Plant Exon and Intron Splicing Enhancers

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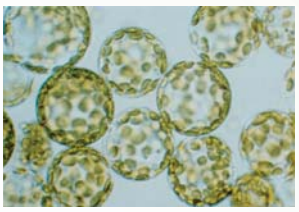
Introduction

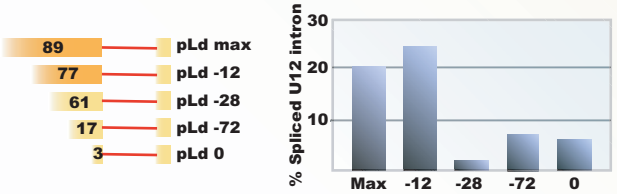
A range of splicing signals exist to regulate splicing of different genes at different times and in different cells. Short intronic and exonic sequence elements either boost (enhancers) or limit (silencers) the use of nearby splice sites. Splicing enhancers are also likely to function in regulation of plant genes by alternative splicing. Although SR proteins have been widely studied in plants, little is known about splicing enhancer signals.

A plant exon splicing enhancer improves U12 intron splicing

Vertebrate U12-dependent introns generally splice less efficiently than the common U2-dependent introns. In studies of plant U12 introns, an *Arabidopsis Luminidependens* intron spliced ~3X more efficiently than other U12 introns.



Plasmid constructs containing the *Ld* U12 intron were prepared in plant expression cassettes with variable amounts of exon sequence and transfected into tobacco protoplasts. RT-PCR analysis revealed the requirement for the upstream exon 10 for enhanced U12 intron splicing.



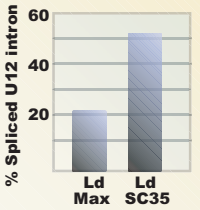
Deletion analysis of the upstream exon showed a significant drop in splicing efficiency with the removal of a 16nt region (between -12 and -28 deletions) of exon 10.

The region between the -12 and -28 deletions enhances Ld U12 intron splicing. This is one of three regions that contain sequences with homology to SR binding sites defined in vertebrate systems. We are currently mutating the -12/-28 region to define the ESE.

Creation of an SC35 binding site improves U12 intron splicing

The more efficient splicing of the Ld U12 intron requires an ESE and presumably a binding site for SR protein(s). To examine whether the Ld U12 exon could be used to screen for potential enhancer sequences, an SC35 site was created in the upstream exon 16-23nt fom the U12 intron 5' splice site.

TTCCACCAAGAAACACATGC TTGGTTCAAATCCATCGT
TTCCACCAAGAAACACATGCTCTTGGTTCAAATCCATCGT
TGCTCTTG SC35

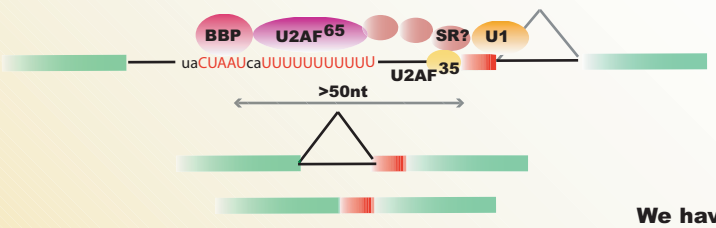


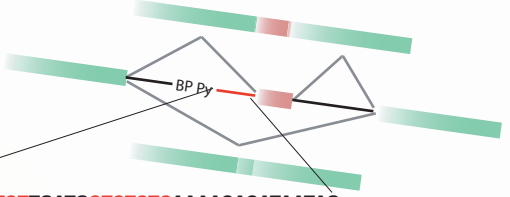
This resulted in a 2 fold enhancement of splicing of the U12 intron. Thus, potential enhancer sequences can be tested in this sensitive splicing assay.

Intron splicing signals enhance mini-exon inclusion

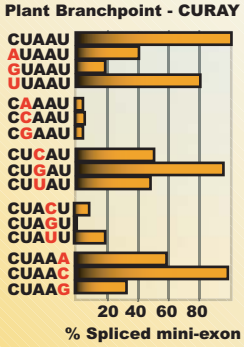
Constitutive splicing of the potato invertase mini-exon 2 (9nt long) requires a strong branchpoint and polypyrimidine tract located more than 50nt upstream of the mini-exon. These signals can also splice synthetic mini-exons, including mini-exons of even 1nt. Disruption of these essential splicing signals leads to mini-exon skipping.

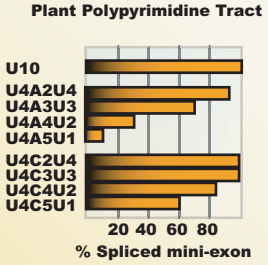
We have shown that intron 2 is spliced first such that exon bridging interactions are thought to link the bp/pY tract to the 5' splice site of intron 2 to promote removal of the intron. Intron 1 is then removed.



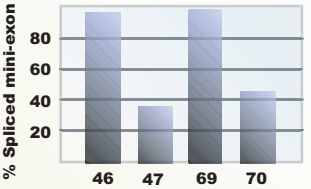


Inv46 GATGATCTCTCTTGATGCTCTCTCAAAACACATAATAG
Inv47 GATGATCACACATGATGCACACACAAAACACATAATAG
Inv69 GATGATCACACATGATGCTCTCTCAAAACACATAATAG
Inv70 GATGATCTCTCTTGATGCACACACAAAACACATAATAG





We have further examined other sequences in the region between the polypyrimidine tract and the 3' splice site. This region is 38nt long and contains of two GATG/pyrimidine repeats. Mutations were made to the pyrimidine region of the two repeats both singly (Inv 69 and 70) and as a double mutation (Inv47).



Mutation of the downstream repeat led to a reduction of mini-exon inclusion to about 40% and onset of exon skipping.

The downstream repeat acts as an intron splicing enhancer, is important for efficient mini-exon splicing and may bind a factor required for establishing the exon bridging interaction.

Weakening the splicing signals leads to mini-exon skipping.

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