Alternative splicing and nonsense mediated decay in Arabidopsis

Craig G Simpson,[1] Maria Kalyna,[2] Dominika Lewandowska,[1] Branislav Kusenda,[2] John Fuller,[1] Linda Milne,[1] Jim McNicol,[3] Gillian Clark,[1] Andrea Barta[2] and John WS Brown[1, 4]

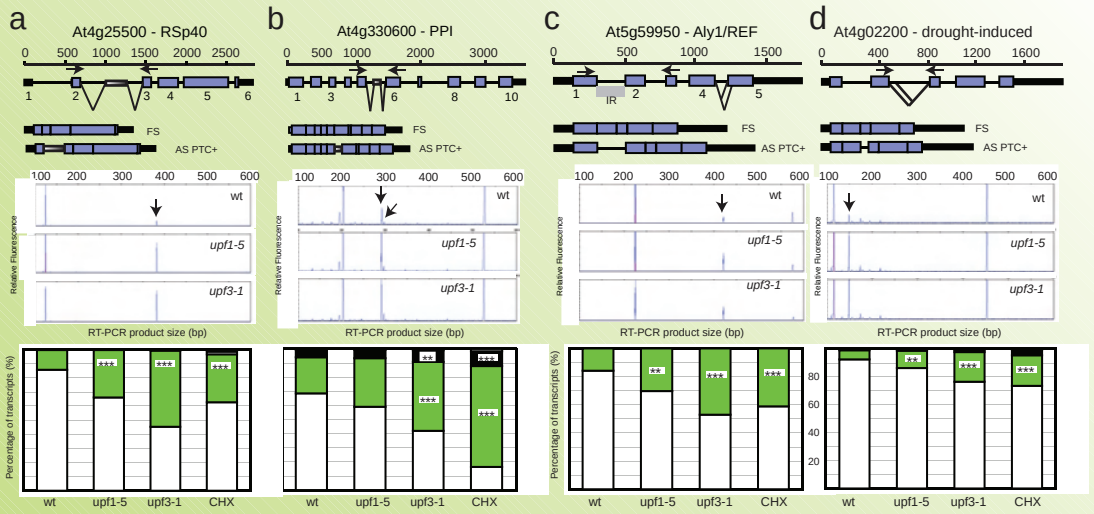
1. Genetics, SCRI, Invergowrie, Dundee, UK. DD2 5DA.
2. Max F Perutz Laboratories, Medical University of Vienna, Dr. Bohr-Gasse 9/3, A-1030 Vienna, Austria
3. Biomathematics and Statistics Scotland, SCRI, Invergowrie, Dundee, UK DD2 5DA
4. Division of Plant Sciences, University of Dundee @ SCRI, Invergowrie, Dundee, UK DD2 5DA

Introduction

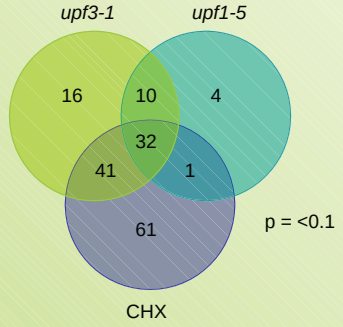
Functional links between alternative splicing (AS) and nonsense mediated decay (NMD) in plants have been shown for genes encoding SR splicing factors and circadian clock proteins (Schöning et al., 2007, 2008; Palusa and Reddy, 2010). PTC-containing (PTC+) transcripts, which are targets for NMD, either 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). We studied a panel of naturally occuring alternative splicing events from around 300 plant genes in mutants of the NMD pathway to examine the relationship between AS and NMD in plants.

Disruption of NMD leads to changes in alternative splicing

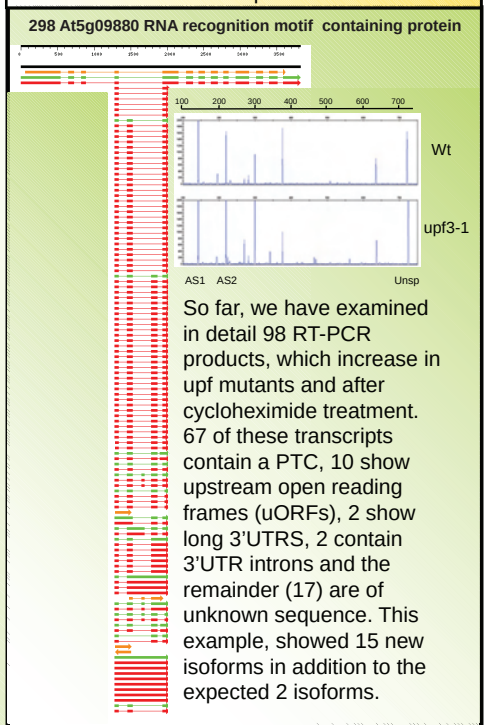
We monitored quantitative changes in 951 alternatively spliced isoforms by RT-PCR using two severe but viable mutant alleles, *upf1-5* and *upf3-1* and by treatment with cycloheximide (NMD and translational inhibitor). NMD sensitive transcripts increase in abundance in the *upf* mutants and when translation is blocked.



- 638 transcripts show no significant change
- 313 transcripts (~33%) change significantly in the NMD mutants and after CHX treatment.
- 165 peaks increase in the *upf* mutant background and after CHX treatment (~17%)
- *upf3-1* is a stronger allele than *upf1-5*.
- CHX treatment has a greater effect than *upf* mutants

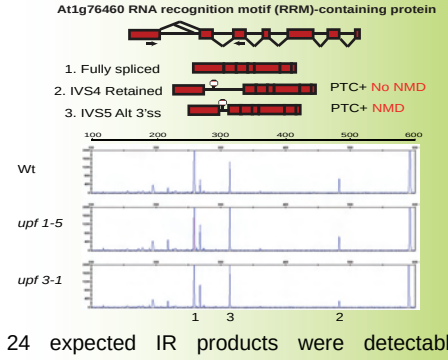


NMD disruption leads to detection of novel AS transcripts



So far, we have examined in detail 98 RT-PCR products, which increase in upf mutants and after cycloheximide treatment. 67 of these transcripts contain a PTC, 10 show upstream open reading frames (uORFs), 2 show long 3'UTRS, 2 contain 3'UTR introns and the remainder (17) are of unknown sequence. This example, showed 15 new isoforms in addition to the expected 2 isoforms.

Retained intron are insensitive to NMD



24 expected IR products were detectable between 2 and 100% of the total transcripts.

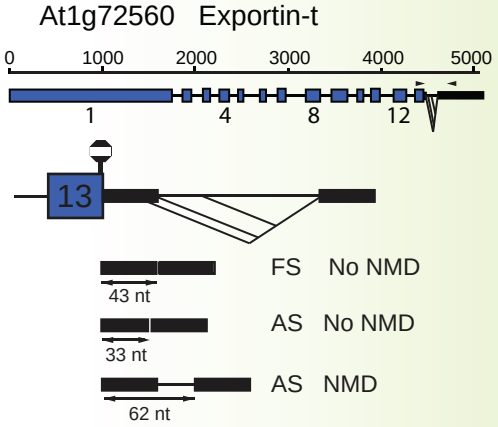
Half of the detectable IR transcripts have a PTC that may lead to a truncated protein.

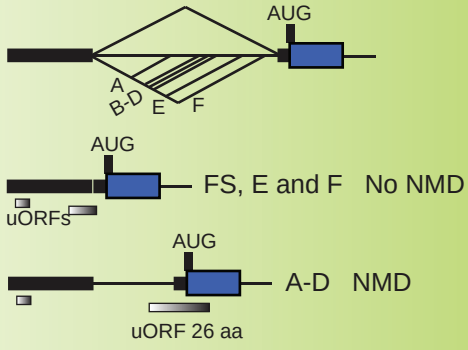
2 were in frame, No NMD expected
5 changed the C-terminus of protein (NMD not expected) 4/5 show No NMD
3 were in the 5'UTR. IR leaves uORFs;
2/3 show no NMD.
Remaining 14 in coding region are PTC+.
12/14 show no NMD.
One of the transcripts that shows NMD has a second AS event that creates a PTC and NMD.

Comparison of spliced and IR transcripts in the wild-type plant with the upf mutants show no significant change in the ratio of spliced to IR transcript in the NMD mutants, indicating that virtually all IR transcripts are insensitive to NMD.

Alternative splicing in UTRs affects NMD

Alternative splicing in the 3'UTR alters the distance between splice junction and authentic translation stop. We find transcripts with less than 44nt between these features are insensitive to NMD while transcripts greater than 61nt are subject to NMD.





Alternative splicing in the 5'UTR affect the presence/absence, length and position of uORFs and NMD.

In six cases NMD sensitive transcripts had uORFs, which overlap the authentic translation start suggesting a mechanism for uORFs to trigger NMD.

Summary

- ~17% of AS transcripts tested in the NMD mutant background and after CHX treatment are subject to NMD.
- A third of the new AS products discovered have been characterised in detail. The majority contain a PTC.
- AS transcripts that arise through intron retention are insensitive to NMD.
- Longer 5' and 3'UTRs through AS are sensitive to NMD.