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Review

Nonsense-mediated mRNA decay in *Saccharomyces cerevisiae*

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Abstract

Cell survival depends on the precise and correct production of polypeptides. Eukaryotic cells have evolved conserved proofreading mechanisms to get rid of incomplete and potentially deleterious proteins. The nonsense-mediated mRNA decay (NMD) pathway is an example of a surveillance mechanism that monitors premature translation termination and promotes degradation of aberrant transcripts that code for nonfunctional or even harmful proteins. In this review we will describe our current knowledge of the NMD pathway, analyzing primarily the results obtained from the yeast *Saccharomyces cerevisiae*, but establishing functional comparisons with those obtained in higher eukaryotes. Based on these observations, we present two related working models to explain how this surveillance pathway recognizes and selectively degrades aberrant mRNAs. © 2001 Published by Elsevier Science B.V.

Keywords: mRNA decay; Translation; Premature termination codon; Downstream sequence element; Heterogeneous nuclear ribonucleoprotein

1. Introduction

The rate of protein synthesis in eukaryotic cells is determined by multiple regulatory events at different levels. Although transcription serves as the initial process at which gene expression can be controlled, it has become clear that mRNA turnover also plays a major role in determining the final level of the protein product. In eukaryotic cells, mRNA decay rates can vary by more than 50-fold. In *Saccharomyces cerevisiae*, for example, some mRNAs have half-lives of about 60 min, while very unstable messages exhibit half-lives as short as 1 min (Peltz and Jacobson, 1993). Several studies have suggested that mRNA decay rates can be modulated in response to environmental cues (Ross, 1995; Gonzalez and Martin, 1996; Jarzembowski and Malter, 1997; Scheffler et al., 1998). In addition, mRNA turnover plays a role in the gene-specific silencing induced by double-stranded RNA (Fire, 1999). Moreover, the expression of many highly regulated transcripts involved in cellular proliferation and differentiation is controlled by mRNA stability. Altered control of their mRNA turnover rates has been shown to result in aberrant gene expression and disease, including cancer, immunological disorders and coronary disease (Schuler and Cole, 1988; Raymond et al., 1989; Greenberg et al., 1990; Taylor et al., 1996; Jarzembowski and Malter, 1997). While the importance of mRNA turnover to the regulation of gene expression has gained much interest in recent years, only now are the mechanisms that control mRNA decay rates being unraveled (for review, see Schwartz and Parker, 2000; McCarthy, 1998; Jacobson and Peltz, 2000).

2. mRNA decay occurs through multiple pathways

Several pathways of mRNA decay have been described. These mechanisms potentially provide different alternatives by which transcript half-lives are controlled. In yeast, one major mRNA turnover pathway is initiated with poly(A) shortening to an oligo(A) tail. Recent studies have identified the Pan2/Pan3 complex and the Ccr4 and Caf1 transcription

Abbreviations: DSE, downstream sequence element; eIF, eukaryotic initiation factor; eRF, eukaryotic release factor; *HRP1*, heterogeneous nuclear ribonucleoprotein 1; NMD, nonsense-mediated mRNA decay; ns, nonsense-containing; *PGK1*, phosphoglyceratekinase 1; poly(A), polyadenylate; poly(U), polyuridylate; RNP, ribonucleoprotein; STE, stabilizer element; uORF, upstream open reading frame; *UPF*, up-frameshift; UTR, untranslated region; wt, wild-type

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factors as part of a large complex that possesses deadenylating activity (Boeck et al., 1996; Brown and Sachs, 1998; Tucker et al., 2001). After poly(A) shortening, transcripts are cleaved one or two nucleotides from their 5′ ends by the *DCP1* gene product (Stevens, 1988; Beelman et al., 1996; LaGrandeur and Parker, 1998). Additional factors involved in decapping have been recently identified, including Dcp2, Vps16, Pat1 and the Lsm protein complex (Dunckley and Parker, 1999; Zhang et al., 1999; Hatfield et al., 1996; Bonnerot et al., 2000; Tharun et al., 2000; Bouveret et al., 2000). Decapped and deadenylated transcripts are subsequently digested exonucleolytically by the 5′ to 3′ exoribonuclease Xrn1p (Decker and Parker, 1993; Hsu and Stevens, 1993; Muhlrad et al., 1994). Alternatively, some yeast transcripts can also be degraded 3′ to 5′ after deadenylation (Muhlrad et al., 1995; Jacobs et al., 1998). A third mode of mRNA decay for some vertebrate transcripts may be triggered by a site-specific endonucleolytic cleavage (Brown et al., 1993; Binder et al., 1994; Chernokalskaya et al., 1998; Cunningham et al., 2000; Wang and Kiledjian, 2000a,b). Another pathway of mRNA decay in yeast has been demonstrated for transcripts that contain a premature translation termination codon (Jacobson and Peltz, 2000). This process is referred to as nonsense-mediated mRNA decay (NMD) and will be the main focus of this review.

Recent evidence suggests that mRNA turnover pathways are conserved between yeast and higher eukaryotes. For example, many mammalian mRNAs are deadenylated prior to further degradation of the transcript (Shyu et al., 1991; Couttet et al., 1997). In addition, several homologues of proteins involved in yeast mRNA decay have been identified in mammals. These include the Xrn1p (Bashkirov et al., 1997), factors involved in the NMD pathway (Perlick et al., 1996; Applequist et al., 1997; Czaplinski et al., 1998; Page et al., 1999; Mendell et al., 2000; Serin et al., 2001) and the exosome complex that is responsible for 3′ to 5′ degradation (Jacobs et al., 1998). Mammalian homologues of the yeast Lsm proteins and the Dcp2 protein have recently been identified (Dunckley and Parker, 1999; Achsel et al., 1999), however, their functions in the regulation of decapping remains to be determined.

3. mRNA turnover and translation are intimately connected

The data obtained from studies of several mRNA turnover pathways indicate that the processes of mRNA turnover and translation are intimately connected. These findings include: (a) inhibition of translational elongation by cycloheximide, as a consequence of tRNA mutations, and by the presence of secondary structure that prevents ribosome scanning, can reduce mRNA decay rates (Herrick et al., 1990; Beelman and Parker, 1994; Peltz et al., 1992; Muhlrad et al., 1995; Zuk and Jacobson, 1998); (b) instability elements are located within mRNA coding regions and their destabilizing function depends on ribosome translocation (He et al., 1993; Aharon and Schneider, 1993); and (c) premature translational termination can enhance mRNA decay rates (reviewed in Jacobson and Peltz, 2000). In addition, an emerging topic establishes that the relationship between mRNA turnover and translation is controlled by the translation initiation efficiency of any given transcript. Recent evidence has suggested that the decapping step of mRNA turnover is controlled by a competition between translation initiation factors and the decapping enzyme (Muhlrad and Parker, 1999a,b; Vilela et al., 2000; Schwartz and Parker, 1999, 2000). Data from these experiments have shown that the mRNA cap structure is the site for both decapping and the assembly of the eIF-4F complex (cap-binding complex), which enhances translation initiation. The general idea is that mRNAs that are translated efficiently are not available to deadenylating and decapping factors that activate rapid mRNA decay due to the binding of the translation initiation complex. Conversely, the binding of the mRNA by factors involved in turnover can reduce the translational efficiency of the transcript. A clear understanding of this relationship will play a dominant role in the elucidation of how cellular mRNA levels are controlled in order to regulate gene expression.

4. NMD ensures a high level of accuracy

As mentioned above, a clear example of the relationship between translation and mRNA turnover is the observation that nonsense mutations in a gene can reduce the steady-state levels of the mRNA transcribed from that gene via the NMD pathway (Czaplinski et al., 1999; Jacobson and Peltz, 2000). This cellular process is an example of a surveillance mechanism that eliminates aberrant mRNAs that contain nonsense mutations within the protein coding region (Hilleren and Parker, 1999; Hentze and Kulozik, 1999; Maquat, 2000). The NMD pathway has been observed in all eukaryotic cells examined (Hentze and Kulozik, 1999; Maquat, 2000; Maquat and Carmichael, 2001; Pulak and Anderson, 1993; Hall and Thein, 1994; Bedwell et al., 1997; Li and Wilkinson, 1998) and it seems to have evolved as a quality control mechanism to ensure that translation termination occurs at the appropriate location within the mRNA. Transcripts harboring premature nonsense codons are degraded rapidly, preventing the synthesis of incomplete and potentially deleterious proteins that could dominantly interfere with the normal functioning of the cell. The phenomenon of NMD is shown in Fig. 1. The PGK1 transcript in the yeast *S. cerevisiae* is very stable with a half-life of greater than 45 min. Insertion of a premature termination codon, however, reduces its decay rate to less than 3 min.

Although NMD was first identified as a pathway that degrades nonsense-containing transcripts, additional results have shown that intron-containing pre-mRNAs that enter the cytoplasm (He et al., 1993), mRNAs that are subjected

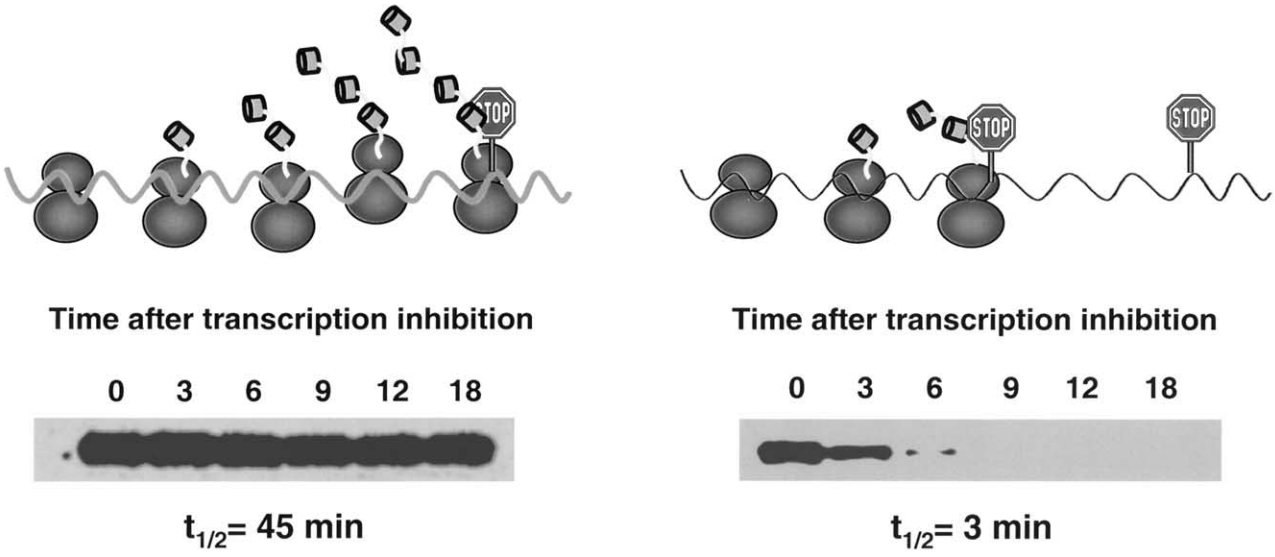

Fig. 1. The effect of a premature termination codon on the decay of the PGK1 mRNA. Decay rates for the mRNAs encoded by the wild-type and the nonsense-containing PGK1 alleles were determined by Northern blot analysis of RNAs isolated at different times after transcription was inhibited by a shift from 24 to 37°C in a yeast strain harboring a temperature-sensitive RNA polymerase II.

to leaky scanning (Welch and Jacobson, 1999), mRNAs harboring upstream open reading frames (uORFs) (Cui et al., 1995; Vilela et al., 1998, 1999; Ruiz-Echevarria and Peltz, 2000), and transcripts with extended 3′ UTRs (Muhlrad and Parker, 1999a,b; Das et al., 2000) are also substrates of this turnover pathway. More recently, results obtained from oligo DNA arrays have shown that more than 225 of the approximately 6000 transcripts of *S. cerevisiae* are affected by inactivation of the NMD pathway (Lelivelt and Culbertson, 1999). In addition, this surveillance mechanism appears to have a role in the regulation of telomeric length (Lew et al., 1998), the levels of kinetochore subunits (Dahlseid et al., 1998), and the maintenance of the double-stranded RNA interference phenotype as demonstrated in *Caenorhabditis elegans* (Domeier et al., 2000).

5. NMD, a translational-dependent mechanism, occurs via a deadenylation-independent decapping of the transcript

In the yeast, *S. cerevisiae*, the activity of the NMD pathway depends on the recognition of the premature stop codon by the translational machinery. Many studies indicate that the NMD pathway in yeast is a translational-dependent event. The following results support this conclusion. (1) NMD is inhibited by drugs and mutations that block translation initiation and elongation (Herrick et al., 1990; Peltz et al., 1992; Zhang et al., 1997; Welch and Jacobson, 1999; Zuk and Jacobson, 1998). (2) Nonsense-containing mRNAs are associated with polysomes whose size reflects the position of the premature termination codon within the open reading frame (He et al., 1993). (3) NMD can be prevented by nonsense-suppressing tRNAs (Losson and Lacroute, 1979; Gozalbo and Hohmann, 1990). (4) The rapid turnover of nonsense-containing mRNAs resumes immediately after the drug cycloheximide is washed out from the growth medium (Zhang et al., 1997). (5) The NMD pathway is functional only after at least one translation initiation/termination cycle has been completed (Ruiz-Echevarria et al., 1998a,b). (6) Factors essential for NMD interact with the translation termination release factors eRF1 and eRF3 (Czaplinski et al., 1998; Wang et al., 2001; see below).

As mentioned previously, the degradation of most wild-type mRNAs occurs via a deadenylation-dependent decapping followed by a 5′ to 3′ degradation of the main body of the transcript (Stevens, 1988; Beelman et al., 1996; LaGrandeur and Parker, 1998; Decker and Parker, 1993; Hsu and Stevens, 1993; Muhlrad et al., 1994). In contrast, the turnover of nonsense-containing mRNAs is deadenylation-independent, entering the predominant 5′ to 3′ decay pathway with an intact poly(A) tail (Muhlrad et al., 1994; Hagan et al., 1995; Beelman et al., 1996). These nonsense-containing mRNAs are then decapped by Dcp1p, followed by a 5′ to 3′ degradation of the body of the transcript by the Xrn1p exoribonuclease.

6. Characterization of trans-acting factors involved in NMD

In yeast, mutants that inactivate the NMD pathway were initially isolated in a genetic screen to identify allosuppressors of the *his4-38* frameshift mutation (Culbertson et al., 1980). Subsequent screens designed to identify genes that affect programmed −1 ribosomal frameshifting efficiencies or suppressors of upstream initiation codons identified additional components of the NMD pathway (Hampsey et al.,

1991; Pinto et al., 1992; Dinman and Wickner, 1994; Cui et al., 1995, 1996, 1998, 1999; He and Jacobson, 1995). Analysis of the genes identified in these studies demonstrated that mutations in the *UPF1*, *UPF2/NMD2*, *UPF3*, *MOF2/SUI1*, *MOF5*, *MOF8*, *PRT1* and *HRP1/NAB4* genes result in the stabilization of nonsense-containing mRNAs while having no effect on the stability of most wild-type transcripts (Leeds et al., 1991, 1992; Cui et al., 1995, 1996, 1998, 1999; He and Jacobson, 1995; Welch and Jacobson, 1999; Gonzalez et al., 2000). An example of the effect of inactivation of the NMD pathway is shown in Fig. 2. The nonsense-containing PGK1 mRNA is a substrate of the NMD pathway. As a consequence, this transcript is stabilized in strains harboring mutations in the *UPF1*, *UPF2*, *UPF3* and *HRP1* genes while the decay rates of the wild-type MFA2 and OLE1 mRNA were not altered.

The Upf1, Upf2, and Upf3 proteins have been the most extensively investigated components of the NMD pathway in the yeast *S. cerevisiae* (for review, see Czaplinski et al., 1999; Jacobson and Peltz, 2000). The *UPF1* gene encodes a protein with a predicted mass of 109 kDa, and it contains a cysteine- and histidine-rich region near its amino terminal domain and all the motifs required to be a member of the superfamily group I helicases (Altamura et al., 1992; Koonin, 1992; Leeds et al., 1992; Atkin et al., 1997). In addition, the Upf1p has been purified and demonstrates RNA binding, RNA-dependent ATPase and RNA helicase activities (Czaplinski et al., 1995, 1999; Weng et al., 1996a,b, 1998). *UPF2* encodes an acidic protein with a predicted mass of 127 kDa (Cui et al., 1995; He and Jacobson, 1995). *UPF3* encodes a basic 45 kDa protein harboring several nuclear localization signals and nuclear export sequences and it has been previously shown that it shuttles from the nucleus to the cytoplasm (Lee and Culbertson, 1995; Shirley et al., 1998). The Upf1, Upf2 and Upf3 have been shown to interact and form a complex (He and Jacobson, 1995; Weng et al., 1996a,b; He et al., 1997). Based on these results, the existence of a surveillance complex required for the degradation of nonsense-containing transcripts consisting of at least Upf1p, Upf2p, and Upf3p has been suggested. Single or multiple mutations of the *UPF* genes have the same effect on mRNA decay, further suggesting that these proteins function in a complex.

Interestingly, mutations in the *UPF1* gene not only result in stabilization of nonsense-containing RNAs, but they also lead to suppression of certain nonsense alleles (Leeds et al., 1992; Weng et al., 1996a,b; Czaplinski et al., 1998). Nonsense suppression occurs when a near cognate tRNA successfully competes with the translation termination factors at a nonsense codon and amino acid incorporation occurs rather than premature termination. Under normal conditions the presence of a nonsense codon in an essential gene would cause premature termination and complete loss of gene function and lethality. However, when nonsense suppression occurs sufficient levels of the full-length protein are produced that may allow restoration of normal cell growth. A role for the Upf1 protein in translation termination first became evident when a set of mutations was isolated in the *UPF1* gene that separated the mRNA decay function from its activity in modulating premature termination (Weng et al., 1996a,b). Subsequent studies have shown that Upf1p interacts with the termination release factors eRF1 and eRF3 (Czaplinski et al., 1998, 1999).

Further evidence for an active role of these proteins in modulating the translation of nonsense-containing transcripts has been provided by experiments showing that deletion of either *UPF2* or *UPF3* can also lead to a nonsense suppression phenotype (Maderazo et al., 2000; Wang et al., 2001). In addition, more recent evidence indicates that Upf2p and Upf3p indeed function to enhance the termina-

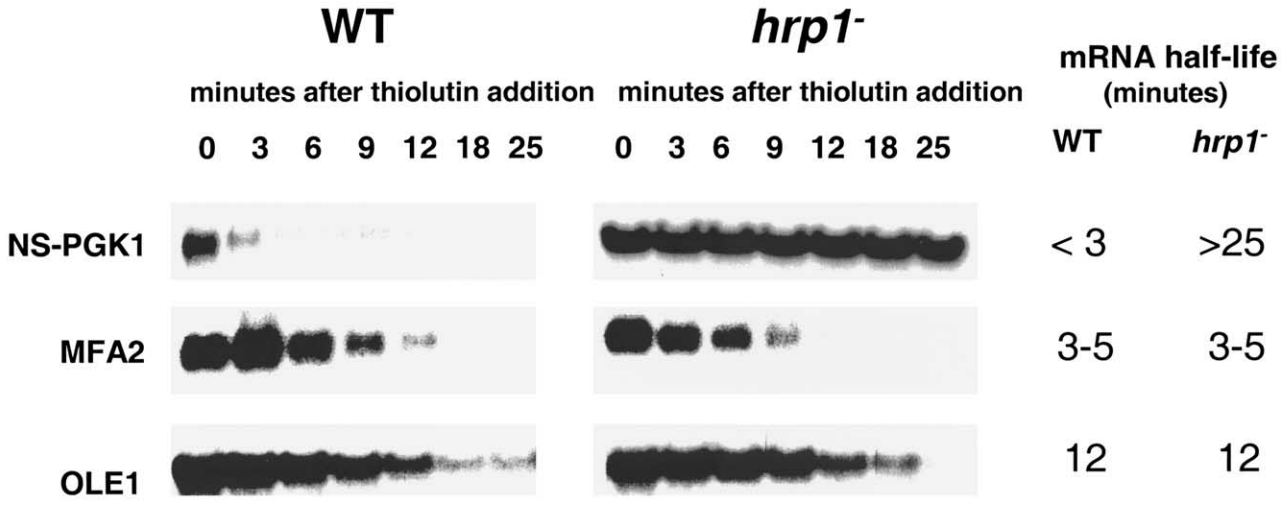

Fig. 2. Mutations in *HRP1* specifically affect the NMD pathway. The effects of a temperature-sensitive *hrp1*$^{-}$ allele on the decay rates of a nonsense-containing PGK1mRNA and the wild-type MFA2 and OLE1 transcripts are shown. The cell cultures were incubated for 1 h at 37°C to inactivate the temperature-sensitive *hrp1*$^{-}$ allele. Following this incubation, thiolutin was added to block transcription, total RNA was isolated and the mRNA decay rates were determined by Northern blotting (Gonzalez et al., 2000).

tion efficiency of the premature stop codon through interactions with release factor eRF3 (Wang et al., 2001). Interestingly, neither Upf2p nor Upf3p interact with eRF1. In fact, through *in vitro* competition experiments, it has been demonstrated that Upf2p, Upf3p and eRF1 actually compete with each other for binding to eRF3. All together, these results suggest that upon recognition of the premature stop codon, the ribosome pauses allowing the assembly of various 'transient' complexes involving the Upf proteins and the termination release factors that modulate translation termination before the mature surveillance complex becomes committed to scanning and degrading the aberrant RNA (see below).

7. Cis-acting sequences involved in NMD

7.1. Instability elements

A necessary decision point in the NMD pathway is the discrimination of a premature translation termination event from a normal one. It has been proposed that the translation termination event triggers the assembly of the surveillance complex (Czaplinski et al., 1998; Wang et al., 2001). This is followed by determining whether the translation termination was premature, leading to decay of the aberrant transcript. One possibility is that the distance between the termination codon and the poly(A) site determines whether the mRNA becomes a substrate of the NMD pathway (Muhlrad and Parker, 1999a,b). However, several studies using the 5′ UTR of the GCN4 mRNA as a model system demonstrated that increasing the distance from the termination codon to the poly(A) site did not trigger NMD (Ruiz-Echevarria and Peltz, 1996). Rather, these results showed that a nonsense codon is recognized as aberrant due to downstream sequence elements (DSE) located 3′ of a premature termination codon (Peltz et al., 1993; Zhang et al., 1995). Transcripts containing a premature termination codon, but lacking a DSE, are not degraded by the NMD pathway; introduction of a DSE 3′ of the GCN4 uORF1, however, activates the NMD pathway (Ruiz-Echevarria and Peltz, 1996). Thus, the signal required for activation of NMD appears to be a dual element consisting of a premature termination codon and a 3′ DSE.

The sequences in the DSE important for activation of the NMD pathway have been characterized in several transcripts and have been reviewed elsewhere (Zhang et al., 1995; Czaplinski et al., 1999; Jacobson and Peltz, 2000). These experiments revealed that the DSE is a degenerate sequence that can be present in multiple copies in a given mRNA (Zhang et al., 1995; Ruiz-Echevarria and Peltz, 1996). At least three different regions within the PGK1 transcript are functional DSEs (Zhang et al., 1995). Deletion analyses within this transcript identified a repeated sequence (TGYYGATGYYYY, referred to as the sequence motif) which is essential for the DSE to be functional (Zhang et al., 1995). Using this weak consensus sequence, other DSEs have been identified in many yeast transcripts.

The importance of the DSE has been further analyzed using the leader region of the *GCN4* gene. These experiments have indicated that: (1) DSEs are only functional after completion of one translation initiation/termination cycle; (2) the initiation and termination phases of translation are required to trigger NMD but the elongation phase is dispensable; (3) DSEs are functional when located within 150 nucleotides 3′ of the stop codon; and (4) DSEs are not functional if located within the protein coding region which is scanned by the ribosomes during the normal process of translation (Ruiz-Echevarria and Peltz, 1996; Ruiz-Echevarria et al., 1998a,b).

7.2. Stabilizer elements

Previous results have shown that the NMD pathway is not activated in some transcripts due to the lack of DSEs in these mRNAs (Ruiz-Echevarria and Peltz, 1996). However, more recent results have demonstrated that other transcripts harbor specific sequences, defined as stabilizer elements (STEs), that inactivate NMD. Two different STE classes have been identified. The first type is located within the protein coding region and must be translated to exert its negative effect (Peltz et al., 1993; Hagan et al., 1995). A second type has been identified in the GCN4 leader region (Ruiz-Echevarria et al., 1998a,b). This STE inactivates the NMD pathway when positioned downstream of the termination codon and upstream of the DSE. A similar element has been recently identified in the YAP1 mRNA and also appears to have an inactivating effect on NMD (Ruiz-Echevarria and Peltz, 2000). Both STEs bind the poly(U)-binding protein, Pub1p, and fail to function in *pub1Δ* strains. Taken together, these observations suggest that Pub1p can bind to the STE and dominantly interfere with the activity of the NMD pathway.

8. The yeast hnRNP-like protein Hrp1/Nab4 may be a 'marker' protein that triggers NMD

A key question in understanding the NMD pathway is to explain how the DSEs function to distinguish a normal termination codon from a premature termination codon. Based on the observations that the sequence of a DSE is flexible and must be positioned downstream of the nonsense codon to promote NMD, the current thinking is that interaction of a RNA-binding protein(s) with the DSE leads to an aberrant RNP structure that promotes rapid decay of the mRNA.

We have recently addressed this issue and our results have identified the RNA-binding protein, Hrp1/Nab4, as a factor directly involved in modulating the activity of the NMD pathway (Gonzalez et al., 2000). Mutations in the *HRP1/NAB4* gene stabilized nonsense-containing transcripts without affecting the decay rates of several wild-

type mRNAs. Electrophoretic mobility shift assays indicated that Hrp1p binds specifically to a DSE identified in the PGK1 transcript. A mutation in *HRP1* that resulted in the stabilization of nonsense-containing mRNAs abolished the affinity of the Hrp1p for the DSE. Furthermore, Hrp1p was found to interact with both Upf1p and Upf2p, two important components of the NMD pathway (Gonzalez et al., 2000; Gonzalez and Peltz, unpublished data). These results suggest that the DSE 3′ of a premature termination codon is critical to promote NMD because it can promote an interaction with proteins such as Hrp1p/Nab4p, marking the mRNA as aberrant.

9. Insights from higher eukaryotes

The effects of NMD have also been observed in many nonsense-containing transcripts in higher eukaryotes including fungi, plants, *C. elegans* and humans (Pulak and Anderson, 1993; Mendell et al., 2000; for review see Maquat, 2000). In *C. elegans*, seven genes (smg-1–smg-7) are required for NMD. Mutations in these genes stabilize normally recessive nonsense-containing unc-54 mRNAs. Consequently, the nonsense-containing transcripts allow the synthesis of truncated dominant-negative unc-54 protein which causes abnormalities in muscle structure and function (Pulak and Anderson, 1993). In several human disorders, in particular, the medical importance of the NMD pathway has been well reported in recent years (Hall and Thein, 1994; Bedwell et al., 1997; Li and Wilkinson, 1998; Maquat, 2000). For example, cystic fibrosis and Duchenne muscular dystrophy can be caused by mutations that generate premature termination codons. In addition, a dominant form of β-thalassemia is associated with a premature termination codon in the last exon of the β-globin transcript (Hall and Thein, 1994). Under normal conditions, β-globin nonsense-containing transcripts are substrates of the NMD pathway, however, under these circumstances they escape NMD and are able to promote disease.

Numerous experiments have indicated that while some mammalian nonsense-containing mRNAs are subjected to NMD in the cytoplasm, others may be degraded while still in association with the nucleus (i.e. prior to their transport from the nucleus to the cytoplasm; see Maquat, 2000; Lykke-Andersen et al., 2000). While the cellular location for mammalian NMD is still an open debate, there is vast evidence that the process of splicing plays a very important role in NMD. This importance has been demonstrated by inserting an intron downstream of a normal stop codon which subjects this normal transcript lacking a premature termination codon to NMD, indicating that an intron can function as a DSE (Carter et al., 1996; Thermann et al., 1998). Thus, like yeast, the signal for NMD in mammalian cells is also a bipartite sequence consisting of a premature termination codon and a 3′ DSE (Nagy and Maquat, 1998; Hentze and Kulozik, 1999; Maquat, 2000). Based on recent studies, a rule for premature termination codon position within intron-containing genes has been established. According to this rule, premature termination codons must be located more than 50–55 nucleotides upstream of the 3′-most exon–exon junction to promote NMD (Cheng et al., 1994; Thermann et al., 1998; Zhang et al., 1998a,b; Sun and Maquat, 2000; Maquat, 2000). Consistent with these observations, normal termination codons are located within the final exon of the mRNA (i.e. do not reside upstream of an exon–exon junction) and therefore do not activate NMD.

The proteins involved in the NMD pathway have also been investigated in *C. elegans* and humans. In *C. elegans*, three of the seven *smg* genes have been recently cloned and characterized: smg-2 encodes a phosphoprotein homologous to the yeast Upf1p (Page et al., 1999), smg-4 is homologous to the yeast Upf3p (Serin et al., 2001) and smg-7 encodes a novel protein with no obvious yeast homologue (Cali et al., 1999). A human homologue of Upf1 has been identified (Perlick et al., 1996; Applequist et al., 1997) and recent results suggest that it has similar enzymatic characteristics to the yeast counterpart (Bhattacharya et al., 2000). In addition, like smg-2, it is a phosphoprotein (Pal et al., 2001) and functions in mammalian cells to control the stability of nonsense-containing transcripts (Sun et al., 1998). Consistent with the view that the Upf1 protein is involved in the termination event, recombinant HUpf1 was found to cause anti-suppression *in vitro* and this effect is independent of its ATPase and helicase activities (Bhattacharya and Peltz, unpublished data). Human homologues of *UPF2* and *UPF3* have also recently been identified (Mendell et al., 2000; Lykke-Andersen et al., 2000; Serin et al., 2001). Interestingly, when these proteins were fused to MS2-coat protein and expressed together with a β-globin mRNA harboring MS2-binding sites in its 3′ UTR, they were both found to promote rapid degradation similar to that triggered by a premature termination codon (Lykke-Andersen et al., 2000). All together, these results strongly suggest that the NMD pathway is evolutionary conserved.

10. Models for NMD

Based on the results described above and other results, two related working models have been proposed for the mechanism of NMD in the yeast *S. cerevisiae*. The first model suggests that concurrent with and following transcription, the mRNA is processed and packaged into an RNP that is transported from the nucleus to the cytoplasm. During or immediately after transport, ribosomes become associated with the RNP and begin translation. The translating ribosome displaces the proteins that bound the RNA in the nucleus, such as Hrp1/Nab4, and remodels the RNP to a cytoplasmic RNP. According to this model, the successful completion of the first round of translation will trigger a conformational rearrangement in the RNP such that the 5′ and 3′ ends become linked. This step is critical for the

successful transition from a nuclear RNP to a cytoplasmic RNP, which will, at least in part, determine the translatability and stability of the mRNA. The presence of a nonsense mutation, however, causes the first ribosome to prematurely terminate translation (Fig. 3). The assembly of a termination/pre-surveillance complex, composed of at least the translation termination factors (eRF1 and eRF3) and *UPF1*, is thought to occur at this point (Czaplinski et al., 1998, 1999; Jacobson and Peltz, 2000; Wang et al., 2001). An interaction between these proteins facilitates the activity of the termination factors and promote their dissociation from the complex. Our current thinking suggests that this step enhances the RNA binding and ATPase activities of Upf1p, which can advance a post-termination surveillance complex (*UPF/NMD* factors) downstream to search 3′ of the nonsense codon. Previous results have demonstrated that the UPF/NMD factors are present at very different levels in yeast cells (Maderazo et al., 2000). Therefore, the Upf proteins are thought to function at different stages of the NMD pathway and might not be all associated with the termination/surveillance complex at the same time. If the post-termination surveillance complex encounters a signal such as a DSE bound to a protein, the transcript is recognized as aberrant due to an incomplete transition from a nuclear RNP to a cytoplasmic RNP. These DSE-binding proteins can be factors such as Hrp1p/Nab4p, Upf3p or other yet unidentified proteins (Gonzalez et al., 2000; Shirley et al., 1998; Culbertson, 1999). These proteins may directly bind the RNA sequence or may interact with the RNA as a consequence of other RNA processing events. The aberrant transcript is then rapidly decapped and the body of the mRNA is subsequently degraded by a $5' \rightarrow 3'$ exoribonuclease.

Similarly, in mammalian NMD, a spliceable intron may function like a DSE as a consequence of the splicing event leaving a mark on the RNA at the exon–exon junction. This

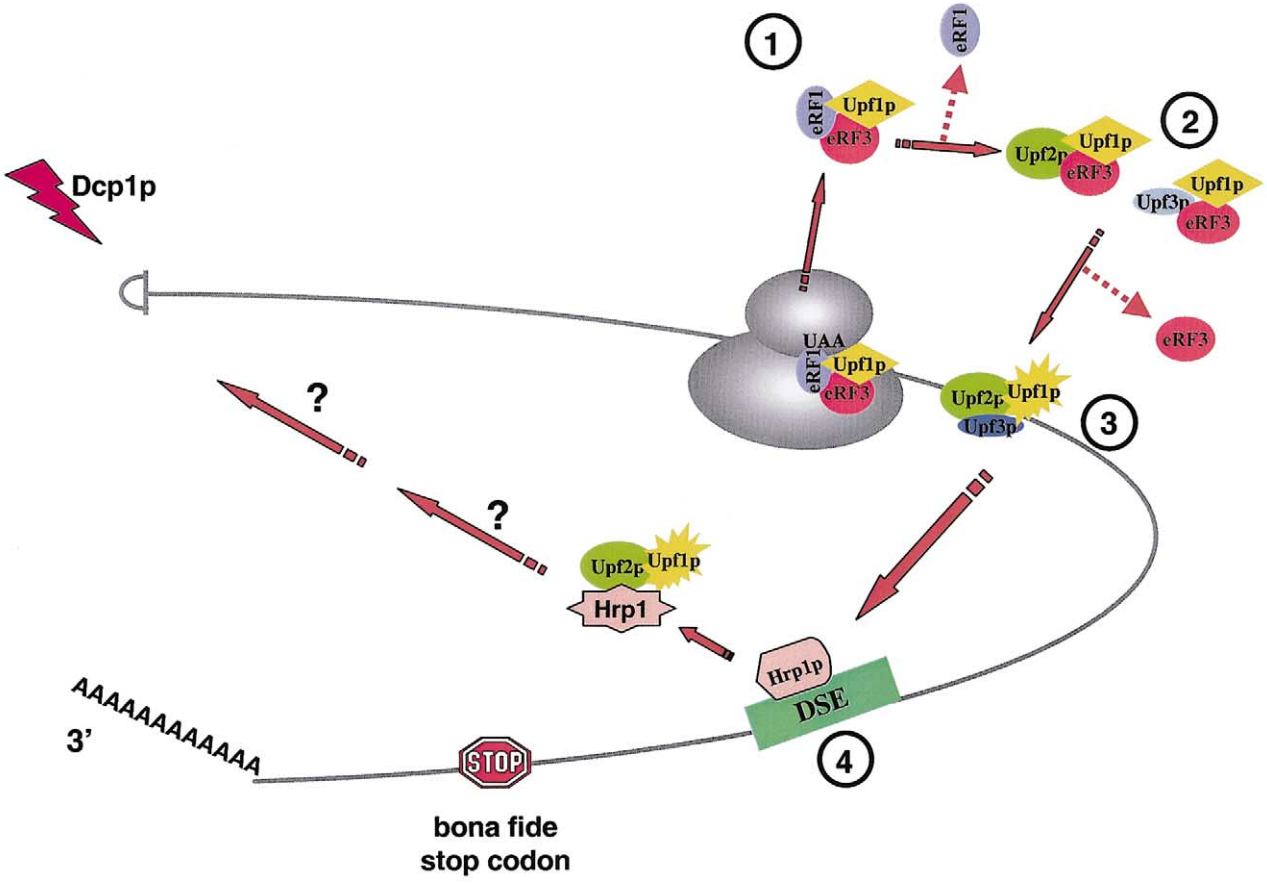

Fig. 3. Model to explain how the NMD pathway occurs in *S. cerevisiae*. NMD occurs during cytoplasmic RNP remodeling. An mRNA is exported to the cytoplasm as an RNP complex with nuclear RNA-binding proteins attached, such as Hrp1. During the initial rounds of translation the attached nuclear proteins are displaced by the ribosomes and complete transition to a cytoplasmic RNP is achieved. A premature termination codon prevents RNP remodeling and activates NMD. The translating ribosome pauses at a premature termination codon and signals to the eRF1-eRF3 complex to bind to the A site. (1) The Upf1p becomes associated with the eRF1-eRF3 termination/pre-surveillance complex during the termination process. After hydrolysis of the peptidyl-tRNA bond, eRF1 dissociates from the ribosome. (2) Dissociation of eRF1 allows Upf2p (or Upf3p) to bind the eRF3-Upf1p complex. (3) Rearrangement of the complex: Upf2p (or Upf3p) joins the complex and displaces eRF3 to form the mature post-termination surveillance complex. Because the ribosome failed to displace Hrp1 (or other yet unidentified DSE marker factors) from the DSE, the surveillance complex can recognize the DSE marker complex as a signal that RNP remodeling is incomplete. Subsequently, the aberrant transcript is rapidly decapped and the body of the mRNA is degraded by a $5' \rightarrow 3'$ exoribonuclease.

mark could be used by the degradation machinery to discriminate between premature termination and normal stop codons. Such a mark is most likely a RNA-binding protein that associates with the mRNA during the early steps of RNP biogenesis and subsequently shuttles between the nucleus and the cytoplasm (Carter et al., 1996; Thermann et al., 1998; Zhang et al., 1998a,b; Hilleren and Parker, 1999; Culbertson, 1999; Lykke-Andersen et al., 2000). In fact, recent results have demonstrated that the splicing process alters mRNP protein composition (Le Hir et al., 2000a,b). Using novel *in vitro* cross-linking and RNase protection assays, several proteins have been detected that associate with mRNA exon–exon junctions only as a consequence of splicing. Immunoprecipitation experiments have identified at least five different proteins in this complex: SRm160, a nuclear matrix-associated splicing factor; DEK and RNPS1, two general splicing factors; and Y14 and REF, two proteins that interact with the mRNA export factor TAP. More recently, Y14 has been shown to shuttle from the nucleus to the cytoplasm and associate preferentially with mRNAs produced by splicing but not with intron-containing pre-mRNAs (Kataoka et al., 2000). Similarly, hUpf3 has been shown to shuttle from the nucleus to the cytoplasm and binds to nascent transcripts at or near exon–exon junctions as a consequence of the splicing process (Lykke-Andersen et al., 2000). Similar to the yeast Hrp1p, hUpf3 might function as a marker protein in mammalian cells. Thus, it might associate with nascent transcripts in the nucleus, remain bound during nuclear export and interact with other components of the surveillance complex (such as Upf1 and Upf2) to promote NMD. Taken together, these findings suggest that, in mammalian cells, pre-mRNA splicing can mark a transcript in the nucleus with proteins that persist in the cytoplasm and determine the fate of the mRNA. Because very few yeast transcripts undergo pre-mRNA splicing, the signal that a proper transition from a nuclear RNP to a cytoplasmic RNP has occurred may involve other pre-mRNA processing events such as 5′ and 3′ end formation. In this light, it is interesting to note that *HRP1/NAB4* has been implicated in 3′ end cleavage and polyadenylation (Kessler et al., 1997; Minvielle-Sebastia et al., 1998). In sum, these results suggest that RNA-binding proteins 3′ of the termination event determine whether a RNA will be a substrate for the NMD pathway.

This first model can be applied to all the NMD substrates, except those transcripts with extended 3′ UTRs (reviewed in Hilleren and Parker, 1999; Jacobson and Peltz, 2000). An alternate model that includes this class of substrates argues that the DSE is a defective 3′ UTR created by the presence of a premature termination codon (Hilleren and Parker, 1999; Jacobson and Peltz, 2000). While the first model suggests that as a consequence of premature termination in a nonsense-containing RNA the translating ribosome fails to displace DSE-binding proteins, such as Hrp1/Nab4, the second model suggests that the DSE triggers NMD because it lacks a factor(s) normally present in a 3′ UTR derived from a wild-type transcript. More recent results, however, suggest that transcripts with extended 3′ UTRs may harbor DSE-like sequences in this region that can promote rapid turnover of the aberrant transcript via the NMD pathway (Das et al., 2000). The identification of DSEs in the extended 3′ UTRs of these transcripts will further support the first model. Current research efforts are trying to discern between these two related possibilities in order to further understand the mechanism of NMD in yeast.

11. Perspectives

For the past several years the NMD pathway has been studied intensively. All together, the NMD pathway has uncovered a strong link between translation and mRNA turnover. It has been shown that factors involved in the NMD pathway also have important roles in translation-related processes such as translation termination and frame-shifting (Cui et al., 1996, 1998, 1999; Ruiz-Echevarria et al., 1998b). More recent results strongly suggest an intimate link between the processes of mRNA turnover, pre-mRNA processing and nucleocytoplasmic transport. The identification of factors such as *HRP1/NAB4*, human Upf3, Y14 and REF proteins, that move with the mRNA from the nucleus to the cytoplasm, will help us understand how multiple nuclear processes influence the cytoplasmic fate of a transcript. Further identification and characterization of DSE/exon–exon junction binding factors will aid in our understanding of the NMD pathway. Future experiments will address the precise mechanism by which these proteins function to trigger decapping of nonsense-containing mRNAs and how they serve to coordinate this cellular cross-talk. Finally, and most importantly, further understanding of the NMD pathway in yeast may hold valuable answers to the study of cellular mechanisms involved in the onset of many human genetic disorders.

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