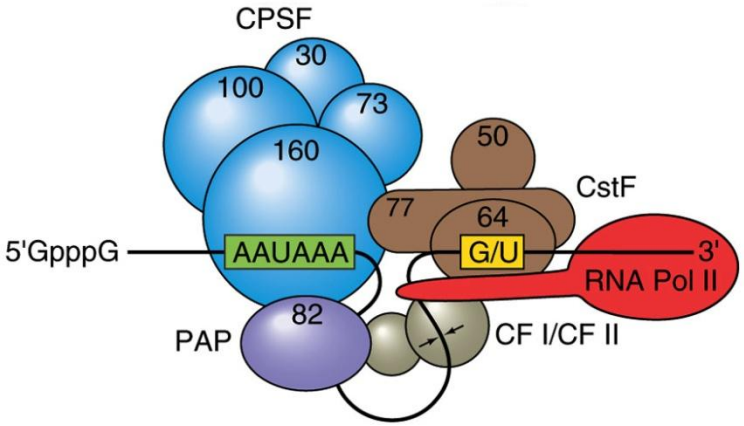
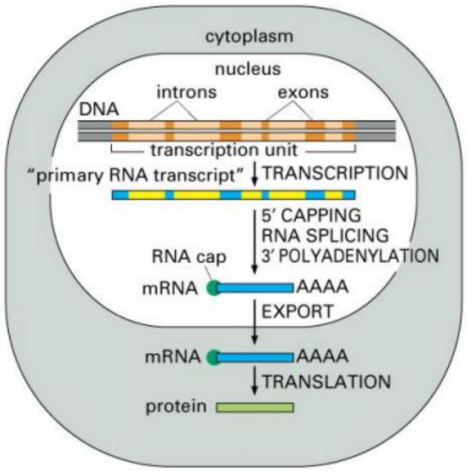




Post Transcriptional Modification

Prokaryotes: RNA transcribed from DNA template and used immediately in protein synthesis

Eukaryotes: Primary transcript (hn RNA) must undergo certain modifications to produce mature mRNA (active form) for protein synthesis.

"Post-transcriptional modification is a set of biological processes common to most eukaryotic cells by which an primary RNA transcript is chemically altered following transcription from a gene to produce a mature, functional RNA molecule that can then leave the nucleus and perform any of a variety of different functions in the cell."

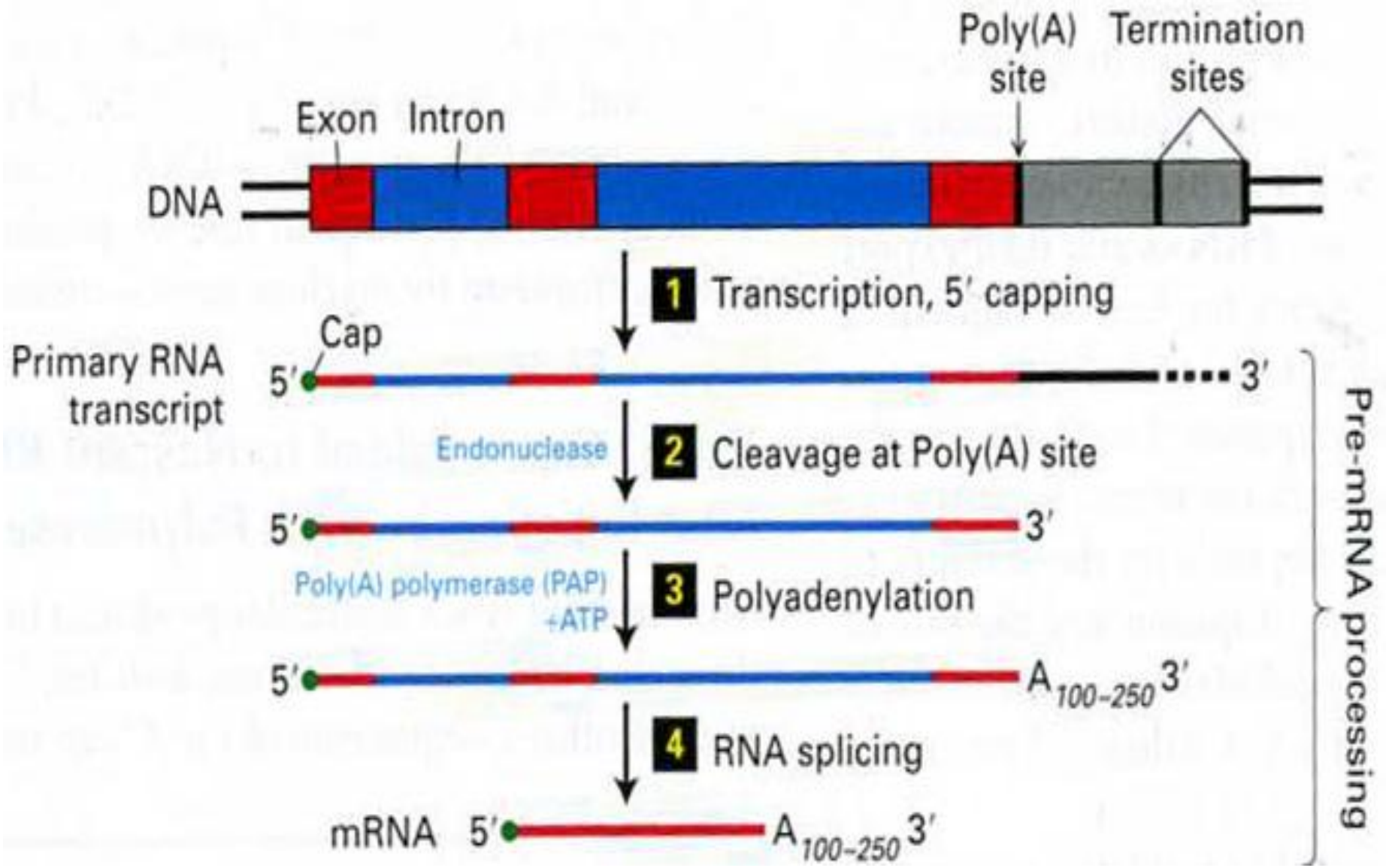
Post Transcriptional Modifications

- **Post transcriptional modifications are also responsible for changes in rRNA, tRNA and other special RNA like srpRNA, snRNA, snoRNA, miRNA etc.**

Important Post Transcriptional Modifications for Production of Mature mRNA

1. 5’ Capping
2. 3' maturation (Cleavage & Polyadenylation)
3. Splicing
4. Transport of RNA to Cytoplasm
5. Stabilization/Destabilization of mRNA

Likely order of events in producing a mature mRNA from a pre-mRNA.



5’ RNA Capping

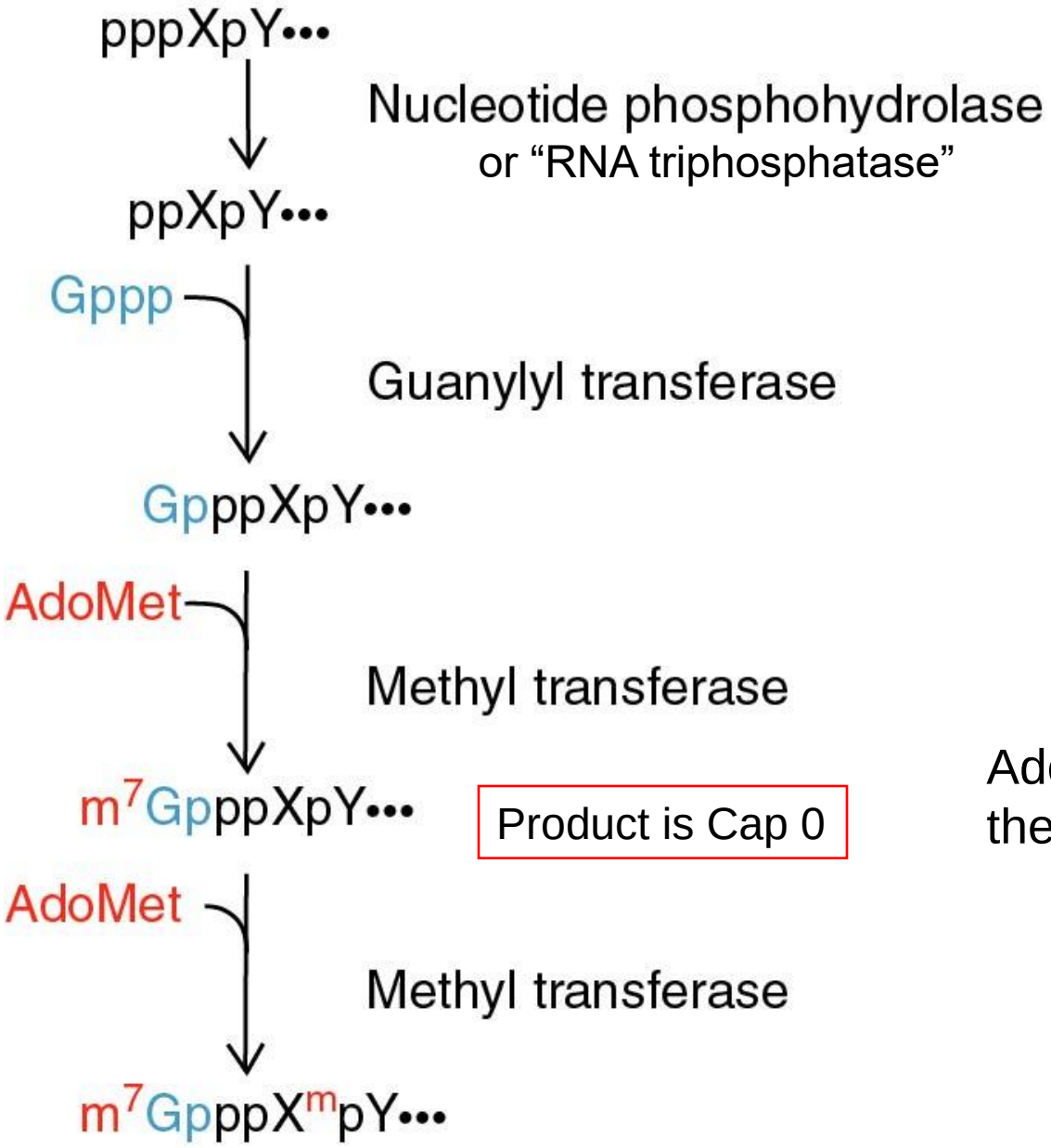
1. **Occurs before the pre-mRNA is 30 nt long.**

2. **The modification that occurs at the 5' end of the primary transcript is called the 5' cap.**

3. **In this modification, a 7-methylguanylate residue is attached to the first nucleotide of the pre-mRNA by a 5'-5' linkage.**

4. **The 2'-hydroxyl groups of the ribose residues of the first 2 nucleotides may also be methylated.**

Order of events and enzymes in 5’ Capping



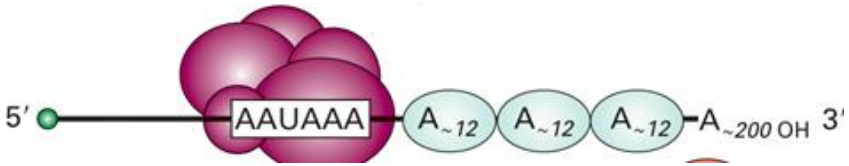
AdoMet = S-adenosylmethionine, the methyl donor

5’ Cap Functions

Cap provides:

1. Protection from some ribonucleases*
2. Enhanced translation*
3. Enhanced transport from nucleus
4. Enhanced splicing of first intron for some pre-mRNAs

*Also functions of the polyA-tail



Polyadenylation

1. String of adenine nucleotide (-AAAAAAA-3’) added at 3’ end of primary transcript is known as polyadenylation
2. Most cytoplasmic mRNAs have a polyA tail (3’ end) of 50-250 Adenylates
   – a notable exception is histone mRNAs
3. Discovered in 1971 (J. Darnell et al.)
4. Added post-transcriptionally by an enzyme, PolyA polymerase(s)
5. It involves 2 steps **a): Cleavage of RNA at 3’ end**
   b): Addition of adenine residue

Specific Sequences for 3' Polyadenylation

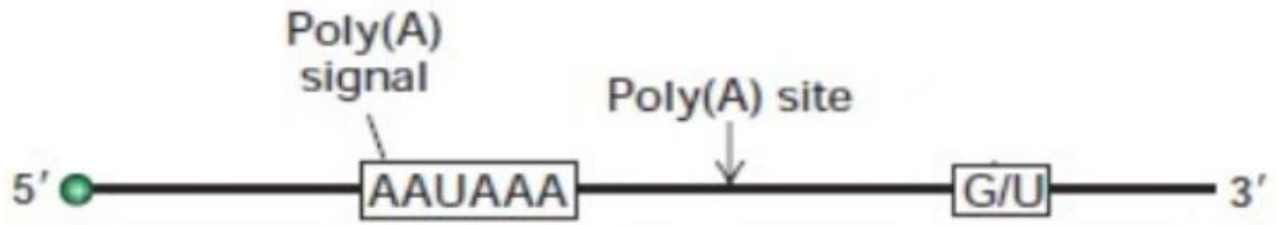
1. A cleavage sequence **CA**.
2. Poly adenylation signal sequence.

AAUAAA

Located 10-30 nucleotides upstream to the cleavage site. Highly conserved.

3. **GU** rich sequence present 20-40 nucleotides

downstream to the cleavage site.



Polyadenylation: The Proteins

James Manley

Proteins required for cleavage and polyadenylation new transcript.

Proteins required for efficient cleavage of pre-mRNA:

1. **CPSF** (cleavage & polyadenylation specificity factor), binds the AAUAAA
2. **CstF** (cleavage stimulation factor) binds to the G/U rich region cooperatively with CPSF
3. **CFI and CFII** (cleavage factors I and II), RNA-binding proteins
4. **PAP (PolyA Polymerase)**
5. **nRNAP II** (the CTD of the very large RPB1 subunit) stimulates cleavage

Functions of the PolyA-Tail

1. **Promotes mRNA stability (protection from ribonucelase activity)**

   - De-adenylation (tail shortening) can trigger rapid degradation of the RNA

2. **Enhances translation**

   - promotes recruitment by ribosomes

   - bound by a polyA-binding protein in the cytoplasm, PAB1

   - synergistic stimulation with Cap!

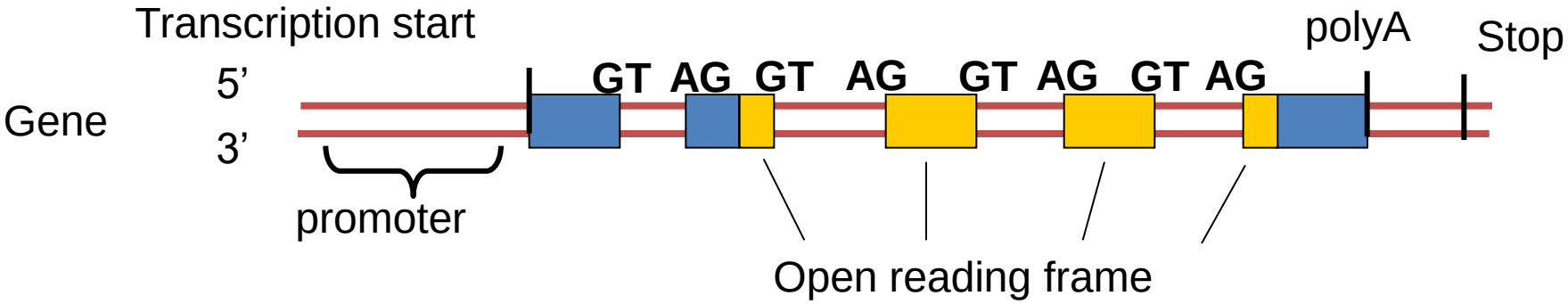
Splicing of mRNA

Eukaryotic gene, the coding sequence (exon) are separated/interrupted by non coding sequences (intron)

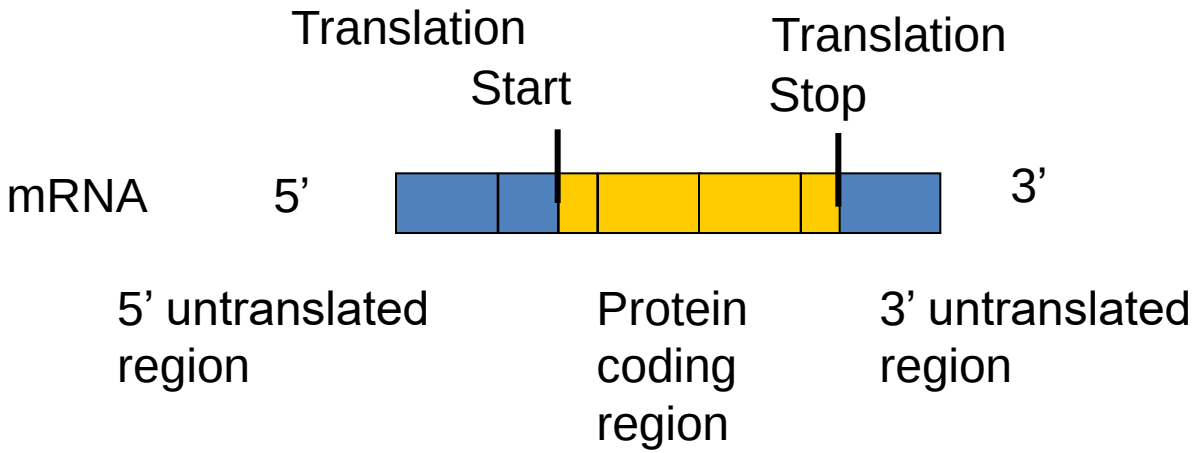
- EXONS –coding sequence, transcribed and translated. Coding for amino acids in the polypeptide chain.
  Vary in number ,sequence and length. A gene starts and ends with exons.(5' to 3').
  Some exon includes untranslated(UTR)region.

- INTRONS- coding sequences are separated by non-coding sequences called introns.
  Any nucleotide sequence that are removed when the primary transcript is processed to give the mature RNA are called introns.

Split Genes (Intron & Exon)



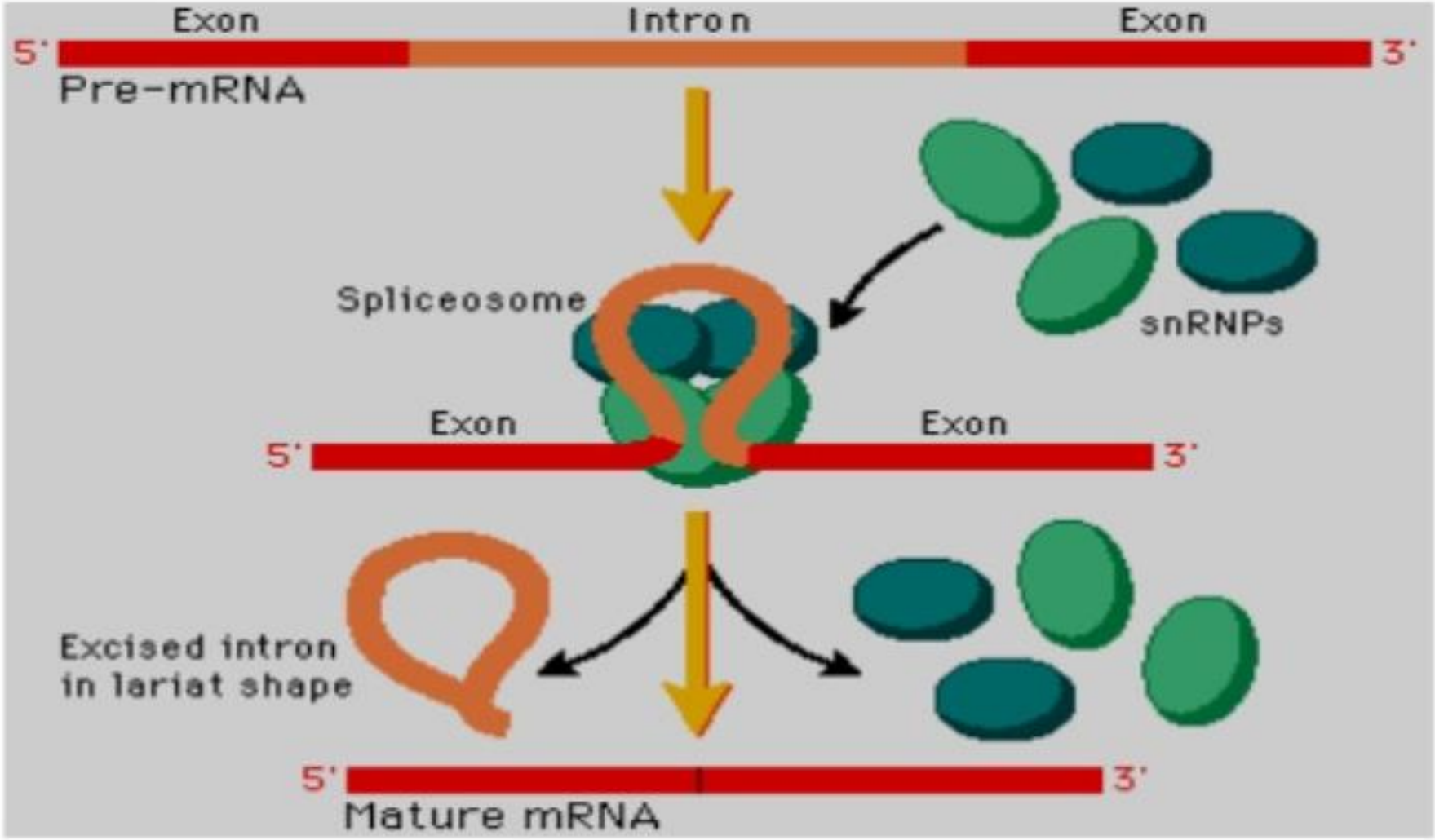
Initial exon
Internal exon
Internal coding exon
Terminal exon



Splicing (Removal of Introns)

- **Removal of introns (Splicing)**
- Introns or intervening sequences are the RNA sequences which do not code for the proteins.
- These introns are removed from the primary transcript in the nucleus, exons (coding sequences) are ligated to form the mRNA molecule, and the mRNA molecule is transported to the cytoplasm.
- The molecular machine that accomplishes the task of splicing is known as the **spliceosome.**
- Small nuclear RNA molecules that recognize splice sites in the pre-mRNA sequence.
- The excised intron is released as a "lariat" structure, which is degraded

Spliceosome



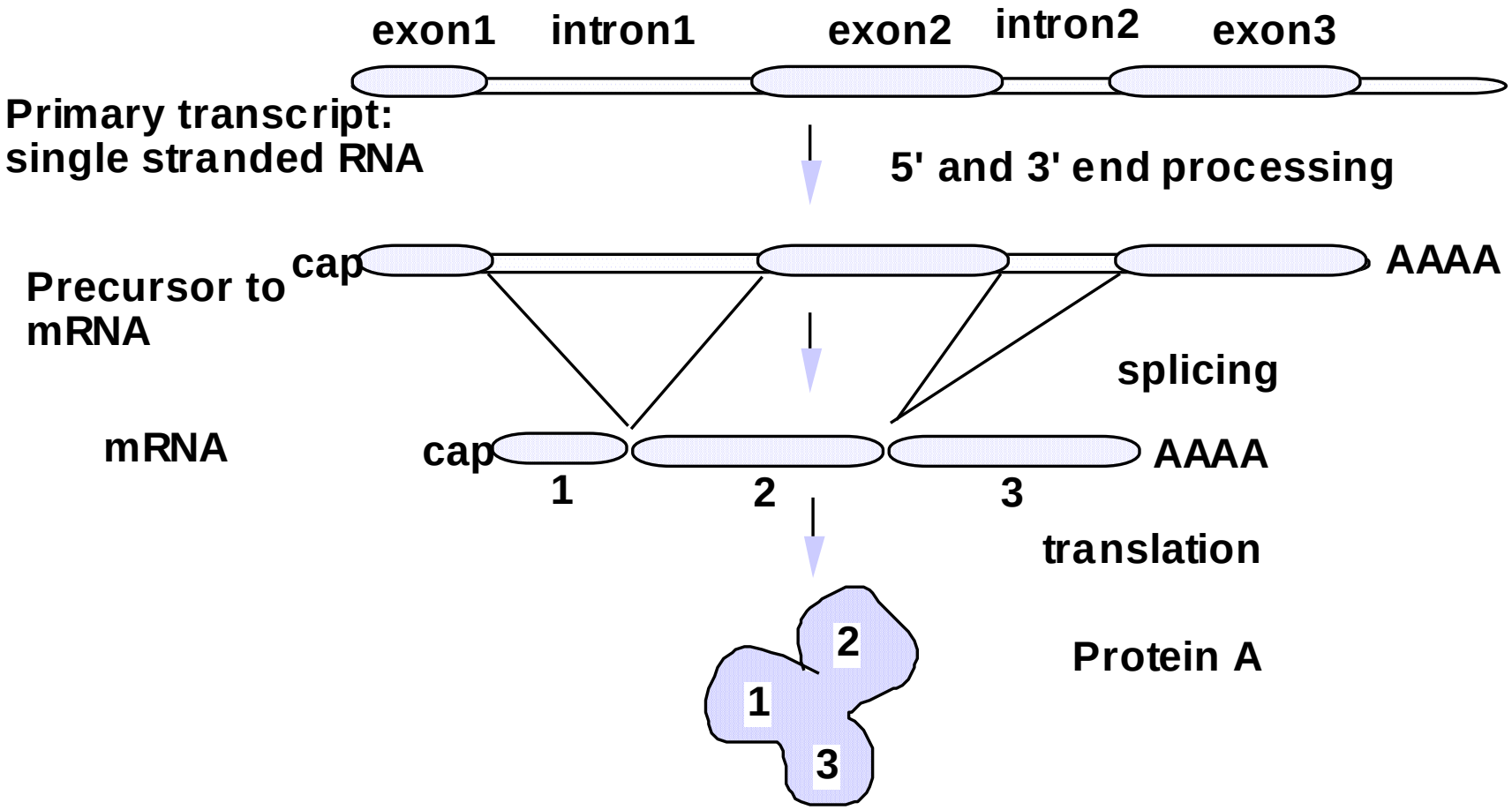
Alternative Splicing

- The pre-m RNA molecules from some genes can be spliced in two or more alternative ways in different tissues.

- This produces multiple variations of the m RNA and thus diverse set of proteins can be synthesized from a given set of genes .

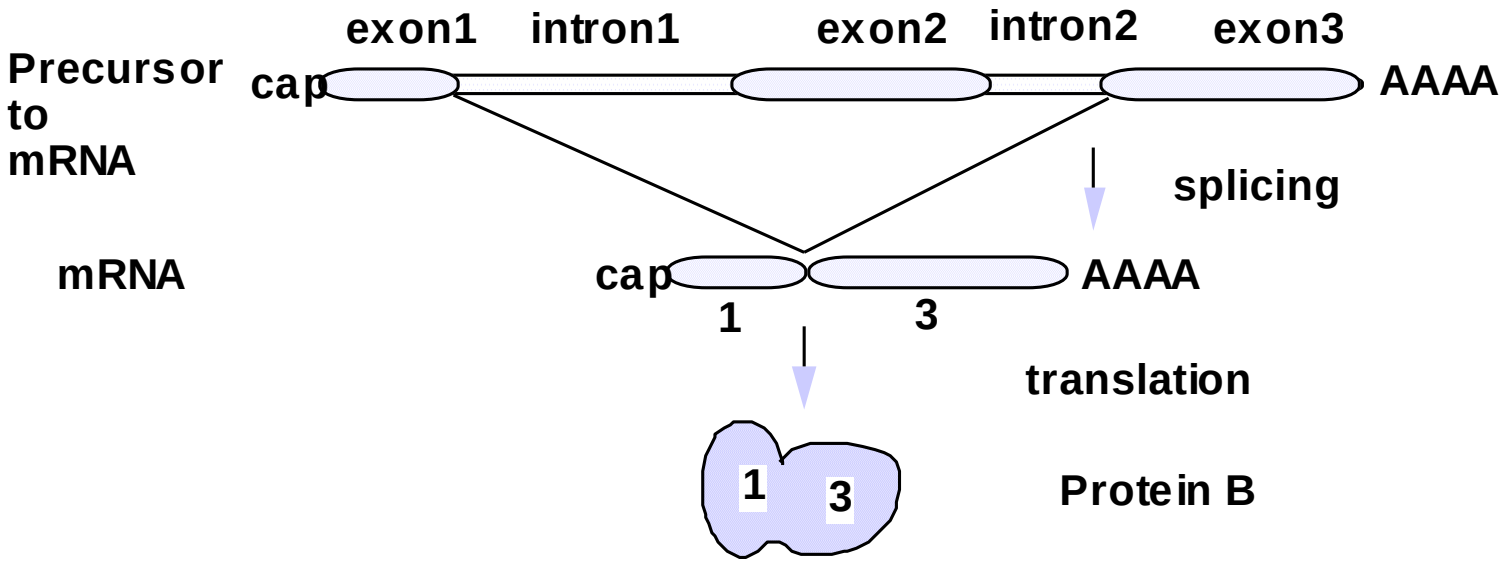
Alternative splicing can generate multiple polypeptides from a single gene (Protein A)

The mRNA for Protein A is made by splicing together exons 1, 2 and 3:



Alternative splicing can generate multiple polypeptides from a single gene (Protein B)

Or, by an alternative pathway of splicing that skips over exon2, Protein B can be made:



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