

# Alternative splicing and nonsense-mediated decay controls expression of important regulatory genes in Arabidopsis

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## Introduction

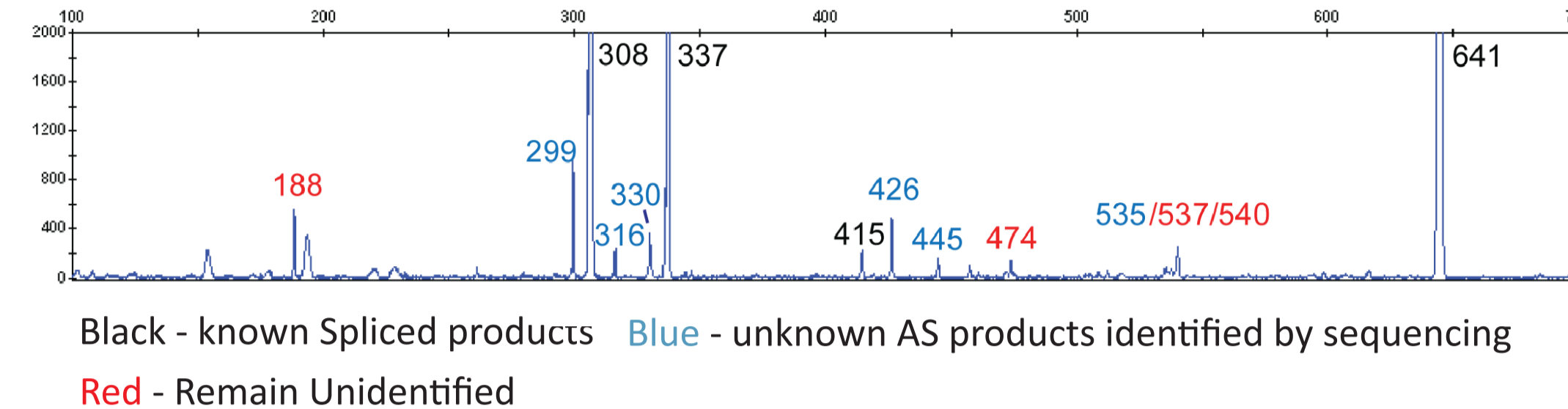
Alternative splicing (AS) coupled to nonsense-mediated decay (NMD) is a post-transcriptional mechanism for regulating gene expression. In the absence of splicing sensitive microarrays in plants, we have used a high-resolution AS RT-PCR based panel to measure relative isoform level changes in endogenous AS genes when NMD was impaired in Arabidopsis NMD factor mutants *upf1-5* and *upf3-1* and by Cycloheximide.

## NMD Targets of Endogenous Alternatively Spliced Genes

We performed high-resolution RT-PCR using a panel of 298 primer pairs in 270 endogenous genes and identified around 950 AS products. AS genes represent mainly important transcription factors, RNA binding proteins and stress related proteins.

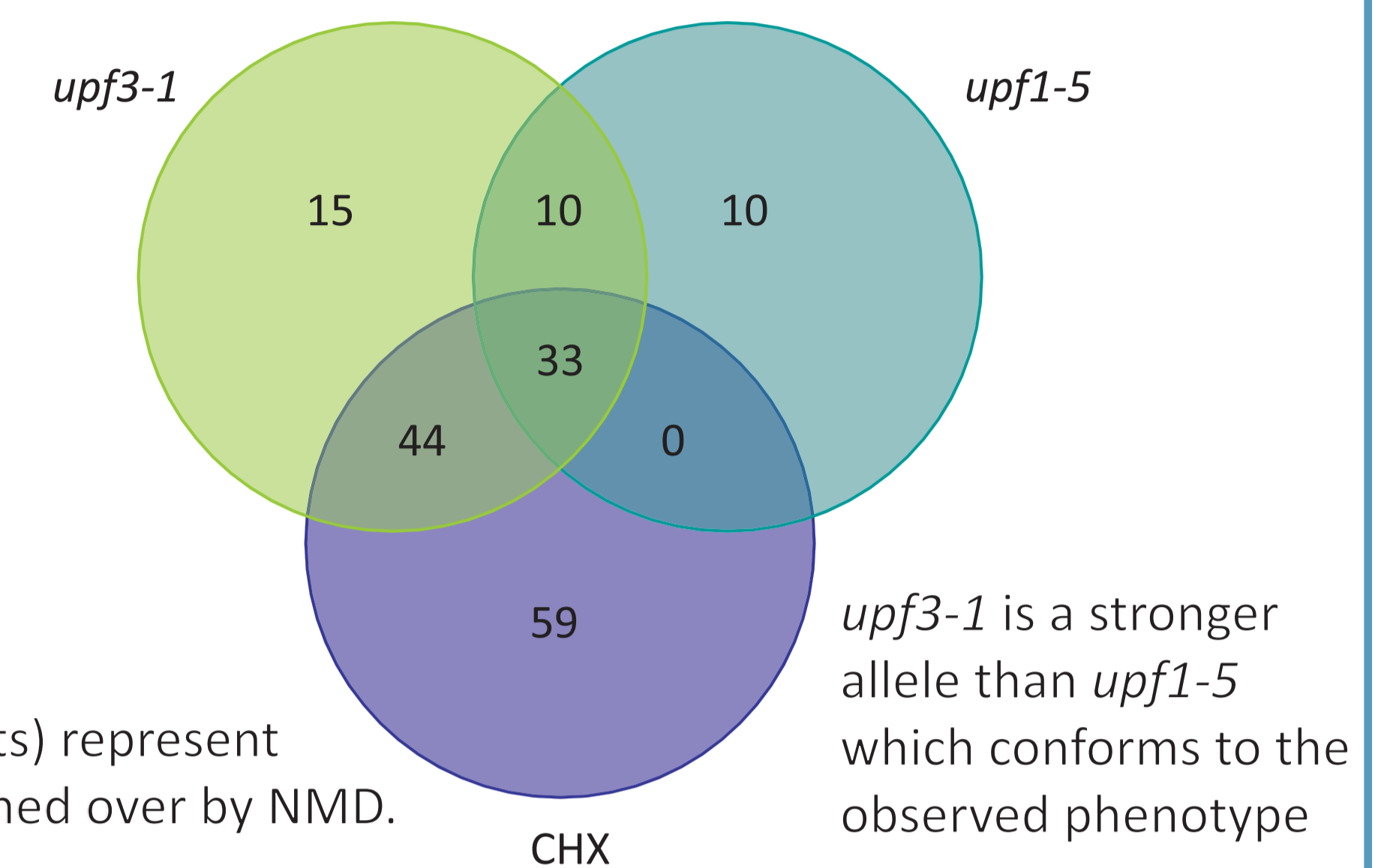
350 new products discovered - many sequenced.

Salt Overly Sensitive - Calcineurin B-like protein 4



165 products (17% of total transcripts) represent naturally occurring AS transcripts turned over by NMD.

We expect NMD sensitive alternatively spliced products to become prevalent within the NMD mutant backgrounds or as a result of CHX treatment.



## NMD mutants and Cycloheximide treatment impair NMD to different extents

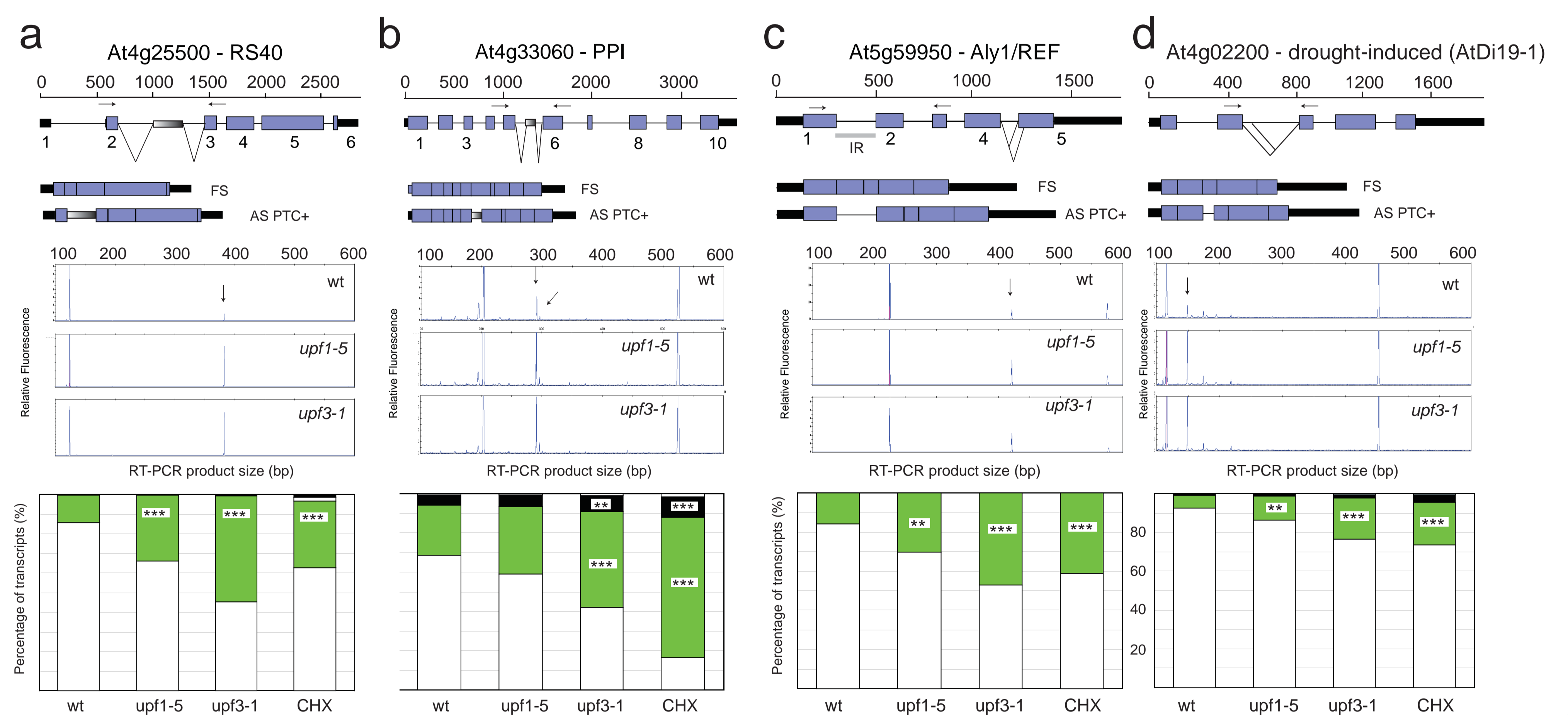
NMD transcripts show a significant increase in abundance in the *upf* mutants and when translation is blocked by CHX.

121 genes showed at least one isoform up-regulated in the mutant and CHX treated plants, suggesting around 45% of the selected genes show AS/NMD. Considering 42% of plant genes show AS (Filichkin, 2010) suggests that around 18% of plant genes show AS/NMD.

4 examples of alternative splicing events that introduce premature termination codons and increase significantly in the treatments are shown.

RS40, PPI and Aly1/Ref show that AS/NMD transcripts represent a significant proportion of the transcripts of a gene in wild type plants.

The NMD transcripts are detectable in wild type plants and increase in abundance to different extents in the *upf* mutants/CHX.

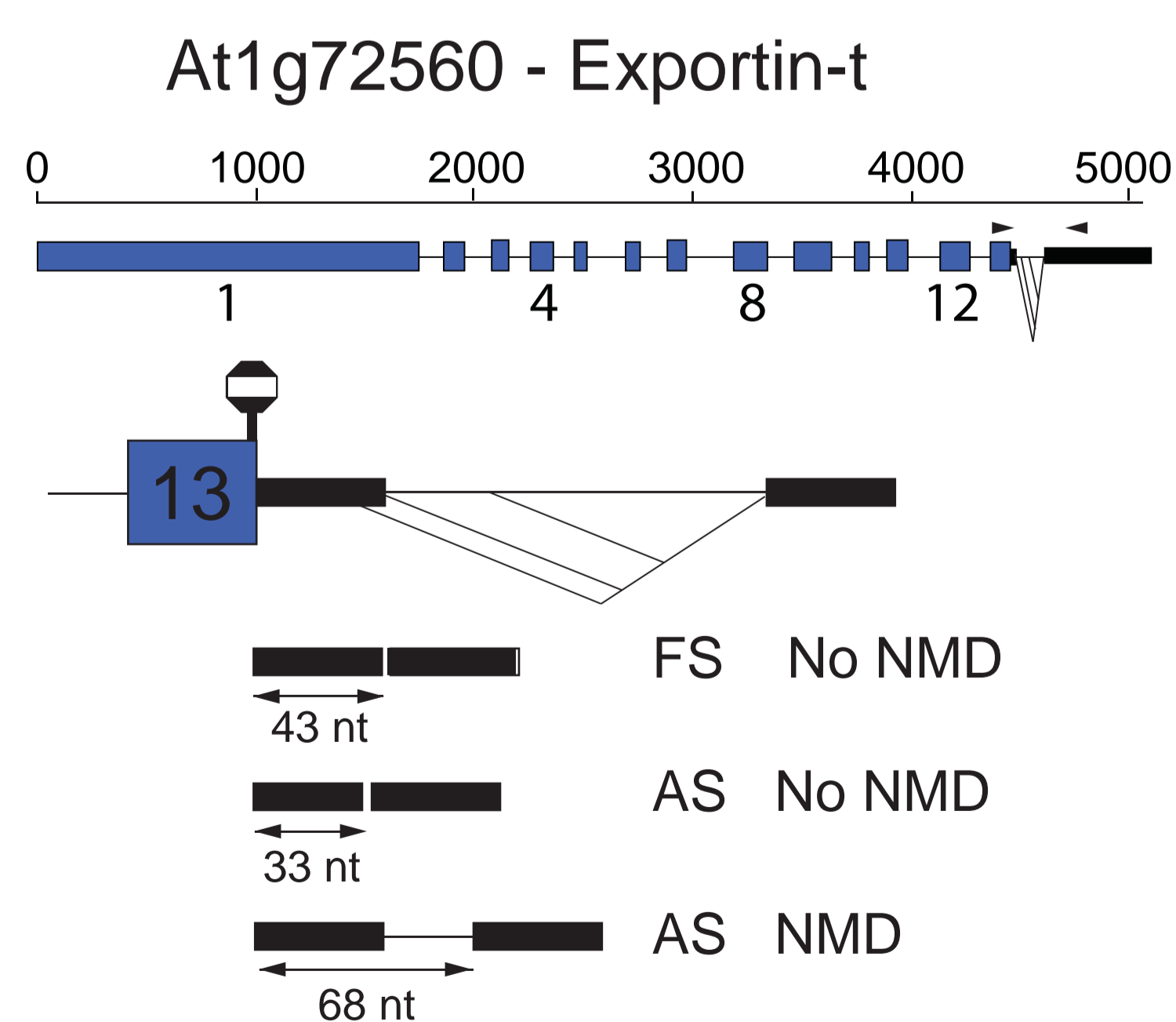


## Features of alternatively spliced transcripts sensitive to NMD

All of the alternatively spliced transcripts sensitive to NMD in the mutants and 11 transcripts from the cycloheximide treatment were characterised in terms of whether they contained PTCs, had splice junctions downstream of the authentic stop codon or PTCs, had long 3' UTR sequences or contained an upstream open reading frame (uORF).

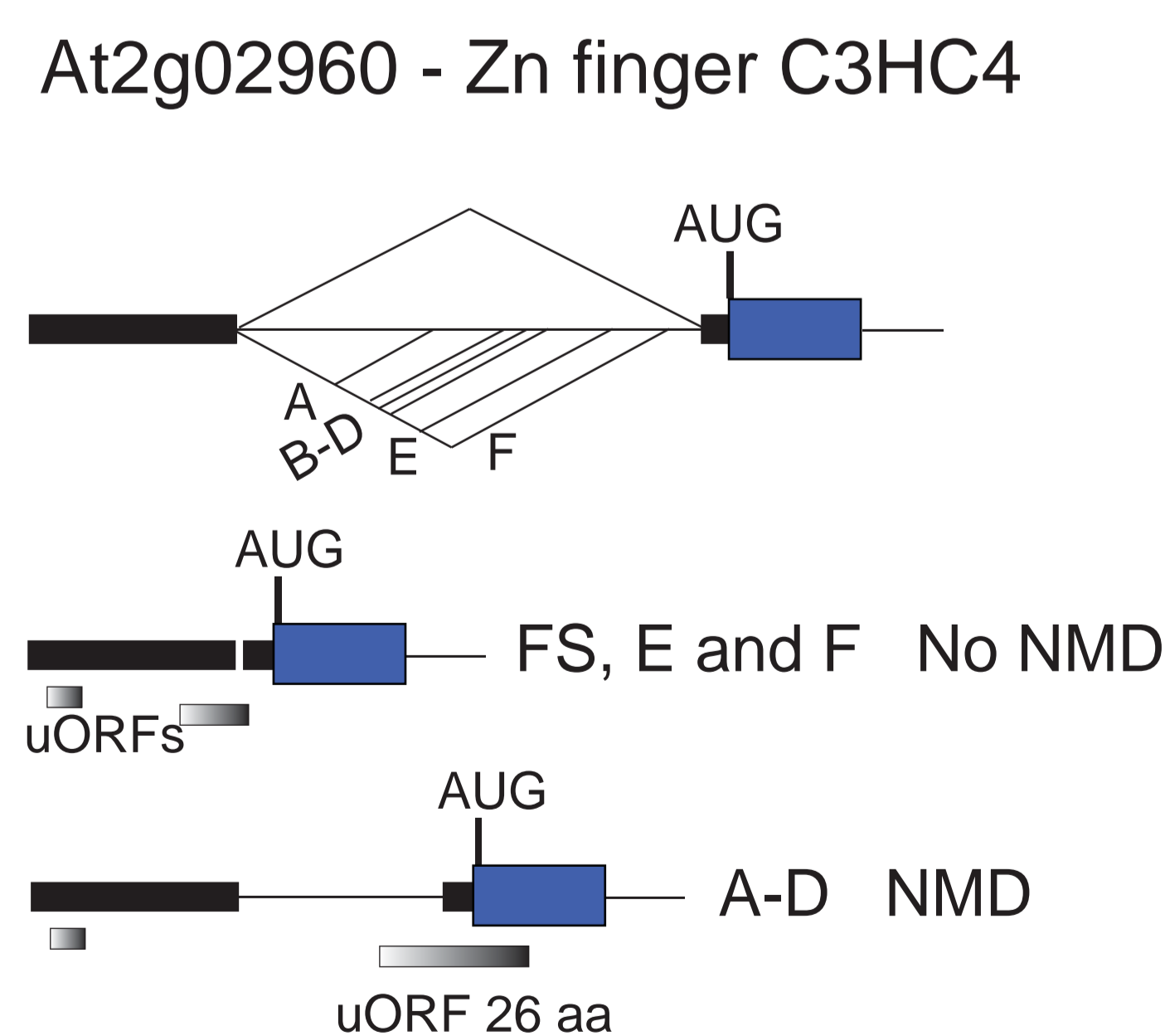
### AS in 3'UTRs modulate NMD

The position of PTCs defines the lengths of 3'UTRs, which can trigger NMD. 2 genes show variable length 3'UTRs through alternative splicing but have the same authentic termination codon. In the example below the longer transcript (68nt) was sensitive to NMD while the shorter products (33 and 43nt) were not.



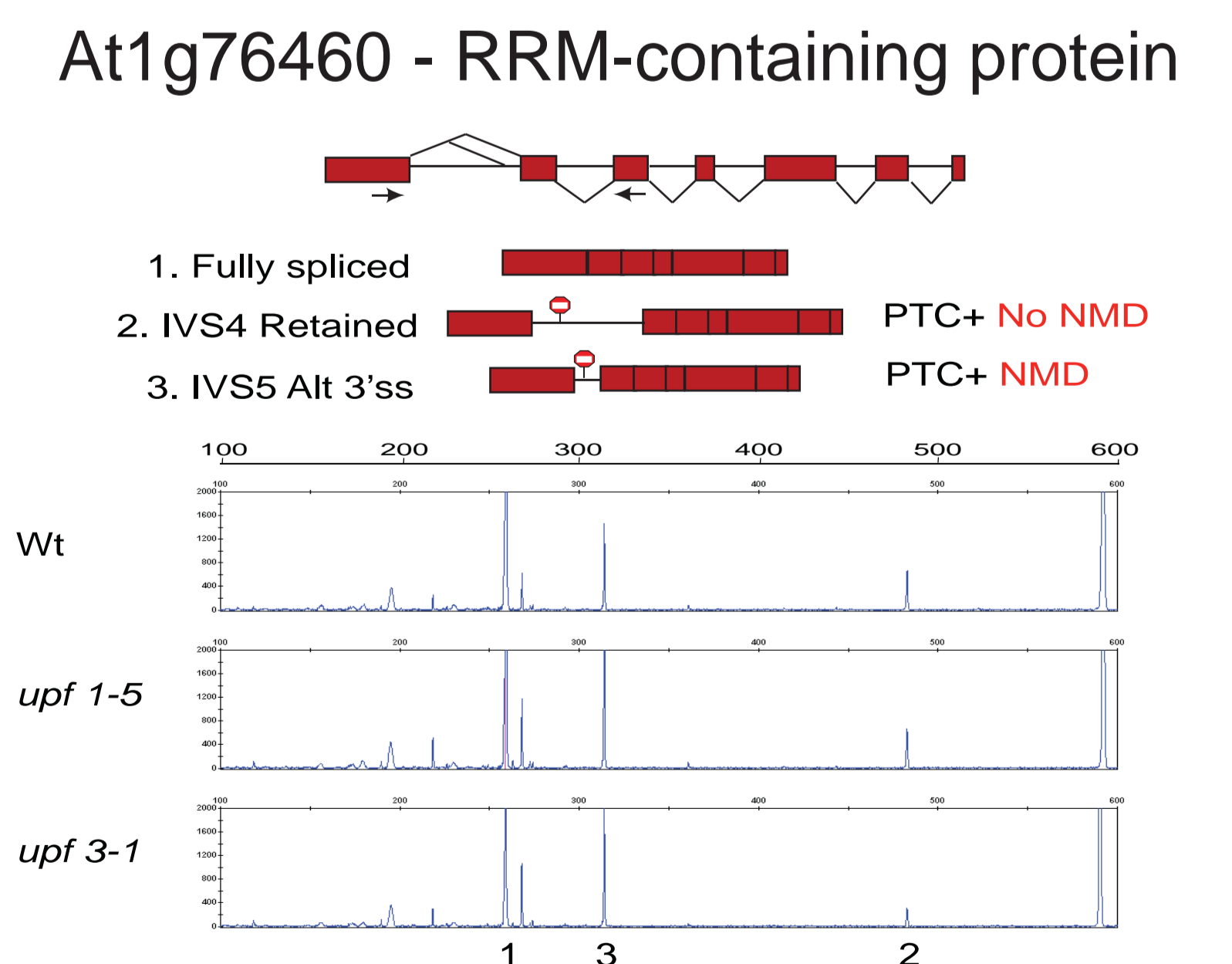
### uORFs overlapping start codons induce NMD

AS in the 5'UTR affect the presence/absence length and position of uORFs and NMD. 7 genes had uORFs that overlapped the authentic translation start suggesting a mechanism for uORFs as a trigger for NMD.



### Retained introns are not sensitive to NMD

19 intron retention AS events had PTCs with downstream splice sites or long 3'UTRs but did not increase in abundance in the *upf* mutants and/or cycloheximide treatment suggesting that they are not turned over by the NMD pathway.



## Conclusions

- We demonstrate a previously unknown prevalence of AS/NMD in plants and also find many new AS events in the genes studied.
- Alternative splicing in 5' and 3'UTRs affects transcript levels and uORFs overlapping the start codon trigger NMD.
- Intron retention events do not trigger NMD despite possessing all the features that induce NMD.
- NMD sensitive transcripts are readily detected in wild type plants, often representing a significant proportion of the total transcripts.