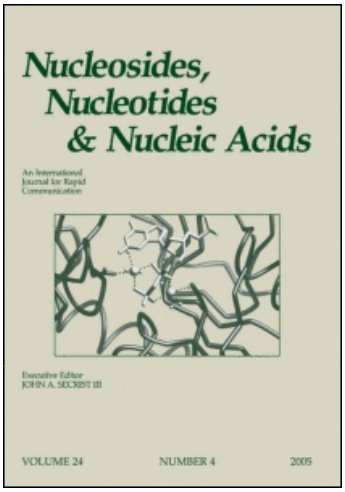

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Oligonucleotide Mediated Mutational Analysis of Recognition Sequence Effects on Lariat Formation During Messenger RNA Splicing in Yeast

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OLIGONUCLEOTIDE MEDIATED MUTATIONAL ANALYSIS OF RECOGNITION SEQUENCE EFFECTS ON LARIAT FORMATION DURING MESSENGER RNA SPLICING IN YEAST

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Abstract. In the yeast Saccharomyces cerevisiae the 5' and the 3' splice junctions and the internal branch acceptor site (TACTAAC-box) are highly conserved intron elements. They represent favourable targets for oligonucleotide mediated site directed mutagenesis.

With the help of site directed mutagenesis (1) we investigated the effects of sequence and positioning of signal sequences within the intron of the yeast actin gene on the efficiency of mRNA splicing. We have previously demonstrated (2) that in yeast the 5' and 3' splice sites are recognized independently, but always in conjunction with the branch site. S. cerevisiae not only follows the "GT/AG-rule" (conserved dinucleotide sequences at the 5' and 3' boundaries consist of GT and AG respectively) of higher eukaryotes (3) but also exhibit a full consensus at the branch site (TACTAAC). From our experiments we had learned that a cryptic branch site with the sequence TACTAAG (located 14 bp upstream) can be activated if the TACTAAC-box is deleted (4), although it is an inefficient substitute, which is not used under normal circumstances. A mutation of the TACTAAC-box to a TAATAAC sequence generates a poorly utilized signal and the cryptic TACTAAG becomes activated again. The contributions to splicing efficiency deriving from the TACTAAG and the TAATAAC signals (47% of wild type efficiency) are more than additive when compared to their individual contributions (9% and 8% respectively). This suggests that interaction of splicing components with either one of the signals facilitates the interaction with the other. A variety of analytical approaches delivered the information about the splicing mechanisms. The methods include splicing dependent β-galactosidase ex-

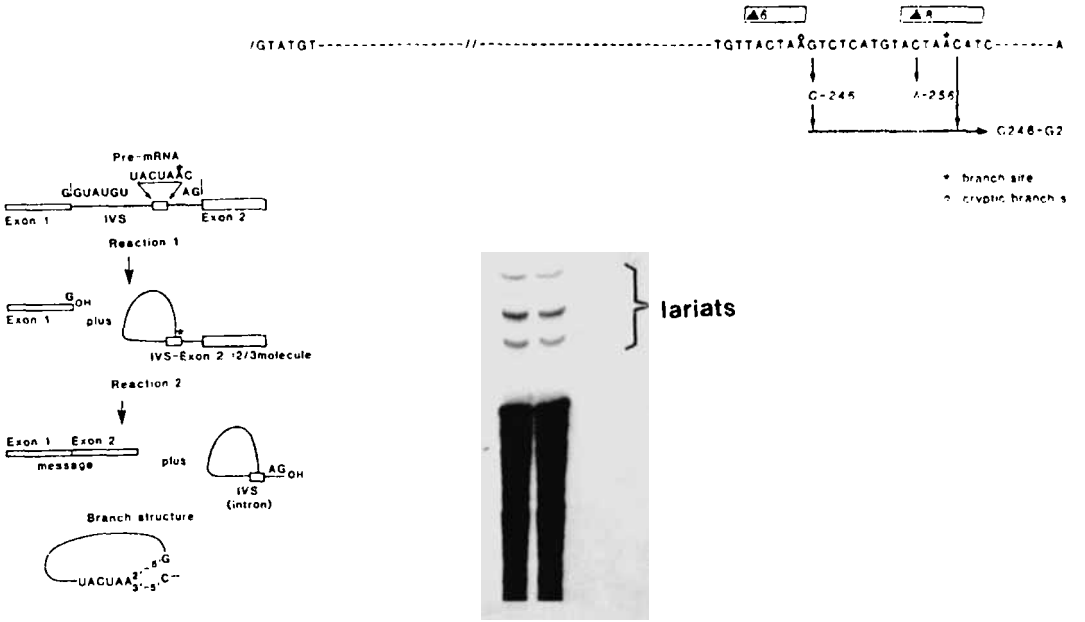

FIGURE 1

pression (5), in vitro splicing assays (6), in vivo and in vitro RNA analyses (2,7).

In the mutant C 246 the cryptic branch point has been mutated to a strong TACTAAC signal. Both sites are then used with comparable frequency for lariat formation, indicating that recognition does not include a unidirectional scanning mechanism along the intron. The overall splicing efficiency dropped to 85% of the wild type rate.

At present our interests are focused on the mutant C 246-G 260, where cryptic and real TACTAAC-box have been interchanged. The autoradiograph of the in vitro splicing reaction shows the unusual presence of three lariat bands. This mutation creates an additional AG signal and a good branch acceptor site where the weak signal used to be. While the exact identity of the lariat products is under investigation, this preliminary result strongly indicates that alternative splicing in yeast is possible.

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