CONCEPT: mRNA PROCESSING

● After transcription, eukaryotic **pre-mRNA,** which is a newly transcribed mRNA, must be processed to become mRNA

□ Processing occurs in the _____ and is required before moving to the cytosol

- Distinguishes mRNA from other RNAs

- *Exosomes* are a protein complex that degrades RNA that isn't in the final mRNA form

□ The **C-terminal domain** of RNA polymerase II carries _____ responsible for RNA processing

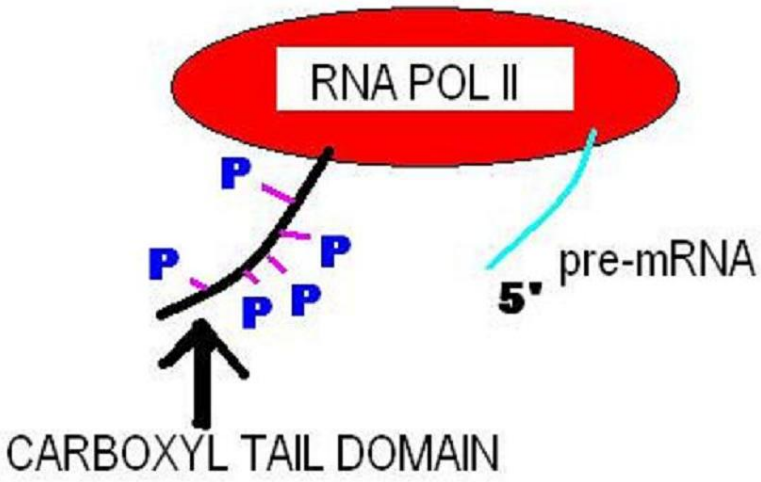
- RNA polymerases I and III lack this domain, and so their transcripts do not undergo the same processing

□ Processing occurs during transcription

□ **Heterogeneous ribonucleoprotein particles (hnRNPs)** are formed by RNA binding to numerous proteins

- Prevent RNA from forming secondary structures

EXAMPLE: Simple drawing of the RNA polymerase II C-terminal domain



5’ RNA Capping

● RNA capping occurs at the 5’ end of the transcript

□ Three enzymes work to cap the 5’ end of the transcript with a special _____ guanine

- Phosphatase removes phosphate from 5’ end

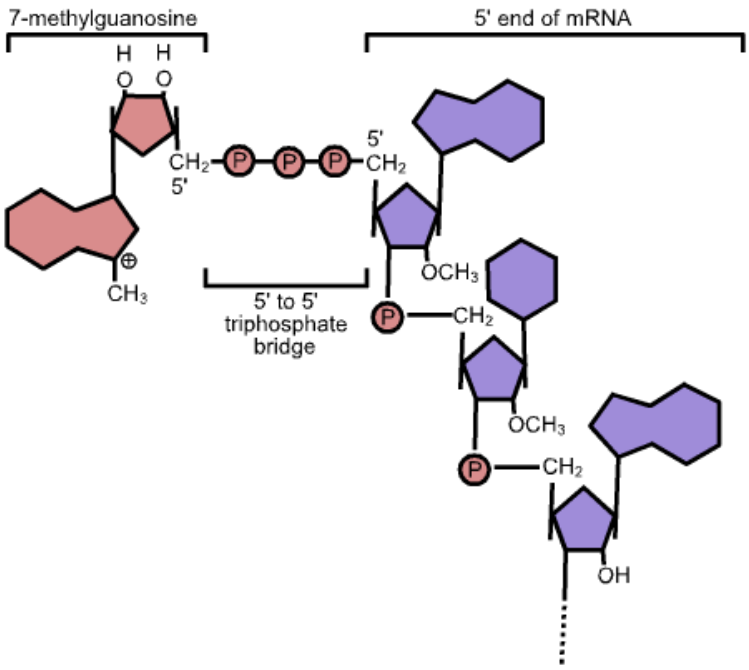
- Guanyl transferases add a guanine mono-phosphate (GMP) in a unique way (binds 5’ to 5’)

- Methyl transferases add a methyl group to the guanine at position 7 on the ribose sugar ring

□ 5’ capping occurs after RNA polymerase has transcribed ~25 nucleotides of RNA

□ Prevents enzymes that _____ RNA (*RNAses)* from destroying the transcript

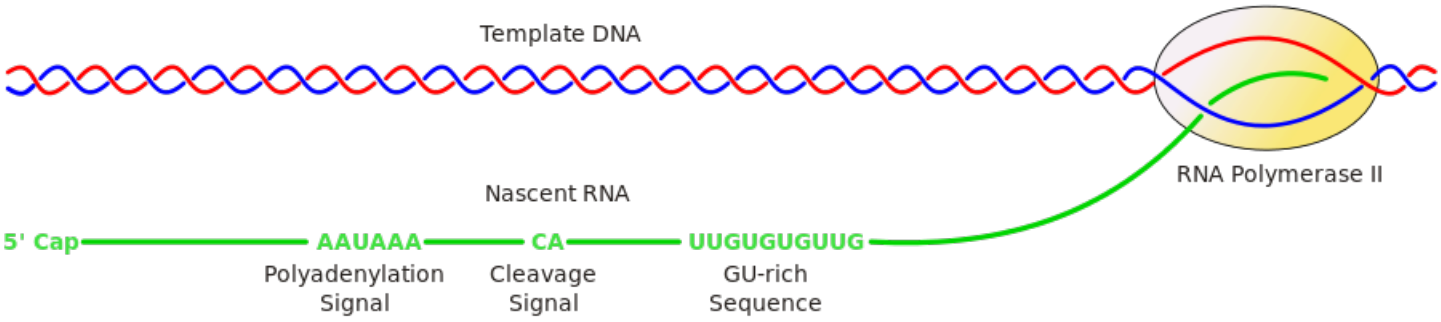
EXAMPLE: The 5’ cap structure



3’ Polyadenylation

- **RNA poyladenylation** (addition of repeating A nucleotides) occurs at the 3’ end of the transcript
  - □ RNA is _____ at a specific sequence
    - Two cleavage signal sequences exist: AAUAAA (upstream) and a GU rich region (downstream)
    - *Cleavage stimulatory factors* interact with the GU sequence (CPSF and CStF)
  - □ The *poly-A polymerases (PAP)* begins adding a linear sequence of adenine nucleotides to the cleavage site
    - Begins at an AU rich site located at end of cleavage site
    - First 12 As are slowly assembled, but then the next 200-250 go much faster
    - Requires no template

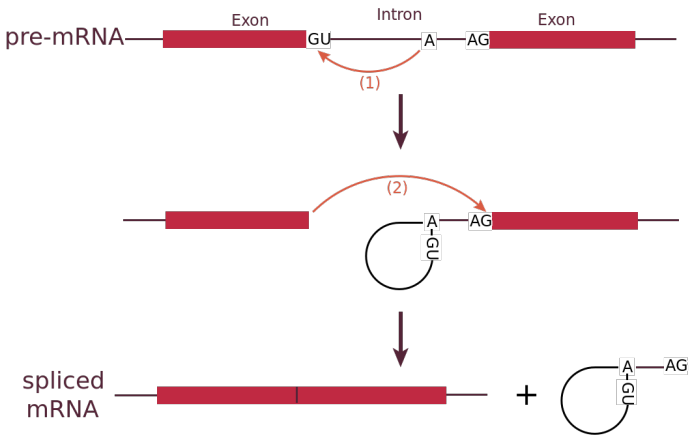
EXAMPLE: Polyadenylation signals at the 3’ end of the transcript



Overview of RNA Splicing

- Introns (non coding segments) must be _____ from the pre-mRNA
  - □ **RNA splicing** removes introns from pre-mRNA
    - Introns vary greatly in size, but exons (coding sequences) average 150 nucleotides long
  - □ Introns contain specific sequences between 30-40 nucleotides long that _____ for splicing
    - 5' splice site begins with a GU sequence (more common) or AU sequence (less common)
    - 3' splice site ends with an AG sequence (more common) or AC sequence (less common
    - Branch point are special sequences located several dozen nucleotides upstream from 3' end
  - □ Improper splicing accounts for 15% of genetic disorders

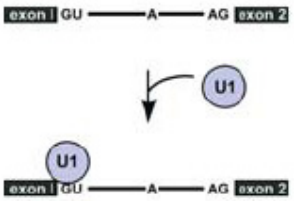
EXAMPLE: Overview of RNA splicing



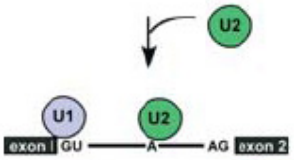
  - □ The **spliceosome** complex is responsible for splicing most RNA
    - Composed of **small nuclear RNA** (snoRNA), which are divided into five groups (U1, U2, U4, U5, U6)
    - The **small nuclear ribonucleotproteins** (snRNPs) are formed by 6-10 splicing proteins bind to snoRNAs
    - The snRNPs form _____ of spliceosome and recognize the splice sequences

7 Steps of Splicing

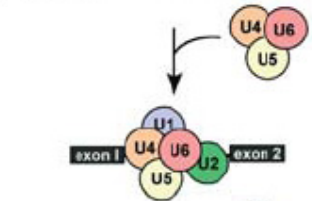
1. The snRNP (U1 snoRNA) binds to the mRNA at the 5' splice site



2. A snRNP (U2 snoRNA) binds to the branch point sequence



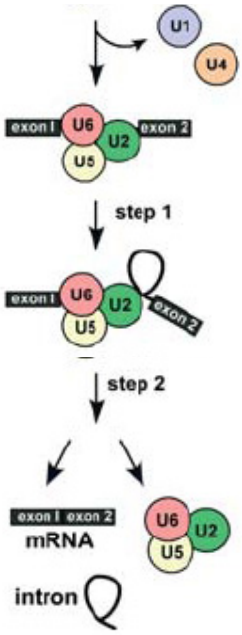
3. Other proteins are recruited to form the full spliceosome complex



4. The pre-mRNA is cleaved at 5' splice site and looped to the branch point sequence (called *lariat)*

5. The pre-mRNA is cleaved at 3' splice site by a transesterfication reaction

6. The two exon ends are joined together



7. The **exon junction complex** is recruited to the newly joined exons to assist in nuclear export

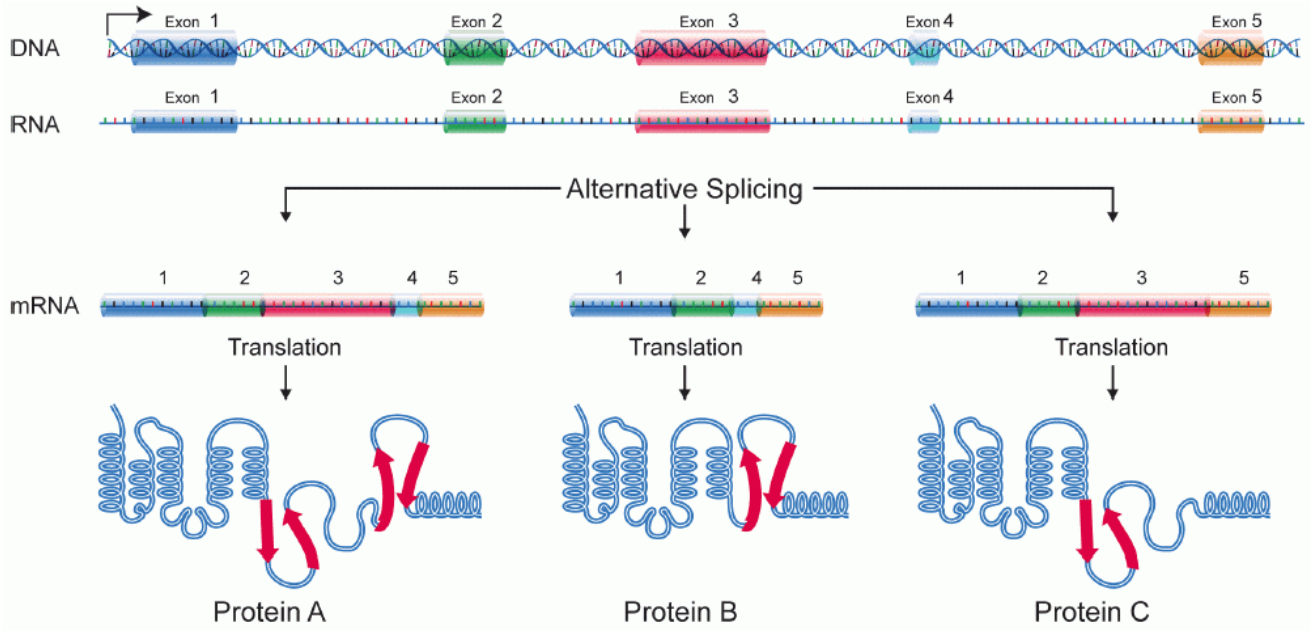
□ **RNA-RNA rearrangements** occur during most of these steps

- Interaction between the RNA and spliceosome are disrupted and differently reformed

Splicing Forms

- **Self-splicing** introns can splice _____ a spliceosome
  - □ These introns fold into complex secondary structures
  - □ Introns contain specific sequences between 30-40 nucleotides long that signal for splicing
    - 5' splice site begins with a GU sequence (more common) or AU sequence (less common)
- Regulation of _____ is a form of regulating of gene expression
  - □ **Alternative splicing** combines different combinations of exons to create different forms of the same protein
  - □ Condensed chromatin can slow the rate of transcription and pre-mRNA processing
    - *Exon skipping* can occur if the process is moving quickly
    - Specific histone modifications can recruit proteins to RNA to control transcription or processing

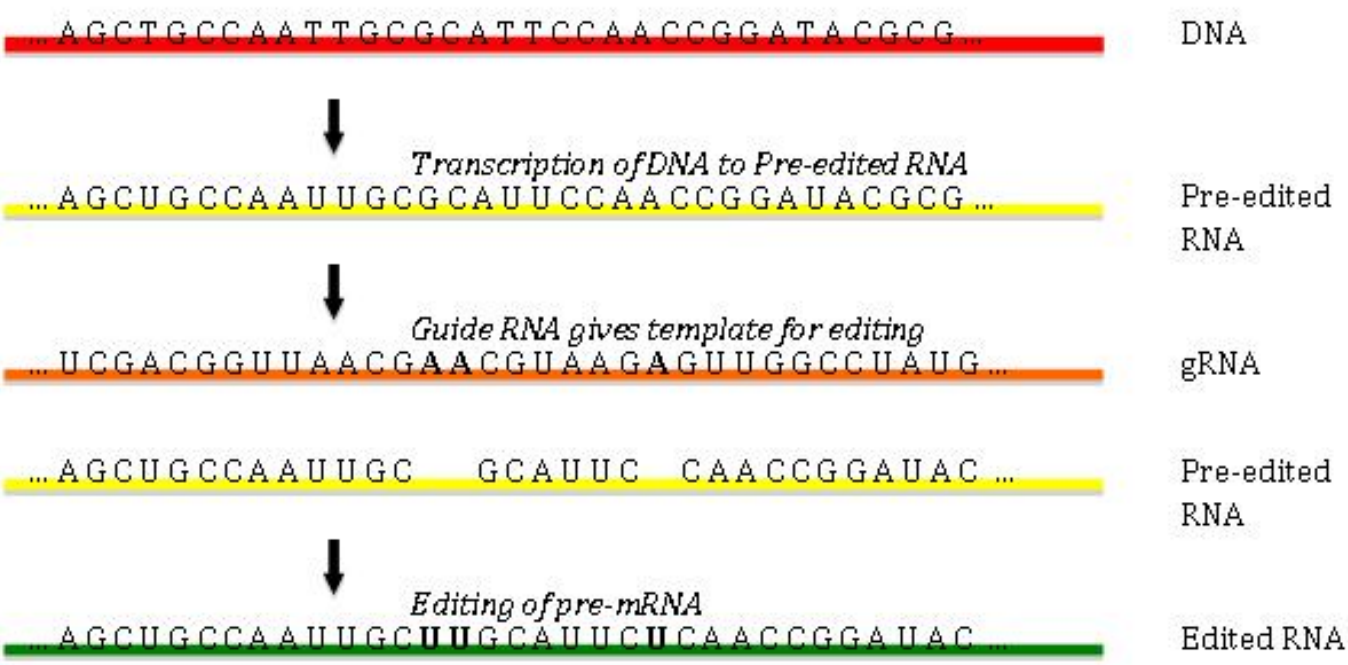
EXAMPLE: Model of alternative splicing



RNA Editing

- **RNA editing** changes or alters the pre-mRNA nucleotide sequence
    - □ Can insert or remove multiple nucleotides in a pre-mRNA transcript
    - □ Deamination (remove an amino group) of specific nucleotides can occur
        - *Uridine* forms from a deaminated cytosine
        - *Inosine* forms from a deaminated adenine
    - □ **Guide RNAs** control RNA editing

EXAMPLE: RNA editing results in the addition of multiple Uracil's to the transcript



PRACTICE

1. Which of the following is NOT a processing event pre-mRNA undergoes to become mRNA?
    a. The addition of an extended sequence of repeating A nucleotides at the 3' end of the transcript
    b. The addition of a methylated guanine at the 5' end of the transcript
    c. Splicing out exons to connect the introns
    d. Removal of 1+ nucleotides in the pre-mRNA transcript

2. Which snoRNA is the first to bind to the 5' splice site during splicing?
    a. U1
    b. U2
    c. U3
    d. U4

3. Which of the following is not a sequence that is required for splicing?
    a. 5’ GU sequence
    b. 3’ AG sequence
    c. Branch Point
    d. Splice Sequence

4. Guide RNAs are responsible for what?
    a. Guiding the spliceosome to the correct splicing sequence
    b. Determining the position of RNA editing
    c. Adding the poly-A tail to the mRNA
    d. Adding the 5’ cap to the mRNA

5. RNA processing occurs where in the cell?
    a. Nucleus
    b. Cytoplasm
    c. Endoplasmic Reticulum
    d. Golgi