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Genetics

The Upf3 protein is a component of the surveillance complex that monitors both translation and mRNA turnover and affects viral propagation

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ABSTRACT **The nonsense-mediated mRNA decay pathway functions to degrade aberrant mRNAs that contain premature translation termination codons. In *Saccharomyces cerevisiae*, the Upf1, Upf2, and Upf3 proteins have been identified as *trans*-acting factors involved in this pathway. Recent results have demonstrated that the Upf proteins may also be involved in maintaining the fidelity of several aspects of the translation process. Certain mutations in the *UPF1* gene have been shown to affect the efficiency of translation termination at nonsense codons and/or the process of programmed −1 ribosomal frameshifting used by viruses to control their gene expression. Alteration of programmed frameshift efficiencies can affect virus assembly leading to reduced viral titers or elimination of the virus. Here we present evidence that the Upf3 protein also functions to regulate programmed −1 frameshift efficiency. A *upf3*-Δ strain demonstrates increased sensitivity to the antibiotic paromomycin and increased programmed −1 ribosomal frameshift efficiency resulting in loss of the M_1 virus. Based on these observations, we hypothesize that the Upf proteins are part of a surveillance complex that functions to monitor translational fidelity and mRNA turnover.**

Numerous studies have demonstrated that the processes of mRNA turnover and translation are directly linked (1–4). One example that couples these two processes is the fact that premature translation termination promotes rapid turnover of the transcript (1, 2, 4–6). This process has been termed nonsense-mediated mRNA decay (NMD). The NMD pathway has been observed in all eukaryotic systems examined and appears to have evolved as a surveillance mechanism to ensure that transcripts containing premature nonsense codons are rapidly degraded, thus preventing synthesis of incomplete and potentially deleterious proteins.

The NMD pathway has been extensively investigated in the yeast *Saccharomyces cerevisiae* (1, 2, 4, 5). *Trans*-acting factors involved in this process have been identified and characterized. Mutations in the *UPF1*, *UPF2*, and *UPF3* genes were shown to selectively stabilize mRNAs containing early nonsense mutations without affecting the decay rate of most wild-type mRNAs (7–11). Studies using the two-hybrid system demonstrated that the Upf1p, Upf2p, and Upf3p interact and form a complex (10, 12–14). A human gene has been recently identified, *RENT1*, that is highly homologous to the *UPF1* gene. A yeast/human hybrid of these two proteins is partially functional in yeast cells, suggesting that NMD is an evolutionarily conserved pathway (15, 16).

In addition to their role in the NMD pathway, recent results indicate that the Upf proteins may be also involved in modulating certain aspects of the translation process (12, 13, 17). Deletion of any one of the *UPF* genes results in a nonsense suppression phenotype (8, 9). Importantly, mutations in the *UPF1* gene have been isolated that separate its ability to promote NMD from its function in modulating translation termination at nonsense codons (12, 13). A unique allele of the *UPF1* gene was also isolated in a screen for mutations that enhance programmed −1 ribosomal frameshifting (17, 18). Taken together, these results suggest that the Upfp complex may be part of a surveillance complex that functions to monitor the accuracy of several processes in translation and in mRNA decay.

Programmed −1 ribosomal frameshifting, a unique alteration in translational fidelity, is used predominantly by certain RNA viruses to induce elongating ribosomes to shift reading frame in response to specific mRNA signals (19–22). The L-A double-stranded RNA (dsRNA) virus of yeast utilizes a programmed −1 ribosomal frameshift event for the production of a Gag-pol fusion protein. The M_1 satellite virus of L-A encodes a secreted killer toxin, and its dsRNA genome is encapsidated and replicated by using the L-A virus encoded gene products (20). Preservation of the appropriate efficiency of ribosomal frameshifting is critical for maintenance of the yeast M_1 dsRNA virus (23). Changes in the efficiency of programmed −1 ribosomal frameshifting by as little as 2- to 3-fold can promote loss of the M_1 virus. Therefore, the ability of the cell to maintain the M_1 virus can be used as an assay to monitor the ability of cells to preserve the accuracy of the translational reading frame. A screen for cellular mutations that increased the programmed −1 ribosomal frameshift efficiency identified mutations in nine chromosomal *mof* genes (for maintenance of frame; refs. 18, 20, and 23). As described above, characterization of the *mof4-1* mutation demonstrated that it is allelic to *UPF1* (17). Interestingly, although *mof4-1* strains demonstrated increased programmed −1 ribosomal frameshifting efficiency and promoted loss of the M_1 virus, a *upf1*-Δ strain did not demonstrate any of these phenotypes (17). It is possible that mof4-1 represents a gain of function mutation or that it alters the Upfp complex such that it results in an increased efficiency of −1 ribosomal frameshifting.

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Abbreviations: NMD, nonsense-mediated mRNA decay; dsRNA, double-stranded RNA; mof, maintenance of frame; β-gal, β-galactosidase; snRNA, small nuclear RNA.
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The observation that the *mof4-1* allele of the *UPF1* gene affected programmed −1 ribosomal frameshifting and M_1 maintenance suggested that other proteins that are part of the surveillance complex may also be involved in modulating the maintenance of translational reading frame. The results presented here demonstrate that the Upf3 protein is involved in modulating −1 programmed frameshift efficiency. A *upf3*-Δ strain shows an increase in programmed −1 ribosomal frameshifting efficiency and a concomitant lose of the ability to maintain the M_1 virus. Based on these results, the role of Upfp as part of the surveillance complex that modulates translation and mRNA turnover will be discussed.

METHODS

Strains, Plasmids, Media, and General Methods. The yeast strains used in this study are listed in Table 1. Plasmids pF8 and pTI25 were previously described (24) and are shown in Fig. 1. Plasmid pmof4BE carrying the *mof4-1* allele was as described (9). Yeast media, transformations, cytoductions of L-A and M_1 into rho-o strains by using strains 3164 and 3165 as cytoduction donors, β-galactosidase (β-gal) assays and paromomycin sensitivity assays were performed as described (9, 17, 23, 25–27).

Cloning of *UPF3*. The *UPF3* gene was cloned following the strategy used to clone the *UPF2* gene (9). Briefly, a yeast strain harboring the *upf3*-Δ, *his4-38* and *SUF1-1* alleles was transformed with a yeast genomic library and screened for cells carrying a plasmid that could overcome the allosuppressor phenotype of the *upf3*-Δ. Subsequent subcloning and sequence analysis identified the sequence of the *UPF3* gene that was previously reported (11).

Killer Assays, Frameshifting Assays, and Extraction and Analysis of Total Nucleic Acids. The killer assay was carried out as described (23) by replica plating colonies onto 4.7MB plates newly seeded with a lawn of 5 × 47 killer indicator cells. Killer activity was observed as a zone of growth inhibition around the colonies. To quantitate loss of killer activity, colonies that had been identified as killer$^+$ were restreaked for single colonies and the percentage of killer$^-$ colonies were determined. The efficiencies of −1 frameshifting were determined as described (17, 28) by using the 0-frame control (pTI25) and −1 reporter (pF8) plasmids.

The abundance of L-A and M_1 (+) RNAs were monitored by RNA blotting analysis as described (17, 26). RNA abundance of the *lacZ* −1 frameshift reporter mRNA and U3 small nuclear RNA (snRNA) was determined by ribonuclease protection assays essentially as described (29).

RESULTS

A *upf3*-Δ Strain Demonstrates an Increased Efficiency of Programmed −1 Ribosomal Frameshifting. *mof4-1* is a unique allele of the *UPF1* gene that specifically increases programmed −1 ribosomal frameshifting efficiency and promotes loss of the M_1 satellite virus (17). A *upf1*-Δ strain, however, does not demonstrate these phenotypes. We hypothesized that other factors of the putative surveillance complex, including the Upf2 or Upf3 proteins, could also affect programmed −1 ribosomal frameshifting. Therefore, we investigated whether isogenic strains harboring deletions of the *UPF* genes demonstrated increased ribosomal frameshifting efficiencies.

Methods to measure efficiencies of programmed ribosomal frameshifting *in vivo* have been described previously (17, 24, 28). A series of *lacZ* reporter plasmids were used (Fig. 1) in which a translational start codon is followed by the *Escherichia coli lacZ* gene. Plasmid pTI25 serves as the 0-frame control because *lacZ* is in the 0-frame with respect to the translational start site (Fig. 1). In plasmid pF8, an L-A derived programmed −1 ribosomal frameshift signal is cloned into the polylinker and the *lacZ* gene is in the −1 frame with respect to the translational start site (Fig. 1). Therefore, in this construct the *lacZ* gene will be translated only if the ribosome shifts frame

Table 1. Strains used in this study

Strain	Genotype	Ref.
HFY1200	MAT**a** *ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 UPF3*	14
HFY870	MATa *ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 upf1::HIS3 NM2 UPF3*	14
HFY1300	*MATα ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 nmd2::HIS3 UPF3*	14
HFY861	MAT**a** *ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 NMD2 upf3::HIS3*	14
HFY3000	*MATα ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 upf1::URA3 nmd2::HIS3 UPF3*	14
HFY872	MAT**a** *ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 upf1-1::URA3 NMD2 upf3::HIS3*	14
HFY874	MAT**a** *ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 UPF1 nmd2::URA3 upf3::HIS3*	14
HFY883	MAT**a** *ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 upf1::LEU2 nmd2::URA3 upf3::HIS3*	14
HYF870mof4	MAT**a** *ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 upf1::HIS3 NMD2 UPF3 pmof4BE*	This study
HFY872mof4	MAT**a** *ade2-1 his3-11, 15 leu2-3, 112 trp1-1 ura3-1 can1-100 upf1-1::URA3 NMD2 upf3::HIS3 pmof4BE*	This study
3164	MAT**a** *kar1-1 arg1L-AHN M1 K$^+$*	23
3165	*MATα kar1-1 arg1 thr(1,x) L-AHN M1 K$^+$*	18
5X47	MAT**a**/*MATα his1/+trp/+ura3/+K$^-$R$^-$*	23

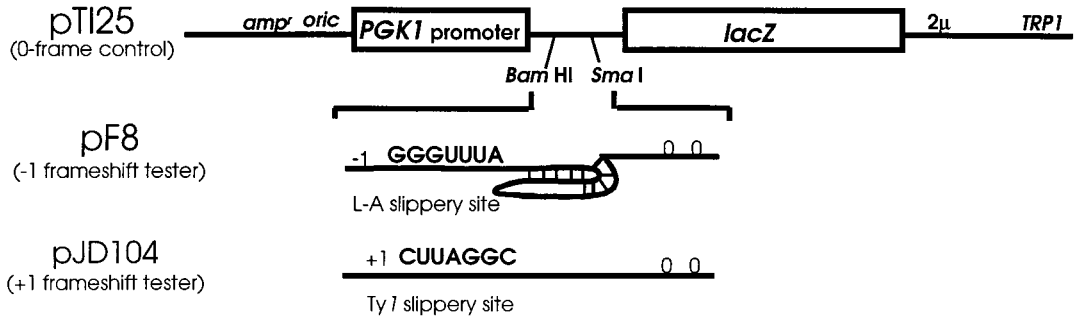

FIG. 1. Schematic diagram of the vectors used to measure programmed −1 ribosomal frameshift efficiencies *in vivo*. Transcription is driven from the *PGK1* promoter and uses the *PGK1* translation initiation codon. In pTI25, the bacterial *lacZ* gene is in the 0-frame with respect to the start site. In plasmid pF8, the *lacZ* gene is positioned 3′ of the L-A virus frameshift signal and in the −1 frame relative to the translation start site.

in the −1 direction. The +1 frameshift reporter plasmid, pJD104 (Fig. 1), contains the *lacZ* gene inserted 3′ of a programmed +1 ribosomal frameshift signal derived from the Ty1 retrotransposable element of yeast. In this construct, the *lacZ* gene will be translated only if the ribosome shifts frame in the +1 direction. The efficiency of −1 ribosomal frameshifting is calculated by determining the ratio of β-gal activities measured in cells harboring the −1 frameshift reporter plasmid, pF8, to those harboring the 0-frame control plasmid, pTI25, and multiplying by 100%. Similarly, the +1 ribosomal frameshift efficiency is calculated based on the pJD104 to pTI25 β-gal ratios.

Frameshift efficiency was measured in isogenic yeast strains harboring deletions of different *UPF* genes (Table 2). The results of these experiments demonstrated that the apparent efficiency of programmed −1 ribosomal frameshifting were slightly greater in *upf1*-Δ and *upf2*-Δ strains than in wild-type cells (Table 2). As discussed below, the small increase in −1 programmed frameshifting was not sufficient to promote loss of the M_1 virus. In contrast, the efficiency of programmed −1 ribosomal frameshifting in *upf3*-Δ cells was 3.4-fold higher than wild-type cells (Table 2) and was sufficient to promote loss of the M_1 virus (see below). None of the *upf*-Δ strains demonstrated a significant increase in the apparent efficiency of programmed +1 ribosomal frameshifting, as measured by the levels of β-gal activity (data not shown). Taken together, these results indicated that, analogous to a *mof4-1* strain, the *upf3*-Δ strain specifically alters −1 ribosomal frameshifting.

The Abundance of the Frameshift Reporter Transcript Is Equivalent in the *upf*-Δ Strains. The −1 frameshift reporter transcripts used in these assays have short protein coding regions 5′ of the frameshift site followed by sequences that code for a reporter protein that is out of frame with the translation initiation site of the 5′ ORF. The apparent changes in ribosomal frameshifting efficiencies could result from changes in the abundance of the LacZ −1 frameshift reporter mRNA that the translational machinery may recognize as a nonsense-containing mRNA (see ref. 17). Deletion of the *UPF* genes could lead to stabilization of the −1 frameshift reporter transcript, resulting in increased synthesis of the β-gal reporter protein. To address whether a *upf3*-Δ strain accumulates the reporter transcript to a greater extent than *upf1*-Δ or *upf2*-Δ strains, the abundance of the LacZ −1 frameshift reporter mRNA was determined by RNase protection analysis. As a loading control, we also determined the abundance of the U3 snRNA. The results indicated that the abundances of the *LacZ* frameshift reporter mRNA, normalized to the U3 snRNA, were equivalent in isogenic wild-type, *upf1*-Δ, *upf2*-Δ, and *upf3*-Δ strains (Fig. 2). Therefore, the increased programmed −1 ribosomal frameshifting efficiency observed in a *upf3*-Δ, when compared with the *upf1*-Δ or *upf2*-Δ strains, was not a consequence of stabilizing the reporter transcript to a greater extent than in the other *upf*-Δ strains. Therefore, a *upf3*-Δ strain also demonstrates a mof phenotype in that it increases the efficiency of −1 ribosomal frameshifting independently of its ability to stabilize nonsense mRNAs.

The M_1 Killer Virus Is Not Maintained in a *upf3*-Δ Strain. Changing the efficiency of −1 ribosomal frameshifting alters the ratio of Gag to Gag-pol proteins available for viral particle

Table 2. Programmed −1 ribosomal frameshifting and M_1 virus maintenance of strains harboring a single deletion of a *UPF* gene

Strain (genotype)	% −1 ribosomal frameshifting*	Killer maintenance†
UPF+ (HFY1200)	2.5	+
upf1-Δ (HFY870)	4.5	+
upf2-Δ (HFY1300)	3.9	+
upf3-Δ (HFY861)	8.4	−

*The −1 ribosomal frameshift efficiency (%) was determined by the ratio of β-gal activity in a strain harboring the −1 ribosomal frameshifting reporter plasmid to the activity in the same strain harboring the 0-frame control plasmid.

†L-AHN and M_1 were introduced into the strains by cytoduction and the maintenance (+) or loss (−) of M_1 dsRNA was analyzed by the killer plate assay and Northern blot analyses as described.

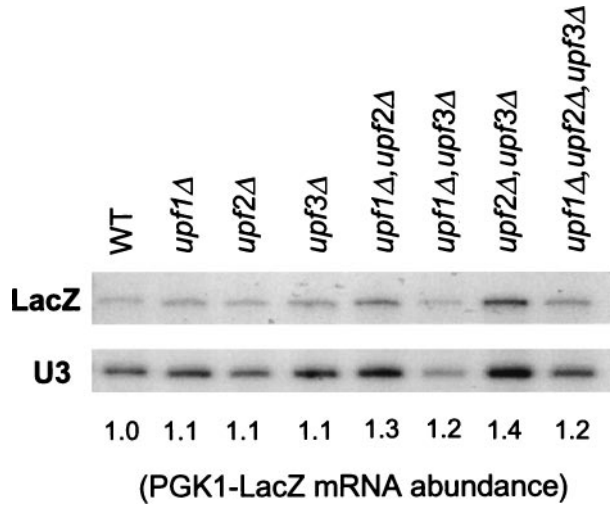

FIG. 2. A *upf3*-Δ strain increases programmed −1 ribosomal frameshifting independently of its ability to promote stabilization of nonsense-containing transcripts. The abundance of the PGK1–LacZ −1 reporter mRNA in the different *upf* deletion strains was determined by RNase protection analysis. The abundance of the U3 snRNA was used as an internal control for loading. The abundance of the reporter transcript in the wild-type strain was taken arbitrarily as 1.0.

assembly, consequently interfering with viral propagation (17, 18, 23, 28). Therefore, we next asked whether a *upf3*-Δ strain was able to maintain the L-A and/or the M_1 satellite virus as an independent assay to monitor changes in the −1 ribosomal frameshift efficency. The L-A and M_1 viruses were introduced by cytoduction into isogenic wild-type, *upf1*-Δ, *upf2*-Δ, and *upf3*-Δ strains, and these cells were grown and replica plated onto a lawn of cells sensitive to the killer toxin. Cells maintaining the M_1 virus secrete the killer toxin, creating a ring of growth inhibition (17, 23, 28). The results of this assay demonstrated that the wild-type, *upf1*-Δ and *upf2*-Δ strains maintained the killer phenotype, whereas the *upf3*-Δ strain lose the ability to maintain the killer phenotype (Fig. 3*A*; Table 2). Consistent with previous results, cells harboring the *mof4-1* allele of the *UPF1* gene were also unable to maintain the killer phenotype (17).

To determine whether lack of the killer phenotype was a consequence of a virus maintenance defect rather than interference with production of the killer toxin, total nucleic acids were extracted from the wild-type, *upf1*-Δ, *upf2*-Δ, and *upf3*-Δ strains and analyzed by Northern blot analysis by using L-A and M_1 (+)-strand RNA-specific probes. The results demonstrated that the 1.8-kb M_1 dsRNA was absent in the *mof4-1* and *upf3*-Δ cells but present in *upf1* and *upf2* mutant and the wild-type strains (Fig. 3*B*). These results support the hypothesis that deleting the *UPF3* gene alters the efficiency of −1 ribosomal frameshifting interfering with the propagation of M_1 satellite virus.

The *upf3*-Δ Strain Demonstrates Increased Sensitivity to Paromomycin. Strains harboring mutations that diminish translational fidelity are hypersensitive to the aminoglycoside antibiotic paromomycin, a drug that is thought to increase the frequency of misreading in yeast (30, 31). Previous results demonstrated that cells harboring the *mof4-1* allele of the *UPF1* gene showed an increased sensitivity to paromomycin relative to the isogenic wild-type strain (17). We determined whether a *upf3*-Δ strain also demonstrates increased sensitivity to this antibiotic. Paromomycin sensitivity was monitored in isogenic wild-type and *upf3*-Δ strains by determining the zone of growth inhibition around a disc containing 1 mg of paromomycin placed onto a lawn of cells. The results demonstrated that, analogous to a *mof4-1* strain, a *upf3*-Δ strain was more sensitive to paromomycin than the isogenic wild-type strain (Fig. 4*A*). Neither the *upf1*-Δ nor *upf2*-Δ strains demonstrate hypersensitivity to paromomycin (ref. 17 and data not shown).

We determined whether increased doses of paromomycin can modulate the −1 frameshifting efficiency of the *upf3*-Δ strain. Isogenic wild-type and *upf3*-Δ strains harboring plasmids pF8 (−1 frameshift reporter construct) or pTI25 (0-frame control), were grown in liquid media in the presence of different concentrations of the drug, and the β-gal activity was determined, normalizing to the number of cells used in the assay. The results indicated that the β-gal activity from *upf3*-Δ cells carrying pF8 (−1 frameshift reporter construct) increased continuously with increased concentrations of paromomycin. However, the β-gal activity was unaffected in wild-type cells containing pF8 or in any of the strains carrying pTI25 (0-frame control construct) (Fig. 4*B*).

The Increased −1 Programmed Frameshifting and Killer Virus Maintenance Defect Phenotypes of *upf3*-Δ and *upf3*-Δ *mof4-1* Strains Are Equivalent. The results described above indicated that a *upf3*-Δ strain has similar phenotypes as *mof4-1* cells. Because the *mof4-1* allele of the *UPF1* gene, but not deletion of the *UPF1* gene, affected programmed −1 ribosomal frameshifting and M_1 maintenance, we hypothesized that the mof4-1p could alter the function of the Upf3p. Thus,

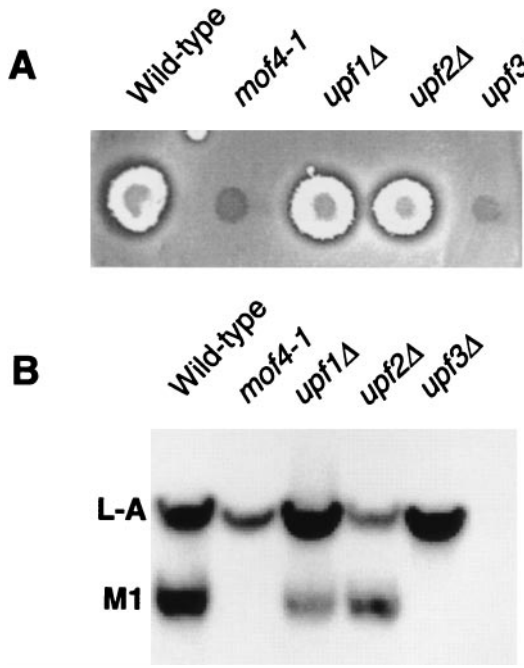

FIG. 3. A *upf3*-Δ strain cannot maintain the M_1 killer virus. (*A*) Killer assay of *upf* mutant strains. Colonies of these strains were grown onto a lawn of cells that are sensitive to the secreted killer toxin produced by the M_1 virus. Killer activity was observed as a zone of growth inhibition around the colonies. (*B*) Total RNAs were isolated from the same strains and analyzed by Northern blotting for the presence of L-A and M_1 viral RNAs.

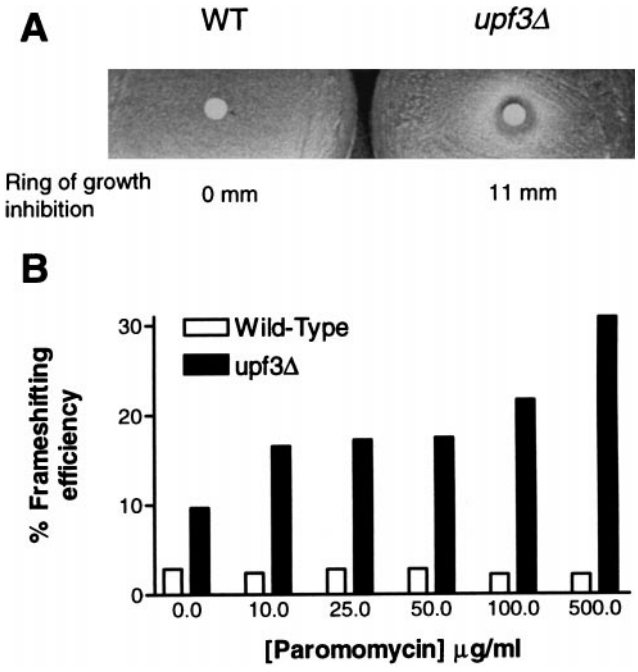

FIG. 4. Effects of paromomycin in a *upf3*-Δ strain. (*A*) A *upf3*-Δ strains shows increased sensitivity to paromomycin. Sensitivity to this drug was determined in isogenic wild-type and *upf3*-Δ strains. Cells were plated in yeast extract/peptone/dextrose (YPD) medium and a disc containing 1 mg of paromomycin was placed on the lawn of cells. The diameter of the zone of growth inhibition was determined after the plates were incubated at 30°C for 2 days. (*B*) Isogenic wild-type and *upf3*-Δ strains were transformed with either −1 frameshift tester or 0-frame control plasmids. Increasing concentrations of paromomycin were added to cells inoculated at 0.1 OD_{600}/ml and grown at 30°C for 4 h. The efficiency of −1 ribosomal frameshifting was calculated by determining the ratio of β-gal activities measured on cells harboring the −1 frameshift reporter plasmid to those harboring the 0-frame control plasmid, grown under the same concentration of paromomycin, and multiplying by 100%.

a *mof4-1 upf3*-Δ strain should have the same programmed −1 frameshifting and killer phenotypes as a *upf3*-Δ strain. We monitored both the programmed −1 ribosomal frameshifting efficiency and virus maintenance phenotypes in isogenic *mof4-1*, *upf3*-Δ, and *mof4-1 upf3*-Δ strains as described above. The programmed −1 ribosomal frameshifting efficiencies observed in *mof4-1*, *upf3*-Δ and *mof4-1 upf3*-Δ strains were equivalent, and all these strains lacked the killer phenotype (Table 3).

The Programmed Frameshifting and Killer Phenotypes of a *upf3*-Δ Allele Are Independent of the Other *upf*-Δ Alleles. The epistatic relationships between *upf1*-Δ, *upf2*-Δ and *upf3*-Δ were examined with regard to both −1 ribosomal frameshifting efficiencies and killer maintenance. Both programmed −1 ribosomal frameshifting and killer phenotypes were monitored as described above in isogenic *UPF*$^+$, *upf1*-Δ *upf2*-Δ, *upf1*-Δ *upf3*-Δ, *upf2*-Δ *upf3*-Δ, and *upf1*-Δ *upf2*-Δ *upf3*-Δ strains. All of the strains harboring the *upf3*-Δ, independent of the presence or absence of the other *UPF* genes, had increased −1 ribosomal frameshifting efficiencies, equivalent to that harboring deletion of the *UPF3* gene (Table 3). Conversely, *upf1*-Δ *UPF3*$^+$, *upf2*-Δ *UPF3*$^+$, and *upf1*-Δ *upf2*-Δ *UPF3*$^+$ strains did not demonstrate an increase in programmed −1 frameshifting efficiencies sufficient to promote loss of the killer phenotype (Tables 2 and 3). Taken together, these results suggest that the Upf3p is the central component of the Upfp complex that modulates programmed frameshifting.

DISCUSSION

The Upf Proteins Are Part of the Surveillance Complex That Monitors Both mRNA Turnover and Translation. Recent results indicate that the factors involved in the NMD pathway play additional roles in modulating several aspects of the translation process. Genetic and biochemical studies of the Upf1p suggest that it is a multifunctional protein that acts both in NMD and in modulating the translation termination process (12, 13, 32). Furthermore, mutations in the *UPF* genes affect programmed frameshift. The *mof4-1* allele of the *UPF1* gene demonstrates an increase in programmed −1 ribosomal frameshifting efficiency and is unable to maintain the M_1 killer virus (17). In addition, *mof2-1* mutants manifest increased programmed −1 ribosomal frameshift efficiency (33). The *mof2-1* mutant is allelic to the *SUI1* gene (33), which was previously shown to play a role in translation initiation start site selection. Interestingly, *mof2-1* mutant strains also demonstrate accumulation of nonsense-containing transcripts (Y. Cui and S.W.P., unpublished results). These results suggest that the surveillance complex, including factors involved in NMD, may also be involved in monitoring other steps in the translation process. The results presented here indicate that the Upf3p, in addition to its role in NMD, is also probably part of the putative surveillance complex involved in maintaining appropriate translational reading frame.

Table 3. Programmed −1 ribosomal frameshifting and M_1 virus maintenance of strains harboring multiple mutations of *UPF* genes

Genotype (strain)	% ribosomal frameshifting*	Killer maintenance
UPF$^+$ (HFY1200)	2.5	+
upf3-Δ (HFY861)	8.4	−
mof4-1 (HFY870mof4)	7.0	−
mof4-1 upf3-Δ (HFY872mof4)	8.0	−
upf1-Δ *upf2*-Δ (HFY3000)	3.2	+
upf1-Δ *upf3*-Δ (HFY872)	7.2	−
upf2-Δ *upf3*-Δ (HFY874)	9.2	−
upf1-Δ *upf2*-Δ *upf3*-Δ (HFY883)	8.0	−

*Programmed −1 ribosomal frameshifting efficiency and M_1 virus maintenance was determined as described in the legend of Table 2.

The Upf3p Is the Key Factor That Links the Upfp Complex to Programmed −1 Ribosomal Frameshifting. The results presented here indicate that the Upf3p has a function in ensuring appropriate maintenance of translational reading frame. Monitoring the programmed ribosomal frameshifting and M_1 virus maintenance profiles of cells harboring deletions of the *UPF1*, *UPF2*, or *UPF3* genes demonstrated that a *upf3*-Δ strain affected programmed −1 frameshift efficiency and virus maintenance (Tables 2 and 3). The notion that the Upf3p is the central component of the Upfp complex that modulates programmed frameshifting is supported by the observation that a *mof4-1 upf3*-Δ strain has the same programmed −1 ribosomal frameshift and killer phenotypes as a *mof4-1* strain (Table 2). In addition, the *mof4-1* allele of the *UPF1* gene, but not a *upf1*-Δ allele, affected programmed −1 ribosomal frameshifting and killer maintenance, suggesting that Upf1p does not directly influence the maintenance of the translational reading frame. We hypothesize that the *mof4-1* allele of the Upf1p alters the Upfp complex such that the Upf3p can no longer function to help maintain the correct translational reading frame, resulting in an increased programmed −1 ribosomal efficiency which consequently leads to loss of the M_1 satellite virus. Although we strongly favor this model, our results do not completely rule out the possibility that mof4–1p functions independently of Upf3p. The fact that a *mof4-1 upf3*-Δ strain shows the same phenotype as a strain harboring a single mutant could also be explained if both proteins function by affecting the same target that is located downstream of both, mof4p and Upf3p.

The Upfp Complex May Be Part of a Surveillance Complex That Function as a Translational Checkpoint. As described above, mutations in the *UPF* genes can result in altered translation termination phenotypes, increased programmed frameshifting and stabilization of nonsense-containing transcripts (refs. 12, 13, and 17; reviewed in refs. 2 and 5). This result suggests that the Upfp complex, perhaps as part of a larger surveillance complex, functions as a "translational checkpoint." Analogous to cell cycle control checkpoints, the *UPF* genes are not essential, but ensure that the processes that they are involved in occur with high fidelity.

An important question is what triggers the activity of the surveillance complex. We hypothesize that a paused ribosome may be a key event that promotes assembly of this complex, which can subsequently monitor these processes. Both programmed frameshifting and translation termination involve a ribosomal pause (refs. 34 and 35; reviewed in ref. 36). We suggest that the interaction of the Upfp with the translation termination release factors eRF1 and eRF3 of a paused ribosome helps promote the assembly of the Upfp complex (32, 37). We suggest that this interaction leads to enhanced translation termination and subsequent degradation of nonsense-containing transcripts (32). In the case of programmed −1 ribosomal frameshifting, the RNA pseudoknot following the slippery site promotes a ribosomal pause (36, 38), which may also trigger assembly of the surveillance complex. This complex, or a subset of the Upf proteins, may help the ribosome maintain the appropriate translational reading frame. The goal of our future experiments will be to determine how these factors interact with the translational machinery to promote decay of nonsense-containing mRNAs, enhance termination, and help maintain the ribosome in the appropriate translational reading frame.

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