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Genetics

Identification of an additional gene required for eukaryotic nonsense mRNA turnover

(*Saccharomyces cerevisiae*/mRNA decay/translation/gene expression)

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ABSTRACT **Loss of function of any one of three *UPF* genes prevents the accelerated decay of nonsense mRNAs in *Saccharomyces cerevisiae*. We report the identification and DNA sequence of *UPF3*, which is present in one nonessential copy on chromosome VII. Upf3 contains three putative nuclear localization signal sequences, suggesting that it may be located in a different compartment than the cytoplasmic Upf1 protein. Epitope-tagged Upf3 (FLAG-Upf3) does not cofractionate with polyribosomes or 80S ribosomal particles. Double disruptions of *UPF1* and *UPF3* affect nonsense mRNA decay in a manner indistinguishable from single disruptions. These results suggest that the Upf proteins perform related functions in a common pathway.**

Several genes have been identified in *Saccharomyces cerevisiae* and *Caenorhabditis elegans* that are required for the accelerated rate of decay that occurs when translation terminates prematurely because of frameshift or nonsense mutation (1–5). Nonsense mRNA decay has been observed in a wide range of eukaryotic organisms and may contribute to the etiology of disease processes in humans. A form of β-thalassemia common in human Mediterranean populations has been shown to result from an amber (UAG) nonsense mutation that reduces β-globin mRNA accumulation and may exacerbate the symptoms of the disease (6). The effects of nonsense mutations that arise in somatic cells could also be exacerbated because rapid decay ensures complete loss of function of a mRNA that might otherwise produce some functional product (3).

In *S. cerevisiae*, mutations in *UPF1, UPF2*, and *UPF3* prevent nonsense mRNA decay (1, 2, 4, 5). They were isolated as allosuppressors of *his4-38*, a +1 frameshift mutation in the *HIS4* gene that causes premature translational termination (7). *UPF1* codes for a 109-kDa protein that contains putative RNA binding domains, suggesting the potential for direct interaction with mRNA (2). *UPF1* behaves like a soluble factor that associates with polyribosomes, but is much less abundant than individual ribosomes (8). *UPF2* codes for a 126-kDa protein that functions in the cytoplasm (4, 5). The *UPF2* gene was identified among clones retrieved by a two-hybrid screen using *UPF1* DNA as bait, indicating that the Upf1 and Upf2 proteins may interact physically (4).

To understand how Upf3 might be related to Upf1 and Upf2, we have cloned the *UPF3* gene, determined the DNA sequence,† and shown that the gene product is not essential for growth. Phenotypic analyses of single and double mutants suggest that both genes may be required in the same pathway.

MATERIALS AND METHODS

Strains, Plasmids, Genetic Techniques, and Media. The following strains of *S. cerevisiae* were used: PLY100 (*MAT***a** *ura3-52 trp1-7 leu2-3,-112*), PLY107 (*MATα his4-38 SUF1-1 ura3-52 leu2 trp1-Δ1 lys1-1*), PLY140 (*MAT***a** *his4-38 SUF1-1 upf3-1 trp1-1*), BSY12 (*MATα his4-38 SUF1-1 upf3-1 ura3-52 trp1-1*), BSY202 (*MATα his4-38 upf3-1 ura3-52 leu2-2 trp1⁻ rpb1-1*), BSY1001 (*MATα trp1-Δ1 his4-38 SUF1-1 upf3-Δ1 ura3-52 lys1-1 leu2*), BSY1044 (*MAT***a** *ura3-52 trp1-7 leu2-3,-112 upf3-Δ2*), BSY1077 (*MAT***a** *ura3-52 leu2-3,-112 trp1-7 upf3-Δ2 esp1-1*), BSY1088 (*MATα ura3-52 leu2-3 ade6*), BSY2103 (*MAT***a** *ura3-52 trp1-7 leu2-3,-112, upf3-Δ2 rpb1-1* [YRpPL81]), BSY2111 (*MATα ura3-52 upf3-Δ2 trp1 leu2-3,-112*), BSY2015 (*MAT***a** *ura3-52 trp1-7 leu2-3,-112 upf3-Δ2* [YRpPL81]), BSY2115 (*MATα ura3-52 upf1-Δ1 upf3-Δ1 trp1 his4-38 leu2-3,-112 lys1-1 rpb1-1*), and BSY2116 (*MAT***a** *ura3-52 upf1-Δ1 upf3-Δ1 trp1 leu2-3,-112 lys1-1 rpb1-1*). Isogenic strains were used that differ only by the absence (−) or presence (+) of extrachromosomal *UPF3*.

The following plasmids were used: YCpBSL1 (*CEN4, URA3*, and a *Sau3*AI–*Sau3*AI insert carrying *UPF3*), YCpBSL2 (*CEN4, URA3*, and a *Cla* I–*Sau3*AI insert carrying *UPF3*), YEpBSL2 (2-μm plasmid origin, *URA3, Bam*HI–*Sal* I insert containing *UPF3*), pBSL1 (pUC19 containing a *Bam*HI–*Sal* I insert carrying *UPF3*), YCpBSL4 (*CEN4, TRP1, Bam*HI–*Sal* I insert containing *UPF3*), YEp[F]BSL2 (2-μm plasmid origin, *URA3*, and a *Bam*HI–*Sal* I insert carrying *FLAG-UPF3*), YCp[F]BSL4 (same as YCpBSL4 except that it carries *FLAG-UPF3* instead of *UPF3*), pBSL12 (pUC18 containing a *Bgl* II–*Hin*fI insert carrying *upf3-Δ1*), pBSL321 (pUC18 containing a *Bgl* II–*Hin*fI insert carrying *upf3-Δ2*), and YRpPL81 (*TRP1, ARS1*, and a *his4-38*, -UAA–*lacZ* gene fusion; see Fig. 4*A*).

Standard genetic methods and media for yeast were used (9, 10). Yeast transformation was by the method of Ito *et al.* (11). Standard bacterial methods and media were used (12).

Nucleic Acid Methods. Yeast chromosomal DNA was prepared by the method of Hoffman and Winston (13). Plasmid DNA isolation and DNA and RNA blotting were performed as described (14). To determine gene copy number, Southern hybridization was performed in 6× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7)/0.5% SDS at 65°C. The filter was washed in 1× SSC/0.1% SDS for 30 min at room temperature and then for 60 min at 65°C. The DNA sequence was determined by the method of Sanger *et al.* (15). mRNA half-lives were determined by the temperature-shift method for blocking transcription in strains carrying *rpb1-1* (1).

Allosuppression. The ability of *upf3* alleles to confer allosuppression of *his4-38* in the presence of the tRNA frameshift suppressor *SUF1-1* has been described (1, 2). Growth was assayed by using synthetic dextrose medium lacking histidine (SD−His) at 37°C in strains carrying *his4-38* and *SUF1-1*. In this assay, *UPF3* confers lack of growth, whereas mutations that cause loss of *UPF3* function allow growth.

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Abbreviations: NLS, nuclear localization signal; ORF, open reading frame.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession number L41153).

Polyribosome Analysis. Polyribosomes were fractionated on 12 ml of 15–50% continuous sucrose gradients (1, 16, 17). RNA was extracted from the fractions and analyzed by Northern blotting using an *Escherichia coli lacZ* probe made from a 2.4-kb *Cla* I–*Bam*HI fragment of the *lacZ* gene. Data are expressed as a percentage of total counts derived by quantitative determination of radioactivity in each band normalized to total counts across all fractions.

Immunodetection. The FLAG epitope (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) was added to the N-terminus of Upf3 by inserting DNA that codes for FLAG after the first ATG in *UPF3* by inverse PCR (18). The reaction was primed with 40- and 45-nucleotide oligomers that contain fused FLAG and 5′ *UPF3* sequences that bracket the translation initiation codon. Anti-FLAG antibodies were from IBI (Kodak). Protein extraction and immunoblotting are as described (19).

RESULTS

Loss of *UPF3* Function Inactivates Nonsense mRNA Decay. HIS4 mRNA has a half-life of 18–20 min (1, 2). The mRNA encoded by *his4-38* contains a +1 frameshift that causes premature termination at a UAA codon (7). In strain BSY202(+), which carries *UPF3*, the his4-38 mRNA half-life is between 2 and 3 min (Fig. 1*A*). In strain BSY202(−), which carries *upf3-1*, the his4-38 mRNA half-life increases to ≈12 min. These results indicate that *UPF3* is required for rapid decay of frameshift and nonsense mRNAs.

We tested other nonsense mutations to see if they were suppressed by *upf3-1* and by *upf3*-null mutations described below. In addition to suppression of *leu2-2* (UGA), *his4-166* (UGA), and *leu2-1* (UAA) (reported previously; ref. 2), we found that *tyr7-1* (UAG) and *met8-1* (UAG) are also suppressed. For all suppressible *his4* and *leu2* alleles, suppression has been shown to result from a change in the half-life and accumulation of mRNA (refs. 1 and 2 and this paper). Some mutations were not suppressed, including *ade2-1* (UAG) and *his4-713* (+1C). The premature stop codon in *his4-713* is near the 3′ end of the *HIS4* coding region (7). Like many other 3′-proximal nonsense mutations (20), *his4-713* does not affect the turnover rate (1).

Analysis of a cross between strains PLY140 and PLY107 shows that the changes in nonsense mRNA accumulation are linked to *upf3-1* (Fig. 1 *B* and *C*). The segregation of *upf3-1* was followed by using allosuppression of his4-38 mRNA in the presence of the tRNA frameshift suppressor *SUF1-1*. In each meiotic tetrad, two spores were His^+ (*upf3-1*) and two were His^- (*UPF3*) at 37°C (Fig. 1*C*). mRNA levels were determined by Northern blotting of total RNA from strains PLY140 and PLY107 and from all four spores of four tetrads. As shown for one tetrad (Fig. 1 *B* and *C*), the two spores that grew at 37°C on SD−His medium had significantly higher levels of mRNA than the two spores that failed to grow. Growth at 37°C cosegregated with increased mRNA abundance in all four tetrads.

The *UPF3* Gene Codes for a 44.9-kDa Protein. The *UPF3* gene was cloned by screening a yeast genomic library for plasmids that complement the recessive *upf3-1* mutation in strain BSY12. A plasmid called YCpBSL1 was rescued from one transformant into *E. coli* by selecting for ampicillin resistance. YCpBSL1 contains a 10-kb yeast genomic DNA insert. A 2.7-kb *Bam*HI–*Sal* I fragment that complements *upf3-1* was used to determine the DNA sequence (Fig. 2 *A* and *C*). A single open reading frame (ORF) of 1161 bp was found that lacked the TACTAAC sequence indicative of an intron. The predicted product is a 44.9-kDa protein of 387 amino acids. Three regions of the amino acid sequence contain basic arginine/lysine-rich stretches that resemble bipartite nuclear localization signal (NLS) sequences found in nucleoplasmin and other proteins that are targeted to the nucleus (21). Two are located near the N terminus at amino acids 15–31 and 58–74 and contain the sequences Lys-Lys-Xaa_{10}-Arg-Gly-Lys-Ser-Lys and Arg-Arg-Xaa_{10}-Asn-Tyr-Lys-Arg-Lys, respectively. A third is located near the C terminus at amino acids 284–300 and contains the sequence Lys-Lys-Xaa_{10}-Pro-Lys-Lys-Lys-Arg.

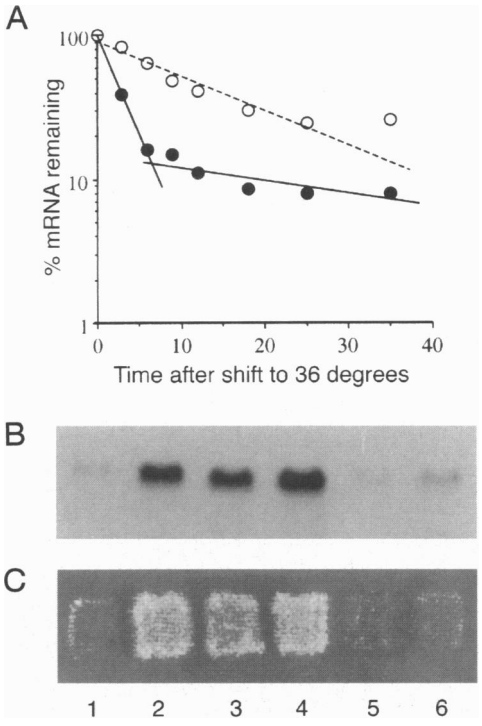

FIG. 1. Effect of *upf3-1* on nonsense mRNA decay. (*A*) The half-life of his4-38 mRNA was determined in strains BSY202(+) (*UPF3*) (●) and BSY202(−) (*upf3-1*) (○). The half-lives were determined by quantitative Northern hybridization analysis at 0, 3, 6, 9, 12, 18, 25, and 35 min following the termination of transcription that occurs when *rpb1-1* strains are shifted to 36°C (1, 2). The decay rate was calculated from the plot of percent RNA remaining vs. time; data points within the first phase of decay were used. It has not been determined whether the second apparent decay phase is of functional significance or whether it is caused by residual transcription due to leakiness of *rpb1-1*. (*B*) Northern blot (2) showing the relative accumulation of his4-38 mRNA in strains PLY107 (lane 1), PLY140 (lane 2), and the four spores of a tetrad derived from a PLY140 × PLY107 cross (lanes 3–6). The blot was probed with *ACT1* DNA (actin) to control for loading differences. Actin mRNA levels were the same in all spores (data not shown). (*C*) Growth rates of the strains analyzed in *B* were compared after 2 days of incubation at 37°C on SD−His medium.

FLAG-UPF3, which codes for an epitope-tagged allele of *UPF3*, was analyzed to estimate the size of the gene product. In the allosuppression assay, growth at 37°C on SD−His/−Ura medium was inhibited to the same extent in strains carrying either *FLAG-UPF3* or *UPF3*, indicating that the FLAG-Upf3 protein is functional. The multicopy plasmids YEp[F]BSL2 and YEpBSL2, containing *FLAG-UPF3* and *UPF3*, respectively, were transformed separately into strain BSY1001. A Western blot (Fig. 3*A*) shows that FLAG-Upf3 was detected in the region of the gel where 45- to 50-kDa proteins migrate. Using differential cellular fractionation, we also detected FLAG-Upf3 in a derivative of strain BSY2111 that carries the *FLAG-UPF3* gene on a single-copy centromeric plasmid (Fig. 3*B*). FLAG-Upf3 was found primarily in the sedimentable fraction after a 20-min centrifugation at 12,130 × *g* in 0.15 M NaCl. FLAG-Upf3 was solubilized from the pellet by extracted in 1 M NaCl. In this procedure, polyribosomes and 80S ribosomal particles are located in the nonsedimentable fraction (not shown) and therefore do not cofractionate with FLAG-Upf3.

***UPF3* Is a Single-Copy, Nonessential Gene on Chromosome VII.** The *UPF3* copy number was determined by genomic Southern blotting with a ^{32}P-labeled 1.59-kb *Nde* I–*Hin*I restriction fragment containing the *UPF3* ORF plus 5′ and 3′

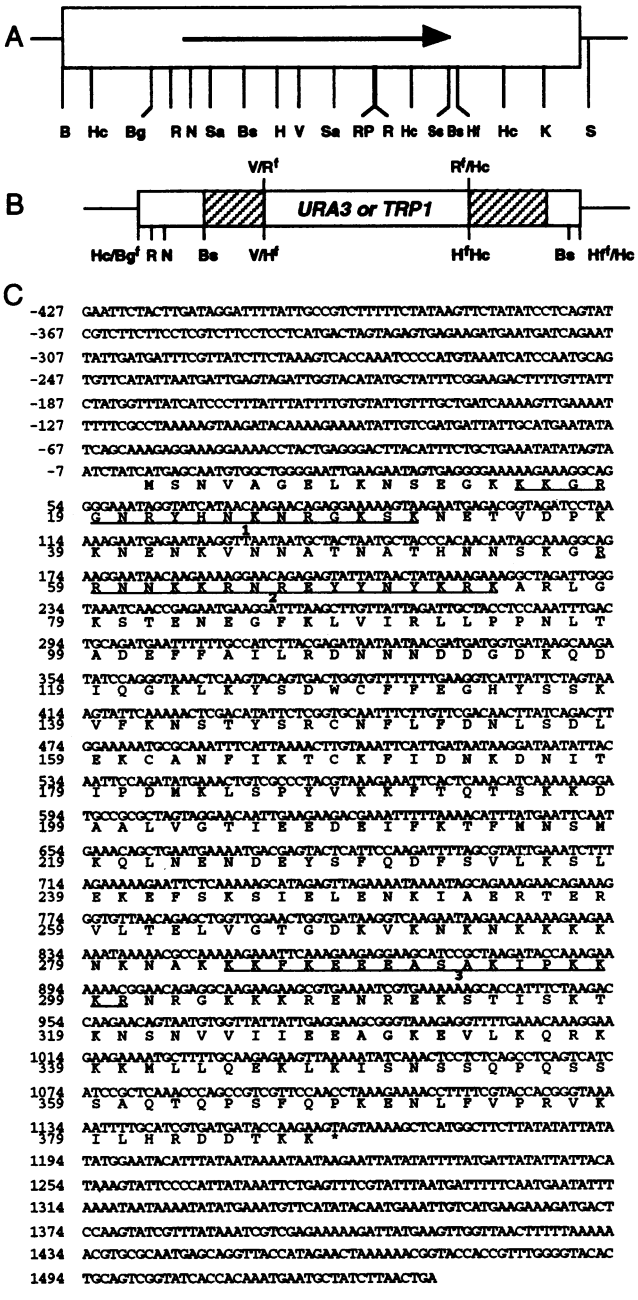

FIG. 2. (*A*) Restriction map of the 2.7-kb *Bam*HI–*Sal* I fragment carrying *UPF3*. The open box denotes yeast genomic DNA inserted in the vector (solid lines). The arrow indicates the position and direction of transcription of the *UPF3* gene. Restriction sites are as follows: B, *Bam*HI; Hc, *Hin*cII; Bg, *Bgl* II; R, *Eco*RI; N, *Nde* I; Ss, *Ssp* I; Bs, *Bsp*HI; H, *Hin*dIII; V, *Eco*RV; P, *Pvu* II, Hf, *Hin*fI; K, *Kpn* I, and S, *Sal* I. (*B*) Structure of *upf3-Δ1* and *upf3-Δ2*, which carry *TRP1* and *URA3* insertions, respectively. A slash denotes a blunt-end ligation. V/R^f and R^f/Hc show the junctions of the *TRP1* insertion. V/H^f and H^f/Hc show the junctions of the *URA3* insertion. Bgf, Hf^f, R^f, and H^f denote the restriction sites corresponding to *Bgl* II, *Hin*fI, *Eco*RI, and *Hin*dIII that were blunt-ended by the Klenow fragment. Hatched areas indicate the locations of the *UPF3* coding sequences. (*C*) DNA sequences of the *UPF3* gene and the corresponding amino acid sequence. The A of the first ATG in the ORF is designated the +1 nucleotide. The three regions that match the consensus for the bipartite NLS sequence are underlined and labeled 1, 2, and 3.

flanking DNA. Using stringent hybridization and washing conditions, we observed two bands when genomic DNA was digested with the restriction enzymes *Hin*dIII or *Pvu* II. For *Bgl* II or *Kpn* I, one band was observed (data not shown). By

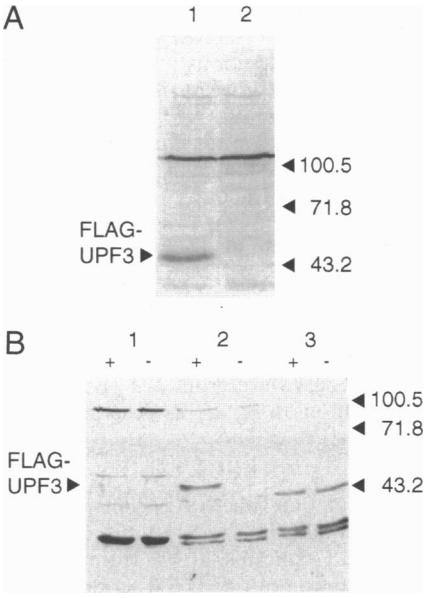

FIG. 3. Immunological detection of FLAG-Upf3. The Upf3 protein was tagged at the N terminus with the FLAG epitope. (*A*) Total protein extracts were prepared from strain BSY1001 separately transformed with the multicopy plasmids YEp[F]BSL2 (*FLAG-UPF3*) and YEpBSL2 (*UPF3*). Equal amounts of the protein extracts were loaded in lane 1 (FLAG-Upf3) and lane 2 (Upf3) and fractionated by SDS/7.5% PAGE. The proteins were analyzed by Western blotting with FLAG M2 monoclonal antibodies (mAbs). (*B*) BSY2111 transformants containing *FLAG-UPF3* on plasmid YCp[F]BSL4 (lanes +) and a control plasmid (YCpBSL4) that lacks *FLAG-UPF3* (lanes −) were fractionated as described (22). Fractions were separated by SDS/10% PAGE and analyzed with FLAG M2 mAbs as follows: supernatant from 12,130 × *g* centrifugation for 20 min in 0.15 M NaCl (lane 1), supernatant from a 1.0 M NaCl extraction of the sedimentable fraction (lane 2), and supernatant from a 2% deoxycholate extraction of the sedimentable fraction (lane 3). Sizes are in kDa.

comparing the position of each band with the position predicted from the restriction map (Fig. 2*A*), the results show that there is only one copy of the *UPF3* gene per haploid genome.

To examine the phenotype of complete loss of function, the *UPF3* gene was disrupted by replacing a 424-bp *Eco*RV–*Hin*cII fragment with either the *TRP1* or the *URA3* genes (*upf3-Δ1* and *upf3-Δ2*, respectively; Fig. 2*B*). The disruptions were introduced by gene replacement into strains BSY1001 and BSY1044. After gene disruption, both strains were viable, with no reduction in growth rate in YPD (yeast extract/peptone/dextrose) medium. When they were grown in SD−His medium, the extent of growth inhibition was similar to strains carrying *upf3-1*, indicating that *upf3-1* confers a null phenotype. Since there are no additional *UPF3* gene copies, the Upf3 protein is not essential for growth.

In tetrads from a cross between strains BSY1001 and PLY140, 21 of 21 segregated 2 Trp^+: 2 Trp^- spores, indicating that the *upf3-Δ1* allele (*upf3::TRP1*) follows Mendelian segregation; 21 of 21 tetrads also segregated 4 His^+: 0 His^- spores at 37°C on SD−His medium (parental ditype). Since no recombination between *upf3-Δ1* and *upf3-1* was detected, the two mutations are genetically linked. This verifies that the original clone is the *UPF3* gene.

The *UPF3* gene was mapped to chromosome VII by probing chromosomes separated on a CHEF (contour-clamped homogeneous electrophoretic field) gel with radioactively labeled YCpBSL2 plasmid DNA. An ordered array of bacteriophage λ–genomic yeast DNA clones (23) was then probed with a labeled 1.7-kb *Nde* I–*Kpn* I fragment from plasmid pBSL1. Results indicate that *upf3* is located between *CEN7* and *spt6* on the right arm of chromosome VII. We analyzed 110 tetrads

Table 1. Accumulation of his4-38 and HIS4 mRNA in single and double mutant strains carrying *upf1-Δ1* and *upf3-Δ1*

Strain	Transcript	Relative abundance* *upf⁻/UPF⁺*
BSY2115 (1^+, 3^-)	his4-38	3.2 ± 0.3
BSY2115 (1^-, 3^+)	his4-38	3.0 ± 0.2
BSY2115 (1^-, 3^-)	his4-38	2.9 ± 0.1
BSY2116 (1^+, 3^-)	HIS4	1.3
BSY2116 (1^-, 3^+)	HIS4	1.2
BSY2116 (1^-, 3^-)	HIS4	1.2

The *UPF1* and *UPF3* genes were introduced into strain BSY2115 (*his4-38 upf1-Δ1 upf3-Δ1*) and BSY2116 (*HIS4 upf1-Δ1 upf3-Δ1*) on multicopy plasmids. The nomenclature (1^+), (1^-), (3^+), and (3^-) denotes whether wild-type or mutant alleles of *UPF1* and *UPF3* are present in each strain.

*The relative abundance of his4-38 or HIS4 mRNA in each strain was determined by measuring mRNA accumulation (2) and comparing it with that observed in the isogenic strain carrying *UPF1* and *UPF3* genes on one plasmid. The mRNAs were detected by Northern blotting with a radiolabeled probe from the *HIS4* coding region. The extent of accumulation was determined by assaying radioactivity with a Betagen blot analyzer. Error bars are based on three repeat experiments. The blots were reprobed with ACT1 (actin) mDNA to standardize the amount of RNA loaded in each lane.

from a three-point cross (BSY1077 × BSY1088) heterozygous for the chromosome VII markers *upf3-Δ2* (scored at Ura^+), *ade6*, and *esp1*. Map distances were as follows: *upf3–ade6*, 9 centimorgans (cM) (90 parental ditype, 20 tetratype); *upf3–esp1*, 21.3 cM (63 parental ditype, 47 tetratype); and *ade6–esp1*, 33 cM (47 parental ditype, 2 nonparental ditype, 61 tetratype). The most likely gene order is *CEN7-ADE6-UPF3-ESP1*.

Accumulation of Nonsense mRNA in *upf1/upf3* Double Mutants. Northern blotting was used to examine his4-38 mRNA accumulation in a haploid double mutant carrying null mutations in both *UPF1* and *UPF3*. Strains were made genetically isogenic by transforming strain BSY2115 (*upf1-Δ1 upf3-Δ1*) with multicopy plasmids carrying the relevant wild-type *UPF* genes (Table 1). Single disruptions of *UPF1* or *UPF3* resulted in a 3-fold increase in his4-38 mRNA accumulation. Nearly identical results were obtained when both genes were simultaneously disrupted. When HIS4 mRNA accumulation was examined in a similar set of isogenic derivatives of strain BSY2116, we found that accumulation was unaffected by *UPF1* or *UPF3*. We conclude that the effects of loss of Upf1 and Upf3 function are nearly identical, nonadditive, and specific to mRNAs containing a premature stop codon.

Decay of Nonsense mRNA Produced from a *his4-lacZ* Gene Fusion. We assessed how loss of Upf3 function affects the decay of a *his4-lacZ* nonsense mRNA in which translation was previously shown to terminate efficiently (1) because of multiple premature stop codons in all three reading frames near the 5′ end of the fused *his4-lacZ* ORF (Fig. 4*A*). In the isogenic strains BSY2015(+)(*UPF3*) and BSY2015(−)(*upf3-Δ2*), both of which carry an integrated copy of the *his4-38*,−UAA/*lacZ* fusion, no β-galactosidase activity was detected by a qualitative assay (24) after 16 hr of development. This shows there is no translational readthrough into the *lacZ* coding region regardless of whether *UPF3* or *upf3-Δ2* is present.

The half-life of the fusion mRNA was measured in strains BSY2103(+) and BSY2103(−) (Fig. 4*B*) (for methods, see the legend to Fig. 1*A*). In BSY2103(+), the half-life of the 3.6-kb fusion mRNA is about 5 min compared with a half-life of 28 min in BSY2103(−). An 8.4-kb transcriptional readthrough product detected on the Northern blots exhibited a similar change in half-life (data not shown). This indicates that loss of *UPF3* function stabilizes the fusion mRNA. Loss of *UPF3* function had no effect on the half-life of a *HIS4-lacZ* fusion that contains an uninterrupted reading frame with no premature stop codons (data not shown).

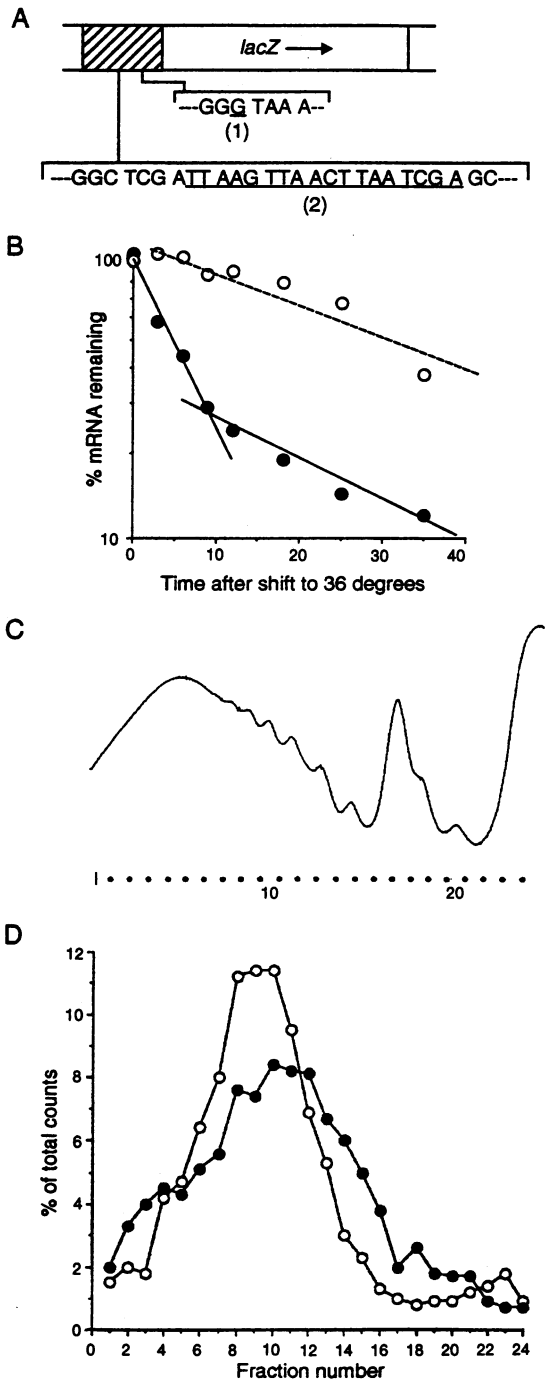

FIG. 4. Behavior of a nonsense mRNA that terminates translation efficiently. (*A*) Structure of *his4-38*,−UAA/lacZ. The *his4* region is hatched. The +1G insertion in *his4-38* is underlined and labeled (1). A linker insertion containing multiple stop codons in each reading frame is underlined and labeled (2). (*B*) The half-life of the fusion mRNA determined in strains BSY2103(+) (*UPF3*) (●) and BSY2103(−) (*upf3-Δ2*) (○). The half-lives were determined as described in Fig. 1*A*. (*C*) The distribution of polyribosome peaks in sucrose gradients was determined by monitoring the A_{260} absorption profile. The A_{260} profiles for the two strains were nearly identical. Only the profile for strain BSY2103(+) is shown. (*D*) Distribution of the 3.6-kb his4-38,−UAA/lacZ fusion mRNA determined by Northern blotting of fractions from the sucrose gradients in *C* using a 2.4-kb *Cla* I–*Bam*HI *lacZ* probe. RNA was extracted from strains BSY2103(+) ($UPF3^+$) (●) and BSY2103(−) (*upf3-Δ2*) (○). As a control, the blots were stripped and reanalyzed with an *ACT1* (actin) probe. Actin mRNA peaked in fractions containing larger polyribosomes than those corresponding to his4-38,−UAA/lacZ (not shown).

Polyribosomes isolated from strain BSY2103(+) and BSY2103(−) were fractionated by centrifugation through 15%–50% sucrose gradients (Fig. 4*C*). The distribution of the 3.6-kb fusion mRNA was determined by Northern blotting across the gradient (Fig. 4*D*). The fusion mRNA is distributed in a similar manner across fractions that contain polyribosomes regardless of the presence or absence of *UPF3* function.

DISCUSSION

The general pathway for mRNA decay in yeast involves a sequence of temporally ordered events, including the shortening of the poly(A) tail, removal of the 5′ cap, and exonucleolytic digestion in the 5′ to 3′ direction (25). The nonsense mRNA decay pathway shares common steps but has the unusual feature that the temporal requirement for poly(A) shortening is bypassed and the 5′ decapping reaction occurs in the presence of a long poly(A) tail (26). The Upf1, Upf2, and Upf3 proteins may in some way contribute, either directly or indirectly, to the decoupling of poly(A) tail structure from the remaining steps in the general decay pathway.

We recently established that Upf1 is cytoplasmic and is associated with actively translating polyribosomes (8). The Upf1 sequence contains several signature motifs that give clues to its function, including a cysteine-rich region that may bind zinc and an NTP-binding/RNA helicase-like domain, suggesting a potential for direct interaction with RNA (2). Since the Upf2 protein physically interacts with Upf1 (4), it appears likely that these two proteins are part of a complex that associates with polyribosomes. Upf2 has been reported to contain a bipartite NLS sequence, but overexpression of a UPF2 peptide fragment has been found to inhibit nonsense mRNA decay only when localized to the cytoplasm, indicating that at least one function of Upf2 is executed in the cytoplasm (4). This does not preclude the possibility that Upf2 resides in both the nucleus and the cytoplasm.

Mutations in *UPF3* have phenotypes similar to mutations in *UPF1* and *UPF2*. They suppress frameshift and nonsense mutations in a variety of genes and have similar effects on nonsense mRNA accumulation and decay (refs. 1, 2, 4, and 5 and this paper). We examined the translation and stability of a *his4-lacZ* fusion that produces a nonsense mRNA that terminates translation efficiently at sites upstream of the *lacZ* coding region. Upf1 (1) and Upf3 both promote decay of this nonsense mRNA, indicating that the increase in decay rate does not correlate with the extent of readthrough past a premature stop codon. Strains that are double null for *UPF1-UPF2* (5) or *UPF1-UPF3* have nonadditive effects on the accumulation of nonsense mRNA. Although other interpretations are possible, the most likely explanation of these phenotypes is that the products of *UPF* genes act in a common pathway leading to accelerated mRNA decay.

Like Upf2, the Upf3 polypeptide contains lysine/arginine-rich sequences that resemble the bipartite NLS sequence known to target proteins to the nucleus. Of all known proteins containing a bipartite NLS, 95% are targeted to the nucleus, whereas most of the remainder are secreted outside the cell or targeted to other organelles (27). Given that nuclear transport of mRNA and nonsense mRNA decay may be coupled, as proposed for animal cells (28), the functional significance of NLSs in *UPF2* and *UPF3* needs to be examined further. The finding that FLAG-Upf3 can be separated from polyribosomes and 80S ribosomal particles by differential cellular fractionation provides an additional incentive to determine the cellular location of Upf3. Unfortunately, the FLAG epitope proved unsuitable for immunolocalization. The intensity of background fluorescence made it impossible to distinguish the FLAG-Upf3 signal.

None of the known Upf proteins identified in yeast are essential for viability. Also, it was reported that mutations in the *smg* genes in *C. elegans*, whose products are required in nonsense mRNA decay in this organism, have some effects on development but are not lethal (3). Without knowing the exact functions of any of these genes, it seems likely that the nonsense mRNA decay pathway itself is dispensable for viability. Nonetheless, it may confer some advantages to eukaryotic organisms. It has been suggested that the pathway may serve to minimize the concentration of truncated polypeptides that accumulate through errors in gene expression, thereby reducing the chances that they could act in a deleterious fashion as poison subunits (29). The pathway also appears to control the expression of some natural mRNAs and might serve a second purpose in the regulation of specific genes (1).

Although the general effects of inactivation of the pathway on growth, viability, and development are subtle, it appears likely that nonsense mRNA decay influences the phenotypes of germ-line nonsense mutations found in the human population and may also influence the phenotypes of nonsense mutations that arise in somatic cells. All three known genes required for nonsense mRNA decay in yeast have now been cloned and characterized (refs. 1, 4, and 5 and this paper). Our efforts are currently focused on unraveling the mechanism of decay and identifying the natural mRNA targets, at which point the purpose of this pathway should become more clear.

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1. Leeds, P., Peltz, S. W., Jacobson, A. & Culbertson, M. R. (1991) *Genes Dev.* **5,** 2303–2314.
2. Leeds, P., Wood, J. M., Lee, B.-S. & Culbertson, M. R. (1992) *Mol. Cell. Biol.* **12,** 2165–2177.
3. Pulak, R. & Anderson, P. (1993) *Genes Dev.* **7,** 1885–1897.
4. He, F. & Jacobson, A. (1995) *Genes Dev.* **9,** 437–454.
5. Cui, Y., Hagan, K. W., Zhang, S. & Peltz, S. W. (1995) *Genes Dev.* **9,** 423–436.
6. Baserga, S. J. & Benz, E. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2935–2939.
7. Donahue, T. F., Farabaugh, P. J. & Fink, G. R. (1981) *Science* **212,** 455–457.
8. Atkin, A. L., Altamura, N., Leeds, P. & Culbertson, M. R. (1995) *Mol. Biol. Cell* **6,** 611–625.
9. Sherman, F., Fink, G. R. & Hicks, J. (1982) *Methods in Yeast Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
10. Gaber, R. F. & Culbertson, M. R. (1982) *Genetics* **101,** 345–367.
11. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
12. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
13. Hoffman, C. S. & Winston, F. (1987) *Gene* **57,** 267–272.
14. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
16. Hutchison, H. T., Hartwell, L. H. & McLaughlin, C. S. (1969) *J. Bacteriol.* **99,** 807–814.
17. Sachs, A. B. & Davis, R. W. (1989) *Cell* **58,** 857–867.
18. Hemsley, A., Arnheim, N., Toney, M. D., Cortopassi, G. & Galas, D. J. (1989) *Nucleic Acids Res.* **17,** 6545–6551.
19. Ursic, D. & Culbertson, M. R. (1991) *Mol. Cell. Biol.* **11,** 2629–2640.
20. Peltz, S. W., Brown, A. H. & Jacobson, A. (1993) *Genes Dev.* **7,** 1737–1754.
21. Dingwall, C. & Laskey, R. A. (1991) *Trends Biochem. Sci.* **16,** 478–481.
22. Ursic, D., DeMarini, D. J. & Culbertson, M. R. (1995) *Mol. Gen. Genet.*, in press.
23. Link, A. J. & Olson, M. V. (1991) *Genetics* **127,** 681–698.
24. Breeden, L. & Nasmyth, K. (1985) *Cold Spring Harbor Symp. Quant. Biol.* **50,** 643–650.
25. Muhlrad, D., Decker, C. J. & Parker, R. (1994) *Genes Dev.* **8,** 855–866.
26. Muhlrad, D. & Parker, R. (1994) *Nature (London)* **370,** 578–581.
27. Robbins, J., Dilworth, S. M., Laskey, R. A. & Dingwall, C. (1991) *Cell* **64,** 615–623.
28. Belgrader, P., Cheng, J. & McQuat, L. E. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 482–486.
29. Hodgkin, J., Papp, A., Pulak, R., Ambros, V. & Anderson, P. (1989) *Genetics* **123,** 301–313.