Regulation of gene expression by alternative splicing and nonsense-mediated decay

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Alternative splicing (AS) is an important mechanism that increases proteome complexity and regulates gene expression. AS generates more than one mRNA from a single gene through the selection and utilization of different splice sites resulting in the production of proteins with different functions (e.g. activity, localization, interactions).

It is estimated that more than 35% of plant genes undergo alternative splicing. AS affects many aspects of plant development and physiology and is important in plant responses to biotic and abiotic stress.

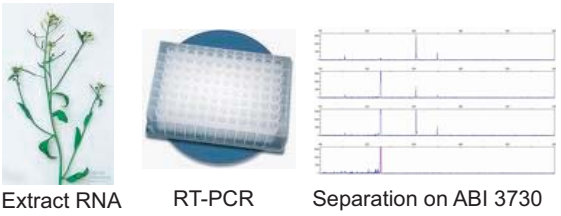
We have established an RT-PCR panel that accurately measures changes in multiple alternative splicing events simultaneously. We are using this system to investigate the effects on AS in mutants and over-expression lines of proteins involved in mRNA biogenesis (e.g. cap binding complex, splicing factors – SR and PTB proteins and degradation factors).

We have also examined the link between AS and nonsense-mediated decay (NMD) - a surveillance pathway that degrades mRNAs containing premature termination codons (PTC). As UPF1 and UPF3 are key proteins involved in NMD, changes in AS were monitored in *upf1-5* and *upf3-1* mutant plants and wild type plants. The mutants are defective in NMD such that PTC-containing transcripts accumulate to higher levels. 692 transcripts have been analyzed using the RT-PCR panel and about 20% of them may be turned over by NMD.

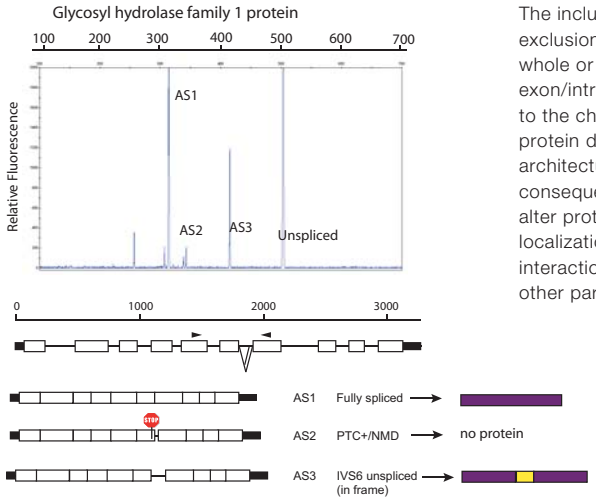
Our analysis shows that there is much more alternative splicing than currently expected and that AS regulates expression of many transcripts via NMD.

RT-PCR platform to study different AS events simultaneously

RT-PCR was carried out on extracted RNA using 292 gene specific primer pairs spanning known AS events. In the PCR reaction, one primer pair was fluorescently labelled and PCR was carried out over 24 cycles (in the linear amplification range). AS products were separated according to their transcript length by an ABI 3730 sequencer, identified by size and the ratio of the alternatively spliced products was calculated. Three biological reps were performed to determine statistically significant changes in AS in the conditions/mutants tested.



Alternative splicing broadens transcriptome and proteome diversity



The inclusion or exclusion of the whole or part of the exon/intron can lead to the changes in protein domain architecture and consequently can alter protein activity, localization or interactions with other partners.

Alternative splicing as a post-transcriptional gene expression control

Some regulated AS events can introduce PTCs into mRNAs which are then degraded by the NMD pathway. Interplay between AS and NMD can function as a quantitative post-transcriptional gene regulator. About 20% of transcripts analyzed in this study are turned over by NMD.