

The product of the yeast *UPF1* gene is required for rapid turnover of mRNAs containing a premature translational termination codon

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mRNA decay rates often increase when translation is terminated prematurely due to a frameshift or nonsense mutation. We have identified a yeast gene, *UPF1*, that codes for a *trans*-acting factor whose function is necessary for enhanced turnover of mRNAs containing a premature stop codon. In the absence of *UPF1* function, frameshift or nonsense mutations in the *HIS4* or *LEU2* genes that normally cause rapid mRNA decay fail to have this effect. Instead, the mRNAs decay at rates similar to the corresponding wild-type mRNAs. The stabilization of frameshift or nonsense mRNAs observed in *upf1*⁻ strains does not appear to result from enhanced readthrough of the termination signal. Loss of *UPF1* function has no effect on the accumulation or stability of *HIS4*⁺ or *LEU2*⁺ mRNA, suggesting that the *UPF1* product functions only in response to a premature termination signal. When we examined the accumulation and stability of other wild-type mRNAs in the presence or absence of *UPF1*, including *MAT α 1*, *STE3*, *ACT1*, *PGK1*, *PAB1*, and *URA3* mRNAs, only the *URA3* transcript was affected. On the basis of these and other results, the *UPF1* product appears to participate in a previously uncharacterized pathway leading to the degradation of a limited class of yeast transcripts.

[Key Words: *Saccharomyces cerevisiae*; *UPF1* gene; mRNA stability; premature stop codon]

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Nonsense mutations that generate a premature translational termination signal often reduce the steady-state accumulation of the corresponding mRNA (Brown 1989; Peltz et al. 1990). In a study of the yeast *URA3* gene, it was shown that the extent of reduced mRNA accumulation depends on the position of the nonsense mutation (Losson and Lacroute 1979). Mutations near the 5' end of the transcript were shown to have a greater destabilizing effect than mutations near the 3' end. Furthermore, introduction of an amber tRNA suppressor restabilized *ura3* nonsense mRNA, indicating that the turnover rate is determined in part by the relative efficiencies of termination versus readthrough of the stop codon. These studies suggested that the turnover rate of nonsense mRNA is probably related to some aspect of its translation rather than to a potential change in mRNA structure that might result from the presence of a nonsense mutation.

Similar studies in higher eukaryotes have proven more difficult to interpret. In some cases, the introduction of a premature stop codon into a gene has been linked to

increased cytoplasmic turnover (Maquat et al. 1981; Barker and Beemon 1991). However, other studies suggest that nonsense mutations may cause changes in nuclear processing and/or transport, and these changes, rather than cytoplasmic mRNA degradation, may be primarily responsible for decreased steady-state mRNA levels (Humphries et al. 1984; Takeshita et al. 1984; Urlaub et al. 1989; Cheng et al. 1990).

Here, we report the characterization of mutations in the yeast *Saccharomyces cerevisiae* that specifically stabilize mRNAs containing a premature translational termination signal. The mutations arose in a strain containing *his4-38*, a +1 frameshift mutation near the 5' end of the *HIS4* transcript that causes translational termination at an adjacent downstream stop codon (Donahue et al. 1981). In this study we show that the *his4-38* mutation results in a four- to fivefold decrease in mRNA stability.

In addition to *his4-38*, the strain used to select mutants also contained *SUF1-1*, which codes for a glycine tRNA frameshift suppressor that promotes a low level of readthrough of the frameshift mutation by decoding a 4-base codon (Mendenhall et al. 1987). Strains carrying both *his4-38* and *SUF1-1* have a His⁺ phenotype at 30°C, but at 37°C the cells remain His⁻. This provides the

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Table 1. Yeast strains

| Strain | Genotype |
|--------|--|
| PLY36 | <i>MATα upf1-2 his4-38 SUF1-1 met14</i> |
| PLY36A | <i>MATα upf1-2 his4-38 suf1⁺ met14</i> |
| PLY102 | <i>MATα upf1-Δ1 ura3-52 trp1-7 leu2-3, -112</i> |
| PLY149 | PLY102 [YRpPL14] |
| PLY150 | PLY102 [YRpPL15] |
| PLY171 | PLY102 [YRpPL81] |
| PLY153 | <i>MATα upf1-Δ1 ura3-52 trp1-Δ1 rpb1-1</i> |
| PLY154 | <i>MATα upf1-Δ1 ura3-52 trp1-Δ1 rpb1-1 his4-38 leu2-1</i> |
| PLY155 | <i>MATα upf1-Δ1 ura3-52 trp1-Δ1 rpb1-1 his4-38 SUF1-1 leu2-1</i> |
| PLY167 | <i>MATα upf1-Δ2 ura3-52 trp1-Δ1 rpb1-1 his4-713 leu2-3, -112 lys2-20</i> |
| PLY172 | <i>MATα upf1-Δ1 ura3-52 trp1-7 rpb1-1 leu2-3, -112 [YRpPL14]</i> |
| PLY173 | <i>MATα upf1-Δ1 ura3-52 trp1-7 rpb1-1 leu2-3, -112 [YRpPL15]</i> |
| PLY176 | <i>MATα upf1-Δ1 ura3-52 trp1-7 rpb1-1 leu2-3, -112 [YRpPL81]</i> |
| PLY177 | <i>MATα upf1-Δ2 ura3-52 trp1-Δ1 rpb1-1 his4-539 leu2-3</i> |

Names in brackets refer to plasmids carrying *HIS4-lacZ* fusion genes that have been integrated into the chromosome at the *trp1* locus.

basis for selection of *upf* (up frameshift) mutations, which confer a modest increase in gene expression sufficient to render the cells His⁺ at 37°C (Culbertson et al. 1980).

Our results indicate that recessive loss of function of a protein product encoded by *UPF1*, one of the genes identified in the selection described above, confers a His⁺ phenotype at 37°C in *his4-38 SUF1-1* strains through re-stabilization of *his4-38* mRNA. We show that enhanced mRNA stability is not a function of changes in translational readthrough of the frameshift mutation or the downstream stop codon that it generates. On the basis of these and other experiments, we propose that the wild-type *UPF1*⁺ gene product performs an important function in a previously unknown pathway leading to destabilization of transcripts containing a premature translational termination codon.

Results

Mutations conferring a *Upf*⁻ phenotype (Culbertson et al. 1980) potentially could affect translational fidelity or mRNA metabolic processes such as transcription, processing, or stability. Our approach in identifying those mutations that specifically affected mRNA stability was, first, to screen the mutants for elevated accumulation of *his4-38* mRNA. This led to the identification, cloning, and molecular analysis of the *UPF1* gene, which is not essential for vegetative growth (Leeds 1991).

Mutations in UPF1 cause increased accumulation of his4-38 mRNA

The haploid strains used in this study (Table 1) all con-

tain either *upf1-2* or a deletion of *UPF1* designated *upf1- Δ 1* or *upf1- Δ 2* (see Materials and methods). These deletions result in complete loss of *UPF1* function and confer the same phenotype as *upf1-2* (Leeds 1991). Derivatives of these strains that contain *UPF1*⁺ on a plasmid are denoted by the strain number followed by a plus sign (+) in parenthesis. Derivatives that contain only a vector are denoted by the strain number followed by a minus sign (-).

The extent of mRNA accumulation was initially estimated by densitometric scanning of autoradiographs from Northern blots. *his4-38* mRNA accumulation was normalized to actin mRNA accumulation, which is unaffected by the *SUF1* or *upf1* mutations. We measured the extent of *his4-38* mRNA accumulation in strains PLY36(+) and PLY36(-) (Table 1). We found a 3.8-fold increase in *his4-38* mRNA accumulation in PLY36(-) compared with the *UPF1*⁺ derivative PLY36(+) (Fig. 1A, lanes 1 and 2). Because the presence or absence of a *UPF1*⁺ gene is the only genetic difference between the two strains, the increase is presumed to be a consequence of the *upf1-2* mutation in PLY36(-). We also examined *his4-38* mRNA accumulation in a similar set of strains [PLY36A(+) and PLY36A(-)] that lacked *SUF1-1* (Table 1). We found a 3.2-fold increase in *his4-38* mRNA accumulation in PLY36A(-) compared with the *UPF1*⁺ derivative PLY36A(+) (Fig. 1A, lanes 3,4). This increase is also a consequence of the *upf1-2* mutation.

We found that *SUF1-1* causes a 1.4-fold increase in the relative accumulation of *his4-38* mRNA (Fig. 1A, cf. lanes 1,3). Thus, *upf1-2* has a much more pronounced effect on accumulation than does the tRNA suppressor, and *upf1-2* appears to affect mRNA accumulation in a manner independent of *SUF1-1*. For this reason, the effects of the tRNA suppressor on mRNA accumulation were not studied further. All subsequent experiments reported below were performed with *suf1*⁺ strains that also carry either *upf1- Δ 1* or *upf1- Δ 2*.

Loss of UPF1 function stabilizes his4-38 mRNA, whereas HIS4⁺ mRNA is unaffected

The increased accumulation of *his4-38* mRNA in *Upf*⁻ strains could be the result of changes in either the rate of synthesis or decay of the mRNA. To measure decay

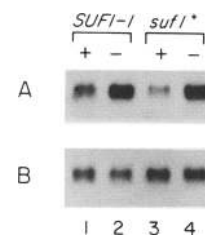


Figure 1. Northern blot analysis of *his4-38* strains. Total RNA (10 μ g/lane) is from strains PLY36(+) (lane 1), PLY36(-) (lane 2), PLY36A(+) (lane 3), and PLY36A(-) (lane 4). Filters were hybridized with probes derived from *HIS4* (A) and *ACT1* (B).

rates, strains were constructed that carry *rpb1-1*, a mutation in a gene coding for a subunit of RNA polymerase II that causes temperature-sensitive growth (Nonet et al. 1987). Shifting strains to the restrictive temperature (36°C) results in rapid cessation of polymerase II transcription, allowing for direct measurement of the decay rates of specific mRNAs with a quantitative Northern blotting procedure on the basis of direct radioisotope counting (Herrick et al. 1990; see Materials and methods).

The accumulation and rate of decay was determined for *HIS4*⁺ and *his4-38* mRNA isolated from strains that carry the chromosomal deletion *upf1-Δ1*. Two of the strains carry the *HIS4*⁺ allele [PLY153(+)] and PLY153(-), and two carry *his4-38* [PLY154(+)] and PLY154(-)] (Table 1).

HIS4⁺ mRNA accumulation is not affected by loss of *UPF1* function (Fig. 2A; Table 2). Also, the half-life of *HIS4*⁺ mRNA in PLY153(+) is nearly identical to its half-life in PLY153(-) (18 vs. 17 min, respectively). In *UPF1*⁺ strains the accumulation of *his4-38* mRNA is reduced three- to fourfold compared with *HIS4*⁺ mRNA. The half-life is reduced from 17–18 min to 4 min (Fig. 2B; Table 2). Deletion of the *UPF1* gene causes a three- to fourfold increase in *his4-38* mRNA accumulation, which is accompanied by an increase in mRNA half-life from 3–4 min to 14 min (Fig. 2B; Table 2).

These results suggest that the accelerated mRNA de-

cay rate resulting from the presence of the *his4-38* frameshift mutation depends on *UPF1* function. When *UPF1* is deleted, the decay rate, although still slightly reduced, is similar to the wild-type *HIS4* decay rate. Also, the net changes in mRNA accumulation can be explained by changes in mRNA half-life without assuming any changes in transcription rates. If deletion of *UPF1* caused any such change, we should have seen an increase in the accumulation of *HIS4*⁺ mRNA when *UPF1* was deleted, but no increase was observed.

Loss of *UPF1* function stabilizes a limited class of mRNAs

We examined mRNAs encoded by wild-type, nonsense, and frameshift alleles for a number of different genes to determine whether accumulation and stability were affected by loss of *UPF1* function (Table 2; Fig. 2). Wild-type transcripts were tested that vary in half-life over a wide range, including *ACT1* and *PGK1* mRNAs (30–60 min) as well as *STE3* and *MATα1* mRNAs (4 min). With the exception of the *URA3* transcript, which is discussed below, none of the wild-type mRNAs tested showed differences in accumulation or turnover in *UPF1*⁺ compared with *upf1-Δ1* strains (Table 2; Fig. 2A).

In strains carrying a functional *UPF1*⁺ gene, mRNAs transcribed from two nonsense alleles (*his4-539*, *leu2-1*)

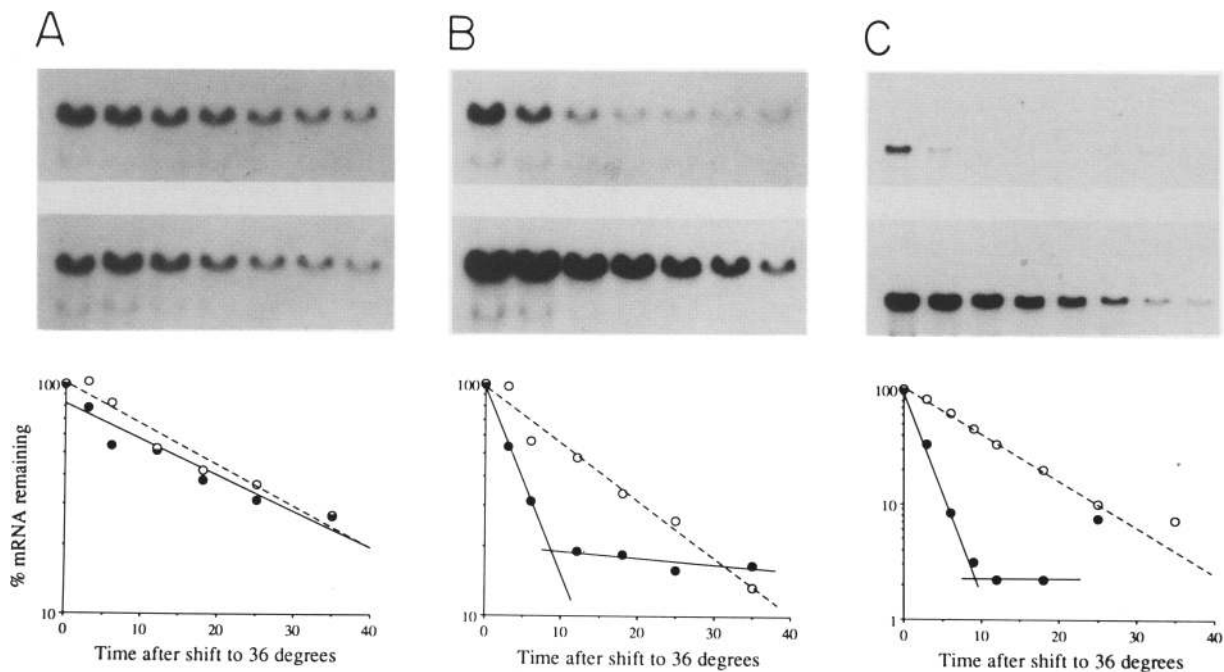


Figure 2. Decay rates of *his4* transcripts. The upper autoradiographs are derived from *UPF1*⁺ strains; the lower autoradiographs are from *upf1-Δ1* or *upf1-Δ2* strains. (A) Decay rate of the *HIS4*⁺ transcript measured in strains PLY153(+) and PLY153(-); (B) decay rate of the *his4-38* transcript measured in strains PLY154(+) and PLY154(-); (C) decay rate of the *his4-539* transcript measured in strains PLY177(+) and PLY177(-). (A,B) Time points were taken 0, 3, 6, 12, 18, 25, and 35 min after the cultures were shifted to 36°C; (C) there is an additional 9-min time point. Solid circles and lines refer to *UPF1*⁺; open circles and dashed lines refer to *upf1-Δ1*.

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Table 2. Half-lives and accumulations of endogenous mRNAs

| Strain | Transcript ^a | Relative abundance [(<i>upf1</i> ⁻ / <i>UPF1</i> ⁺) _{t=0}] | Half-life (min) | |
|-----------|--------------------------------|---|--------------------------|--------------------------|
| | | | <i>UPF1</i> ⁺ | <i>upf1</i> ⁻ |
| I | | | | |
| PLY154 | <i>his4-38</i> | 4.3 | 4 | 14 |
| PLY177 | <i>his4-539</i> | 4.8 | 2 | 8 |
| PLY154 | <i>leu2-1</i> | 3.4 | 4 | 16 |
| PLY177 | <i>leu2-3</i> | 4.4 | 3 | 7 |
| II | | | | |
| PLY153 | <i>HIS4</i> | 0.9 | 18 | 17 |
| PLY167 | <i>his4-713</i> | 0.8 | 14 | 15 |
| PLY153 | <i>LEU2</i> | 1.0 | 14 | 14 |
| PLY153 | <i>MATα1</i> | 1.2 | 4 | 4 |
| PLY153 | <i>STE3</i> | 0.8 | 4 | 4 |
| PLY153 | <i>ACT1</i> | 1.1 | 37 | 34 |
| PLY153 | <i>PGK1</i> | 0.9 | 60 | 60 |
| PLY153 | <i>PAB1</i> | 1.2 | 13 | 11 |

^aThe nucleotide locations of the first in-frame termination codons (relative to the start of translation) for the transcripts that contain premature stop codons are *his4-38*, 178; *leu2-3*, 256; and *his4-713*, 2275. The *his4-539* mutation has been mapped upstream of the *his4-17* mutation, which is located at nucleotide position 688 (Fink and Styles 1974; Donahue et al. 1982). The location of the stop codon in *leu2-1* is not known. The *HIS4*⁺-coding region is 2397 bp (Donahue et al. 1982); the *LEU2*⁺-coding region is 1092 bp (Andreadis et al. 1984). All strains contain *suf1*⁺.

and two frameshift alleles (*his4-38*, *leu2-3*) all exhibit at least a three- to fourfold decrease in accumulation and mRNA half-life compared with the *HIS4*⁺ or *LEU2*⁺ transcripts (Table 2). In *upf1- Δ 1* or *upf1- Δ 2* strains the accumulation and half-life of the nonsense and frameshift mRNAs always increases but never exceeds that of the corresponding wild-type mRNA (Table 2; Fig. 2).

However, not all frameshift mutations affect the stability of mRNA. In strains containing a *UPF1*⁺ gene we found that the extent of accumulation and rate of turnover of mRNA encoded by the frameshift allele *his4-713* (14-min half-life) was similar to *HIS4*⁺ mRNA (17-min half-life; Table 2). Furthermore, *his4-713* mRNA accumulation and turnover was unaffected by loss of *UPF1* function. This exceptional behavior may be the result of the location of the frameshift mutation in *his4-713*. The single nucleotide insertion results in premature translational termination near the 3' end of *HIS4* mRNA 120 nucleotides upstream of the normal termination codon (Donahue et al. 1981). The *his4-38*, *his4-539*, and *leu2-3* mutations are all known to map in the 5' third of their respective mRNAs, whereas the position of *leu2-1* has not been established (Fink and Styles 1974; Donahue et al. 1981, 1982; I. Edelman and M. Culbertson, unpubl.). Similar position-dependent effects on mRNA stability have been reported for nonsense mutations in the *URA3* gene (Losson and Lacroute 1979).

URA3⁺ mRNA shows a 1.9-fold (± 0.1) increase in accumulation in strain PLY153(-) compared with PLY153(+) (Tables 1 and 2; Fig. 3). When decay rates were determined (Fig. 3) it was found that *URA3* mRNA had a 2-min half-life in PLY153(-) compared with a 3-min half-life in PLY153(+). Thus, the increased accumulation of *URA3* mRNA resulting from loss of *UPF1*

function is not the result of a corresponding increase in *URA3* mRNA half-life. An increase in the rate of transcription is the most likely explanation for the increase in accumulation. Detailed studies presented elsewhere support this conclusion (see Discussion).

Changes in mRNA stability due to loss of UPF1 function are unrelated to the extent of productive translational readthrough

To monitor productive translation downstream of a stop codon, integrative plasmids were constructed in which *lacZ* was fused downstream of a fragment containing the *HIS4* promoter and part of the *HIS4*⁺ or *his4-38* reading frame (Fig. 4A and 4B; see Materials and methods). A third fusion, designated *his4-38*, -*UAA* : *lacZ*, contains multiple UAA stop codons in all three reading frames inserted just upstream of the *his4-38* frameshift mutation (Fig. 4C). Haploid strains PLY149, PLY150, and PLY171 carry a single, chromosomally integrated copy of the *HIS4*⁺ : *lacZ*, *his4-38* : *lacZ*, and *his4-38*, -*UAA* : *lacZ* fusions, respectively.

Strains containing the *HIS4*⁺ : *lacZ* fusion exhibit relatively high levels of β -galactosidase activity, producing a dark-blue color in in situ X-gal assays after 15 min (Fig. 4A). Quantitative assays of several transformants confirmed that there is no increase in β -galactosidase activity in *upf1- Δ 1* strains relative to *UPF1*⁺ strains [activity (*upf1- Δ 1*/*UPF1*⁺) = 0.94 ± 0.04]. Antibodies directed against β -galactosidase detect a single 140-kD band on Western blots, which is similar to the predicted molecular mass of 136 kD for the fusion protein (Fig. 4D, lanes 2,3). This band is absent from strain PLY102, which does not contain a *lacZ* fusion plasmid (Fig. 4D, lane 1).

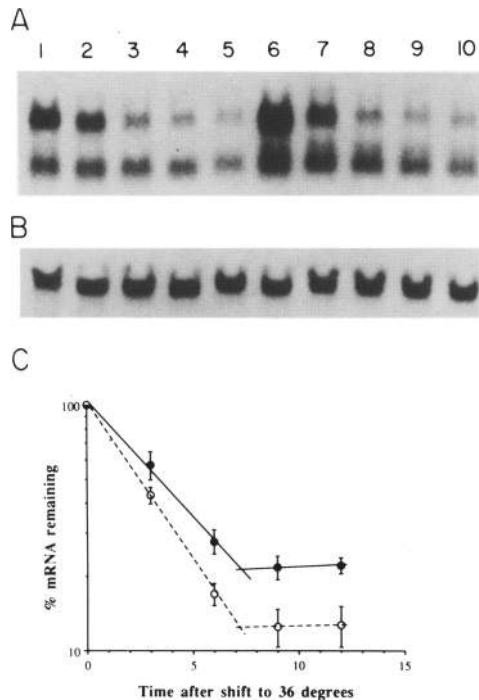


Figure 3. Decay rate of *URA3* mRNA. Decay rates are shown for strains PLY153(+) (lanes 1–5) and PLY153(–) (lanes 6–10). Time points were taken 0, 3, 6, 9, and 12 min after cultures were shifted to 36°C. (A) *URA3*⁺ mRNA decay. Hybridization to a *URA3* probe produces two bands in each of these strains. The strains carry both the *ura3-52* mutation and a wild-type copy of *URA3* inserted into *UPF1* to construct *upf1-Δ1* (see Materials and methods). The upper band comigrates with the 1-kb wild-type *URA3* transcript; the lower band comigrates with an mRNA seen in *ura3-52* strains. Both mRNAs appear to be under the control of the *URA3* transcription promoter, because growth in the presence of 6-azauracil induces both transcripts (Losson and Lacroute 1981; data not shown). (B) The filter shown in A rehybridized with an *ACT1* probe. (C) Decay rate of the 1-kb wild-type *URA3* transcript (upper band in A). Data points and error bars were derived from three independent hybridization experiments. Solid circles and lines refer to *UPF1*⁺; open circles and dashed lines refer to *upf1-Δ1*.

Decay-rate experiments with strains that contain *lacZ* fusions are shown in Figure 5. The fusion alleles each produce a 3.6-kb and an 8.4-kb transcript that hybridize to a *lacZ* probe (see legend to Fig. 5). Both transcripts show the same pattern of response to changes in *UPF1* expression. The transcripts associated with the *HIS4*⁺ : *lacZ* fusion are not stabilized in a *upf1-Δ1* background (Fig. 5A; Table 3). On the basis of the β-galactosidase assays, Western blots, and mRNA accumulation and half-life measurement, we conclude that there is no significant difference in expression of the *HIS4*⁺ : *lacZ* fusion protein in *UPF1*⁺ and *upf1-Δ1* strains.

Strains carrying the *his4-38* : *lacZ* fusion produce much less β-galactosidase activity than the *HIS4*⁺ : *lacZ* fusion does, although some blue color was detected in both the *UPF1*⁺ and *upf1-Δ1* strains after 16 hr of devel-

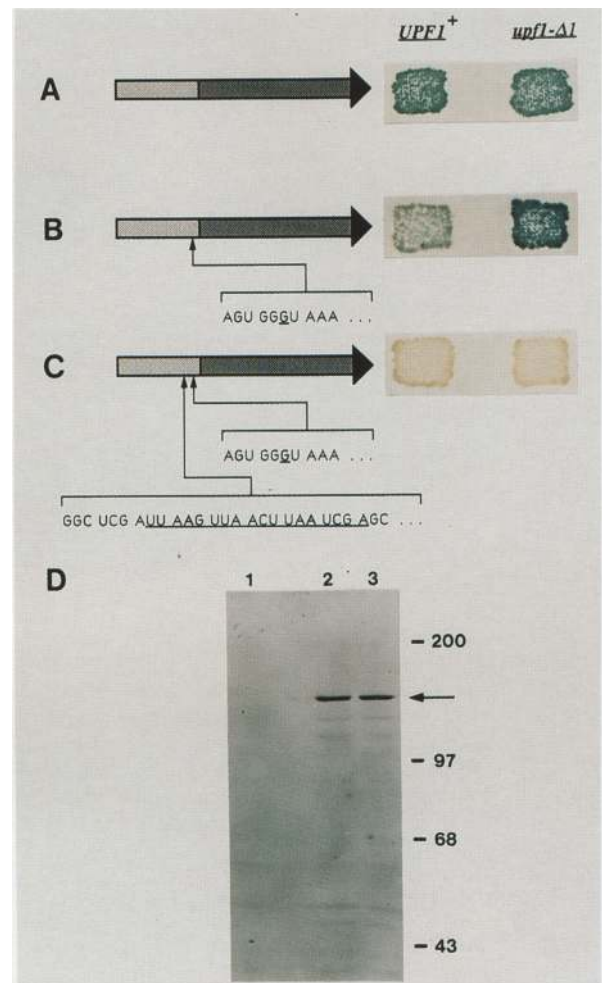


Figure 4. β-Galactosidase activity in *lacZ* fusion strains. The light portions of the arrows left of each panel denote *HIS4* sequences; the dark portions represent *lacZ*. Transcription is in the direction indicated. *UPF1*⁺ and *upf1-Δ1* derivatives of strains containing the *lacZ* fusion alleles were constructed as described in Materials and methods. Underlined sequences represent insertions defining the *his4*[−] mutations. (A) *HIS4*⁺ : *lacZ* [strains PLY149(±)]. A 1.5-kb fragment containing 1 kb of the 5′-untranslated region of *HIS4*⁺ and the first 0.5 kb of the *HIS4*⁺ open reading frame was fused in-frame to *lacZ* in the plasmid PMC1790. The β-galactosidase filter assay shown at right was allowed to develop for 15 min. (B) *his4-38* : *lacZ* [strains PLY150(±)]. Construction details are identical to A except that the fragment introduced into PMC1790 contains the *his4-38* mutation. The β-galactosidase filter assay was allowed to develop for 16 hr. (C) *his4-38*, −UAA : *lacZ* [strains PLY171(±)]. Construction details are the same as A except that the *his4* fragment contains both the *his4-38* mutation and an oligonucleotide insertion at a unique *XhoI* site 80 nucleotides upstream of the *his4-38* mutation. The β-galactosidase filter assay was allowed to develop for 16 hr. (D) Western blot analysis of *HIS4*⁺ : *lacZ* strains with antibodies directed against β-galactosidase. Equal amounts of total protein from strains PLY102 (lane 1); PLY149(+) (lane 2); and PLY149(−) (lane 3) were loaded on each lane. The arrow indicates the single band that is present in lanes 2 and 3 but absent in lane 1.

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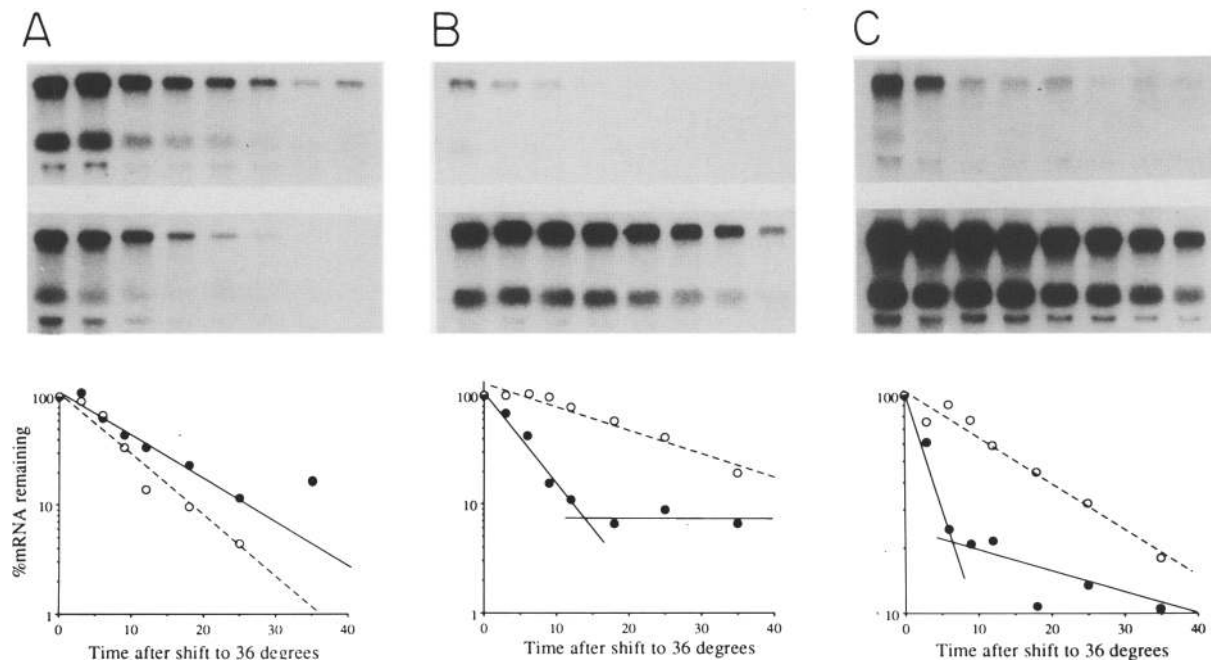


Figure 5. Decay rates of the *lacZ* gene fusion transcripts. Half-lives were measured in strains PLY172(\pm) (*HIS4*⁺ : *lacZ*) (A); PLY173(\pm) (*his4-38* : *lacZ*) (B); and PLY176(\pm) (*his4-38*, -*UAA* : *lacZ*) (C). The upper autoradiograph in each section is derived from the *UPF1*⁺ variant of these strains; the lower autoradiograph is derived from the *upf1*- Δ 1 variant. A *lacZ* probe detects two major RNA species of ~8.4 kb (upper band) and ~3.6 kb (lower band) in each strain. Hybridization to a single-stranded probe has shown that both of these RNAs are transcribed in the direction expected if initiation were occurring at the *HIS4* promoter. The size of the 3.6-kb band is consistent with transcription initiating at the normal *HIS4* site and terminating close to the 3' end of the *lacZ* sequences. The 8.4-kb band hybridizes to *TRP1* sequences, which are located downstream of the *lacZ* fusion alleles. The larger mRNA is probably produced by transcription initiating at the *HIS4* site and proceeding through *lacZ*, pMC1790 bacterial sequences, and *TRP1* with transcription terminating at the normal *TRP1* termination site. Half-lives were measured for both the 3.6- and 8.4-kb transcripts (see Table 3), but only data from the larger transcript are plotted below. Time points were taken 0, 3, 6, 9, 12, 18, 25, and 35 min after the cultures were shifted to 36°C. Solid circles and lines refer to *UPF1*⁺; open circles and dashed lines refer to *upf1*- Δ 1.

opment (Fig. 4B). The activity was too low to obtain quantitative data, but qualitatively, the results of the in situ filter assay suggest that β -galactosidase activity is higher in the *upf1*- Δ 1 strain than in the *UPF1*⁺ strain. The *his4-38* mutation exhibits a low level of leakiness that is enhanced phenotypically in strains lacking *UPF1* function.

Both the steady-state level and the half-life of *his4-*

38 : *lacZ* mRNA increase approximately fourfold in *upf1*- Δ 1 strains (Fig. 5B; Table 3). The half-life of the *his4-38* : *lacZ* transcript was also measured in strains derived from PLY150, which does not contain *rpb1-1*, by inhibiting transcription with thiolutin (Herrick et al. 1990). Results were consistent with those obtained by inhibiting transcription at the restrictive temperature in strains carrying *rpb1-1*.

Table 3. Half-lives and accumulations of *lacZ* fusion mRNAs

| Strain | Transcript | Relative abundance [(<i>upf1</i> - Δ 1/ <i>UPF1</i> ⁺) _{t=0}] | Half-life (min) | |
|--------|---|--|--------------------------|--------------------------|
| | | | <i>UPF1</i> ⁺ | <i>upf1</i> - Δ 1 |
| PLY172 | <i>HIS4</i> ⁺ : <i>lacZ</i> | 0.7 | 8 | 5 |
| | | 0.7 | 6 | 5 |
| PLY173 | <i>his4-38</i> : <i>lacZ</i> | 4.4 | 4 | 14 |
| | | 4.1 | <3 | 13 |
| PLY176 | <i>his4-38</i> , - <i>UAA</i> : <i>lacZ</i> | 4.9 | 3 | 14 |
| | | 7.0 | <3 | 21 |
| PLY150 | <i>his4-38</i> : <i>lacZ</i> | 5.0 | 4 | 20 |
| | | ND | 3 | 17 |

Values on the first line of each entry are derived from measurements of the 8.4-kb (upper) band seen on the autoradiograms in Fig. 4. Data on the second line of each entry are from the 3.6-kb (lower) band. Strain PLY150 does not carry the *rpb1-1* mutation. Half-lives were measured by inhibiting transcription with thiolutin (see Materials and methods). (ND) Not done.

Increased leakiness in the *upf1*⁻ strain detected by assaying β -galactosidase could be the result of enhanced translational readthrough or direct stabilization of *his4-38* mRNA leading to an increased steady-state mRNA concentration. We distinguished between these possibilities by examining the *his4-38, -UAA : lacZ* fusion. Strains carrying this fusion produce undetectable levels of β -galactosidase regardless of the presence or absence of a functional *UPF1* gene (Fig. 4C). These results indicate that the leaky phenotype of *his4-38 : lacZ* fusion is abolished completely when UAA stop codons are introduced upstream of the *his4-38* mutation. There is no detectable translational readthrough of this fusion mRNA.

The *his4-38, -UAA : lacZ* transcript accumulates in a *upf1- Δ 1* strain to the same extent as the *his4-38 : lacZ* transcript (Fig. 5C). Half-life measurements confirm that the observed fourfold increase in steady-state accumulation of mRNA is associated with a similar increase in mRNA stability (Fig. 5C; Table 3). The combined results suggest that mRNA stabilization resulting from loss of *UPF1* function is unrelated to and independent of the level of readthrough of frameshift mutations or stop codons in the mRNA.

Changes in mRNA stability due to loss of UPF1 function are not the result of ribosome bypass of a stop codon

The β -galactosidase assays described above measure the extent of translationally productive readthrough of a termination codon but do not, by themselves, constitute a measure of how effectively ribosomes can bypass a stop codon and remain bound to the mRNA. We tested the extent to which ribosomes might bypass a stop codon, migrate downstream, and confer stabilization through a passive protection mechanism. To determine whether this was occurring, we examined the distribution of the *his4-38, -UAA : lacZ* transcript on polyribosomes in both *UPF1*⁺ and *upf1- Δ 1* derivatives of strain PLY176. Polyribosomes from both strains were fractionated by centrifugation through sucrose gradients, and the distribution of ribosomes on the fusion mRNA was determined by Northern hybridization analysis of RNA extracted from each fraction (Fig. 6). As a control, filters were also hybridized to an *ACT1* probe (which detects actin mRNA). The results indicate that loss of *UPF1* function does not substantially change the extent of ribosome loading on the stabilized fusion mRNA. Given the magnitude of the change in mRNA half-life, the potential change in ribosome loading should have been detected if it were occurring. Therefore, we conclude that the changes in mRNA stability resulting from loss of *UPF1* function are not the result of an enhanced rate of ribosome bypass beyond a translation termination codon.

Discussion

The mechanism responsible for destabilization of tran-

scripts containing premature termination signals is not understood. Formally, premature stop codons are *cis*-acting elements that promote accelerated mRNA decay rates. In this study we present data supporting the conclusion that the *UPF1* gene product is a *trans*-acting component of a degradative pathway that acts specifically on the class of mRNAs containing premature stop codons.

Originally, *upf1*⁻ mutations were isolated by selecting for enhanced suppression of the *his4-38* frameshift mutation in the presence of a tRNA frameshift suppressor, *SUF1-1*. By using a clone of *UPF1*, strains were constructed that lack the tRNA suppressor and contain a null *UPF1* allele constructed in vitro and introduced into yeast by gene replacement. We have shown that the *his4-38* transcript is stabilized in these strains. The increase in mRNA half-life causes a corresponding increase in steady-state mRNA level. We have also observed a stabilizing effect on the mRNA produced by a *his4-38 : lacZ* fusion; the nonsense alleles, *his4-539* and *leu2-1*; and the frameshift mutation, *leu2-3*. With the exception of *leu2-1*, all of the mutations generate translation-termination codons in-frame toward the 5' end of the respective coding regions. The position of the premature stop codon in *leu2-1* is unknown.

In contrast, the *his4-713* frameshift mutation, which generates a termination codon in-frame near the 3' end of the coding region, produces a relatively stable transcript in *UPF1*⁺ strains compared with wild-type *HIS4* mRNA. Furthermore, the half-life of *his4-713* mRNA is not altered by loss of *UPF1* function. Additional wild-type mRNAs that have a broad range of inherent decay rates were also examined, and the turnover rates of these mRNAs were unaltered in strains lacking *UPF1*⁺ function.

Taken together, these results suggest that the *UPF1* product promotes the specific degradation of mRNAs in which translation is interrupted prematurely. The interdependence of translation and mRNA stability in this system suggests that the *UPF1* product could have a primary mode of action in mRNA degradation or in translation. In the latter case, the changes in mRNA half-life could represent a secondary consequence of loss of a translational component.

Data from gene fusions between the *his4* promoter/coding region and *lacZ* show that even in the absence of a tRNA suppressor, there is a low level of translational readthrough of the *his4-38* mRNA. Therefore, it seemed possible that the *UPF1* mutations might act by enhancing this low-level readthrough, allowing ribosomes that bypass the stop codon to restabilize the mRNA. A mechanism of this type can be used to explain the results of studies of *ura3* mutants, where a tRNA nonsense suppressor was shown to stabilize an mRNA that is otherwise destabilized by a premature stop codon (Losson and Lacroute 1979).

Our results show that this mechanism does not explain the stabilizing effect of *UPF1* mutations. In a gene fusion where readthrough is abolished completely by the presence of multiple stop codons introduced in the *HIS4*

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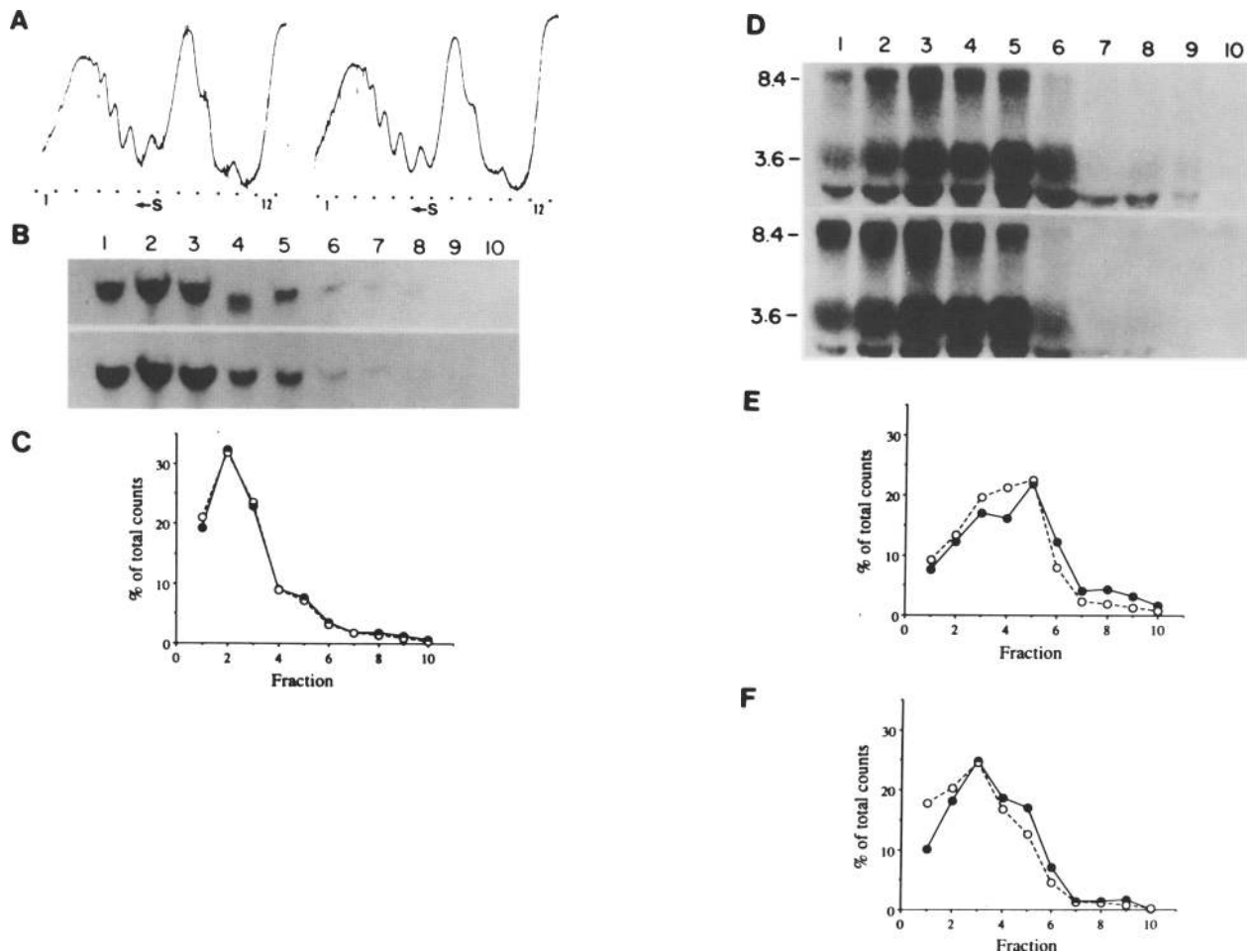


Figure 6. Distribution of *his4-38, -UAA : lacZ* mRNA on polyribosomes in strains PLY176(+) and PLY176(-). Extracts containing polyribosomes were fractionated on 12-ml 15–50% continuous sucrose gradients. Fractions of 1 ml were collected from the bottom of the gradient and used for Northern analysis. Only the first 10 fractions are shown in the autoradiographs, no hybridizing material was detectable in fractions 11 and 12. Graphs were plotted by counting the activity in each band and normalizing to total counts in fractions 1–10 for that particular band. Solid circles and lines refer to *UPF1*⁺; open circles and dashed lines refer to *upf1-Δ1*. (A) The absorption profile of gradients containing polyribosomes isolated from PLY176(+) (left) and PLY176(-) (right) monitored at 260 nm. (B) Hybridization with an *ACT1* probe. The upper autoradiograph is derived from PLY176(+); the lower is derived from PLY176(-). (C) Distribution of counts hybridized to the bands shown in B. (D) Hybridization with a *lacZ* probe. The upper autoradiograph is derived from PLY176(+) and has been exposed five times longer than the lower autoradiograph, which is derived from PLY176(-). Band sizes are given in kilobases. (E) Distribution of counts hybridized to the 3.6-kb band. (F) Distribution of counts hybridized to the 8.4-kb band. The above experiments were repeated with RNA derived from strain PLY172 to determine the distribution of ribosomes on *HIS4*⁺ : *lacZ* mRNA. In this case, the peak activity hybridizing to the 8.4- and 3.6-kb bands was found in fraction 2 (data not shown).

region, the corresponding mRNA is stabilized still by loss of *UPF1* function. In addition, the tRNA suppressor *SUF1-1*, whose primary mode of action is to promote readthrough of frameshift mutations, does not, by itself, fully stabilize the *his4-38* transcript. The apparent disagreement between our study and that involving the stabilization of *ura3* nonsense alleles by nonsense suppressors is probably due to the fact that the tRNA amber nonsense suppressors used in the study of *ura3* were more efficient in promoting translational readthrough than is *SUF1-1*.

Thus, stabilization of *his4-38* mRNA in strains lack-

ing *UPF1* function is not an indirect consequence of increased levels of mistranslation. We also tested the possibility that loss of *UPF1* function results in bypass of premature stop codons in a manner that is translationally nonproductive but still affords the mRNA protection from degradation. A bypass mechanism of this type does not occur to an extent that can be detected as a major change in the distribution of ribosomes on the *his4-38, -UAA : lacZ* fusion transcript in strains deleted for *UPF1*.

Our studies with various fusions between *his4* and *lacZ* also suggest a rationale for how loss of *UPF1* func-

tion causes a phenotype that resembles translational suppression. Although loss of *UPF1* function does not confer a His⁺ phenotype in strains containing *his4-38*, our studies with gene fusions indicate that it does promote a low level of suppression as measured by β -galactosidase activity. Previous studies indicate that a minimum of 6% of wild-type *HIS4* product is needed to confer a leaky His⁺ phenotype (Gaber and Culbertson 1984), so less than this amount of functional product must be produced by loss of *UPF1* function in *his4-38* strains. Given that *his4-38* is itself slightly leaky, the low level of suppression might be accounted for solely by an increase in steady-state level of the *his4-38* transcript. Therefore, in the original selection for *upf* mutations in strains containing the tRNA suppressor *SUF1-1*, enhancement of suppression (translational allosuppression) by a *upf1* mutation might result from the combined effects of mRNA stabilization and tRNA-mediated translational readthrough.

Our results show that in a *upf1*⁻ background, the half-lives of mRNAs derived from the *his4* and *leu2* nonsense and frameshift alleles are never greater than the half-lives of the wild-type *HIS4* and *LEU2* transcripts. Coupled with the finding that the stability of wild-type mRNAs is generally unaffected by *UPF1*, these results suggest that the upper limit on stability that can be achieved by combining a *UPF1* loss-of-function mutation with a nonsense mutation is equivalent to the half-life of the wild-type mRNA. An exception to this rule was found when mutations that generate premature nonsense codons were introduced into the *his4* region of a *his4 : lacZ* fusion. The stabilities of the corresponding transcripts in *upf1-Δ1* strains exceeded that of the *HIS4*⁺ : *lacZ* fusion transcripts. This unexpectedly low stability of *HIS4*⁺ : *lacZ* mRNA could be explained by postulating the existence of a sequence element in *HIS4* or *lacZ* that destabilizes the mRNA in a manner independent of *UPF1* function. Such an element has been shown to exist in the yeast *MATα1* gene (Parker and Jacobson 1990). Because an element of this type is active only when translated, it might destabilize the *HIS4*⁺ : *lacZ* transcript but have no effect on transcripts containing premature stop codons.

Important clues regarding *UPF1* function may come from our studies of the endogenous *URA3*⁺ transcript, which exhibits excess accumulation in *upf1*⁻ strains but shows no change in mRNA stability. This result is unusual in several respects. *URA3*⁺ mRNA is the only wild-type transcript analyzed in this study whose accumulation is affected by loss of *UPF1* function. Because mRNA stability is unchanged, the effect appears to be mediated by a transcriptional mechanism.

More detailed analyses suggest that the effect on *URA3* is transcriptional and is indirectly mediated by the *PPR1* gene product, a transcriptional activator of *URA3* (S. Peltz, P. Leeds, J.M. Wood, A. Atkin, M.R. Culbertson, and A. Jacobson, in prep.). *PPR1* encodes an inherently unstable mRNA (Losson et al. 1983), which we have shown is stabilized by loss of *UPF1* function (S.W. Peltz, P. Leeds, M.R. Culbertson, and A. Jacobson,

unpubl.). The higher rate of *URA3* transcription may therefore be the result of increased expression of *PPR1*.

An unusual feature of *PPR1* mRNA may explain its inherent instability and the stabilization that results from loss of *UPF1* function. The leader region of the transcript contains two contiguous AUG codons, followed by a stop codon, three codons later (Losson et al. 1983). Therefore, it may contain a premature stop codon that normally targets the mRNA for degradation by the *UPF1*-mediated pathway. According to this reasoning, *UPF1* may function in a specialized degradation pathway that controls the stabilities of a select subclass of wild-type mRNAs that contain a naturally occurring premature termination signal. An investigation of the effects of *UPF1* on mRNAs of this type is currently in progress.

Because the *UPF1* product is required for degradation of a particular class of mRNAs, it is possible that *UPF1* is the structural gene for a ribonuclease. Alternatively, *UPF1* may code for a positive effector of ribonuclease activity. DNA sequence analysis of the *UPF1* gene has revealed a potential zinc-dependent nucleic acid-binding domain as well as a region of sequence similarity to the yeast *SEN1* gene (Leeds 1991; D.J. Demarini, M. Winey, D. Ursic, F. Webb, and M.R. Culbertson; P. Leeds, J.M. Wood, B. Lee, and M.R. Culbertson; both in prep.). *SEN1* is required for tRNA splicing endonuclease activity but is unlikely to be the structural gene for a catalytic subunit of the endonuclease (D. Ursic and M.R. Culbertson, unpubl.). The presence of a nucleic acid-binding domain suggests that the *UPF1* product may function through a direct interaction with RNA, a model that is currently being tested.

Materials and methods

Strains and genetic techniques

The *S. cerevisiae* strains used in this study are described in Table 1. Strains were constructed using standard yeast genetic techniques (Sherman et al. 1979). Media for growth of yeast was described by Gaber and Culbertson (1982). Yeast transformations were performed by using the method of Ito et al. (1983). Bacterial methods, nomenclature, and media were described by Miller (1972). The *Escherichia coli* strains used for plasmid preparations were MC1066A (Sandbaken and Culbertson 1988) and JM109 (Yanisch-Perron et al. 1985).

Nucleic acid methods

Yeast chromosomal DNA was prepared by the method of Sherman et al. (1979). Plasmid DNA was prepared from *E. coli* by the method of Birnboim and Doly (1979) or Holmes and Quigley (1981). DNA was sequenced by the method of Sanger et al. (1977).

RNA used to study steady-state mRNA accumulation was isolated by the method of Lindquist (1981). RNA used to measure decay rates was isolated by hot phenol extraction. Cell pellets suspended in 0.6 ml of buffer A (50 mM sodium acetate, 10 mM EDTA) containing 1% SDS and 1% diethylpyrocarbonate were extracted twice with buffer A-saturated phenol. Extractions were done at 65°C for 5 min. The aqueous phase was then extracted once with phenol/chloroform and precipitated twice

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with ethanol. The RNA pellet was suspended in water and the concentration was measured by absorbance at 260 nm.

Plasmids

The vectors YCp50, YCpMS38, YEp13, and pMC1790 are described by Rose et al. (1987), Sandbaken and Culbertson (1988), Broach et al. (1979), and Casadaban et al. (1983), respectively.

A copy of *UPF1*⁺ can be obtained on either a 4.2-kb *EcoRI*–*Bam*HI fragment or a 4.6-kb *Clal*–*Bam*HI fragment (Leeds 1991). (The EMBL/GenBank DNA sequence accession number for *UPF1* is M76659.) YCpPL63 was constructed by inserting the 4.2-kb *UPF1*⁺ fragment into *EcoRI*/*Bam*HI-digested YCp50, which also contains *URA3*⁺. YCpPL53 was constructed by inserting the 4.6-kb *UPF1*⁺ fragment into *Clal*/*Bam*HI-digested YCpMS38, which also contains *TRP1*⁺. YEpPL55 was constructed by ligating a *Bam*HI linker on the *Clal* end of the 4.6-kb *UPF1*⁺ fragment and inserting the end-modified fragment into *Bam*HI-digested YEp13, which also contains *LEU2*⁺.

Plasmids YIp309 and B134 contain the 5'-untranslated region and the *HIS4A*-coding region of *HIS4* and *his4-38*, respectively. Digestion of each plasmid with *Bal*I and *Clal* releases a 1.5-kb fragment. The *Bal*I site is 1036 bases upstream of the start of translation, whereas the *Clal* site in *HIS4A* is located 340 bases downstream of the site of the *his4-38* mutation. A *Bam*HI linker (CGGATCCG) was ligated into the *Clal* site of each plasmid following treatment with Klenow enzyme. The resulting *Bal*I–*Bam*HI fragment from YIp309 was inserted into *Sma*I/*Bam*HI-digested pMC1790 to construct the *HIS4*⁺ : *lacZ* fusion plasmid YRpPL14 (Fig. 4A). The same procedure was used to construct the *his4-38/lacZ* fusion plasmid YRpPL15, except that the *Bal*I–*Bam*HI fragment was derived from B134 (Fig. 4B). The structures of both plasmids were verified by DNA sequence analysis.

The plasmid YRpPL81, which contains the *his4-38*, –*UAA* : *lacZ* gene fusion, was constructed as follows. YRpPL15, which contains the *HIS4A* region, including the *his4-38* mutation, was digested with *Xho*I at a unique site 96 nucleotides downstream of the start of translation and 80 nucleotides upstream of the *his4-38* mutation. The resulting linear fragment was treated with Klenow enzyme to produce blunt ends. The nucleotide linker TTAAGTTAACTTAA (Suppressible Reading Frame Termination Linker; Pharmacia) was added in with the blunt-ended fragment and ligated by T4 ligase.

The DNA fragment used to make the *HIS4* hybridization probe consisted of a 2.4-kb *Pvu*II fragment from plasmid B137 (*his4-519*, *URA3*). The *LEU2* probe was made from a 2.3-kb *Sal*I–*Xho*I fragment from YEp13 (Broach et al. 1979). The *lacZ* probe was from a 3.0-kb *Bam*HI fragment from pMC1871 (Shapiro et al. 1983). All other hybridization probes are described by Herrick et al. (1990).

Strain construction

The alleles designated *upf1-Δ1* and *upf1-Δ2* are chromosomal deletions of *UPF1* constructed by substituting a 1.1-kb DNA fragment containing *URA3* for *UPF1* sequences. The deletion in *upf1-Δ1* includes the 5' three-fifths of the *UPF1* open reading frame and 187 nucleotides of the 5'-untranslated region. The deletion in *upf1-Δ2* has the same 5' end point but includes the entire *UPF1* translated region and 189 nucleotides of the 3'-untranslated region. Both deletions confer the same recessive phenotype as the original *upf1-Δ2* allele (Leeds 1991).

Strain PLY36A was constructed as follows. Plasmid pMM54 (*suf1*⁺, *URA3*; Mendenhall et al. 1987) was linearized by digestion with *Bgl*III, which cuts in the *suf1*⁺-flanking region, and

used to transform strain PLY36 (Table 1). *Ura*⁺ transformants all contained the chromosomal copy of *SUF1-1* and were therefore *His*⁺ at 30°C due to suppression of *his4-38*. One transformant, containing a copy of pMM54 stably integrated at the *SUF1* locus, was plated on media containing 5-fluoro-orotic acid to select for *Ura*[–] colonies (Boeke et al. 1984). These colonies were then screened for loss of *SUF1-1*, which results in a *His*[–] phenotype. The loss of *SUF1-1* in one of these strains, denoted PLY36A, was confirmed by crossing it to a *suf1*⁺ *his4-38* strain and verifying that the resulting tetrads contained only *His*[–] spores.

Both PLY36 and PLY36A carry the recessive allele *upf1-2*. *UPF1*⁺ derivatives of these two strains were constructed by transforming them with YCpPL63 (*UPF1*⁺, *URA3*⁺). *upf1-2* derivatives were obtained by transforming with the vector YCp50 (*URA3*⁺).

Strains PLY149, PLY150, and PLY171 were constructed as follows. Plasmids YRpPL14, YRpPL15, and YRpPL81 each contain a *his4* : *lacZ* fusion gene. Strain PLY102 was transformed with these plasmids after each was linearized by digestion with *Bgl*III, which cuts in the *TRP1*-flanking region. Transformants carrying YRpPL14, YRpPL15, and YRpPL81 were designated PLY149, PLY150, and PLY171, respectively (Table 1). Tetrad analysis showed that in each case the plasmid had integrated into the chromosome and the site of integration was linked tightly to the *trp1* locus. This was confirmed by Southern hybridization analysis with *lacZ* DNA as a probe, which also verified that a single copy of each plasmid was present in the chromosome. The linker used in the construction of the *his4-38*, –*UAA* : *lacZ* allele in YRpPL81 contains a *Hpa*I site that can be detected in strain PLY171 by Southern analysis. Hybridization of our *HIS4* probe to *Hpa*I-digested genomic DNA from strain PLY150 yields a 3.8-kb restriction fragment from the integrated *his4-38* : *lacZ* fusion allele. Hybridization of the same probe to *Hpa*I-digested DNA from strain PLY171 yields a 2.9-kb and an 0.9-kb restriction fragment, which are the sizes expected to result from *Hpa*I cleavage within the linker.

Strains PLY149, PLY150, and PLY171 each carry the recessive allele *upf1-Δ1*. *UPF1*⁺ derivatives of these strains were constructed by transforming them with YEpPL55 (*UPF1*⁺, *LEU2*⁺). *upf1-Δ1* derivatives were obtained by transforming with the vector YEp13 (*LEU2*⁺).

The strains used to measure mRNA decay rates all carry *rbp1-1* and either *upf1-Δ1* or *upf1-Δ2* (Table 1). *UPF1*⁺ derivatives of these strains were constructed by transforming them with either YCpPL53 (*UPF1*⁺, *TRP1*⁺) or YEpPL55 (*UPF1*⁺, *LEU2*⁺). *upf1*[–] derivatives were obtained by transforming with either YCpMS38 (*TRP1*⁺) or YEp13 (*LEU2*⁺).

mRNA decay rates

Decay rates of individual mRNAs were measured by the methods of Herrick et al. (1990) and Parker et al. (1991). Cultures of strains carrying the temperature-sensitive polymerase II allele *rbp1-1* were shifted rapidly from 25°C to 36°C by the addition of an equal volume of media heated to 48°C. Aliquots of cells were removed at specific times and spun down, and the cell pellets were frozen in dry ice/ethanol. RNA was prepared and fractionated on denaturing 1% agarose gels, transferred to nitrocellulose, and hybridized to radiolabeled DNA probes. The absolute amount of radiolabeled probe hybridized to specific bands was quantitated on a Betascope Blot Analyzer (Betagen, Waltham, MA). Cricket graph (Cricket Software, Malvern, PA) was used to generate mRNA decay curves, which were plotted as the log of the percentage of activity remaining in a particular band (relative to the activity at time zero) versus time at 36°C. Lines were

generated by using a least-squares fit. As noted previously (Herrick et al. 1990; Parker et al. 1991), decay curves of some transcripts were biphasic, with a second, slower-decaying component comprising 5%–20% of the total RNA. Although the cause of the biphasic decay is unclear, the small amount of slow component is, in all cases, insufficient to make a significant contribution to the overall decay rate.

Decay rates in strain PLY150 were measured by inhibiting transcription with the drug thiolutin (3 μ g/ml) (Herrick et al. 1990; Parker et al. 1991). Time points were taken following addition of the drug and analyzed as described above.

Protein isolation and Western blots

Cell pellets containing $\sim 10^9$ cells were washed once with lysis buffer [50 mM Tris-HCl (pH 7.4), 0.25 M NaCl, 0.1% (vol/vol) NP-40, 5 mM EDTA] and resuspended in 1.6 ml of lysis buffer containing 1.0 mM phenylmethylsulfonyl fluoride and 1.0 mg/liter each of chymostatin, leupeptin, pepstatin, antipain, and aprotinin. The cells were transferred to two 1.5-ml tubes that were then filled to the top with cold, acid-washed glass beads (425–600 μ m; Sigma). Cells were disrupted by four 20-sec pulses on a Mini-Beadbeater (Biospec Products, Inc.). Protein concentrations in the supernatant were quantitated by absorbance at 280 nm and equal amounts from each strain were separated by SDS-PAGE. Following transfer of proteins to nitrocellulose (Towbin et al. 1979), immunoblotting was performed as described by BRL (Immunoselect instruction manual, no. 9590SA) with mouse anti- β -galactosidase and alkaline phosphatase coupled to anti-mouse IgG.

Polyribosome analysis

Polyribosomes were prepared and fractionated as described by Hutchison (1969) and Sachs and Davis (1989). The experiment involved comparison of profiles obtained from different sucrose gradients. We found that variation between gradients, as measured by UV monitoring, was minimized by preparing the gradients according to the method of Luthe (1983). Fractions were extracted three times with phenol/chloroform and ethanol-precipitated. The pellet was resuspended in water, and half of each fraction was loaded on denaturing 1% agarose gels, transferred to nitrocellulose, and hybridized to radiolabeled DNA probes. The activity of individual bands was measured with a Betascope Blot Analyzer (Betagen, Waltham, MA).

β -Galactosidase assays

Activity was quantitated by using the whole-cell ONPG assay of Miller (1972) as modified by Guarente (1981). Qualitative X-gal assays were done by the method of Breeden and Nasmyth (1985).

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