Altered Splicing of a Mini-exon by Over-expression of *Arabidopsis* PTB-like Proteins

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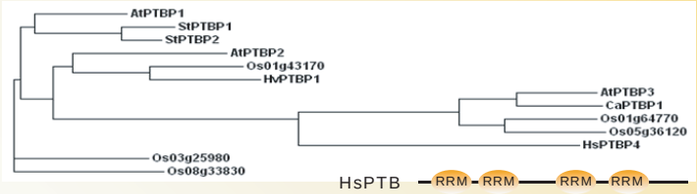
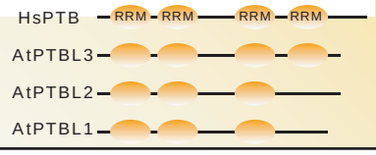
Introduction

Human polypyrimidine tract binding proteins (PTBs) are established negative regulators of splicing. We describe the development of *in vivo* plant splicing reporter systems that allows us to measure the effect of over-expression of PTB-like proteins and other plant regulators of splicing.

Plant orthologues of human PTB.

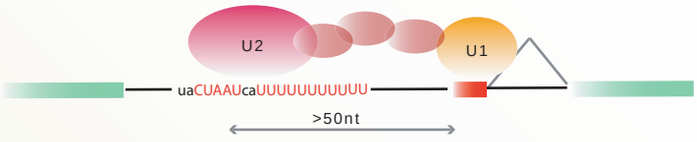
Plants contain families of PTB-like proteins with similarity to human PTB4. PTB-like proteins were identified in *Arabidopsis*, rice, potato, barley and chickpea, and they fall into three distinct groups. The similarity with HsPTB4 lies mainly in the RRMs. The *Arabidopsis* sequences have identity between 25 and 35% compared to the human PTB protein.





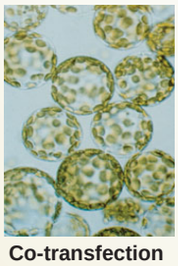
Of the three *Arabidopsis* genes, AtPTBL3 has the best overall match with the human PTB protein because it contains 4 RRM domains. The other two members of the family AtPTBL1 and 2 lack the C-terminal RNA binding domain.

Development of plant splicing reporter systems

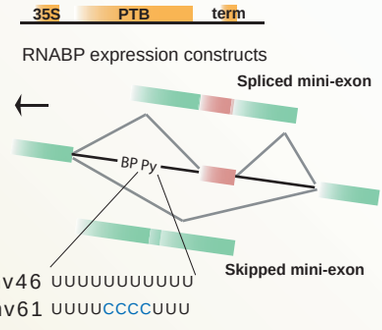


Constitutive splicing of the potato invertase mini-exon 2 (9nt long) requires a strong branchpoint and polypyrimidine tract located more than 50nt upstream of the mini-exon. Weakening the polypyrimidine tract leads to mini-exon skipping and factors that interact with this region are also expected to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

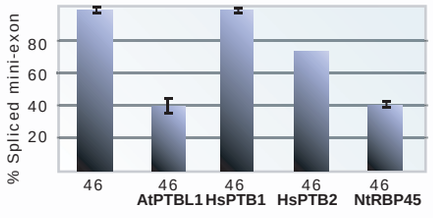
To examine whether these plant PTB-like proteins affect splicing we have developed a reporter system based around invertase mini-exon splicing.

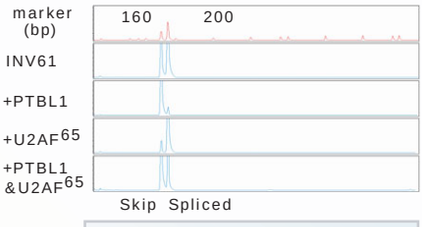


Co-transfection of protoplasts

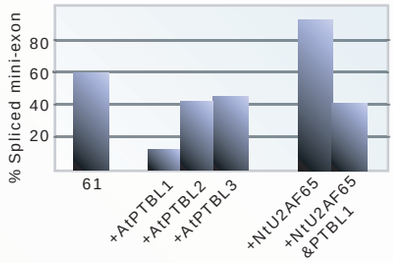


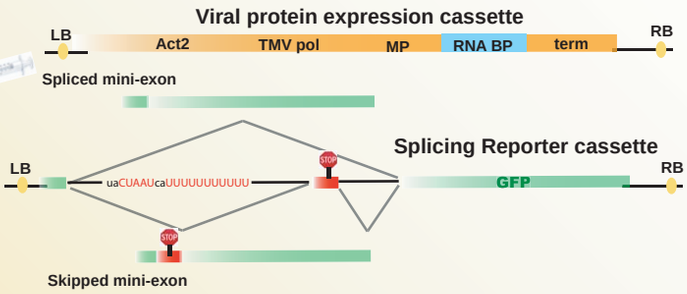
Using a construct that constitutively includes the mini-exon (Inv46), we measured the ability of PTBL1, two human PTB cDNAs (Gooding and Smith) and RBP45 to alter splicing of the mini-exon by transfecting expression cassettes into tobacco protoplasts. With the exception of human PTB1, the over-expression of all the RRM containing proteins led to skipping of the mini-exon.



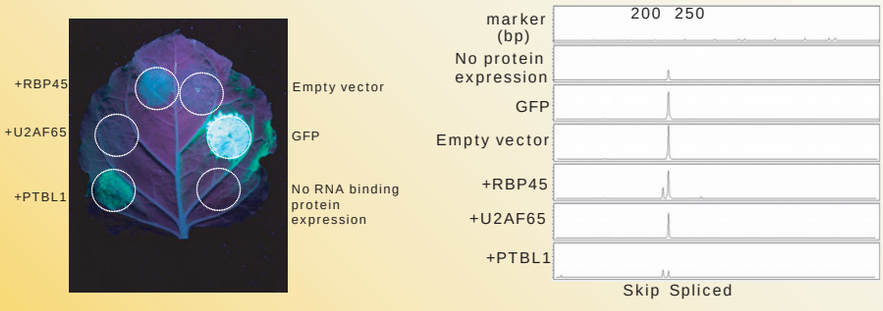


PTB proteins are antagonistic to U2AF65 which binds to the polypyrimidine tract. To test this we weakened the polypyrimidine tract (Inv61) which results in 60:40 splicing:skipping of the mini-exon. Overexpression of the three PTBL genes led to a reduction in splicing. PTBL1 gave the most significant result leading to a mean of 15.5% mini-exon splicing. Over-expression of U2AF65 increased splicing of the mini-exon with the weakened polypyrimidine tract. When both PTBL1 and U2AF65 were co-expressed with the splicing reporter, mini-exon splicing was intermediate to that found between PTBL1 and U2AF65 alone indicating that the two proteins operate antagonistically.





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 plant cells with agrobacterium containing the expression cassettes allowed us to visualise the effect of expressing PTBL1, U2AF65 and RBP45 with the splicing reporter. Both PTBL1 and RBP45 show activation of GFP expression indicating altered splicing of the mini-exon. RNA extraction and RT-PCR from leaf discs around the transformed tissue confirm the increase in mini-exon skipping. This reporter is being developed as a genetic screen for proteins that negatively affect splicing.



We are currently characterising PTBL insertion mutant lines and making PTBL1 and PTBL3 over-expression transgenic lines, which will be used to screen the *Arabidopsis* RT-PCR alternative splicing panel to identify altered splicing in endogenous genes.