# BIOC 385: M11.T02-Miesfeld

Assigned Reading: *Biochemistry* Chapter 24.3

THE UNIVERSITY
OF ARIZONA

# Mechanisms of Eukaryotic RNA Processing

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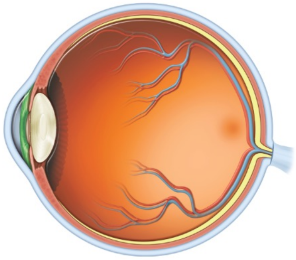
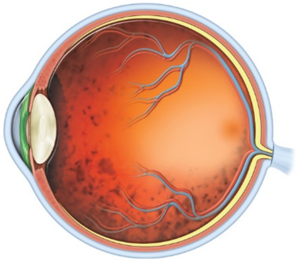
# The Big Picture

- RNA processing converts primary transcripts into mature mRNA, tRNA, and rRNA molecules that are essential for translation.
- Processing requires ribozymes, protein–RNA complexes, and RNA polymerase II coordination, ensuring transcript maturation.

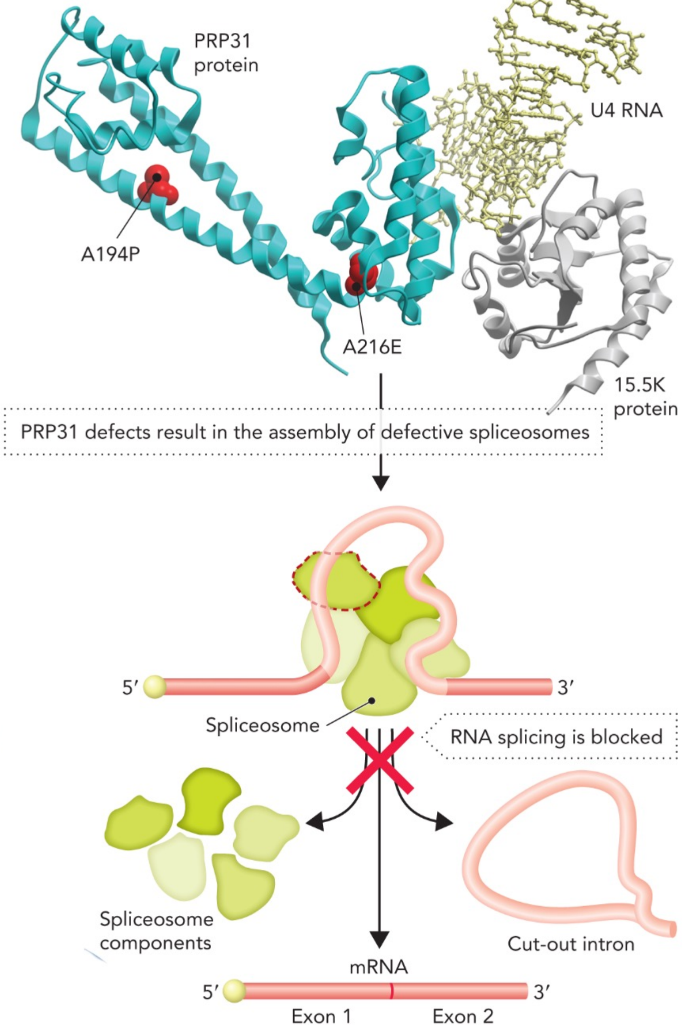

Normal view


Normal retina


View with retinitis pigmentosa

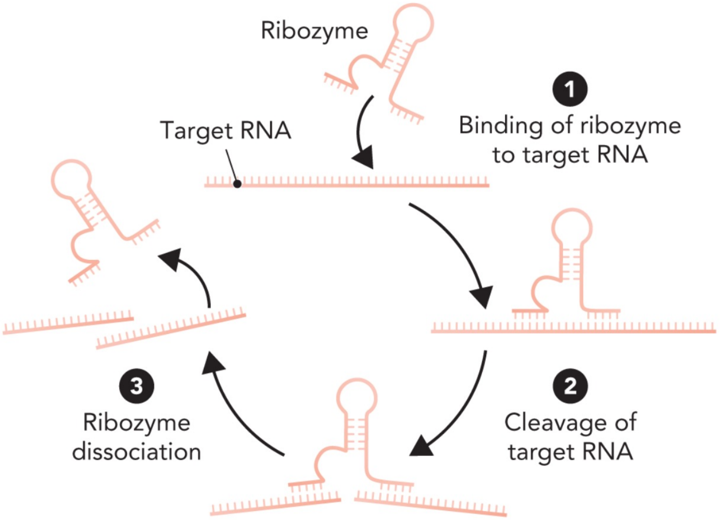

Retina with retinitis pigmentosa

Mutations in the splicing protein PRP31 are responsible for retinitis pigmentosa



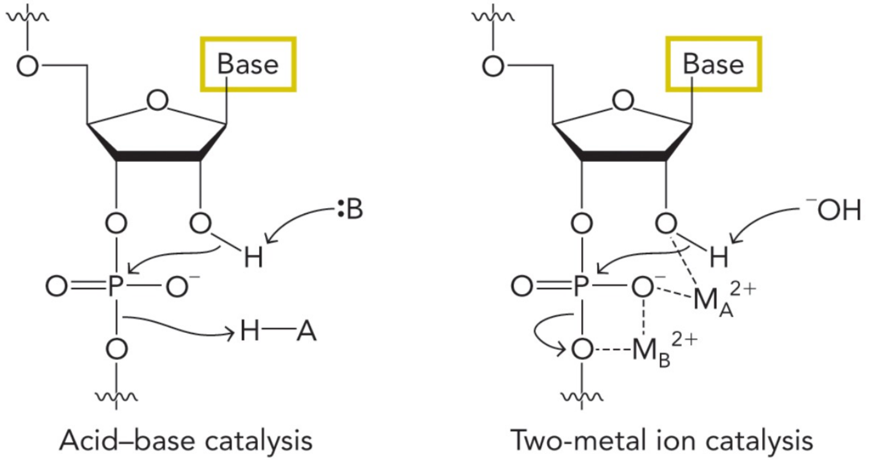
# Ribozymes are RNA Catalysts

- Ribozymes catalyze intramolecular or intermolecular cleavage of RNA substrates and are regenerated after each catalytic cycle.
- The discovery of ribozymes provided evidence for the RNA world hypothesis, showing that RNA serves as both genetic material and a catalyst in biochemical reactions.



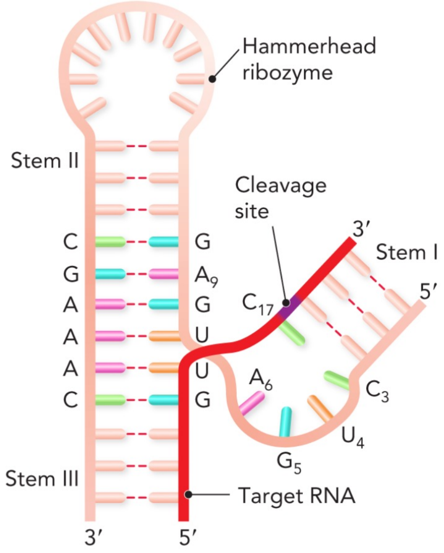
# Mechanisms of Ribozyme-Mediated Cleavage

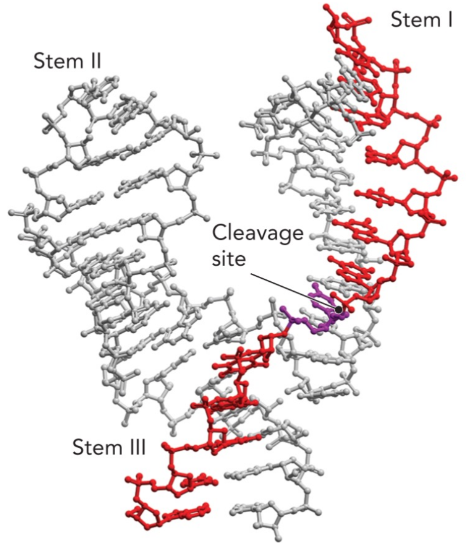
- Ribozymes cleave RNA using general acid–base catalysis or two-metal ion catalysis, mechanisms that are similar to protein enzymes.
- These strategies highlight the chemical versatility of RNA, which can mediate precise phosphodiester bond cleavage without protein cofactors.



# Structure of the Hammerhead Ribozyme

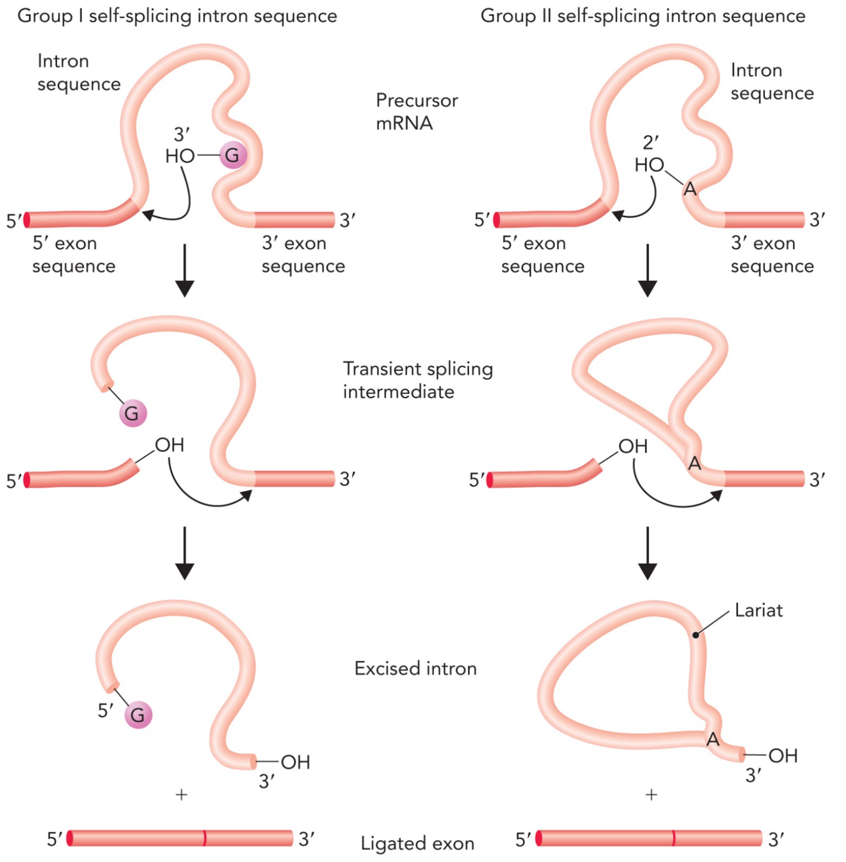
- Hammerhead ribozymes form base-paired stems that catalyze trans cleavage of target RNA substrates.
- The conserved tertiary structure of hammerhead ribozymes demonstrates how RNA folds into active sites for catalysis.





# Self-Splicing Introns: Group I and Group II

- Group I introns require an exogenous guanosine to initiate splicing, generating a linear excised intron.
- Group II introns form a lariat through attack by a branch-point adenosine, a mechanism similar to that of spliceosomes.



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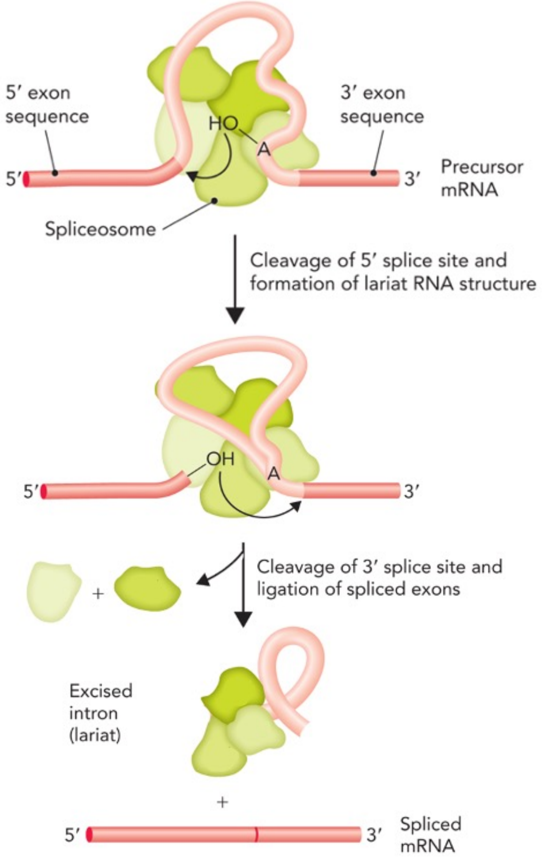
# Spliceosome-Mediated RNA Splicing

- Spliceosomes remove introns through transesterification reactions that are similar to group II intron self-splicing.
- RNA-binding proteins in spliceosomes provide structural support, ensuring accuracy and efficiency of intron removal.



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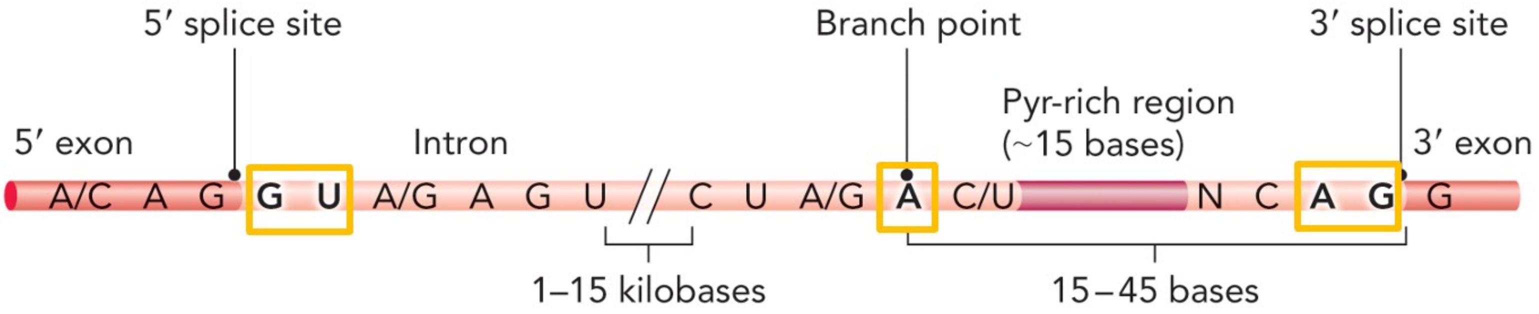
# Conserved RNA Sequences at Splice Sites

- Introns in pre-mRNA contain conserved 5′ GU, branch-point A, and 3′ AG sequences that define splice junctions.
- These short motifs are critical recognition sites for spliceosome assembly and catalysis of lariat formation.



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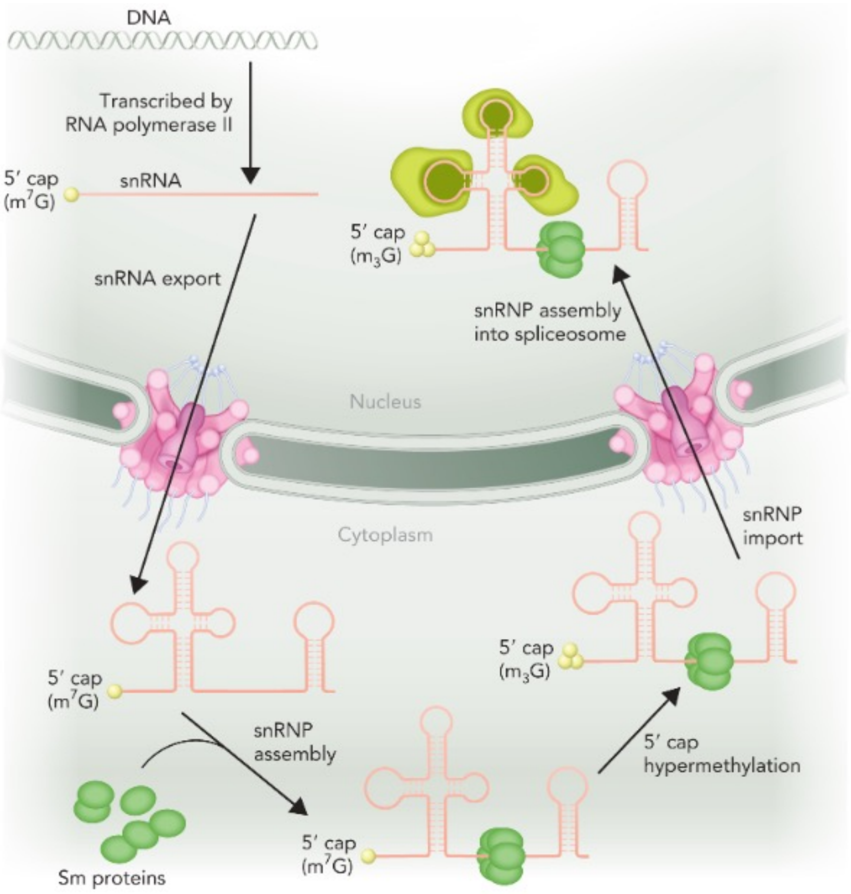
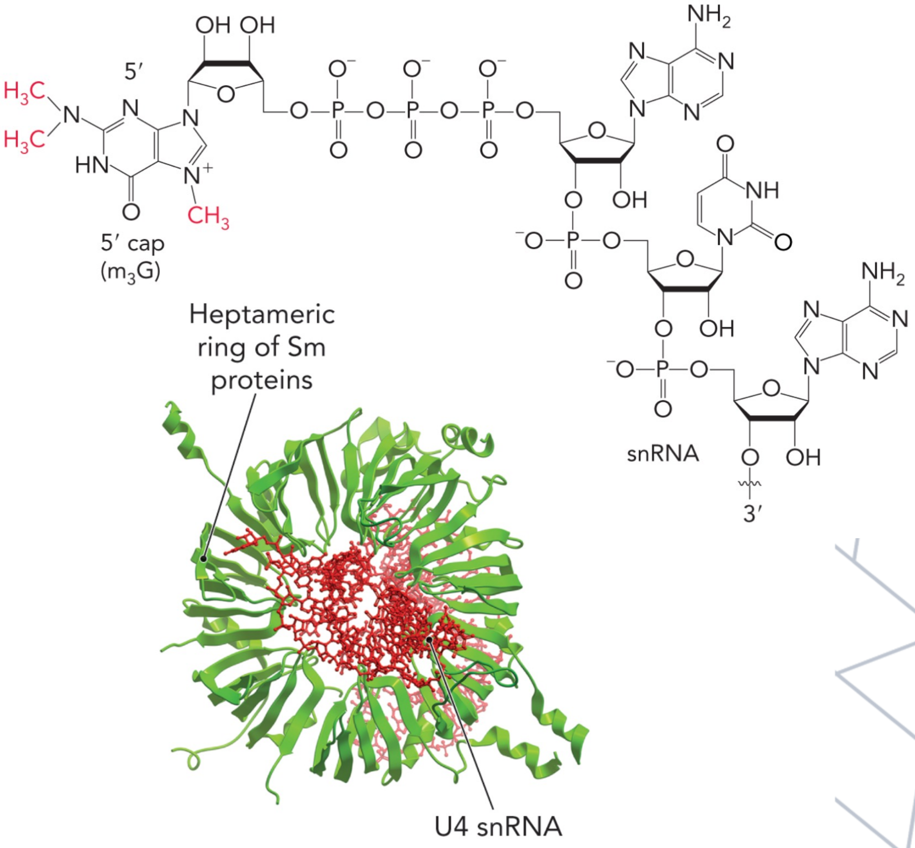
# Spliceosome Assembly Pathway

- snRNAs are capped, exported, assembled with Sm proteins in the cytoplasm, and then reimported into the nucleus as mature snRNPs.
- This cyclical pathway ensures spliceosome components are properly modified and assembled for accurate pre-mRNA splicing.



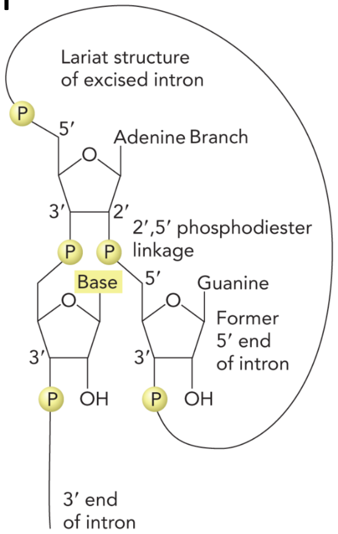
# Structure of snRNP Complexes

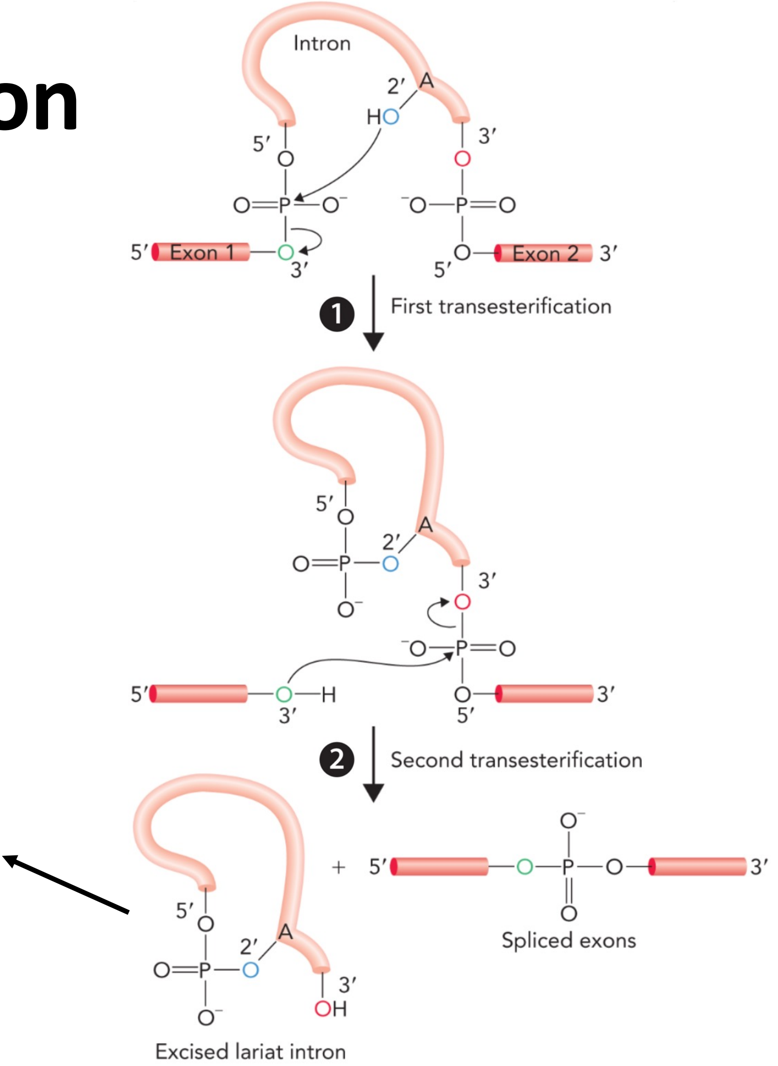
- The U4 snRNP structure reveals the central Sm protein ring with bound snRNA, stabilized by a trimethylguanosine cap.
- The m³G cap modification protects snRNAs from degradation, ensuring their stability during splicing cycles.



# Spliceosome Transesterification

- In step one, the branch-point adenine 2′-OH attacks the 5′ splice site, forming the intron lariat.
- In step two, the free 3′-OH of the upstream exon attacks the downstream exon, completing intron removal and exon ligation.





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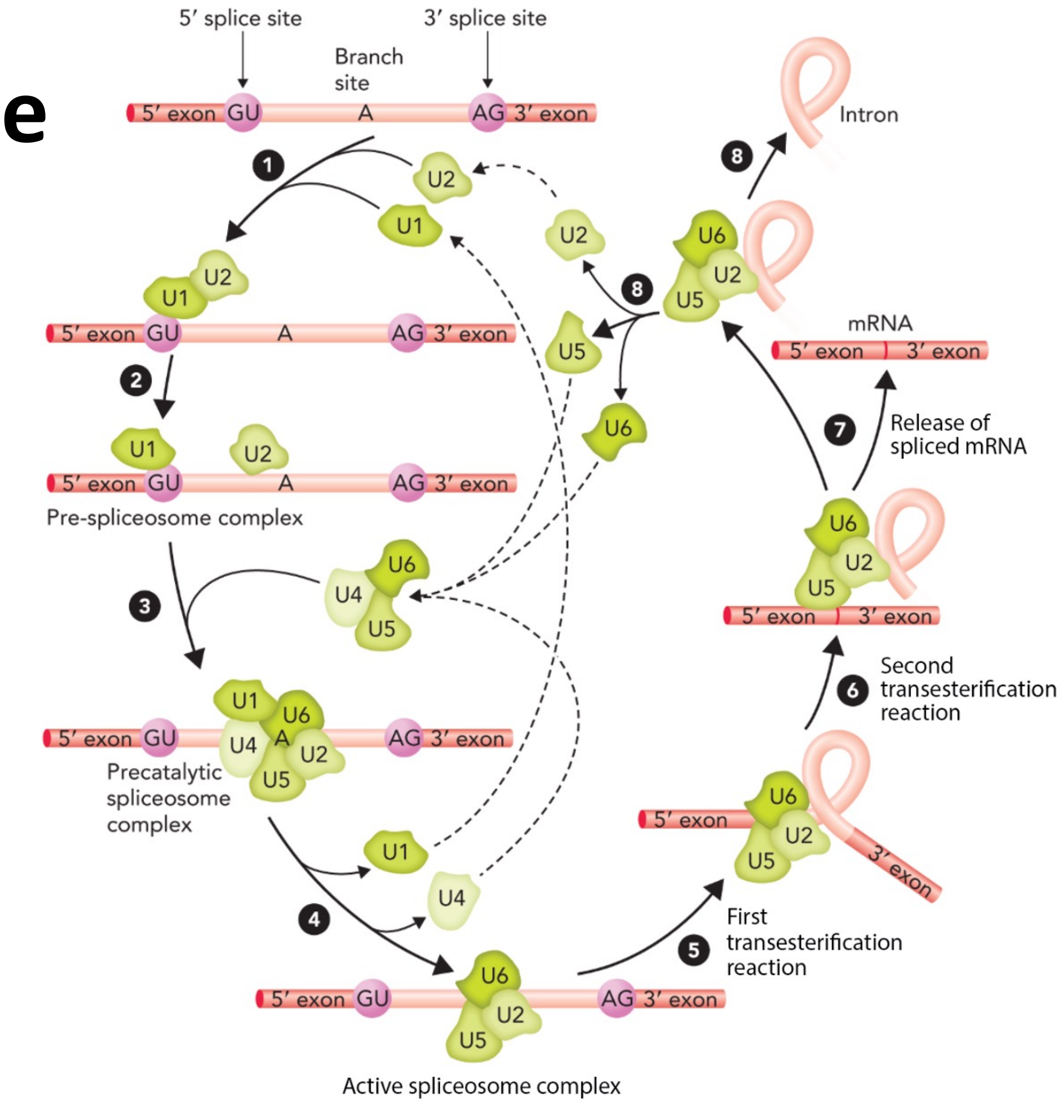
# The Spliceosome Cycle

- Sequential binding of U1, U2, and the U4/U5/U6 tri-snRNP rearranges RNA–RNA base pairing to activate catalysis.
- Base pairing of snRNAs with splice-site sequences ensures precise alignment of donor and acceptor sites during splicing.



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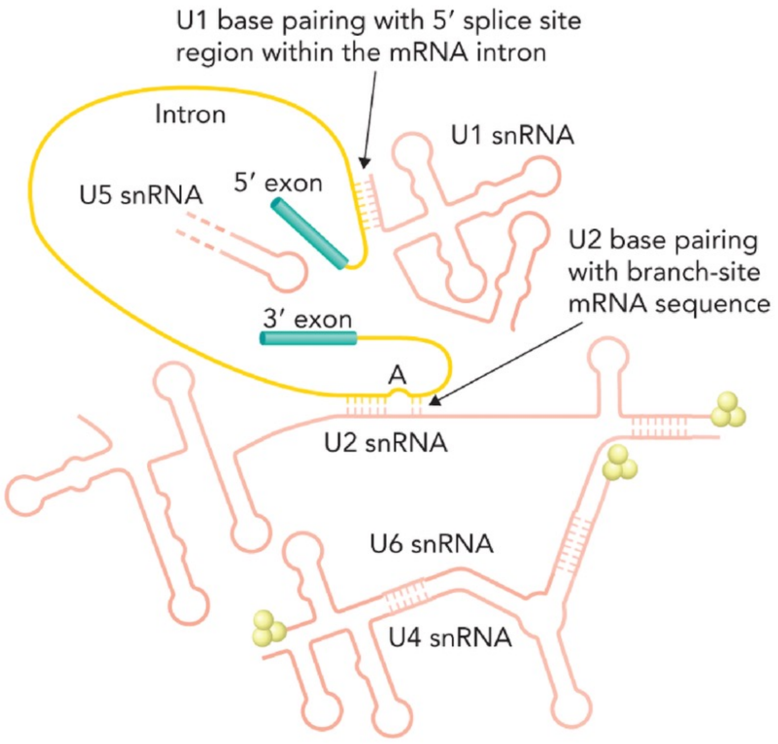
# RNA-RNA Base Pairing Within the Spliceosome

- There are multiple intrastrand and interstrand base pairs form between the snRNA molecules within the spliceosome complex.
- The U1 snRNA directly base-pairs with sequences in the 5‘ donor site, whereas the U2 snRNA base-pairs with residues in the branch site.
- The hypermethylated snRNA $m_3G$ cap is represented by yellow spheres.



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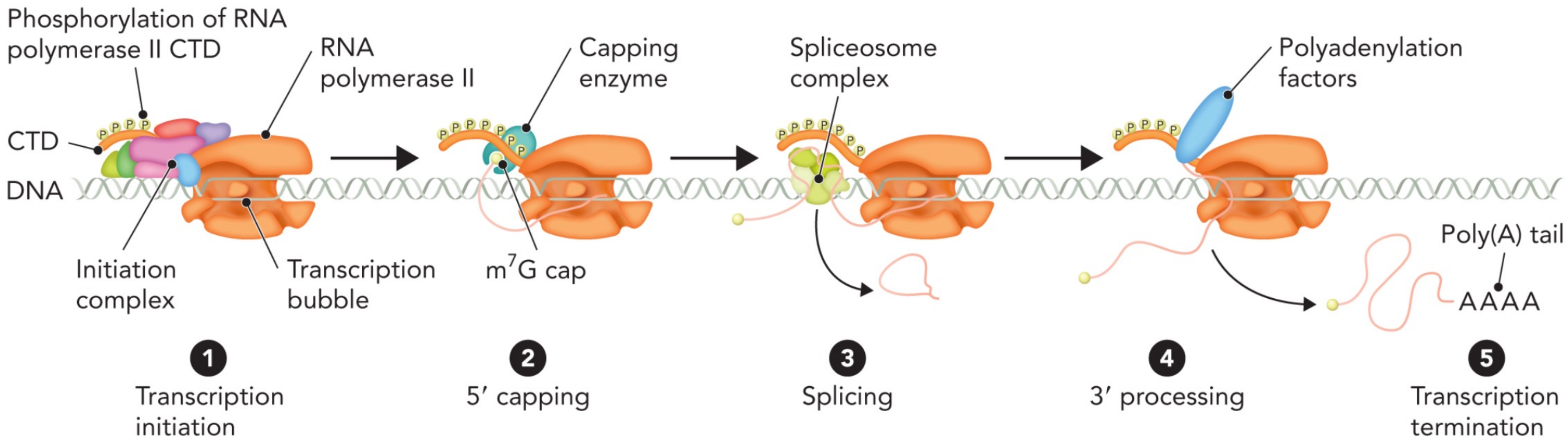
# RNA Pol II Coordinates mRNA Processing

- The CTD of RNA Pol II recruits enzymes for 5′ capping, splicing, and 3′ polyadenylation, integrating transcription with processing.



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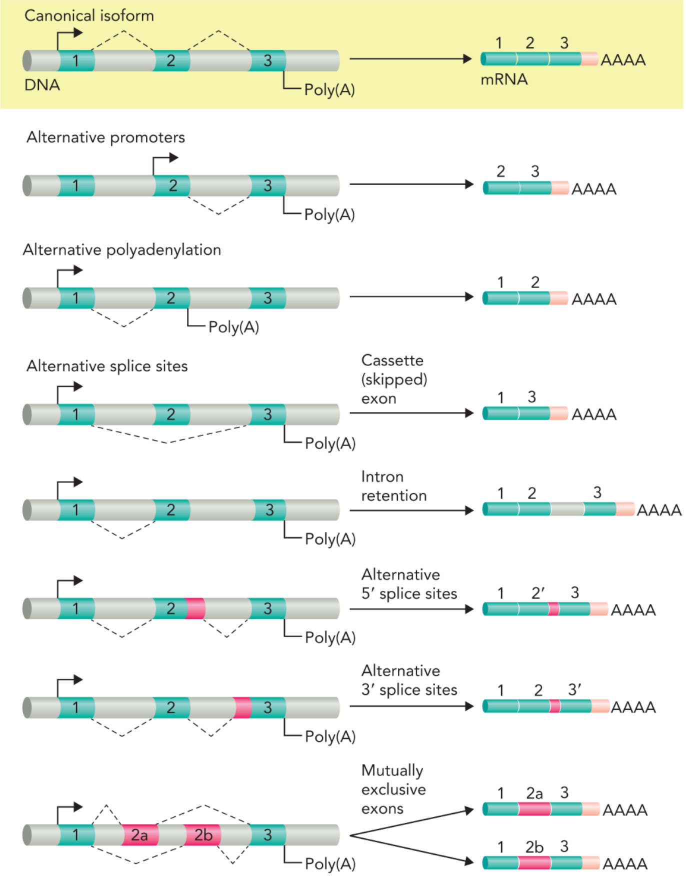
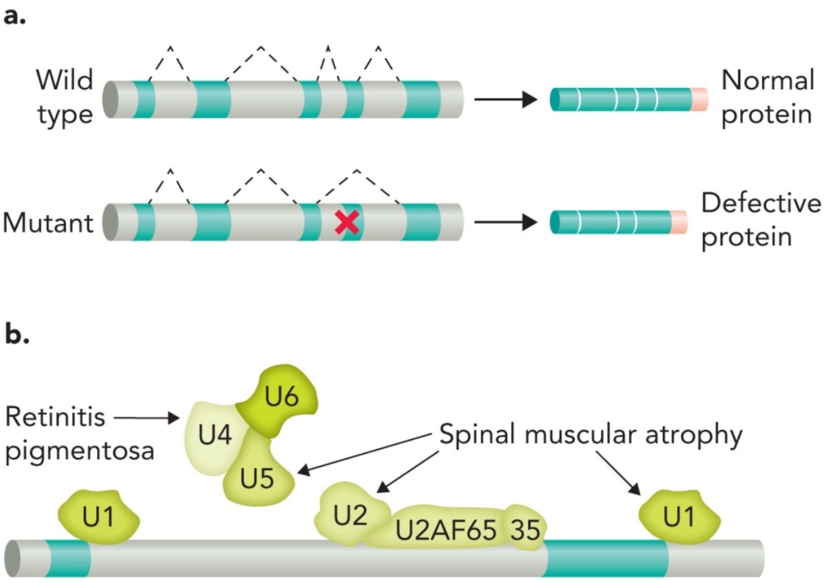
# Alternative RNA Splicing Generates Diversity

- Protein diversity in eukaryotes reflects the use of alternative promoters, polyadenylation sites, and alternative splicing.
- The canonical transcription, splicing, and polyadenylation pattern is shown at the top, and the predicted coding sequence of the resulting mRNA transcript is shown at the right.
- Translation of each mRNA results in a unique protein.



# RNA Splicing Defects Cause Disease

- Splicing defects that cause disease can be due to defects in splicing recognition sequences (cis mutations) or in components of spliceosome complexes (trans mutations).
- Trans mutations in the snRNA or protein components of spliceosomes have been identified in the human diseases retinitis pigmentosa and spinal muscular atrophy.



# Key Concepts to Guide Your Learning

- Ribozymes are enzymes that contain a catalytically active RNA; the hammerhead ribozyme is a catalytic RNA that self-cleaves and cleaves target RNA molecules.
- Group I and group II self-splicing intron reactions do not require proteins; Group II intron self-splicing and spliceosome-mediated splicing give rise to an excised lariat intron structure that is degraded.
- Eukaryotic precursor mRNA introns processed by spliceosomes are flanked by conserved sequences at the 5ʻ and 3ʻ splice sites; the branch site is ~15–45 nucleotides upstream of the 3ʻ splice site and can contain a polypyrimidine tract.
- Alternative splicing of mRNA can increase genomic complexity but can also cause disease if defective; DNA mutations can lead to a gain or loss of splice sites, resulting in alternative splicing and production of aberrant proteins.