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A NOVEL C/A-RICH EXONIC SPLICING ENHANCER IS ENRICHED BY AN ITERATIVE SELECTION PROCEDURE PERFORMED *IN VIVO*

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Many exons contain auxiliary splicing elements referred to as splicing enhancers. Most enhancers identified thus far contain a purme-rich motif. We propose that additional splicing enhancers with different sequence motifs are likely to be present in vertebrate exons. To identify novel splicing enhancers, we have established a transient transfection scheme to select for exon sequences that enhance exon inclusion *in vivo.* Our approach is modeled on the *in vitro* SELEX procedure and, to our knowledge, is the first application of an iterative procedure to select for RNA sequences that enhance RNA processing in vertebrate cells. Synthetic DNA fragments containing 13 random positions were ligated into the middle exon of a three exon minigene. The middle exon is skipped in the absence of a positive-acting splicing element. Sequences that enhance exon inclusion are "captured" in the mRNA, amplified by RT-PCR, and cycled through multiple rounds of ligation-transfection-amplification. Two predominant classes of enhancers were enriched after three rounds. One is a purine-rich motif that resembles previously identified splicing enhancers. Isolation of purine-rich enhancers validates the *in vivo* selection approach. The second motif consists of a novel C/A-rich sequence. Selected C/A-rich sequences enhanced splicing of a heterologous exon indicating that the activity of this motif is independent of the minigene used for selection. Enhancer activity was reproduced *in vitro* using HeLa nuclear extracts. Point mutations within the C/A motifs disrupted enhancer activity *in vivo* and *in vitro* demonstrating that enhancer activity is sequence-specific. Enhancer-dependent splicing is competed *in vitro* by RNAs containing unmodified enhancers but not by RNAs containing mutations in the C/A-rich motif demonstrating that enhancer activity is mediated by sequence-specific interaction between the C/A-rich motif and *trans-acting* factors required for splicing. Interestingly, the C/A-rich enhancer resembles the 13 nucleotide *Drosophila* doublesex *(dsx)* repeat element. One copy of the *dsx* repeat was one of the strongest enhancers tested in our minigene. Sequence requirements for *dsx* enhancer activity is the same in vertebrate and Drosophila cells. We are investigating the factors that mediate enhanced splicing of C/A-rich and *dsx* enhancers in vertebrate cells.