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Upf1p, Nmd2p, and Upf3p Regulate the Decapping and Exonucleolytic Degradation of both Nonsense-Containing mRNAs and Wild-Type mRNAs

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In Saccharomyces cerevisiae, rapid degradation of nonsense-containing mRNAs requires the decapping enzyme Dep1p, the 5′-to-3′ exoribonuclease Xrn1p, and the three nonsense-mediated mRNA decay (NMD) factors, Upf1p, Nmd2p, and Upf3p. To identify specific functions for the NMD factors, we analyzed the mRNA decay phenotypes of yeast strains containing deletions of DCP1 or XRN1 and UPF1, NMD2, or UPF3. Our results indicate that Upf1p, Nmd2p, and Upf3p regulate decapping and exonucleolytic degradation of nonsense-containing mRNAs. In addition, we show that these factors also regulate the same processes in the degradation of wild-type mRNAs. The participation of the NMD factors in general mRNA degradation suggests that they may regulate an aspect of translation termination common to all transcripts.

mRNA degradation, an important aspect of gene expression, is a regulated process that is often linked to mRNA translation (30, 59). Specific pathways of mRNA decay have been studied in several experimental systems and have been most extensively characterized in the yeast Saccharomyces cerevisiae (5, 30). In yeast, wild-type mRNAs are primarily degraded by a 5′-to-3′ deadenylation-dependent mechanism in which the initial nucleolytic event is the shortening of the poly(A) tail to an oligo(A) length of 10 to 15 nucleotides. After poly(A) shortening, transcripts are decapped by the product of the DCP1 gene (Dep1p) and digested exonucleolytically by the 5′-to-3′ exoribonuclease, Xrn1p (6, 9, 37). Although this decay pathway appears to comprise the predominant mode of mRNA degradation, recent evidence indicates that mRNAs can also be degraded by a 3′-to-5′ mechanism that requires the products of the SKI2, SKI3, and SKI8 genes, as well as Ski6p (also known as Rrp41) and Rrp4p, two 3′-to-5′ exonucleases of the exosome (46, 29, 67).

mRNAs containing a premature termination codon are degraded by the nonsense-mediated mRNA decay pathway (26, 31, 54). This type of mRNA decay has been observed in all eukaryotic cells so far examined and may, in part, serve as a surveillance mechanism that eliminates aberrant mRNAs (14, 22, 50, 57). Degradation of nonsense-containing mRNAs is deadenylation independent, proceeds from decapping by Dep1p to Xrn1p-catalyzed 5′-to-3′ decay without prior poly(A) shortening (6, 21, 47, 65). Nonsense-mediated mRNA decay also requires at least three additional trans-acting factors: Upf1p, Nmd2p (Upf2p), and Upf3p. Mutations or deletions of one or more of the genes encoding these factors (UPF1, NMD2 [UPF2], and UPF3) generally lead to the same phenotype: the selective stabilization of nonsense-containing mRNAs with no apparent effect on the stability of most wild-type mRNAs (10, 23, 38, 40–42, 52, 54, 68).

The UPF1, NMD2, and UPF3 genes and their products, have been characterized extensively. UPF1 encodes a 109-kDa protein that has two putative Zn fingers near its N terminus and seven conserved motifs common to the members of RNA/DNA helicase superfamily 1 (1, 36, 41). Upf1p has been purified from yeast cells and shown to possess nucleic acid-binding activity as well as nucleic acid-dependent ATPase and helicase activities (12). Nmd2p, a 127-kDa acidic protein, and Upf3p, a 45-kDa basic protein, both contain putative bipartite nuclear localization signals (23, 38). However, both proteins, as well as Upf1p, are primarily localized in the cytoplasm and appear to be polyribosome associated (3, 4, 23, 44, 53; F. He and A. Jacobson, unpublished data). Nmd2p interacts with Upf3p and Upf1p, and the latter, in turn, can also interact with itself, the release factors Sup35p and Sup45p, and Nmd1p, Nmd3p, and Dbp2p (7, 13, 23–25; A. Bond, D. Mangus, F. He, and A. Jacobson, submitted for publication; F. He and A. Jacobson, submitted for publication).

Although the identification and characterization of Upf1p, Nmd2p, and Upf3p have provided insight into the mechanism of nonsense-mediated mRNA decay, the precise functions of these factors remain unknown. For example, a role in regulating translational termination and fidelity is suggested by the interaction of Upf1p with Sup35p and Sup45p (13) and by the occurrence of allosuppression, omnipotent suppression, and 1 frameshifting phenotypes in upf1, nmd2, and upf3 mutants (11, 17, 39, 43, 61; He and Jacobson, submitted). Whether the translational functions of these proteins dictate their mRNA decay functions (or vice versa) remains to be determined, but it is noteworthy that the two roles of Upf1p have been separated by distinct mutations (69, 70; He and Jacobson, submitted) and by overexpression (43; He and Jacobson, submitted). Likewise, little is known about the epistatic and regulatory interactions of the respective factors. DCP1, XRN1, UPF1, NMD2, and UPF3 are all required for degradation of non-
sense-containing mRNAs, but the only dissection of their regulatory interactions has been a study demonstrating that

The role of these factors in regulating a general aspect of translation was studied. To address these basic issues, we constructed a set of yeast strains that contain single or multiple deletions of the DCP1, XRNI, UPF1, NMD2, and UPF3 genes and analyzed mRNA decay phenotypes in these strains. We show that (i) deletions of UPF1, NMD2, or UPF3 lead to increased accumulation of capped nonsense-containing mRNAs, regardless of Xrn1p function; (ii) deletions of these genes in xrn1Δ cells differentially affect the accumulation of decapped nonsense-containing mRNAs, as well as capped and decapped wild-type mRNAs; and (iii) deletions of these genes in dcp1Δ cells differentially affect the accumulation of capped nonsense-containing and wild-type mRNAs. Our data indicated that Upf1p, Nmd2p, and Up3p can regulate decapping and exonucleolytic degradation of both nonsense-containing mRNAs and wild-type mRNAs and suggest that these effects may be a consequence of the roles played by these factors in regulating a general aspect of translation termination.

MATERIALS AND METHODS

General methods. Preparation of standard yeast media and methods of cell culture were as described (58). Transformation of yeast was done by the high-

Plasmids. Plasmids used in this study included (i) pHF2095, which contains the xrn1::ADE2 allele in pBluescript KSHI (+); (ii) pRP716 (a gift from Roy Parker, University of Arizona), which contains the dcp1::URA3 allele in pBluescript; (iii) pHF2105, which contains the MER2 gene in YEpplac112, and (iv) pHF1083, pHF1085, and pHF1463, which contain the ADH1-HA-UPF1::NMD2, or UPF1::NMD2 alleles in YEpplac112, respectively.

Construction of the xrn1::ADE2 allele. The plasmid pHF2095, which carries the xrn1::ADE2 allele, was constructed in two steps. First, a 514-bp PCR-derived NotI-BglII fragment containing the promoter and 5′ untranslated region of the ADH1 gene was cloned into the NotI-BglII sites of pBluescript KSII (+); (ii) pRP716 (a gift from Roy Parker, University of Arizona), which contains the dcp1::URA3 allele in pBluescript; (iii) pHF2105, which contains the MER2 gene in YEpplac112, and (iv) pHF1083, pHF1085, and pHF1463, which contain the ADH1-HA-UPF1::NMD2, or UPF1::NMD2 alleles in YEpplac112, respectively.

To address these basic issues, we constructed a set of yeast strains that contain single or multiple deletions of the DCP1, XRNI, UPF1, NMD2, and UPF3 genes and analyzed mRNA decay phenotypes in these strains. We show that (i) deletions of UPF1, NMD2, or UPF3 lead to increased accumulation of capped nonsense-containing mRNAs, regardless of Xrn1p function; (ii) deletions of these genes in xrn1Δ cells differentially affect the accumulation of decapped nonsense-containing mRNAs, as well as capped and decapped wild-type mRNAs; and (iii) deletions of these genes in dcp1Δ cells differentially affect the accumulation of capped nonsense-containing and wild-type mRNAs. Our data indicated that Upf1p, Nmd2p, and Up3p can regulate decapping and exonucleolytic degradation of both nonsense-containing mRNAs and wild-type mRNAs and suggest that these effects may be a consequence of the roles played by these factors in regulating a general aspect of translation termination.

Yeast strains. The yeast strains used in this study are listed in Table 1. Strains containing deletions of XRNI or DCP1 were constructed by gene replacement (60). A Nor1-SalI fragment containing the xrn1::ADE2 allele isolated from pHF2095 or a DraI-ClaI fragment containing the dcp1::URA3 allele isolated from pRP716 was used for yeast transformation. Ade2+ or Ura+ transformants were selected, and the disruption was confirmed by PCR analysis of genomic DNA.

TABLE 1. Yeast strains used in this study

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RESULTS

Nonsense-containing mRNAs that accumulate in upf1Δ, nmd2Δ, or upf2Δ cells are full-length and capped. To define the functional roles and interrelationships of Upf1p, Nmd2p, Upf3p, Xrn1p, and Dcp1p, we first determined the 5' ends and cap status of two nonsense-containing mRNAs that were stabilized in otherwise isogenic cells harboring deletions of the genes encoding these factors. Primer extension and anti-m7G immunoprecipitation assays were utilized to characterize the CYH2 and MER2 pre-mRNAs, two endogenous substrates of the nonsense-mediated mRNA decay pathway (22). These pre-mRNAs are normally very unstable, and their 5' ends were barely detectable in wild-type cells (Fig. 1A). However, CYH2 pre-mRNA transcripts accumulated in upf1Δ, nmd2Δ, upf3Δ, and dcp1Δ strains, and all had identical 5' ends, including a major transcript that initiated at nucleotide −18 and two minor species with ends at nucleotides −22 and −27 (Fig. 1A). In contrast, the predominant transcripts that accumulated in xrn1Δ cells had 5' ends at nucleotides +1, −3, and −16. Comparable results were obtained with the MER2 pre-mRNA. This transcript exhibited identical 5' ends at nucleotides −27, −36, −44, −67, and −70 in upf1Δ, nmd2Δ, upf3Δ, and dcp1Δ cells but had 5' ends at nucleotides −23, −33, −55, −59, and −64 in xrn1Δ cells (Fig. 1A).

Since the CYH2 and MER2 pre-mRNAs present in upf1Δ, nmd2Δ, or upf2Δ cells all had the same 5' ends as those in the dcp1Δ strain, it seemed likely that they accumulated as capped species. To test this directly, anti-m7G antibodies were used to separate the CYH2 and MER2 pre-mRNAs into capped and uncapped fractions that were subsequently analyzed by Northern blotting. These experiments showed that approximately 90% of the CYH2 pre-mRNA present in cells harboring a dcp1Δ mutation or upf1Δ, nmd2Δ, or upf3Δ mutations was in the capped fraction (Fig. 1B and Table 2). In xrn1Δ cells, however, only 10% of the CYH2 pre-mRNA transcripts were in the capped fraction and the remainder were in the decapped fraction (Fig. 1B and Table 2). Analyses of the cap status of the MER2 pre-mRNA in the same strains provided identical results (data not shown). These data indicate that full-length, capped CYH2 and MER2 pre-mRNAs accumulate in cells lacking Upf1p, Nmd2p, or Upf3p and suggest that these factors promote efficient decapping of nonsense-containing transcripts.

Nonsense-containing mRNAs that accumulate in xrn1Δ cells are decapped, shortened at their 5' ends, and generated by partial Rat1p digestion. As shown in Fig. 1, the CYH2 and MER2 pre-mRNAs that accumulate in xrn1Δ cells are uncapped and shortened at their 5' ends. The structure of these RNAs suggests that they may be decay intermediates, possibly arising by either of two general mechanisms. In the first, decapping could proceed by the usual Dcp1p-mediated event, but be followed by inefficient 5'-to-3' exonucleolytic digestion by a nuclease other than Xrn1p. In the second, the premature nonsense codon could trigger decapping by a 5'-proximal Dcp1p-independent endonucleolytic cleavage. To distinguish between these possibilities, we first determined whether the appearance of the atypical RNA species is dependent on Dcp1p. To this end, we constructed a dcp1Δ xrn1Δ double mutant and examined the 5' ends and 5'-cap status of the CYH2 and MER2 pre-mRNAs that accumulated in this strain. As shown in Fig. 1A, in dcp1Δ xrn1Δ cells all putative decay intermediates are absent and both pre-mRNAs have the same 5' ends as do their counterparts in dcp1Δ cells. Moreover, approximately 90% of the CYH2 and MER2 pre-mRNAs present in dcp1Δ xrn1Δ cells are capped (Fig. 1B and data not shown). These results indicate that the RNA species peculiar to xrn1Δ cells must arise from decapping by Dcp1p, thereby ruling out the second possibility. To test the first possibility directly, we sought to identify a 5'-to-3' exonuclease that might generate the respective truncated RNAs.

Biochemical analyses of 5'-to-3' exonuclease activities in yeast identified only two such activities. One, as noted above, is encoded by the XRNI gene (37), and the other is encoded by the RAT1 (also known as KEM1) gene (2, 35). Since sequence analyses indicated that Rat1p is the only protein in the yeast genome that shares significant homology with Xrn1p (data not shown), we reasoned that Rat1p might be responsible for the production of the putative decay intermediates in xrn1Δ cells. To test this notion, we used the temperature-sensitive rat1-1 allele and constructed a rat1-1 xrn1Δ strain. Due to loss of Rat1p function, this strain ceased growth within 2 h after being shifted to 37°C (2). The effect of inactivation of Rat1p on the production of mRNA decay intermediates in xrn1Δ cells was analyzed by Northern blotting and primer extension. The results presented in Fig. 2A indicate that, when grown at 24°C, rat1-1 xrn1Δ cells accumulate the same shortened species of CYH2 pre-mRNA as do xrn1Δ cells (i.e., RNA species with 5' ends at nucleotides +1, −3, and −16 [Fig. 2A, lane 0]). However, when shifted to 37°C for 1, 2, or 4 h, the rat1-1 xrn1Δ cells (i) stabilized the CYH2 pre-mRNA (Fig. 2B); (ii) accumulated full-length CYH2 pre-mRNAs with 5' ends at nucleotides −18, −22, and −27 (Fig. 2A); and (iii) greatly reduced the levels of shortened RNA species (Fig. 2A). These results demonstrate that Rat1p is responsible for the production of the unusual CYH2 and MER2 RNAs detected in xrn1Δ cells. Taken together, the data indicate that inactivation of Xrn1p leads to the accumulation of decay intermediates for nonsense-containing mRNAs and that these decay intermediates arise from decapping by Dcp1p and incomplete 5'-to-3' exonucleolytic digestion by Rat1p.

In xrn1Δ cells, incomplete Rat1p digestion also leads to the accumulation of decay intermediates of wild-type mRNAs. Decapped and 5' shortened species of wild-type PKG1 and RPS5A mRNAs have been identified in xrn1Δ cells previously (28, 49). Since Xrn1p plays a general role in mRNA degradation, we reasoned that the accumulation of decay intermediates in xrn1Δ cells might be a general phenomenon. To evaluate this possibility further, we analyzed the 5' ends and cap status of additional wild-type transcripts that accumulated in xrn1Δ cells. As shown in Fig. 3A, ADH1, URA5, and CUP1 mRNAs that accumulated in wild-type; upf1Δ, nmd2Δ, upf3Δ, or dcp1Δ strains all had identical 5' ends. The ADH1, URA5, and CUP1 mRNAs had major transcription start sites at nucleotides −39 and −30, −267 and −255, and −70 and −61, respectively. In
contrast, novel species of each of these mRNAs accumulated in \(*m\Delta\) cells, including those with 5' ends at nucleotides −38, −37, −28, −27, and −23 for \(ADH1\) mRNA, nucleotides −260 and −248 for \(URA5\) mRNA, and nucleotides −66, −57, −51, −43, −37, and −32 for \(CUP1\) mRNA. Consistent with these primer extension data, anticap immunoprecipitation experiments showed that transcripts accumulating in the wild-type, \(upf1\Delta\), \(nmd2\Delta\), \(upf3\Delta\), and \(dcp1\Delta\) strains were largely in the capped fraction and those accumulating in \(*m\Delta\) cells were predominantly in the decapped fraction (data not shown; see also Fig. 6). Two observations indicate that these shortened and decapped species of wild-type mRNAs arise in \(*m\Delta\) cells by the same mechanism that generated decay intermediates of nonsense-containing mRNAs. First, the 5’-shortened species

**FIG. 1.** Analysis of the 5' ends and cap status of nonsense-containing mRNAs that accumulate in yeast strains defective in nonsense-mediated mRNA decay. (A) Analysis of the 5' ends of the \(CYH2\) and \(MER2\) pre-mRNAs by primer extension. Total RNA was isolated from yeast strains of the indicated genotypes. Radiolabeled primers (\(CYH2-IN4\) or \(MER2-2\)) were annealed to aliquots (20 μg) of each RNA sample and extended by avian myeloblastosis virus reverse transcriptase. DNA sequencing reactions with the same primers (run in lanes G, A, T, and C) were used to determine the positions of the primer extension products. The major transcriptional start sites (positions noted are relative to the initiation codon) for both pre-mRNAs are indicated by arrows. The atypical extension products detected in RNA from \(*m\Delta\) cells are denoted by asterisks. (B) Analysis of the 5’ cap status of the \(CYH2\) pre-mRNA by anti-m\(^7\)G immunoprecipitation. Aliquots (10 μg) of total RNA isolated from the indicated yeast strains were immunoprecipitated using polyclonal anti-m\(^7\)G antibodies. RNA comprising the supernatant (S) (uncapped) and pellet (P) (capped) fractions, as well as an aliquot of the input RNA (I), were analyzed by Northern blotting, using a \(CYH2\) probe. The positions of the \(CYH2\) pre-mRNA and mRNA are indicated. Quantitation of this experiment is summarized in Table 2. WT, wild type.
of ADH1, URA5, and CUP1 mRNAs detected in the xrn1Δ strain are absent in dcp1Δ xrn1Δ cells (Fig. 3A). Second, although rat1Δ xrn1Δ cells grown at the permissive temperature accumulated 5′-shortened species of URA5 mRNA, these cells did not accumulate the shortened transcripts when shifted to the nonpermissive temperature for 1, 2, or 4 h (Fig. 3B).

Cells harboring both xrn1Δ and upf1Δ, nmd2Δ, or upf3Δ mutations accumulate nonsense-containing mRNAs that are full length and capped. The experiments shown in Fig. 1 indicated that Upf1p, Nmd2p, and Upf3p may play a role in the decapping of nonsense-containing mRNAs (see above). To test this idea further, we constructed xrn1Δ upf1Δ, xrn1Δ nmd2Δ, and xrn1Δ upf3Δ double mutants and analyzed the 5′ ends and relative abundance of capped and uncapped CYH2 pre-mRNAs in these strains. In contrast to xrn1Δ cells, in which the CYH2 pre-mRNA was principally present as an uncapped species, xrn1Δ upf1Δ, xrn1Δ nmd2Δ, and xrn1Δ upf3Δ cells all exhibited substantially increased levels of this RNA in the capped fraction (Fig. 4A and Table 2). Consistent with their increased levels of capped CYH2 pre-mRNAs, the doubly mutant strains also contained higher levels of full-length transcripts (Fig. 4B). However, in contrast to xrn1Δ dcp1Δ cells, which accumulated only the full-length and capped transcripts (Fig. 1), the xrn1Δ upf1Δ, xrn1Δ nmd2Δ, and xrn1Δ upf3Δ strains also retained significant amounts of decapped and 5′-shortened CYH2 pre-mRNA (Fig. 4A and B and Table 2). Since inactivation of Upf1p, Nmd2p, or Upf3p in xrn1Δ cells leads to an increased accumulation of full-length and capped nonsense-containing mRNAs without completely eliminating the accumulation of decapped and 5′-shortened RNAs, it is likely that these factors regulate but do not catalyze decapping of nonsense-containing transcripts.

In xrn1Δ cells, deletions of UPF1, NMD2, or UPF3 differentially affect the accumulation of decapped nonsense-containing mRNAs. As shown in Fig. 4C, comparable amounts of the CYH2 pre-mRNA accumulated in XRN1 cells that contain deletions of UPF1, NMD2, or UPF3. However, in an xrn1Δ background, deletions of the same genes affected the levels of the CYH2 pre-mRNA to different extents. The level of the CYH2 pre-mRNA was highest in the xrn1Δ upf1Δ strain, intermediate in the xrn1Δ upf3Δ strain, and lowest in the xrn1Δ nmd2Δ strain (Fig. 4C and Table 2). Analyses of anticyc immunoprecipitation assays indicate that these differences are largely a reflection of variations in the accumulation of decapped transcripts, i.e., xrn1Δ upf1Δ, xrn1Δ nmd2Δ, and xrn1Δ upf3Δ strains accumulated levels of capped CYH2 pre-mRNA that differed by at most 30%, but varied more than twofold in their levels of the decapped version of the same transcript (Fig. 4A and Table 2). Much like the variations seen in the distribution of unfractinated CYH2 pre-mRNA, the level of decapped transcripts was highest in xrn1Δ upf1Δ cells, intermediate in xrn1Δ upf3Δ cells, and lowest in xrn1Δ nmd2Δ cells. Consistent with these variations in the levels of decapped transcripts, primer extension analyses revealed the same relationships between strains for the accumulation of CYH2 pre-mRNA decay intermediates (Fig. 4B). Comparable analyses of the levels of total, capped, and decapped MER2 pre-mRNA in these strains yielded essentially identical results (data not shown). Since deletions of UPF1, NMD2, and UPF3 in xrn1Δ cells have differential effects on the accumulation of decapped nonsense-containing mRNAs, it appears that Upf1p, Nmd2p, and Upf3p may also regulate the degradation of decapped nonsense-containing mRNAs.

In xrn1Δ cells, deletions of UPF1, NMD2, or UPF3 also have a differential effect on the accumulation of capped and decapped wild-type mRNAs. In our analyses of xrn1Δ cells it became apparent that deletions of UPF1, NMD2, and UPF3 also had differential effects on the steady-state level of the CYH2 mRNA. These differences were comparable to the effects seen with the CYH2 pre-mRNA, such that levels of the mature mRNA were highest in xrn1Δ upf1Δ cells, intermediate in xrn1Δ upf3Δ cells, and lowest in xrn1Δ nmd2Δ cells (Fig. 4C).

### Table 2. Effects of single or multiple deletions of the UPF1, NMD2, and UPF3 genes on the accumulation of total, capped, and decapped CYH2 pre-mRNA and mRNA

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a The northern blot in Fig. 4C was used to measure the levels of the CYH2 pre-mRNA and mRNA in each sample. Northern blots in Fig. 1B and 4A were used to determine the percentage of capped and uncapped transcripts.

b Numbers represent the relative abundance of the transcripts and are the ratios of RNA in each mutant strain to that in the wild-type strain, after normalizing to the SCR1 internal control. The level of transcripts in the wild-type strain was arbitrarily defined as 1 unit.

c Relative levels of capped and decapped transcripts and were obtained by multiplying the corresponding number in the Total column by the percentage of capped or decapped transcripts, respectively.

d NA, not applicable.
To determine whether the variations seen in these strains were restricted to the CYH2 mRNA, we examined the accumulation of seven other wild-type mRNAs that represented a broad range of inherent stabilities. As shown in Fig. 5, for all but the PGK1 mRNA (see below), levels were highest in the xrn1Δ upf1Δ strain, intermediate in the xrn1Δ upf3Δ strain, and lowest in the xrn1Δ nmd2Δ strain. The level of the PGK1 mRNA, in contrast, was highest in the xrn1Δ nmd2Δ strain, intermediate in the xrn1Δ upf3Δ strain, and lowest in the xrn1Δ upf1Δ strain (Fig. 5 and Table 3). These results indicate that, in xrn1Δ cells, deletions of UPF1, NMD2, or UPF3 affect the accumulation of wild-type mRNAs differentially and in an mRNA-specific manner.

To determine whether the differences in the levels of wild-type mRNAs reflected selective effects on capped or uncapped transcripts, we characterized the respective RNA samples by anticap immunoprecipitation and primer extension. Control experiments demonstrated that deletion of UPF1, NMD2, or UPF3 in XRN1 cells had no significant consequences for the accumulation of capped or decapped wild-type mRNAs (Fig. 6). The parental strains (XRN1, UPF1, NMD2, and UPF3), as well as the individual upf1Δ, nmd2Δ, and upf3Δ strains, accumulated primarily capped transcripts for each mRNA examined. For example, in both wild-type and single-deletion strains, approximately 90% of the CYH2 and PGK1 mRNAs, and 70% of the URA5 and TCM1 mRNAs, were in the capped fraction (Fig. 1A and 6A; Tables 2 and 3). As expected, deletion of only the XRN1 gene led to substantial increases in the levels of decapped transcripts for all mRNAs examined and, for all but the PGK1 mRNA, large decreases in the levels of capped transcripts (Fig. 4A and 6B; Tables 2 and 3).

In contrast to the effects of the single deletions, inactivation of both XRN1 and UPF1, NMD2, or UPF3 affected the accumulation of capped wild-type transcripts differentially and in an mRNA-specific manner. Deletions of UPF1 or NMD2 in xrn1Δ cells led to increases in the accumulation of capped URA5, TCM1, and CYH2 mRNAs. However, the levels of capped PGK1 transcripts decreased in xrn1Δ upf1Δ cells and increased in xrn1Δ nmd2Δ cells. In all cases, simultaneous deletion of UPF3 and XRN1 did not affect the levels of capped wild-type transcripts significantly (Fig. 4A and 6B; Tables 2 and 3).
Deletions of XRN1 and UPF1, NMD2, or UPF3 also had differential effects on the accumulation of decapped wild-type mRNAs. The levels of decapped CYH2, URA5, and TCM1 mRNAs were highest in xrn1Δ upf1Δ cells, intermediate in xrn1Δ upf3Δ cells, and lowest in xrn1Δ nmd2Δ cells (Fig. 4A and 6B; Tables 2 and 3). These observations were consistent with primer extension analyses which demonstrated that the levels of mRNA decay intermediates paralleled the levels of decapped transcripts in the respective strains (Fig. 6C and data not shown). Unlike the results obtained with the CYH2, URA5, and TCM1 mRNAs, the levels of decapped PGK1 transcripts did not vary substantially in the double mutant strains (Fig. 6B and Table 3).

Collectively, the differential effects of xrn1Δ upf1Δ, xrn1Δ nmd2Δ, and xrn1Δ upf3Δ mutations on the levels of capped and decapped CYH2, URA5, TCM1, and PGK1 mRNAs indicate that, in addition to their functions in nonsense-mediated mRNA decay, Upf1p, Nmd2p, and Upf3p also have roles in regulating the decapping and degradation of wild-type mRNAs.

The function of Upf1p is epistatic to those of Nmd2p and Upf3p in the degradation of nonsense-containing and wild-type mRNAs. Cells harboring xrn1Δ upf1Δ, xrn1Δ nmd2Δ, or xrn1Δ upf3Δ mutations contained similar levels of capped but different levels of decapped CYH2 pre-mRNA (Fig. 4A and Table 2). By constructing a set of xrn1Δ strains harboring double and triple deletions of UPF1, NMD2, and UPF3, we were able to exploit the differences in the levels of decapped CYH2 pre-mRNA to determine the epistatic relationships of Upf1p, Nmd2p, and Upf3p. These experiments showed that all strains containing a upf1Δ allele (i.e., xrn1Δ upf1Δ nmd2Δ, xrn1Δ upf1Δ upf3Δ, and xrn1Δ upf1Δ nmd2Δ upf3Δ strains) accumulated the same level of decapped transcripts as did the xrn1Δ upf3Δ strain. The xrn1Δ nmd2Δ upf3Δ strain accumulated the same level of decapped transcripts as the xrn1Δ upf3Δ strain but differed from the level in the xrn1Δ nmd2Δ strain (Fig. 4A and Table 2). Primer extension analyses showed the same relationships among UPF1, NMD2, and UPF3 mutations for accumulation of CYH2 pre-mRNA decay intermediates (Fig. 4B). These results indicate that, at least with regard to effects on the abundance of decapped nonsense-containing mRNAs in xrn1Δ cells, the function of Upf1p is epistatic to Upf3p, and that of Upf3p is epistatic to Nmd2p.

Similar analyses with the CYH2, URA5, PGK1, and TCM1 mRNAs allowed us to examine the epistatic relationships of these factors that pertained to effects on the levels of capped and decapped wild-type mRNAs (Fig. 5 and 6 and Table 3). These studies showed that, in the regulation of the levels of capped wild-type mRNAs, the function of Upf1p is always epistatic to Nmd2p and Upf3p. However, the epistatic relationships of Nmd2p and Upf3p are mRNA specific. For the CYH2, URA5, and TCM1 mRNAs, the function of Nmd2p is
FIG. 4. In xrn1Δ cells, single or multiple deletions of UPF1, NMD2, or UPF3 differentially affect the levels of decapped nonsense-containing transcripts. (A) Analysis of the levels of CYH2 pre-mRNAs by anti-m\(^7\)G immunoprecipitation. Total RNA was isolated from yeast strains of the indicated genotypes, capped mRNAs were immunoprecipitated as in Fig. 1B, and each sample was analyzed by Northern blotting, using a CYH2 probe. Lanes I, S, and P designate input, supernatant, and pellet, respectively. (B) Analysis of the levels of CYH2 pre-mRNA decay intermediates. Primer extension analysis of the CYH2 pre-mRNA was performed on total RNA from each yeast strain as in Fig. 1A. The major transcriptional start sites of the CYH2 pre-mRNA and the 5' ends of its decay intermediates are indicated by arrows and asterisks, respectively. Total RNA from the upf3Δ strain was used as a control. (C) Northern analysis of the steady-state levels of CYH2 pre-mRNA. Total RNA was isolated from yeast strains of the indicated genotypes and analyzed by Northern hybridization. The SCR1 RNA (20), which is transcribed by RNA polymerase III, was used as an internal control. WT, wild type. In panels A and C, the CYH2 DNA probe used was the same as in Fig. 1B. Quantitation of this experiment is summarized in Table 2.
epistatic to Upf3p, but for the PGK1 mRNA, the function of Upf3p is epistatic to Nmd2p (Fig. 4A and 6B; Tables 2 and 3). With regard to the effects on decapped mRNAs, the function of Upf1p was found to be epistatic to Upf3p and Upf3p was found to be epistatic to Nmd2p (Fig. 4A and 6B; Tables 2 and 3).

**Overexpression of UPF1 in xrn1Δ cells decreases the levels of mRNA decay intermediates.** As described above, Upf1p, Nmd2p, and Upf3p influence the steady-state levels of decapped nonsense-containing and wild-type mRNAs in xrn1Δ cells. Of the three factors, Upf1p appears to play the most significant role in this regulatory event because all xrn1Δ strains in which Upf1p is retained (i.e., xrn1Δ nmd2Δ, xrn1Δ upf3Δ, and xrn1Δ nmd2Δ upf3Δ strains) accumulate lower levels of total and decapped transcripts than the xrn1Δ upf1Δ strain (Fig. 4C and 5; Tables 2 and 3). One possible explanation for this observation is that Upf1p promotes exonucleolytic degradation of decapped mRNAs. To test this idea, we examined the consequences of UPF1 overexpression on the accumulation of total mRNA and mRNA decay intermediates in xrn1Δ cells. These experiments showed that introduction of a high-copy-number plasmid expressing the UPF1 gene into

**FIG. 5.** Single or multiple deletions of UPF1, NMD2, or UPF3 in xrn1Δ cells differentially affect the levels of wild-type (WT) mRNAs. Total RNA was isolated from yeast strains of the indicated genotypes and analyzed by Northern blotting, using the SCR1 RNA as an internal control. Quantitation of the results for the URA5, TCM1, and PGK1 mRNAs is summarized in Table 3.
TABLE 3. Effects of single or multiple deletions of UPF1, NMD2, and UPF3 on the accumulation of total, capped, and decapped wild-type mRNAs

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*The Northern blot in Fig. 5 was used to measure the total levels of the UR45, TCM1, and PGK1 mRNAs in each sample. Northern blots in Fig. 6A and B were used to determine the percentage of capped and uncapped transcripts. The relative levels of total, capped, and decapped RNAs were calculated as explained in footnotes b and c to Table 2.*

In *dcplΔ* cells, deletions of *UPF1*, *NMD2*, and *UPF3* differentially affect the accumulation of capped nonsense-containing and wild-type mRNAs. Experiments described above indicated that Upf1p, Nmd2p, and Upf3p promote, but do not catalyze, decapping of nonsense-containing mRNAs and also regulate the degradation of decapped transcripts of any type. Our epistasis analyses, as well as the experiments of Fig. 7, demonstrated that the latter effect could be attributable to increased efficiency of 5’-to-3’ exonuclease digestion. Since exonuclease digestion of decapped transcripts has also been shown to occur by a 3’-to-5’ mechanism (29) we sought to determine whether Upf1p, Nmd2p, and Upf3p have a role in modulating this pathway. Accordingly, we constructed *dcplΔ upf1Δ*, *dcplΔ nmd2Δ*, and *dcplΔ upf3Δ* strains, rationalizing that the inhibition of decapping that would occur in such strains would eliminate 5’-to-3’ decay. Indeed, all of the transcripts that accumulated in these strains were capped (Fig. 5A and data not shown). Northern analyses of RNAs isolated from these strains demonstrated that the abundance of the nonsense-containing CYH2 pre-mRNA and the wild-type CYH2 and TCM1 mRNAs were uniformly lowest in the *dcplΔ* strain, intermediate in the *dcplΔ upf3Δ* and *dcplΔ* strains, and highest in the *dcplΔ nmd2Δ* strain (Fig. 5B, Table 4, and data not shown). These results imply that Upf1p, Nmd2p, and Upf3p can also regulate 3’-to-5’ exonuclease decay differentially, with Upf1p having apparent negative regulatory capability in *dcplΔ* cells.

**DISCUSSION**

Upf1p, Nmd2p, and Upf3p regulate decapping of nonsense-containing and wild-type mRNAs. In the yeast *S. cerevisiae* the rapid degradation of nonsense-containing mRNAs proceeds from deadenylation-independent removal of the 5’ cap by the decapping enzyme, Dcp1p, to 5’→3’ digestion of the remainder of the mRNA by the exoribonuclease Xrn1p (6, 21, 47). This decay pathway also requires the activities of three additional trans-acting factors, Upf1p, Nmd2p, and Upf3p (10, 22, 23, 25, 38, 40, 41, 52, 54). Previous studies showed that mutations in any of the respective *UPF* or *NMD* genes led to the selective stabilization of nonsense-containing mRNAs but did not identify a mechanistic basis for such stabilization. Here, we show that loss of Upf1p, Nmd2p, or Upf3p inhibits the decapping of nonsense-containing mRNAs. This conclusion follows from experiments showing that inactivation of any or all of these factors, in *XRN1* or *xrn1Δ* cells, leads to the accumulation of capped CYH2 and *MER2* pre-mRNAs (Fig. 1, 2, and 4). Moreover, since *dcplΔ* strains accumulate only capped nonsense-containing transcripts, and all *xrn1Δ* strains containing single or multiple deletions of *UPF1*, *NMD2*, or *UPF3* still accumulate some decapped transcripts, our data indicate that Upf1p, Nmd2p, and Upf3p regulate but do not catalyze decapping of nonsense-containing mRNAs.

Although Upf1p, Nmd2p, and Upf3p were originally identified as factors that only regulated nonsense-containing mRNAs, our data indicate that these factors can, under some circumstances, also affect decapping of wild-type mRNAs. Several observations support this conclusion. First, in *xrn1Δ* cells, inactivation of these factors differentially alters the levels of all capped wild-type transcripts examined (Fig. 4A and 6B; Tables 2 and 3). Second, the effects of inactivation of Upf1p, Nmd2p, and Upf3p on the accumulation of capped transcripts in *xrn1Δ* cells are mRNA specific, suggesting that certain mRNA features can influence the activities of these factors. Third, even in *XRN1* cells, deletions of these genes lead to small but repro-
ducible increases in the levels of some wild-type mRNAs (Fig. 5).

Although decapping of nonsense-containing and wild-type mRNAs requires the same decapping enzyme, our data also indicate that the functions of Upf1p, Nmd2p, and Upf3p affect decapping of both classes of mRNAs differently. For example, Upf1p, Nmd2p, and Upf3p are all required to promote efficient decapping of nonsense-containing mRNAs. In contrast, in xrn1Δ cells, it appears that Upf1p and Nmd2p affect normal decapping of wild-type mRNAs, while Upf3p seems to have no effect on this activity. In addition, inactivation of Upf1p, Nmd2p, and Upf3p affects the levels of capped nonsense-containing transcripts dramatically but only affects the levels of capped wild-type transcripts modestly.

Rat1p functions in cytoplasmic mRNA degradation. Rat1p, one of two 5'-to-3' exoribonucleases in yeast, is predominantly localized to the nucleus in the steady state and has an essential nuclear function (33, 35). Consistent with these characteristics, the protein is involved in the formation of the 5' ends of 5.8S rRNA and some snoRNAs (45, 55). Here, we have demonstrated that Rat1p also functions in 5'-to-3' exonucleolytic degradation of decapped yeast mRNAs, at least in the absence of Xrn1p. Inactivation of Xrn1p leads to the accumulation of nonsense-containing and wild-type mRNAs that lacked the cap structure and several 5' nucleotides. Two key observations indicate that these mRNA decay intermediates arise from decapping by Dcp1p and 5' trimming by Rat1p. First, in contrast to the xrn1Δ strain, the dcp1Δ xrn1Δ strain accumulated only full-length, capped mRNAs, indicating that formation of the decay intermediates in xrn1Δ cells requires the activity of Dcp1p. Second, mRNA decay intermediates present in rat1-1 xrn1Δ cells grown at the permissive temperature disappeared after a shift to the nonpermissive temperature, indicating that the formation of these decay intermediates in xrn1Δ cells also requires the activity of Rat1p.

The ability of Rat1p to degrade cytoplasmic mRNAs in a 5'-to-3' direction in the absence of Xrn1p is consistent with two earlier observations. Muhlrad and Parker (47) showed that xrn1Δ cells can still accumulate low levels of 5'-to-3' decay intermediates of the nonsense-containing and wild-type PGK1 mRNAs. Further, Johnson (33) identified several dominant alleles of the RAT1 gene that cause mislocalization of Rat1p to the cytoplasm and complement the mRNA turnover defect of xrn1Δ cells. Surprisingly, however, while the 5' ends of the mRNA decay intermediates generated by Rat1p in vivo suggest a distributive activity for this enzyme, earlier in vitro analyses indicated that Rat1p had processive activity (56).

Upf1p, Nmd2p, and Upf3p regulate exonucleolytic degradation of nonsense-containing and wild-type mRNAs. After decapping, the remainder of a nonsense-containing or wild-type mRNA was isolated from yeast strains of the indicated genotypes, and anticap immunoprecipitation was carried out as in Fig. 1B. DNA probes specific for URA5, TCM1, and PGK1 were used for Northern analysis of the respective RNA fractions. Lanes 1, S, and P represent input, supernant, and pellet, respectively. Quantitation of this experiment is summarized in Table 3. (C) Analysis of the levels of URAS5 mRNA decay intermediates. Primer extension analysis of the URAS5 mRNA was performed on total RNA from each indicated yeast strain, as in Fig. 3A. Total RNA isolated from the upf3Δ strain was used as a control. The major transcriptional start sites of the URAS5 mRNA and the 5' ends of its decay intermediates are indicated by arrows and asterisks, respectively.
transcript is eliminated by exonucleolytic digestion. In wild-type cells, decapped transcripts are principally degraded in a 5'-to-3' direction by Xrn1p (28, 47, 48). However, in xrn1Δ cells, decapped transcripts are degraded in both the 5'-to-3' and 3'-to-5' directions (47, 49). Our observation that inactivation of Rat1p in xrn1Δ cells eliminates the formation of 5'-to-3' mRNA decay intermediates but does not increase the levels of all mRNAs examined (Fig. 2 and 3) supports this conclusion further. Our analyses of xrn1Δ cells provide several lines of evidence that Upf1p, Nmd2p, and Upf3p can also regulate exonucleolytic degradation of decapped nonsense-containing and wild-type mRNAs, including the following: (i) inactivation of Upf1p, Nmd2p, or Upf3p differentially affects total mRNA levels only in xrn1Δ cells, but not in XRN1 cells (Fig. 4 and 5); (ii) inactivation of these factors differentially affects the accumulation of decapped transcripts and mRNA decay intermediates; and (iii) the phenotypes caused by inactivation of these factors exhibit epistatic relationships.

Our epistatic analysis indicates that Upf1p plays a positive role in promoting exonucleolytic degradation and that Nmd2p and Upf3p function by regulating the activity of Upf1p (see below). However, in the experiments reported here, the xrn1Δ strain always accumulated higher levels of decapped transcripts than the xrn1Δ upf1Δ strain. This indicates that Upf1p can also play a negative role in exonucleolytic degradation. The dual roles of Upf1p may reflect differential regulation of two different pathways: positively on 5'-to-3' decay and negatively on 3'-to-5' decay. Two observations support this conclusion. First, when 5'-to-3' exonucleolytic degradation is partially blocked by inactivation of Xrn1p, overexpression of Upf1p reduces the accumulation of 5'-to-3' mRNA decay intermediates (Fig. 7B). Second, when 5'-to-3' exonucleolytic degradation is completely blocked by inactivation of Dcp1p, inactivation of Upf1p leads to decreased accumulation of capped mRNAs (Fig. 8A and Table 4). These apparently conflicting roles of Upf1p could be explained if the positive function reflected an indirect consequence of enhancing ribosome release at termination codons (31, 43) and the negative function reflected a regulatory interaction with a component(s) of the 3'→5' pathway (see below).

Functional relationships of Upf1p, Nmd2p, and Upf3p. The differential effects on the accumulation of decapped transcripts engendered by inactivation of Upf1p, Nmd2p, or Upf3p in xrn1Δ cells not only led us to conclude that these factors have different roles in regulating exonucleolytic degradation but also allowed us to determine their respective functional relationships. Our data indicate that the function of Upf1p is epistatic to Nmd2p and Upf3p and that the function of Upf3p is epistatic to that of Nmd2p. We interpret these relationships to suggest that Nmd2p and Upf3p regulate the activity of Upf1p, a conclusion consistent with our earlier analyses of nonsense suppression in upf and nmd cells (43) and with several observations in this study. Here, we show that (i) in an xrn1Δ background, all strains that contain UPF1, except the UPF or NMD wild-type strain, accumulate lower levels of decapped transcripts than strains lacking UPF1, indicating that Upf1p plays a more direct role in regulating exonucleolytic degradation than Nmd2p or Upf3p; (ii) the xrn1Δ upf1Δ nmd2Δ, xrn1Δ upf1Δ upf3Δ, and xrn1Δ upf1Δ nmd2Δ upf3Δ mutant strains accumulate the same level of decapped tran-

FIG. 7. In xrn1Δ cells, overexpression of the UPF1 gene decreases total mRNA levels as well as the levels of mRNA decay intermediates. (A) Northern analysis of total mRNA. xrn1Δ upf1Δ, xrn1Δ nmd2Δ, and xrn1Δ upf3Δ strains were transformed with a single-copy (S.C.) or a high-copy-number (H.C.) plasmid harboring the UPF1 gene. Total RNAs isolated from the resulting yeast strains, as well as the xrn1Δ upf1Δ strain, were analyzed by Northern hybridization, using probes for the CYH2, URA5, GCN4, and CUP1 mRNAs. The SCR1 RNA was used as an internal control. (B) Analysis of mRNA decay intermediates. Primer extension analysis of the CYH2 pre-mRNA and the URA5 mRNA was performed on each of the RNAs used in panel A. Radiolabeled primers CYH2-IN4 and URA5-1 were used for both reverse transcription and DNA sequencing reactions. The major transcriptional start sites and the 5' ends of decay intermediates are indicated by arrows and asterisks, respectively. The yeast strains labeled 1 to 7 correspond to those in panel A.

scripts as an \textit{xrn1} strain, indicating that, in the absence of \textit{Upf1p}, the presence of \textit{Nmd2p}, \textit{Upf3p}, or both, has no additional effects and that the functions of \textit{Nmd2p} and \textit{Upf3p} must operate through \textit{Upf1p}; (iii) \textit{UPF} or \textit{NMD} wild-type, \textit{xrn1 Nmd2} \textit{upf3}, and \textit{xrn1 Nmd2 upf3} strains accumulate different levels of decapped transcripts, demonstrating that the presence of \textit{Nmd2p}, \textit{Upf3p}, or both, has different effects on the activity of \textit{Upf1p}; (iv) \textit{xrn1 Nmd2} cells accumulate a lower level of decapped transcripts than \textit{xrn1 Nmd2 upf3} cells, indicating that, in the absence of \textit{Nmd2p}, \textit{Upf3p} enhances the function of \textit{Upf1p}; and (v) \textit{xrn1 Nmd2 upf3} cells accumulate the same level of decapped transcripts as \textit{xrn1 upf3} cells but a lower level than \textit{UPF} or \textit{NMD} wild-type cells. This last observation establishes that

![FIG. 8](image_url)

\textbf{FIG. 8.} Deletion of \textit{UPF1}, \textit{NMD2}, or \textit{UPF3} in \textit{dcp1Δ} cells differentially affects the levels of the \textit{CYH2} pre-mRNA and mRNA. (A) Analysis of 5’ cap status. Total RNA was isolated from yeast strains of the indicated genotypes and anti-m7G immunoprecipitation was performed as in Fig. 1B. Lanes I, S, and P represent input, supernatant, and pellet samples, respectively. (B) Northern analysis of total mRNA. Total RNA from yeast strains of the indicated genotypes was isolated and analyzed by Northern hybridization, using the \textit{SCR1} RNA as an internal control. WT, wild type. In both panels A and B, the \textit{CYH2} probe used was the same as in Fig. 1B. Quantitation of this experiment is summarized in Table 4.

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<td>17.80</td>
<td>3.10</td>
<td>1.05</td>
<td>0.93</td>
<td>0.12</td>
</tr>
<tr>
<td>\textit{dcp1Δ upf3Δ}</td>
<td>12.20</td>
<td>10.60</td>
<td>1.60</td>
<td>0.70</td>
<td>0.60</td>
<td>0.10</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The Northern blot in Fig. 8B was used to determine levels of the \textit{CYH2} pre-mRNA and mRNA. The relative abundance of the different RNAs was determined as explained in footnotes \textsuperscript{b} and \textsuperscript{c} Table 2.

\textsuperscript{b} NA, not applicable.
Nmd2p has no effect on Upf1p in the absence of Upf3p and that, in the presence of Upf3p, Nmd2p negatively regulates Upf1p.

In xrn1Δ cells, inactivation of the UPF and NMD factors also had differential effects on the accumulation of capped wild-type transcripts. Using these phenotypes to determine the functional relationships of Upf1p, Nmd2p, and Upf3p, we found the same functional relationships among the factors, i.e., that the function of Upf1p was epistatic to Nmd2p and Upf3p for all mRNAs examined. However, these analyses revealed that the epistatic relationships of Nmd2p and Upf3p are mRNA-specific. Interestingly, our data indicate that the functional relationships of these factors in controlling the accumulation of capped PGK1 mRNA are the same as those regulating exonucleolytic degradation. It remains to be determined what these two sets of events have in common.

Reconciliation of the diverse functions of Upf1p, Nmd2p, and Upf3p. The data presented in this paper demonstrate that Upf1p, Nmd2p, and Upf3p function in the regulation of mRNA decapping and in both modes of exonucleolytic decay. These mRNA degradative events involve multiple factors, including Dcp1p, Xrn1p, Rat1p, and the components of the exosome (6, 28, 29, 46, 48), none of which have been shown to have significant physical interactions with the products of the UPF and NMD genes. How, then, could Upf1p, Nmd2p, and Upf3p function as such general regulators of mRNA decay? Since it is unlikely that these factors regulate the activities of all of the degradative enzymes directly, it seems reasonable to consider the possibility that they exert their regulatory effects by controlling substrate availability to the decay pathways.

Other than regulating the stability of nonsense-containing mRNAs, the principal function ascribed to the UPF and NMD gene products has been the regulation of translation termination fidelity and/or efficiency. This conclusion follows from experiments showing that (i) mutations or deletions of the UPF1, NMD2, and UPF3 genes promote omnipotent nonsense suppression and allosuppression (10, 11, 41, 43, 69, 70); (ii) nonsense suppression in upf and nmd mutations is directly attributable to effects on translation termination, not mRNA decay (43, 69, 70); and (iii) Upf1p interacts with the polypeptide release factors, Sup35p and Sup45p, both in vitro and in vivo (13). While translation termination is generally defined as release of the completed polypeptide from the peptidyl-tRNA in response to a stop codon, it is clear, at least in prokaryotes, that the event is considerably more complex and must include at least one more step in which ribosomes are dissociated from the mRNA (32). Moreover, the participation of the initiation factors eIF3 and IF3 in the dissociation process (18, 34, 66) suggests that disassembly of the termination complex may prepare the ribosome for recycling to the next round of translation initiation on the same mRNA or a different mRNA. The possibility that this event may influence the subsequent translation or stability of the mRNA in question is suggested by experiments showing that (i) mutations in eIF3 can lead to the selective stabilization of nonsense-containing mRNAs (68); (ii) premature translation termination can decrease the translational efficiency of an mRNA (51); and (iii) Upf1p interacts with Nmd3p, a 60S ribosome-associated factor that may have a role in subunit association and dissociation (7, 15, 16, 18, 27, 73).

These observations, and the suggestion that proper termination of translation can only occur in the context of interactions between a terminating ribosome and a specific RNP domain or set of factors localized 3′ to a normal stop codon (8, 26, 31), lead us to propose that the direct regulatory effects of Upf1p, Nmd2p, and Upf3p on translation termination can explain at least some of their effects on mRNA decay. In this model, Upf1p is thought to utilize its ATPase and helicase activities to promote ribosome release or a conformational change among the components of the termination complex (26, 31). If interactions with factors bound 3′ to the termination site influence Upf1p’s activity, then the efficiency of the latter event, and/or the subsequent translational competence of the ribosome, may differ with normal versus premature stop codons. In turn, the altered competence of the ribosome for an additional round of initiation may render the mRNA more susceptible to decapping (51) and inefficient ribosome release may decrease the efficiency of 5′→3′ exonucleolytic decay. This model does not accommodate our observations on the effects of upf and nmd mutations on 3′→5′ decay, leading us to suggest further that the apparatus involved in the latter mode of decay may be influenced by factors involved in effecting proper termination at the normal end of an open reading frame. As presented, this model also does not explain why inactivation of Xrn1p renders several of the decay phenotypes detectable or more pronounced. One plausible explanation is that inactivation of Xrn1p leads to increased accumulation of decapped transcripts for most mRNAs and these decapped transcripts may sequester some component(s) involved in translation initiation and/or termination, thereby making decapping of wild-type mRNAs more dependent on the function of Upf1p, Nmd2p, and Upf3p.

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REFERENCES
To address the regulation of mRNA decay, recent studies have identified key components that contribute to the degradation of unstable transcripts. The Upf1p, Nmd2p, and Upf3p proteins play crucial roles in this process. Upf1p controls the degradation of aberrant mRNAs, Nmd2p promotes mRNA turnover, and Upf3p is involved in the recognition of non-sense-mediated mRNAs. These factors interact with the exosome complex containing KEM1 and XRN1/XRN2/XRN3, which are essential in the surveillance of mRNA decay.

In summary, the interplay between translation termination and mRNA decay is tightly regulated by Upf1p, Nmd2p, and Upf3p proteins. These proteins work in concert with other factors such as the exosome complex to ensure that only functional mRNAs are translated, thereby maintaining cellular homeostasis.


