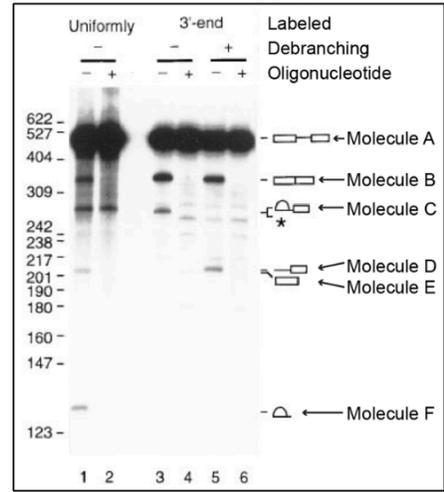


Question 7 (20 pts). In addition to the spliceosome studied in class, which is known as the major spliceosome, there is a secondary set of machinery known as the minor spliceosome that acts upon a subset of transcripts. Knowledge of how the major spliceosome works was also instrumental in understanding the minor spliceosome. Here we will compare and contrast mechanisms utilized by the major and minor spliceosomes.

Part I: Researchers combined purified components of the minor spliceosome with unspliced mRNA (Molecule A), and monitored the formation of intermediates and products by separating them on a gel and visualizing using autoradiography. In lanes 1-2, the unspliced mRNA is radioactively labeled at every alpha phosphate (Uniformly); in lanes 3-6, only the 3' end is radioactively labeled (3'-end). In lanes 5-6, a debranching enzyme is included in the reaction. In lanes 2,4, and 6, an oligonucleotide was also included that is complementary to something in the reaction mixture. (Note: Molecule E is only observed in lane 1, and Molecule D is only observed in lane 5; they just happen to run at roughly the same place on the gel.)



7.1: Based on the molecules noted on the right-hand side of the reaction, write the letters that correspond to the molecules that are the intermediates and products of the splicing reaction from the minor spliceosome. These all match what we would expect for the major spliceosome. Each arrow represents a strand transfer (0.5 pts each)

Molecule A → Molecules and → Molecules and

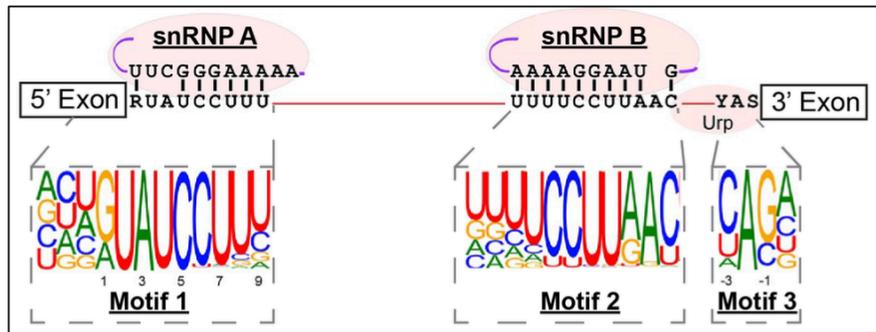
7.2: What bond is broken by the debranching enzyme to generate a linear product? Be specific. (0.75 pts)

7.3: Why is Molecule F visible in lane 1, but not in lane 3? Explain briefly (1.5 pts)

7.4: What translation-dependent process discussed in class would be most likely to degrade unspliced mRNAs in cells? (0.75 pts)

7.5: What is the impact of the oligonucleotide on the splicing reaction? Explain briefly, noting two lanes and the bands that correspond to specific molecules in your answer (1 pt)

Part II: Researchers used sequence analysis to study the features of genes typically acted upon by the minor spliceosome, yielding the sequence logo that is shown to the right. This analysis revealed three sets of sequences (Motif 1-3) and two snRNA bindings sites (snRNP A and B). The one-letter code S denotes G or C.



7.6 What is the name of the functional equivalent of snRNP A in the major spliceosome, as discussed in class? (0.75 pt)

7.7: What is the name of the functional equivalent of snRNP B in the major spliceosome, as discussed in class? (0.75 pt)

7.8 What is the name of the functional equivalent of Motif 1 in the major spliceosome, as discussed in class? (0.75 pt)

7.9 What is the name of the functional equivalent of Motif 2 in the major spliceosome, as discussed in class? (0.75 pt)

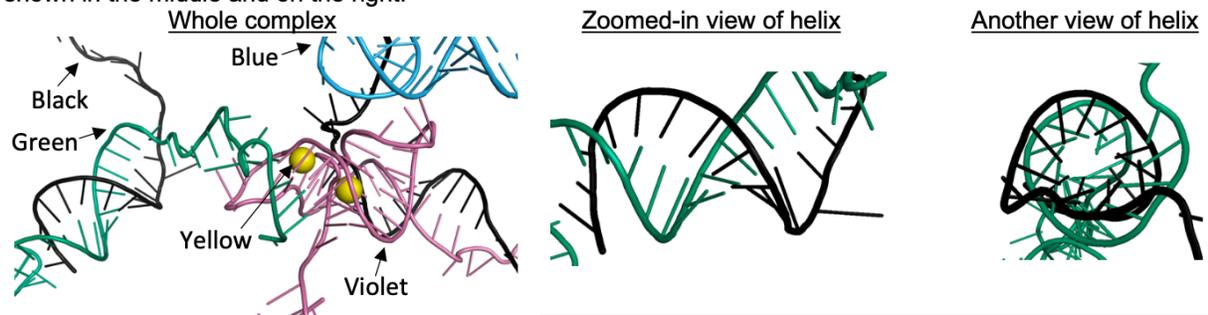
7.10 What is the name of the functional equivalent of Motif 3 in the major spliceosome, as discussed in class? (0.75 pt)

7.11: From the sequence logo and secondary structure diagram shown above, does the minor spliceosome recognize specific sequences in the 5' exon? How does this differ from the equivalent interaction in the major spliceosome, as discussed in class? Limit your answer to one sentence. (1.5 pts)

7.12: Draw an asterisk directly below the nucleotide that acts as the nucleophile for the first step of the splicing reaction on the image at the start of part II. (0.5 pt)

7.13: How did you identify the nucleotide that acts as the nucleophile in question 7.12? Limit your answer to one sentence, and name the specific secondary structure feature that led you to this conclusion. (1.5 pts)

Part III: Researchers solved a structure of the minor spliceosome prior to the first strand transfer event, which proceeds by the same mechanism as in the major spliceosome. Here the snRNA B, U5 snRNA, U6 snRNA, pre-mRNA, and divalent cations are all shown in different colors. A zoomed-in image of the green/black helix is shown in the middle and on the right.



7.14: What is the conformation of the black/green helix? What 3 features led you to this conclusion? (2 pts)

7.15: Write the name of the molecule (snRNA B, U5 snRNA, U6 snRNA, or pre-mRNA) that corresponds to the following colors in the structure. (0.5 pts each)

Blue

Green

Violet

Black

7.16: What is the identity of the black nucleotide in the middle image that is not involved in Watson-Crick base pairing? (0.75 pt)

7.17: Is this complex ready to catalyze the first strand transfer event? If so, what features led you to this conclusion? If not, what rearrangements are needed to activate this complex? Answer in 1-2 sentences. (2 pts)