Identification and characterization of genes that are required for the accelerated degradation of mRNAs containing a premature translational termination codon

Ying Cui,1 Kevin W. Hagan,1 Shuang Zhang,2 and Stuart W. Peltz1–3

1Department of Molecular Genetics and Microbiology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey and 2the Program in Microbiology and Molecular Genetics, Rutgers University, Piscataway, New Jersey 08854 USA

In both prokaryotes and eukaryotes nonsense mutations in a gene can enhance the decay rate of the mRNA transcribed from that gene, a phenomenon described as nonsense-mediated mRNA decay. In yeast, the products of the UPF1 and UPF3 genes are required for this decay pathway, and in this report we focus on the identification and characterization of additional factors required for rapid decay of nonsense-containing mRNAs. We present evidence that the product of the UPF2 gene is a new factor involved in this decay pathway. Mutation of the UPF2 gene or deletion of it from the chromosome resulted in stabilization of nonsense-containing mRNAs, whereas the decay of wild-type transcripts was not affected. The UPF2 gene was isolated, and its transcript was characterized. Our results demonstrate that the UPF2 gene encodes a putative 126.7-kD protein with an acidic region at its carboxyl terminus [-D-E]n found in many nucleolar and transcriptional activator proteins. The UPF2 transcript is 3600 nucleotides in length and contains an intron near its 5' end. The UPF2 gene is dispensable for vegetative growth, but upf2Δ strains were found to be more sensitive to the translational elongation inhibitor cycloheximide than UPF2+. A genetic analysis of other alleles proposed to be involved in nonsense-mediated mRNA decay revealed that the UPF2 gene is allelic to the previously identified sua1 allele, a suppressor of an out-of-frame ATG insertion shown previously to reduce translational initiation from the normal ATG of the CYC1 gene. In addition, we demonstrate that another suppressor of this cycl mutation, sua6, is allelic to upf3, a previously identified lesion involved in nonsense-mediated mRNA decay.

[Key Words: RNA degradation; UPF2; nonsense mutations; translation]

Received October 12, 1994; revised version accepted December 5, 1994.

Differences in the decay rates of individual mRNAs can have profound effects on the overall levels of expression of specific genes. In eukaryotic cells the decay rates of mRNAs can differ from each other by >50-fold (for review, see Ross 1988; Peltz et al. 1991, 1993a; Peltz and Jacobson 1993; Sachs 1993). In addition, the decay rates of certain mRNAs are modulated depending on either the stage of the cell cycle, cell type or stage of differentiation and external factors such as hormonal levels, nutritional needs and environmental stresses (for review, see Cleveland and Yen 1989; Atwater et al. 1990; Peltz et al. 1991). The mechanisms that control these processes are largely unknown.

We have been studying mRNA turnover in the yeast Saccharomyces cerevisiae. Our results, as well as results from other laboratories, strongly indicate that mRNA turnover and translation are intimately linked processes and that understanding their relationship is critical to the understanding of mRNA decay (Stimac et al. 1984; Graves et al. 1987; Cleveland 1988; Gay et al. 1989a,b; Peltz et al. 1989, Parker and Jacobson 1990, Wisdom and Lee 1991; Bernstein et al. 1992, Laird-Offringa 1992, Peltz et al. 1992; Aharon and Schneider 1993; for review, see Pelsy and Lacroute 1984; Peltz et al. 1993a). One clear example of the relationship between translation and mRNA decay is the effect of nonsense mutations on the abundances and decay rates of mRNAs. In both prokaryotes and eukaryotes nonsense mutations in a gene can accelerate the decay rate of the mRNA transcribed from that gene 10- to 20-fold (Losson and Lacroute 1979; Losson and Lacroute 1979; Maquat et al. 1981; Pelsy and Lacroute 1984;
Baumann et al. 1985; Nilsson et al. 1987; Daar and Maquat 1988; Urlaub et al. 1989; Cheng et al. 1990; Gozalbo and Hohmann 1990; Leeds et al. 1991; Barker and Berman 1991; Gaspar et al. 1991; Baserga and Benz 1992, Lim et al. 1992, Cheng and Maquat 1993, Peltz et al. 1993a. We use the term nonsense-mediated mRNA decay to describe this phenomenon [Peltz et al. 1993a]. We have begun to identify the cis-acting sequences and trans-acting factors involved in this decay pathway [Leeds et al. 1991, 1992; Peltz et al. 1993a; for review, see Peltz et al. 1994].

A genetic screen for translational frameshift suppressors led to the identification of a class of mutant alleles termed upf [for up-frameshift; Culbertson et al. 1980; Leeds et al. 1992]. Subsequent analysis of these alleles demonstrated that mutations in either the UPF1 or UPF3 genes led to the specific stabilization of nonsense-containing mRNAs [Leeds et al. 1991, 1992, Peltz et al. 1993a]. For example, an amber codon that terminates translation of the PGK1 transcript after 5% of its protein-coding region has been translated decreased its mRNA half-life from 60 min or greater to 3 min or less in a wild-type strain [Peltz et al. 1993a]. In a upf1 strain the decay rate of the same mRNA is 60 min or greater [Peltz et al. 1993a]. Similar observations have been found in strains containing mutations in the UPF3 gene [K. Hagan and S.W. Peltz, unpubl.].

Experiments investigating the role of the UPF2 gene in nonsense-mediated mRNA decay were ambiguous [Leeds et al. 1992]. The results presented here identify and characterize the UPF2 gene and demonstrate that similar to the UPF1 and UPF3 genes, the product of the UPF2 gene is a necessary component for nonsense-mediated mRNA decay. Furthermore, we demonstrate that the previously identified sua1 lesion [Hampsey et al. 1991; Pinto et al. 1992a] is allelic to upf2 and that sua6 is allelic to upf3 [Pinto et al. 1992a,b].

Results

The abundance of nonsense-containing mRNAs is increased in strains harboring the upf2-1 allele

We wanted to determine whether the level of nonsense-containing mRNAs was increased in strains harboring a mutant upf2 allele. The abundances of the his4-38 mRNA and CYH2 precursor were monitored in upf1-1, upf2-1, and wild-type strains (see Table 1). The his4-38 allele is a single G insertion in the HIS4 gene that generates a +1 frameshift and a UAA nonsense codon in the triplet adjacent to the insertion, resulting in rapid decay of this mRNA in wild-type cells [Leeds et al. 1991]. The inefficiently spliced CYH2 precursor is stabilized 5- to 10-fold in a upf1-1 strain because the introns near the 5' end contain an in-frame nonsense codon [He et al. 1993]. RNAs from wild-type, upf2-1, and upf1-1 cells were isolated, and the abundances of the CYH2 precursor and his4-38 mRNA were determined by RNA blotting analysis involving hybridization to radioactive probes complementary to these RNAs. As shown in Figure 1, the abundances of the CYH2 precursor and the his4-38 transcript were low in wild-type cells and could be detected only after overdevelopment of the film but increased at least fivefold in both the upf1-1 and upf2-1 mutant strains. These results indicate that the product of the UPF2 gene is involved in nonsense-mediated mRNA decay.

Isolation of the UPF2 gene

Strains harboring the upf2-1 allele were isolated on the basis of its ability to act as an allosuppressor of the his4-38 frameshift mutation [Culbertson et al. 1980, Leeds et al. 1992]. At 30°C but not 37°C, the his4-38 frameshift allele is suppressed by SUFI-1, which encodes a glycine tRNA capable of reading a 4-base codon [Mendenhall et al. 1987]. Mutations in the UPF genes, including UPF2, allow cells harboring his4-38 SUFI-1 to grow at 37°C. The wild-type UPF2 gene was isolated by transformation of a yeast strain harboring the upf2-1, his4-38, and SUFI-1 alleles with a yeast genomic library and screening for cells that could no longer grow on medium lacking histidine at 37°C. From 5000 colonies, nine colonies containing the single-copy plasmids were no longer capable of growing at 37°C [Fig. 2A]. The strains harboring the nine plasmids identified above could overcome the allosuppressor phenotype of the upf2 mutation. We next wanted to determine whether the loss of the allosuppressor phenotype corresponded with decreased abundance of the nonsense-containing mRNAs. Therefore, we determined whether the abundance of the CYH2 precursor in cells harboring these plasmids was decreased compared with cells harboring only the upf2 allele. RNAs were isolated from wild-type cells, upf2-1 cells, and upf2-1 cells harboring the plasmids identified above, and the abundance of the CYH2 precursor was determined as described above. The results demonstrate that only one of the nine strains harboring the plasmids identified above has a reduced abundance of the CYH2 precursor [Fig. 2B, lane 5]. Strain YPF2-5 that had lost the plasmid after being plated on medium containing 5-FOA was now able to grow at the higher temperature [data not shown]. The plasmid pYCpA5 was isolated and transformed again into a mutant upf2 strain [upf2-1 his4-38 SUFI-1] and restested for inhibition of growth at 37°C. The plasmid that was transferred into the upf2-1 strain prevented the cells from growing at 37°C [data not shown]. These results demonstrate that the modulation of the frameshift suppression was a consequence of the plasmid containing the yeast gene. The plasmid pYCpA5 harboring the putative UPF2 gene [Fig. 2B, lane 5] was characterized further. The genes from the other plasmids that abrogate the translational effects of the upf2 mutation have not been characterized.

A restriction map of the genomic 13.7-kb DNA fragment containing the plasmid pYCpA5 harboring the putative UPF2 gene was constructed [Fig. 3A]. Plasmid subclones of the genomic DNA were prepared [Fig. 3B], and their ability to affect the allosuppressor phenotype [data not shown] and the abundance of the CYH2 precurs-
Table 1. Strains used in this study

| Strain  | Genotype                        | Source, reference, or derivation |
|---------|---------------------------------|---------------------------------|
| PLY36   | MATα his4-38 SUFI-1 upf1-2 ura3-52 met14 | Leeds et al. (1991), (1992) |
| PLY18   | MATα his4-38 SUFI-1 ura3-52 trp1-1 leu2-3 | Leeds et al. (1991), (1992) |
| PLY136  | MATα his4-38 SUFI-1 upf2-1 ura3-52 | this study |
| YGC110  | MATα his4-38 SUFI-1 upf2-1 ura3-52 | Leeds et al. (1991), (1992) |
| PLY139  | MATα his4-38 SUFI-1 upf3-1 ura3-52 | PLY36 derivative |
| YGC10− | MATα his4-38 SUFI-1 upf1-2 ura3-52 met14 [YCplac33] | PLY36 derivative |
| YGC10+ | MATα his4-38 SUFI-1 upf1-2 ura3-52 met14 [YCp33UPF1] | PLY36 derivative |
| YGC112 | MATα his4-38 SUFI-1 UPF2::URA3 ura3-52 trp1-1 leu2-3 | this study |
| RY262  | MATα rpbl-1 his4-519 ura3-52 | Beate Schwer's laboratory |
| YGC14+ | MATα rpbl-1 his4-519 ura3-52 [YCplac33] | this study |
| YGC14− | MATα rpbl-1 his4-519 UPFI::URA3 ura3-52 | this study |
| YGC116 | MATα rpbl-1 his4-519 UPFI::URA3 ura3-52 | this study |
| RY262  | MATα rpbl-1 his4-519 UPFI::URA3 ura3-52 | this study |
| YGC118 | MATα his4-38 SUFI-1 UPF2::URA3 ura3-52 | this study |
| YGC120 | MATα his4-38 SUFI-1-upf2-1 UPF2::URA3 ura3-52 met14 | this study |
| YGC12EF2 | MATα his4-38 SUFI-1 UPF2::URA3 ura3-52 trp1-1 leu2-3 [YEpUPF2] | YGC112 derivative |
| T16    | MATα cyc1-5000 cyc7-67 ura3-52 leu2-3,112 cyh2 S UA + | Hampsey et al. (1991); Pinto et al. (1992) |
| YIP15−4A | MATα cyc1-362 arg4-17 leu2-3,112 sual | Hampsey et al. (1991); Pinto et al. (1992) |
| YIP15−4B | MATα cyc362 his3-Δ1 ura3-52 su2a | Hampsey et al. (1991); Pinto et al. (1992) |
| YIP15−4D | MATα cyc1-362 arg4-17 ura3-52 sual sua2 | Hampsey et al. (1991); Pinto et al. (1992) |
| YIP13−11A | MATα cyc1-362 arg4-17 his3-Δ1 ura3-52 sua3 | Hampsey et al. (1991); Pinto et al. (1992) |
| YIP16−4D | MATα cyc1-362 his3-Δ1 ura3-52 sua4 | Hampsey et al. (1991); Pinto et al. (1992) |
| YIN192  | MATα cyc1-1019 cyc7-67 ura3-52 leu2-3,112 cyh2' sual5-1 | Hampsey et al. (1991); Pinto et al. (1992) |
| YIN−8A  | MATα cyc1-362 ura3-52 leu1-12 his5-2 (trp51) sua6 | Hampsey et al. (1991); Pinto et al. (1992) |

DNA sequence analysis of the UPF2 gene

The sequence of the DNA fragment harboring the putative UPF2 gene in plasmid pYCpAp7.1 was determined. The sequence of a 5.015-kb DNA fragment from 100 bp upstream of the first Clal site to ~1400 nucleotides downstream of the EcoRI site was determined (Fig. 4). Our initial inspection of the sequence identified a 1091-amino-acid open reading frame (ORF), but subsequent analysis demonstrated a perfect splice site branchpoint consensus sequence 33 bp upstream of the ORF and the consensus acceptor site (Fig. 4, 5'-TACTAAC-3', between 65 and 71 bp; for review, see Rymond and Rosbash 1993). The location of the splice donor site could not be identified by computer analysis. Subsequent analysis of the UPF2 mRNA indicated that the transcript contained a short intron very near the 5' end and encoded a 1089-amino-acid protein (Fig. 4, see below). These findings are consistent with the observation that in the yeast S. cerevisiae the introns are usually found near the 5' end of the mRNA.

The predicted peptide sequence of the Upf2 protein (Upf2p) was used to search the SWISSPROT and nonredundant protein sequence data bases by use of the GCG program. With the exception of its carboxyl terminus, Upf2p had no significant homology to other proteins.
The carboxyl terminus of the Upf2p (amino acids 838–995) were highly homologous to a group of nucleolar or nuclear acidic proteins (with an identity score of 30% and a similarity score of 90%), including nucleolin (Maridor et al. 1990; Srivastova et al. 1990), nucleolar phosphoprotein B23 (Chang et al. 1988), nucleolar transcription factor upstream binding factor (UBF) (O’Mahony et al. 1992; Voit et al. 1992), and yeast RNA polymerase III subunit RPC31 (Mosrin et al. 1990). The first three nucleolar proteins are known to be critical factors in ribosomal biogenesis. Within this region of homology all of these proteins have a conserved acidic amino acid stretch that is considered to be a casein kinase II phosphorylation site and is thought to be important for the function of these proteins (Chan et al. 1986; Chang et al. 1988; O’Mahony et al. 1992; Voit et al. 1992). The acidic region of the yeast RNA polymerase III gene has been demonstrated to be essential for its trans-activation function (Mosrin et al. 1990; O’Mahony et al. 1992; Voit et al. 1992). Although the role of the acidic region in the Upf2p is not known, on the basis of previous studies, we suggest that its role is probably involved in regulating the phosphorylation status of the protein which, in turn, is in some way necessary for maintenance of the protein–protein interactions. A search for other protein motifs by use of the PROSITE data base (v. 2/94) did not reveal any other known conserved motifs.
haploid strain demonstrates that the putative
ysis Isee Materials and methods (Table 2, item 3), whereas the putative upf2Δ strain was unable to complement the strain harboring the upf2-1 allele (Table 2, item 4). In addition, the CYH2 precursor abundance was high in the diploid prepared from a cross between the upf2-1 and upf2Δ (Table 2, item 4), whereas the cross between upf1-2 and upf2Δ reduced the intron-containing RNA fivefold (Table 2, item 3). These results demonstrate that the gene we have identified encodes the UPF2 gene.

Analysis of the UPF2 transcript

We have analyzed the structure of the UPF2 transcript by a variety of techniques. The UPF2 transcript was visualized by RNA blotting analyses of RNAs isolated from upf2Δ cells harboring either a centromere or 2μ plasmid containing the UPF2 gene or a plasmid lacking the UPF2 gene. The results demonstrated that the UPF2 mRNA was ~3600 nucleotides in length and was absent from a upf2Δ strain (Fig. 5A). The UPF2 gene contains splicing consensus elements near the putative 5′ end of the UPF2 gene (Fig. 4). A PCR strategy was employed to identify the exon/intron boundaries of the UPF2 transcript. Total RNA was isolated, and cDNA from the RNA was prepared by reverse transcription. DNA primers that traversed the putative exon/intron boundaries were used in a PCR. A DNA fragment corresponding to the cDNA fragment from a spliced mRNA was isolated and sequenced (Fig. 5B,C). The DNA fragment described above was absent if the RNAs utilized in the reaction were prepared from a temperature-sensitive splicing-defective prp2 strain (Table 1, RLP2) shifted to the nonpermissive temperature [data not shown] or if reverse transcriptase was left out of the reaction mixture (Fig. 5B, lane 2). The 390-bp DNA fragment was sequenced, and the results indicate that the UPF2 mRNA is spliced and that the splice site donor and splice site acceptor is located at 7 and 120 bp, respectively (Figs. 4 and 5). The splicing branchpoint is situated at 65–71 bp (Fig. 4).

The complementation of the mutant alleles were determined by replica plating of the diploids onto media lacking histidine at 30°C or 37°C. Complementation occurs if the diploids are not able to grow at 37°C on media lacking histidine. A upf1-2 strain was able to complement a strain harboring the disruption of the putative upf2 gene (Table 2, item 3), whereas the putative upf2Δ strain was unable to complement the strain harboring the upf2-1 allele (Table 2, item 4). The complementation of the mutant alleles were determined by replica plating of the diploids onto media lacking histidine at 30°C or 37°C.

The gene that complements the upf2-1 allele encodes the UPF2 gene

To determine whether the gene that we have isolated encodes the UPF2 gene, a genomic disruption of the UPF2 gene was constructed (Fig. 3B, pKOF2). Haploid yeast cells harboring the putative UPF2 gene disruption were constructed and confirmed by Southern blot analysis (see Materials and methods). The viability of the haploid strain demonstrates that the putative UPF2 gene was not essential for vegetative growth (Table 2, items 1 and 2). To determine whether the genomic disruption was able to complement the upf2-1 allele, strain YGC112 containing the his4-38 SRF1-1 alleles and the genomic disruption of the putative UPF2 gene was crossed with strains harboring the his4-38 and SRF1-1 alleles that contained either the upf1-2 or upf2-1 alleles

Figure 3. Identification of the putative UPF2 gene. (A) A restriction map of yeast genomic DNA harboring the putative UPF2 gene. The open reading frame (ORF) of the putative UPF2 gene is shown. The shaded region in the schematic represents a portion of the pYCp50 vector. The restriction enzymes used were (S) SalI, (E) EcoRI, (Asp) Asp718, (C) ClaI, (Bs) BstXI, (X) XhoI, (B) BamHI, (N) NruI, (Xh) Xhol, (Sp) SphI, (P11) PvuII. (B) Determination of the region of the yeast genomic fragment harboring the putative UPF2 gene and construction of a upf2 disruption plasmid. The DNA from the yeast genomic fragment was cleaved with the restriction enzymes shown in the figure and various subclones were prepared by insertion of these DNA fragments into pYCplac33. plasmid pKOF2 was made to delete the UPF2 gene from the yeast chromosome and was constructed as described in Materials and methods. (C) Identification of the DNA fragment harboring the putative UPF2 gene. The plasmids described in B were transformed into FLY136 and the abundance of the CYH2 precursor and mRNA were determined as described in Fig. 1.
Cui et al. used in isolation of the sequence is shown in the single-letter code. Shaded nucleotides represent the consensus splice site sequence. The oligonucleotides

```
1 -874
-976
-262 ta~at~aq~atgatattagggttattaataggtttacaattatataatttatgtgataattatcacttgatacgaattgatggagcctgcttctttttt
-161
-364
-466
-568
-772
-160 tttttttcactttcttggcagtcactgaaaaactgcattcgaatacaggtttgagaaactaatga@gcccatattactttacaatgaacagt~ac~atc~ac
-59
1065
1575
1371
1677 gaatataaggaattaatggaaaaaatggtccaactaatcaaggataaaaaaaatgataggcaattgaacatgaacatgaaaagcgccttagaaaacataatt
1778
2187
2085 attgagcgaggcttagaaattaacgattatggacaaaacatgcatagaatatcaaatgtcagatacttaactgaaatattcaactttgaaatgataaaatcc
2186
2391
2289
2595 attcacaacggtaaggagagtgctgttcctatcgagtcaatcaccgaagatgatgaggatgaagatgatgaaaacgacgatggtgtcgatttactaggagaa
2696
2799 gaggatgagcagagcttagaaattaacgattatggacaaaacatgcatagaatatcaaatgtcagatacttaactgaaatattcaactttgaaatgataaaatcc
2900
2697
2901
3003
3513
3513 ctgctaaatgttttgaataaaaatgatgacgtaattgccgctgttgagatcataagtggacttcatcaaaggttcaatggccgatttactagtccgctttta
452
284 K L L P I F I R F K T S A I T L G E F F K L E I P E L E G A S N D D
317
420 T Q D W S K L P V Y S R F I A T S K N Y S F E I N Y L D N
453
521 G A F L Q A F E N P S V D I E S E R D E L Q R I T R V K G N L R V F
567
```

Figure 4. Sequence of the UPF2 gene. The UPF2 gene was sequenced as described in Materials and methods. The predicted amino acid sequence is shown in the single-letter code. Shaded nucleotides represent the consensus splice site sequence. The oligonucleotides used in isolation of the UPF2 cDNA by PCR are bold and underlined. Boldface double-underlined nucleotides are the oligonucleotides for primer extension. Nucleotides marked by open circles or carets indicate the major or minor transcription start sites, respectively. The bold amino acid sequences (838–995) make up the acidic amino acid-rich region homologous to nucleolin.

resulting in an out-of-frame ATG codon upstream of the CYC1 protein-coding region [Stiles et al. 1981]. The cycl1-362 mutation is therefore analogous to an amino-terminal nonsense mutation. Suppressors of the cycl1-
Trans-acting factors in nonsense-mediated mRNA decay

Figure 5. Analysis of the UPF2 mRNA.

[A] RNA blotting analysis of the UPF2 transcript. RNAs were prepared from yeast strain YGC112 [upf2A; lane 1], from yeast strain PLY136 containing the single-copy plasmid YCpAp7.1 [lane 2] or strain YGC112 containing the high copy plasmid YEpAp7.1 [lane 3]; an RNA blot was prepared as described above, and the membrane was hybridized with radiolabeled a 1.7-kb BamHI–EcoRI DNA fragment harboring the UPF2 open reading frame (see Fig. 3).

(B,C) Identification of the intron–exon junction of UPF2 RNA. Total yeast RNA (30 μg) from PLY18 containing the high copy plasmid pYEpAp7.1 was reverse transcribed by use of hexamers as described in Materials and methods. The product of reverse transcription was subjected to PCR with primers b and c that we hypothesized to be near the 5' and 3' splice junctions (see Materials and methods). The PCR product was electrophoresed on a 1.5% agarose gel and is shown in B. Marker lane (M), 1-kb marker. (Lane 1) The PCR product in which the reaction mixture contained reverse transcriptase [RT]; (lane 2) the PCR product in which the reaction mixture did not contain RT. (C) DNA sequence analysis of the 390-bp PCR product. The 390-bp DNA fragment from PCR was isolated and sequenced as described in Materials and methods. The arrow at left depicts the region where the two exons were joined. The sequence below the gel represents the DNA and protein sequences at the exon joining region. [D] Primer extension analysis of the UPF2 transcript. Total yeast RNA prepared from strain YGC112 harboring pYEpAp7.1 plasmid was used in a reverse transcription reaction (see Materials and methods). Lanes 1–4 are the results of DNA sequencing reactions by use of the same primer as for the primer extension analysis [Materials and methods]. (Lane 5) Fifty micrograms of RNA in the reaction; (lane 6) 30 μg of RNA used in the reaction. The mark at right indicates the length of the reverse transcription product. See Fig. 4 for the location of the transcription start site in the UPF2 gene.

362 (called sua for suppressor of upstream ATG) have been isolated [sua1–sua6] and sua7 and sua8 have been characterized and shown to affect transcription start site selection [Hampsey et al. 1991; Pinto et al. 1992a,b]. The mechanism of suppression for the other sua alleles has not been determined. In our search for other trans-acting factors involved in nonsense-mediated mRNA decay, we hypothesized that a subclass of the sua alleles would be involved in this decay pathway. Therefore, we asked whether any of the sua alleles affect the abundance of nonsense-containing mRNAs and whether they are allelic to the previously identified upf alleles. As described above, we utilized the abundance of the CYH2 precursor as an assay to determine whether the sua alleles affected the activity of the nonsense-mediated mRNA decay pathway. RNAs were isolated from strains harboring either wild-type or mutant sua alleles, and the CYH2 precursor and mRNA abundances were determined by RNA blotting analysis. The abundance of the CYH2 precursor relative to the CYH2 mRNA abundance was increased in strains containing either sua1 or sua6 allele but not in strains harboring the other sua alleles [Fig. 6A]. These results indicate that the products from the SUA1 and SUA6 genes are involved in nonsense-mediated mRNA decay.

Complementation analysis was performed to determine whether the sua1 or sua6 mutations were allelic to any of the upf alleles. Strains harboring either the sua1 or sua6 alleles were transformed with centromere-based plasmids harboring either the UPF1 or UPF2 gene, and the abundance of the CYH2 precursor was determined and compared with the RNA abundance of the CYH2 precursor in a wild-type SUA+ strain. The abundance of the CYH2 precursor decreased fivefold when a strain harboring the sua1 allele was transformed with a plasmid harboring the UPF2 gene [Fig. 6B, lanes 3, 4]. In addition,
Table 2. Growth characteristics and CYH2 precursor and mRNA levels in crosses between strains harboring upf2Δ with either upf2-1 or upf1-2 alleles

| Item number | Strain                                | Growth* 30°C | CYH2 precursor/ CYH2 mRNAb |
|-------------|---------------------------------------|--------------|---------------------------|
| 1           | YGC112 (upf2Δ)                        | +            | 0.54                      |
| 2           | YGC114 (upf2Δ)                        | + N.D.       | 0.64                      |
| 3           | YGC112 × PLY36 (upf2Δ × upf1-2)       | +            | 0.05                      |
| 4           | YGC112 × PLY136 (upf2Δ × upf1-2)      | +            | 0.40                      |

*Growth was determined on media lacking histidine. Yeast strains were grown on YPD media and replica plated onto yeast medium lacking histidine. The growth was followed for 4 days. (+) Strains able to grow on media lacking histidine, (−) cells unable to grow on this medium. (N.D.) Not determined.

bThe ratios of the levels of the CYH2 precursor and CYH2 mRNA were determined as described in Fig. 1 and in Materials and methods. The CYH2 precursor and mRNAs were quantitated by use of a Bio-Rad densitometer.

A ufp2Δ strain failed to complement a strain harboring a sua1 allele as determined by the high CYH2 precursor level in the diploid cell (Fig. 6B, lanes 13, 14), indicating that SUA1 and UPF2 are the same gene. The sua6 allele was not complemented by any of the identified UFP genes, indicating that it was not UPF1 or UPF2 (Fig. 6B, lanes 7–12). Crossing the sua6 mutant cell with a strain harboring upf3-1 allele demonstrated that the abundance of the CYH2 precursor was high in a sua6/upf3 diploid cell. Tetrad analysis from seven sua6/upf3 diploid strains also confirmed that the levels of CYH2 precursor in all spores were the same as observed in the parental upf3 or sua6 strain (one example of these results is shown in Fig. 6B, bottom), indicating that SUA6 is the same gene as UPF3.

Deletion of the UPF2 gene from the yeast chromosome stabilizes nonsense-containing mRNAs without affecting the decay of wild-type transcripts

We next determined whether the high steady-state levels of nonsense-containing mRNAs in a upf2Δ strain resulted from stabilizing their transcripts. The mRNA decay rates of wild-type and nonsense-containing mRNAs were determined in UPF2+ and upf2Δ strains that also harbored the temperature-sensitive allele of RNA polymerase II (rpB1-1). The decay rates of the wild-type and nonsense-containing mRNAs were determined by RNA blotting analyses of RNA isolated at different times after inhibition of transcription by a shift of the culture to the nonpermissive temperature (36°C). The results of these experiments demonstrate that the nonsense-containing his4-519 and CYH2 precursor RNAs were stabilized 10- and 8-fold, respectively (Fig. 7; Table 3) compared with UPF2+ cells, whereas the half-lives of the wild-type CYH2, MATa1, LEu2, and TIF4631, were the same in either UPF2+ or UPF2Δ strains (Table 3). These results demonstrate that the UPF2 gene is involved in nonsense-mediated mRNA decay.

Figure 6. sua1 and sua6 affect the abundance of the CYH2 precursor. (A) Wild-type and sua-containing strains were grown. RNAs were prepared and the abundances of CYH2 precursor and mRNA were assayed by RNA blotting as described in Fig. 1. The results from sua alleles are shown in lanes 2–8 (the captions at top describe the sua allele tested). Cells harboring the wild-type UPF2 (lanes 1, 9) or the upf2-1 allele (lane 10) are shown as controls. (B) Determination of whether sua1 or sua6 is allelic to the UPF allelic. Plasmid pYCplac33 harboring either the UPF1 or the UPF2 gene, or just vector, were transformed individually into strains harboring either sua1 or sua6. The strains were grown in medium lacking uracil, RNAs were prepared, and the abundance of CYH2 precursor and mRNA was assayed by RNA blotting as described in Fig. 1. Lanes 1–6 are results from strains harboring one of the various plasmids described. Lanes 13–14 are results from a sua1 diploid yeast strain prepared by crossing sua1 strain with the upf2A strain [see Material and methods for description of the strain construction]. Lanes 15–16 are results from upf2A strain harboring either wild-type UPF2 plasmid or vector alone, respectively. Lanes 7–12 show the results from sua6 strain transformed with different plasmids as described. (Bottom) A depiction of results from an RNA blotting assay (hybridized with a radioactive CYH2 probe) with RNAs isolated from a sua6/upf3 diploid cell (which was prepared from a cross between sua6 strain and upf3-1 strain). In addition, a total of seven tetrads were analyzed, and one example of RNA analysis from the spores of a single tetrad after sporulation of the diploid cell is shown here.
were quantitated, and the mRNA half-lives were determined from the semi-log plot of the percent of mRNA remaining versus the time after the temperature was shifted.

We wanted to determine whether a strain harboring a
utilized the abundance of the
do not affect cell viability or increase the stability
alleles and
in combination with other upf alleles would either affect cell viability or exacerbate the stabilization of nonsense-containing mRNAs. Strains harboring upf1Δ upf2Δ alleles and upf2Δ upf3-1 alleles were constructed and were shown to be viable with no apparent growth defect (Table 1; data not shown). As described above, we utilized the abundance of the CYH2 precursor as an assay to determine the effects of these mutations on the activity of the nonsense-mediated mRNA decay pathway. RNAs from upf1Δ, upf2Δ, upf3-1, upf1Δ upf2Δ, and upf2Δ upf3-1 strains were isolated, and the CYH2 precursor and mRNA abundances were determined by RNA blotting analysis. A summary of these results is shown in Table 4. The results indicate that strains harboring pairwise combinations of the upf2 allele with the other upf alleles were viable but did not increase the abundance of the CYH2 precursor further in these cells (Table 4).

Strains harboring a genomic disruption of the UPF2 gene were slightly sensitive to cycloheximide

Previous experiments have demonstrated that, compared to wild-type cells, upf1Δ strains are sensitive to the translation elongation inhibitor cycloheximide but not to paromomycin [Leeds et al. 1992], a drug that decreases translational fidelity during elongation [Palmer et al. 1979; Singh et al. 1979]. We wanted to determine the drug sensitivity of strains harboring a deletion of the UPF2 gene to these drugs. Isogenic wild-type, upf1Δ, upf2Δ, or upf1Δ upf2Δ strains were grown, and discs containing either paromomycin or cycloheximide were placed onto the plate and the growth of these cells were monitored. By comparing the zone of growth inhibition around the disc containing the drug, the antibiotic sensitivity of these strains can be assessed. We found that upf1Δ, upf2Δ, and upf1Δ upf2Δ strains were slightly more sensitive to cycloheximide than wild-type cells (the diameter of the inhibited growth zone was 3.72 cm for wild-type, 4.26 cm for upf1Δ, 4.32 cm for upf2Δ, and 4.23 cm for upf1Δ upf2Δ) but have the same sensitivities to paromomycin as a wild-type UPF1+ UPF2+ strain (the diameter of the inhibited growth zone was 1.10 cm for wild-type, 1.09 cm for upf1Δ, 1.07 cm for upf2Δ, and 1.10 cm for upf1Δ upf2Δ). The sensitivity of the upf1Δ upf2Δ strain to cycloheximide was not greater than strains harboring either of the individual mutant upf alleles. These results suggest that the product of the UPF2 gene, either directly or indirectly, may alter ribosome structure.

Discussion

A large body of experiments has demonstrated a strong relationship between the processes of translation and mRNA turnover. Cis-acting sequences that promote instability of mRNAs have been identified in the protein-coding regions as well as in 3'-untranslated regions of transcripts, and recent results have demonstrated that on-going translation is required for these elements to promote mRNA decay [Graves et al. 1987; Cleveland 1988; Gay et al. 1989a, b; Shyu et al. 1989; Wisdom and Lee 1991; Heaton et al. 1992; Herrick and Jacobson 1992; Laird-Offringa 1992; Peltz et al. 1992, 1993; Aharon and Schneider 1993; Caponigro et al. 1993; Herrick and Ross 1994]. The role of translation in determining mRNA decay rates is not indirect, and at least for a subset of instability elements, the sequences that promote mRNA turnover must be actively translated to induce mRNA decay [Graves et al. 1987; Cleveland 1988; Gay et al. 1989a, b; Parker and Jacobson 1990; Wisdom and Lee 1991;
These proteins are thought to be involved in ribosomal biogenesis, and their acidic regions contain phosphorylation sites that are demonstrated to be important for their functions. It suggests that the acidic amino acid rich region near the carboxyl terminus of Upf2p might be involved in protein–protein interactions modulated by phosphorylation.

As far as we can determine, upf2Δ strains are phenotypically identical to strains harboring the upf1Δ allele. upf1Δ strain and upf2Δ strains have the following similar characteristics: (1) The stabilities of nonsense-containing transcripts are increased in these strains compared with wild-type cells; (2) the mRNA decay rates of wild-type mRNAs are, for the most part, unaffected in these strains; (3) upf1Δ and upf2Δ strains are both slightly sensitive to the translation elongation inhibitor cycloheximide, whereas they do not show any sensitivities to paromomycin, a drug that decreases translational fidelity during elongation; (4) neither the UPF1 or the UPF2 gene is essential for vegetative growth under the growth conditions used, because haploid cells harboring either upf1Δ or upf2Δ alleles were viable with no apparent growth defect; (5) both upf1Δ and upf2Δ alleles can function as omnipotent suppressors [Leeds et al. 1992; Table 2, item 2]. Furthermore, strains harboring both upf1Δ and upf2Δ alleles or upf2Δ and upf3-1 were also viable with no apparent growth defect and did not further alter the abundance of the CYH2 precursor when compared with each of the individual upf alleles. Taken together, these results suggest that UPF1, UPF2, and UPF3 are involved in the same pathway.

Laird-Offringa 1992; Aharon and Schneider 1993; Peltz et al. 1993; Schiavi et al. 1994).

Studies on the nonsense-mediated mRNA decay pathway have been particularly fruitful in the identification of genes whose products are involved in mRNA turnover. In the yeast S. cerevisiae the products from the UPF1, UPF2 (SUA1) and UPF3 (SUA6) genes are involved in controlling the abundance of nonsense-containing mRNAs [Culbertson et al. 1980; Leeds et al. 1991, 1992, Pinto et al. 1992, Peltz et al. 1993, results presented here]. The UPF1, UPF2, and UPF3 genes elevate the concentration of nonsense-containing mRNAs in cells by increasing their half-lives [Leeds et al. 1991, 1992; Peltz et al. 1993, results presented here]. Mutations in the UPF1 gene have been identified and characterized [Leeds et al. 1991, 1992; Peltz et al. 1993], the identification and characterization of the UPF2 gene is described here, whereas the UPF3 (SUA6) gene has not yet been characterized. Furthermore, in Caenorhabditis elegans, seven smg alleles identified as extragenic suppressors of myosin heavy-chain B mutations increase the abundance of nonsense-containing myosin transcripts while not affecting the abundance of wild-type mRNAs [Hodgkin et al. 1989; Pulak and Anderson 1993]. At present, the cloning of the smg genes has not been reported.

Sequencing of the UPF2 gene and characterization of its 3600-nucleotide transcript suggest that it encodes a protein with a predicted molecular mass of 126.7 kD [Fig. 4]. The polypeptide sequence located at its carboxyl terminus has a long stretch of acidic amino acids consisting of aspartic acid and glutamic acid repeats similar to amino acid sequences found in the nucleolin, nucleolar phosphoprotein B23, as well as nucleolar transcription factor UBF [Fig. 4; the homologous region is shown]. These proteins are thought to be involved in ribosomal biogenesis, and their acidic regions contain phosphorylation sites that are demonstrated to be important for their functions. It suggests that the acidic amino acid rich region near the carboxyl terminus of Upilp2p might be involved in protein–protein interactions modulated by phosphorylation.

Table 4. Multiple mutations in the UPF genes are not additive in affecting the abundance of the CYH2 precursor/ mRNAs

| Strain          | Genotype       | CYH2 precursor/ CYH2 mRNA |
|-----------------|----------------|--------------------------|
| YGC14*          | UPF1+ UPF2+ UPF3+ | 0.05                     |
| Y52             | upf1Δ          | 0.31                     |
| YGC114          | upf2Δ          | 0.52                     |
| PLY139          | upf3-1         | 0.43                     |
| YGC116          | upf1Δ upf2Δ    | 0.38                     |
| YGC118          | upf2Δ upf3-1   | 0.58                     |

The mRNA abundances of the CYH2 precursor and mRNA were determined in the strains shown and as described in Materials and methods. The abundances of the CYH2 precursor and mRNA in the various strains were quantitated, and their ratios were determined.
Materials and methods

Strains, media, and general methods

The yeast strains used in this study are listed in Table 1. The E. coli DH5α strain was used to amplify plasmid DNA. Yeast media was prepared as described (Rose et al. 1990). Yeast transformations were performed by the lithium acetate method (Schiestl and Gietz 1989). Tetrad analysis was performed as described (Rose et al. 1990).

Materials

Restriction enzymes were obtained from Boehringer Mannheim, New England Biolabs, and BRL. Radioactive nucleotides were obtained from either NEN [γ-32P]ATP) or Amersham [α-32P]dCTP). Oligonucleotides used in these studies were purchased from the UMDNJ-RWJ DNA synthesis center.

Isolation and characterization of the UPF2 gene

The plasmids pYCp50 (Ausubel et al. 1992), pYCplac33, and pYCplac112 (Gietz and Sugino 1988) were used in these studies. The UPF2 gene was cloned from a pYCp50 yeast genomic library (purchased from ATCC) that was prepared from a partial Sau3A digest. Strain PLY136 was transformed with this library and a total of 5000 Ura+ transformants were screened by replica-plating onto minimal media lacking uracil and histidine and grown at either 30° or 37°C for 4–5 days. Colonies that grew at 30°C but not at 37°C on minimal media lacking uracil and histidine were tested and nine strains harboring plasmids were isolated (YPF2-1 to YPF2-9). To confirm that the growth phenotype of the upf2 strains harboring plasmids was a consequence of the plasmids, a 5-FOA selection for the plasmids loss was performed (Rose et al. 1990). The plasmid pYCpA5 was isolated from strain YPF2-5 and propagated in E. coli.

Subcloning of the UPF2 gene

A restriction map of the yeast genomic DNA fragment in pYCPA5 was prepared (Fig. 3A). Plasmid pYCPA5Δβ is a derivative of pYCPA5 in which the BamHI DNA fragment was deleted (Fig. 3B). This plasmid was constructed by cleaving of pYCPA5 with the enzyme BamHI, isolation of the 9.9-kb DNA fragment, ligation, and amplification in E. coli. The following subclones of the yeast genomic DNA fragment in pYCPA5 were prepared by isolation of various DNA fragments and insertion of them into the yeast centromere plasmid pYCplac33 (see restriction map of the yeast genomic DNA fragment Fig. 3A): pYCPA3.5 (3.5-kb BamHI–BamHI DNA fragment), pYCPA5.0 (5.0-kb Asp718–EcoRI DNA fragment), pYCPAX6.6 (6.6-kb XhoI–XhoI DNA fragment), pYCPAp7.1 (7.1-kb Asp718–XhoI DNA fragment), and pYCPA5.4 (5.4-kb XstI–XhoI DNA fragment). The multiplicity plasmid pYEPUPF2 was constructed by isolation of the 7.1-kb Asp718–XhoI DNA fragment from plasmid pYCPA5 and insertion of this fragment into pYEpLac12 (Fig. 3). This plasmid was constructed into the upf2α strain YGG112 and strain PLY18 and used in the analysis of the UPF2 transcript. pYCPA5.0 was constructed by isolation of the Asp718–EcoRI DNA fragment from pYCPA5.0 (Fig. 3A) and insertion of it into pYCP18. The GenBank accession number for the sequence of the UPF2 gene is U12137.

Preparation of the UPF2 knockout allele

pKOF2 was prepared to delete the UPF2 gene from the yeast chromosome. pKOF2 was prepared by cleaving pPUCA5.0 with the restriction enzyme ClaI and replacing the 2.3-kb of the UPF2 gene (nucleotide –1076 to 1288 containing UPF2 transcription initiation site and of UPF2 coding region; see Figs. 3A, B and Fig. 4) with a 1.57-kb DNA fragment harboring the URA3 gene.

Preparation of a UPF1 knockout allele

pKOM was prepared to delete the UPF1 gene from the yeast chromosome. First, pPUC19-UPF1 was constructed by insertion of the 4.2-kb EcoRI–BamHI DNA fragment harboring the UPF1 gene (Leeds et al. 1992) into pPUC19. Plasmid pKOM was then prepared by cleavage of pPUC19-UPF1 with MuniI and BstXI replacement of this 2.9-kb DNA fragment (base pairs 494–3426 in the UPF1 gene map, Leeds et al. 1992) with a DNA fragment harboring the URA3 gene imbedded between two HisG cassettes (Alani et al. 1987).

Preparation of a strain harboring genomic disruption of UPF1

Plasmid pKOM was digested with BamHI and EcoRI, the 4.6-kb DNA fragment harboring the upf1::HisG-URA3-HisG disruption was transformed into strain YR262 (Table 1), and cells harboring the UPF1 disruption were selected by plating on medium containing 5-FOA to select for strains that lost the URA3 gene as a consequence of recombination between the HisG cassettes. The deletion of the UPF1 gene from the yeast chromosome was confirmed by DNA blotting analysis of BamHI/EcoRI-digested genomic DNA. A radioactively labeled DNA fragment from the flanking sequences of the UPF1 gene was used as the probe. The results of the Southern blotting analysis confirmed that the UPF1 gene was deleted from the yeast chromosome (data not shown).

Preparation of a strain harboring genomic disruption of UPF2

Plasmid pKOF2 was digested with Asp718 and EcoRI and the 5-kb DNA fragment harboring the upf2::URA3 disruption was introduced into the yeast strains YR262, Y527, PLY36, PLY139, and PLY18 (see Table. 1), and transformants were selected on medium lacking uracil. Deletion of the UPF2 gene from the yeast chromosome was confirmed by Southern blotting of BamHI/EcoRI-digested genomic DNA as described above. A radioactively labeled 1.7-kb BamHI–EcoRI DNA fragment containing the UPF2 gene-coding region was used as a probe. The results of this analysis confirmed that the UPF2 gene was deleted from the yeast chromosome (data not shown).

mRNA decay measurements, RNA preparation, and RNA analysis

mRNA decay rates were determined as follows: Cells were grown to mid-log phase (OD600 = 0.7–1.0) at 24°C, centrifuged, resuspended in 18 ml of the same medium, and incubated at 24°C for 10 min. Transcription was inhibited by thermal inactivation of RNA polymerase II by shifting the concentrated culture to 36°C by addition of 18 ml of medium preheated to 54°C. After the temperature shift, the culture was maintained at 36°C and aliquots (4 ml) were removed at various times. Upon removal of an aliquot, cells were collected by rapid centrifugation, the supernatants were removed by aspiration, and the cell pellets were frozen quickly in dry ice. Routinely, cells were frozen within 15 sec after removal of the culture aliquot. Total yeast RNA was isolated as described previously (Herrick et al. 1990; Parker et al. 1991). Equal amounts (usually 20–40 μg) of total
RNA from each time point of an experiment were analyzed by RNA blotting (Thomas 1980). Gels were stained with ethidium bromide before and after blotting to assess the efficiency of RNA transfer and to confirm the equal loading of RNA. Hybridizations with probes prepared by random priming [see below] were performed as described previously (Herrick et al. 1990). RNA blots were hybridized to a plasmid containing the Rous sarcoma virus transcript. The other radioactive probes used to monitor the decay of mRNAs were the following: a 0.6-kb EcoRI–HindIII fragment from the CYH2 gene; a 4-kb SphI–SacI fragment from the HIS4 gene; a 1.5-kb SalI–BstEI fragment from the LEU2 gene; a 1.6-kb EcoRV–HindIII fragment from MATa1 gene; and a 5-kb HindIII–HindIII fragment from the TIF4631 gene were radiolabeled by random priming.

Acknowledgments

This work was supported by a grant [GM48631-01] from the National Institutes of Health and an American Cancer Society Junior Investigator Award given to S.W.P. We thank Peter Leeds, Beate Schwer, and Michael Hampsey for a number of yeast strains used in these studies. We are indebted to Nahum Sonenberg for the plasmid harboring the TIF4631 gene. We thank Feng He and Allan Jacobson for communicating results prior to publication. We are grateful to Kevin Czaplinski, Allan Jacobson, and Beate Schwer for critical reading of the manuscript.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

References

Aharon, T. and R.J. Schneider. 1993. Selective destabilization of short-lived mRNAs with the granulocyte-macrophage colony-stimulating factor AU-rich 3′ noncoding region is mediated by a cotranslational mechanism. Mol. Cell. Biol. 13: 1971–1980.

Alani, E., L. Cao, and N. Kleckner. 1987. A method for gene disruption: Deficient accumulation of mRNA occurs despite normal cytoplasmic stability. Mol. Cell. Biol. 7: 642–654.

Atwater, J.A., R. Wisdom, and I.M. Verma. 1990. Regulated mRNA stability. Annu. Rev. Genet. 24: 519–541.

Ausbuch, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1992. Current protocols in molecular biology. Vol. 2, p. 13.4.7. Wiley/Greene, New York.

Barker, G.F. and K. Beemon. 1991. Nonsense codons within the Rous sarcoma virus gag gene decrease the stability of unspliced viral RNA. Mol. Cell. Biol. 11: 2760–2768.

Baserga, S.J. and E.J. Benz, Jr. 1992. β-Globin nonsense mutation: Deficient accumulation of mRNA occurs despite normal cytoplasmic stability. Proc. Natl. Acad. Sci. 89: 2935–2939.

Baumann, B., M.J. Potash, and G. Kohler. 1985. Consequences of frameshift mutations at the immunoglobulin heavy chain locus of the mouse. EMBO J. 4: 351–359.

Bernstein, P., D. Herrick, R.D. Prokipcak, and J. Ross. 1992. Control of c-myc mRNA half-life in vitro by a protein capable of binding to a coding region stability determinant. Genes & Dev. 6: 642–654.

Brill, S.J. and B. Stillman. 1991. Replication factor A from Sac-
charomycetes cerevisiae is encoded by three essential genes coordinately expressed at S phase. Genes & Dev. 5: 1589–1600.

Caponigro, G., D. Muhlrad, and R. Parker. 1993. A small segment of the MATα1 transcript promotes mRNA decay in Saccharomyces cerevisiae: A stimulatory role for rare codons. Mol. Cell. Biol. 13: 5141–5148.

Chan, P.-K., M. Aldrich, R.G. Cook, and H. Busch. 1986. Amino acid sequence of protein B23 phosphorylation site. J. Biol. Chem. 261: 1688–1782.

Chang, J.-H., T.S. Dumbar, and M.O.J. Olson. 1988. cDNA and deduced primary structure of rat protein B23, a nucleolar protein containing highly conserved sequences. J. Biol. Chem. 263: 12824–12827.

Cheng, J. and L.E. Maquat. 1993. Nonsense codons can reduce mRNA decay. Mol. Cell. Biol. 13: 1892–1902.

Cheng, J., M. Fogel-Petrovic, and L.E. Maquat. 1990. Translation to near the distal end of the penultimate exon is required for normal levels of spliced triosephosphate isomerase mRNA. Mol. Cell. Biol. 10: 5215–5225.

Cleveland, D.W. and T.J. Yen. 1989. Multiple determinants of multiple determinants of frameshift suppression in Saccharomyces cerevisiae. II. Genetic properties of Group II suppressors. Genetics 95: 833–853.

Daar, I.O. and L.E. Maquat. 1988. Premature translation termination mediates triosephosphate isomerase mRNA degradation. Mol. Cell. Biol. 8: 802–813.

Donahue, T.F.P.J. Farabaugh, and G.R. Fink. 1981. Suppressible frameshift mutations affecting the site of ribosome release. Genes & Dev. 10: 7034–7038.

Heaton, B., C. Decker, D. Muhlrad, J. Donahue, A. Jacobson, and R. Parker. 1992. Analysis of chimeric mRNAs identifies two regions within the STE3 mRNA which promote rapid mRNA decay. Nucleic Acids Res. 20: 5365–5373.

Herrick, D. and A. Jacobson. 1992. A segment of the coding region is necessary but not sufficient for rapid decay of the HSP3 mRNA in yeast. Gene 114: 35–41.

Herrick, D.J. and J. Ross. 1994. The half-life of ε-myc mRNA in growing and serum-stimulated cells: influence of the coding and 3′ untranslated regions and role of ribosome translation. Mol. Cell. Biol. 14: 2119–2128.

Hodgkin, L., A. Papp, R. Pulak, V. Ambros, and P. Anderson. 1989. A new kind of informational suppression in the nematode Caenorhabditis elegans. Genetics 123: 301–313.

Laird-Orrígha, I.A. 1992. What determines the instability of ε-myc mRNA? BioEssays 14: 119–124.

Losson, R., D. Parker, and A. Jacobson. 1990. Identification and comparison of stable and unstable mRNAs in Saccharomyces cerevisiae. Mol. Cell. Biol. 10: 2269–2284.

Lim, S.-K., C.D. Sigmund, K.W. Gross, and L.E. Maquat. 1992. A stimulatory role for rare codons. Mol. Cell. Biol. 12: 1149–1161.

Mendenhall, M.D., P. Leeds, H. Fen, L. Mathison, M. Zwick, C. Mosrin, C. M. Riva, M. Beltrame, E. Cassar, A. Sentenac, and P. O'Mahony, D.J., S.D. Smith, W.Q. Xie, and L.I. Rothblum. 1992. Effect of premature termination of translation on mRNA stability. Proc. Natl. Acad. Sci. USA 89: 10091–10095.

Mendenhall, M.D., P. Leeds, H. Fen, L. Mathison, M. Zwick, C. Mosrin, C. M. Riva, M. Beltrame, E. Cassar, A. Sentenac, and P. O'Mahony, D.J., S.D. Smith, W.Q. Xie, and L.I. Rothblum. 1992. Effect of premature termination of translation on mRNA stability. Proc. Natl. Acad. Sci. USA 89: 10091–10095.

Mendenhall, M.D., P. Leeds, H. Fen, L. Mathison, M. Zwick, C. Mosrin, C. M. Riva, M. Beltrame, E. Cassar, A. Sentenac, and P. O'Mahony, D.J., S.D. Smith, W.Q. Xie, and L.I. Rothblum. 1992. Effect of premature termination of translation on mRNA stability. Proc. Natl. Acad. Sci. USA 89: 10091–10095.

Mendenhall, M.D., P. Leeds, H. Fen, L. Mathison, M. Zwick, C. Mosrin, C. M. Riva, M. Beltrame, E. Cassar, A. Sentenac, and P. O'Mahony, D.J., S.D. Smith, W.Q. Xie, and L.I. Rothblum. 1992. Effect of premature termination of translation on mRNA stability. Proc. Natl. Acad. Sci. USA 89: 10091–10095.

Mendenhall, M.D., P. Leeds, H. Fen, L. Mathison, M. Zwick, C. Mosrin, C. M. Riva, M. Beltrame, E. Cassar, A. Sentenac, and P. O'Mahony, D.J., S.D. Smith, W.Q. Xie, and L.I. Rothblum. 1992. Effect of premature termination of translation on mRNA stability. Proc. Natl. Acad. Sci. USA 89: 10091–10095.
nucleotide segment within the coding region of the mRNA encoded by the MATa1 gene are involved in promoting rapid mRNA decay in yeast. Proc. Natl. Acad. Sci. 87: 2780–2784.

Parker, R., D. Herrick, S.W. Peltz, and A. Jacobson. 1991. Measurement of mRNA decay rates in Saccharomyces cerevisiae. In Methods in enzymology: Molecular biology of Saccharomyces cerevisiae [ed. C. Guthrie and G. Fink], pp. 415–423. Academic Press, New York.

Pelsy, F. and F. Lacroute. 1984. Effect of ochre nonsense mutations on yeast URA1 stability. Curr. Genet. 8: 277–282.

Peltz, S.W. and A. Jacobson. 1983. mRNA Turnover in Saccharomyces cerevisiae. In Control of mRNA stability [ed. G. Brawerman and J. Belasco], pp. 291–328. Academic Press, New York.

Peltz, S.W., G. Brewer, V. Groppi, and J. Ross. 1989. The exonucleolytic activity that degrades histone mRNA remains a stable activity throughout the cell cycle. Mol. Biol. Med. 6: 227–238.

Peltz, S.W., G. Brewer, P. Bernstein, R. Kratzke, and J. Ross. 1991. Regulation of mRNA turnover in eucaryotic cells. CRC Crit. Rev. Eukaryotic Gene Expression 1: 99–126.

Peltz, S.W., J.L. Donahue, and A. Jacobson. 1992. A mutation in tRNA nucleotidyltransferase stabilizes mRNAs in Saccharomyces cerevisiae. Mol. Cell. Biol. 12:5776–5784.

Peltz, S.W., A.H. Brown, and A. Jacobson. 1993a. mRNA destabilization triggered by premature translational termination depends on three mRNA sequence elements and at least one trans-acting factor. Genes & Dev. 7: 1737–1754.

Peltz, S.W., C. Trotta, H. Feng, A. Brown, J. Donahue, E. Welch, and A. Jacobson. 1993b. Identification of the cis-acting sequences and trans-acting factors involved in nonsense-mediated mRNA decay. In Protein synthesis and targeting in yeast [ed. M. Tuite, J. McCarthy, A. Brown, and F. Sherman], Springer-Verlag, Berlin/Heidelberg, Germany.

Peltz, S.W., H. Feng, E. Welch, and A. Jacobson. 1994. Nonsense-mediated mRNA decay in yeast. Prog. Nucleic Acid Res. Mol. Biol. 47: 271–298.

Pinto, I., J.G. Na, F. Sherman and M. Hampsey. 1992a. Cis- and trans-acting suppressors of a translation initiation defect at the cyc1 locus of Saccharomyces cerevisiae. Genetics 132: 97–112.

Pinto, I., D.E. Ware and M. Hampsey. 1992b. The yeast SA7 gene encodes a homologue of the human transcription factor TFIIB and is required for normal start site selection in vivo. Cell 68: 977–988.

Pulak, R. and P. Anderson. 1993. mRNA surveillance by the Caenorhabditis elegans smg genes. Genes & Dev. 7: 1885–1897.

Rose, M.D., F. Winston, and P. Hieter. 1990. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Ross, J. 1988. Messenger RNA turnover in eukaryotic cells. Mol. Biol. Med. 5: 1–14.

Rymond, B.C. and M. Rosbash. 1993. Yeast pre-mRNA splicing. In Molecular and cellular biology of the yeast Saccharomyces, vol. 2 [ed. J.R. Broach, J.R. Pringle, and E.W. Jones]. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sachs, A.B. 1993. Messenger degradation in eucaryotes. Cell 74: 413–471.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schiestl, R.H. and R.D. Gietz. 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Curr. Genet. 16: 339–346.

Schiavi, S.C., C.L. Wellington, A.-B. Shyu, C.-Y.A. Chen, M.E. Greenberg, and J.G. Belasco. 1994. Multiple elements in the c-fos protein-coding region facilitate mRNA deadenylation and decay by a mechanism coupled to translation. J. Biol. Chem. 269: 3441–3448.

Shyu, A.-B., M.E. Greenberg, and J.G. Belasco. 1989. The c-fos transcript is targeted for rapid decay by two distinct mRNA degradation pathways. Genes & Dev. 3: 60–72.

Singh, A., D. Ursic, and J. Davies. 1979. Phenotypic suppression and misreading in Saccharomyces cerevisiae. Nature 277: 146.

Srivastova, M., O.W. McBride, P.J., Fleming, H.B. Pollard, and A.L. Burns. 1990. Genomic organization and chromosomal localization of the human nucleolin gene. J. Biol. Chem. 265: 14922–14931.

Stiles, J.L., J.W. Szostak, A.T. Young, R. Wu, S. Consaul, and F. Sherman. 1981. DNA sequence of a mutation in the leader region of the yeast iso-l-cytochrome c mRNA. Cell 25: 277–284.

Stimac, E., V.E. Groppi, Jr., and P. Coffino. 1984. Inhibition of protein synthesis stabilizes histone mRNA. Mol. Cell. Biol. 4: 2082–2090.

Thomas, P.F. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. 77: 5201–5205.

Urlaub, G., P.J. Mitchell, C.J. Ciudad, and L.A. Chasin. 1989. Nonsense mutations in the dihydrofolate reductase gene affect RNA processing. Mol. Cell. Biol. 9: 2868–2880.

Voit, R., A. Schnapp, A. Khun, H. Rosenbauer, P. Hirschman, H.G. Stunnenberg, and I. Grummt. 1992. The nucleolar transcription factor mUFB is phosphorylated by casein kinase II in the C-terminal hyperacidic tail which is essential for transactuation. EMBO J. 11: 2211–2218.

Wisdom, R. and W. Lee. 1991. The protein-coding region of c-myc mRNA contains a sequence that specifies rapid mRNA turnover and induction by protein synthesis inhibitors. Genes & Dev. 5: 232–243.
Identification and characterization of genes that are required for the accelerated degradation of mRNAs containing a premature translational termination codon.

Y Cui, K W Hagan, S Zhang, et al.

*Genes Dev.* 1995, 9: 
Access the most recent version at doi:10.1101/gad.9.4.423

References
This article cites 66 articles, 37 of which can be accessed free at: http://genesdev.cshlp.org/content/9/4/423.full.html#ref-list-1

License

Email Alerting Service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.