Current perspectives on mRNA stability in plants: multiple levels and mechanisms of control

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The control of mRNA stability plays a fundamental role in the regulation of gene expression in plants and other eukaryotes. This control can be influenced by the basal mRNA decay machinery, sequence-specific decay components, and regulatory factors that respond to various stimuli. Important progress has been made towards the identification of some of these elements over the past several years. This is true particularly with respect to *cis*-acting sequences that control mRNA stability, the identification of which has been the focus of much of the initial work in the field. Characterization of mRNA fragments associated with post-transcriptional gene silencing and two plant transcripts that give rise to detectable decay intermediates have provided insight into the mRNA decay pathways. These, and other studies, are indicative of similarities, as well as of interesting differences between mRNA decay mechanisms in plants and yeast – the system that has been used for most of the pioneering work. Future studies in this area, particularly when enhanced by emerging genetic and genomic approaches, have tremendous potential to provide additional knowledge that is unique to plants or of broad significance.

Normal growth and development, as well as the ability to adjust to changing environmental conditions, requires the carefully regulated expression of many genes. Although much of this regulation is exerted at the transcriptional level, post-transcriptional mechanisms also play a fundamental role. For some genes, post-transcriptional mechanisms constitute the predominant form of control in response to a given stimulus. In other cases, an extra level of modulation is provided by post-transcriptional control that increases the flexibility and speed of responses beyond that which can be achieved through transcriptional regulation alone. The control of mRNA stability is one of the most prominent forms of post-transcriptional regulation in eukaryotic cells. The stability of a particular mRNA determines, to a great extent, its steady-state levels, and directly affects the rate of its induction or repression following a change in transcription. Thus, a thorough understanding of how mRNA stability is controlled is essential for elucidating how the abundance of endogenous mRNAs is governed and to optimize the accumulation of transgene mRNAs in plants for biotechnological applications.

The molecular components that control mRNA stability can be considered in three layers (Fig. 1). Recent work in yeast indicates that eukaryotic cells contain RNA-degrading activities and protein cofactors, which appear to constitute the general or basal mRNA decay machinery responsible for the degradation of most stable and unstable mRNAs. Superimposed on this basal machinery are the sequence-specific controls that dictate the inherent stability of various mRNAs, the half-lives of which can vary over a wide range. For those transcripts whose stability changes in response to exogenous or endogenous stimuli, a third layer of control must be evoked. This last layer would transduce the signals elicited by various stimuli into changes in mRNA turnover. In this conceptual framework, investigation of all three layers is critical because of their individual importance and the probable inter-relationships between them. For example, differential control of the stability of a particular mRNA could be mediated by modulating the activity of a sequence-specific recognition factor that interacts with the basal decay machinery.

Most of the recent progress in our understanding of mRNA stability in plants has emerged from studies of sequence-specific recognition of transcripts for rapid and/or regulated decay. This review highlights the current knowledge concerning those nuclear-encoded transcripts that have been studied in the most detail. A discussion about the potential mRNA decay pathways will also be emphasized because interesting data is emerging in this important area that underscores the potential of future research. For a more comprehensive presentation of mRNA stability and post-transcriptional control in plants and other eukaryotes see Refs 1–3 and 4,5, respectively.

Molecular determinants of mRNA stability

The decay rate of transcripts in plants appears to be similar to those observed in other multicellular eukaryotes. Half-lives range from <1 h for unstable messages, to several days for stable transcripts, the average being several hours[3,6]. The decay rates of some transcripts can be rather dynamic, and are modulated by the coordinated integration of internal and external stimuli. What are the molecular determinants that control the half-life of a particular transcript at any time in the cell? In recent years, research has focused mainly on the identification and characterization of structural features of the mRNA molecule, or on the *cis*-acting elements, which influence mRNA decay rates. These studies show that general structural elements found at the ends of virtually all mRNAs, as well as specific sequence elements located within a transcript, can all contribute to the overall stability.

In addition to their role as translational enhancers, the 7-methyl-G cap at the 5′ end and the polyadenylate [poly(A)] tail at the 3′ end increase mRNA stability in transient assays[7]. By electroporating capped or uncapped mRNAs and mRNAs with or without poly(A) tails into tobacco protoplasts, it has been found that the 5′ cap stabilizes reporter transcripts by two- to fourfold

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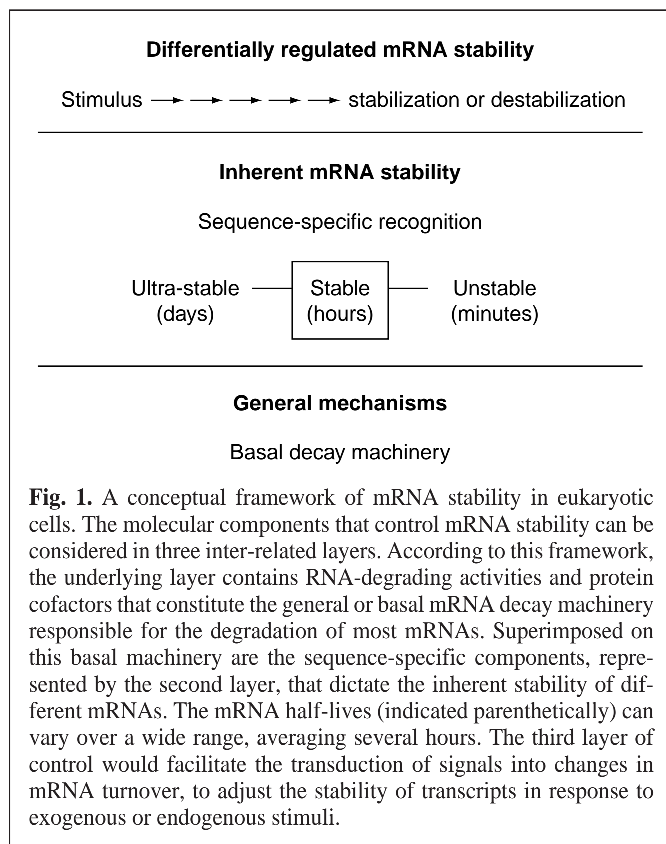

Fig. 1. A conceptual framework of mRNA stability in eukaryotic cells. The molecular components that control mRNA stability can be considered in three inter-related layers. According to this framework, the underlying layer contains RNA-degrading activities and protein cofactors that constitute the general or basal mRNA decay machinery responsible for the degradation of most mRNAs. Superimposed on this basal machinery are the sequence-specific components, represented by the second layer, that dictate the inherent stability of different mRNAs. The mRNA half-lives (indicated parenthetically) can vary over a wide range, averaging several hours. The third layer of control would facilitate the transduction of signals into changes in mRNA turnover, to adjust the stability of transcripts in response to exogenous or endogenous stimuli.

and the poly(A) tail stabilizes reporter transcripts by two- to three-fold[8]. Although it is unclear how the cap and the poly(A) tail protect a transcript from degradation, an appealing model would be that the physical interaction between the cap and the poly(A) tail, via their associated factors [e.g. poly(A)-binding protein, eIF4G, eIF4E or eIF4B], would sequester the ends of the mRNA, protecting them from the action of nucleases[7].

Within the body of the mRNA, specific sequence motifs that are present only in a subset of transcripts can either act constitutively to establish the inherent instability (or stability) of a particular transcript, or they can modulate the stability of an mRNA in response to certain physiological, developmental or environmental cues. Major examples of both classes of stability determinant are discussed in the following sections. Although they are presented separately, it should be noted that the division is organizational rather than biological. Some sequences that appear to affect mRNA decay rates constitutively, might subsequently be found to be regulated under special conditions. Conversely, regulatory sequences might also contribute to inherent stability in the absence of stimuli. The characterization of these sequences is already leading to mechanistic insights into how they are recognized in the cell and how that recognition might be controlled.

Sequence elements that control inherent mRNA stability

DST (downstream) element

The DST (downstream element) was originally identified as a conserved region in the 3′ untranslated region (UTR) of the unstable small auxin-up RNA (*SAUR*) transcripts[9]. It consists of three highly conserved subdomains separated by two variable regions (Fig. 2). When a synthetic dimer of the soybean *SAUR-15A* DST sequence is placed in the 3′ UTR of a reporter transcript, its turnover is significantly faster than that of a spacer or no-insert control in bright yellow-2 tobacco cell line (BY-2) cells[10]. Subsequent mutational analysis has indicated that two conserved subdomains, designated ATAGAT and GTA regions (named after the invariant nucleotides they contain) are critical for DST function. Five- and six-base substitutions in the ATAGAT and GTA regions, respectively, resulted in slower turnover rates in BY-2 cells and higher reporter transcript accumulation in transgenic tobacco plants[11]. Two-base substitution mutations within these two subdomains indicate that the first four bases of the ATAGAT subdomain are critical for instability function in tobacco cell culture. Interestingly, a 2-base substitution in the GTA subdomain inactivates DST function in transgenic tobacco leaves but not in cell culture. This suggests that the DST element might be differentially recognized in different cell types[11].

Detailed studies of *SAUR* gene expression in *Arabidopsis thaliana* have been carried out on the *SAUR-AC1* gene. Examination of chimeric gene expression shows that the promoter region is responsible for auxin induction, and that sequences downstream of the promoter limit mRNA accumulation in an auxin-independent manner[12]. Half-life measurements of the transcripts encoded by chimeric genes show that the 3′ UTR acts as a potent mRNA instability determinant[12] (Fig. 2). Interestingly, the *SAUR-AC1* 3′ UTR contains one canonical DST element and several ATAGAT-like and GTA-like subdomains that might contribute to mRNA instability (Fig. 2). This is intriguing because in previous work two copies of the prototype DST element from *SAUR-15A* were needed to cause instability of a reporter transcript[10]. Further studies are necessary to investigate the contribution that particular sequences make to *SAUR-AC1* mRNA instability, and the importance of context for DST element function. Nevertheless, the novel structure of DST sequences indicates that they might mediate mRNA decay through a pathway that is novel and unique to plants.

AUUUA-repeats

Adenylate/uridylate-rich elements (AREs) represent a common determinant of RNA stability in mammalian cells. Transcripts that contain AREs are selectively targeted for rapid decay[13]. AREs are ~50–150 nucleotides long, usually contain multiple copies of the AUUUA motif and a high content of uridine, and are located in the 3′ UTR of mRNAs encoding a variety of proto-oncoproteins, cytokines and transcription factors[13]. Accordingly, in mammalian systems, AUUUA sequences play important roles in the post-transcriptional regulation of gene expression during processes, such as cell growth, differentiation and the immune response. Because of the significance of AUUUA elements in mammals, a synthetic AUUUA repeat has been tested for the ability to act as an instability determinant in plants. Reporter transcripts containing 11 repeats of the AUUUA motif in their 3′ UTRs are degraded more rapidly in stably transformed tobacco cells and accumulate to a lower level in transgenic tobacco plants than those of the control constructs[14] (Fig. 2). The effect appears to be AUUUA-specific because two other sequences with the same size and A+U content had no effect in parallel experiments. These results indicate that the mRNA decay pathway mediated by AUUUA repeats might be conserved between animals and plants. However, the natural targets of the plant AUUUA-mediated decay pathway remain to be identified. Possible candidates include the *PvPRP1* transcript from *Phaseolus vulgaris* and the *αAmy3* transcript from *Oryzae sativa*. Curiously AUUUA repeats have not been reported to cause instability in yeast.

Nonsense codons

Premature nonsense codons decrease mRNA stability by activating nonsense-mediated decay pathways in several eukaryotic systems. The yeast nonsense-mediated decay pathway (the deadenylation-independent decay pathway; Fig. 3) is the best understood pathway

Sequence element	Description	Experimental evidence	Ref.
Prototype DST element derived from the soybean *SAUR 15A* gene (GGAgactgacATAGATTggaggagacAtTTtGTAtaata)$_2$	~40 bp conserved sequence found in the 3′ UTR of *SAUR* transcripts. Highly conserved residues across different species are shaded and invariant residues underlined. Mutational analysis has identified important residues (bold) for DST function.	The $t_{1/2}$ of a reporter transcript containing a dimer of the element and a control transcript have been measured during an ActD time course in stably transformed BY-2 cells.	10,11
***SAUR-AC1* 3′ UTR** AGTACTATACTACAACATTTCCATATTTTTTTAGATTGTTAGCTAATTT CCCCTGGAGATAATTGTAAATTGTTTCAATGAGAGGAATATACAATA CATAGATCGTAATTGATCAATGCGTATTTGCATGTT	The *SAUR-AC1* 3′ UTR includes one DST element (shaded) and several ATAGAT-like (underline) and GTA-like (upper bar) subdomains that might contribute to its instability function.	The $t_{1/2}$ of chimeric genes have been measured using the Top10 promoter system in stably transformed BY-2 cells.	12
A/U rich element AUUUAAUUUAUUUAUUUAUUUAUUUAUUUAUUUAUUUAUUUAUUUA	AUUUA is a commonly found motif in A/U-rich instability determinants in the 3′ UTRs of mammalian transcripts. Endecamer of AUUUA (dotted lines), but not a related sequence, acts as an instability determinant in plants.	The $t_{1/2}$ has been measured during an ActD time course in stably transformed BY-2 cells.	14
Premature nonsense codons ATG STOP STOP 7mG AAAAAAAA(A)n	The effect of nonsense codons in plants has been tested in naturally occurring and *in vitro*-generated phytohemagglutinin genes.	The $t_{1/2}$ has been measured during an ActD time course in stably transformed BY-2 cells.	19
Light responsive element ATG STOP 7mG AAAAAAAA(A)n (CATT)$_4$	Light-responsive element from *ferredoxin 1* gene spans part of the 5′ UTR and the first third of the coding region. Critical regions for iLRE function are depicted as shaded boxes.	The $t_{1/2}$ has been measured using Top10 promoter in transgenic tobacco plants.	26
***αAmy3* 3′ UTR (seconday structure prediction)** AAAAAAAA(A)n I II III	*αAmy3* 316 bp 3′ UTR involved in sugar repression. Contains AUUUA-like motifs within an A/U-rich context. Subdomains that have been subjected to analysis are indicated (I, II and III). Subdomains I and III are critical for sugar repression and possess a conserved A/U-rich sequence in the loops.	The $t_{1/2}$ has been measured during an ActD time course in rice culture cells.	30,31

Trends in Plant Science

Fig. 2. Summary of sequence elements that control mRNA stability in plants. The *SAUR 15A* DST element and *SAUR-AC1* 3′ UTR are represented as DNA sequences. Abbreviations: $t_{1/2}$, half-life; ActD, actinomycin D; BY-2, bright yellow-2 tobacco cell line (NT-1).

at the molecular and genetic levels. Nonsense-mediated decay is presumably part of a mRNA surveillance system that rapidly removes abnormal mRNAs to prevent the formation of truncated or other potentially detrimental polypeptides[15,16].

Initial evidence that nonsense-mediated mRNA decay occurred in plants came from studies conducted on natural alleles of the soybean Kunitz trypsin inhibitor[17] and from bean phytohemagglutinin A (*PHA*) genes[18]. Cells containing alleles with early stop codons accumulate low levels of mRNA (Refs 17,18) even when transcriptional rates were normal[17]. Generally, nonsense codons affect mRNA abundance in a position-dependent manner, the closer they are to the initiation codon the greater the likelihood of affecting mRNA abundance. The effect of stop codons positioned at a variable distance from the translation start codon of a reporter gene has been directly addressed using the initially isolated *PHA* allele and other *PHA* alleles constructed *in vitro*[19]. Measurements of mRNA decay rates in stably transformed tobacco cell lines demonstrate that transcripts with nonsense codons positioned 20, 40 and 60% of the way through the normal coding region yield highly unstable mRNAs, whereas a transcript with a nonsense codon at 80% is as stable as the wild type. These findings support the idea that plants have a nonsense-mediated decay pathway similar to that found in other eukaryotes.

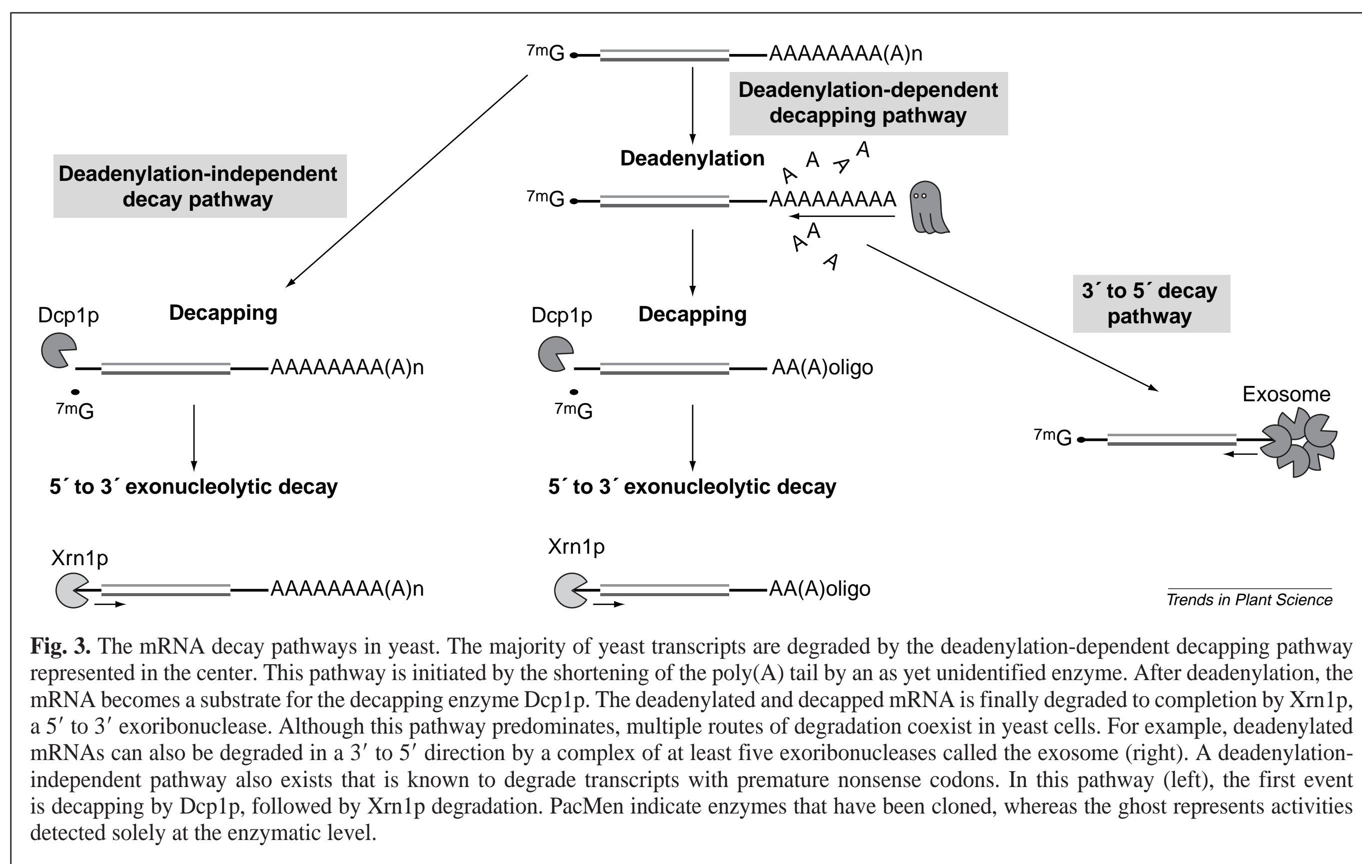

Fig. 3. The mRNA decay pathways in yeast. The majority of yeast transcripts are degraded by the deadenylation-dependent decapping pathway represented in the center. This pathway is initiated by the shortening of the poly(A) tail by an as yet unidentified enzyme. After deadenylation, the mRNA becomes a substrate for the decapping enzyme Dcp1p. The deadenylated and decapped mRNA is finally degraded to completion by Xrn1p, a 5′ to 3′ exoribonuclease. Although this pathway predominates, multiple routes of degradation coexist in yeast cells. For example, deadenylated mRNAs can also be degraded in a 3′ to 5′ direction by a complex of at least five exoribonucleases called the exosome (right). A deadenylation-independent pathway also exists that is known to degrade transcripts with premature nonsense codons. In this pathway (left), the first event is decapping by Dcp1p, followed by Xrn1p degradation. PacMen indicate enzymes that have been cloned, whereas the ghost represents activities detected solely at the enzymatic level.

Instability of mRNA as a cause of limited expression of foreign genes

Problems encountered while attempting to express some foreign genes in plants have further emphasized the importance of understanding post-transcriptional mechanisms of mRNA accumulation. Key examples are the *cry* genes, which encode the *Bacillus thuringiensis* (*Bt*) insecticidal proteins, which have been introduced into plants for crop improvement. It has been known for more than ten years that expression of these genes gives rise to little mRNA in plants, even when they are expressed under the control of strong plant promoters (reviewed in Ref. 20). *Bt* toxin genes typically have a higher A/T content than dicot and monocot coding regions. This feature increases the probability that sequences will be recognized as signals of pre-mRNA processing, mRNA decay or other processes that can affect the structure and accumulation of the transcript when expressed in plants. However, the mechanisms that cause poor mRNA accumulation have been the subject of controversy[20]. Estimates of *Bt* toxin mRNA stability in electroporated protoplasts led to opposite conclusions regarding the contribution of mRNA stability to the poor *Bt* toxin gene expression[21,22]. This discrepancy was solved recently by determining the mRNA decay rates of *Bt* toxin transcripts in stably transformed BY-2 cells[23]. Half-life measurements of transcripts encoded by the wild-type *cryIA(c)* gene and a G/C-rich synthetic version demonstrate that the wild-type mRNA is considerably less stable than the synthetic one. Moreover, transcriptional rate measurements confirm that the poor *Bt* toxin transcript accumulation is not because of differences in transcriptional activity. Subsequent analysis of a set of chimeric genes has shown that the sequences that contribute to low mRNA accumulation are found in multiple positions within the coding region. Interestingly, a comparison of mRNA accumulation in stably transformed tobacco cells and transgenic *Arabidopsis* plants suggests that some of the sequences are differentially recognized in the different plant systems, which emphasizes the need for detailed knowledge of the particular working system when trying to engineer expression of a heterologous gene. These results indicate that one of the major causes of poor *Bt* toxin gene expression is the rapid decay of the wild-type transcripts. Moreover, these results further support the findings that A/U-rich sequences can cause mRNA instability in plants.

Additional mechanistic explanations for the poor accumulation of *Bt*-toxin mRNA in plants have been reported. In particular, sequences within the coding region are recognized as polyadenylation signals in plants, therefore interfering with the production of full-length *Bt*-toxin mRNAs (Ref. 24). The short transcripts resulting from premature polyadenylation are also unstable[23]. Another consequence of the AT-richness of *Bt* toxin genes is the frequent occurrence of codons that are rare in plant genes. A rare codon-rich segment from the *cryIA(c) Bt* toxin gene, or a synthetic sequence of rare codons, are not sufficient to destabilize a reporter gene transcript in tobacco cells, or to limit mRNA accumulation in transgenic plants[25]. However, rare codons might slow translation[20] or enhance the effect of mRNA instability sequences as a consequence of ribosome pausing, as suggested for the unstable *Matα1* transcript from yeast[5], thereby contributing to the overall low abundance of *Bt* toxin protein.

Differential control of mRNA stability

Light modulation

Light regulation at the post-transcriptional level has been well characterized for the pea photosynthetic electron carrier *ferredoxin I* (*Fed-1*) gene (reviewed in Ref. 26). As with many other photosynthetic genes, *Fed-1* expression is induced by light, mRNA levels being fivefold higher in the light than in darkness. Moreover, the mRNA half-life in transgenic tobacco seedlings is twofold higher for transcripts in light versus darkness, demonstrating that light

regulation occurs through a change in mRNA stability[27]. A sequence element that can confer light responsiveness to a reporter gene under the control of a constitutive promoter has been identified within the transcribed region. This internal light regulatory element (iLRE) spans a portion of the 5′ UTR and the first 20 codons of the coding region[26] (Fig. 2). *Fed-1* mRNA is loaded on polyribosomes and accumulates to higher levels in response to light. In addition, nonsense mutations, which block ribosomal progression, abrogate *Fed-1* mRNA accumulation in response to light. These observations prompted a model in which efficient translation of *Fed-1* in the light is associated with increased mRNA stability[26]. It is also possible that some of the nonsense mutations can target the transcript for nonsense-mediated decay. Further mutation analysis of the iLRE has identified two regions that are critical for its function – a CATT repeat in the 5′ UTR and the translation initiation region[28] (Fig. 2). Two different substitution mutations have been made within the CATT repeat that block *Fed-1* mRNA accumulation, one of which affects ribosome loading[28]. The simplest explanation of these findings is that *Fed-1* mRNA is stable in illuminated plants when associated with polyribosomes. In darkness, inefficient translation renders the transcript less stable because of a process involving the CATT repeat located in the 5′ portion of the message[28]. Further studies should help to determine whether the CATT region is a stability or instability determinant, as well as the exact mechanistic relationship between mRNA stability and translation.

Sucrose regulation

The α-amylases are endo-amylolytic enzymes that catalyze the hydrolysis of α-1,4-linked glucose polymers and have an important role in the degradation of starch in higher plants. The expression of the rice α-amylase gene family is coordinately induced by sucrose starvation and suppressed by sucrose availability, a process that depends on both transcriptional and post-transcriptional mechanisms[29]. The sucrose-mediated effect on mRNA stability has been analyzed in detail for one of the most abundant α-amylase genes, *αAmy3*. The mRNA decay rates of *αAmy3* transcripts are ~1.5 h in the presence of sucrose and increase to 6 h in sucrose-starved cells[29]. Examination of chimeric gene expression in stably transformed rice cells shows that the *αAmy3* 3′ UTR is sufficient and probably the major determinant for controlling the stability of *αAmy3* mRNA in response to sucrose availability[30,31]. Further analysis of the 3′ UTR has identified two subdomains, I and III (Fig. 2), that could each function as a sugar-dependent stability determinant[30,31]. In addition, secondary structure analysis predicts extensive duplex formation in the *αAmy3* 3′ UTR, and interestingly, conserved A/U-rich regions have been found in the loop of subdomains I and III (Ref. 30). Whether these A/U-rich regions, or the structural motifs that contain them, are involved in the modulation of mRNA stability in response to sucrose levels remains to be elucidated. Moreover, as in other cases of regulated mRNA stability, it is unclear whether a *trans*-acting factor decreases the transcript turnover rate in the presence of sucrose or speeds up the turnover in its absence. Treatment with the translation inhibitor cycloheximide enhances the accumulation of *αAmy3* transcript in the presence or absence of sucrose[29]. By contrast, cycloheximide does not significantly affect transcriptional rates of *αAmy3*, regardless of whether or not the cells are provided with sucrose[29]. These observations might suggest that labile proteins are involved in *αAmy3* mRNA decay. However, cycloheximide might interfere with the normal decay of the message in other ways, for example, translation of the message might be required for degradation to take place.

Biotic stress

One of the best examples of the modulation of mRNA stability in response to biotic stress (commonly a result of infection from bacteria, fungi or viruses) has been characterized in common bean (*Phaseolus vulgaris*) cells. Fungal elicitor treatment of bean cells results in the down-regulation of the *PvPRP1* gene, which encodes a cell wall proline-rich protein[32]. Direct proof that the major control mechanism of this down-regulation is modulation of mRNA stability has been provided by the observation that *PvPRP1* mRNA half-life in the presence of the elicitor is shorter than in its absence. Moreover, transcriptional rates remain constant regardless of the presence or absence of the elicitor[33]. Subsequent studies have identified a 50 kDa protein (PRP-BP) that can be specifically crosslinked to the 3′ UTR of the *PvPRP1* transcript[34]. Using deletion analysis, the binding site for PRP-BP has been mapped to a 27 nt, U-rich site that contains one copy of the AUUUA motif. It remains to be demonstrated that this binding site is important for the regulation of transcript stability. Nevertheless, the observation that *PvPRP1* mRNA degradation in response to fungal elicitor treatment is preceded by increased PRP-BP-binding activity in bean cells suggests that this protein and the *cis*-element it binds are involved in the regulation[34]. PRP-BP activity *in vitro* is increased by the reducing agents dithiothreitol (DTT) or β-mercaptoethanol and reversibly eliminated with the –SH oxidizing agent diamide, or by the –SH alkylating agent *N*-methylmaleimide[34]. The defense response in bean and many other species is accompanied by the production of active oxygen species and other redox perturbations. Hence, these observations suggest that PRP-BP-binding activity could be modulated by the redox changes that take place during the plant defense response[32,34].

Other stimuli

Hormones play an indisputable role in the regulation of a multitude of physiological and developmental processes in plants. Although it is clear that hormones can influence gene expression at both transcriptional and post-transcriptional levels, a detailed understanding of the molecular basis of hormone action, especially at the post-transcriptional level is lacking. A recent example of hormonal regulation of mRNA stability arose during a study of cytokinin effects on the soybean mRNA *Cim1* (Ref. 35). The predicted Cim1 protein product is related to a group of proteins termed β-expansins, which are involved in cell wall expansion during the vegetative and/or reproductive phases of plant development. *Cim1* mRNA abundance increases 20–60-fold upon the addition of cytokinin to cytokinin-starved soybean suspension cultures. When the half-life of the *Cim1* mRNA was determined following actinomycin D treatment, cytokinin addition to cytokinin-starved soybean cells increased the mRNA half-life of *Cim1* about fourfold[35]. Further experiments have been undertaken with the aim of characterizing the role of protein phosphorylation and dephosphorylation in cytokinin-mediated induction of *Cim1*. The accumulation of the *Cim1* message is stimulated by staurosporine in the absence of cytokinin and inhibited by okadaic acid in the presence of cytokinin. These results suggest a role for protein phosphatases in cytokinin regulation of *Cim1* mRNA abundance[35].

In addition to the examples described above, several other mRNAs have been reported to show modulation of mRNA stability in response to biotic stress, abiotic stress (e.g. cold, heat and salinity) and hormone treatments. In the majority of these cases, conclusions have been drawn after finding a poor correlation between the rate of transcription and mRNA accumulation in response to the stimulus. However, most of this research remains at a preliminary stage and the mechanisms through which this modulation is achieved have not yet been reported (reviewed in Refs 1,3,32,36).

Stable mRNAs in plants

Although it is clear that unstable mRNAs contain instability sequences, no discrete stabilizing determinant has been demonstrated to be responsible for the long half-life of an extremely stable transcript in plant systems. The search for mRNA stabilization sequences has lagged behind those of destabilizing elements in eukaryotes in general, but at least one example has been well characterized in mammals. Studies undertaken with the aim of understanding the mechanism for the selective stabilization of the α-globin message during erythroid cell development identified a pyrimidine-rich sequence in the 3′ UTR that is responsible for the long half-life of this mammal transcript (reviewed in Ref. 37). This finding negates a previous idea that all mRNAs are stable by default rather than by the presence of stabilizing sequences and suggests that stabilizing sequences might exist in other systems as well. Specific mRNA sequences could, for example, contribute to the stability of seed storage protein mRNAs (Ref. 36), as in the case of wild cultivars of oat[38], perhaps by influencing their compartmentalization. Studies conducted in these and other plant systems might provide insights into the mechanisms of mRNA stabilization. Identification of the sequence elements, and the *trans*-acting factors that they interact with, might provide tools to improve transgene expression in crop plants, and should certainly contribute to a more complete understanding of mRNA metabolism.

The mRNA decay pathways

Elucidating the pathways of mRNA decay in plant cells is a critical step in understanding the molecular basis of both inherent and differential regulation of mRNA stability. This is because it is the decay pathways themselves, or in some cases the choice between pathways, which must be modulated to control mRNA levels at any given time. To date, yeast is the only eukaryotic organism in which the mRNA decay pathways have been extensively dissected (Fig. 3) and the main enzymes purified, cloned and characterized. This progress in yeast, which relied on well established genetic and biochemical techniques, has provided useful insights into potential decay pathways in plants and mammals.

Insight from yeast

Main decay pathway in yeast

The most accepted model suggests that many mRNAs in *Saccharomyces cerevisiae* are degraded at different rates using a common mechanism called the deadenylation-dependent decapping decay pathway[5] (Fig. 3). The first step in this pathway involves deadenylation of the transcript, shortening the poly(A) tail to an oligo(A) of ~10–15 residues[39]. The gene(s) responsible for this deadenylating activity have yet to be identified. Once the poly(A) tail has been shortened, the transcript is susceptible to decapping by the enzyme Dcp1p (Refs 40,41). Deadenylation promotes decapping, and, as in the case of deadenylation, decapping of unstable transcripts is faster than decapping of stable transcripts, suggesting a mechanism that is dependent on specific sequences within the mRNA (Ref. 42). Following decapping, the message is degraded by Xrn1p, a processive exoribonuclease that hydrolyses RNA in a 5′ to 3′ direction to completion[43].

The steps in the yeast mRNA decay pathway have been demonstrated using two principal strategies[5]. One approach is the isolation and characterization of mutants defective in the key activities. For example, *xrn1*$^{-}$ strains accumulate decapped and deadenylated full-length mRNAs (Ref. 44). The second approach is the introduction of poly(G) stretches of ~18 residues into reporter genes. These poly(G) sequences create strong secondary structures that can block the action of exonucleases, such as Xrn1p (Ref. 5), thus making the study of decay intermediates possible. In addition to blocking 5′ to 3′ exoribonucleases, such as Xrn1p, poly(G) sequences also block the progression of the 3′ to 5′ exoribonucleases in yeast[45]. Accordingly, poly(G) has been a useful tool for dissecting the secondary pathways of mRNA decay that involve both types of exoribonucleases.

Secondary pathways in yeast

Although the deadenylation-dependent decapping decay pathway is considered the main decay pathway in yeast, alternative pathways also exist. The deadenylation-independent decay pathway (Fig. 3), which is responsible for the degradation of nonsense-containing mRNAs also involves decapping followed by exonucleolytic 5′ to 3′ degradation, with Dcp1p and Xrn1p as the major players[46]. However, this pathway is independent of deadenylation. Another secondary pathway for mRNA decay described in yeast, the 3′ to 5′ decay pathway (Fig. 3), involves the action of a protein complex called the exosome[45,47]. This complex consists of at least five different 3′ to 5′ exonucleases and was first identified as an essential complex for 5.8S rRNA processing. It has only been possible to study this secondary pathway in strains defective for the main pathway, which suggests that it is only of minor importance in mRNA decay, although it could be the main mechanism for degradation of specific transcripts. The existence of endonucleases that can trigger the decay of specific transcripts has also been described. For example, the post-transcriptional feedback regulation of *L2A* mRNA abundance involves the action of an endonucleolytic cleavage as the primary event[48]. In yeast, endonucleases appear to play a relatively minor role in mRNA decay, but they are clearly important in the decay of certain unstable mRNAs in mammalian cells, as in the case of the transferrin receptor and c-*myc* transcripts (reviewed in Ref. 49).

General differences and similarities in plants

Although some features of yeast mRNA decay processes are conserved in plants, such as the accelerated decay of transcripts containing early nonsense codons[19], other evidence suggests that plants (and possibly mammals) might diverge from the main pathway observed in yeast. In plants, mRNAs are generally degraded without the accumulation of detectable intermediates[2], similar to yeast and mammals. However, in contrast with yeast, the insertion of poly(G) tracts into reporter genes does not lead to the detection of intermediates in either stably transformed tobacco cultures or transgenic plants. Insertion of poly(G) tracts into reporter transcripts has also failed to yield intermediates in mammalian systems[50]. These findings might be an indication that the major mRNA degradation pathway in plants differs substantially from that described in yeast. Alternatively, it is possible that small differences are responsible for the lack of intermediates. For example, a plant homolog of Xrn1p might progress through poly(G) tracts, or an RNA helicase activity present in higher eukaryotes might unwind the secondary structure, allowing the exoribonucleases to proceed. In addition, the few known cases where decay intermediates have been found suggest that mechanisms of mRNA decay in plants might differ from the mRNA decay pathways in yeast (Fig. 3).

An analysis of current databases suggests the existence of plant homologs for some of the main players in the yeast mRNA decay pathways (Fig. 3). A BLAST search[51] of the *Arabidopsis* database reveals potential homologs of *Xrn1* (GenBank Accession no. W43714), the exosome 3′ to 5′ exonuclease *Ski6* (Accession no. T43164) and *Dcp1* (Accession no. 3169719)[50]. Additionally, there are several homologs of Pab1p, a poly(A)-binding protein that affects decay in yeast[52], which are expressed in an organ-specific manner in *Arabidopsis*[53,54]. At first approximation, the presence of these potential homologs would appear to indicate that the

corresponding decay mechanisms are mainly conserved in plants. However, to obtain direct evidence for this, the functional significance of these apparent homologies must be addressed.

Naturally-occurring decay intermediates in plants

For most plant mRNAs, no degradation intermediates can be detected on RNA gel blots. However, there are exceptions to this rule, two of which have been well characterized. The first is the *SRS4* mRNA (Fig. 4), which encodes the soybean ribulose-1,5-bisphosphate carboxylase small-subunit. *In vitro* and *in vivo* studies have revealed a set of discrete 5′ and 3′ degradation products that *in vitro* are generated from full-length transcripts. Mapping these fragments demonstrated that many of the 5′ fragments end at a site adjacent to the beginning of a 3′ fragment. This indicates that the fragments are largely generated by endonucleolytic cleavage, probably by a single enzymatic activity, which, according to sequence comparison of the sites of nucleolysis, recognizes secondary structure and not specific sequence elements within the transcript[55]. In contrast with the main pathway in yeast, the endonucleolytic cleavages occur independently of the presence of the 5′ m^7G cap or the 3′ poly(A) tail. However, after the primary endonucleolytic event, the 5′ fragment can be recognized as a deadenylated transcript and can then be a substrate for a 3′ to 5′ exonuclease. The 3′ fragment can be degraded by 5′ to 3′ exonucleases because it lacks the cap structure (Fig. 4). In addition, it is possible that other pathways operate on *SRS4* mRNA because full-length transcripts are found in all the fractions studied, that is, poly(A)$^+$, poly(A)$^-$, cap$^+$ and cap$^-$. Finally, it is important to mention that the degradation intermediates are associated with polysomes[55], an observation that has been obtained in other cases where intermediates are found.

Evidence also indicates that fragments of oat phytochrome A (*PHYA*) mRNA, which can be observed on RNA gel blots, are *in vivo*-generated degradation intermediates. *PHYA* mRNA fragments are present in RNA isolated from a polysome-based *in vitro* system, and are associated with polysomes *in vivo* as well. However, in contrast with *SRS4* mRNA fragments, *PHYA* fragments migrate as a continuous distribution on RNA gel blots. In addition, the majority of *PHYA* mRNA molecules (75%) appears to be degraded by a 5′ to 3′ exonuclease activity before removal of the poly(A) tail, whereas the remaining 25% of the transcripts are deadenylated first, and then degraded by 5′ to 3′ and 3′ to 5′ exonuclease activities (Fig. 4). An alternative model involving a stochastic endonuclease can be used to explain *PHYA* mRNA decay, but this appears less likely because of the continuous distribution of RNA fragments[56].

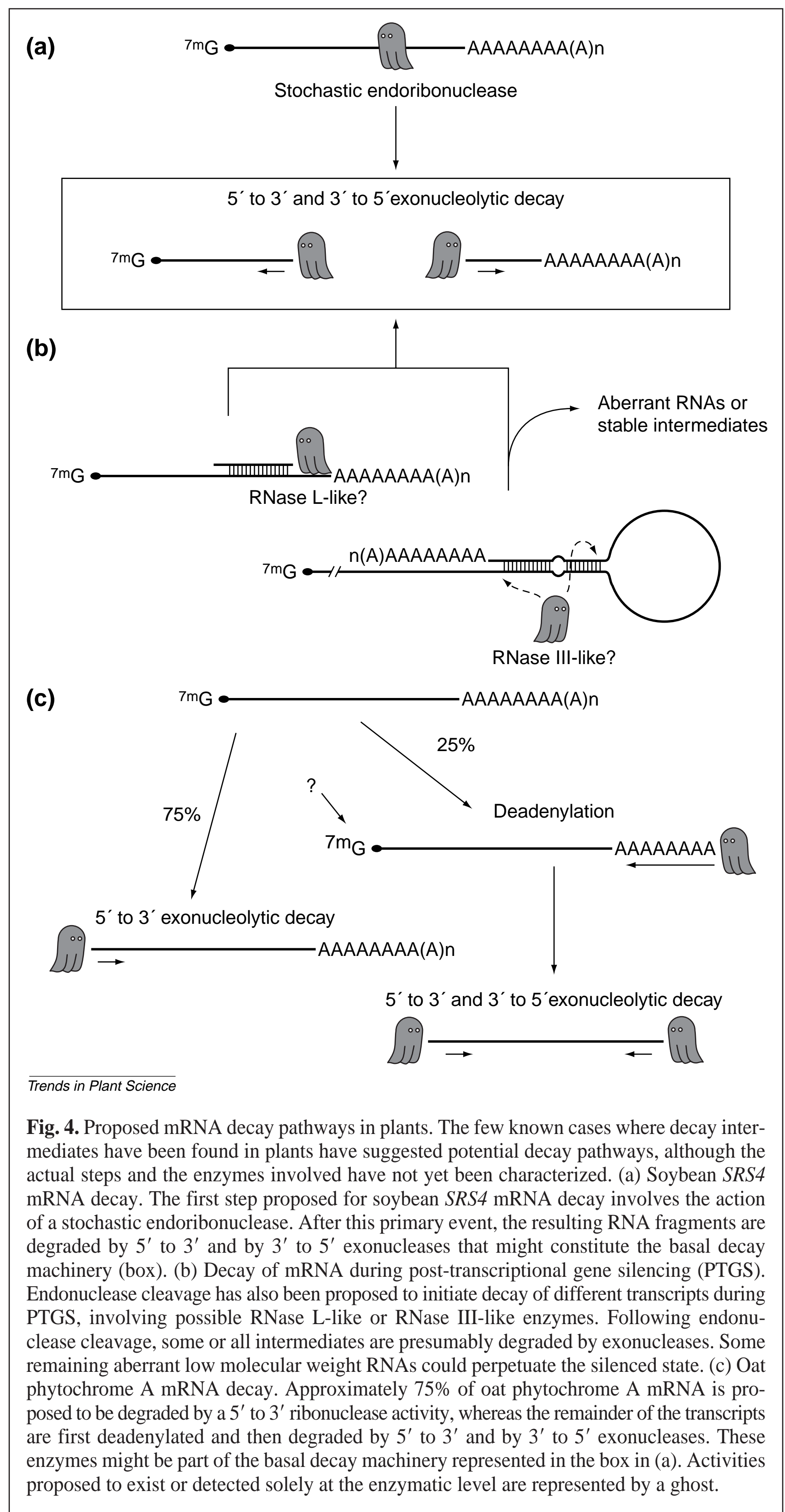

Fig. 4. Proposed mRNA decay pathways in plants. The few known cases where decay intermediates have been found in plants have suggested potential decay pathways, although the actual steps and the enzymes involved have not yet been characterized. (a) Soybean *SRS4* mRNA decay. The first step proposed for soybean *SRS4* mRNA decay involves the action of a stochastic endoribonuclease. After this primary event, the resulting RNA fragments are degraded by 5′ to 3′ and by 3′ to 5′ exonucleases that might constitute the basal decay machinery (box). (b) Decay of mRNA during post-transcriptional gene silencing (PTGS). Endonuclease cleavage has also been proposed to initiate decay of different transcripts during PTGS, involving possible RNase L-like or RNase III-like enzymes. Following endonuclease cleavage, some or all intermediates are presumably degraded by exonucleases. Some remaining aberrant low molecular weight RNAs could perpetuate the silenced state. (c) Oat phytochrome A mRNA decay. Approximately 75% of oat phytochrome A mRNA is proposed to be degraded by a 5′ to 3′ ribonuclease activity, whereas the remainder of the transcripts are first deadenylated and then degraded by 5′ to 3′ and by 3′ to 5′ exonucleases. These enzymes might be part of the basal decay machinery represented in the box in (a). Activities proposed to exist or detected solely at the enzymatic level are represented by a ghost.

RNA decay intermediates during post-transcriptional gene silencing

Recently, other insights into the mechanistic nature of mRNA decay pathways in plants have been obtained from studies of gene silencing. Introducing transgenes or viral genes into plants can

cause inactivation of the introduced gene at the mRNA level, as well as of the endogenous genes with sequence homology to the introduced gene. This inactivation can occur transcriptionally or post-transcriptionally (reviewed in Refs 3,57–59). Post-transcriptional gene silencing (PTGS) has been assumed to involve mRNA degradation because nuclear run-on experiments indicate that the synthesis of the silenced transcript cannot explain the severely diminished mRNA levels that are observed. Recently, a direct demonstration that PTGS is associated with decreased stability of the silenced transcript has been reported for chitinase and β-1,3-glucanase genes in tobacco[60].

Several PTGS models not only predict accelerated degradation of the full-length mRNA, but also the appearance of aberrant low molecular weight (LMW) RNA fragments that are often observed. The nature of these LMW RNA fragments has been carefully analyzed in the case of the tobacco etch virus coat protein (TEV-CP) transcript (*2RC*) PTGS (Ref. 61). The LMW RNAs correspond to 5′ and 3′ fragments of *2RC* that are generated by endonucleolytic cleavage similar to the mechanism proposed for *SRS4* (Fig. 4). Endonuclease cleavage is independent of poly(A) tail shortening, as is the case for *SRS4* degradation. The fact that *2RC* LMW RNAs are enriched in the polysomal fraction might suggest that the endonucleolytic activity is associated with polysomes, and acts after ribosomes are loaded, although translation is not necessary because CHX treatment does not affect the appearance of the LMW RNAs (Ref. 61). Data indicating an association between initiation of translation and silencing-associated mRNA degradation has also been obtained in the study of ACC synthase PTGS in tomato[62], and polysome-associated ribonuclease activities have been reported in plants (reviewed in Ref. 63). However, recent work shows that verrucarin A, a translational inhibitor that dissociates mRNA from polysomes, does not affect the decay rate of silenced chitinase transcripts in tobacco plants[60]. This suggests that the association between mRNA decay intermediates and polysomes might not be of functional significance, at least for some PTGS-associated mRNA degradation processes.

A degradation mechanism, similar to that which occurs during *2RC* PTGS, has been proposed in the PTGS of the β-1,3-glucanase gene, *gn1* (Ref. 64). Degradation intermediates of the full-length *gn1* mRNA have been cloned and analyzed. The results are consistent with fragments being produced by an endonucleolytic cleavage followed by exonucleolytic degradation of the unprotected ends. The degradation mechanism of this transcript is also independent of deadenylation. The presence of specific RNA fragments in silenced plants, as well as of fragments that are common to silenced and non-silenced plants, are observed in *gn1* (Ref. 64) as well as *2RC* PTGS (Ref. 61). This implies that multiple RNA degradation pathways are acting simultaneously in tobacco cells, and that during PTGS the partitioning of the silenced transcripts among these pathways is shifted in favor of a more active, silencing-associated (not necessarily silencing-specific) pathway[64].

Endonucleolytic cleavage of mRNA at specific sites occurs during PTGS of chalcone synthase (*chsA*) in *Petunia*. An attractive cleavage model has been proposed based on the presence of internal complementarity within *chsA* mRNA, cleavage site position and other data[65]. In this model (Fig. 4), intra- or intermolecular RNA–RNA pairing between complementary sequences triggers RNase III-like enzymes to cleave the *chsA* transcript at specific sites. Following the initial cleavage events, this model predicts that some of the resulting RNA fragments will be degraded by the general degradation machinery, whereas others, in particular a 304-base fragment, which spans the 3′ end of the coding region and part of the 3′ UTR, accumulates in silenced tissues[65]. The resistant fragments have the potential to perpetuate the silenced state because they can base pair with full-length *chsA* transcripts in a manner that could make them substrates for cleavage. Recently, direct evidence that dsRNA could trigger gene silencing, presumably by inducing RNA degradation, has been obtained from experiments involving transgenic tobacco and rice plants. Homologous gene expression is inhibited most effectively in plants that are transformed with a construct that produces self-complementary (panhandle) transcripts or in plants that produce both sense and antisense transcripts following a cross of appropriate transgenics[66]. A model involving the action of an RNase L-like enzyme has been proposed[66] (Fig. 4) because, in mammals, RNase L can be artificially recruited to a target site by fusing its cofactor to an oligonucleotide complementary to the target[67]. What would recruit RNase L to sense and antisense hybrids in plants is unclear.

Mechanistic implications and future prospects

The mRNA degradation intermediates observed during PTGS, as well as most of those derived from *SRS4* and *PHYA* mRNAs, are certainly suggestive of differences between major yeast and plant mRNA decay mechanisms. However, to date it is unclear whether these intermediates are generated by a mechanism common to the majority of plant transcripts, or if they represent exceptions derived from minor mechanisms that act on specific transcripts. Accordingly, one can envision at least two scenarios that can reconcile the structure of these intermediates with the existing data.

Perhaps the simplest model would be to postulate that the primary events that trigger transcript decay are different in yeast and plants. In yeast, the primary event is deadenylation followed by decapping. Instead, plants might initiate decay using mainly endonucleases (Fig. 4) or a decapping enzyme (Fig. 4). Once the decay is initiated, Xrn1-like exonucleases and exosome-like complexes could finish the process. This type of model would explain most of the intermediates that accumulate for *SRS4*, *PHYA* and during PTGS. It would also be consistent with the presence of potential homologs of yeast mRNase genes in plant genomes.

An alternative scenario would be that mRNA decay in plants is primarily initiated by deadenylation, as it is in yeast. According to this model, the decay intermediates characterized so far would have to be generated by secondary decay pathways, except for the 25% of *PHYA* mRNA that is deadenylated before being degraded[56] (Fig. 4). Because most yeast and plant transcripts are degraded without the accumulation of prominent intermediates, and the major decay pathway in yeast is deadenylation-dependent, it appears reasonable to envision that a similar major pathway operates in plants. This model is also consistent with the presence of potential homologs of yeast mRNases in plants. In both models, the lack of poly(G) intermediates in plants might be explained by helicases or other cellular factors that can resolve poly(G) secondary structures, or by plant Xrn-like activities that can progress through poly(G) tracts. Now that *Xrn*-like genes of plants have been identified, testing whether Xrn-like activities can progress through poly(G) tracts should be straightforward. Resolving whether or not deadenylation precedes decay for most plant mRNAs also needs to be addressed.

Regardless of which model most closely corresponds to the main mRNA decay pathway in plants, several observations indicate that multiple pathways operate simultaneously. The presence of a variety of prominent decay intermediates for some, but not most, plant mRNAs argues for this, as do the PTGS models involving RNase L-like or RNase III-like activities. Therefore, not only is there a need to identify the major and minor pathways, and the corresponding components of the decay machinery, but there is also a need to determine which mRNAs are degraded by which pathway. Inactivating the

genes for potential mRNases in *Arabidopsis* is one powerful approach for identifying the role of mRNA decay components. Analysis of the decay intermediates that accumulate in such mRNase 'knock-out' (KO) mutants should be instrumental in dissecting the steps in the corresponding decay pathways. Extending the analysis of KOs to include thousands of transcripts by using DNA microarray analysis promises to add another dimension. Because mRNAs degraded by a particular pathway are often elevated in the mutants that knock out that pathway, microarray analysis of the mutants could help categorize transcripts on the basis of their decay.

One of the most significant advances in the study of plant mRNA decay during the past several years is the identification of sequences that cause mRNA instability or that mediate differential regulation of mRNA stability (Fig. 2). The next important step will be to resolve the connections between these sequences and the components of the decay machinery. These connections probably occur via proteins that either bind to or cleave the sequences in question. Some RNA-binding proteins might simply accelerate or block the rate-limiting step of the major decay pathway, be it deadenylation, decapping or endonuclease cleavage. Alternatively, a sequence element could create a site for a new endoribonuclease, or target a transcript for recognition by an alternative decay pathway. Biochemical analysis of the cellular proteins that bind these sequence elements (e.g. PVPRP-BP) is just beginning. In addition, there is tremendous potential for identifying new plant proteins involved in the sequence-specific recognition of transcripts for rapid or regulated decay using genetic approaches.

With respect to the signal transduction pathways that convert internal and external stimuli (e.g. light, hormones and stress) into changes in mRNA stability, one might expect them to include many of the same components that regulate transcription. Indeed, examples where kinases or phosphatases are implicated in the regulation of mRNA stability are already emerging. It remains to be determined whether novel signaling pathways have evolved specifically for the control of mRNA stability. In addition, nuclear– cytoplasmic communications have the potential to be a particularly dynamic and interesting area of study with regard to how mRNA stability is regulated on all levels. Although mRNA decay is primarily assumed to take place in the cytoplasm, the contribution of nuclear RNA decay mechanisms is poorly understood. Further, tagging of mRNAs for rapid or regulated decay in the cytoplasm might occur in the nucleus by binding proteins that remain associated with the transcript following its transport through the nuclear pore. Future studies that investigate the control of mRNA stability from a cell biological perspective should be particularly exciting.

Acknowledgements
We thank James Kastenmayer and Mark Johnson for critical comments on the manuscript. We regret that most of the primary literature could not be cited because of space limitations. Work in our laboratory was funded by grants from the USDA (9801498), NSF (IBN9408052), DOE (FG0291-ER200210) and Michigan State University Research Excellence Fund to PJG.

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