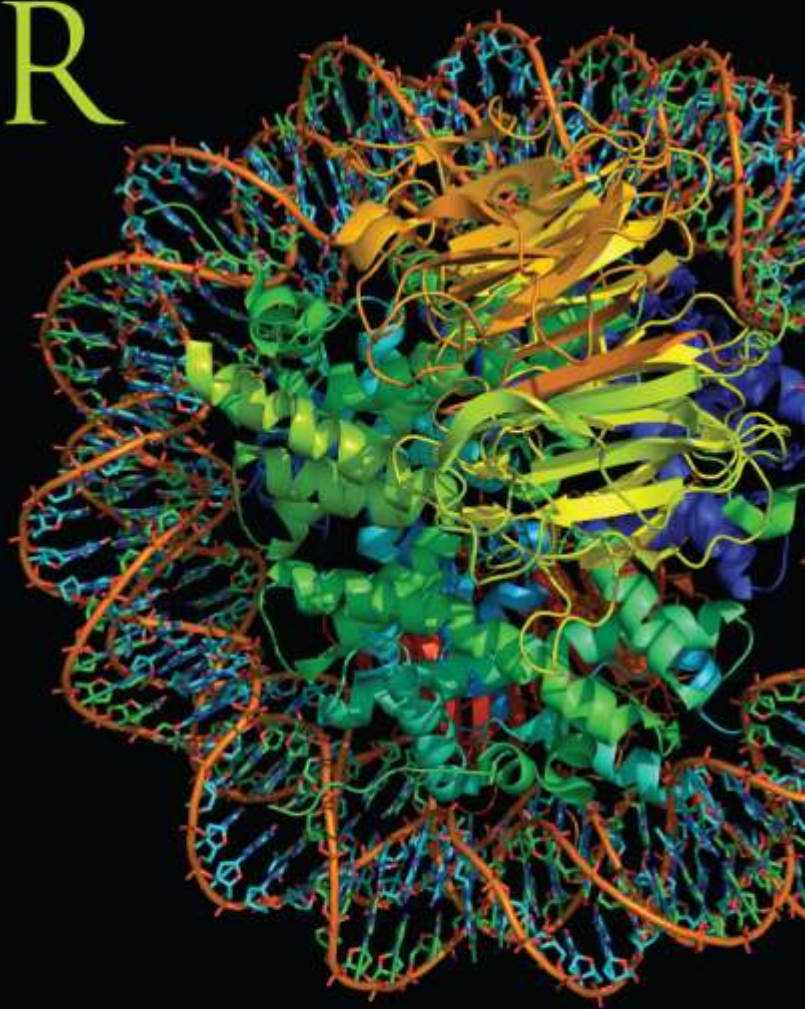
Principles of

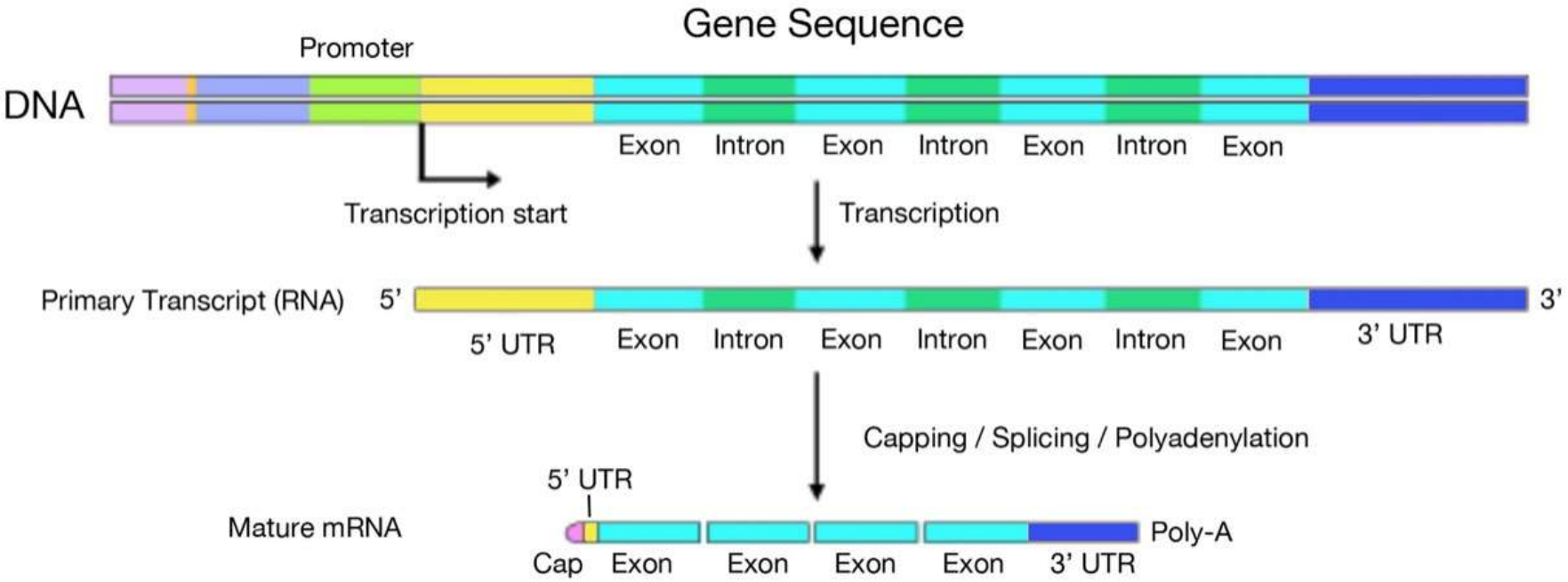
MOLECULAR BIOLOGY

BURTON E. TROPP



Chapter 14
RNA Polymerase II: Cotranscriptional and Posttranscriptional Processes

Eukaryotic RNA Maturation

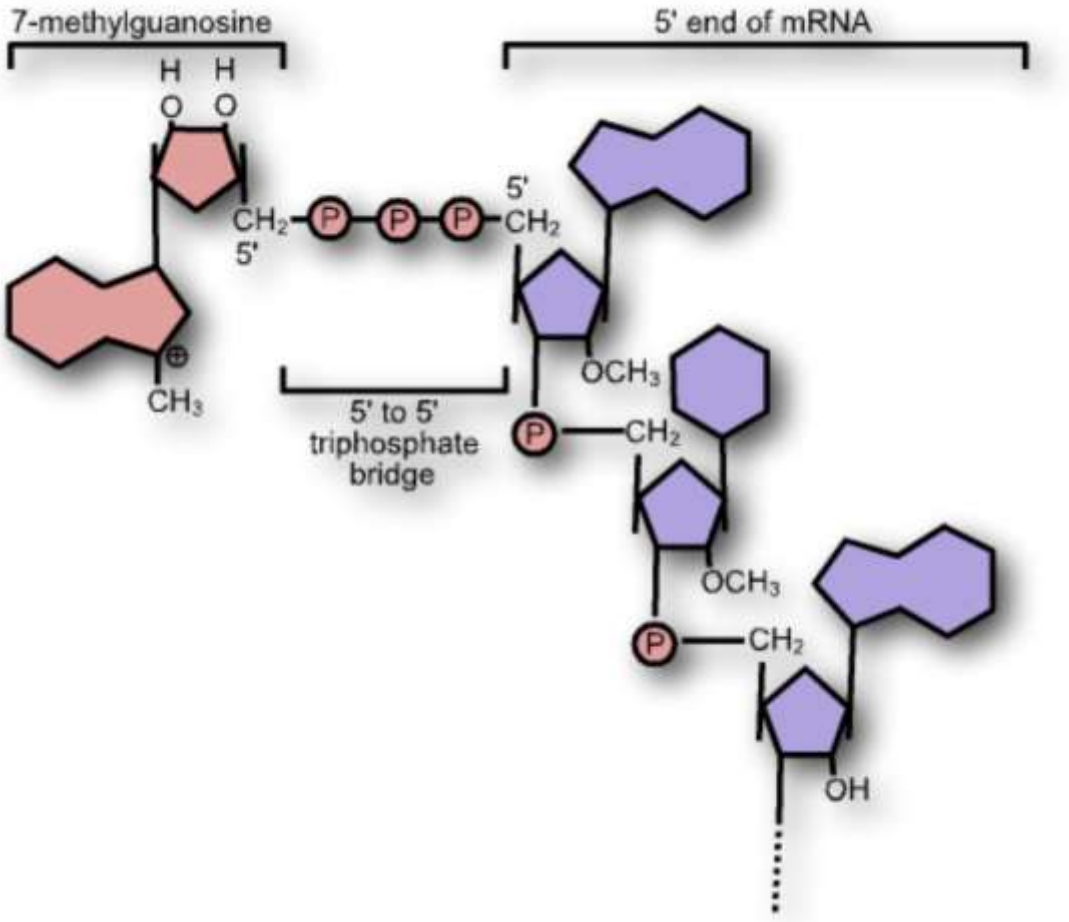


Cap Formation

mRNA molecules have 7-methylguanosine caps at their 5’-ends

- The 5’-m^7G cap forms when nascent transcripts are 20-30 nts long

Eukaryotic mRNA 5’ Cap



Cap Formation

- Virtually all eukaryotic mRNAs have 5’-m^7G caps
- They protect mRNA from digestion by 5’→3’ exonucleases
- Influences subsequent stages of mRNA processing
- Participates in translation

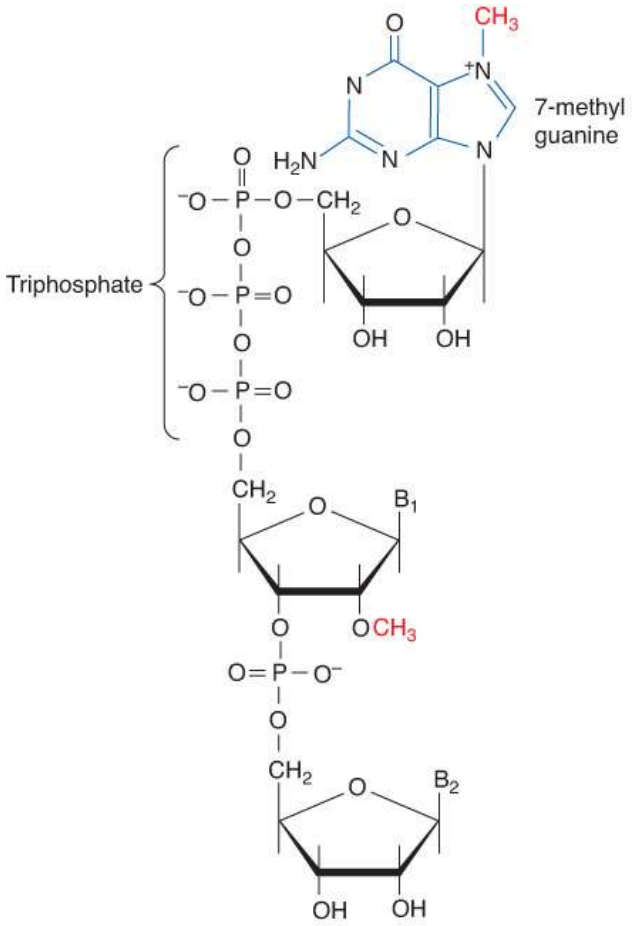


Figure 14.05: Messenger RNA 5’-cap structure.

Cap Formation

- All eukaryotes use the same basic pathway to form 5’-m^7G caps
  - Three enzyme activities work together
  1. **RNA 5’-triphosphatase** cleaves the γ–phosphate from the initiating nucleotide
  2. **Guanylytransferase** transfers the guanylyl group from GTP to the di-phosphate end
  3. **Methyl-transferase** transfers a methyl from S-adenosylmethionine to the N-7 position of the cap guanine

Cap Formation

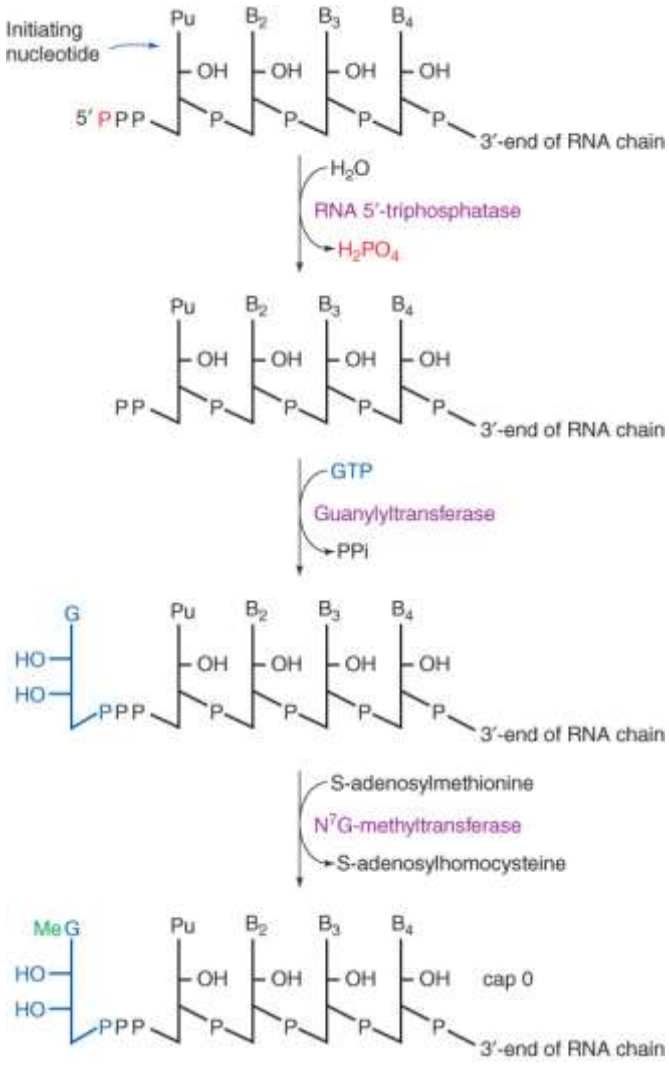


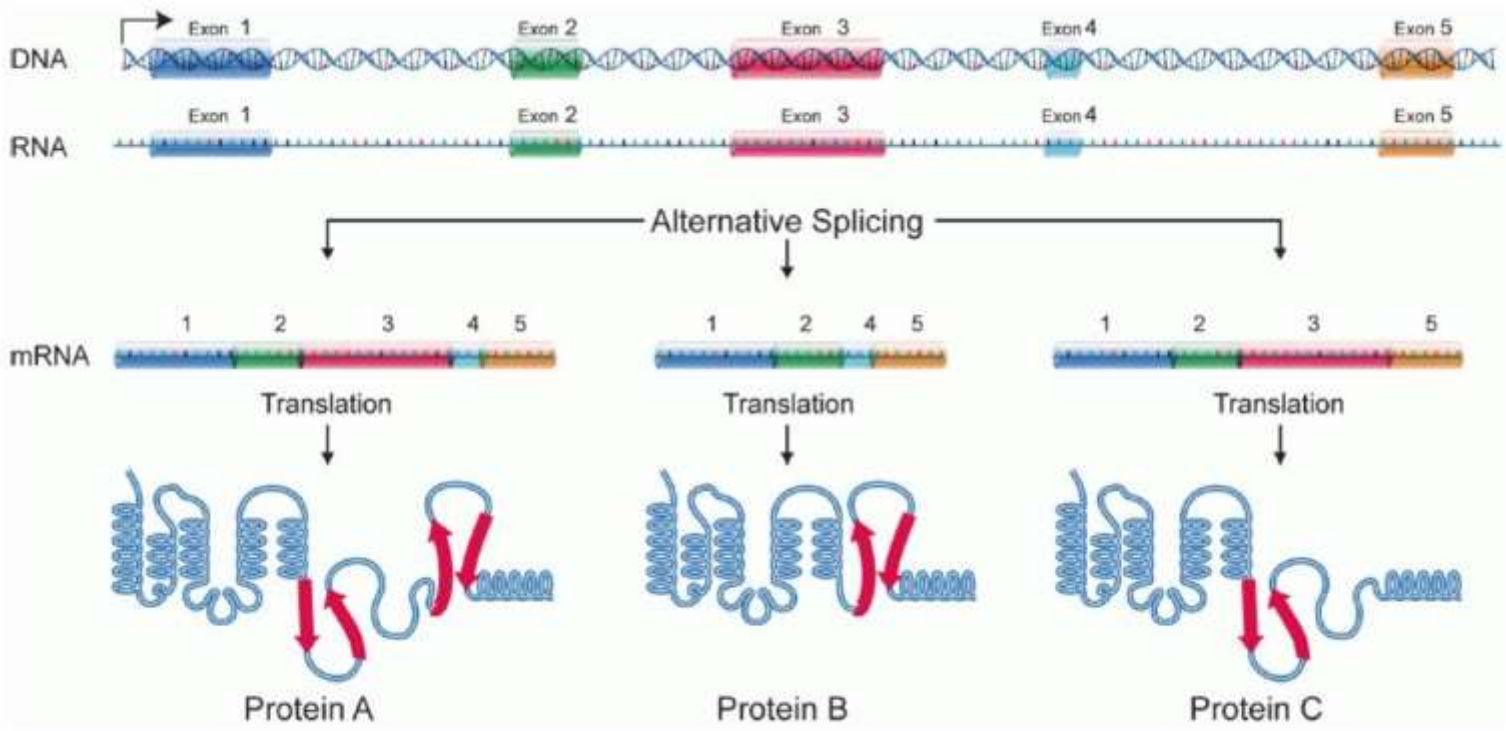
Figure 14.06: Capping pathway. The process takes place in three steps.

Split Genes

A single pre-mRNA can be processed to produce two or more different mRNA molecules

- Splicing may occur so each exon in a pre-mRNA is in the processed mRNA
- Or one combination of exons is incorporated in one mRNA while other combinations are incorporated into other mRNAs
- More than 95% of human pre-mRNAs go through this 2nd process known as **alternative splicing**

Alternative Splicing



Split Genes

Pre-mRNA requires specific sequences for precise splicing to occur

- Precision in splicing is critical
- Requires an unambiguous **5-splice site** and **3’-splice site**

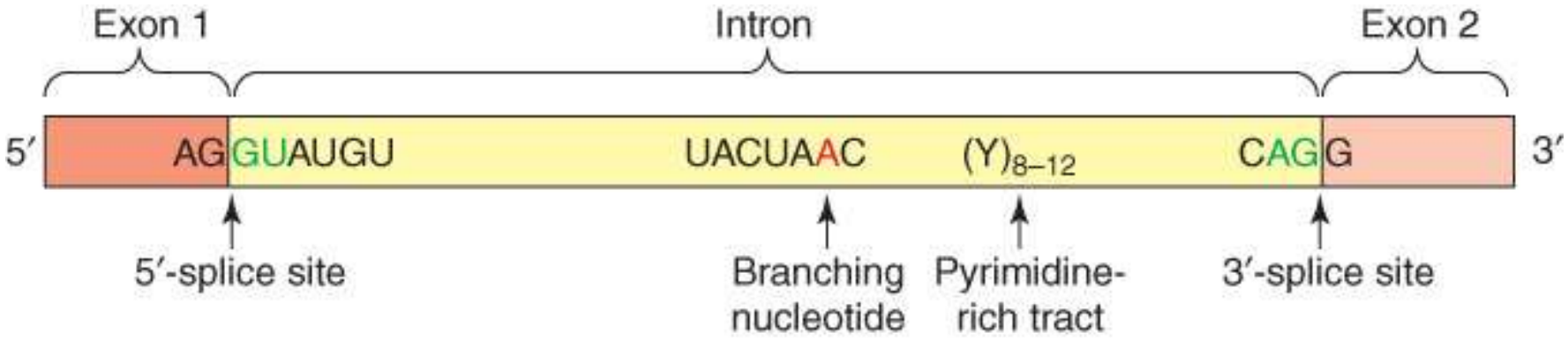


Figure 14.15: Sequences in yeast pre-mRNA that help to define the 5′- and 3′-splice sites. Y denotes a pyrimidine nucleotide.

Split Genes

- Three nearly invariant short sequences are required for splicing in yeast
    1. 5’-splice sequence (AG/**GU**AUGU)
    2. 3’-splice sequence (C**AG**/G)
    3. Branchpoint sequence(UACUAAC)
    4. Many introns have an 8-12 polypyrimidine tract
- Most animal and plant introns have the GU-AG pattern
- 98% of known human introns share this as well

Split Genes

The splicing mechanism that fits the structural and kinetic data best

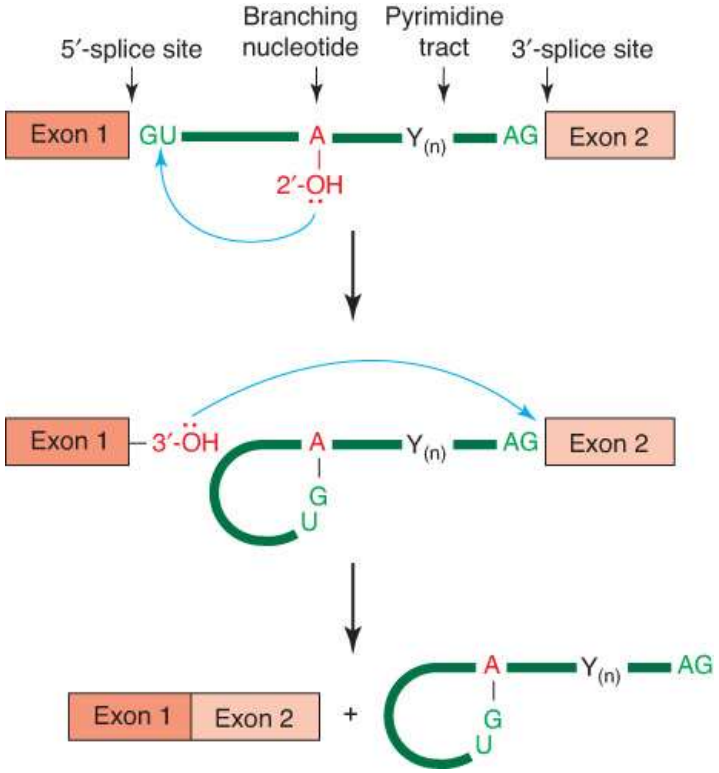


Figure 14.20: The two coordinated transesterification steps in splicing.

Spliceosomes

- Aberrant antibodies which are produced by individuals with certain autoimmune diseases, bind to **small nuclear ribonucleoprotein particles (snRNPs)**
- This observation led to the discovery of the splicing mechanism called the **spliceosome:** the splicing machine that excises introns
  - Made up of uridine-rich small nuclear RNAs
    - U1, U2, U4, U5 and U6 combined with non-snRNP protein factors

Spliceosomes

- RNA and protein may both contribute to the spliceosome's catalytic site
    - Splicing factor 1 (SF1) also called branchpoint binding protein binds to the branchpoint sequence
    - U2AF^{65} binds to the polypyrimidine tract
    - U2AF^{35} binds to the AG dinucleotide at the 3'-splice site
  - U1 snRNP binds to the 5'-splice site
    - Creates the E (early) complex

Spliceosomes

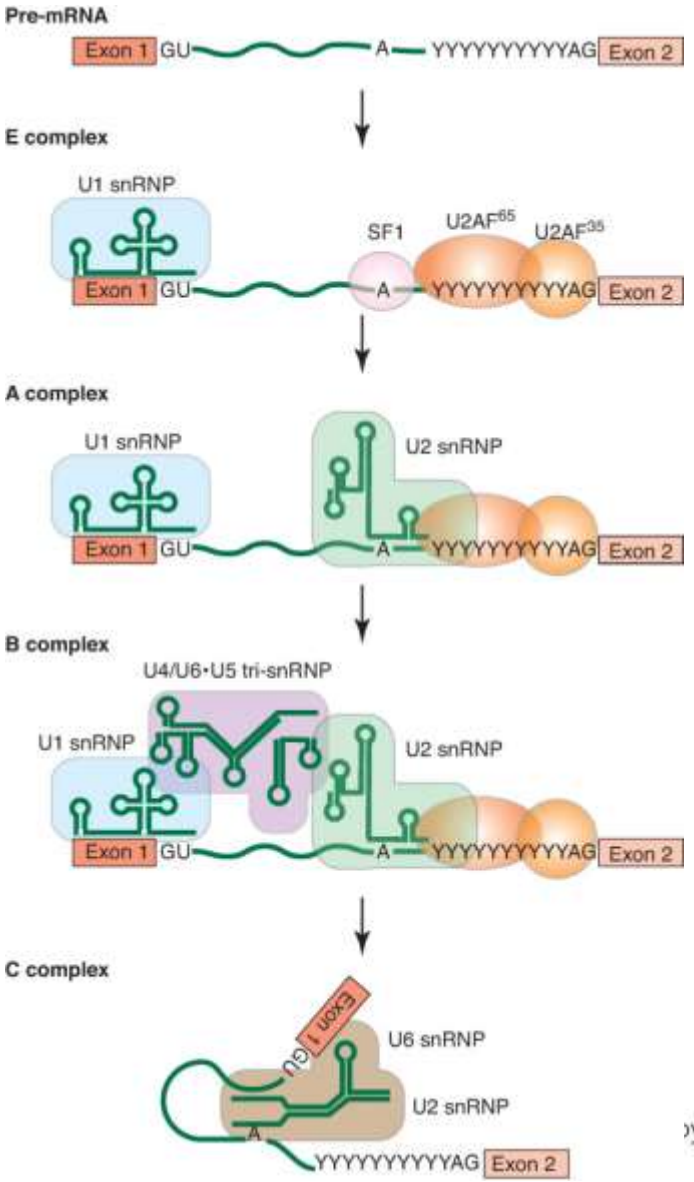


Figure 14.22: Spliceosome assembly. During the first step in stepwise spliceosome assembly, U1 snRNP binds to the 5′-splice site.

(Modified from Hertel, K. J., and Graveley, B. R. 2005. Trends Biochem Sci 30:115–119. Copyright 2005, with permission from Elsevier [http://www.sciencedirect.com/science/journal/09680004].)

Cleavage/Polyadenylation and Transcription Termination

Poly(A) tail synthesis and transcription termination are coupled, cotranscriptional processes

1. **Pre-mRNA cleavage**: Pre-mRNA is cleaved at a site called the cleavage/polyadenylation site or the poly(A)site
2. **Poly(A) addition**: Poly(A) tail is added to the newly generated 3’-end
3. **Transcription termination**: Transcription is terminated downstream from the poly(A) site

Cleavage/Polyadenylation and Transcription Termination

- The poly(A) site in mammals is located between a polyadenylation signal and a U-rich element
  - Requires ≈ 85 different proteins

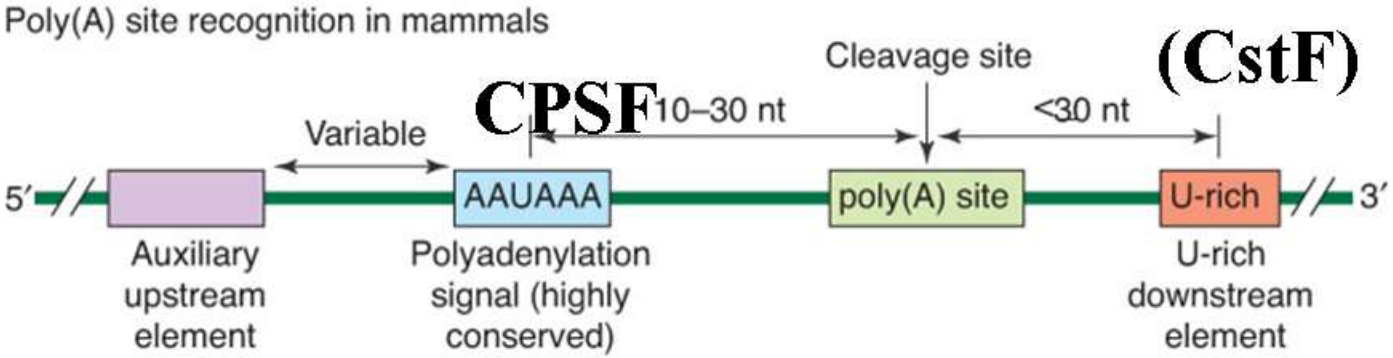


Figure 14.26: Schematic representation of Poly(A) site recognition in mammals.

(Adapted from Gilmartin, G. M. 2005. Genes Dev 19:2517–2521.)

Cleavage/Polyadenylation and Transcription Termination

1. **Cleavage/polyadenylation specificity factor (CPSF)** binds to the poly(A) signal
2. **Cleavage stimulation factor (CstF)** binds to the U-rich sequence and is required for cleavage at the poly(A) site
3. **Cleavage factor I** and **cleavage factor II** are required but little is known about their function
4. **Poly(A) polymerase** add adenylates to the 3’ end and is recruited by CPSF