Alternative splicing and NMD in Arabidopsis

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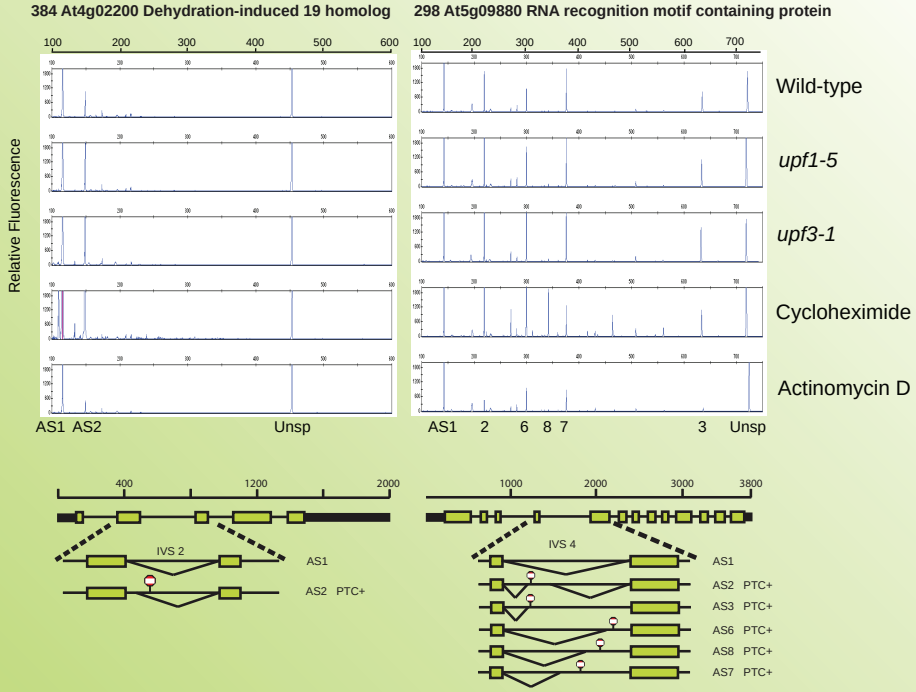
Introduction

The nonsense mediated decay (NMD) pathway recognises and induces degradation of mRNA transcripts containing premature termination codons (PTC) thereby reducing expression of potentially detrimental truncated proteins. NMD is also a key post-transcriptional regulator of normal gene expression affecting large numbers of transcripts. PTC-containing (PTC+) transcripts either exist naturally, arise through mutation or through errors in transcription or splicing, or are produced by alternative splicing. Analyses of human and mouse ESTs estimate that around 20-30% of alternative splicing variants are turned over by the NMD pathway (Lewis et al., 2003; Baek and Green, 2005) although other studies have suggested that direct regulation of transcript levels by NMD may not be widespread (Pan et al., 2006).

Disruption of NMD leads to changes in alternative splicing

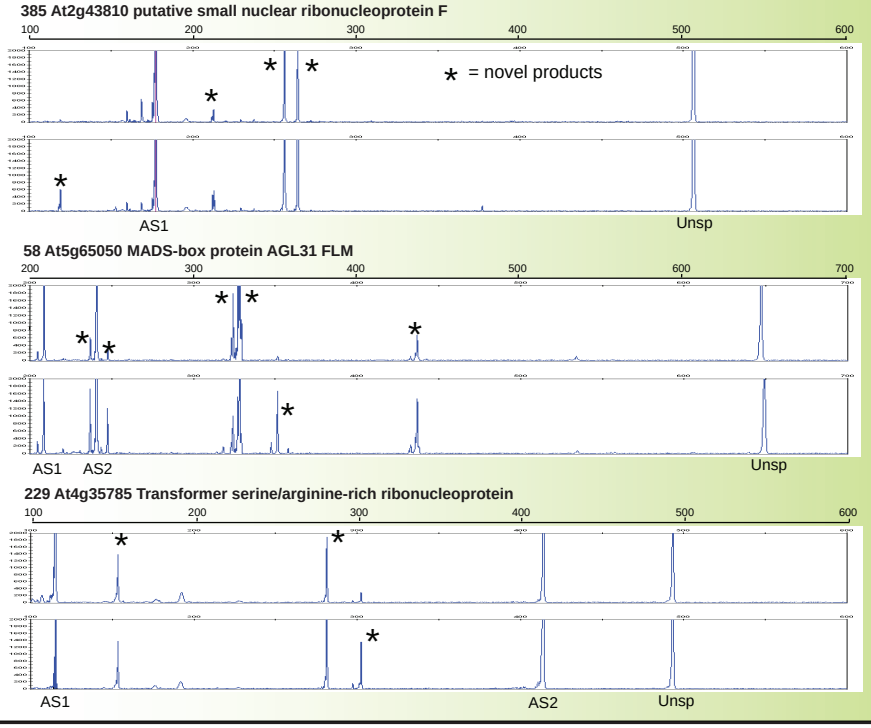
We are analysing an RT-PCR panel of around 300 alternative splicing events to determine the link between NMD and alternative splicing in plants using two severe but viable mutant alleles, *upf1-5* and *upf3-1*.



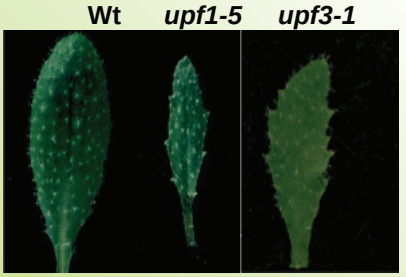
Two examples highlight the changes in alternative splicing found in the *upf* mutant plants and plants treated with cycloheximide and the transcriptional inhibitor, Actinomycin D. PTC-containing transcripts that are usually degraded by NMD increase in abundance in the mutants. The schematic shows established and predicted alternative splicing events in the introns studied and the position of PTCs .

Disruption of NMD leads to production of novel alternatively spliced transcripts

Our analysis of the 264 genes identified 200 novel RT-PCR products. In particular, cycloheximide treatment generated products not seen in the wild-type, untreated plant.



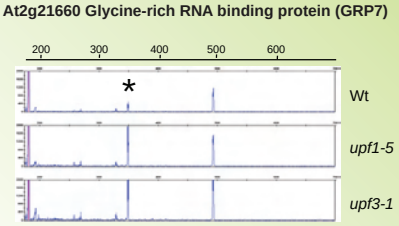
UPF mutants *upf1-5* and *upf3-1*



UPF proteins are key components of the NMD mechanism. UPF1 and UPF3 are conserved in plants and have been shown to function in NMD in *Arabidopsis* (Hori and Watanabe, 2005; Arciga-Reyes et al. 2006). Mutants of UPF1 and UPF3 show a range of vegetative and floral abnormalities, including jagged leaves (shown above), late flowering, fused flowers and seedling lethality.

Analysis of alternative splicing supports a link with NMD in plants

Of 264 genes studied to date a total of 692 potential alternative splicing products have been analysed. 150 (21.7%) of these increase in abundance in the *upf* mutants and after cycloheximide treatment and therefore appear to be NMD-sensitive. These include genes such as the circadian clock gene AtGRP7, which is known to autoregulate using alternative splicing and NMD.



19% and 25% of the genes showed significant changes in known alternative splicing events in the *upf1-5* and *upf3-1* backgrounds compared to the wild-type control and the larger number of genes changing in *upf3-1* compared to *upf1-5* suggests a more severe effect on alternative splicing in this mutant background. This is further supported by the observation that of the genes that show a significant change in the mutants, nearly 80% of these show a greater change in alternative splicing ratos in

References

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Baek and Green (2005) PNAS 102, 12813-12818.
Pan et al. (2006) Genes Dev. 20, 153-158.
Hori and Watanabe (2005) Plant Journal 43, 530-540.
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