Arabidopsis PTB-like 1 (AtPTBL1) negatively regulates splicing inclusion of a plant mini-exon

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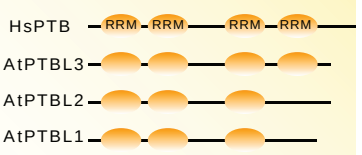
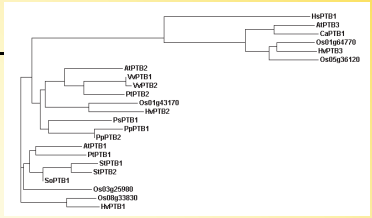
Introduction

Polypyrimidine tract binding proteins (PTBs) are established negative regulators of splicing in humans but virtually nothing is known about splicing suppressors in plants. We describe the development of an *in vivo* plant splicing reporter systems that allows us to measure the effect of over-expression of putative plant regulators of splicing. We then investigated whether an Arabidopsis PTB-like protein can function as a repressor of plant splicing.

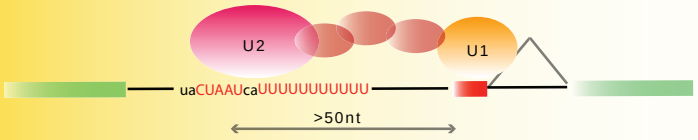
Plant orthologues of human PTB.

Plants contain a family of PTB-like proteins with similarity to human PTB (Hs). PTB-like proteins were identified in Arabidopsis (At), rice (Os), potato (St), barley (Hv), chickpea (Ca), sage (So), grape (Vv), spruce (Ps), poplar (Pt) and the moss Physcomitrella (Pp), and they fall into three distinct groups. The similarity with HsPTB lies mainly in the RRMs. The three Arabidopsis sequences have 20% (AtPTBL1), 15% (AtPTBL2) and 32% (AtPTBL3) peptide sequence identity with the human PTB protein.



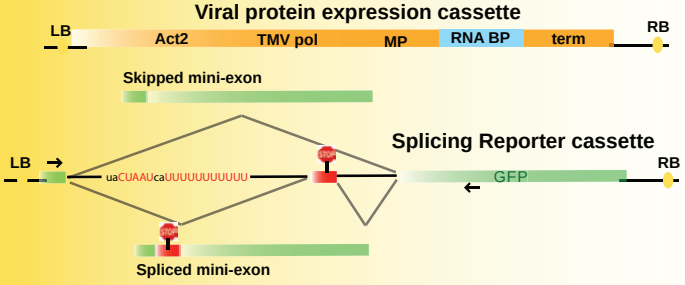


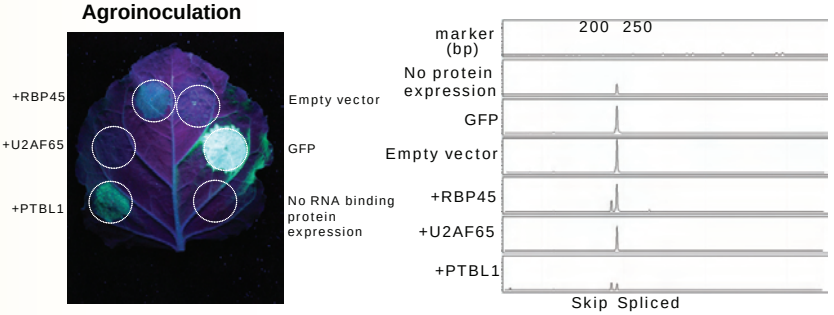
Development of a plant splicing reporter system



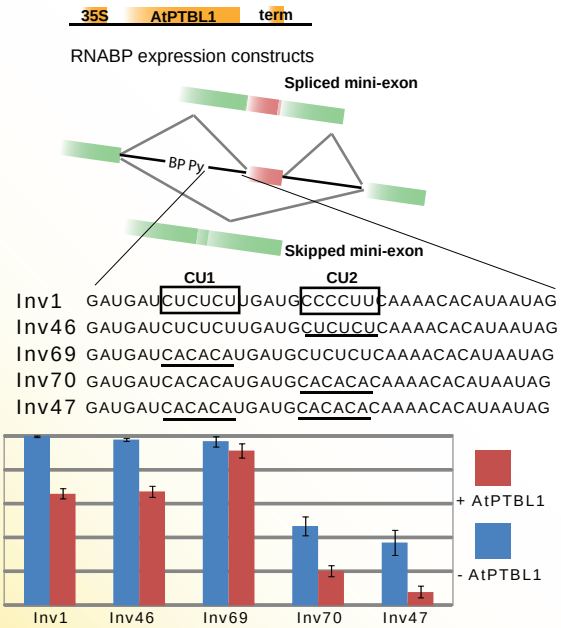
Constitutive splicing of the invertase mini-exon 2 (9nt long) requires a strong branchpoint and polypyrimidine tract located more than 50nt upstream of the mini-exon. Weakening the polypyrimidine tract leads to mini-exon skipping and factors that interact with this region are also expected to influence mini-exon splicing (Simpson et al., 2002).

To examine whether plant PTB-like proteins affect splicing we have developed a generic reporter system based around the invertase mini-exon that allows us to visualise repression of mini-exon splicing. To visualise mini-exon skipping we placed a stop codon in the mini-exon and linked the splicing cassette in frame with GFP such that skipping of the mini-exon will activate GFP expression. Agroinoculation by transient transformation of *N. benthamiana* leaves with agrobacterium containing the expression cassettes allowed us to visualise the effect of co-expressing AtPTBL1, U2AF65 and RBP45 with the splicing reporter. Both AtPTBL1 and RBP45 show activation of GFP expression indicating skipping of the mini-exon. RNA extraction and RT-PCR from leaf discs around the transformed tissue confirm the increase in mini-exon skipping.



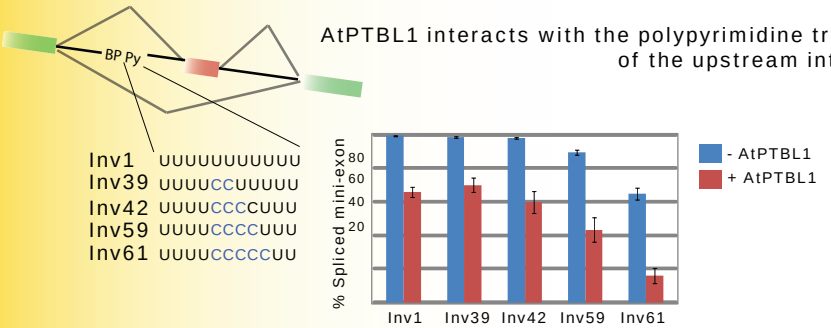


Identification of a new plant intron splicing enhancer



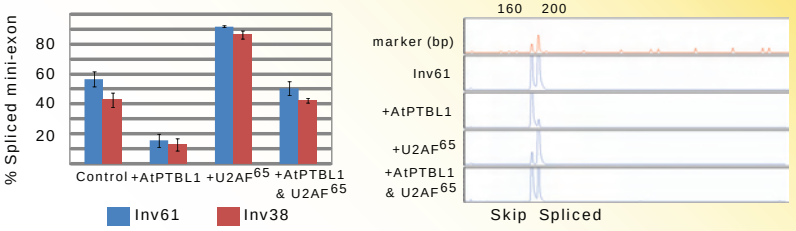
The 38nt region between the polypyrimidine tract and the 3' splice site of the mini-exon have two CU sequences that show similarity to PTB binding sites. Both elements were mutated and the CU element closest to the 3' splice site was found to be important for splicing of the mini-exon, indicating a new, plant intron located, splicing enhancer element. Overexpression of AtPTBL1 resulted in an increase in mini-exon skipping in all mutants except Inv69 indicating that the CU1 element is needed for AtPTBL1 repression.

AtPTBL1 interacts with the polypyrimidine tract of the upstream intron



Mutations of the polypyrimidine tract in the upstream intron have been shown to reduce mini-exon splicing and that C's are better able to compensate for U's than A's . Over-expression of AtPTBL1 showed a dramatic effect on the C series of mutations showing increased exon-skipping with increasing C's. This indicates that AtPTBL1 interacts with the polypyrimidine tract and that the interaction was stronger as the number of C residues increased.

Negative regulation of AtPTBL1 is compensated by U2AF65



PTB proteins are antagonistic to U2AF65, which binds to the polypyrimidine tract. Using polypyrimidine tract mutants that give around equal exon-skipping and exon inclusion (Inv61 and Inv38), over-expression of AtPTBL1 led to induction of exon skipping whileU2AF65 had the opposite effect of enhancing exon inclusion. Over-expression of both led to little change in splicing, suggesting that both are competing with each other and function anatgonistically to each other.