A pervasive theme in development is that dynamic changes in gene expression drive developmental progression; yet in studies of gene expression, the general RNA decay pathways have historically played second fiddle to transcription. However, recent advances in this field have revealed a surprising degree of mRNA specificity for particular branches of these RNA decay pathways. General cytoplasmic mRNA decay typically initiates with deadenylation, following which the deadenylated mRNA can continue decay from the 3'-end through the action of the exosome, or it can undergo 5'-to-3' decay. Functional characterization of exosome subunits using inducible knock-outs uncovered a surprising complexity of molecular phenotypes and RNA substrates. Decay in the 5'-to-3' direction requires decapping, which is carried out by the decapping complex in Processing bodies (PBs). Recent analyses of decapping mutants have also revealed substrate specificity and roles in translational regulation. In addition, recent studies of specialized pathways such as nonsense-mediated decay and silencing reveal interactions with the general RNA decay pathways.

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**Current Opinion in Plant Biology** 2009, 12:96–102

This review comes from a themed issue on Growth and Development
Edited by Charles S. Gasser and Caroline Dean

Available online 5th November 2008
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DOI 10.1016/j.pbi.2008.09.003

**Introduction**

Levels of many mRNAs respond to developmental or environmental signals; however, the extent to which gene expression changes are due to specific responses in general mRNA decay pathways is largely unknown. Nevertheless, major advances over the past two years have given us a broader understanding of RNA decay (through transcriptome-wide approaches) [1**], identified some of the key players in mRNA decay, and revealed specific roles in mRNA turnover [2**,3**].

This review summarizes recent studies of the general cytoplasmic mRNA decay pathways (excluding the miRNA-programmed decay), focusing primarily on studies published since 2006 that reveal mechanistic details of the pathways and those relevant to plant development.

**High-throughput approaches to mRNA decay studies**

Genome-wide expression profiling has contributed to the global understanding of mRNA decay [1**]. Microarrays, used to measure mRNA decay rates in Arabidopsis tissue culture cells after transcriptional inhibition, documented mRNA half-life for more than 13 000 genes. Half-life varied from 0.2 to >24 hours (mean 5.9 hours and median 3.8 hours), and detailed analyses revealed two mechanisms that appear to affect differential decay. First, conserved sequence elements were identified in both 5' -UTRs and 3'-UTRs that correlated with unstable mRNAs or stable mRNAs; patterns of these sequence motifs suggested that they might act combinatorially. Second, genes containing at least one intron gave rise to significantly more stable mRNAs than those of intronless genes, suggesting that the nuclear biogenesis pathway influences stability. Thus mRNA stability appears to be strongly influenced by protein factors that either bind to UTR sequences or are deposited in a splicing-dependent manner.

Array assays often ignore the possibility that per cell RNA content might vary; however, a recent study documented that Arabidopsis cold acclimation is accompanied by a 15% mRNA up-regulation [4*]. Moreover, significant bias would have arisen if this had not been taken into account. Analyses of mRNA decay have another possible confounding problem: transcription inhibition depletes all mRNAs, including the ones encoding the effectors of mRNA decay. Thus, decay measurements are most valid at the earliest time point, and so for the most unstable messages.

**Deadenylation**

Cytoplasmic mRNA degradation generally begins with deadenylation, the shortening or complete removal of the poly(A) tail (Figure 1). Several biochemically distinct complexes that carry out deadenylation in animals and
fungi are conserved in plants: poly(A) ribonuclease PARN, CCR4/CAF1 deadenylase complex, and poly(A) nuclease PAN (Table 1). This diversity of deadenylation machinery might be expected to be highly redundant; however, null alleles of PARN are embryo-lethal in Arabidopsis [5,6]. The CCR4/CAF1 pathway also appears to be functional. CAF1 overexpression led to enhanced growth and disease resistance as well as up-regulation of \(N\) genes, including the ones functioning in signaling and metabolism. The knock-down gave the converse phenotype, small plants with reduced disease resistance [7]. The requirement for both the PARN and the CCR4/CAF1 pathways suggests that these pathways are non-redundant, and that each complex targets specific subsets of mRNAs. In support of this, PARN mutants show increased poly(A) tail length of only a subset of embryo-expressed genes [5] and its expression is induced by stress [8]. However, the extent of deadenylase substrate specificity, and how specificity is achieved, remains unknown.

### 3′–5′ Pathways

Perhaps the major source of the 3′–5′ exonucleolytic activity in eukaryotes is the exosome — an amazingly versatile macromolecular machine that can either firstly, conduct a limited processing of the RNA precursor species, as in the case of 5.8S rRNA, or secondly, completely degrade RNA, as in the course of cytoplasmic mRNA turnover; or thirdly, carry out RNA quality control, whereby it identifies and selectively degrades only defective RNA molecules, for example, misfolded tRNAs — but not the properly matured molecules. The first high-resolution genome-wide atlas of the exosome substrates (in any eukaryote) has been constructed in Arabidopsis [2**]. This was accomplished using conditional RNAi alleles and monitoring changes in the transcriptome kinetically. Thus, the direct effects of exosome inactivation were distinguished from secondary effects. Using this approach, Chekanova et al. identified the major classes of RNA targeted by Arabidopsis exosome, including stable structural RNAs, intermediates of...
the biogenesis of rRNA as well as of microRNA, hundreds of noncoding RNA of unknown function, and hundreds of polyadenylated heterochromatic transcripts. Interestingly, the majority of noncoding RNAs that are targeted by the exosome had not been previously described, indicating the existence of a ‘deeply hidden’ layer of the transcriptome.

Knock-down of the exosome subunits also resulted in the up-regulation of several hundred mRNAs [2]. Because this study was designed to only interrogate the poly(A)+ segment of the transcriptome, and deadenylation is believed to occur before exosome-mediated decay (Figure 1), the exosome knock-down was predicted to cause the accumulation of deadenylated, 3'–truncated mRNA decay intermediates. Thus, finding up-regulation of full length, poly(A)+ species (~200) was unexpected, and suggests the existence of a distinct pathway in which the exosome carries out deadenylation as well as degradation of mRNA body.

Prevailing models of exosome function (in animals and fungi) feature an invariant multisubunit core complex; however the induced knock-down of two different exosome subunits in Arabidopsis caused different (though overlapping) sets of changes [2]. This finding implies that the exosome might be composed of distinct molecular modules that are functionally nonequivalent. Another difference between exosomes of plants and animals is their enzymatic activity; the only active subunits in animals are RRP6 (a nuclear-specific subunit) and RRP44 [9], whereas in Arabidopsis RRP41 is an active phosphorolytic RNase [10]. Determining the significance of exosome complex variability, subunit specificity, differential RNA substrates, and developmental roles, is high priority for future studies.

mRNA decapping

Deadenylated mRNA can also undergo 5'–to-3' decay, which first requires the removal of the mRNAs 5' cap (Figure 1). Cap removal is carried out by the decapping complex; its core components (DCP1 and DCP2) are conserved in plants, and respective mutants have been analyzed. Forward genetic screens led to the identification of dep2 alleles as cotyledon vein patterning mutants (called trident for their forked veins) [3], and reverse genetic approaches also led to the characterization of DCP2 and DCP1 alleles [11,12]. A third component of the decapping core complex, VARICOSE (VCS)/HEDLS, was first identified in plants, and initially

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Table 1

<table>
<thead>
<tr>
<th>Subunit name</th>
<th>Function</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(A) ribonuclease (PARN)</td>
<td>Deadenylation</td>
<td>[5,6]</td>
</tr>
<tr>
<td>CCR4/CAF1</td>
<td>Deadenylation</td>
<td>[7]</td>
</tr>
<tr>
<td>PAN-like</td>
<td>Deadenylation</td>
<td>DAB</td>
</tr>
<tr>
<td>RRP4</td>
<td>Exosome, S1 + KH domain</td>
<td>[2]</td>
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<tr>
<td>RRP40A</td>
<td>Exosome, S1 + KH domain</td>
<td>[2]</td>
</tr>
<tr>
<td>RRP40B</td>
<td>Exosome, S1 + KH domain</td>
<td>[2]</td>
</tr>
<tr>
<td>CSL4</td>
<td>Exosome, S1 + KH domain</td>
<td>[2]</td>
</tr>
<tr>
<td>RRP41</td>
<td>Exosome, RNAse PH domain</td>
<td>[2]</td>
</tr>
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<td>Exosome, RNAse PH domain</td>
<td>[2]</td>
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<td>RRP43</td>
<td>Exosome, RNAse PH domain</td>
<td>[2]</td>
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<td>[2]</td>
</tr>
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<td>Exosome, RNAse PH domain</td>
<td>[2]</td>
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<td>RRP44A</td>
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<td>[2]</td>
</tr>
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<td>RRP44B</td>
<td>Exosome, RNAse II</td>
<td>[2]</td>
</tr>
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<td>[2]</td>
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<td>[2]</td>
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<td>DCP2/TDT</td>
<td>Decapping enzyme</td>
<td>[3,11,12]</td>
</tr>
<tr>
<td>DCP1</td>
<td>Decapping subunit</td>
<td>[11,12]</td>
</tr>
<tr>
<td>VARICOSE</td>
<td>Scaffold for decapping complex assembly, P-body core</td>
<td>[3,11,13]</td>
</tr>
<tr>
<td>XRN4</td>
<td>5'–3' exoribonuclease</td>
<td>1718</td>
</tr>
<tr>
<td>UPF1</td>
<td>Nonsense-mediated decay</td>
<td>[36,37,38]</td>
</tr>
<tr>
<td>UPF2</td>
<td>Nonsense-mediated decay</td>
<td>[36]</td>
</tr>
<tr>
<td>UPF3</td>
<td>Nonsense-mediated decay</td>
<td>[36,37,38]</td>
</tr>
<tr>
<td>SMG-7</td>
<td>Nonsense-mediated decay</td>
<td>[36]</td>
</tr>
<tr>
<td>UBP1</td>
<td>TIA1-like, stress granules</td>
<td>[26]</td>
</tr>
<tr>
<td>UBP47</td>
<td>TIA1-like, stress granules</td>
<td>[26]</td>
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characterized as a vein pattern mutant [13]. The mammalian homolog, called HEDLS, was subsequently implicated in mRNA decapping as a molecular scaffold for DCP1 and DCP2 [13,14,15]. Thus, animal and plant decapping complexes appear to be similar to each other (Arabidopsis VCS, DCP2, and DCP1 also interact and colocalize in the cytoplasm), and they both differ from yeast, which lacks a VCS/HEDLS homolog. Future analyses to determine whether VCS confers additional unique functions to the decapping complex (e.g. miRNA-mediated translational repression) are a high priority.

Decapping is required for normal developmental progression, as vcs, dcp2tdt, and dcp1 mutants all show similar pleiotropic seedling phenotypes (altered cotyledon shape, vascular patterning, and shoot and root meristem progression, as well as weak post-transcriptional gene silencing in Arabidopsis plants containing weak ago1 alleles [22,23]. These effects are accompanied by the RDR-dependent production of small RNAs from endogenous protein-coding loci or transgenes [23,24]. Note that RNA silencing can also be triggered by defects in splicing and mRNA 3′-end formation [23]. Taken together, these results indicate that rapid hydrolysis of abnormal mRNA, including the ones that are decapped or that have aberrant 3′ ends, is essential to prevent RDR-mediated silencing.

**P-bodies: sites for 5′–3′ decay and additional roles?**

Enzymes for 5′–3′ decay become sequestered in dynamic cytoplasmic complexes termed Processing bodies (PBs) [3,11,12,25,26]. The hallmark of PBs is their dependence on an mRNA supply. This is typically shown by cycloheximide treatment, which by ‘freezing’ ribosomes on mRNA halts the supply of RNA decay substrate, thereby inducing the loss of PBs. The vcs mutant lacks PBs, consistent with a role for VCS as a decapping scaffold [3], and xrn4 mutants produce abnormally large PBs, consistent with a role for XRN4 in the hydrolysis of decapped RNAs [26]. PB formation was recently found to be induced by stress treatment (hypoxia) [26]. However, hypoxia-induced PB formation was not evoked in the xrn4 mutant background, suggesting the intriguing possibility that XRN4 might serve a nucleating role for PBs formed under stress. Moreover, because xrn4 mutants do form PBs under nonhypoxic conditions, this result suggests the existence of biochemically distinct PB populations.

In yeast, mRNAs targeted to the PB are not necessarily committed to decay, they can be sequestered for translational arrest, following which they are either degraded or returned to polysomes [27]. Plant PBs have also been implicated in translational arrest, and specifically translational arrest induced by miRNA and siRNAs and requiring VCS, AGO1, and AGO10 [28]). The similar requirement for AGO1, AGO10, and VCS implies that they might all localize to PBs, which would be consistent with PBs of animal cells [29,30]. However, in plants AGO1 has been localized to nuclear D-bodies, not the cytoplasm [31], and AGO10 localization remains unknown. Another possible function for PBs is viral assembly. When brome mosaic virus is assembled in yeast, it accumulates in endoplasmic reticulum-associated PBs [32]. Sequestration in PBs might serve to separate gene expression from the sites of viral assembly.

Another type of mRNA particle characterized in animal cells is called stress granule (SG). SGs appear to contain mRNAs that aborted translational initiation, and the mRNAs fate appears to vary, and might include the transfer to PBs for decay [33]. A recent study documented SG-like granules in plant cells; these contained polyadenylated
RNA, eIF4E, and two other proteins related to animal stress-granule components [26]. These plant SGs localize in close proximity to PBs, supporting a relationship between these two compartments. Another component of plant SGs is the RNA helicase ISE2 [34]. The ise2 mutant was identified in a screen for plasmodesmata defects, and ise2 mutants show both morphological defects and defects in plasmodesmata. These phenotypes suggest a possible role for SGs in development, but it will be important to determine whether the phenotypes are directly caused by altered SG function or are indirect consequences of altered plasmodesmata.

**Specialized decay pathways**

Additional specialized cytoplasmic mRNA decay pathways also exist (Figure 1), for example, nonsense-mediated decay (NMD) is a cytoplasmic surveillance system that identifies old or aberrant mRNAs and targets them for decay. In plants, NMD is activated by either a long 3′-UTR or by a premature termination codon (PTC) in close proximity to an exon junction [35]. Three NMD core proteins (UPF1, UPF2, and UPF3) are conserved and are required for NMD in Arabidopsis [36,37–39]. RNA decay can also be elicited by an internal cleavage that is independent from RISC and thus distinct from small RNA pathways. For example, the decay of CGS1 mRNA (encoding cystathionine γ-synthase, an enzyme responsible for the key regulatory step of methionine biosynthetic pathway) is regulated through feedback. This mRNA’s decay initiates by internal cleavage, which is promoted by ribosomal stalling induced by the methionine metabolite AdoMet [40,41]. Internal cleavage is an especially effective method for the rapid loss of RNA as each fragment presents an unprotected end (Figure 1). But do these pathways have specific roles in development? Evidence suggests that they might: both upf1 and upf3 mutants show severe seedling-lethal phenotypes [38], partial loss-of-function upf1 alleles have altered sugar responses and overaccumulate a subset of mRNAs (especially the ones encoding transcription factors and metabolic enzymes) [42], and NMD play a role in autoregulation of at least two mRNAs: the NMD accessory factor SMG7 [36**] and decay of an alternatively spliced version of the circadian oscillator AtGRP7 [43].

**RNA decay and development: how specific?**

Developmental arrest and vein patterning are common phenotypes of mutants defective in general mRNA decay pathways. A challenge in interpreting these phenotypes is to distinguish between housekeeping and developmental functions. RNA decay is essential for the removal of defective and old RNAs, but we do not know whether developmental signals actively induce destabilization of specific mRNAs. That this is a possibility comes from the identification of ethylene signaling defects in xrn4 mutants [19,20] and the finding that a specific epicuticular wax defect is linked to a mutation in a component of the exosome [44]. Also instructive is the phenotype of vcs (decapping) mutants grown with polar auxin transport inhibitors [13]. The vcs phenotype is partially suppressed in the L.er background and mutants can produce normal-looking leaves (Figure 2). However, the inhibition of polar auxin transport causes vcs-1 leaves to develop devoid of blade, and instead composed entirely of petiole/midrib tissues (Figure 2). This result indicates that VCS and normal auxin signaling function redundantly in the specification of leaf blade. An interesting challenge will be to determine whether the vcs-1 response to altered auxin transport arises due to RNA stability defects or a different P-body function that requires VCS (e.g. translational arrest).
Conclusions
Although our understanding of general mRNA decay pathways is far from complete, several interesting themes are emerging. First, macromolecular complexes once viewed as single invariable entities, the exosome and the P-body, appear to be able to assemble with variable subunit composition, and possibly with different downstream functions [2**,26**]. Second, functional redundancy appears to be less common than models imply. Although an mRNAs decay pathway could use any of three different deadenylation complexes, and then either 3’-to-5’ (exosome) or 5’-to-3’ (decapping/ XRN4), each of these complexes appears to be essential, and to function on discrete subsets of cytoplasmic mRNAs. Thus, the emerging picture is a complex system with a surprisingly high degree of mRNA sub-
strate specificity. Probably the biggest challenge, though, is to identify the proteins that bind mRNA and target them to particular decay pathways, and to understand how the activity of these proteins is regulated. These binding proteins may be the key to understanding developmentally regulated general mRNA decay, and should shed light on both decay pathways and developmental mechanisms.

Acknowledgements
We apologize to those colleagues whose work could not be cited because of space limitations. RNA decay research in the LES lab is supported by the National Science Foundation (0642118), and research on plant gene expression in the DAB lab is supported by USDA, NSF, and US-Israel Bi-
National Science Foundation (072018). RNA decay research in the LES lab is supported by the

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Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


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Together with [19], this paper independently identified XRN4 as an important factor of ethylene signaling.


See annotation to [24**].


This paper makes a strong contribution to our ideas about PB functions in plants by revealing translational regulation as a common regulatory mode for plant miRNAs.


This paper makes a strong contribution to our ideas about PB functions in plants by revealing translational regulation as a common regulatory mode for plant miRNAs.


A study of mutants with altered embryonic fluorescent tracer movement led to the identification of ISE2 as a component of SGs. The accompanying defect in plasmodesmata suggests roles for SGs in development.


The authors describe a clever approach to investigating NMD using VIGS and agro-infiltration. Their analyses revealed the conservation of NMD components UPS1, UPS2, and UPS3, and confirmed that both PTC and long 3’-UTR pathways require UPF1 and 3. Moreover, they also identified SMG-7 as both a component of NMD and as a regulatory target, as SMG-7 mRNA level was regulated in a NMD-based feedback system.


The authors show that the defect in one of the two isoforms of the exosome component RRP45 specifically impairs the synthesis of cuticular wax.