Sequences that enhance plant splicing.

C.G. Simpson, D. Lewandowska, M.S. Liney, G.P. Clark, C.M. Booth & J.W.S. Brown

Plant gene expression involves the transcription of messenger RNAs (mRNAs) that are translated into proteins which regulate all aspects of plant growth and development. Most plant genes contain introns (nonsense sequences) which are removed from mRNAs by splicing. Splicing depends on the recognition of intron signals by a large RNA and protein complex called the spliceosome. The recognition of signals leading to intron removal is a complex process and is an important level at which gene expression is controlled. A wide range of signals have therefore evolved to allow different genes to be regulated at different times or in different cells. In vertebrates, short intronic and exonic sequence elements either boost (enhancers) or limit (silencers) the use of nearby splice sites. Although splicing enhancers likely function in splice site selection in many plant genes, and contribute to the regulation of alternative splicing, such sequences have not yet been described in plants.

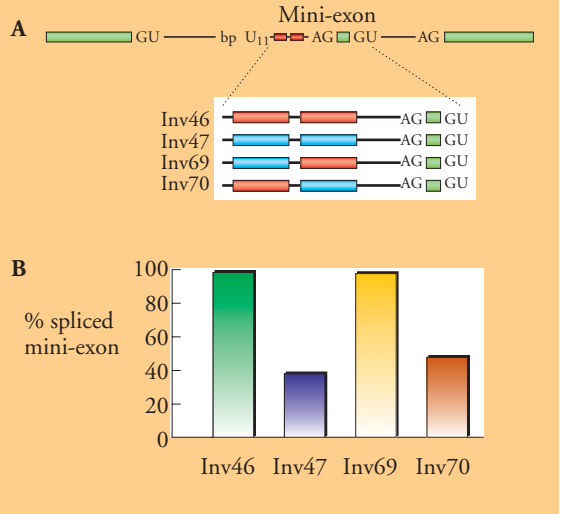

Figure 1 Plant intron splicing enhancer.
A. Two blocks of sequence repeats (red blocks) in the region between the invertase polypyrimidine tract and the invertase mini-exon 3' splice site were mutated (blue blocks) in the construct Inv46, both singly (Inv69 and Inv70) and as a double mutation (Inv47). **B.** Splicing analysis for the four constructs is shown graphically and reveals mutation of the downstream sequence block leads to reduction in splicing of the mini-exon.

Plant Intronic Splicing Enhancer Constitutive splicing of the potato invertase mini-exon 2, which is only 9 nucleotides (nt) long, requires a branchpoint sequence and a polypyrimidine tract located about 50 nt upstream of the mini-exon.[1,2] The sequence between the polyprimidine tract and 3' splice site is 38 nt long and consists of two GATG/pyrimidine repeats. To investigate the importance of these two elements in invertase mini-exon splicing, mutations were made to the pyrimidine region of the two repeats both singly (inv69 and inv70) and as a double mutation (inv47) (Fig. 1A). When the downstream repeat was mutated, mini-exon inclusion was reduced to between 40 and 50% of spliced transcripts (Fig. 1B). This shows that the second of the two repeat sequences is important for enhancing the splicing of the mini-exon and represents a novel intronic splicing enhancer.

Plant Exonic Splicing Enhancer U12-dependent introns are a class of non-abundant introns with non-canonical 5' and 3' splice sites, and branchpoint sequences. In vertebrates, these introns splice less efficiently in comparison to the abundant canonical introns and are thought to regulate the level of expression of the genes in which they are found. In a detailed study of three plant U12 introns we found that splicing in tobacco protoplasts was very poor,

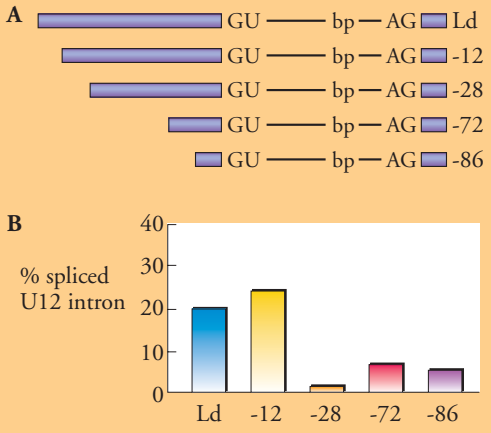

Figure 2 Plant exon splicing enhancer.
A. Schematic shows progressive deletion of the exon upstream of the U12 intron. Introns are shown as a line bordered by GU and AG, with an internal branchpoint bp. Exons are shown as a coloured box. **B.** Splicing analysis of the 5 constructs is shown graphically and reveals the highest level of U12 intron splicing in the Ld and Ld-12 constructs. Deletion of the region between the -12 and -28 constructs shows a significant reduction in U12 splicing.

with the exception of a U12 intron from the *Arabidopsis thaliana* gene *LUMINIDEPENDENS* (*Ld*), which showed that about 50% of transcripts were accurately spliced. Deletion of the surrounding exons showed the upstream exon to be essential for this efficient splicing of the intron. A series of smaller deletions that removed 12, 28, 72 and 86 nt from the upstream region of the exon showed a large reduction in intron splicing efficiency in tobacco protoplasts from the full length exon and -12 deletion to the remaining deletions (Fig. 2A and B). This shows that the region between the -12 and -28 deletions contains a sequence which enhances *Ld* U12 intron splicing. Computer analysis using vertebrate exon splicing enhancer sequences has identified a potential splicing enhancer in the region between -12 and -28.

This is the first time that intron and exon splicing enhancer elements have been characterised in any detail in plants. Such sequences will be important in understanding gene regulation and it will be of great interest to find how common these splicing signals are in different plant species, how diverse the sequences are and the influence they play on both constitutive and alternative plant pre-mRNA splicing in plant growth and development.

References

[1] Simpson,C.G., Hedley,P.E., McQuade,C.M., Lyon,J.M., Clark,G.P., Machray,G.C. & Brown,J.W.S. (1998) *Scottish Crop Research Institute Annual Report* **1997/98,** 71-73

[2] Simpson,CG., Clark,G. Watters,J. & Brown,J.W.S. (2000) *Scottish Crop Research Institute Annual Report* **1999/2000**, 111-113