RNA Degradation in *Saccharomyces cerevisae*

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ABSTRACT All RNA species in yeast cells are subject to turnover. Work over the past 20 years has defined degradation mechanisms for messenger RNAs, transfer RNAs, ribosomal RNAs, and noncoding RNAs. In addition, numerous quality control mechanisms that target aberrant RNAs have been identified. Generally, each decay mechanism contains factors that funnel RNA substrates to abundant exo- and/or endonucleases. Key issues for future work include determining the mechanisms that control the specificity of RNA degradation and how RNA degradation processes interact with translation, RNA transport, and other cellular processes.

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ALL RNA species in eukaryotic cells are subject to turnover, which plays several roles in yeast cells. First, the differential degradation of messenger RNA (mRNAs) can play an important role in setting the basal level of mRNA expression and how that mRNA level is modulated by environmental stimuli. Second, numerous quality control systems degrade aberrant transfer RNA (tRNAs) and ribosomal RNA (rRNAs), as well as aberrant mRNAs, which might otherwise encode a defective protein product. Third, RNA degradation removes the by-products of gene expression, including excised introns and other RNA pieces released during RNA processing. Finally, RNA degradation mechanisms functions in removing intergenic, intragenic, promoter-associated, and antisense RNAs that arise either as regulatory RNAs or transcriptional noise.

Here I review our understanding of the pathways and nucleases of RNA turnover by considering the different classes of RNAs and how they are degraded. Three common themes emerge from this review. First, most RNA degradation mechanisms funnel RNAs to the cytoplasmic Xrn1 or nuclear Rat1 5′ to 3′ nucleases, or to the exosome, which is a conserved cytoplasmic and nuclear complex with both 3′ to 5′ exonuclease activities and an endonuclease cleavage site. Second, where examined, all RNAs are subject to quality control systems where nonfunctional RNAs are more rapidly degraded. Third, the RNA pathways are modulated by environmental inputs and interact with other cellular processes including translation, RNA processing, transcription, and stress responses.

Degradation of mRNA

Cytoplasmic turnover of mRNA

Cytoplasmic degradation of yeast mRNAs occurs by two general pathways, both of which are initiated by shortening of the 3′ poly(A) tail in a process referred to as deadenylation (Muhlrad and Parker 1992; Decker and Parker1993) (Figure 1A). Deadenylation in yeast is carried out by the Pan2/Pan3 complex as well as the by Ccr4/Pop2/Not complex (Brown and Sachs 1998; Tucker *et al.* 2001). Following deadenylation, mRNAs can be subjected to 3′ to 5′ degradation by the exosome (Anderson and Parker 1998). More commonly, mRNAs are decapped by the Dcp1/Dcp2 decapping enzyme and then subjected to 5′ to 3′ degradation by Xrn1 (Hsu and Stevens 1993; Muhlrad *et al.* 1994, 1995; Dunckley and Parker 1999; Van Dijk *et al.* 2002; Steiger *et al.* 2003).

Yeast also contain specialized mRNA decay pathways that act in response to aberrancies in translation (Figure 1B). In these cases, mRNAs can be subject to either deadenylation independent decapping (Muhlrad and Parker 1994), rapid 3′ to 5′ degradation (Van Hoof *et al.* 2002), or endonuclease cleavage (Doma and Parker 2006). The available evidence suggests that these specialized mechanisms function primarily on aberrant mRNAs, although the nonsense-mediated decay (NMD) pathway does degrade a pool of "normal" mRNAs (see section on *mRNA Quality Control Pathways*).

Several observations suggest that, at least during mid-log growth phase, decapping is the predominant pathway of mRNA degradation. First, strains lacking the decapping enzyme grow very slowly or are lethal in some strain backgrounds (Beelman *et al.* 1996; Dunckley and Parker 1999; Giaever *et al.* 2002), while strains defective in cytoplasmic 3′ to 5′ mRNA degradation grow relatively normally (Anderson and Parker 1998; Giaever *et al.* 2002). Second, strains defective in decapping or 5′ to 3′ degradation show changes in both the steady-state levels and decay rates of many mRNAs (Beelman *et al.* 1996; He *et al.* 2003; Van Dijk *et al.* 2011). Finally, the genome-wide mapping of endonuclease sites in mRNAs has revealed that few yeast mRNAs are subject to endonucleolytic degradation (Y. Harigaya and

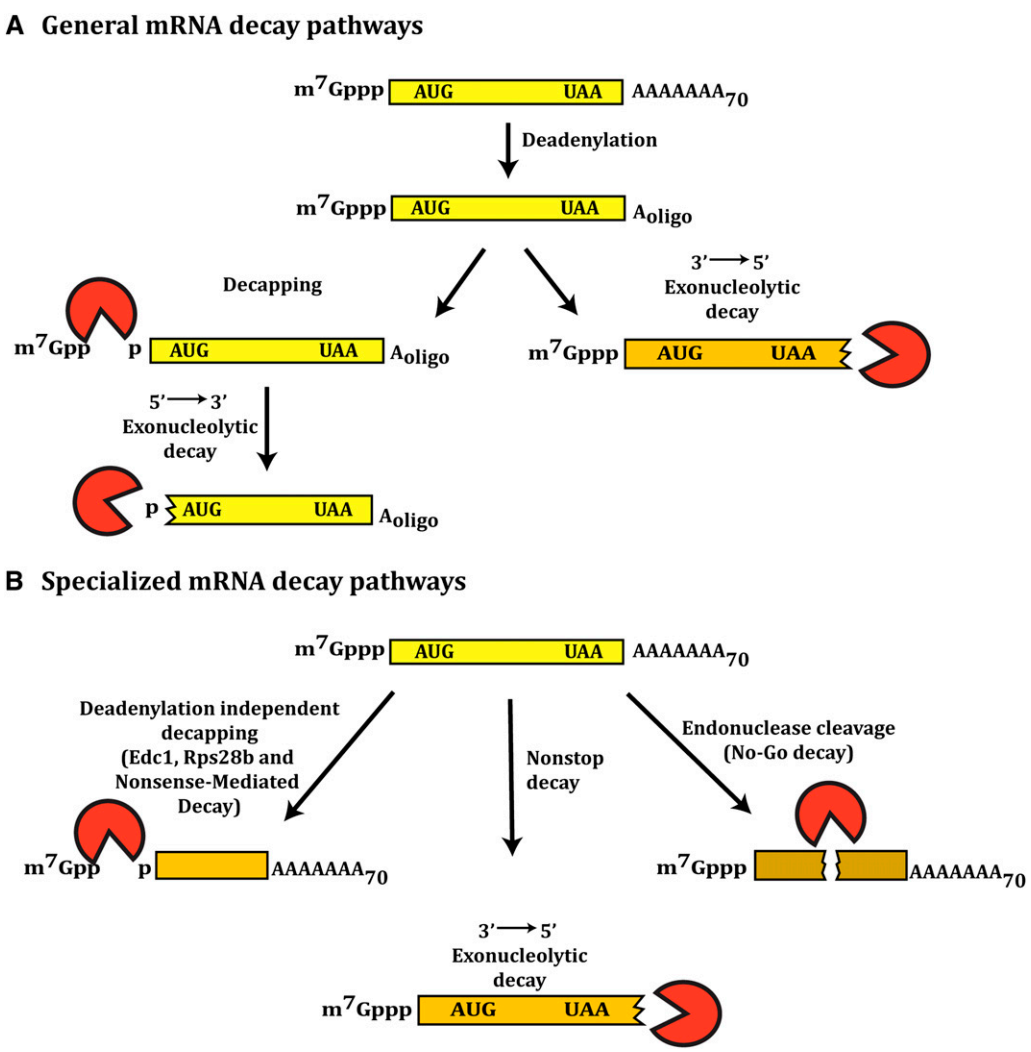

Figure 1 (A) General mRNA decay pathways. (B) Specialized mRNA decay pathways.

R. Parker, unpublished data). However, it should be noted that 3′ to 5′ degradation of mRNAs is just slightly slower than decapping. For example, for the PGK1 and MFA2 mRNAs, computational analysis of experimental data has indicated that 3′ to 5′ decay is 1.5 and 6 times slower than decapping, respectively (Cao and Parker 2001).

As assessed by a variety of different methods (Passos and Parker 2008; Munchel *et al.* 2011), the degradation rates of individual mRNAs can vary by over an order of magnitude. This was first observed in decay rate measurements of groups of mRNAs (Herrick *et al.* 1990; Brown and Sachs 1998) and has now been confirmed by multiple genome-wide studies of mRNA decay rates (Wang *et al.* 2002; Grigull *et al.* 2004; Munchel *et al.* 2011). The decay rates of mRNAs are somewhat clustered by the function of the encoded protein (Herrick *et al.* 1990; Grigull *et al.* 2004; Wang *et al.* 2006; Beilharz and Preiss 2007). Differences in the decay rates of individual mRNAs can arise by differences in deadenylation rates, decapping rates, or the rates of 3′ to 5′ degradation (Cao and Parker 2001; Beilharz and Preiss 2007). For example, the MFA2 mRNA ($t_{1/2}$ = 3′–4′) differs from the relatively stable PGK1 mRNA ($t_{1/2}$ = 30′–45′) by having faster rates of deadenylation (15 adenosines/min compared to 3 adenosines/min), decapping (0.0077 sec^{-1} compared to 0.000462 sec^{-1}) and 3′ to 5′ degradation (0.0012 sec^{-1} compared to 0.0003 sec^{-1}) (Cao and Parker 2001). Thus, to understand differential control of mRNA decay rates, one must consider the processes of deadenylation, decapping, and 3′ to 5′ degradation and how they are regulated.

Deadenylation

Two enzyme complexes catalyze poly(A) shortening (Table 1). The predominant deadenylase is the Ccr4/Pop2/Not complex (Daugeron *et al.* 2001; Tucker *et al.* 2001). This large complex consists of two active 3′ to 5′ exonucleases (Ccr4 and Pop2/Caf1) and includes the Not1, Not2, Not3, Not4, Not5, Caf40, and Caf130 proteins (Denis and Chen 2003). In yeast, at least during mid-log growth, the major deadenylase in this complex is the Ccr4 protein, a member of the ExoIII nuclease family, since mutations in the active site of this enzyme give defects in deadenylation similar to the ccr4Δ strain (Chen *et al.* 2002; Tucker *et al.* 2002). Ccr4 also interacts directly with the Pop2 protein through a leucine-rich-repeat region (Clark *et al.* 2004).

Table 1 General factors involved in mRNA deadenylation

Factor	Function	References
Ccr4/Pop2/Not complex	Major mRNA deadenylase Ccr4 critical catalytic subunit, ExoIII family member Pop2: second catalytic subunit, RNaseD family member Not1: large scaffolding protein Not2–5, Caf130, Caf40: accessory proteins of unknown function	Daugeron *et al.* (2001); Tucker *et al.* (2001, 2002); Chen *et al.* (2002)
Pan2/Pan3 complex	Additional mRNA deadenylase Primarily functions in initial trimming of poly(A) tail Pan2: catalytic subunit; RNaseD family member Interacts with and stimulated by Pab1 Pan3: regulatory subunit	Brown *et al.* (1996); Boeck *et al.* (1996); Brown and Sachs (1998)
Tpa1	Prolyl 4-hydroxylase Binds poly(A) Interacts with eRF1 and eRF3 Required for normal deadenylation and translation termination	Keeling *et al.* (2006); Henri *et al.* (2010); Kim *et al.* (2010)
Pab1	Major poly(A)-binding protein Inhibits Ccr4 deadenylase Stimulates Pan2/Pan3 complex May interact with eRF3 to affect deadenylation Couples deadenylation to decapping	Caponigro and Parker (1995); Boeck *et al.* (1996); Cosson *et al.* (2002); Tucker *et al.* (2002); Hosada *et al.* (2003)
eRF3 (Sup35)	Subunit of translation termination complex Interacts with Pab1 and thereby may influence deadenylation rates	Hosada *et al.* (2003); Funakoshi *et al.* (2007)
Rpb4/Rpb7	Two subunits of RNA polymerase II Required for normal rates of deadenylation May exit from nucleus as part of mRNP to affect cytoplasmic deadenylation	Lotan *et al.* (2005, 2007)

The Pop2/Caf1 protein, a member of the RNaseD family, is a second exonuclease in the Ccr4/Pop2/Not complex (Thore *et al.* 2003). Despite the presence of noncanonical residues in its active site, one report describes Pop2 purified from bacteria as having 3′ exonuclease *in vitro* (Thore *et al.* 2003). However, all of the catalytic activity of Ccr4/Pop2 complexes purified from yeast is dependent on the Ccr4 active site, suggesting that Ccr4 is the critical active deadenylase (Goldstrohm *et al.* 2007). Strains lacking Pop2/Caf1 show a defect in deadenylation, but this is due to Pop2 interacting with and promoting Ccr4 function since mutations in the active site of Pop2 do not alter deadenylation of reporter mRNAs and overexpression of Ccr4 suppresses the deadenylation defects seen in a pop2Δ strain (Tucker *et al.* 2002; Viswanathan *et al.* 2004). Since Pop2/Caf1 has catalytic activity and Pop2 orthologs in other organisms play catalytic roles in deadenylation (Goldstrohm and Wickens 2008), it remains a formal possibility that Pop2 may function as a deadenylase under some conditions or for some mRNAs.

The roles of the Not, Caf40, and Caf130 proteins in deadenylation are not yet clear. One possibility is that they adapt the deadenylase complex to different mRNAs through the action of regulatory proteins. Consistent with this possibility, defects in some of the Not proteins can affect deadenylation of specific mRNAs (Tucker *et al.* 2002). An alternative is that the Not and Caf accessory proteins play roles in other functions of the Ccr4/Pop2/Not complex, which has been suggested to have roles in transcription initiation and elongation (Deluen *et al.* 2002; Swanson *et al.* 2003; Qiu *et al.* 2004; Kruk *et al.* 2011).

A second deadenylase complex consists of the Pan2 and Pan3 proteins, with Pan2, a RNaseD family member, being the catalytic subunit (Boeck *et al.* 1996). Pan2 and Ccr4 appear to be the only major deadenylases since pan2Δ ccr4Δ strains are slow growing and show no deadenylation of reporter mRNAs (Tucker *et al.* 2001). The activity of Pan2 is promoted by Pab1 (Boeck *et al.* 1996), while Pab1 appears to inhibit the action of the Ccr4 complex (Tucker *et al.* 2002). This suggests that mRNAs with Pab1 bound to the poly(A) tail will be resistant to deadenylation by Ccr4/Pop2 but will be substrates for Pan2/Pan3. Thus, the specific deadenylase active on an mRNA will be influenced by the nature of the protein complex on its 3′ poly(A) tail.

The Pan2/Pan3 and Ccr4/Pop2/Not complexes appear to function in a temporal manner on most mRNAs with Pan2/Pan3 first acting to shorten the nascent poly(A) tail from ~90 residues to ~65, although this can vary a bit between different mRNAs (Brown and Sachs 1998). This step appears to happen quickly since the poly(A) tail lengths longer than 70 residues are typically not observed in yeast cells unless Pan2 is inactive (Brown and Sachs 1998). This implies that there is a difference between the accessibility of the 0–65 A residues of the poly(A) tail and the 3′-most 25 nucleotides. Since Pab1 promotes Pan2 activity, one model is that this deadenylation reflects Pab1 bound to the first ~65

residues of the A tail, but the 3′ most region is exposed and thereby rapidly deadenylated by Pan2. Since pan2Δ strains show relatively normal deadenylation of reporter mRNAs (Tucker *et al.* 2001), Ccr4 then appears to be responsible for the continued deadenylation of the mRNA. Since the Ccr4 complex is inhibited by Pab1 (Tucker *et al.* 2002), this phase of deadenylation implies that Pab1 is at least partially dissociating from the poly(A) tail. However, in strains lacking Ccr4 activity, the Pan2 complex can continue to deadenylate mRNAs, although at a slower rate than Ccr4 (Tucker *et al.* 2001). Interestingly, the Pan2 complex stalls at an A tail of ~20–25 residues (Daugeron *et al.* 2001; Tucker *et al.* 2001), which might be a length at which the Pab1 can no longer associate with the mRNA, and therefore Pan2 activity becomes limited (Tucker *et al.* 2001). Interestingly, once the poly(A) tail reaches an oligo(A) length of 10–12 residues, the mRNA can become a substrate for decapping and for binding of the Pat1/Lsm1–7 complex at the 3′ end (Tharun and Parker 2001; Chowdhury *et al.* 2007), which enhances the rate of decapping. This exchange of the Pab1 protein for the Pat1/Lsm1–7 complex is part of the mechanism that allows decapping to be promoted following deadenylation (see below).

Control of deadenylation: Three types of interactions are known to modulate deadenylation rate, either generally or on specific mRNAs. First, because the Ccr4 and Pan2 deadenylases are influenced by the binding of Pab1 to the poly (A) tail, the rate of deadenylation is influenced by features of the Pab1–poly(A) interaction and its dynamics. Because Pab1–mRNA interactions are influenced by translation *per se*, this leads to deadenylation being coupled to aspects of translation. Second, key regulators of deadenylation on specific mRNAs are sequence-specific binding proteins that either directly, or indirectly, recruit the deadenylases to the mRNA to accelerate deadenylation. Finally, deadenylation is regulated in response to environmental cues, including stress and nutrient limitations.

On the basis of the biochemical analyses of deadenylases, a working model for understanding how deadenylation is affected by mRNP dynamics is that when Pab1 is present on the poly(A) tail, the Ccr4 deadenylase is inhibited and Pan2 is stimulated, whereas, when Pab1 dissociates, Ccr4 deadenylation is accelerated and Pan2 deadenylation is inhibited. Consistent with this view, self-association of Pab1 limits its binding to poly(A) and increases Ccr4-dependent deadenylation (Simon and Seraphin 2007; Yao *et al.* 2007). Moreover, strains defective in Pab1 show a defect in the initial rapid Pan2-dependent poly(A) shortening (Caponigro and Parker 1995; Morrissey *et al.* 1999; Simon and Seraphin 2007).

This model suggests that some of the effects of translation on deadenylation can be understood by their effects on Pab1 binding the poly(A) tail. For example, defects in translation initiation caused by a poor AUG context, a stem loop in the 5′ UTR, or mutations in translation initiation factors can increase the rates of deadenylation of yeast mRNAs (Muhlrad *et al.* 1995; Lagrandeur and Parker 1999; Schwartz and Parker 1999). One possibility is that defects in translation initiation either directly or indirectly destabilize Pab1 binding the poly(A) tail. Note that this model also predicts that Pan2-mediated deadenylation would be compromised by decreases in translation initiation. Surprisingly, in temperature-sensitive eIF4E strains, deadenylation of the Gal1 mRNA increases even in a *ccr4*Δ strain, which has been interpreted to suggest that eIF4E can also inhibit Pan2-based deadenylation (Lee *et al.* 2010). However, another possibility is that the "deadenylation" seen in a *cdc33-1 ccr4*Δ strain is not due to Pan2, but may be due to the cytoplasmic exosome (or to an unknown additional deadenylases), which is suggested by the observation that deadenylation is restored in a *ccr4*Δ *pan2*Δ strain when pab1 is mutated (M. Tucker and R. Parker, unpublished observation).

Deadenylation is also affected by aspects of translation termination. For example, premature translation termination accelerates poly(A) shortening as part of the process of NMD (see below and Cao and Parker 2003; Mitchell *et al.* 2003). This accelerated deadenylation may be a consequence of NMD leading to repression of translation and/or to dissociation of Pab1 from the mRNA, since decapping triggered by NMD is independent of the poly(A) tail (Muhlrad and Parker 1994). Similarly, the Tpa1 protein, a proline hydroxylase that binds poly(A) and interacts with translation termination factors, can influence the rate of deadenylation (Keeling *et al.* 2006; Henri *et al.* 2010).

The coupling of translation termination to deadenylation has been suggested to occur through direct interactions of the translation termination factor eRF3 with Pab1 (Cosson *et al.* 2002). This interaction appears to influence mRNA deadenylation since overexpression or deletion of the N-terminal domain of eRF3, where Pab1 interacts, leads to defects in deadenylation and mRNA decay (Kobayashi *et al.* 2004; Funakoshi *et al.* 2007). Since this effect seems to be primarily on the Ccr4 deadenylase (Funakoshi *et al.* 2007), one possibility is that eRF3–Pab1 interactions during translation termination transiently dissociate Pab1 from the poly (A) tail and increase deadenylation. However, it is important to note that translation termination is not required for deadenylation since mRNAs that never initiate translation due to stem-loop structures in their 5′ UTRs still deadenylate rapidly (Beelman and Parker 1994; Muhlrad *et al.* 1995).

Deadenylation also appears to be coupled to the process of transcription. The Rpb4 and Rpb7 subunits of RNA polymerase II are required for optimal deadenylation rates of yeast mRNAs (Lotan *et al.* 2005, 2007), and this has been proposed to occur by Rpb4 and Rbp7 loading on the mRNA during transcription to regulate cytoplasmic function (see *Future Perspectives*).

Control of deadenylation by mRNA-specific features: There are now several examples of specific proteins that bind mRNAs in a sequence-specific manner to control deadenylation. Moreover, in many other cases, 3′ UTR elements

modulate the poly(A) tail length, presumably by deadenylation, and identify a broad role of deadenylation regulation in gene expression (Beilharz and Preiss 2007). For example, the six members of the Puf protein family bind to specific-sequence 3′ UTR elements and regulate ~10% of the yeast transcriptome (Olivas and Parker 2000; Gerber *et al.* 2004; Yosefzon *et al.* 2011). In yeast, the Puf1, Puf3, Puf4, and Puf5 proteins have all been shown to promote deadenylation and degradation of specific subsets of yeast mRNAs (Olivas and Parker 2000; Tadauchi *et al.* 2001; Hook *et al.* 2007; Ulbricht and Olivas 2008). Mechanistic studies have demonstrated that Puf5 promotes deadenylation at least in part by direct interaction with Pop2 and thereby recruitment of the Ccr4 deadenylase (Goldstrohm *et al.* 2006, 2007), although Puf proteins may also recruit the deadenylase complexes through other interactions. In addition, Puf proteins can also repress translation independently of deadenylation and therefore might also promote deadenylation indirectly (Chritton and Wickens 2011).

Other sequence-specific regulators of deadenylation include the Vts1 protein, which binds to a subset of yeast mRNAs through a specific stem-loop structure (Aviv *et al.* 2006) and recruits the Ccr4/Pop2 deadenylase (Rendl *et al.* 2008). Similarly, the Cth1 and Cth2 proteins are zinc-finger RNA-binding proteins that regulate the deadenylation of a subset of mRNAs, perhaps through interactions with Dhh1 that interacts with Pop2 (Puig *et al.* 2005; Pedro-Segura *et al.* 2008). One anticipates that a growing set of mRNA-specific binding proteins will regulate deadenylation either by direct recruitment of the deadenylase complexes or by inhibiting translation initiation and thereby indirectly promoting deadenylation.

Environmental control of deadenylation: Deadenylation is also regulated on a global scale in response to environmental cues. For example, a variety of different stresses lead to a general inhibition of both Ccr4 and Pan2 deadenylation (Hilgers *et al.* 2006). Similar results occur in mammalian cells, suggesting that inhibition of deadenylation is a conserved aspect of the stress response (Gowrishankar *et al.* 2005, 2006). Inhibition of deadenylation during stress does not seem to require mRNAs to assemble in stress granules or P-bodies (see below), since deadenylation is still inhibited by stress in the presence of cyclohexmide (Hilgers *et al.* 2006), which prevents the formation of stress granules and P-bodies (Sheth and Parker 2003; Buchan *et al.* 2008). Deadenylation is also inhibited during stress. Since the stress response often leads to a global decrease in translation initiation, a general inhibition of deadenylation might be required to maintain a stable population of mRNAs. Deadenylation can also be reduced for some mRNAs when Hsp70 function is altered, which might mimic a stress response, although the basis or generality of this effect has not been determined (Duttagupta *et al.* 2003).

Normal rates of deadenylation also appear to be dependent on the activity of the Pkh1 and Pkh2 kinases, which are activated by sphingolipids (Luo *et al.* 2011). This suggests that aspects of mRNA metabolism are modulated in response to lipid signaling. This interpretation is also supported by the observation that, during heat stress, the formation of P-bodies, which are cytoplasmic mRNP aggregates of untranslating mRNAs, in conjunction with the mRNA decapping machinery (see below), requires sphingolipid synthesis, and exogenous phytosphingosine can stimulate P-body formation (Cowart *et al.* 2010). Interestingly, the effect of Pkh1 and Pkh2 on deadenylation rates is observed only in synthetic media, suggesting that this regulation is an integrative readout of both lipid and nutrient availability (Luo *et al.* 2011). An important area of future work will be to understand how deadenylation is regulated both globally and on specific mRNAs in response to environmental cues.

mRNA decapping

Nucleases of decapping and 5′ to 3′ degradation: mRNA decapping is carried out by a complex of the Dcp1 and Dcp2 proteins and is influenced by several other factors (Table 2). Dcp2 is the catalytic subunit and is a member of the Nudix family of pyrophosphatases (Van Dijk *et al.* 2002; Steiger *et al.* 2003). Dcp2 cleaves the cap structure to release m7GDP and a 5′ monophosphate mRNA (She *et al.* 2008). Dcp1 is an EVH family protein (She *et al.* 2004) that interacts with Dcp2 to promote its catalytic activity (Deshmukh *et al.* 2008; She *et al.* 2008). The first 300 amino acids of Dcp2 are sufficient to promote decapping (Dunckley and Parker 1999) and fold into a two-domain structure wherein the N-terminal domain interacts with Dcp1 and the Nudix domain is present in the C-terminal domain (residues 100–245) (She *et al.* 2006, 2008). Dcp2 has a conserved region between 245 and 286, which contains binding sites for the Edc3 protein and possibly other decapping activators (Harigaya *et al.* 2010). Yeast Dcp2 has an extended C-terminal region that is not required for general mRNA decapping. Since Dcp2 can shuttle into the nucleus (Grousl *et al.* 2009) and this region has sites that can enhance transcription (Gaudon *et al.* 1999), one possibility is that this region plays some role in controlling transcription (Shalem *et al.* 2011).

Dcp2's catalytic mechanism is a typical Nudix family reaction wherein Mg++ ions coordinated by a set of glutamic acid residues promote catalysis (Dunckley and Parker 1999; Steiger *et al.* 2003; She *et al.* 2006). Dcp2 catalysis is promoted by the closing of the bi-lobed Dcp2 structure to create a more active enzyme and more stable substrate binding (Deshmukh *et al.* 2008; She *et al.* 2008; Floor *et al.* 2010). Dcp1 is thought to enhance decapping by promoting the formation of this closed and more active structure (Deshmukh *et al.* 2008; She *et al.* 2008). The Dcp1/Dcp2 holoenzyme or Dcp2 alone prefer longer mRNA substrates *in vitro*, which is consistent with Dcp2 containing an extended RNA-binding site and having a reaction mechanism that consists of an initial binding to the substrate followed by sliding to the cap structure (Steiger *et al.* 2003). However,

the presence of structures near the 5′ end is unlikely to inhibit decapping *in vivo* since even mRNAs with poly(G) tracts very near their 5′ end undergo rapid decapping *in vivo* (Muhlrad *et al.* 1994, 1995), presumably because Dcp2 catalysis is not generally rate limiting for decapping *in vivo* (see below).

Yeast cells contain additional decapping enzymes. The Dcs1 and Dcs2 proteins are members of the HIT family of pyrophosphatases and *in vitro* appear to cleave short RNA substrates (Liu *et al.* 2002). One function for Dcs1 in yeast is to cleave the m7GDP produced by decapping to m7GMP (Van Dijk *et al.* 2003), although how the m7GMP is further recycled is not known. Dcs2 can form heterodimers with Dcs1 and inhibit its activity, which occurs as cells enter diauxie (Malys and McCarthy 2006), although the significance of this effect is not clear. The nuclear Rai1 protein is known to function as a endonuclease that can cleave near the 5′ ends of mRNAs, and this has been suggested to function as a quality control mechanism for mRNA capping (Jiao *et al.* 2010).

Following decapping, mRNAs are degraded in a 5′ to 3′ direction by the Xrn1 nuclease (Hsu and Stevens 1993; Muhlrad *et al.* 1994), which prefers mRNA substrates with a 5′ monophosphate (Stevens 2001). Xrn1 has two highly conserved domains that fold into the active region of the enzyme, which is then stabilized by interactions with additional domains (Chang *et al.* 2011; Jinek *et al.* 2011). The active site of Xrn1 couples unwinding of duplexes to the processivity of the enzyme, which explains how it can degrade through structures without a helicase (Jinek *et al.* 2011). A paralog of Xrn1 is Rat1, which is typically localized to the nucleus and functions in nuclear RNA processing and/or degradation pathways (see below). However, Rat1 can substitute for Xrn1 when it is localized to the cytoplasm due to mutation, indicating that no Xrn1-specific protein–protein interactions are required for mRNA degradation (Johnson 1997). Xrn1 is inhibited by the adenosine 3′, 5′ biphosphate (pAp), which is produced by sulfate assimilation (Dichtl *et al.* 1997), and cells can utilize this circuit to limit Xrn1 activity during various responses (Benard 2004; Todeschini *et al.* 2006).

Model of mRNA decapping: A working model for mRNA decapping has three critical steps (Figure 2). First, the 5′ cap structure must be exposed, and therefore the cytoplasmic cap-binding complex consisting of eIF4E and eIF4G needs to be lost from the mRNA. Second, the decapping enzyme must be recruited to the mRNA, which appears to be coordinated with the formation of a larger decapping complex, including the decapping enzyme, Xrn1, and several decapping activators (see below). Third, catalysis by Dcp2 occurs, leading to rapid 5′ to 3′ degradation of the mRNA. Consistent with this model, proteins enhancing decapping can function by interfering with translation initiation factors, by binding RNA and forming scaffolds for assembly of the decapping machinery, or by promoting Dcp2 catalysis. Untranslating mRNPs complexed with the decapping machinery can also aggregate into cytoplasmic RNP granules referred to as P-bodies although the specific role of these macromolecular complexes is not yet clear (see below).

Decapping and translation initiation: Several observations argue that decapping is in competition with translation initiation and that decapping requires the loss of the cap-binding complex. This was first suggested since the cap structure, which is recognized for decapping, also functions in promoting translation initiation by recruiting the eIF4E/eIF4G translation initiation complex. Moreover, when translation initiation is decreased by mutations in translation initiation factors, a poor AUG context, or 5′ UTR structures, there is a concomitant increase in decapping rates (Muhlrad *et al.* 1995; Lagrandeur and Parker 1999; Schwartz and Parker 1999). In addition, the eIF4E cap-binding protein can directly inhibit decapping *in vitro* (Schwartz and Parker 2000). These observations argue that decapping requires the mRNP to exchange the eIF4E/eIF4G cap-binding complex for the decapping enzyme.

Stimulation of decapping by general activators: Several protein factors, referred to as either decapping enhancers or activators, are known to function to stimulate the rate of decapping *in vivo* (Table 2). The core set of proteins affecting decapping includes Dhh1, a DEAD-box helicase, Pat1, Edc1, Edc2, Edc3, Scd6, and the Lsm1–7 complex. Some of these decapping activators promote decapping by inhibiting translation initiation. For example, Dhh1, a member of the DEAD family of ATPases, represses translation *in vitro*, and its overexpression in cells inhibits translation and leads to the accumulation of cytoplasmic mRNP granules (Coller and Parker 2005; Swisher and Parker 2010; Carroll *et al.* 2011). Similarly, Pat1, Scd6, and Stm1, which affect the decapping of some mRNAs (Balagopal and Parker 2009), repress translation both *in vivo* and *in vitro* (Pilkington and Parker 2008; Nissan *et al.* 2010; Balagopal and Parker 2011; Rajyaguru *et al.* 2012).

Decapping activators can inhibit translation at different steps. For example, the Pat1, Dhh1, and Scd6 proteins all appear to block translation before the formation of a 48S pre-initiation complex (Coller and Parker 2005; Nissan *et al.* 2010). For Scd6, this translation repression appears to occur by direct binding to eIF4G and inhibition of the joining of the 43S complex (Rajyaguru *et al.* 2012). In contrast, the Stm1 protein, which promotes decapping of a subset of yeast mRNAs (Balagopal and Parker 2009), inhibits translation after formation of an 80S complex, likely through direct interactions with the ribosome (Balagopal and Parker 2011). An unresolved issue is how inhibition of translation initiation by these factors leads to decapping. One possibility is that, by stalling initiation, it gives more time for dissociation of the translation initiation factors to allow for decapping complexes to associate with the mRNA. Alternatively, such a transition may involve an ordered exchange of factors on the mRNA, which is suggested by decapping activators,

Table 2 Decapping and 5′ → 3′ exonuclease factors

Factor	Function	References
Dcp1/Dcp2	mRNA decapping enzyme Dcp2: catalytic subunit: Nudix family member Releases m7GDP and 5′p-RNA Dcp1: stimulatory subunit, Evh1/WH1 family member Blocked by eIF4E bound to cap	Schwartz and Parker (2000); She *et al.* (2004, 2008); Deshmukh *et al.* (2008)
Xrn1	Major cytoplasmic 5′ to 3′ exonuclease Processive and requires 5′ monophosphate Stimulated by Dcs1/Dcs2	Kenna *et al.* (1993); Poole and Stevens (1995); Van Dijk *et al.* (2003); Malys *et al.* (2004);
Dcs1 (DcpS)/Dcs2	mRNA decapping enzymes with preference for short RNAs Releases m7Gp and ppN− Cleaves m7GDP produced by Dcp1/Dcp2 to m7GMP and P Can affect stress responses	Liu and Kiledjian (2005); Jinek *et al.* (2011)
Rat1	Major nuclear 5′ to 3′ nuclease Paralog of Xrn1 Functions in nuclear RNA processing and decay	Johnson (1997); Xiang *et al.* (2009)
Rai1	Interacts with and stimulates Rat1 Contains mRNA cleavage site Releases m7GpppN− and N− May function in cap quality control	Xue *et al.* (2000); Xiang *et al.* (2009); Jiao *et al.* (2010)
Pat1	Activates general mRNA decapping Serves as scaffolding protein for decapping complexes Both represses translation initiation and stimulates Dcp2 Interacts with Lsm1–7 complex and prefers to bind 3′ end of oligoadenylated mRNA Promotes P-body assembly After deadenylation stabilizes 3′ ends to 3′ trimming Target of PKA kinase	Bouveret *et al.* (2000); Tharun *et al.* (2000); Chowdhury *et al.* (2007); Pilkington and Parker (2008); Nissan *et al.* (2010); Ramachandran *et al.* (2011)
Lsm1–7 complex	Required for efficient decapping Forms heptometric ring complex and binds oligo- or deadenylated mRNAs May promote Pat1 conformational change to activate Dcp2 After deadenylation stabilizes 3′ ends to 3′ trimming	Boeck *et al.* (1998); Bouveret *et al.* (2000); Tharun *et al.* (2000); Chowdhury *et al.* (2007)
Dhh1	Required for efficient decapping of translating mRNAs Member of ATP-dependent DExD/H box RNA helicase family Inhibits translation initiation *in vitro* upstream of 48S complex formation Accumulates in both stress granules and P-bodies Interacts with Dcp2, Pat1, Scd6, Edc3	Coller *et al.* (2001); Fischer and Weiss (2002); Coller and Parker (2005); Swisher and Parker (2009); Nissan *et al.* (2010)
Edc3	RNA-binding protein Binds and directly stimulates Dcp2 Plays major role in aggregation of P-bodies and serves as scaffold for decapping factors Not generally required for mRNA decapping unless Dcp1/Dcp2 is limited	Badis *et al.* (2004); Kshirsagar and Parker (2004); Decker *et al.* (2007); Dong *et al.* (2007); Harigaya *et al.* (2010)
Scd6	RNA-binding protein related to Edc3 Genetic interaction with Edc3 and synthetic decapping defect in *edc3*Δ *scd6*Δ Represses translation by binding eIF4G Interacts with Dhh1, Dcp2, Pat1 May be mRNA-specific decapping/translation regulatory factor	Decourty *et al.* (2008); Nissan *et al.* (2010); Rajyaguru *et al.* (2011)
Edc1/Edc2	Two small RNA-binding proteins Directly bind and stimulate Dcp1/Dcp2	Dunckley *et al.* 2001; Schwartz *et al.* (2003); Neef and Thiele (2009); Borja *et al.* (2011)
Stm1	Ribosome-binding protein Can stimulate Dhh1-dependent decapping Typically required only for subset of mRNAs decapping Stalls 80S complex after translation initiation	Balagopal and Parker (2009, 2011)
Sbp1	Abundant RNA-binding protein Overexpression suppresses *pat1*Δ defects by enhancing Dhh1 function Binds eIF4G to repress translation initiation	Segal *et al.* (2006); Rajyaguru *et al.* (2011)
Tif51A	Translation initiation factor eIF5A Specific mutations inhibit decapping Mechanism is not known	Zuk and Jacobson (1998)
Mrt4, Grc5, Sla2, Ths1	Additional proteins affecting mRNA turnover by unknown mechanism	Zuk *et al.* (1999)

such as Pat1 and Scd6, that can directly interact with translation factors and the decapping enzyme (Nissan *et al.* 2010; Rajyaguru *et al.* 2012). An important area for future research is determining how mRNPs are remodeled to allow decapping complexes to form and degrade the mRNA.

A second role of decapping activators is to promote the assembly of a larger decapping complex. The core set of decapping components shows an extensive network of direct interactions as determined by protein-binding experiments with recombinant proteins and supported by co-ip and two-hybrid analyses (Decker *et al.* 2007; Nissan *et al.* 2010). On the basis of coimmunoprecipitation (co-ip) experiments and the dependence of interactions on RNA, there appear to be two complexes that assemble on mRNAs targeted for decapping. One complex consists of the Pat1 protein, the Lsm1–7 complex, and Xrn1 (Bouveret *et al.* 2000; Tharun *et al.* 2000; Tharun and Parker 2001). This complex is thought to assemble on the 3′ end of deadenylated mRNAs on the basis of its binding specificity *in vitro* (Chowdhury *et al.* 2007) and the exonuclease trimming of the 3′ end of deadenylated mRNAs in *pat1*Δ *or lsm1*Δ strains (Boeck *et al.* 1998; Tharun *et al.* 2000; He and Parker 2001). A second set of interacting proteins consists of the Dcp1, Dcp2, Edc3, or Scd6 and Dhh1, although whether all these factors can associate at the same time remains to be determined. Within and between these complexes, Pat1 and Edc3 appear to play important scaffolding roles and interact with many components of the decapping machinery (Decker *et al.* 2007; Nissan *et al.* 2010).

A third role of decapping activators is to directly stimulate decapping by Dcp2. For example, the Edc3 and Pat1 proteins directly bind Dcp2 and enhance its activity in purified systems (Harigaya *et al.* 2010; Nissan *et al.* 2010). Similarly, the paralogs Edc1 and Edc2, which are high-copy suppressors of temperature-sensitive alleles in Dcp1 or Dcp2 (Dunckley *et al.* 2001), bind RNA and stimulate Dcp2 either in extracts or in reconstituted systems (Schwartz *et al.* 2003; Steiger *et al.* 2003). Edc1, and presumably Edc2 as well, directly bind Dcp1 to stimulate the decapping enzyme by enhancing both Km and kcat of Dcp2 (Borja *et al.* 2011).

Several observations suggest that Dcp2 catalysis is not generally rate limiting for decapping *in vivo*. First, conditional mutations in Dcp1 or Dcp2 that compromise decapping activity *in vitro* do not significantly affect mRNA decapping rates *in vivo* (Tharun and Parker 1999; Dunckley *et al.* 2001; Steiger *et al.* 2003). Second, strains lacking Edc1, Edc2, and/or Edc3 do not show defects in mRNA decay rates *in vivo* unless the decapping ability of Dcp2 is reduced by mutation (Dunckley *et al.* 2001; Kshirsagar and Parker 2004). Third, deletion of the C-terminal domain of Pat1, which stimulated Dcp2 *in vitro*, has only a marginal effect on mRNA decapping rates *in vivo* (Pilkington and Parker 2008; Nissan *et al.* 2010). Taken together, these observations suggest that, at least in mid-log growth, the rate-limiting step in decapping is the translation repression and assembly of a decapping complex on the mRNP.

Several observations suggest that Pat1 and the Lsm1–7 complex function in an mRNP rearrangement that enhances decapping activity. First, *lsm1*Δ strains accumulate mRNA and the decapping machinery in P-bodies, arguing that the Lsm1–7 complex functions at a late stage in decapping after the mRNA has exited translation (Sheth and Parker 2003; Teixeira and Parker 2007). Second, the middle and carboxy-terminal domains of Pat1 interact with themselves, and, while the C-terminal domain is sufficient to bind Dcp2 and activate decapping *in vitro*, its interaction with Dcp2 is blocked by the middle domain of Pat1 (Nissan *et al.* 2010). This raises the possibility that, for Pat1 to interact with Dcp2 and activate decapping catalysis, a conformational change in Pat1 between the middle and C-terminal domains is required. Strikingly, the Lsm1–7 complex appears to interact with both the middle and the C-terminal domain of Pat1 (Pilkington and Parker 2008; Nissan *et al.* 2010). Thus, a working model is that the binding of Pat1 to the mRNA with the Lsm1–7 complex allows for the formation of a binding site for Dcp2 in Pat1's C-terminal domain that is sufficient to activate catalysis.

Several other factors have been identified as promoting decapping (Zuk *et al.* 1999; Table 2). Most notably, mutations in one of the genes encoding eIF5a lead to slower decapping of mRNAs, perhaps because of alteration to translation initiation and/or translation elongation (Zuk and Jacobson 1998; Saini *et al.* 2009). The Stm1 and Sbp1 proteins interact with the ribosome and eIF4G, respectively, and thereby appear to promote the ability of Dhh1 to promote decapping (Segal *et al.* 2006; Balagopal and Parker 2009, 2011; Rajyaguru *et al.* 2012), although the mechanism by which these proteins stimulate Dhh1 function is unknown.

Control of decapping: *Poly(A) tails as inhibitors of decapping:* Several observations argue that the ability of the poly (A) tail to inhibit decapping is partially mediated through the poly(A)-binding protein (Pab1). First, it has been demonstrated in yeast that decapping occurs when the poly(A) tail length has been shortened to an oligo(A) length of ~12 residues (Decker and Parker 1993). This is approximately the minimum length required for Pab1 binding (Sachs *et al.* 1987). Second, in *pab1* mutant strains, decapping is uncoupled from deadenylation (Caponigro and Parker 1995; Morrissey *et al.* 1999). In this case, intermediates in mRNA decay, trapped by inhibiting 5′ to 3′ degradation in *cis* with strong secondary structures, are produced as decapped mRNA fragments with long poly(A) species (Caponigro and Parker 1995). This indicates that, in the absence of Pab1, the absolute requirement for prior deadenylation before decapping is not necessary.

A second reason for decapping occurring after deadenylation is that the Pat1/Lsm1–7 complex prefers to bind oligoadenylated mRNAs *in vitro* (Chowdhury *et al.* 2007) and associates with mRNAs after deadenylation *in vivo* (Tharun and Parker 2001). Thus, a working model is that polyadenylated mRNAs are protected by Pab1 binding and

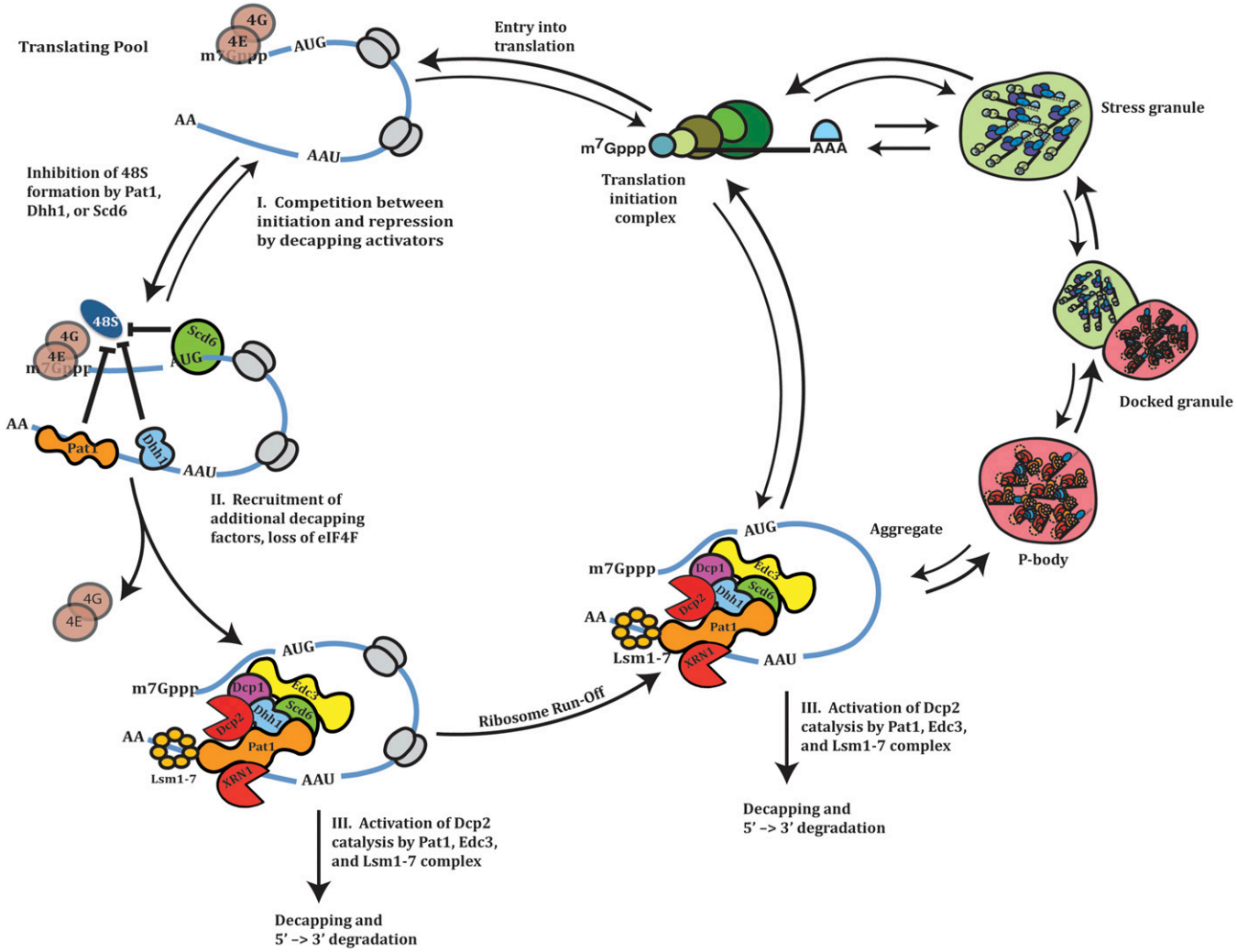

Figure 2 Model for mRNA decapping.

by promoting translation initiation, and deadenylated mRNAs are enhanced for decapping by the Pat1/Lsm1–7 complex binding and promoting translation repression and decapping (Tharun 2009).

Control decapping on specific mRNAs: Differences in mRNA decapping rates appear to arise due to two features of individual mRNAs. First, since decapping is inversely related to translation initiation, features that inherently decrease translation initiation are expected to increase decapping rates. For example, the faster decapping rate of the MFA2 mRNA as compared to the PGK1 mRNA is due to a poor AUG context on the MFA2 mRNA (Lagrandeur and Parker 1999). In addition, mRNAs contain sites for sequence-specific mRNA-binding proteins that enhance decapping. For example, the binding of Puf3 to the Cox17 mRNA enhances decapping of this mRNA following deadenylation (Olivas and Parker 2000).

An important correlation is that many features that increase the decapping rate—including 3′ UTR or coding region elements, AUG context, or 5′ stem-loop structures—also increase deadenylation rate (Muhlrad and Parker 1992; Caponigro *et al.* 1993; Muhlrad *et al.* 1995; Caponigro and Parker 1996; Lagrandeur and Parker 1999; Olivas and Parker 2000). This suggests that a critical aspect of controlling cytoplasmic mRNA function is an exchange of mRNAs between mRNPs complexed with translation factors and protected from decay and an mRNP associated with decay factors and having reduced translation rate and enhanced deadenylation and decapping. A key issue for future work is determining the specifics of these mRNPs and how transitions between both states occur.

Decapping of specific mRNAs can also be triggered independently of deadenylation. For example, the Edc1 mRNA is decapped without prior deadenylation, and this is due to a poly(U) tract in the 3′ UTR that sequesters the poly(A) tail and renders it nonfunctional (Muhlrad and Parker 2005). Alternatively, in an autoregulatory loop, the Rps28b mRNA assembles a decapping complex through Rps28 binding to a specific stem loop in its 3′ UTR that allows decapping to proceed independently of deadenylation (Badis *et al.* 2004).

An unresolved issue is how many different modes of decapping exist in yeast cells and therefore how the diversity of mRNA decay rates is achieved.

Decapping can also be negatively regulated on specific mRNAs. For example, the Khd1 protein binds to and limits the decapping on the Mtl1 mRNA (Mauchi *et al.* 2010). Since Khd1 can bind eIF4G and inhibit translation (Paquin *et al.* 2007), this may allow for the formation of a mRNP complex that is limited for translation, but protected from decapping. Since Khd1 can affect mRNA localization (Hasegawa *et al.* 2008), such a mechanism may be important in keeping mRNAs in a stable, but untranslating, state while the mRNAs localize to specific regions of the cell. This may be a common mechanism of mRNA control since multiple translation repressors that can affect decapping also bind directly to eIF4G and can repress translation (Rajyaguru *et al.* 2012). Similarly, the Pub1 protein binds to, and stabilizes, a significant subset of yeast mRNAs, presumably by inhibiting decapping, although this has not been directly demonstrated (Ruiz-Echevarria and Peltz 2000; Duttagupta *et al.* 2005).

mRNA decapping and P-bodies: The mRNA degradation machinery can also be concentrated in specific cytoplasmic mRNP aggregates referred to as P-bodies (Sheth and Parker 2003; see Figure 2). P-bodies are aggregates of untranslating mRNAs that are associated with the mRNA decapping machinery, and to a lesser extent, with the deadenylases (Parker and Sheth 2007). P-bodies are proportional to the pool of untranslating mRNA associated with the decapping machinery (Teixeira *et al.* 2005). Consistent with this view, if mRNAs are trapped in polysomes, P-bodies decrease, whereas decreases in translation initiation increase the pool of mRNAs in P-bodies (Teixeira *et al.* 2005). Moreover, when mRNA decay is limited at the actual step of decapping or 5′ to 3′ degradation, P-bodies increase (Sheth and Parker 2003; Teixeira and Parker 2007). mRNAs within P-bodies can return to translation (Brengues *et al.* 2005), and this may occur through the transition of mRNAs from P-bodies to stress granules, which are aggregates of untranslating mRNAs assocatied with translation initation factors and RNA-binding proteins (reviewed in Buchan and Parker 2009). Note that the putative transfer of an mRNA from a P-body to a stress granule would correspond to an exchange of the mRNA decapping machinery for translation initiation factors (Buchan *et al.* 2008). This constitutes an "mRNA cycle" wherein mRNAs can exit translation either for degradation or to eventually return (Figure 2), which may play a role in the regulation of both translation and mRNA degradation (Balagopal and Parker 2009). Interestingly, recent genomic analyses have identified at least 400 different mRNAs, compromising a large percentage of the transcriptome, that can recycle from repression to translation upon stress relief (Arribere *et al.* 2011).

An unresolved issue is the role of P-body formation *per se* in the decapping of mRNAs. The aggregation of individual mRNPs into larger P-bodies is largely dependent on the YjeF domain of the Edc3 protein (Decker *et al.* 2007), although P-bodies can still form to some extent on the basis of a "prion" domain on Lsm4 (Decker *et al.* 2007; Reijns *et al.* 2008) and some Pat1-dependent aggregation (Buchan *et al.* 2008). How these aggregation motifs affect mRNA decay rates is unclear. In one report, strains lacking Edc3 and/or the aggregation domain of Lsm4 did not show any changes in the decay rates of the MFA2 mRNA (Decker *et al.* 2007), although in another report a strain lacking the Lsm4 aggregation motif did show a modest change in MFA2 mRNA decay rates (Reijns *et al.* 2008). The current simplest interpretation is that the formation of large P-body aggregates is not required for mRNA decapping but might affect the rate of decapping either in certain conditions or for subsets of mRNAs.

Relationship of decapping to ongoing translation elongation: An unresolved issue is how ongoing translation elongation affects the decapping of yeast mRNAs. Some evidence argues that decapping can be inhibited by elongating ribosomes. First, inhibition of translation elongation by chemicals such as cycloheximide or sodarin leads to decreases in the rate of decapping (Beelman and Parker 1994; Cereghino *et al.* 1995; S. Jain and R. Parker, unpublished observation). However, this could also be due to indirect effects since cycloheximide can stabilize mRNAs that are never translated as well (Beelman and Parker 1994). Second, the rate of decapping of NMD substrates is proportional to the length of the ORF (Cao and Parker 2003). Moreover, in some cases, shortening the length of the ORF can lead to faster decay of specific mRNAs, although this could be due to loss of specific stabilizing sequences (Heaton *et al.* 1992). These results raise the possibility that mRNAs harboring elongating ribosomes have slower rates of decapping than ribosome-free mRNAs.

In contrast, several observations argue that decapping can occur while ribosomes are still associated with mRNAs. First, in the presence of cycloheximide mRNAs are seen to be shortened from their 5′ ends and degraded in a 5′ to 3′ manner to internal sites that are thought to be stalled ribosomes, although this rate is slower than normal rates of decapping (Beelman and Parker 1994; Cereghino *et al.* 1995). Similarly, mRNAs with strong stalls in translation elongation have been argued to generate mRNA fragments by decapping and 5′ to 3′ degradation to the stalled ribosomes (Hu *et al.* 2009), but more recent studies argue that these mRNA fragments may be produced by an endonuclease cleavage triggered by stalled ribosomes, referred to as no-go decay (NGD; see below) (D. Muhlrad and R. Parker, unpublished data). Additional evidence for decapping occurring on mRNAs engaged in elongation is that decapped mRNAs, either in wild-type cells or in *xrn1*Δ strains, appear to associate with polysomes, suggesting they are decapped while bound to elongating ribosomes (Hu *et al.* 2009). Taken together, the best current interpretation is that decapping can occur while mRNAs are associated with ribosomes, but that ribosomes may also play a role in limiting the rates of decapping.

Regulation by signal transduction paths: Multiple signal transduction pathways impinge on mRNA decapping. First, Ste20 has been observed to phosphorylate Dcp2 and affect its assembly into P-bodies. Ste20 phosphorylation of Dcp2 also affects the degradation of certain mRNAs (Yoon *et al.* 2010). Second, Pat1 is a target of PKA, and phosphorylation of Pat1 limits its ability to assemble into P-bodies (Ramachandran *et al.* 2011), although how this affects specific protein interactions or mRNA decay is not clear. Third, when the growth regulatory Tor kinase is inhibited, the activation of the Rim15 kinase leads to phosphorylation of the paralogous Igo1 and Igo2 proteins, which then interact with Dhh1 and stabilize mRNAs required for entry into G0 (Talarek *et al.* 2010). One anticipates that other signal transduction pathways will regulate the mRNA turnover machinery. On the basis of genome-wide studies Dhh1, Edc1, Edc3, Pop2, Ccr4, Xrn1, Dcp2, and Dcp1 are also known to be phosphoproteins and could be targets of such signal transduction pathways.

3′ to 5′ mRNA degradation

The second pathway of mRNA decay following deadenylation is 3′ to 5′ degradation, which is catalyzed by the exosome and various cofactors (Table 3) (Anderson and Parker 1998). The exosome is a multiprotein complex consisting of 10 main proteins, including six members of the RNase PH protein family and three small RNA-binding proteins (Allmang *et al.* 1999), which together form a ring structure analogous to bacterial PNPase (Liu *et al.* 2006), and the Rrp44/Dis3 protein, which has both an exonuclease and endonuclease domain (Lebreton *et al.* 2008; Schaeffer *et al.* 2009). In addition to its roles in the cytoplasm, the exosome is involved in numerous nuclear RNA processing and degradation processes (see below and reviewed in Lykke-Andersen *et al.* 2009). In the nucleus, the exosome is also associated with Rrp6, another 3′ to 5′ exonuclease, Rrp47, and Mpp6 (Mitchell *et al.* 2003; Milligan *et al.* 2008; Synowsky *et al.* 2009), which have roles in nuclear function of the exosome (see below).

Despite the similarity of the core ring domain to active exonucleases, the only active nuclease sites in the exosome appear to be present in the Rrp44/Dis3 protein (Liu *et al.* 2006; Dziembowski *et al.* 2007). The exosome is then thought to function by the core ring structure serving as a binding platform for proteins targeting the exosome to various substrates and to channel the RNA to the active sites of Rrp44/Dis3 (Bonneau *et al.* 2009).

For the degradation of cytoplasmic mRNA, the exosome requires the Ski proteins (Anderson and Parker 1998; Van Hoof *et al.* 2000b; Araki *et al.* 2001). The Ski7 protein appears stably bound to the cytoplasmic exosome through the Ski4 subunit (Van Hoof *et al.* 2002). The Ski2, Ski3, and Ski8 proteins form a separate protein complex (Brown *et al.* 2000; Wang *et al.* 2005). The Ski2/3/8 complex interacts with Ski7 (Araki *et al.* 2001; Wang *et al.* 2005), and this interaction, which appears to occur between Ski7 and the Ski3 and Ski8 proteins, is required for 3′ to 5′ degradation of mRNAs. The Ski2 protein is an ATPase of the RNA helicase family and presumably utilizes the energy of ATP hydrolysis to unwind substrates and/or dissociate bound proteins to deliver the RNA to the exosome. Interestingly, RNA processing and degradation by the nuclear exosome require the related ATPase Mtr4, suggesting that this is a general feature of exosome function (reviewed in Lykke-Andersen *et al.* 2009).

There are several unresolved issues with regards to 3′ to 5′ degradation of mRNAs. For example, the role of the Ski complex is not well understood. Moreover, although mRNAs can have differences in their rates of 3′ to 5′ degradation (Cao and Parker 2001), the features of mRNAs that dictate different rates of 3′ to 5′ degradation are not understood. In mammalian cells, sequence-specific RNA-binding proteins can recruit the exosome directly to mRNAs, and similar events might exist in yeast cells (*e.g.*, Chen *et al.* 2001). Finally, whether some mRNAs are normally degraded by the exosome or whether 3′ to 5′ degradation is the major pathway of cytoplasmic mRNA under some growth conditions is not resolved.

Other mRNA decay pathways

Some observations imply that there will be two additional mechanisms by which yeast mRNAs are degraded. First, the vacuolar nuclease Rny1 can degrade tRNAs, rRNAs, and even small nuclear RNA (snRNAs) (Thompson and Parker 2009; N. Luhtala and R. Parker, unpublished observations). Given this, one anticipates some mRNAs will also be degraded by Rny1 either during the process of autophagy or specific targeting of some mRNAs to the vacuole or because Rny1 can enter the cytosol and degrade mRNAs under some conditions (Thompson and Parker 2009). A second nuclease that probably targets mRNAs during stress is Nuc1. Nuc1 is a general nuclease that is localized to the mitochondria, but during stress or in high cell densities is released to the cytosol through the mitochondrial porins and then is transported to the nucleus where it can play a role in apoptosis (Buttner *et al.* 2007). Interestingly, Nuc1 is also known to target the mRNAs produced by the double-stranded RNA killer virus, suggesting that it could also target some, or all, cytoplasmic mRNAs under these conditions (Liu and Dieckmann 1989). A potential role of Nuc1 in mRNA decay is suggested by its negative genetic interactions with Ski2, -3, -7, -8, and Xrn1 (Costanzo *et al.* 2010).

mRNA Quality Control Pathways

Cytoplasmic quality control

Several cytoplasmic quality control mechanisms degrade eukaryotic mRNAs that are defective in translation (Doma and Parker 2007). An emerging principle is that aberrant

Table 3 Exosome and associated proteins involved in 3′ to 5′ degradation of RNAs

Component	Features	Reference
Core exosome	Six RNasePH domain proteins (no active sites) Rrp41, Rrp42, Rrp43, Rrp45, Rrp46, Mtr3 3 RNA-binding subunits (Rrp4, Rrp40, Csl4) One catalytic subunit, Rrp44, with both endo and exo active sites Functions in both RNA processing and degradation in cytoplasm and nucleus	Lykke-Andersen *et al.* (2011)
Cytoplasmic cofactors		
Ski7	Binds Csl4 subunit of core Required for 3′ to 5′ decay of mRNAs GTPase domain required for non-stop decay Interacts with Ski2/Ski3/Ski8 complex	Van Hoof *et al.* (2000b, 2002); Wang *et al.* (2005)
Ski2/Ski3/Ski8 complex	Required for 3′ to 5′ mRNA decay Ski2 is member of ATPase RNA helicase family Ski8 is WD40 protein Ski3 may function as scaffold	Anderson and Parker (1998); Brown *et al.* (2000); Araki *et al.* (2001); Wang *et al.* (2005)
Nuclear cofactors		
Rrp6	3′ to 5′ exonuclease of RNAseD family Associated with nuclear exosome Required for RNA processing and decay of RNAs in the nucleus Functions in retention of aberrant mRNAs at sites of transcription	Butler and Mitchell (2011)
Rrp47 (Lrp1)	RNA-binding protein Required for RNA processing and nuclear RNA decay Associated with nuclear exosome	Butler and Mitchell (2011)
Mpp6	RNA-binding protein Associated with nuclear exosome Required for RNA processing and nuclear RNA decay	Milligan *et al.* (2008)
Tramp complexes (Trf4 or Trf5)	Consist of Mtr4 with 1 noncanonical poly(A) polymerase (Trf4 or Trf5) and 1 RNA-binding protein (Air1 or Air2) Required for several RNA-processing and nuclear RNA decay pathways Can promote processing/degradation in poly(A)-dependent and -independent manners by recruiting exosome to substrates	Houseley and Tollervey (2006); San Paolo *et al.* (2009); Butler and Mitchell (2011)

mRNAs are distinguished from the normal mRNAs by adaptor proteins that interact with the translation machinery and direct the aberrant mRNA into a degradation pathway. Key issues for each quality control pathway are the biological role, the specificity of distinguishing normal from aberrant mRNAs, and the mechanism by which mRNAs are recognized and degraded.

Nonsense-mediated decay: NMD is an mRNA quality control system that degrades mRNAs with aberrant translation termination. NMD was first described as a system that degrades mutant mRNAs with premature translation termination codons (Losson and Lacroute 1979). However, NMD degrades a wide variety of mRNAs that have aberrant translation termination events. Such substrates include mRNAs with long 3′ UTRs that alter the relationship of the poly(A) tail to the stop codon (Muhlrad and Parker 1999a; Kebaara and Atkin 2009; Deliz-Aguirre *et al.* 2011), mRNAs with alternative translation initiation sites that that are out of frame with the main ORF and lead to premature termination (Welch and Jacobson 1999), mRNAs with upstream ORFs (Gaba *et al.* 2005; Guan *et al.* 2006), pre-mRNAs that contain introns with stop codons (He *et al.* 1993; Sayani *et al.* 2008), and mRNAs with frameshifts, where a proportion of the ribosomes are shifted into alternative reading frames containing premature termination codons (Belew *et al.* 2011). In addition, one anticipates that errors in transcription or mis-splicing that introduce premature stop codons will generate substrates for NMD at a low level across many different genes.

Consistent with this wide range of substrates, several genomic analyses have revealed that NMD targets a wide range of different mRNAs. As such, NMD is not just a quality control system but is also utilized by cells to degrade a subset of "normal" mRNAs, particularly those involved in cell-surface dynamics and chromosome structure (Lelivelt and Culbertson 1999; He *et al.* 2003; Guan *et al.* 2006). For many of these mRNAs it is not clear why they are substrates of NMD. One possibility is that they are lacking features that specify proper translation termination and as such are targeted by NMD (see below).

Substrates for NMD are identified by the action of the interacting Upf1, Upf2, and Upf3 proteins (reviewed in Baker and Parker 2004). The recognition of an mRNA by the NMD pathway has several effects on the metabolism of the mRNA. Specifically, the mRNA is targeted for enhanced deadenylation (Muhlrad and Parker 1994; Cao and Parker 2003; Mitchell and Tollervey 2003), rapid deadenylation-

independent decapping (Muhlrad and Parker 1994), slightly increased rates of 3′ to 5′ degradation after deadenylation (Cao and Parker 2003; Mitchell and Tollervey 2003), and translation repression (Muhlrad and Parker 1999b). Consistent with NMD targeting mRNAs for translation repression, when decapping or 5′ to 3′ degradation is blocked, NMD substrates accumulate as repressed mRNAs in P-bodies in an Upf1-dependent manner (Sheth and Parker 2006).

NMD has been suggested to be coupled to degradation of the nascent peptide in an Upf1-dependent manner (Kuroha *et al.* 2009). In this manner, not only would the mRNA be degraded, but any potential dominant-negative peptides produced would also be rapidly destroyed. Interestingly, Upf1 has been suggested to have ubiquitin ligase activity, and mutations that affect this activity alter the process of NMD for RNA degradation (Takahashi *et al.* 2008), although the sites of these mutations would also be predicted to disrupt Upf1 interaction with Upf2, which is known to be required for NMD (He *et al.* 1996, 1997; Clerici *et al.* 2009). However, how general NMD stimulated protein decay remains to be established since a peptide from a different NMD mRNA substrate shows the same decay rates in wild-type and upf1Δ cells (Muhlrad and Parker 1999b).

Transcripts appear to be targeted for the diverse effects of NMD in two steps (Figure 3). Several observations suggest that, in an initial step, Upf1, which is a member of the SF1 protein superfamily of nucleic acid helicases (Fairman-Williams *et al.* 2010), interacts with the translation termination complex and alters the nature of translation termination (reviewed in Baker and Parker 2004). The most direct evidence is that a ribosome toeprint generated at a normal translation termination codon is distinct from the toeprint of a ribosome terminating at a premature termination codon in a manner dependent on Upf1p (Amrani *et al.* 2004). In addition, the Upf1, -2, and -3 proteins co-immunoprecipitate with the eukaryotic translation termination factors eRF1 and/or eRF3 (Czaplinski *et al.* 1998; Wang *et al.* 2001). Although *upf1*Δ mutants can show increased rates of stop-codon readthrough, this effect appears to be due to stabilization and increased expression in the *upf1*Δ strain of a magnesium transporter, which increases intracellular Mg++ and leads to increased miscoding of stop codons (Johansson and Jacobson 2010). Interestingly, a defect in *upf2*Δ and *upf3*Δ strains in stop codon readthrough can be suppressed by overexpression of Upf1, suggesting that Upf1 directly affects translation, independently of Upf2 and Upf3, of mRNAs with some stop codons, perhaps by inhibiting translation initation (Muhlrad and Parker 1999b; Maderazo *et al.* 2000). Consistent with this view, Upf1 can associate with polysomes independently of Upf2 and Upf3 (Atkin *et al.* 1997). The *upf2*Δ and *upf3*Δ strains may have decreased Upf1 function since in these strains Upf1 accumulates in P-bodies, and therefore the majority of Upf1 may not be available to affect translation termination (Sheth and Parker 2006). Moreover, because NMD substrates accumulate in P-bodies independently of Upf2 and Upf3, the simplest model is that Upf1 is sufficient to repress translation of the mRNA (Sheth and Parker 2006).

A second step in NMD appears to be the interaction of Upf2 and Upf3 with Upf1, and this triggers the degradation of the mRNA. This conclusion is supported by the Upf2 and Upf3 independent effects of Upf1 on translation readthrough and targeting of NMD substrates to P-bodies (Maderazo *et al.* 2000; Sheth and Parker 2006). Upf3 is an RNA-binding protein that is proposed to load on mRNAs in the nucleus (Shirley *et al.* 2002). Upf2 interacts with both Upf3 and Upf1, and its binding to Upf1 reduces the Upf1–RNA interaction and enhances the helicase activity of Upf1 (Chakrabarti *et al.* 2011). This suggests that, following translation termination altered by Upf1, interaction of Upf2 with Upf1 would enhance Upf1 catalytic properties and lead to mRNP rearrangements that trigger mRNA degradation, possibly by rearrangements of the mRNP or by altering the fate of the terminating ribosome (Ghosh *et al.* 2010). Consistent with that model, mutations inactivating the ATPase activity of Upf1 also accumulate mRNAs in P-bodies (Sheth and Parker 2006).

A key issue is how the specificity of NMD is determined. In principle, an mRNA will be targeted for NMD on the basis of the nature of translation termination (is it "aberrant" or not?) and whether Upf2/Upf3 can influence Upf1 after altered termination. Thus, the specificity of NMD is determined by factors that influence translation termination and whether Upf2/Upf3 is associated with the mRNA after termination. One factor that contributes to proper translation termination is Pab1, which is known to interact with the translation termination factors, and, when tethered to the mRNA near a premature stop codon, can prevent NMD on that mRNA (Amrani *et al.* 2004). However, strains lacking Pab1, as well as poly(A)-mRNAs, still show Upf1-dependent degradation of mRNAs with premature stop codons, indicating that additional factors also contribute to NMD targeting, although whether those factors influence Upf1 effects on termination or effect a downstream step in NMD is not resolved (Caponigro and Parker 1995; Meaux *et al.* 2008). Thus, a key issue is determining what other features of an mRNA influence the nature of translation termination and Upf2/Upf3 interaction with the mRNA. Although currently controversial, one potential contribution is elements in coding regions, sometimes referred to as downstream sequence elements (reviewed in Gonzalez *et al.* 2001), that might recruit Upf3 and Upf2 to mRNAs, and, if Upf2 and Upf3 are not removed by elongating ribosomes, might lead to triggering NMD after upstream termination.

The multistep process of NMD in yeast is also revealed by the observation that 5′ proximal stop codons trigger faster mRNA degradation than stop codons farther into the ORF (Losson and Lacroute 1979; Peltz *et al.* 1993; Cao and Parker 2003). Strikingly, as judged by Upf1-promoted deadenylation, all premature stop codons are recognized as aberrant, but the position of the stop codon simply dictates differences in the actual rates of Upf1-promoted decapping

(Cao and Parker 2003). Although the molecular mechanism that leads to distal stop codons leading to slower rates of decapping is not known, it does demonstrate that there are multiple steps in the targeting of an mRNA for NMD.

Additional factors can also influence the process of NMD in yeast. Both Upf1 and Upf2 are phosphorylated (De Pinto *et al.* 2004; Wang *et al.* 2006), and phosphorylation of Upf2 may affect NMD (Wang *et al.* 2006). In addition, strains lacking the Ebs1 protein, which is homologous to the metazoan NMD factor Smg7 and contains a 14-3-3 domain for binding phosphoproteins, show partial defects in NMD (Luke *et al.* 2007). Interestingly, Ebs1 is also regulated by the NMD pathway, which might provide a feedback regulatory loop for maintaining active NMD under some conditions (Ford *et al.* 2006).

No-go decay: A second quality control system for mRNA translation, NGD, leads to endonucleolytic cleavage of mRNAs with strong stalls in translation elongation (Doma and Parker 2006; reviewed in detail in Harigaya *et al.* 2010). After such cleavage, the 3′ mRNA fragment is degraded by Xrn1, and the 5′ fragment is degraded primarily by the cytoplasmic exosome (Doma and Parker 2006). No-go decay can occur at a wide range of translation elongation stalls, including strong stem loops, rare codons, polyLys or polyArg runs, sites of depurination, and possibly at frameshift sites (Doma and Parker 2006; Gandhi *et al.* 2008; Chen *et al.* 2010; Kuroha *et al.* 2010; Letzring *et al.* 2010; Belew *et al.* 2011). To date, no specific mRNAs that are predominantly degraded by NGD have been identified, and the suggestion is that NGD primarily functions to degrade aberrant or damaged mRNAs, which could be produced by chemicals or ultraviolet light exposure (Y. Harigaya and R. Parker, unpublished data). The endonuclease(s) that cleaves the mRNA during NGD has not been identified.

At some translation stalls, NGD is promoted by the Dom34 and Hbs1 proteins, which are paralogs of the translation termination factors eRF1 and eRF3 (Doma and Parker 2006). Structural analyses indicate that Dom34 and Hbs1 fold and interact similarly to eRF1 and eRF3, respectively (Lee *et al.* 2007; Graille *et al.* 2008; Chen *et al.* 2010; Van Den Elzen *et al.* 2010). Moreover, Dom34 and Hbs1 bind the ribosome in the empty A site (Becker *et al.* 2011). This similarity to translation termination complexes suggested that Dom34 and Hbs1 function at elongation stalls to terminate translation, which has been demonstrated using reconstituted systems from both yeast and mammals (Shoemaker *et al.* 2010; Pisareva *et al.* 2011). However, under some conditions, or at very strong translation pauses, Hbs1 and Dom34 are not required for NGD (Passos *et al.* 2009; Chen *et al.* 2010; Kuroha *et al.* 2010).

On the basis of these observations, a working model for NGD can be proposed (Figure 4). During translation elongation, the ribosome can be paused for a variety of reasons. If the A site is empty during a prolonged elongation stall, it allows for a Dom34/Hbs1/GTP complex, rather than cognate aminoacyl-tRNA, to interact with the A site in the stalled ribosome, leading to dissociation of the peptide and tRNA or a peptide-tRNA conjugate, while maintaining the ribosome on the mRNA. At this stage, three events can occur. First, the mRNA can be cleaved at the vicinity of the "terminated" ribosome. Although the identity of the nuclease is unknown, it is likely to be physically associated with the stalled ribosome. Note that the mRNA cleavage could possibly occur before the release of the peptide-tRNA conjugate. Second, the ribosomes can be released by an unknown mechanism, which may or may not be similar to ribosome recycling at a regular termination codon. Release of the ribosomes is predicted to limit cleavage of the mRNA by preventing the recruitment of the nuclease. Third, the released nascent peptide or peptide tRNA-conjugate would be subject to ubiquitin-proteasome-mediated degradation, possibly in conjunction with peptide-tRNA hydrolysis.

Non-stop decay: Another mechanism of mRNA quality control is the rapid degradation of mRNAs that do not contain translation termination codons, which is referred to as non-stop decay (NSD) (Frischmeyer *et al.* 2002; Van Hoof *et al.* 2002) (Figure 5). Such mRNAs naturally occur due to use of polyadenylation sites within open reading frames, which are estimated to occur at ~10% of the transcriptional events (Van Hoof *et al.* 2002). In addition, such non-stop mRNAs could also arise due to mutations, readthrough of stop codons due to PSI+ that limits the translation termination factor function (Wilson *et al.* 2005), or endonuclease cleavage sites with the open reading frame.

The process of NSD appears triggered by a ribosome reaching the 3′ end of the mRNA and being unable to terminate translation. In this situation, the mRNA is rapidly degraded in a process that requires the exosome and the Ski7, Ski2, Ski3, and Ski8 proteins. NSD is distinguished from 3′ to 5′ decay of mRNAs by requiring the GTPase domain of Ski7, which is similar to Ef1a and is thought to interact with the ribosome (Van Hoof *et al.* 2002). NSD is also mechanistically different from normal 3′ to 5′ decay of mRNAs in that it can utilize either endonuclease or exonuclease activity of Rrp44, whereas 3′ to 5′ decay of normal mRNAs appears to require the exonuclease activity of Rrp44 (Schaeffer and Van Hoof 2011). Given this, the prevailing model of NSD is that a stalled ribosome at the 3′ end of the mRNA is recognized by the Ski7 protein, which then recruits the Ski2/Ski3/Ski8 complex and the exosome to degrade the mRNA in a 3′ to 5′ direction. In the absence of Ski7 or the exosome, nonstop mRNAs are subject to accelerated decapping and 5′ to 3′ degradation (Inada and Aiba 2005), perhaps because of the failure to recycle terminating ribosomes for continued efficient translation initiation. An unresolved issue is how the ribosome is removed from the mRNA and if Ski7 hydrolyzes GTP to promote its dissociation.

The peptides produced by non-stop mRNAs are also subject to rapid degradation in a proteasome-mediated manner. In this case, two different ubiquitin ligases have

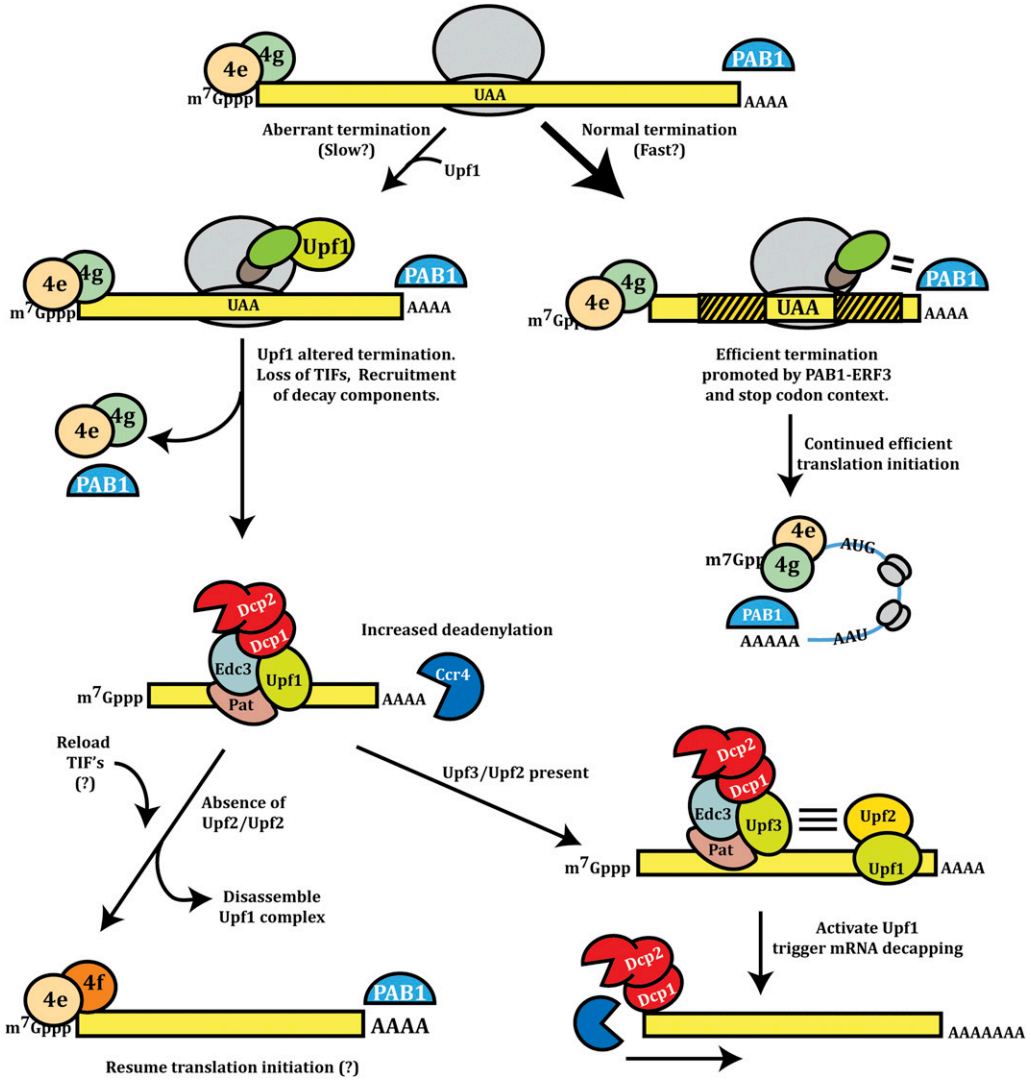

Figure 3 Model for the nonsense-mediated decay.

been proposed to function. Two reports have suggested that the Ltn1 protein, which is an E3 ubiquitin ligase that interacts with ribosomes, is required for rapid decay of the nascent peptide (Wilson *et al.* 2007; Bengtson and Joazeiro 2010). In a second study, it has been suggested that Not4 promotes the ubiquitination and degradation of such nascent peptides (Dimitrova *et al.* 2009), although whether this is due to differences in the reporter constructs used is yet to be resolved. In either case, the rapid degradation of the nascent and aberrant polypeptide would ensure that only proteins of the proper length are produced.

The specific features of the mRNAs may affect how they become substrates for NSD or other mRNA quality control pathways. For example, while NSD mRNAs generated by poly(A) addition within the coding region require the GTPase domain of Ski7 for their degradation, non-stop mRNAs generated by a ribozyme within the coding region do not (Meaux and Van Hoof 2006). One possibility is that this difference is due to the specific loading of proteins during nuclear polyadenylation that affect NSD in the cytosol. Alternatively, it could be that mRNAs with translated poly(A) tails are subject to a hybrid type of mRNA decay that involves aspects of both NGD and NSD. This possibility is suggested by the fact that poly(A) tracts, which encode for lysine, can stall elongating ribosomes and trigger NGD (Ito-Harashima *et al.* 2007; Kuroha *et al.* 2010). An interesting area for future research will be to determine how specific types of mRNAs are recognized and targeted for these quality control systems.

Quality control of nuclear mRNA processing

Numerous quality control systems target mRNA defective in pre-mRNA splicing, polyadenylation, or mRNA export. These nuclear quality control systems prevent the function of the aberrant mRNA by triggering nuclear degradation or by nuclear export leading to cytoplasmic degradation. In addition, aberrant or unprocessed nuclear mRNAs can also be retained within the nucleus. Nuclear retention may be important both to give time for RNA processing to be completed and to allow for a kinetically disfavored nuclear degradation pathway to degrade the RNA (see below).

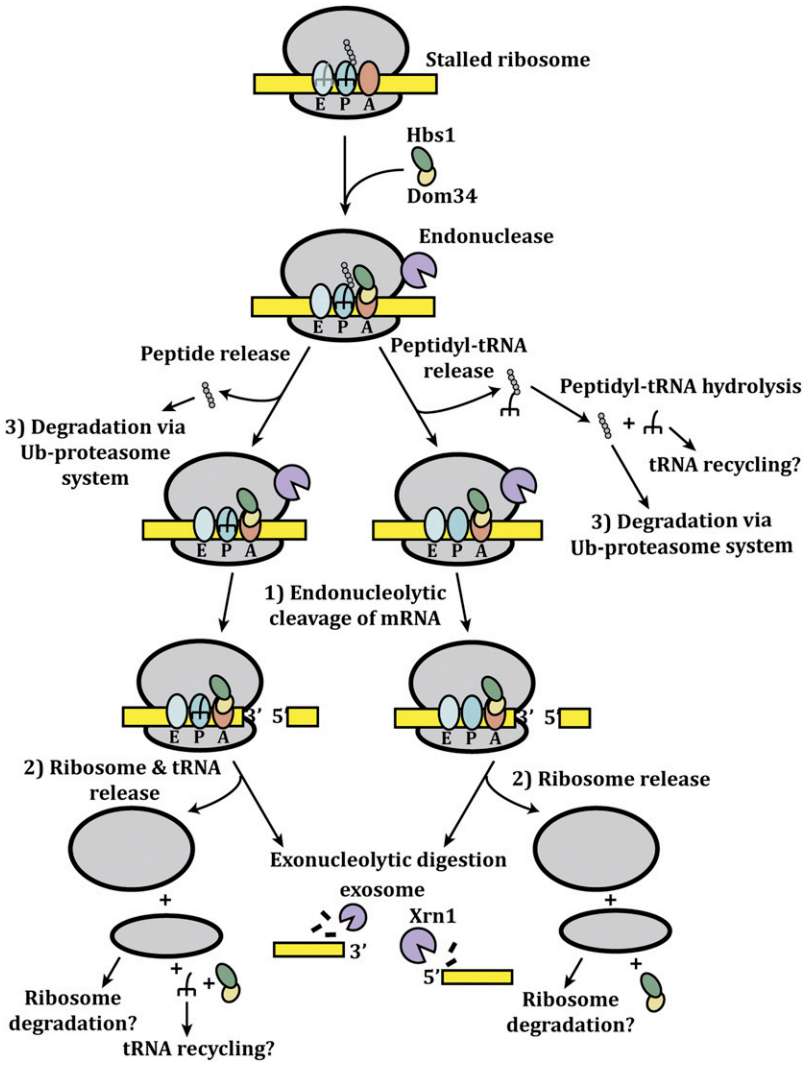

Figure 4 Model for no-go decay.

Examples of nuclear retention of aberrant mRNAs include the retention of mRNAs with defects in polyadenylation (Hilleren *et al.* 2001; Jensen *et al.* 2001). Interestingly, these aberrant RNAs are retained in the vicinity of the gene (Thomsen *et al.* 2003), which has the potential to have feedback effects on transcription.

Quality control of pre-mRNA splicing: Several RNA degradation systems contribute to degrading unspliced or mis-spliced pre-mRNAs (Figure 6). For the Rps22b and Rpl18a pre-mRNAs, the intron contains a cleavage site for the yeast RNAseIII enzyme, Rnt1, and endonuclease cleavage within the intron reduces the pool of both pre-mRNA and the excised intron (Danin-Kreiselman *et al.* 2003). This reaction appears to occur in the nucleus due to Rnt1 being concentrated in the nucleus, and the observation that the pre-mRNA cleavage products are degraded by the nuclear Rrp6 and Rat1 nucleases (Danin-Kreiselman *et al.* 2003). Nuclear pre-mRNA degradation has also been proposed for pre-mRNAs that fail to enter splicing or are trapped as lariat intermediates (Bousquet-Antonelli *et al.* 2000).

In contrast to nuclear degradation, multiple experiments suggest that unspliced pre-mRNA are exported to the cytoplasm and degraded. This was first suggested by the observation that the CYH2 pre-mRNA was exported to the cytoplasm and degraded by NMD (He *et al.* 1993). Genome-wide analysis has shown that a number of pre-mRNAs with weak splicing signals are exported to the cytosol and then degraded by NMD (Sayani *et al.* 2008). In addition, in strains with defective splicing machinery, NMD is seen to degrade pre-mRNAs from even more genes (Kawashima *et al.* 2009). Bioinformatics analysis suggests that this is an effective way to monitor introns since intron sequences contain an overrepresentation of translation termination signals and would tend to channel pre-mRNAs that enter translation into NMD (reviewed in Egecioglu and Chanfreau 2011). NMD would also be expected to degrade errors in splice site choice that lead to frameshifting during translation and thereby to premature translation termination.

Unspliced pre-mRNAs can also be degraded in the cytoplasm independently of NMD (Hilleren and Parker 2003), perhaps because the retained intron represses translation

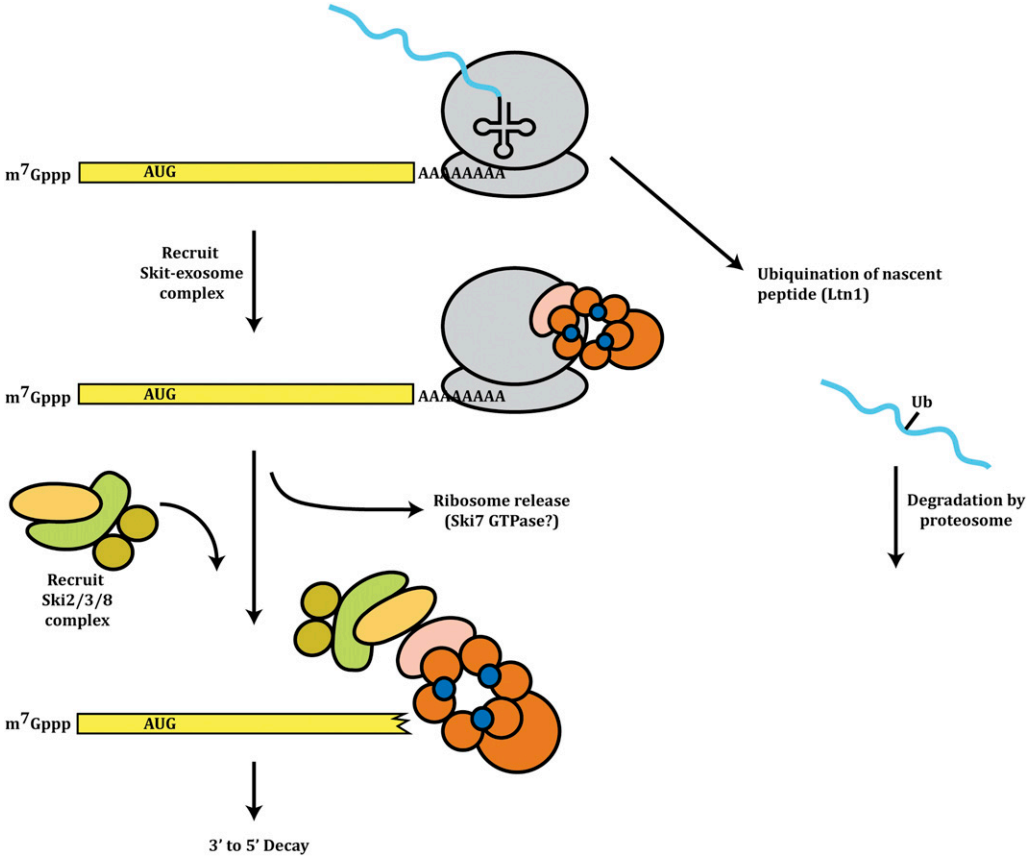

Figure 5 Model for non-stop decay.

initiation and untranslated mRNAs in yeast tend to be rapidly deadenylated and decapped (Muhlrad *et al.* 1995).

Pre-mRNAs can also be degraded after the formation of the lariat intermediate. In this case, when the lariat intermediate is stuck—either due to mutations in the intron (Hilleren and Parker 2003; Mayas *et al.* 2010) or to errors in 5′ splice site choice or stochastic events in endogenous genes (Y. Harigaya and R. Parker, unpublished data)—the 3′ intron–exon lariat is debranched, exported to the cytoplasm, and degraded by Xrn1 or the cytoplasmic exosome. Moreover, the Prp43 ATPase is required for this discard pathway, presumably to facilitate spliceosome disassembly, thereby allowing export (Mayas *et al.* 2010).

Unspliced pre-mRNAs from reporter mRNAs can also be retained at the nuclear pore by the Mlp1 and Mlp2 proteins (Galy *et al.* 2004), although this system appears to part of a more general quality control system for mRNA export that is not limited to pre-mRNAs (Vinciguerra *et al.* 2005).

Quality control of 3′ end generation: Multiple types of mutations lead to defects in mRNA 3′ end generation and polyadenylation. For example, mutations in proteins required for recognition of the polyadenylation site or the poly(A) polymerase Pap1 can alter the site of 3′ end formation and, in the case of *pap1* alleles, lead to the production of unadenylated mRNAs (Patel and Butler 1992; Mandart and Parker 1995). Similarly, defects in mRNA export factors or in the Tho complex, which couples transcription and mRNP assembly, lead to hyperadenylation of mRNAs, perhaps due to a failure to recycle mRNP proteins to the nucleus (Hilleren and Parker 2001; Jensen *et al.* 2001; Libri *et al.* 2002).

Several studies suggest that yeast mRNAs with aberrant 3′ ends are retained at the site of transcription (Hilleren *et al.* 2001; Jensen *et al.* 2001; Thomsen *et al.* 2003). Moreover, this retention appears to be due to the absence of a proper poly(A) tail since mRNAs where the 3′ end is generated by a ribozyme are often retained (Libri *et al.* 2002) unless a poly(A) tract is encoded 5′ of the site of ribozyme cleavage (Dower *et al.* 2004).

The retention of aberrant mRNAs with aberrant ends requires the 3′ to 5′ exonuclease activity of the nuclear exosome and is reduced in *rrp6*Δ strains, or by mutations in the exonuclease active sites of Rrp6 or Dis3/Rrp44 (Hilleren *et al.* 2001; Libri *et al.* 2002; Thomsen *et al.* 2003; Assenholt *et al.* 2008). The role of the exosome suggested that these aberrant mRNAs might be degraded in the nucleus by the nuclear exosome. However, in some cases the actual levels of mRNAs do not increase in the *rrp6*Δ strains, although the mRNAs are no longer nuclear retained, suggesting that the role of the exosome might be to allow retention, and not degrade, the mRNAs (Hilleren *et al.* 2001). In contrast, in other studies it has been suggested that the exosome degrades these mRNAs since, in

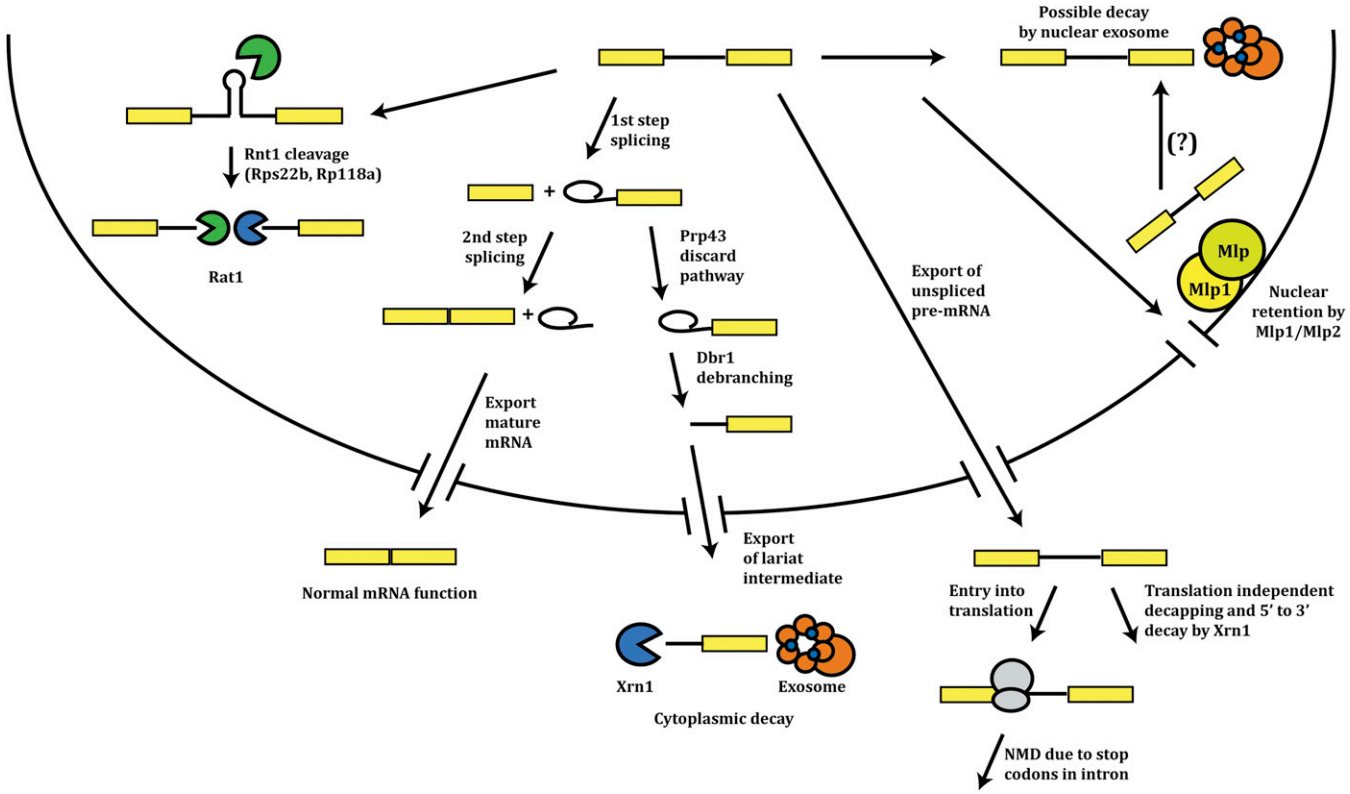

Figure 6 Mechanisms of degradation for unspliced pre-mRNAs.

strains defective in the THO complex, the 3′ end of some mRNAs is reduced compared to the 5′ end, implying that some mRNAs have been degraded in a 3′ to 5′ direction (Libri *et al.* 2002).

In principle, mRNAs generated in mutants with defects in 3′-end formation might be subject to three different fates and that population studies might give misleading results due to different populations behaving in different ways. For example, in export or THO mutant strains a subset of the transcripts is produced with normal 3′-end generation and poly(A) tails, and these appear to undergo normal export, deadenylation, and cytoplasmic decapping (Hilleren and Parker 2001; Libri *et al.* 2002). A second population of mRNAs are those that are produced with either hypo- or hyperadenylated 3′ ends. Strikingly, the observable population of both hyperadenylated and hypoadenylated mRNAs is quite stable, suggesting that these mRNAs are retained within the nucleus and are stable in that state (Mandart and Parker 1995; Hilleren and Parker 2001; Rougemaille *et al.* 2007). Finally, it may be that there is a pool of mRNAs that are recognized as aberrant and subjected to extremely rapid degradation (Rougemaille *et al.* 2007). Since these mRNAs are proposed to degrade very rapidly, it would not be possible to measure their decay rates from steady state as the steady-state pool will consist only of mRNAs that have escaped this rapid degradation. An important issue in future work is determining if such rapid degradation does occur and determining its mechanism.

Intergenic, Intragenic, Promoter-Associated, and Antisense RNAs

From genome-wide analysis it is now clear that yeast cells produce numerous untraditional transcripts. Such transcripts include intergenic and intragenic transcripts (referred to as cryptic unstable transcripts, stable unannotated transcripts, or Xrn1-sensitive unstable transcripts); antisense RNAs; transcripts associated with or overlapping promoter regions (referred to as promoter-associated RNAs); and RNAs from heterochromatin, including telomeric, centromere, and rRNA spacer regions (Olivas *et al.* 1997; Wyers *et al.* 2005; Houseley *et al.* 2007, 2008; Luke *et al.* 2008). These transcripts can be produced from bidirectional transcription from promoters of known genes (Neil *et al.* 2009; Xu *et al.* 2009) or from distinct promoters (Rhee and Pugh 2012) and have been suggested to have a variety of functions. For example, some antisense RNAs, or their act of transcription, can play a role in modulating the expression of the corresponding sense gene via histone modification (Camblong *et al.* 2007; Berretta *et al.* 2008; Houseley *et al.* 2008; Geisler *et al.* 2012). At least in the case of the GAL1 mRNA, the degradation of an antisense RNA appears to be required for efficient induction of the corresponding sense transcript

(Geisler *et al.* 2012). This might be a general feature of genes induced by environmental stimuli since antisense RNAs stabilized in *xrn1*Δ and *dcp2*Δ strains are often antisense to induced genes (Van Djik *et al.* 2011; Geisler *et al.* 2012), An important and unresolved future issue will be determining why degradation of this antisense RNA is required for efficient gene induction.

In other cases, transcripts that overlap the promoter, or even the ORF, can alter the transcription of the associated mRNA (*e.g.*, Martens *et al.* 2004; Thiebaut *et al.* 2008; Huang *et al.* 2010; Toesca *et al.* 2011). Finally, since some of these RNAs are associated with ribosomes and can contain short ORFs, it seems likely that some of these RNAs encode short polypeptides (Olivas *et al.* 1997; Thompson and Parker 2007; Wilson and Masel 2011). Because many of these RNAs are most easily detected in strains defective in RNA degradation pathways, it is thought that many such RNAs are highly unstable.

This set of RNAs is subject to a variety of different and overlapping RNA degradation pathways. For example, many intergenic, antisense, and promoter-associated transcripts increase in levels and/or show increased stability in *xrn1*Δ, *dcp1*Δ, or *dcp2*Δ strains, suggesting that they are subject to degradation by decapping and Xrn1 action (Thompson and Parker 2007; Berretta *et al.* 2008; Van Dijk *et al.* 2011; Geisler *et al.* 2012). A significant number of intergenic RNAs are also increased in strains defective in NMD, suggesting that these RNAs might enter translation and then be recognized as aberrant RNAs and degraded by NMD (Thompson and Parker 2007; Toesca *et al.* 2011). Some of these RNAs also appear to be degraded in a mechanism dependent on RNaseP (Marvin *et al.* 2011).

Numerous studies have also shown that intergenic, intragenic, promoter-associated, and antisense RNAs increase in levels in strains defective in the nuclear exosome, most commonly the Rrp6 subunit, leading to the suggestion that these RNAs are subject to nuclear 3′ to 5′ degradation (Wyers *et al.* 2005; Davis and Ares 2006; Thompson and Parker 2007; Milligan *et al.* 2008; Lardenois *et al.* 2011). However, examination of RNA decay rates has shown that while the steady-state levels of RNA actually increase for many RNAs in *rrp6*Δ strains, the actual decay rates increase and appear dependent on Xrn1 (Thompson and Parker 2007), although some reports indicate an increased half-life in *rrp6*Δ strains (Wyers *et al.* 2005). One possibility is that *rrp6*Δ strains show increased transcription, but this model is inconsistent with nuclear run-on and RNA polymerase II chromatin immunoprecipitation experiments (Wyers *et al.* 2005; Rougemaille *et al.* 2007). An alternative is that the nuclear degradation of such transcripts occurs extremely rapidly and cannot be measured at steady state, an issue that remains to be rigorously resolved in future work.

The reduction in cryptic unstable transcript and promoter-associated RNA levels by the nuclear exosome is facilitated by the TRAMP complexes (Lacava *et al.* 2005; Wyers *et al.* 2005; Egecioglu *et al.* 2006; Houseley *et al.* 2007; San Paolo *et al.* 2009; Callahan and Butler 2010). The TRAMP complexes consist of one of two related RNA-binding proteins, Air1 and Air2, the Mtr4 RNA helicase, and one of two noncanonical poly(A) polymerases, Trf4 or Trf5 (Lacava *et al.* 2005; Vanacova *et al.* 2005; Wyers *et al.* 2005). Analogous to the situation in bacteria, the TRAMP complex can promote 3′ to 5′ degradation of RNAs by the addition of poly(A) tails to their 3′ end, which is then thought to promote the targeting of those RNAs to the exosome (Kadaba *et al.* 2004; Lacava *et al.* 2005; Vanacova *et al.* 2005; Wyers *et al.* 2005). However, in some cases the TRAMP complex can also promote exosome-mediated RNA degradation independently of polyadenylation activity and may in that case serve as a scaffolding complex to recruit the exosome to the RNA (Houseley *et al.* 2007; Rougemaille *et al.* 2007; San Paolo *et al.* 2009). The TRAMP complexes also serve important roles in targeting the exosome for degradation or processing of small nucleolar RNAs (snoRNAs), snRNAs, tRNA, and other RNA species (see below).

The targeting of RNAs to the TRAMP and exosome complexes appears to be modulated in part by the Nrd1 and Nab3 RNA-binding proteins. The Nrd1 and Nab3 proteins bind to specific elements in nascent transcripts (Creamer *et al.* 2011; Jamonnak *et al.* 2011; Wlotzka *et al.* 2011) and can direct termination of transcription for snoRNAs, snRNAs, some mRNAs, and numerous intergenic transcripts (Arigo *et al.* 2006; Thiebaut *et al.* 2006; Carroll *et al.* 2007; Rondon *et al.* 2009; Kim and Levin 2011). Nrd1 and Nab3 associate with the carboxy-terminal doman of RNA polymerase II when phosphorylated at serine 5 and serine 7 (Gudipati *et al.* 2008; Vasiljeva *et al.* 2008; Kim *et al.* 2010) and may act preferentially on shorter transcripts as serine 5 and 7 phosphorylation can be lost with ongoing elongation (Komarnitsky *et al.* 2000; Schroeder *et al.* 2000). Nrd1 and Nab3 then appear to recruit the exosome to degrade intergenic transcripts or to promote processing of snoRNAs and snRNAs (Arigo *et al.* 2006; Thiebaut *et al.* 2006; Grzechnik and Kufel 2008; Vasiljeva *et al.* 2008). This effect is not limited to RNA polymerase II transcripts as Nab3 and Nrd1 can also target RNA polymerase III transcripts for polyadenylation and degradation (Wlotzka *et al.* 2011).

Decay of tRNA, rRNAs, snRNAs, and snoRNAs

Most functional noncoding RNAs such as rRNAs, tRNAs, snRNAs, and snoRNAs are quite stable, although they are probably degraded at some rate even under optimal growth conditions. However, these RNAs are clearly subject to increased turnover during nutrient limitations (see below) and are also subject to a variety of quality control systems to ensure their proper biogenesis and function.

tRNAs

Three mechanisms can target tRNAs for degradation. First, when tRNAs have defects in their maturation and/or

modification, pre-tRNAs can be adenylated by TRAMP complexes for targeting to the nuclear exosome (Kadaba *et al.* 2004, 2006; Vanacova *et al.* 2005). Similarly, defects in 3′-end processing of pre-tRNAs can also lead to adenylation by TRAMP complexes (Copela *et al.* 2008; Ozanick *et al.* 2009). Adenylation of defective tRNAs is thought to provide a single-stranded extension of RNA that the nuclear exosome can then load on and then degrade through the structured tRNA.

A second mechanism of tRNA decay occurs when tRNAs are defective in their modifications and/or stability of the combined acceptor and T stems. For example, in *trm4*Δ *trm8*Δ strains, which produce undermodified tRNA[Val(AAC)], the tRNA[Val(AAC)] is degraded more rapidly (Alexandrov *et al.* 2006). This degradation utilizes the 5′ to 3′ exonucleases Rat1 and Xrn1 (Chernyakov *et al.* 2008). Moreover, mutations that weaken the stability of the combined acceptor and T stems of the tRNA increase degradation (Whipple *et al.* 2011). This suggests a model wherein the strength of the tRNA structure at the 5′ end dictates whether it can be subject to 5′ to 3′ degradation.

The stability of the 5′ stem loop in tRNAs can also affect whether tRNAs receive an additional CCA addition, which can promote their degradation (Wilusz *et al.* 2011). This occurs when tRNAs have a weak acceptor stem and the first two nucleotides of the tRNA are guanosines. In this case, the tRNA appears to adopt an alternative fold whereby the CCA addition enzyme can add a second CCA, which then appears to promote degradation of the tRNA, perhaps by providing a 3′ single-stranded region for the recruitment of the exosome (Wilusz *et al.* 2011). Strains defective in the CCA addition enzyme also produce tRNAs with shortened 3′ ends that are degraded more rapidly (Aebi *et al.* 1990), although the specific mechanism is not yet known. One anticipates that the stability of tRNAs will also be influenced by tRNA-binding proteins (*e.g.*, eEF1 and synthetases) that will limit nuclease accessibility.

A final mechanism of tRNA cleavage and degradation involves the nuclease Rny1. Rny1 is an RNaseT2 family member that is secreted and targeted to membrane-bound compartments, most notably the vacuole (Macintosh *et al.* 2001; Thompson and Parker 2009). Rny1 has been shown to cleave tRNAs in the anticodon loop, at least during various stresses and high cell density (Thompson *et al.* 2008; Thompson and Parker 2009). Rny1 may be released from membrane compartments to degrade tRNAs during stress, although it remains possible that tRNAs are also imported into the vacuole, or other membrane-bound compartment, by some type of autophagy-related process for degradation by Rny1.

rRNA decay

To date, three types of RNA degradation have been described to occur to rRNAs. First, when rRNA processing or assembly into ribosomes is altered by a variety of mutations, the defective rRNA is targeted for degradation by polyadenylation by TRAMP complexes and recruitment of the nuclear exosome (*e.g.*, Allmang *et al.* 2000; Kuai *et al.* 2004; Fang *et al.* 2005; Lacava *et al.* 2005; Dez *et al.* 2006; Kadaba *et al.* 2006). Polyadenylated rRNA species can also be detected in strains defective in nuclear exosome function, suggesting that a certain percentage of "normal" rRNA transcripts, either due to errors in transcription, processing, or assembly, are subject to this nuclear RNA degradation pathway.

Ribosomes that are accurately assembled but fail to function are exported to the cytoplasm and degraded by a process referred to as nonfunctional ribosomal decay. For 25S rRNAs that are defective in peptidyl transferase activity, the 60S subunit appears to be recognized as aberrant prior to assembly into 80S complexes and targeted for degradation by ubiquitination in a process dependent on the ubiquitin ligase Rtt101 and its associated protein Mms1 (Lariviere *et al.* 2006; Fujii *et al.* 2009). The exact nuclease(s) that degrades these defective 25S RNAs is not yet known, although the exosome may have some role (Cole *et al.* 2009).

For the small ribosomal subunit, mutations in the decoding site of the 18S rRNA lead to 40S complexes that can assemble into 80S complexes on the translation start site, but appear to be blocked for subsequent elongation (Lariviere *et al.* 2006). In this case, the degradation of the defective 18S rRNAs utilizes the same Dom34/Hbs1 complex that is involved in the release of ribosomes stalled by defects in the mRNA (NGD; see above), which leads to degradation by the cytoplasmic exosome as well as Xrn1 (Cole *et al.* 2009).

rRNAs are also subject to degradation during stress or nutrient limitations. For example, in response to oxidative stress or entry into stationary phase, the rRNAs are fragmented to some extent (Mroczek and Kufel 2008; Thompson *et al.* 2008). This degradation is at least partially dependent on the Rny1 nuclease, which is concentrated in vacuoles (Thompson and Parker 2009). One possibility is that these stress conditions lead to release of Rny1 from the vacuole and cleavage of cytoplasmic rRNAs (Thompson and Parker 2009). Since the Nuc1 nuclease is also released from mitochondria under these conditions (Buttner *et al.* 2007), it might also play some role in rRNA degradation during stress. Another possibility is that rRNAs are targeted to vacuoles under these conditions by selective autophagy. Consistent with that model, based on following GFP-tagged ribosomal proteins, ribosomes have been inferred to be targeted to vacuoles during nitrogen starvation in a process referred to as ribophagy, which requires ubiquitination and de-ubiquitination (Kraft *et al.* 2008; Ossareh-Nazari *et al.* 2010). Ribosomes may also be targeted for degradation in the vacuole by a piecemeal microautophagy of the nucleus, wherein regions of the nucleus are directly targeted to the vacuole by invagination of the nuclear envelope into the vacuolar lumen (Roberts *et al.* 2003).

snRNAs/snoRNAs

Several observations suggest that defects in snRNP or snoRNP assembly lead to degradation of these RNA species.

For example, mutations in the Naf1 protein, which is required for the assembly of H/ACA snoRNPs, lead to the loss of that class of snoRNAs (Fatica *et al.* 2002; Dez *et al.* 2002). Similarly, mutations in the SM-binding sites of yeast U1, U2, and U5 or the telomerase RNA and TLC1 lead to decreased levels of these RNAs (Jones and Guthrie 1990; Seipelt *et al.* 1999; Seto *et al.* 1999). Although effects on transcription have not been ruled out, the simplest model is that the failure to properly assemble the snoRNP/snRNPs leads to their accelerated degradation by a yet-to-be-determined degradation pathway.

Defects in U6 snRNA biogenesis also give increased decay of RNA. For example, an internal deletion within the U6 snRNA leads to unstable transcripts, which are adenylated and degraded by the nuclear exosome (Kadaba *et al.* 2006). Similarly, defects in the Lsm2–8 complex, which binds the 3′ end of the U6 snRNA, lead to decreased levels of the U6 snRNA (Pannone *et al.* 2001, 1998; Luhtala and Parker 2009), although the mechanism of U6 degradation in this case is not known. Taken together, these observations argue that systems exist to degrade snRNAs and snoRNAs that fail to be properly assembled into RNPs.

Degradation of RNAs in Mitochondria

tRNAs, rRNAs, and mRNAs are also transcribed within the mitochondria of yeast cells in 13 complex transcription units. These transcripts are subject to RNA processing, including splicing of group I and group II introns, endonuclease cleavage, and 5′ and 3′ trimming. The biogenesis of mitochondrial mRNAs typically involves a 5′ endonucleolytic cleavage, often followed by 5′ to 3′ trimming of the exposed 5′ end to specific sites and a 3′ endonuclease cleavage just downstream of a so-called dodecamer element (Dieckmann and Staples 1994). The dodecamer element is proposed to bind an unknown protein factor and may play a role in translation and/or stabilization of the mRNAs (reviewed in Gagliardi *et al.* 2004). In contrast to cytoplasmic mRNAs, mitochondrial mRNAs in yeast are not polyadenylated.

The major functional RNAse in the mitochondria mechanism is termed the mitochondrial degradasome or mtEXO and consists of a 1:1 ratio of Dss1, a member of the RNaseII family, and Suv3, a DEVH family member. Strains defective in Suv3 or Dss1 show accumulation of mitochondrial mRNAs, indicating a defect in their turnover (Dziembowski *et al.* 2003; Malecki *et al.* 2008). Suv3 and Dss1 mutants also accumulate excised introns, indicating that mtEXO is required for their degradation (Szczesny *et al.* 2011). Interestingly, such strains also accumulate incompletely processed mitochondrial rRNAs, which has led to the suggestion that the mtEXO also functions in a quality control system for the degradation of rRNAs or mRNAs that are not completely processed. The activity of the mtEXO on mitochondrial mRNAs may be limited by the 3′ features of mRNAs, possibly including a factor binding the dodecamer element (Min and Zassenhaus 1993).

The activity of mtEXO is due to the concerted action of the Suv3 helicase activity and the Dss1 nuclease in response to a RNA substrate. For example, Suv3 has intrinsic ATPase activity, but, in a complex with Dss1, the ATPase actitivy is enhanced by a single-stranded nucleic acid substrate (Malecki *et al.* 2007). Moreover, the helicase activity of Suv3 is dependent on Dss1 (Malecki *et al.* 2007). Similarly, the exonuclease activity of Dss1 is increased by Suv3 and made dependent on ATP (Malecki *et al.* 2007). These observations suggest a working model whereby Suv3 acts as an ATP-dependent motor to feed RNA into the active site of Dss1 (Szczesny *et al.* 2011).

There is also a 5′ to 3′ exonuclease activity in mitochondria that is dependent on the Pet127 protein (Wiesenberger and Fox 1997; Fekete *et al.* 2008), although whether Pet127 is actually a nuclease remains to be determined. The Pet127-dependent 5′ to 3′ exonuclease may also be able to stimulate and/or degrade mitochondrial RNAs since dominant mutations in, or overexpression of, Pet127 partially suppresses the defects seen in *suv3* or *dss1* mutants (Wegierski *et al.* 1998; Chen *et al.* 1999). Strains lacking Pet127 also show misregulation of some mitochondrial mRNAs, although whether this is due to misprocessing of their 5′ ends or due to disruption of normal mRNA degradation pathways is not yet clear (Wiesenberger and Fox 1997; Fekete *et al.* 2008).

Two additional nucleases have been localized to mitochondria but do not appear to affect RNA degradation. These include Nuc1, which is the major DNAse/RNAse in mitochondria by enzymatic activity (Dake *et al.* 1988; Vincent *et al.* 1988), and Rex2, which is a 3′ to 5′ exonuclease that is found both in the mitochondria (Hanekamp and Thorsness 1999) and in the nucleus, where it plays a role in the trimming of 5S and 5.8S rRNAs and U4 and U5 snRNA (Van Hoof *et al.* 2000a). Although no clear role for these nucleases has been identified in yeast mitochondrial RNA degradation, it is notable that *nuc1*Δ and *rex2*Δ strains show a negative genetic interaction for growth, suggesting some degree of functional overlap (Costanzo *et al.* 2010).

Future Perspectives

There are several areas of importance in the future study of RNA turnover in yeast cells. For example, although many RNA degradation pathways, enzymes, and cofactors have been identified, a precise understanding of their biochemical function and mechanisms of action will need further experimentation, including the development of robust *in vitro* systems for mechanistic studies. Moreover, one anticipates that there are yet-to-be-identified nucleases that play roles in RNA degradation.

It will also be important to understand how RNA degradation systems interface with other cellular processes. Although this is a general issue, three examples stand out. First, the tight inverse coupling between translation and mRNA degradation highlights that an understanding of the regulation of mRNA function will require mechanistic

insight into how mRNAs transition between mRNP states capable of translation or deadenylation and mRNA decapping. A second, and related issue, is that an understanding of mRNA degradation will require a broader understanding of mRNP biogenesis, dynamics, and how the specific proteins bound to individual mRNAs act, either in isolation or in combination, to modulate translation and mRNA degradation.

A third area of interest will be in determining the relationship between mRNA degradation and transcription. This issue is of interest since several observations suggest that transcription and mRNA degradation are coupled. For example, *dcp1*Δ strains were observed to have prolonged decay rates of reporter mRNAs without a corresponding increase in steady-state levels, suggesting alterations in transcription (Muhlrad and Parker 1999b). In addition, strains lacking the decapping activator Edc1 were unable to induce new transcription during a shift in carbon source (Schwartz *et al.* 2003). Moreover, the Rpb4 and Rpb7 subunits of RNA polymerase II were shown to shuttle to the cytoplasm and to affect the decay of mRNAs (Lotan *et al.* 2005, 2007; Selitrennik *et al.* 2006) in a manner coupled to their recruitment to RNA polymerase II (Goler-Baron *et al.* 2008). This has led to the suggestion that transcription of new mRNAs is dependent on decay of pre-existing mRNAs in the cytosol, which would be an elegant feedback mechanism to couple mRNA biogenesis and degradation (Dori and Choder 2007). More recently, it has been suggested that the rate of mRNA degradation can be determined by the promoter (Bregman *et al.* 2011; Trcek *et al.* 2011), possibly through the loading of proteins on the nascent transcript in a promoter-dependent manner. Given these new-found connections between mRNA biogenesis and degradation, it will be important to determine how the mechanisms by which these two processes are coupled, whether this is general to most mRNAs and even other RNA classes, and the how such coupling is used by the cell to regulate gene expression in response to environmental cues.

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