

P033 The U6 small nuclear RNA directs exon alignment during pre-messenger RNA splicing
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Removal of introns from pre-messenger RNA and correct ligation of coding exons is catalyzed by the spliceosome, a large RNA-protein complex. Exon ligation requires precise alignment of the exon ends to be joined. During pre-messenger RNA splicing in the yeast *Saccharomyces cerevisiae* the U6 snRNA contributes to catalysis in the spliceosome while the U5 snRNA loop 1 holds the exons in the correct orientation for ligation. It is not known how the variable exons are positioned precisely with U5 loop 1. Here we show that reconstitution of functional spliceosomes with U6 mutants A59U or A51U results in the misalignment of the 3' exon with U5 loop 1. The 5' exon interaction with U5 loop 1 is not affected by these U6 mutations. In addition, mutations in U6 that inhibit the second step of splicing are synthetic lethal with deletions in U5 loop 1 whereas a mutation in U6 that inhibits the first step is not. Our data indicate that the U6 snRNA is responsible for aligning the 3' exon with the 5' exon on U5 snRNA loop 1 for the second catalytic step of pre-messenger RNA splicing.