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Gene Products That Promote mRNA Turnover in *Saccharomyces cerevisiae*†

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We showed previously that the increased rate of mRNA turnover associated with premature translational termination in the yeast *Saccharomyces cerevisiae* requires a functional *UPF1* gene product. In this study, we show that the *UPF1* gene codes for a 109-kDa primary translation product whose function is not essential for growth. The protein contains a potential zinc-dependent nucleic acid-binding domain and a nucleoside triphosphate-binding domain. A 300-amino-acid segment of the UPF1 protein is 36% identical to a segment of the yeast SEN1 protein, which is required for endonucleolytic processing of intron-containing pre-tRNAs. The same region is 32% identical to a segment of Mov-10, a mouse protein of unknown function. Dominant-negative *upf1* mutations were isolated following in vitro mutagenesis of a plasmid containing the *UPF1* gene. They mapped exclusively at conserved positions within the sequence element common to all three proteins, whereas the recessive *upf1-2* mutation maps outside this region. The clustering of dominant-negative mutations suggests the presence of a functional domain in UPF1 that may be shared by all three proteins. We also identified *upf* mutations in three other genes designated *UPF2*, *UPF3*, and *UPF4*. When alleles of each gene were screened for effects on mRNA accumulation, we found that the recessive mutation *upf3-1* causes increased accumulation of mRNA containing a premature stop codon. When mRNA half-lives were measured, we found that excess mRNA accumulation was due to mRNA stabilization. On the basis of these results, we suggest that the products of at least two genes, *UPF1* and *UPF3*, are responsible for the accelerated rate of mRNA decay associated with premature translational termination.

In a wide variety of organisms, mRNAs transcribed from genes containing nonsense or frameshift mutations accumulate to a much lesser extent than do the corresponding wild-type mRNAs. In *Saccharomyces cerevisiae*, the introduction of a premature stop codon into a transcript causes a reduction in mRNA half-life that leads to a decrease in steady-state mRNA accumulation (29, 34, 48). The introduction of an efficient tRNA nonsense suppressor, which promotes read-through and restores translation of the mRNA, prevents the decline in stability and accumulation caused by premature translational termination (34). These results suggest the existence of a mechanism that serves to adjust the intrinsic rate of mRNA decay according to the ability of the mRNA to be translated. The underlying molecular basis for such a mechanism has not yet been established.

To further study how mRNA turnover is related to premature translational termination, we took advantage of a selection scheme capable of yielding mutations that uncouple the two processes. The mutations were obtained in a strain containing *his4-38*, a +1 frameshift near the 5′ end of the *HIS4* transcript that causes translational termination at an adjacent downstream stop codon (7, 13). The *his4-38* mutation results in a four- to fivefold decrease in mRNA stability (29). The strain used to select mutations also contained *SUF1-1*, which codes for a glycine tRNA frameshift suppressor that promotes inefficient read-through of the *his4-38* frameshift mutation by decoding a four-base codon (36). The *SUF1-1* tRNA suppressor does not restabilize *his4-38* mRNA to a significant extent, probably because of a low efficiency of suppression (29). Strains carrying both *his4-38* and *SUF1-1* produce an unstable *his4* mRNA and are phenotypically His^+ at 30°C but His^- at 37°C. Up frameshift (*upf*) mutations were obtained by selecting for growth at 37°C, which can result from a change in any process that leads to increased expression of the *his4* gene (29).

In this study, we report that four unlinked genes, designated *UPF1* through *UPF4*, have been identified in a collection of mutations that confer the Upf^- phenotype described above. To date, the *UPF1* gene has been studied in the greatest detail. Using a *upf1* null allele, we have reported elsewhere that *his4* or *leu2* mRNAs containing premature stop codons fail to show the characteristic destabilization relative to wild-type *HIS4* or *LEU2* mRNA (29). On this basis, we have proposed that *UPF1* is required for destabilization. Cells carrying *his4-38*, *SUF1-1*, and a *upf1* mutation are His^+ at 37°C as a result of increased accumulation of a defective *his4* mRNA rendered translatable by the *SUF1-1* tRNA suppressor.

The specific function performed by the *UPF1* gene product is still unclear. *UPF1* apparently acts in response to a signal generated by premature translational termination. This view is supported by the observation that loss of *UPF1* function affects only mRNAs containing a premature stop codon. The half-life and accumulation of a number of wild-type mRNAs, including *HIS4*, *LEU2*, *MATα1*, *STE3*, *ACT1*, *PGK1*, and *PAB1*, are not affected (29). We have considered the possibility that *UPF1* encodes a translation factor whose function has secondary consequences for mRNA stability. To test whether UPF1 is a translation factor that inhibits read-through of stop codons, thereby preventing ribosomes from migrating past the stop codon and protecting the mRNA from degradation, we analyzed gene fusions and polyribosome profiles (29). Our results showed that loss of *UPF1* function does not enhance read-through, indicating that wild-type *UPF1* probably does not inhibit read-through.

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TABLE 1. Yeast strains

Strain	Genotype	Source
PLY22	*MATa his4-38 SUF1-1 ura3-52 met14*	This study
PLY36	*MATα his4-38 SUF1-1 ura3-52 met14 upf1-2*	This study
PLY89	*MATα leu2-3 ura3-52 UPF1* [YIpPL46]	This study
PLY82	*MATα leu2-1 ura3-52*	This study
PLY97	*MATa/MATα ura3-52/ura3-52 trp1/TRP1 tyr7-1/TYR7 LEU2/leu2-3*	This study
PLY144	*MATa upf1-Δ2 ura3-52 trp1-Δ1 his4-166 ade2-1 leu2-2*	This study
PLY145	*MATα upf1-Δ2 ura3-52 trp1-Δ1 his4-166 tyr7-1*	This study
PLY146	*MATα upf1-Δ2 ura3-52 trp1-Δ1 tyr7-1 leu2-2*	This study
PLY147	*MATα upf1-Δ2 ura3-52 trp1-Δ1 lys2-1 met8-1*	This study
PLY148	*MATα upf1-Δ2 ura3-52 trp1-Δ1 lys2-1 tyr7-1 leu2-1*	This study
PLY153	*MATα upf1-Δ1 ura3-52 trp1-Δ1 rpb1-1*	This study
PLY172	*MATa upf1-Δ1 ura3-52 trp1-7 leu2-3,112 rpb1-1* [YRpPL14]	This study
PLY180	*MATa his4-38 SUF1-1 ura3-52 leu2-3,112*	This study
PLY56-15B	*MATa lys7 ura3-52 his4-38 SUF1-1 leu2 trp5*	This study
PLY81-20A	*MATa upf1-Δ2 ura3-52 trp1-1 his4-38*	This study
PLY85-4C	*MATα upf1-Δ2 ura3-52 trp1-Δ1 leu2-3*	This study
PLY96-6D	*MATa his4-38 SUF1-1 upf1-2*	This study
PLY102-6D	*MATa his4-38 SUF1-1 trp1-1*	This study
PLY102-10C	*MATα his4-38 SUF1-1 trp1-1*	This study
DBY703	*MATα his3-Δ1 trp1-289 ura3-52* [cir^0]	D. Botstein
RLY190	*MATa ura3-52 met6 rna1*	I. Edelman
K398-4D	*MATa spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*	R. Esposito
K399-7D	*MATa spo11 ura3 his2 leu1 lys1 met4 pet8*	R. Esposito
K396-11A	*MATa spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*	R. Esposito
BSY-200	*MATα his4-166 upf3-1 rpb1-1 ura3-52 trp1*	This study
BSY-201	*MATα his4-166 leu2-2 rpb1-1 ura3-52 upf3-1 trp1*	This study
BSY-202	*MATα his4-38 leu2-2 upf3-1 rpb1-1 ura3-52 trp1*	This study
BSY-300	*MATa leu2-3,112 upf3-1 rpb1-1 ura3-52 trp1*	This study

However, *UPF1* could still function in some aspect of translation not detectable in read-through assays. Alternatively, *UPF1* could play a more direct role in mediating mRNA stability and might participate in the degradation process itself.

To gain more information about *UPF1*, we have cloned the gene, determined the DNA sequence, characterized the transcript, and constructed and analyzed new mutations in the *UPF1* gene. Our results indicate that the UPF1 protein contains several structural elements that are likely to be important for function. We show that *UPF1* is a member of a previously unknown gene family whose products appear to be involved in different RNA metabolic pathways (see also reference 8). We have also extended our genetic analyses by identifying a second gene, *UPF3*, that affects mRNA accumulation and stability in a manner similar to that of *UPF1*.

MATERIALS AND METHODS

Strains and genetic techniques. The *S. cerevisiae* strains used in this study are described in Table 1. Strains were constructed by using standard yeast genetic techniques (56). Medium for growth of yeast was described by Gaber and Culbertson (18). Yeast transformations were performed by the method of Ito et al. (23). Bacterial methods, nomenclature, and media were described by Miller (38). The *Escherichia coli* strains used were MC1066A (53) and JM109 (64).

Nucleic acid methods. Yeast chromosomal DNA was prepared by the method of Sherman et al. (56). Plasmid DNA was prepared from *E. coli* by the method of Birnboim and Doly (4) or Holmes and Quigley (22). DNA was sequenced by the method of Sanger et al. (54).

RNA used in Northern (RNA) hybridization analysis was isolated by the method of Lindquist (30). Cell pellets from 5 ml of cells at an optical density at 600 nm of 0.4 were resuspended in 150 μl of extraction buffer (0.1 M Tris [pH 7.5], 0.1 M LiCl, 0.01 M dithiothreitol), 30 μl of 10% sodium dodecyl sulfate, 150 μl of phenol, and 150 μl of chloroform. Then 0.42 g of cold acid-washed beads (425 to 600 μm; Sigma) was added, and the cells were disrupted by vortexing for 5 min. Following centrifugation, the supernatant was extracted three times with phenol-chloroform and precipitated four times with ethanol. Pellets were resuspended in water, and RNA concentrations were measured by A_{260}. mRNA half-life was determined by using RNA prepared by hot phenol extraction (29).

Measurement of mRNA decay rate. Decay rates of individual mRNAs were measured by the methods of Herrick et al. (20) and Parker et al. (47). Cultures of strains carrying the temperature-sensitive polymerase II allele *rpb1-1* were rapidly shifted from 25 to 36°C by addition of an equal volume of medium heated to 48°C. Aliquots of cells were removed at specific times and centrifuged, 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, Mass.). Cricketgraph (Cricket Software, Malvern, Pa.) was used to generate mRNA decay curves, which were plotted as log of 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.

Plasmid and strain construction. The plasmid vectors YCp50, YCpMS38, and YIp5 are described by Rose et al. (52), Sandbaken and Culbertson (53), and Struhl et al. (59), respectively. Plasmid EC402, which carries the *URA3* gene and the 2μm origin of replication, was obtained from E. Craig. The *UPF1* gene was cloned from a yeast DNA library

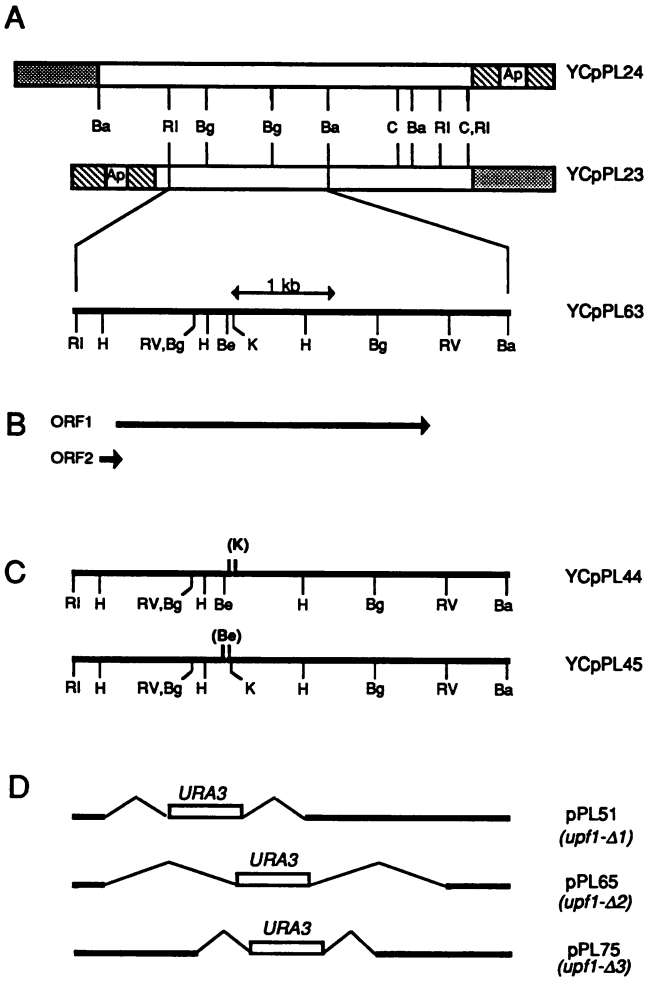


FIG. 1. *UPF1* restriction maps. (A) Restriction map of two independent clones of *UPF1* and the common 4.2-kb *Eco*RI-*Bam*HI fragment capable of complementing *upf1-2*. Shaded and cross-hatched areas represent YCp50 sequences and are included to show that the fragments in YCpPL23 and YCpPL24 are in opposite orientations in the vector. (B) Location of ORF1 and ORF2. ORF1 corresponds to the *UPF1* gene. Transcription is in the direction indicated by the arrows. (C) Location of the *Kpn*I and *Bst*EII sites that were used to make disruptions in *UPF1*. (D) *UPF1* deletions. Heavy lines represent *UPF1* sequences; light lines delineate the deleted regions that were replaced with *URA3*. Restriction sites: Ba, *Bam*HI; Be, *Bst*EII; Bg, *Bgl*II; C, *Cla*I; H, *Hin*dIII; K, *Kpn*I; RI, *Eco*RI; RV, *Eco*RV.

containing partial *Sau*3A fragments inserted in the *Bam*HI site of YCp50 (52).

The integrative plasmid YIpPL46 was used to determine whether the cloned DNA fragment carrying the putative *UPF1* gene integrates by homologous recombination at the *upf1* locus. pPL46 was constructed by inserting a 4.2-kb *Bam*HI-*Eco*RI fragment carrying the *UPF1* gene (Fig. 1) into *Bam*HI-*Eco*RI-digested YIp5. The multicopy plasmid YEpPL29 was used for chromosome mapping of the *UPF1* gene and for isolating dominant-negative mutations in *UPF1*. YEpPL29 was constructed by inserting a 4.6-kb *Bam*HI-*Cla*I fragment containing the *UPF1* gene (Fig. 1) into *Bam*HI-*Cla*I-digested EC402. YCpPL53 was used to analyze the ability of *UPF1* deletions to confer nonsense or frameshift suppression. YCpPL53 was constructed by inserting the 4.6-kb *Bam*HI-*Cla*I fragment into *Bam*HI-*Cla*I-digested YCpMS38. The multicopy plasmid YEpPL55 carrying *UPF1* has been described previously (29).

Plasmid YCpBS3 contains *UPF3* in YCp50 (28a). Strains BSY-200, BSY-201, BSY-202, and BSY-300 each contain *upf3-1*. Strains carrying *UPF3*$^+$ were constructed by introducing YCpBS3 into each of these strains. The *upf3-1* strains were constructed by introducing the vector YCp50.

Plasmids pPL51, pPL65, and pPL75 contain insertions and deletions within the *UPF1* coding sequence (Fig. 1D). pPL51 is a derivative of YIp5 that contains the *upf1-Δ1* allele. *upf1-Δ1* was constructed by deleting two adjacent *Hin*dIII fragments that contain part of the *UPF1* coding sequence (nucleotides −187 to +1726) and inserting a 1.1-kb *Hin*dIII fragment carrying the *URA3* gene. pPL65 is a derivative of pBR322 that contains the *upf1-Δ2* allele. *upf1-Δ2* was constructed by deleting the *Hin*dIII-*Eco*RV fragment that contains the entire *UPF1* coding sequence (nucleotides −187 to a position 189 nucleotides beyond the stop codon). The *Eco*RV site was converted to a *Hin*dIII site by using a *Hin*dIII linker, and the *URA3* gene on the 1.1-kb *Hin*dIII fragment was inserted. pPL75 is a derivative of YIp5 that contains the *upf1-Δ3* allele. *upf1-Δ3* was constructed by deleting the *Bgl*II-*Bgl*II fragment that contains the internal one-third of the *UPF1* coding sequence (nucleotides +724 to +2435) and inserting the 1.1-kb *Hin*dIII fragment carrying the *URA3* gene.

Mapping the site of chromosomal integration. YIpPL46, a derivative of YIp5 carrying the *UPF1* gene on the *Bam*HI-*Eco*RI fragment, was linearized by digestion with *Bgl*II. This removes a 1,711-bp fragment within the *UPF1* coding sequence. Strain PLY36, which carries the recessive *upf1-2* mutation (Table 1), was transformed with the linear plasmid to a Ura$^+$ phenotype. The resulting colonies failed to grow on medium lacking histidine at 37°C, indicating that the transformants contained a wild-type *UPF1* gene. One Ura$^+$ transformant was crossed with strain PLY96-6D (Table 1). Tetrads derived from the cross all contained two Ura$^+$ temperature-sensitive and two Ura$^-$ temperature-independent spore colonies (35 parental ditype, 0 nonparental ditype, 0 tetratype), indicating genetic linkage between the *upf1-2* mutation and the site of plasmid integration.

Genomic deletion of *UPF1*. pPL65 was codigested with *Eco*RI and *Bam*HI, which releases a fragment containing *URA3* plus 5′ and 3′ sequences that flank *UPF1*. This fragment was introduced into PLY97, a homozygous *ura3-52* diploid strain, by selecting for Ura$^+$ transformants. Genomic DNA from a Ura$^+$ transformant was codigested with *Bam*HI and *Eco*RI and probed by Southern blotting with a labeled DNA fragment containing *UPF1* flanking sequences. Two bands were detected, the 4.2-kb band corresponding to the wild-type *UPF1* gene and a 2.0-kb band corresponding to the marked deletion. Analysis of each of four spore colonies obtained following sporulation of this strain showed that two Ura$^+$ colonies contained the 2.0-kb band whereas two Ura$^-$ colonies contained the 4.2-kb band (data not shown).

Nonsense and frameshift suppression. To assay for *upf1*-mediated nonsense and frameshift suppression, strains PLY144 through PLY148 (Table 1) were constructed. Each strain carries a deletion of *TRP1* gene (*trp1-Δ1*), a *UPF1* deletion marked with *URA3* (*upf1-Δ2*; Fig. 1D), and a subset of the nonsense or frameshift mutations listed in Table 2. The identities of the nonsense mutations were determined by demonstrating that they were suppressible by one of the *SUP4* nonsense suppressor genes carried on plasmids pPM689 (UAA), pPM600 (UAG), and pPM609 (UGA) (55).

The identity of the *leu2-3* frameshift mutation in strain PLY85-4C was determined by demonstrating that it is suppressible by *SUF1-1*. Strains PLY144 through PLY148 were transformed either with YCpPL53, which carries *UPF1* and *TRP1*, or with YCpMS38, which carries only *TRP1*. The relative extent of suppression was determined by comparing growth on appropriate media of genetically similar strains that contain or lack a functional *UPF1* gene.

2μm mapping. Chromosome mapping of the *upf1* locus was done by the method of Falco et al. (16). Plasmid pP129, which contains *UPF1*, *URA3*, and a fragment containing the origin of replication from the yeast 2μm plasmid, was linearized by digestion with *Bgl*II and used to transform DBY703, a haploid [cir^0] strain lacking 2μm DNA. Ura^+ transformants carrying a stably integrated copy of the plasmid were mated to the [cir^+] strains K398-4D, K399-7D, K396-11A, and RLY190 (Table 1). The resulting diploids lost the integrated plasmid and became Ura^- at high frequency. Ura^- colonies were screened for the coincident appearance of phenotypes conferred by recessive markers in the strains.

Antibiotic sensitivity. Genetically similar strains differing only in the expression of *UPF1* were used to test for sensitivity to antibiotics. Cultures grown to stationary phase were diluted to an optical density at 600 nm of 0.4 to 0.6, and 0.3-ml aliquots were spread on plates. A 3/8-in. (ca. 1-cm)-diameter analytical filter (Schleicher & Schuell) was placed on each plate. Either 50 μl of 50-mg/ml paromomycin sulfate (Warner-Lambert Co., Ann Arbor, Mich.) or 25 μl of 0.2-mg/ml cycloheximide (Sigma) was placed on each filter. Plates were incubated for 3 days. The extent of sensitivity to each antibiotic was estimated from the diameter of the zone of growth inhibition surrounding the filter.

Transcript mapping. Primer extension analysis was performed by using a modification of the procedure described by Kingston (27). An oligonucleotide primer that was complementary to nucleotides +67 to +106 of the *UPF1* open reading frame (ORF) (Fig. 2) was synthesized by the University of Wisconsin Biotechnology Center. Total RNA (55 μg) or poly(A)$^+$ RNA (3 μl) was incubated with 5′-end-labeled primer in 20 μl of annealing buffer (0.3 M NaCl, 10 mM Tris-HCl [pH 7.5], 2 mM EDTA) for 3 h at 30°C. Then $MgCl_2$ was added to a concentration of 10 mM, dithiothreitol was added to 2 mM, and each of the four deoxynucleoside triphosphates (dNTP's) was added to 1 mM. Following extension of the primer (27), reaction products were analyzed on a 6% polyacrylamide sequencing gel along with the products of a DNA sequencing reaction labeled with [α-^{35}S]dATP (>1,000 Ci/mmol; Amersham). Identical results were obtained when the sequencing reaction was labeled with [α-^{32}P]dATP (400 Ci/mmol; Amersham).

A 301-bp oligonucleotide used in S1 nuclease protection experiments was constructed by extending the primer described above to the *Hin*dIII site upstream of the start of the *UPF1* ORF (Fig. 1A). Reactions were carried out by the method of Greene and Struhl (19). S1 reaction products were run alongside a sequencing reaction labeled with [α-^{32}P]dATP.

Isolation of dominant mutations. Plasmid YEpPL29 was mutagenized in vitro with hydroxylamine according to the method of Rose and Fink (51) and used to transform strain PLY22 (Table 1). Cultures containing transformed cells were allowed to grow overnight in liquid synthetic medium lacking uracil prior to plating on solid medium. Ura^+ transformants capable of growth at 37°C on medium lacking both uracil and histidine were selected. In all cases, loss of the mutagenized plasmid resulted in reversion to a His^- phenotype at 37°C.

The mutations were localized within *UPF1* by a gene conversion assay similar to that described by Sandbaken and Culbertson (53). Plasmids carrying the mutations were digested either with *Kpn*I or *Bgl*II (Fig. 1), and the digests were used to transform a yeast strain carrying the wild-type *UPF1* gene. The frequency of conversion of the mutation on the plasmid to wild type was used as a measure of the distance of a mutation from a given restriction site. All of the dominant mutations resulted in a low frequency of conversion following *Kpn*I digestion and a high frequency of conversion following *Bgl*II digestion, which indicated that the mutations were near the 3′-proximal *Bgl*II site. The DNA sequence of each plasmid was determined starting from the 3′ end of the *UPF1* ORF.

Seven of the plasmids contained a single base substitution in the region between the 3′ end of the gene and the *Bsu*36I site at position +1191. Each of these plasmids was codigested with *Bsu*36I and *Bam*HI, and the 2.4-kb fragment containing the mutation was used to replace the wild-type *Bsu*36I-*Bam*HI fragment in YEpPL29. The resulting plasmids, which contain only one base change in the *UPF1* coding region, were used to transform PLY22. All transformants were His^+ at 37°C, indicating that the single substitution was sufficient to confer a dominant phenotype.

The strains used to assay the strength of suppression of the dominant alleles were constructed as follows. PLY180 was transformed with YEp13 or YEpPL55 to produce strains carrying a single copy or multiple copies of *UPF1*$^+$, respectively. Each of these strains was then transformed with plasmids carrying the *UPF1-D* allele in either YCp50 or EC402 to produce strains that carry either a single copy or multiple copies of the dominant allele.

The recessive *upf1-2* mutation was cloned by the method of a gap repair (45). Strain PLY36 (Table 1) was transformed with *Bam*HI-digested YCpPL23 plasmid DNA. Temperature-independent transformants were identified and cloned in *E. coli*. The DNA sequence was determined starting at the 5′ end of the *UPF1* ORF.

Nucleotide sequence accession number. The GenBank accession number for the *UPF1* sequence is M76659.

RESULTS

Isolation of the *UPF1* gene. The *UPF1* gene was cloned by screening wild-type yeast genomic DNA fragments in a YCp50 library (52) for those that complemented the recessive mutation *upf1-2*. In strains that carry *his4-38*, *SUF1-1*, and *upf1-2*, complementation by a wild-type gene was expected to result in lack of growth at 37°C on medium lacking histidine. Strain PLY36 (Table 1) was transformed to a Ura^+ phenotype with library DNA, and the resulting colonies were tested for growth at 37°C. Among 10,000 Ura^+ transformants screened, two were His^- at 37°C. In both cases, loss of the plasmid restored growth at 37°C. Restriction maps of the plasmids rescued from these strains (YCpPL23 and YCpPL24) indicated that the two yeast DNA inserts share a common region but are in opposite orientation in the vector (Fig. 1A). Retransformation of strain PLY36 with either YCpPL23 or YCpPL24 yielded only Upf^+ transformants. Verification that these plasmids carried the *UPF1* gene was obtained by demonstrating that the cloned inserts recombine into the yeast genome at a site that is genetically linked to the *upf1-2* mutation (Materials and Methods).

The smallest subclone able to complement *upf1-2* was a 4.2-kb *Bam*HI-*Eco*RI fragment that was inserted into YCp50 to produce YCpPL63 (Fig. 1A). A five-nucleotide insertion was constructed in YCpPL63 by digesting the DNA at a

unique *Bst*EII site and by filling in the 5′ overhang with Klenow enzyme prior to religation (Fig. 1C). The resulting plasmid, YCpPL45, was unable to complement *upf1-2*, indicating that a region essential to *UPF1* function had been disrupted. Plasmid YCpPL44 contains a similar disruption at a unique *Kpn*I site and is also unable to complement *upf1-2*.

The *upf1* locus maps on chromosome 13. The 2μm mapping method of Falco and Botstein (15) was used to assign *UPF1* to a chromosome. One diploid strain constructed as described in Materials and Methods showed a moderate rate of homozygosis of *lys7*. Among 90 Ura$^-$ colonies, 23 were Lys$^-$. *lys7* is located 25 centimorgans from the centromere on the right arm of chromosome 13 (40). A second diploid strain exhibited a high rate of homozygosis of *rna1-1*, which confers temperature-sensitive growth. Among 129 Ura$^-$ colonies, 127 were temperature sensitive. *rna1* is located at the distal tip of the right arm of chromosome 13. These results suggested that *upf1* is located between *lys7* and *rna1*.

The map position of *upf1* was confirmed by tetrad analysis, which established genetic linkage between *lys7* and a copy of the *URA3* gene integrated at the *upf1* locus. In a cross between strain PLY89 and PLY56-15B (Table 1), the *lys7-URA3* map interval was 30 centimorgans (53 parental ditype, 1 nonparental ditype, 67 tetratype). In addition, *URA3* was not centromere linked. Thus, the most likely gene order is *CEN13-lys7-upf1-rna1*. The omnipotent nonsense suppressor locus *sup113* also maps in the *lys7-rna1* interval 28 centimorgans from *lys7* (43, 44), but we have not analyzed crosses between *upf1* and *sup113* to prove that they are allelic.

DNA sequence analysis. Nucleotide sequence data were obtained for the region of YCpPL63 extending from the *Eco*RI site to about 500 nucleotides from the *Bam*HI site (Fig. 2). This region includes a 2,913-bp ORF (ORF1; Fig. 1B) that could potentially code for a 109-kDa product. The *Kpn*I and *Bst*EII sites used to construct disruptions that eliminate *UPF1* function are both located in ORF1. ORF1 is therefore equivalent to the *UPF1* gene. A pyrimidine-rich sequence followed by CAAG is present upstream of ORF1. This motif often precedes the translational start of yeast genes (11, 39). The codon bias calculated from the derived ORF1 amino acid sequence is 0.21, indicating that the codon usage for *UPF1* is typical of the random usage characteristic of yeast genes that are expressed at a low level (2).

Significant similarity was found between the amino acid sequences derived from *UPF1* and the amino acid sequences derived from the yeast *SEN1* gene (8) and the mouse *Mov-10* gene (41). Three blocks of conserved sequences were identified (regions I, II, and III; Fig. 3).

Region I, which extends from residues 426 to 439 in UPF1, contains a consensus sequence (G-x-x-x-x-G-K-S/T) for one element found in proteins that bind and hydrolyze ATP or GTP (Fig. 4A) (9). The similarity between UPF1, SEN1, and Mov-10 in region I extends to residues other than those defined by the consensus. A second element required for NTP binding and hydrolysis (D-x-x-G) is present in UPF1 at position 519 (which lies outside region I, II, or III) and is also found in the SEN1 and Mov-10 sequences. A third element (N-K-x-D), which is specific to GTP-binding proteins, is present in the UPF1 sequence at position 545 (which also lies outside region I, II, or III). However, the 22 amino acid residues in UPF1 located between the second and third elements of region I are fewer than the 40 to 80 residues typically seen in other GTP-binding proteins. Imperfect matches with the N-K-x-D motif are found in SEN1 (x-K-x-D) and Mov-10 (N-x-x-D).

Region II consists of 15 amino acid residues (Fig. 4B). Eleven are conserved between UPF1 and SEN1, and nine are conserved between UPF1 and Mov-10. In all three proteins, region II is located between the first and second elements for nucleotide binding. Region III, the longest region of structural similarity (Fig. 3), includes amino acid residues 537 to 842 of UPF1 and is 36% identical to residues 1433 to 1742 in SEN1 and 32% identical to residues 622 to 950 in Mov-10.

The amino terminus of UPF1 contains a cysteine-rich region that can be organized to form potential zinc-binding sites (3, 62). SEN1 and Mov-10 lack this region. Amino acids 62 to 84 contain six cysteine residues in an arrangement similar to that found in several yeast proteins, including the GAL4 protein, which has been shown to bind zinc (Fig. 4C) (24). A second potential zinc-binding region, Cys-X_2-Cys-X_{22}-Cys-X_3-Cys, begins at residue 122. The dipeptides Ser-Pro and Thr-Pro, which are repeated in the region flanking zinc finger domains of several proteins (61), are repeated three times upstream of the UPF1 cysteine-rich region.

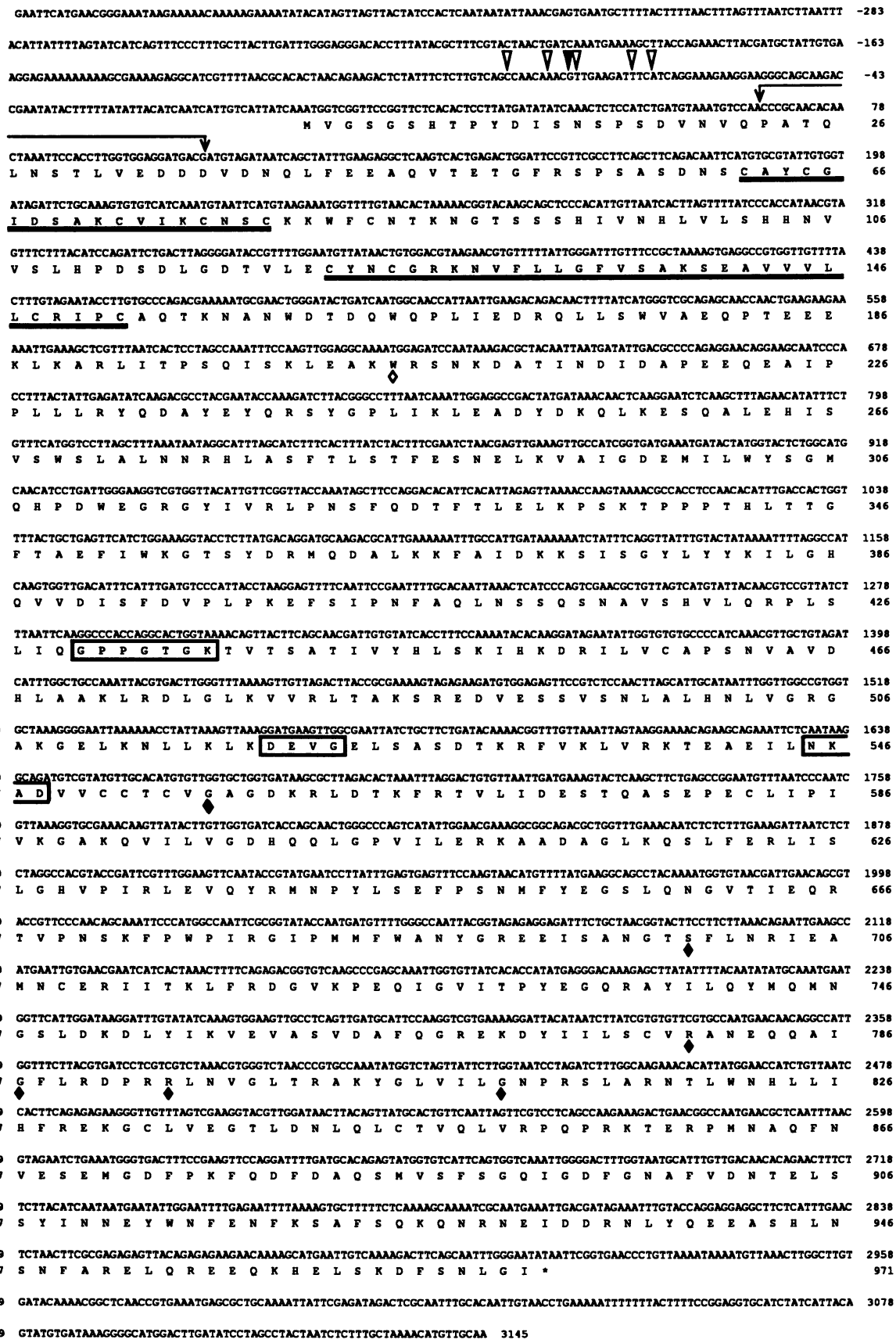
Analysis of the *UPF1* transcript. A probe derived from the internal *Bgl*II fragment of ORF1 (Fig. 1) hybridizes to a single 3.1-kb band on a Northern blot of total RNA (Fig. 5A). The observed transcript length is similar to that predicted from the known length of ORF1. A 20-fold increase in band intensity was observed when RNA was prepared from a strain containing *UPF1* on the multicopy plasmid YEpPL29.

Several features of the DNA sequence prompted us to determine the site of mRNA transcription initiation. Analysis of the sequence revealed a second ORF, designated ORF2 (Fig. 1B), that extends in an alternate frame from ORF1 starting at nucleotide −203 and ending at +30. The potential start codons of ORF1 and ORF2 are both preceded by the sequence ATCAA. In addition, the sequence TAC TAAC, the consensus for lariat formation during mRNA splicing in yeast cells, is located just upstream of the start of ORF2 at nucleotide −217.

A 40-mer complementary to nucleotides +67 to +106 of the ORF1 sense strand (Fig. 2) was annealed to both total RNA and poly(A)$^+$ RNA and extended by reverse transcriptase. The lengths of the extended products indicate a major transcription initiation site at nucleotide −80 (Fig. 5B). Several closely spaced minor start sites were also detected. This result indicates that ORF2 is probably not included in the transcript.

S1 nuclease protection assays were performed by using a 301-bp oligomer constructed by extending the oligomer used for primer extension analysis to the *Hin*dIII site at nucleotide −194. The pattern of protection from S1 nuclease was similar to the pattern of fragments obtained by primer extension (Fig. 5C). In addition, the lengths of the major bands were the same. Except for the possibility of a very short intron whose presence might not be detected in the S1 protection assay, our results suggest that *UPF1* is devoid of intervening sequences.

The *UPF1* product is not essential for vegetative growth. To determine whether *UPF1* is required for growth, a diploid strain heterozygous for *UPF1* and the deletion *upf1-Δ2* (Materials and Methods) was sporulated, and tetrads were analyzed. Each tetrad contained four viable spores that produced two Ura$^+$ and two Ura$^-$ colonies. Southern analysis confirmed that the two Ura$^-$ colonies carried the wild-type *UPF1* allele while the two Ura$^+$ colonies carried *upf1-Δ2*. In addition, Southern hybridization was performed by using genomic yeast DNA digested separately with seven different enzymes that cut outside the region complementary



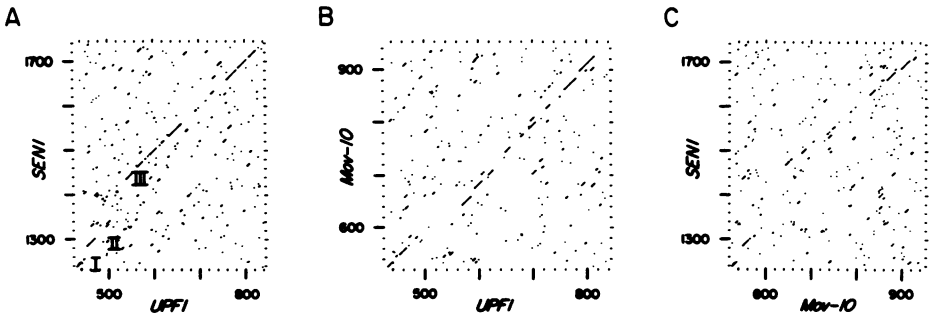


FIG. 3. Amino acid sequence comparison of UPF1, SEN1, and Mov-10. Sequences were analyzed by using the COMPARE and DOTPLOT programs (10) with a stringency of 6 in a window of eight amino acids. Regions I, II, and III correspond to UPF1 amino acid residues 426 to 439, 452 to 466, and 537 to 842, respectively. Amino acid residues are numbered on each axis.

to a *UPF1* DNA probe. We never detected more than a single band in each lane (data not shown). These results indicate that *UPF1* is probably a single copy gene that is not required for vegetative growth. However, we cannot rule out the possible existence of a gene that performs the same function as *UPF1* but fails to hybridize to a *UPF1* probe.

***UPF1* deletions confer omnipotent suppression.** Haploid strains carrying *his4-38*, *suf1*$^+$, and *upf1-Δ2* were His$^-$ at all temperatures, indicating that the *upf1* deletion fails to suppress *his4-38*. By contrast, a haploid strain carrying *his4-38*, *SUF1-1*, and *upf1-Δ2* was His$^+$ at both 30 and 37°C. The *upf1-Δ2* deletion therefore confers the same phenotype as do point mutations such as *upf1-2* (see below). These results prompted us to test other nonsense and frameshift mutations for their ability to be suppressed by *upf1* loss-of-function mutations (Table 2). We identified a number of suppressible mutations, including one UAA (*leu2-1*), two UAG (*tyr7-1*, *met8-1*), and two UGA (*leu2-2*, *his4-166*) nonsense mutations as well as the frameshift mutation *leu2-3*. Mutations in *UPF1* have been shown to stabilize the *leu2-1* and *leu2-3* transcripts, resulting in a correspondingly higher steady-state level of these mRNAs (29). Stabilization of the *leu2-2* and *his4-166* mRNAs by *UPF1* mutations has also been observed (data not shown). The effect of *UPF1* on *tyr7-1* and *met8-1* mRNAs has not yet been determined.

The *upf1-Δ2* allele that was used in the suppression tests described above contains a deletion of the *upf1* coding sequence plus several hundred nucleotides of 5′ and 3′ flanking sequences. To eliminate the possibility that deletion of a flanking region was responsible for suppression, these tests were repeated with *upf1-Δ3* in which only part of the coding sequence is deleted and flanking sequences remain intact (Fig. 1D). Identical results were obtained.

Dominant-negative mutations map in region III of the UPF1 protein. The original *UPF1* mutations isolated by in vivo selection were recessive (7). To continue the genetic analysis of *UPF1*, dominant-negative alleles were sought (see Materials and Methods). By isolating and mapping such mutations, we reasoned that it might be possible to define a functional domain and correlate its location with the various motifs identified in the DNA sequence (21).

Fifteen independent dominant mutants were analyzed (Table 3). DNA sequence analysis revealed that in seven of the mutants, a single base change was sufficient to confer the dominant phenotype. These changes cause amino acid substitutions at six positions clustered in region III (Fig. 2; Table 3). When the *UPF1*, *SEN1*, and *Mov-10* sequences were simultaneously aligned (Fig. 6), it was found that six of the seven mutations produced an amino acid substitution at a position that is conserved in all three proteins. The exceptional mutation, *UPF1-D1*, caused a substitution at Gly-556, which is conserved between *UPF1* and *SEN1* only. Even among alleles that contained multiple base changes, at least one change always produced a substitution in a conserved residue within region III. For comparison, we also determined the DNA sequence of one of the original recessive mutations, *upf1-2* (7, 29). Unlike the dominant mutations, *upf1-2* resides outside of region III at nucleotide +615 (Fig. 2). The mutation changes the UGG Trp-205 codon to a UGA nonsense codon.

When *UPF1* is deleted from strain PLY180, *his4-38* mRNA is stabilized, leading to an increase in its steady-state level (29). Similarly, when plasmids carrying the dominant-negative alleles were introduced into PLY180, the steady-state level of *his4-38* mRNA increased (Table 3). Since the dominant mutations were phenotypically identical to recessive loss-of-function alleles, we inferred that the dominant mutations might act in a negative manner by interfering with wild-type *UPF1* function. To test this model, we estimated the extent of wild-type *UPF1* function by assaying for suppression, using strains in which the relative dosages of mutant and wild-type *UPF1* genes were varied (Table 3).

The mutations *UPF1-D1* (Gly-556→Asp), *UPF1-D2* (Ser-699→Phe), *UPF1-D4* (Arg-779→Cys), *UPF1-D6* (Arg-794→Cys), and *UPF1-D7* (Gly-810→Arg) were placed on both single-copy and multicopy plasmids and analyzed in strains that carry a single copy of the wild-type *UPF1* gene. In general, suppression conferred by each of these mutations

FIG. 2. Sequence of the *UPF1* gene and the corresponding amino acid sequence. The closed arrowhead indicates the major transcriptional start site; the open arrowheads indicate the minor start sites. Translation is assumed to initiate at the first AUG within the transcript; numbering of the amino acid sequence begins at the corresponding methionine residue. The sequence of the oligonucleotide used for primer extension analysis is bracketed by a thin horizontal line. The cysteine-rich elements are underlined, and the three elements matching the GTP-binding consensus are boxed. Sites of dominant mutations are marked with closed diamonds. The site of the recessive *upf1-2* mutation is marked with an open diamond.

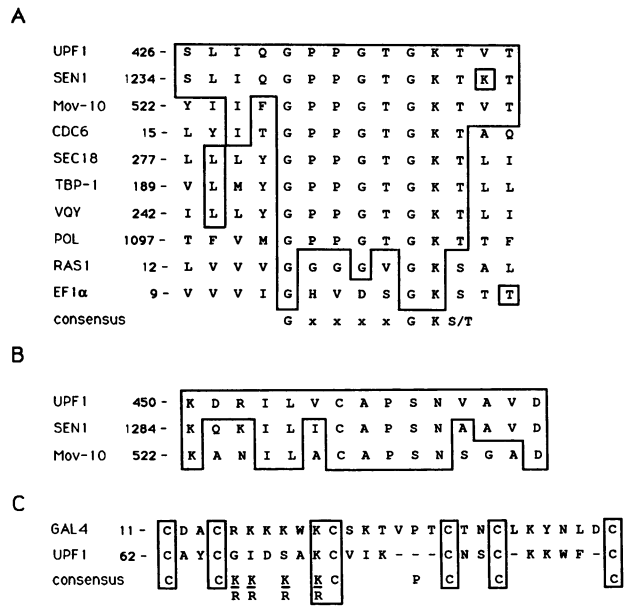


FIG. 4. Potential functional domains in UPF1. (A) The region of UPF1 containing a match to the first element in the NTP-binding consensus (region I; Fig. 3A) (9) aligned with the corresponding element in several other genes. Sources: CDC6, Lisziewicz et al. (31); SEC18, Eakle et al. (14); TBP-1, Nelbock et al. (42); VQY, Koller and Brownstein (28); POL, Snijder et al. (58); RAS1, Powers et al. (50); EF1α, Cottrelle et al. (6). (B) Comparison of region II (Fig. 3A) in UPF1, SEN1, and Mov-10. (C) Part of the cysteine-rich region of UPF1 compared with the region of GAL4 that codes for a zinc cluster (46). The residues in the consensus sequence are conserved in several other *S. cerevisiae* transcriptional activators, including those encoded by *PPR1* (25), *AGR2* (37), *MAL63* (26), *LEU3* (17), *UGA3* (1), and *HAP1* (49). HAP1 has a histidine residue at the position of the sixth cysteine in the consensus; all other residues are conserved.

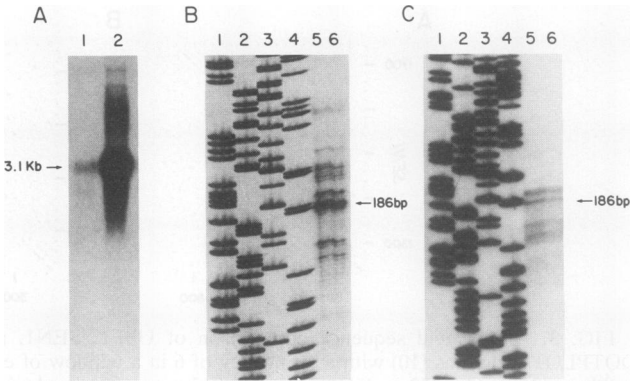


FIG. 5. Analysis of the *UPF1* transcript. (A) Northern analysis of total RNA from strain PLY22 (lane 1) and PLY22 containing YEpPL29 (lane 2). YEpPL29 is a multicopy plasmid that carries the wild-type *UPF1* gene. The filter was hybridized to a radiolabeled probe derived from the internal *Bgl*II fragment of *UPF1* (Fig. 1A). (B) Primer extension analysis of the *UPF1* transcript. RNA was isolated from PLY172 transformed with YEpPL55, a multicopy plasmid that carries the wild-type *UPF1* gene (29). Primer was annealed to 3 μg of poly(A)$^+$ RNA (lane 5) or 55 μg of total RNA (lane 6). The sequencing reaction run on lanes 1 to 4 was used to determine the sizes of the extended oligonucleotides. The oligonucleotide corresponding to the primary transcription start site is 186 nucleotides in length and is indicated by an arrow. Identical results were obtained by using RNA derived from a strain containing a single genomic copy of *UPF1* (data not shown). (C) S1 nuclease protection analysis of the *UPF1* transcript. In this assay, 500,000 cpm (lane 5) or 200,000 cpm (lane 6) of the labeled 301-nucleotide fragment described in Materials and Methods was annealed to 5 μg of poly(A)$^+$ RNA. The source of the RNA is the same as panel B. The band corresponding to a 186-nucleotide protected fragment is indicated with an arrow.

was more efficient when the dominant allele was present in multiple gene copies. When the relative concentration of the wild-type gene product was increased by placing *UPF1* on a 2μm plasmid, the efficiency of suppression was visibly diminished in strains carrying *UPF1-D2*, *UPF1-D4*, *UPF1-D5*, and *UPF1-D6* but was not diminished in strains carrying *UPF1-D1* or *UPF1-D7*. We infer from this analysis that at least some of the mutant proteins interfere with wild-type *UPF1* function. Since the mutations causing the interference all map in region III, this region appears to contain an important functional domain.

Use of antibiotic sensitivity to monitor translation in Upf$^-$ strains. Since *UPF1* could have a primary function in mRNA turnover or in translation, we used antibiotics that interact with ribosomes to monitor translation in vivo. Strains carrying the *upf1-Δ2* null allele were examined for their ability to grow in the presence of two antibiotics, paromomycin and cycloheximide.

Paromomycin increases the translational error rate during elongation in yeast cells, and mutations that affect translational fidelity often confer increased sensitivity to this drug (35, 53, 57, 60). We find that strains carrying a wild-type *UPF1* gene grow at the same rate as do strains carrying *upf1-Δ2* in the presence of paromomycin (Fig. 7A and B). This result is consistent with the results of previous studies (29) suggesting that *UPF1* does not cause a change in the intrinsic rate of translational error during elongation.

Compared with strains carrying the wild-type *UPF1* gene, strains that carry *upf1-Δ2* exhibit increased sensitivity to cycloheximide, an antibiotic that inhibits translational elongation by binding to the 60S ribosomal subunit (Fig. 7C and D). One possible interpretation is that the *UPF1* gene product affects ribosome structure either directly or indirectly.

Mutations in other genes that confer a Upf$^-$ phenotype. Mutations that confer a Upf$^-$ phenotype (growth in the absence of histidine at 37°C in strains carrying *his4-38* and *SUF1-1*) were shown previously to map at two unlinked loci, *UPF1* and *UPF2* (7). We used the same selection scheme in

TABLE 2. Suppression of nonsense and frameshift mutations

Allele	Type of mutation	Suppression[a] by:	
		upf1-Δ2	*upf3-1*
leu2-1	UAA	+	+
ade2-1	UAA	−	NT
tyr7-1	UAG	+	NT
met8-1	UAG	+	NT
leu2-2	UGA	+	+
his4-166	UGA	+	+
lys2-1	UGA	−	NT
leu2-3	+1 G/C	+	NT

[a] Suppression was scored as positive (+) if visible growth was observed within 2 to 7 days at 30°C on medium lacking histidine. Suppression was scored as negative (−) if there was no observable growth after 7 days. NT, not tested.

TABLE 3. Dominant-negative mutations in the *UPF1* gene

Dominant allele[a]	Amino acid change in *UPF1*	Corresponding residue[b] in:		Relative strength of suppression[c]			*his4-38* mRNA accumulation ratio[d]
				Single-copy *UPF1*$^+$		Multicopy *UPF1*$^+$	
				UPF1-D		*UPF1-D*	
		SEN1	*Mov-10*	Single copy	Multicopy	Multicopy	
UPF1-D1 (4)	Gly-556 →Asp	Gly-1453	Val-631	+	++	++	3.1
UPF1-D2 (2)	Ser-699 → Phe	Ser-1596	Ser-794	+/−	++	−	3.0
UPF1-D3 (1)	Arg-779 → Gly	Arg-1678	Arg-883	NT[e]	++	NT	NT
UPF1-D4 (2)	Arg-779 → Cys	Arg-1678	Arg-883	+	++	+/−	3.3
UPF1-D5 (2)	Gly-787 → Asp	Gly-1687	Gly-898	NT	++	+	2.9
UPF1-D6 (3)	Arg-794 → Cys	Arg-1694	Arg-905	−	+	−	NT
UPF1-D7 (1)	Gly-810 → Arg	Gly-1710	Gly-922	−	++	++	2.7

[a] The alleles listed contain only one amino acid substitution. Numbers in parentheses indicate the number of independent times the identical amino acid substitution occurred either by itself or in mutants containing more than one amino acid substitution.

[b] Determined by using the PILEUP program for multiple sequence alignment (Fig. 6) (10).

[c] Determined by comparing growth on medium lacking histidine at 37°C for 3 days, using strains that carry *his4-38* and *SUF1-1* (see Materials and Methods). −, no observable growth; +/−, limited growth was observed; + and ++, the relative extents of vigorous growth (++ is equivalent to growth of a strain isogenic to PLY180 but containing a deletion of *UPF1*).

[d] Determined by measuring steady-state *his4-38* mRNA levels on Northern blots. The numerator is the amount of *his4-38* mRNA in strains containing multiple copies of *UPF1-D* and a single copy of *UPF1*$^+$. The denominator is the amount of *his4-38* mRNA in a strain containing only a single copy of *UPF1*$^+$.

[e] NT, not tested.

this study to isolate additional spontaneous *upf* mutations in strains PLY102-6D and PLY102-10C.

Forty-six independent mutations were analyzed. In diploids heterozygous for each *upf* mutation and the corresponding wild-type allele, 38 were recessive and 8 were dominant. The recessive mutations fell into three complementation groups. Twenty-one mutations in one group failed to complement *upf1-2* and are therefore probably alleles of the *UPF1* gene. Sixteen mutations in a second group failed to complement *upf2-1*, indicating probable allelism with the *UPF2* gene. One isolate [strain PLY102-10C(32A)] contained a mutation that complemented both *upf1-2* and *upf2-1*. The mutation, designated *upf3-1*, segregated in crosses as a single Mendelian factor and was shown to be unlinked to *his4*, *SUF1*, *upf1*, and *upf2*.

Six of the eight dominant mutations were linked to *his4*. The two remaining strains, PLY102-10C(35A) and PLY102-10C(40A), carried single gene mutations that were unlinked to *his4*, *SUF1*, *upf1*, *upf2*, or *upf3* but were closely linked to each other. These mutations therefore define a fourth locus designated *UPF4*.

mRNA accumulation and decay in Upf$^-$ mutants. We screened strains carrying mutations in *upf2*, *upf3*, and *UPF4* by Northern blotting to determine whether they resulted in excess mRNA accumulation compared with Upf$^+$ strains. Our preliminary results with *upf2* and *upf4* mutations were inconclusive and will require further study. However, *upf3-1* was a promising candidate for a second gene affecting mRNA stability (Table 4). *his4-38* mRNA showed an accumulation ratio (*upf3-1*/*UPF3*$^+$) of 3.5. We also examined two other mRNAs, *his4-166* and *leu2-2*, both of which contain a UGA nonsense mutation. These mutations were chosen because we had observed that *upf3-1* suppresses them to histidine and leucine prototrophy, respectively (Table 2). The accumulation ratios were 4.7 for *his4-166* and 2.2 for *leu2-2*. The accumulation ratios for the wild-type *HIS4* and *LEU2* genes were both 1.2, indicating that the *upf3-1* mutation had little effect on accumulation of these mRNAs.

To determine whether *upf3-1* strains exhibit excess accumulation of *his4-38*, *his4-166*, and *leu2-2* mRNAs due to changes in the rate of mRNA turnover, the half-lives of the mRNAs were measured (Table 4; Fig. 8). The half-life ratios

FIG. 6. Alignment of common amino acid sequences for the UPF1, SEN1, and Mov-10 proteins. The sequences begin at residues 425, 1221, and 510 respectively. Relative numbers are shown above each line. The consensus sequence shows residues that are common to at least two of the three proteins. Residues found in only two of the three proteins are typed in lowercase letters; those that are common to all three proteins are shaded and typed in bold uppercase letters. The sites of dominant negative mutations are indicated by arrows. The conserved glycine residue at position 426 that is changed to aspartic acid in the *sen1-1* allele is marked with an asterisk (8).

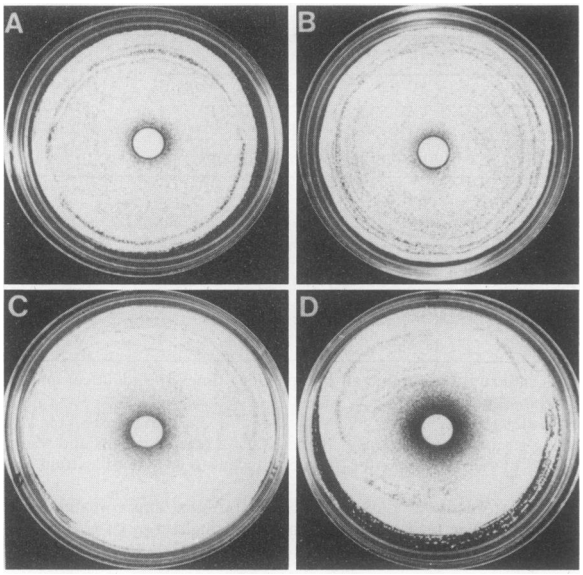


FIG. 7. Drug sensitivity of strains carrying a null allele of *UPF1*. (A) Strain PLY153 transformed with YCpPL53 (*TRP1*$^+$ *UPF1*$^+$). The filter contains 2.5 mg of paromomycin sulfate. (B) Strain PLY153 transformed with YCpMS38 (*TRP1*$^+$). The filter contains 2.5 mg of paromomycin sulfate. (C) Strain PLY153 transformed with YCpPL53 (*TRP1*$^+$ *UPF1*$^+$). The filter contains 0.25 mg of cycloheximide. (D) Strain PLY153 transformed with YCpMS38 (*TRP1*$^+$). The filter contains 0.25 mg of cycloheximide.

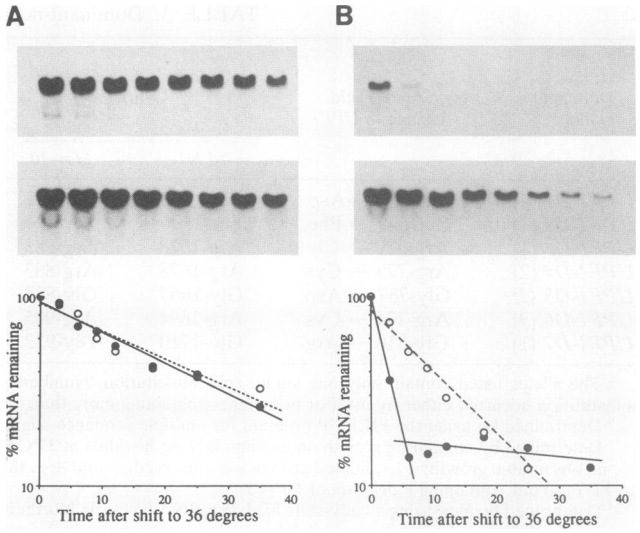


FIG. 8. Effect of *upf3-1* on mRNA decay. The autoradiograms show the mRNA levels detected by Northern analysis at time intervals following the shift of *rpb1-1* strains to 36°C (see Materials and Methods). Decay rates were calculated from plots of percent mRNA remaining versus time. The upper autoradiograms are derived from *UPF3*$^+$ strains; the lower autoradiograms are derived from *upf3-1* strains. Time points were taken at 0, 3, 6, 9, 12, 18, 25, and 35 min following the shift to 36°C. Solid circles and lines represent strains carrying the *UPF3*$^+$ gene; open circles and dashed lines represent strains carrying *upf3-1*. In panel A, the decay rate of *HIS4*$^+$ mRNA is shown for two isogenic strains, BSY-300(+), which carries the *UPF3*$^+$ gene, and BSY-300(−), which carries the *upf3-1* mutation. In panel B, the decay rate of *his4-166* mRNA is shown for two isogenic strains, BSY-201(+), which carries the *UPF3*$^+$ gene, and BSY-201(−), which carries the *upf3-1* mutation. See Materials and Methods for details of strain construction.

measured for *his4-166* mRNA (4.5) and *leu2-2* mRNA (2.2) are close to the accumulation ratios of 4.7 and 2.2, respectively. The half-life ratio of 5.5 measured for *his4-38* mRNA is somewhat larger than the 3.5-fold accumulation ratio. The half-life ratios calculated for the wild-type *HIS4* (Fig. 8) and *LEU2* mRNAs were both close to 1.0, consistent with the observation that the steady-state levels of these mRNAs are unaffected by *upf3-1*. Overall, the data in Table 4 support the conclusion that the *upf3-1* mutation confers increased stability to mRNAs containing a signal for premature translational termination.

DISCUSSION

Nonsense mutations that cause premature translational termination generally result in mRNA destabilization (29, 34). By selecting for improved expression of the yeast *HIS4* gene (7), we have identified mutations that restabilize mRNAs containing a premature translational termination codon (7, 29).

Approximately half of the mutations obtained map in the *UPF1* gene, which has been cloned by screening a yeast DNA library for plasmids that complement the recessive *upf1-2* allele. DNA sequence analysis and 5′-end mapping of the transcript indicate that *UPF1* corresponds to an uninterrupted 2,913-bp ORF that potentially codes for a 109-kDa protein product. By analyzing gene disruptions, we find that loss of *UPF1* function has no detectable effect on growth. Since no additional sequences similar to *UPF1* can be detected in the yeast genome by Southern hybridization, we

TABLE 4. Effect of *upf3-1* on mRNA accumulation and half-life

Strain[a]	mRNA[b]	Accumulation ratio,[c] *upf3-1*/*UPF3*$^+$	Half-life (min)		Half-life ratio, *upf3-1*/*UPF3*$^+$
			upf3-1	*UPF3*$^+$	
BSY-201	*his4-166*	4.7	9.4	2.1	4.5
BSY-202	*his4-38*	3.5	12.0	2.2	5.5
BSY-300	*HIS4*$^+$	1.2	20.0	20.3	1.0
BSY-201	*leu2-2*	2.2	6.5	3.0	2.2
BSY-200	*LEU2*$^+$	1.2	33.0	32.0	1.0

[a] Half-lives and accumulations were measured in isogenic *UPF3*$^+$ and *upf3-1* strains constructed from the listed strains (see Materials and Methods).
[b] The identities of *his4-166* and *leu2-2* in strain BSY-201 were confirmed by showing that they are suppressible by a *SUP4* UGA suppressor (Materials and Methods). The identity of *his4-38*, which generates a premature UAA codon, was confirmed by showing that it is suppressible by the glycine tRNA frameshift suppressor *SUF1-1*.
[c] Determined by measuring steady-state mRNA levels on Northern blots, using the strains listed. mRNA accumulations were normalized to actin mRNA accumulation, which is not affected by *upf3*.

presume that the function of the *UPF1* gene product is not essential for growth.

However, loss of *UPF1* function does confer a detectable phenotype in that UAA, UAG, and UGA nonsense mutations as well as +1 frameshift mutations distributed among several functionally unrelated genes are suppressed. We have shown that suppression of nonsense and frameshift mutations in the *his4* and *leu2* genes is a consequence of mRNA restabilization (29). To our knowledge, this is the first example of a yeast translational suppressor that acts by causing a change in mRNA stability.

Translational suppressors of this type have traditionally been thought to act by causing changes in translational fidelity. Recessive loss-of-function mutations in *UPF1* probably do not alter translational fidelity because they do not enhance read-through of stop codons (29). This conclusion is supported by the observation in this study that deletions of *UPF1* do not confer sensitivity to paramomycin, an antibiotic that induces translational misreading (35, 53, 57, 60). We conclude that *UPF1* may function in some aspect of translation unrelated to fidelity or may function directly in controlling the intrinsic decay rate of mRNA.

Several features of the predicted *UPF1* amino acid sequence provide insight regarding the potential function of the gene product. Near the amino terminus of the protein, there are two potential zinc-binding domains. One of these domains contains six cysteine residues in an arrangement similar to that found in the yeast GAL4 protein, which regulates transcription of genes involved in galactose utilization (24). Using two-dimensional nuclear magnetic resonance spectroscopy, Pan and Coleman (46) have shown that the six cysteines in GAL4 coordinate binding of two zinc atoms to form a DNA-binding domain referred to as a zinc cluster. The *UPF1* gene product resembles GAL4 in that a conserved lysine is present adjacent to the third cysteine, but the spacing between cysteine residues is different. Also, there are other amino acid residues conserved among GAL4-like transcription factors that are not found in the UPF1 protein. The *UPF1* gene product also contains a second cysteine-rich sequence near the amino terminus that could potentially bind zinc.

Despite the similarity between UPF1 and GAL4, we think it unlikely that UPF1 plays a role in transcription, since loss of UPF1 function confers no detectable effect on the transcription of yeast genes (29). Alternatively, we suggest that the potential zinc-binding region of the UPF1 gene product may mediate interaction with RNA, since several zinc-binding proteins are now known to interact with RNA. For example, the yeast methionine initiator tRNA synthetase and the translation initiation factor eIF-2β both contain zinc-binding domains involved in an interaction with tRNA and mRNA, respectively (5, 12). Mutations in or near the zinc-binding domains of these proteins alter or inactivate the functions of these proteins, probably by disrupting the protein-RNA complexes. We are currently examining the possibility that UPF1 interacts with RNA and that the putative zinc-binding domains mediate the interaction.

A segment spanning the central and C-terminal portion of UPF1 shares significant sequence similarity with the yeast SEN1 protein (8) and the mouse Mov-10 protein (9). The *SEN1* gene product is required in tRNA splicing, as evidenced by a mutation, *sen1-1*, that confers temperature-sensitive growth, a deficiency of in vitro tRNA-splicing endonuclease activity, and the accumulation of unspliced precursor tRNAs in vivo (63). The *SEN1* gene product is essential for growth but is probably not a catalytic subunit of the endonuclease (8). The *Mov-10* gene was identified as a site for chromosomal integration of Moloney murine leukemia provirus, but the function of the gene product is unknown (41).

The amino acid segment shared by these proteins includes but is not limited to region I (Fig. 2 to 4), which contains one of three sequence motifs found in other proteins that bind and hydrolyze ATP or GTP (9). Two of the consensus elements found in UPF1 are known to mediate interactions with NTP-bound phosphate. The sequence surrounding the first element is highly conserved among UPF1, SEN1, and Mov-10. A third element that matches the consensus for GTP-specific binding (N-K-x-D) is present in UPF1, but the spacing between this element and the other two elements is shorter than that of any known GTP-binding protein. A data base search uncovered five other genes that contain segments resembling region I (Fig. 4A), but none of these genes share similarity with the *UPF1/SEN1/Mov-10* gene family outside of region I.

Although it has not yet been determined experimentally whether the UPF1 protein binds NTP, we envision a potential role for NTP binding and hydrolysis analogous to that found for many other soluble translation factors. The nucleotide-binding domain could provide a molecular switch that permits UPF1 to cycle between active and inactive conformations in response to the state of translation of mRNA. Alternatively, the energy derived from NTP hydrolysis might play a more direct role in promoting the process of mRNA degradation itself. In either case, additional factors responsible for the recycling of NTP may exist, and it should be possible to identify such factors through further genetic analysis.

Region III of UPF1 is defined not only by sequence similarity to the SEN1 and Mov-10 proteins but also by dominant-negative *UPF1* mutations and the temperature-sensitive *sen1-1* mutation (8), all of which are located at conserved positions within the region (Fig. 2). Some of the dominant-negative mutations are phenotypically sensitive to the relative in vivo concentrations of mutant and wild-type proteins (Table 3), suggesting that they may compete for interaction with some as yet unidentified component of the system (21). We envision, for example, the possibility that functionally defective mutant proteins bind to a nucleic acid (probably mRNA or rRNA) with the same affinity as does the wild-type protein such that overproduction of the mutant protein displaces the wild-type protein, leading to loss of UPF1 function. Both genetic and biochemical experiments to test this model are in progress.

The sequence similarity between UPF1 and SEN1 is even more striking considering that both gene products perform important and perhaps analogous functions in different RNA metabolic pathways. The *SEN1* gene product is required for the activity of an endonuclease essential to tRNA splicing but does not appear to be an endonuclease itself (8). UPF1 is required for the activation of a nuclease activity that degrades mRNAs with premature stop codons. By analogy to *SEN1*, *UPF1* may not encode the nuclease itself. While the region of UPF1 and SEN1 sequence similarity may represent a signature of common function, we know that the two gene products act in separate RNA pathways because strains carrying a deletion of *UPF1* have wild-type levels of tRNA-splicing endonuclease activity (19a). Further experiments will be required to decipher what the common signature means biochemically.

The complexity of the system is further highlighted by the identification of a second gene, *UPF3*, that also appears to

encode a product important in mRNA degradation. Thus far our studies indicate that the recessive *upf3-1* mutation confers the same phenotype as does a loss-of-function mutation in *UPF1*. Considering that *UPF1* is not essential for growth, it is possible that *UPF3* performs the same function as does *UPF1*. However, the results of Southern blotting with *UPF1* probes indicate that the *UPF3* gene is sufficiently dissimilar that cross-hybridization is precluded. Alternatively, *UPF3* could perform a different function in the *UPF1*-mediated degradation pathway or it might act through a different pathway that is independent of *UPF1* function. It should be possible to address these issues by cloning the *UPF3* gene and by analyzing double mutants in which both gene products are functionally absent or altered.

Perhaps the most important question regarding the function of *UPF1* and *UPF3* is why wild-type cells have a specialized system to degrade only those mRNAs unable to participate in full-length translation. One potential answer may come from the observation that *UPF1* affects the turnover rate of *PPR1* mRNA (48a), which codes for a yeast transcriptional activator (32, 33). The *PPR1* mRNA coding region is preceded by a leader sequence containing an upstream ORF (25) whose potential translation might cause the equivalent of premature translational termination. We are currently investigating the possible role of upstream ORF sequences in targeting mRNA for *UPF1*-mediated degradation.

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