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# The salicylic acid dependent and independent effects of NMD in plants

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**Keywords:** Arabidopsis, nonsense-mediated mRNA decay, salicylic acid, conserved upstream open reading frame

In eukaryotes, nonsense-mediated mRNA decay (NMD) targets aberrant and selected non-aberrant mRNAs for destruction. A recent screen for mRNAs showing increased abundance in Arabidopsis NMD-deficient mutants revealed that most are associated with the salicylic acid (SA)-mediated defense pathway. mRNAs with conserved peptide upstream open reading frames (CpuORFs or CuORFs) are hugely overrepresented among the smaller class of NMD-regulated transcripts not associated with SA. Here we show that the common phenotypes observed in Arabidopsis NMD mutants are SA-dependent, whereas the upregulation of CpuORF-containing transcripts in NMD mutants is independent of SA. We speculate that CpuORFs could allow the conditional targeting of mRNAs for destruction using the NMD pathway.

In addition to its role in the destruction of aberrant mRNAs, nonsense-mediated mRNA decay (NMD) modulates gene expression by degrading endogenous transcripts bearing specific target features.<sup>1–6</sup> Despite the presence of an NMD mechanism in all eukaryotes, few mRNAs have been identified as conserved NMD targets across evolution.<sup>4,5</sup> The features that target a transcript to NMD are also not fully defined. In plants some, but not all, long 3'UTRs, 3' UTR-located introns and upstream open reading frames (uORFs) result in NMD.<sup>7–12</sup> In order to understand the role of NMD in plant gene regulation it is important to identify the transcripts that are targeted by NMD, the features that subject them to NMD and the effect of impairing NMD on growth and development.

Microarrays were used to identify 206 'common NMD transcripts' that are coordinately upregulated in three Arabidopsis NMD mutants (*upf1-5*, *upf3-1* and *smg7-1*).<sup>13</sup> These transcripts represent both direct and indirect NMD targets. Meta-analyses reveal that most of these transcripts (183) respond to pathogens in an SA dependent manner, whereas only 23 do not respond to pathogens or exogenously applied SA.<sup>13</sup> Furthermore, Arabidopsis NMD mutants are partially resistant to *Pseudomonas syringae* pathovar tomato DC3000 (DC3000) and accumulate more SA than their wild-type cohorts in the presence or absence of pathogen.<sup>13</sup> These findings suggest that one role for NMD in plants could be to facilitate a rapid pathogen response by changing the stability of pathogen responsive transcripts.

## NMD Mutant Phenotypes in Arabidopsis

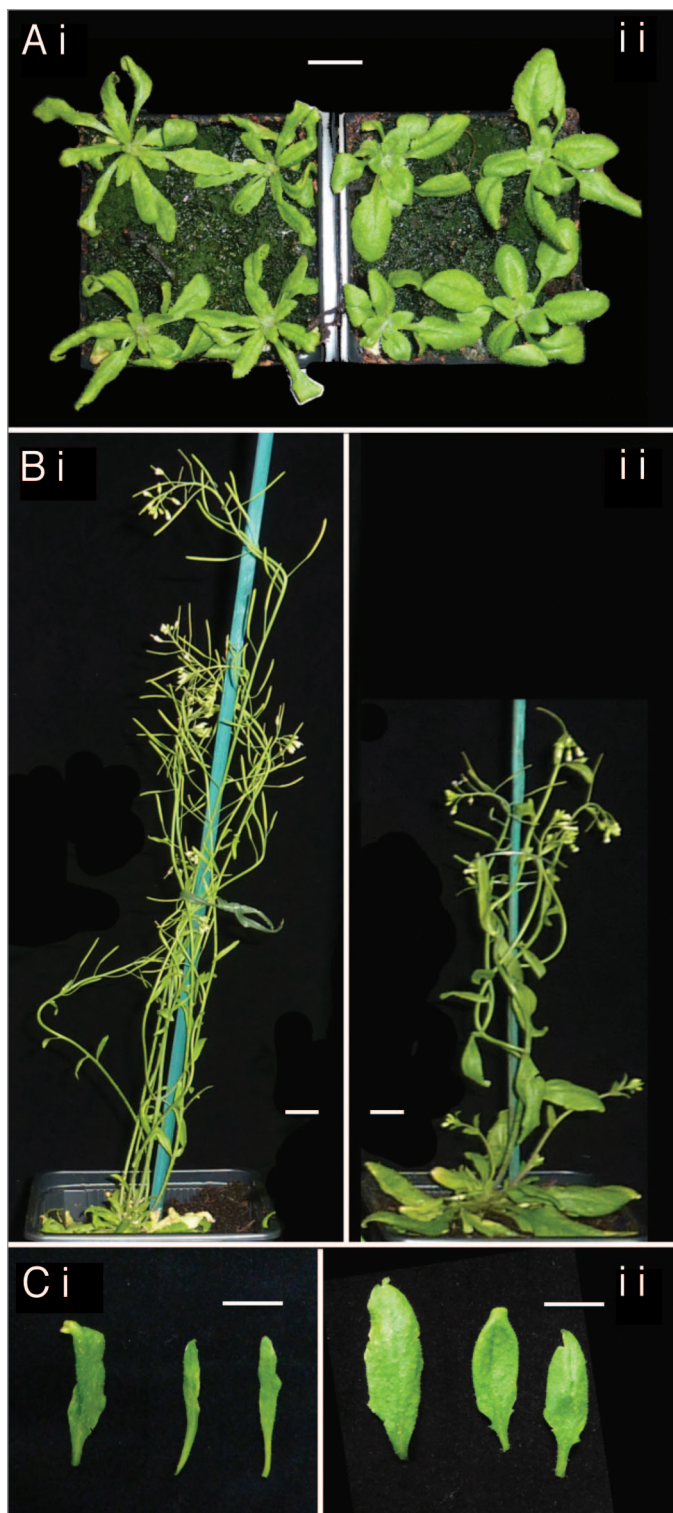
Plants deficient in different NMD effectors display similar phenotypes.<sup>8,9,14,15</sup> The thin, twisted leaves of NMD mutants

resemble SA accumulating mutants,<sup>16</sup> suggesting that some aspects of the NMD mutant phenotype may be caused by the constitutive pathogen response. To disentangle the effects of SA and NMD, NMD mutants were studied in an SA-deficient background. *sid2-1* mutants are incapable of accumulating SA in response to pathogens.<sup>17</sup> The NMD impaired *upf1-5* mutant exhibits the typical NMD phenotype of thin leaves with twisted petioles.<sup>9</sup> However, *upf1-5 sid2-1* double mutants appear wild-type, with wider, flatter leaves (Fig. 1). This suggests that the distinctive leaf-shape phenotype of Arabidopsis NMD mutants is caused by accumulation of SA, consistent with a previously published finding that disruption of *PAD4*, which is upstream of *SID2*, also suppresses the NMD mutant phenotype.<sup>14</sup>

## CpuORFs as Targets for NMD

While it is likely that many of the 183 SA-responsive transcripts are indirectly affected by NMD, we previously suggested that the remaining 23 transcripts that do not respond to pathogens are enriched for direct NMD targets.<sup>13</sup> Eight of these 23 transcripts have CpuORFs; short open reading frames in the leader sequences that are conserved between Arabidopsis and rice.<sup>13,18</sup> This is a striking overrepresentation given that only 44 Arabidopsis genes show this feature.<sup>18</sup> Comparisons with other species reveal that 82 Arabidopsis genes harbour CpuORFs.<sup>18–20</sup> Of the 77 CpuORF-containing genes represented on the ATH1 GeneChip microarray, 49% are upregulated in at least one NMD mutant line (Fig. 2, Table S1). Enrichment for CpuORFs among NMD targets suggests that this feature is recognized by NMD.

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**Figure 1.** Phenotypes of NMD mutant plants with and without a functional *ICS1* allele. All plants shown are homozygous for the mutant *upf1-5* allele and therefore impaired in NMD. (i) Wild-type *ICS1*. (ii) Homozygous for the *sid2-1* mutant allele of *ICS1*. Plants are 3 weeks (A), and 6 weeks (B and C) old. The white scale bar indicates 1 cm.

Many uORFs are not recognized by NMD and it is unclear why CpuORFs should differ from other uORFs in this respect.

Although CpuORFs tend to be among the longest uORFs,<sup>18,21</sup> length does not account for the differential representation of uORFs and CpuORFs in the list of NMD-regulated genes. CpuORFs are over-represented among the common NMD genes, even as a proportion of transcripts with a uORF exceeding 42 codons ( $p < 0.05$ ). This suggests that the conserved sequence of the CpuORF may be important for recognition by NMD. In diverse organisms, nascent peptides of uORFs exert translational control of downstream ORFs by ribosome stalling at the uORF, sometimes in response to changing cellular conditions.<sup>22-24</sup> Perhaps the short peptides encoded by the CpuORFs of NMD-sensitive transcripts expose the transcript to NMD by stalling the ribosome at the CpuORF, decreasing the efficiency of re-initiation of translation at the downstream ORF or making the transcript vulnerable to a change in cellular competence for translation reinitiation. Since recognition by NMD would depend on the translation of the CpuORF, this provides a mechanism by which individual transcripts could be targeted to, or protected from, NMD in a condition dependent manner. There is a precedent for this; in *Saccharomyces cerevisiae*, the *CPA1* transcript harbours an uORF that encodes arginine attenuator peptide (AAP). In the presence of arginine, nascent AAP stalls ribosomes at the uORF targeting the transcript to NMD and downregulating *CPA1* expression.<sup>25</sup>

Publicly available microarray data indicates that CpuORF-containing transcripts are mainly independent of SA and could therefore represent direct NMD targets. Meta-analyses across multiple microarray experiments show no association between CpuORFs and responses to SA. Furthermore, a specific search for transcripts that show increased abundance in *smg7-1* mutants that are compromised in their ability to accumulate SA (*smg7-1 pad4-1* vs *pad4-1*; *PAD4* being a gene that mediates numerous responses to pathogens including SA accumulation via *ICS1*<sup>26,27</sup>) reveals 340 such transcripts (fold change  $> 1.5$ ,  $p > 0.05$ ). This list includes 16 CpuORF-containing transcripts, 8 of which are also commonly upregulated in the *upf1-5*, *upf3-1* and *smg7-1* NMD mutants. The *PAD4*-independence of the upregulation of these CpuORF transcripts supports the view that they are targeted directly by NMD, rather than responding indirectly via elevated SA.

Here we provide further evidence that CpuORFs are direct NMD targets in Arabidopsis. The high degree of conservation of CpuORFs suggests that they could also be direct NMD targets in other species. The presence of a CpuORF in the leaders of specific mRNAs could provide a mechanism whereby those mRNAs are conditionally moved into and out of the influence of NMD, raising the possibility that environmental and cellular conditions could act through CpuORFs to effectively alter the expression of the associated major transcripts.

#### Disclosure of Potential Conflicts of Interest

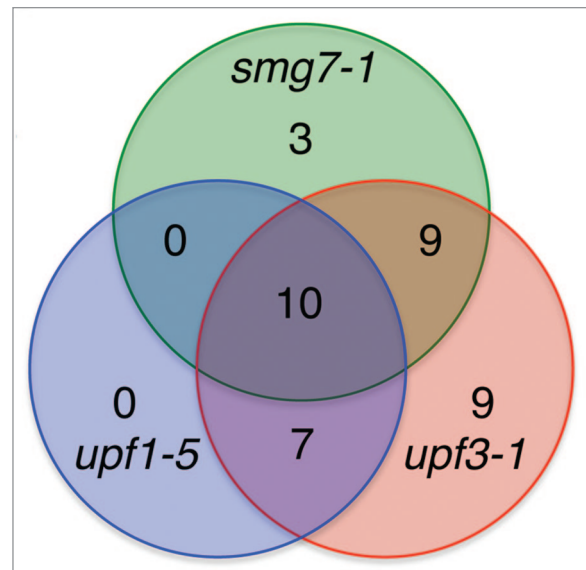
No potential conflicts of interest were disclosed.

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**Figure 2.** Upregulation of CpuORF-containing transcripts in Arabidopsis plants deficient in NMD. The universe list for the Venn diagram is Arabidopsis loci that are represented on the ATH1 GeneChip microarray and that have CpuORFs that are conserved between Arabidopsis and another plant species (77 transcripts, Hayden and Jorgensen, 2007, Tran, Schiltz and Bauman, 2008 and Takahashi et al., 2012).<sup>18-20</sup> Each circle represents transcripts that are upregulated at least 1.5-fold,  $p < 0.05$ .



gratefully acknowledge Karel Riha for making the array data 'NASCARRAYS-455' publicly available. Array data for *pad4-1* mutants was obtained from the NASCArrays repository.<sup>28</sup>

#### Supplemental Material

Supplemental material may be downloaded here:

[www.landesbioscience.com/journals/psb/article/21960/](http://www.landesbioscience.com/journals/psb/article/21960/)

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