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mof4-1 is an allele of the *UPF1/IFS2* gene which affects both mRNA turnover and –1 ribosomal frameshifting efficiency

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The *mof4-1* (*m*aintenance *o*f *f*rame) allele in the yeast *Saccharomyces cerevisiae* was isolated as a chromosomal mutation that increased the efficiency of –1 ribosomal frameshifting at the L-A virus frameshift site and caused loss of M_1, the satellite virus of L-A. Here, we demonstrate that strains harboring the *mof4-1* allele inactivated the nonsense-mediated mRNA decay pathway. The *MOF4* gene was shown to be allelic to *UPF1*, a gene whose product is involved in the nonsense-mediated mRNA decay pathway. Although cells harboring the *mof4-1* allele of the *UPF1* gene lose the M_1 virus, mutations in other *UPF* genes involved in nonsense-mediated mRNA decay maintain the M_1 virus. The *mof4-1* strain is more sensitive to the aminoglycoside antibiotic paromomycin than a *upf1*Δ strain, and frameshifting efficiency increases in a *mof4-1* strain grown in the presence of this drug. Further, the *ifs1* and *ifs2* alleles previously identified as mutations that enhance frameshifting were shown to be allelic to the *UPF2* and *UPF1* genes, respectively, and both ifs strains maintained M1. These results indicate that *mof4-1* is a unique allele of the *UPF1* gene and that the gene product of the *mof4-1* allele affects both –1 ribosomal frameshifting and mRNA turnover.

Keywords: frameshifting/mRNA decay/nonsense mutation/ribosome/translation

Introduction

Maintenance of the correct reading frame is fundamental to the integrity of the translation process and, ultimately, to cell growth and viability. However, in the last 10 years, a number of cases of directed ribosomal frameshifting have been reported in viruses, including retroviruses, coronaviruses, the L-A double-stranded RNA (dsRNA) virus and the Ty family of viruses in yeast, the dsRNA virus of *Giardia lamblia*, (+) single-stranded RNA viruses of plants and the bacteriophage T7. In addition, programed frameshifting has been utilized by a number of bacterial transposons, as well as in a few bacterial cellular genes and the ornithine decarboxylase antizyme gene in mammals (for reviews, see Chandler and Fayet, 1993; Dinman, 1995; Farabaugh, 1995; Hayashi and Murakami, 1995). Frameshifting events typically produce fusion proteins, in which the N- and C-terminal domains are encoded by two distinct, overlapping open reading frames (ORFs). The study of these ribosomal frameshifts is important both because of their critical role in animal and plant pathogens, and because of the information they provide about the mechanisms by which the reading frame is normally maintained.

The dsRNA L-A virus in the yeast *Saccharomyces cerevisiae* has two ORFs. The 5′ *gag* gene encodes the Gag protein and the 3′ *pol* gene encodes a multifunctional protein domain required for viral RNA packaging and replication. A –1 ribosomal frameshift event is responsible for the production of the Gag–pol fusion protein. M_1, a satellite dsRNA virus of L-A which encodes a secreted killer toxin, is encapsidated and replicated using the gene products synthesized by the L-A virus (reviewed in Dinman, 1995). The combination of L-A and M_1 constitute the yeast 'killer' virus system.

The efficiency of ribosomal frameshifting determines the relative ratios of Gag to Gag–pol fusion protein available for viral particle morphogenesis, and changes in ribosomal frameshift efficiencies have major effects on the ability of cells to propagate viruses which use ribosomal frameshifting (reviewed in Dinman, 1995). Screens for mutations that increased the programed –1 ribosomal frameshift efficiencies in yeast cells identified chromosomal mutations that are called *mof* (for *m*aintenance *o*f *f*rame; Dinman and Wickner, 1992, 1994; Dinman, 1995) and *ifs* (*i*ncreased *f*rame*s*hifting; Lee *et al.*, 1995). The screen originally used to identify the *mof* mutants utilized a construct in which the *lacZ* gene was inserted downstream of the L-A –1 ribosomal frameshift signal and in the –1 reading frame relative to a translational start site. Similarly, the *ifs* mutations were identified using a reporter construct in which the *CUP1* gene was used as a reporter and inserted downstream of a −1 ribosomal frameshift signal from the mouse mammary tumor virus and in the −1 reading frame relative to a translational start site (Lee *et al.*, 1995). The assays for *mof* and *ifs* mutants relied upon identifying cells with higher β-galactosidase activities or cells demonstrating increased resistance to media containing copper. The inference was that the efficiency of −1 ribosomal frameshifting was increased in these mutants.

The frameshift reporter transcripts used in these screens have short protein-coding regions 5′ of the frameshift site, followed by a sequence that codes for the reporter proteins that are out-of-frame with the 5′ ORF. Since ribosomal frameshifting is inefficient (i.e. 2- to 8-fold increase) in these mutants, it is conceivable that the translation apparatus may 'see' these reporter transcripts as nonsense-containing mRNAs. Transcripts containing premature translation termination codons are degraded rapidly in a cell, in a process that is referred to as nonsense-mediated

mRNA decay (reviewed in Peltz *et al.*, 1994; Maquat, 1995; Weng *et al.*, 1996a). Reduced mRNA levels or decreased stability of nonsense-containing transcripts have been observed in both prokaryotes and eukaryotes (reviewed in Peltz *et al.*, 1994; Maquat, 1995; Jacobson and Peltz, 1996). Chromosomal mutations that stabilize nonsense-containing mRNAs by inactivating the nonsense-mediated mRNA decay pathway have been identified and characterized in the yeast *S.cerevisiae*. Mutations in the *UPF1*, *UPF2* and *UPF3* genes elevate the concentration of nonsense-containing mRNAs in cells by increasing their half-lives without affecting the decay rates of most wild-type transcripts (Leeds *et al.*, 1991, 1992; Peltz *et al.*, 1993; Cui *et al.*, 1995; Hagan *et al.*, 1995; He and Jacobson, 1995). Seen in this light, the increased β-galactosidase activity or copper resistance observed in *mof* or *ifs* strains may result from mutations that increase the intracellular concentrations of these nonsense-containing reporter transcripts.

The results presented here demonstrate that *mof4-1* is a unique allele of the *UPF1* gene that affects both the nonsense-mediated mRNA decay pathway and programed −1 frameshifting. We demonstrate that, in contrast to the *ifs* alleles and the other *upf1* and *upf2* mutations, the *mof4-1* allele of *UPF1* is special in that it promotes loss of the M_1 dsRNA virus. Furthermore, *mof4-1* strains are sensitive to paromomycin, a drug affecting translation fidelity (Palmer *et al.*, 1979; Singh *et al.*, 1979), and increasing dosages of paromomycin lead to enhanced efficiency of programed −1 frameshifting in a *mof4-1* strain, but not in a wild-type $MOF4^+/UPF1^+$ strain or a *mof4Δ/upf1Δ* strain. To our knowledge, the product of the *UPF1* gene is the first example of a multifunctional protein that can modulate both translation and mRNA turnover. These results are a clear example of how the processes of translation and mRNA decay are integrated.

Results

Nonsense-containing mRNAs accumulate in a mof4-1 strain

To determine the effect of the *mof4-1* mutation on the nonsense-mediated mRNA decay pathway, the abundances of the CYH2 precursor and mRNA, which encodes a ribosomal protein, were monitored. The inefficiently spliced CYH2 precursor, which contains an intron near the 5′ end, is a naturally occurring substrate for nonsense-mediated mRNA decay and has been demonstrated to be a reliable indicator of the activity of this decay pathway (He *et al.*, 1993; Cui *et al.*, 1995; Hagan *et al.*, 1995). The status of the nonsense-mediated mRNA decay activity in cells can be determined easily by comparing the ratio of the abundance of the CYH2 precursor to the CYH2 mRNA on an RNA blot. Wild-type, *upf1Δ* and *mof4-1* strains were grown to mid-log phase, RNAs were isolated, and the abundance of transcripts was determined by Northern blotting. The abundance of the CYH2 precursor was low in wild-type cells but increased at least 5-fold in a *upf1Δ* strain (Figure 1A). In the *mof4-1* mutants, the abundance of CYH2 precursor was elevated to a level similar to that observed in a *upf1Δ* strain (Figure 1A). The abundance of the nonsense-containing mini-PGK1 transcript, which is extremely sensitive to the *UPF1* status of the cell (Zhang *et al.*, 1995), was also monitored and its abundance was increased 10-fold in both *mof4-1* and *upf1Δ* strains as compared with wild-type cells (Figure 1B). The abundance of the CYH2 mRNA and wild-type PGK1 mRNA was equivalent in all strains (Figure 1B), indicating that they are probably degraded by a different mRNA decay pathway (Peltz *et al.*, 1994).

We also examined whether the programed −1 ribosomal frameshift *lacZ* reporter transcript, which was used to monitor frameshifting efficiency in the genetic screen to identify the *mof* mutants, was sensitive to the nonsense-mediated mRNA decay pathway. The abundance of the mRNA encoded from p315-JD85-ter (containing the *lacZ* gene in −1 reading frame; see schematic of reporter construct in Figure 1C and Materials and methods) was determined by Northern blot analysis in wild-type, *mof4-1* and *upf1Δ* cells (Figure 1C). The results demonstrate that the level of the LacZ reporter mRNA was elevated 2- to 3-fold in both *mof4-1* and *upf1Δ* strains compared with wild-type cells, indicating that this reporter mRNA is sensitive to the nonsense-mediated mRNA decay pathway.

To confirm that the increase in mRNA abundance observed in a *mof4-1* strain was a consequence of inactivating the nonsense-mediated mRNA decay pathway, the half-lives of the CYH2 precursor, the −1 LacZ fusion transcript and the CYH2 mRNA were examined. The decay rates were determined in a strain harboring a temperature-sensitive RNA polymerase mutant (*rpb1-1*) and containing either the wild-type *UPF1* gene, a *upf1Δ* or the *mof4-1* allele (Hagan *et al.*, 1995) by blotting analyses of RNAs isolated at different times after inhibiting transcription by shifting the culture to the non-permissive temperature (36°C). The results of these experiments are summarized in Table I and demonstrate that the CYH2 precursor and the −1 LacZ fusion transcripts were stabilized equally in either a *upf1Δ* or a *mof4-1* strain (Table I).

mof4-1 and ifs2-1 are alleles of the UPF1 gene

We next tested whether *mof4-1* is allelic to any of the previously characterized *UPF* genes. A *mof4-1* strain (strain JD474-3D, Table II) was mated with a *upf1Δ* (strain YGC106) or a *upf2Δ* (strain YGC112) strain, and the CYH2 precursor abundance was monitored in diploid cells. The CYH2 precursor abundance was low in the *mof4-1*×*upf2Δ* diploid cells (data not shown) but was increased in *mof4-1*×*upf1Δ* diploids equivalent to that observed in a haploid *upf1Δ* or *mof4-1* strain (Figure 1D, lane 8). A strain harboring the *mof4-1* allele was transformed with centromere-based plasmids harboring either the *UPF1* gene, the *UPF2* gene or vector alone, and the abundance of the CYH2 precursor was monitored in each strain. In the *mof4-1* strain containing the single copy plasmid harboring the *UPF1* gene, the abundance of the CYH2 precursor was reduced to wild-type levels (Figure 1D, lanes 1–2 and 7), whereas the vector plasmid alone or the single copy plasmid harboring the *UPF2* gene did not reduce the abundance of the CYH2 precursor (Figure 1D, lanes 3–4 and 5–6). Furthermore, the *UPF1* gene was able to reduce the efficiency of −1 ribosomal frameshifting in a *mof4-1* strain to wild-type levels, as determined by the ratio of β-galactosidase activity from strains harboring the frameshift reporter plasmid pJD107 (the *lacZ* gene in −1 reading frame) to the zero frame

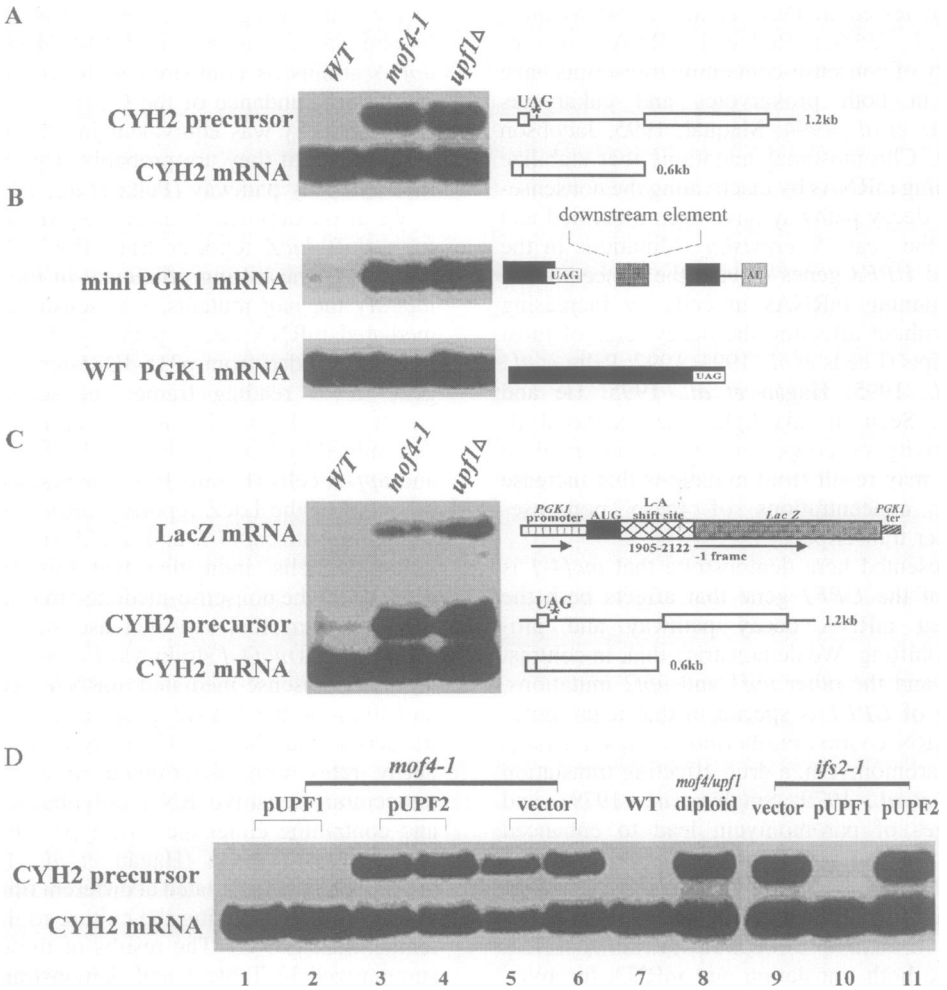

Fig. 1. Characterization of the mRNA abundance of nonsense-containing mRNAs in the *mof4-1* strain. The mRNA abundance for the CYH2 precursor, CYH2 mRNA (**A**), nonsense-containing mini-PGK1 and wild-type PGK1 mRNAs (**B**) were determined by RNA blot analysis of total RNAs from wild-type (strain JD61), *mof4-1* (strain JD474-3D) and *upf1*Δ (strain YGC106) cells. The cells were grown to mid-log phase and total RNA subsequently was isolated (see Materials and methods). The RNA blot containing 20 μg of RNA per lane was hybridized with a radiolabeled CYH2 or PGK1 probe. Schematic representation of the CYH2 precursor and its spliced product, the nonsense-containing mini-*PGK1* allele and the wild-type *PGK1* gene are shown to the right of the autoradiogram. (**C**) The abundance of the frameshift reporter LacZ mRNA (transcribed from the plasmid p315-JD85-ter) was determined as described above. The strains used in these experiments were a *upf1*Δ strain (strain YGC106) transformed with pYCp33UPF1 (a plasmid harboring the *UPF1* gene; WT), pmof4XAE (a plasmid harboring the *mof4-1* allele; see Materials and methods; *mof4-1*) or pYCplac33 vector (*upf1*Δ). Radiolabeled CYH2 and LacZ probes were used to hybridize the nitrocellulose membrane. The frameshift reporter *lacZ* construct (p315-JD85-ter) is shown schematically. (**D**) The mRNA abundance of the CYH2 precursor was determined from the *mof4-1* or *ifs2-1* strains transformed with pYCp33UPF1 (pUPF1), pYCp33UPF2 (pUPF2), or with the vector, as well as a diploid cell from a cross between *mof4-1* and *upf1*Δ strains (JD474–3D×YGC106; *mof4/upf1*).

control plasmid pJD108 (the *lacZ* gene in zero reading frame; data not shown). These results indicate that *MOF4* is allelic to *UPF1*.

We further wanted to determine whether the *ifs1-1* and *ifs2-1* mutations recently isolated by Lee and colleagues (Lee *et al.*, 1995) also affected the nonsense-mediated mRNA decay pathway and whether they are allelic to the *UPF* genes. The CYH2 precursor was stabilized in both the *ifs1* and *ifs2* mutants to a level equivalent to that observed in *upf1*Δ or *upf2*Δ strains (Figure 1D and data not shown). The *IFS1* gene was cloned and sequenced (Lee *et al.*, 1995), and our comparison of *IFS1* and *UPF2* sequences demonstrated that they are identical (Cui *et al.*, 1995; He and Jacobson, 1995). We have also determined that *ifs2* and *mof4-1* are in the same complementation group as determined by the β-galactosidase assay (data not shown). By assaying the programed –1 frameshifting

Table I. The mRNA decay rates in isogenic *upf1*Δ, *mof4-1* and wild-type *UPF1* strains

mRNA	$t_{1/2}$ (min)[a]		
	*upf1*Δ	*mof4-1*	*UPF1*$^+$
LacZ (–1) reporter	28.7	27.1	12.2
CYH2 precursor	11.3	9.7	1.6
CYH2 mRNA	45.1	43.5	43.8

[a]The temperature shift experiments were performed in strain Y46 (Table II) which harbors *rpb1-1* and *upf1*Δ alleles. The single copy plasmids containing either the wild-type *UPF1* gene, the *mof4-1* allele or the vector were transformed into this strain and the –1 frameshifting reporter construct (pRS314-JD85-ter) was also co-transformed in. The decay rates for these mRNAs were measured as described in Materials and methods. These measurements were performed at least three times and did not vary by >15%.

Table II. Strains used in this study

Strain	Genotype	Reference
YGC106	*MATa ade2,3 ura3 leu2 his7 can1 sap3 upfΔ::hisG*	this study
Y109	*MATa leu2-1::pJD85 ura3 his4 mof4Δ::ura3 K⁻*	this study
JD742-2D	*MATα leu2-1::pJD85 ura3 his3,4* L-AHN M1 K^+	this study
cross JD830	JD742-2D×JD474-5A	this study
$Y52^-$	*MATα rpb1-1 his4-519 ura3-52 upf1Δ::hisG*	Cui *et al.* (1995)
Y46	*MATα rpb1-1 his4-519 ura3-52 trp1-Δ1 upf1Δ::hisG*	Weng *et al.* (1996b)
PLY36	*MATα his4-38 SUF1-1 ura3-52 met14 upf1-2*	Leeds *et al.* (1991)
PLY136	*MATa his4-38 SUF1-1 ura3-52 upf2-1*	Leeds *et al.* (1991)
JD61	*MATa his4-644 leu2-1::pJD85 K⁻*	Dinman and Wickner (1994)
JD474-3D	*MATα leu2-1::pJD85 ura3 his4 mof4-1 K⁻*	Dinman and Wickner (1994)
JD474-5A	*MATa leu2-1::pJD85 ura3 his4 trp1 ade2 mof4-1 K⁻*	Dinman and Wickner (1994)
1074	*MATa kar1-1 leu1 L-AHN M1 K⁺*	Dinman and Wickner (1992)
3164	*MATa kar1-1 arg1 L-AHN M1 K⁺*	Dinman and Wickner (1992)
3165	*MATα kar1-1 arg1 thr(1,x) L-AHN M1 K⁺*	Dinman and Wickner (1994)
5X47	*MATa/MATα his1/+ trp1/+ ura3/+ K⁻ R⁻*	Dinman and Wickner (1994)
ifs1	*MATa cup1Δ::ura3 ura3-52 his3-Δ200 ade2 lys2 trp1 leu2 ifs1-2 K⁻*	Lee *et al.* (1995)
ifs2	*MATa cup1Δ::ura3 ura3-52 his3-Δ200 ade2 lys2 trp1 leu2 ifs2-1 K⁻*	Lee *et al.* (1995)

efficiencies in strains harboring the *ifs* alleles using the *lacZ* reporter constructs described above (pJD107 and pJD108), both *ifs* strains had –1 ribosomal frameshifting efficiencies ~2-fold greater than wild-type cells. The increase in programed –1 frameshifting efficiencies of the *ifs2-1* strain and its effect on CYH2 precursor stabilization can be corrected by a single copy *UPF1* gene but not by a *UPF2* gene (Figure 1D, lanes 9–11; data not shown). These results indicate that *IFS2* is allelic to *UPF1*.

Identification of the mof4-1 lesion in the UPF1 gene

We next sought to identify the mutation(s) that caused the *mof4-1* phenotype. Utilizing the appropriate primers, PCR products corresponding to either the 5′ one-third or the 3′ two-thirds of the *UPF1* gene from the *mof4-1* strain were isolated, and hybrid genes between the wild-type *UPF1* and the *mof4-1* allele were prepared (Figure 2A). In addition, the complete *UPF1* gene from a *mof4-1* strain was also synthesized by PCR (Materials and methods). These plasmids were transformed into a *upf1Δ* strain ($Y52^-$, Table II) and the CYH2 precursor abundance was determined in these strains (Figure 2B). The CYH2 precursor was abundant in cells containing a hybrid in which the 5′ segment of the wild-type *UPF1* gene was replaced with the 5′ fragment from the *mof4-1* allele (Figure 2, pmof4AE_{1-2}), or in cells containing plasmid pmof4BE_{1-2}, which encodes the complete *mof4-1* allele of the *UPF1* gene. The abundance of CYH2 precursor was low in cells harboring plasmid pmof4AB_{1-2}, which contains the hybrid *UPF1* gene in which the 3′ two-thirds of the gene was replaced with the DNA fragment from the *mof4-1* allele (Figure 2, pmof4AB_{1-2}). These results indicate that the mutation in the *mof4-1* allele is located within the 5′ one-third of the *UPF1* gene.

The DNA sequence of the 5′ region of the *mof4-1* allele (nt –83 to 1469) was determined from plasmids pmof4AE_1 and pmof4BE_1 (Figure 2A). Comparison of this sequence with wild-type *UPF1* revealed a single G→A mutation at nucleotide 586 in the cysteine/histidine-rich region, changing a cysteine codon at codon 62 to a tyrosine. The *mof4-1* clones from both plasmids pmof4AE_2 and pmof4BE_2 also contained the same G→A mutation (data not shown). To confirm that the identified Cys→Tyr mutation resulted in the *Mof4* phenotype, a 900 bp *Bst*XI–*Asp*718 DNA fragment from the wild-type *UPF1* gene was replaced with an analogous DNA fragment from either plasmid pmof4AE_1 or pmof4BE_1 harboring the *mof4-1* mutation (Figure 2B, pmof4XAE and pmof4XBE). Cells harboring the above hybrid *UPF1* gene had the same three phenotypes as the *mof4-1* strain, including: (i) elevated abundance of CYH2 precursor and frameshift reporter LacZ mRNA (Figures 2B and 1C); (ii) inability to propagate the M_1 killer virus (Table IV, #5); and (iii) increased sensitivity to paromomycin (Figure 4A, #3; Table IV, #5). The last two phenotypes of the *mof4-1* strain will be discussed further in the following sections.

Unlike upf or ifs alleles, the mof4-1 allele of UPF1 increases –1 ribosomal frameshifting efficiency and causes loss of the M_1 virus

The efficiency of –1 ribosomal frameshifting in the various mutant forms was measured by using the *lacZ* reporter construct described above. A *upf1Δ* strain (YGC106) containing the *lacZ* frameshift reporter construct in the zero or –1 frame relative to the translation start site (p315-JD86-ter or p315-JD85-ter) was transformed with a single copy plasmid harboring either the wild-type *UPF1* gene, the *mof4-1* allele or the vector alone. The β-galactosidase activities in these strains were monitored, and the percentage of –1 frameshifting was calculated. Cells containing the *mof4-1* allele had a –1 ribosomal frameshifting efficiency of 6.4%. *UPF1* and *upf1Δ* cells had an efficiency of 1.4 and 3.1%, respectively. Interestingly, the ~2-fold increase in –1 ribosomal frameshifting observed in *upf1Δ* cells is very similar to that reported for the *ifs2-1* mutants (Lee *et al.*, 1995). The 2-fold increase in programed frameshifting efficiency in these strains corresponds to the 2- to 3-fold stabilization of the frameshift reporter transcript (Figure 1C). The elevated level of –1 frameshifting in *mof4-1* cells, however, was not due solely to the stabilization of the LacZ frameshift reporter mRNA, since the abundance of the LacZ frameshift reporter mRNA was equivalent in both *mof4-1* and *upf1Δ* cells (Figure 1C). Thus, the higher expression level of the *lacZ* gene product in the –1 reading frame in *mof4-1* cells as compared

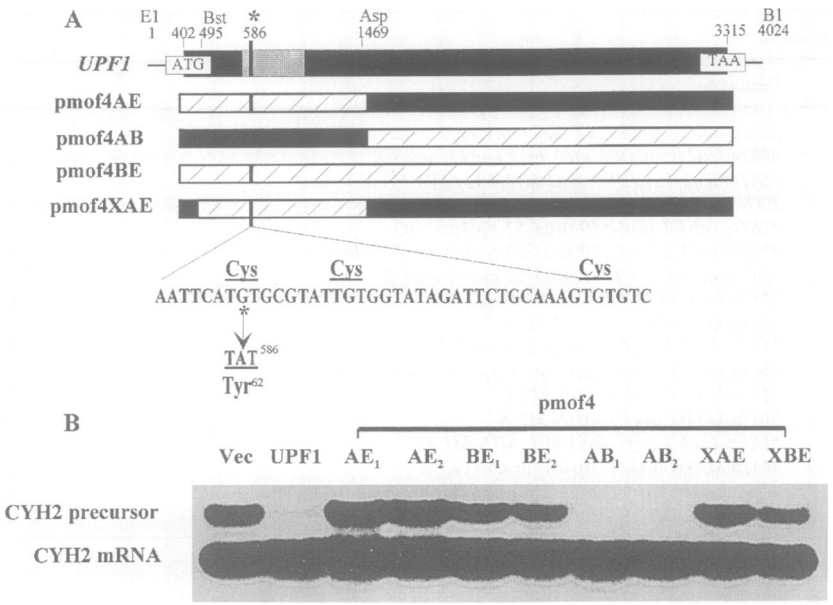

Fig. 2. Identification and characterization of the lesion in the *mof4-1* allele. Hybrid genes between the wild-type *UPF1* and the *mof4-1* alleles schematically represented in (**A**) were constructed, transformed into a *upf1*Δ strain (Y52$^-$) and CYH2 precursor abundance was determined by RNA blotting analysis as described in Figure 1. An autoradiogram of this analysis is shown in (**B**). The black rectangle in (A) represents sequences from the wild-type *UPF1* gene while the hatched rectangle represents sequences from the *mof4-1* allele. The cysteine/histidine-rich region of the *UPF1* gene is represented by a gray rectangle in the wild-type *UPF1* gene. The dark vertical line represents the location of the mutation within the *mof4-1* allele. The *mof4-1* allele was sequenced and the sequence change is shown. For each hybrid allele shown in (A) two identical constructs were prepared from different PCRs and are designated with the subscript 1 or 2 in (B) (see Materials and methods). The restriction endonucleases represented in (A) are: E1 (*Eco*RI), Bst (*Bst*XI), Asp (*Asp*718), B1 (*Bam*HI).

with cells harboring a *upf1*Δ mutation must result from something other than stabilization of the reporter mRNA, suggesting that the *mof4-1* allele increases the efficiency of programed –1 ribosomal frameshifting.

The contribution of frameshift suppression independent of the programed –1 frameshifting signal in a *mof4-1* strain was also examined. The ratio of β-galactosidase activities where the *lacZ* gene is in the –1 frame but lacks the ribosomal –1 frameshift signal (pTI24; Dinman *et al.*, 1991) was determined with regard to pTI25, the zero frame control. We found that a *mof4-1* strain was able to enhance frameshift suppression from the *lacZ* fusion transcript synthesized from pTI24 from 0.01 to 0.22%, a 22-fold increase in β-galactosidase activity compared with wild-type cells. Although this represents a significant increase in frameshift suppression, it is far below the 8% efficiency of programed ribosomal –1 frameshifting observed in *mof4-1* cells. A 600-fold increase in frameshift suppression in *mof4-1* cells would have had to be observed to account for the increase in β-galactosidase activity generated from the *lacZ* reporter construct containing the programed –1 frameshift signal. Therefore, frameshift suppression at the termination codon does not account for the apparent increase in the efficiency of programed –1 ribosomal frameshifting in *mof4-1* cells.

The results above have demonstrated that the recessive *mof4-1* allele increased the efficiency of programed –1 frameshifting. The changes in ribosomal frameshifting efficiencies have been shown to have major effects on the ability of cells to propagate the M_1 killer virus (Dinman and Wickner, 1992, 1994). Cells harboring the *mof4-1* allele lose the ability to propagate the M_1 dsRNA virus Therefore, we next examined whether mutations that inactivate the nonsense-mediated mRNA decay pathway affect the maintenance of the M_1 dsRNA virus. L-A and M_1 were introduced by cytoduction into strains harboring the *mof4-1*, *upf1*Δ, *upf1-2*, *upf2-1*, *upf2*Δ, *ifs1-1* or *ifs2-1* alleles, and these cells were replica plated onto a lawn of cells that are sensitive to the killer toxin. Cells maintaining the M_1 virus secrete the killer toxin, creating a ring of growth inhibition (Dinman and Wickner, 1992). The results from these experiments are shown in Figure 3A and summarized in Table III. Only cells harboring the *mof4-1* allele were unable to maintain the killer phenotype (Figure 3A; Table III). *MOF4/UPF1* and *mof4*Δ*/upf1*Δ strains were both able to propagate the M_1 dsRNA virus, as demonstrated by the zone of growth inhibition around these colonies (Figure 2A). Similarly, cells harboring the *upf1*, *upf2*, *ifs1* and *ifs2* alleles maintained M_1 (Table III). Consistent with the loss of killer phenotype, the 1.8 kb M_1 dsRNA was absent in the *mof4-1* cells but present in all other *upf* or *ifs* mutants, as demonstrated by RNA blot analysis (see Materials and methods; results not shown).

Three separate sets of experiments demonstrate that the increased –1 ribosomal frameshifting efficiency and the loss of M_1 dsRNA virus are the consequence of the *mof4-1* allele rather than a secondary mutation within the cell. First, a single copy *UPF1* gene introduced into *mof4-1* cells on a centromere plasmid rescued the ability of *mof4-1* cells to maintain M_1, while the vector-transformed cells had no affect (see Figure 3A; Table IV, compare #1 with #2). Second, deleting the *UPF1/MOF4* gene from the chromosome in cells harboring the *mof4-1* allele of the *UPF1* gene restored the ability of these cells to propagate M_1 (see Figure 3A; Table IV #3). Third, tetrad analysis of cells harboring the *mof4-1* allele crossed with a *MOF*$^+$ L-A$^+$ M_1^+ strain was performed to determine whether the loss of the killer phenotype co-segregated with the increased β-galactosidase

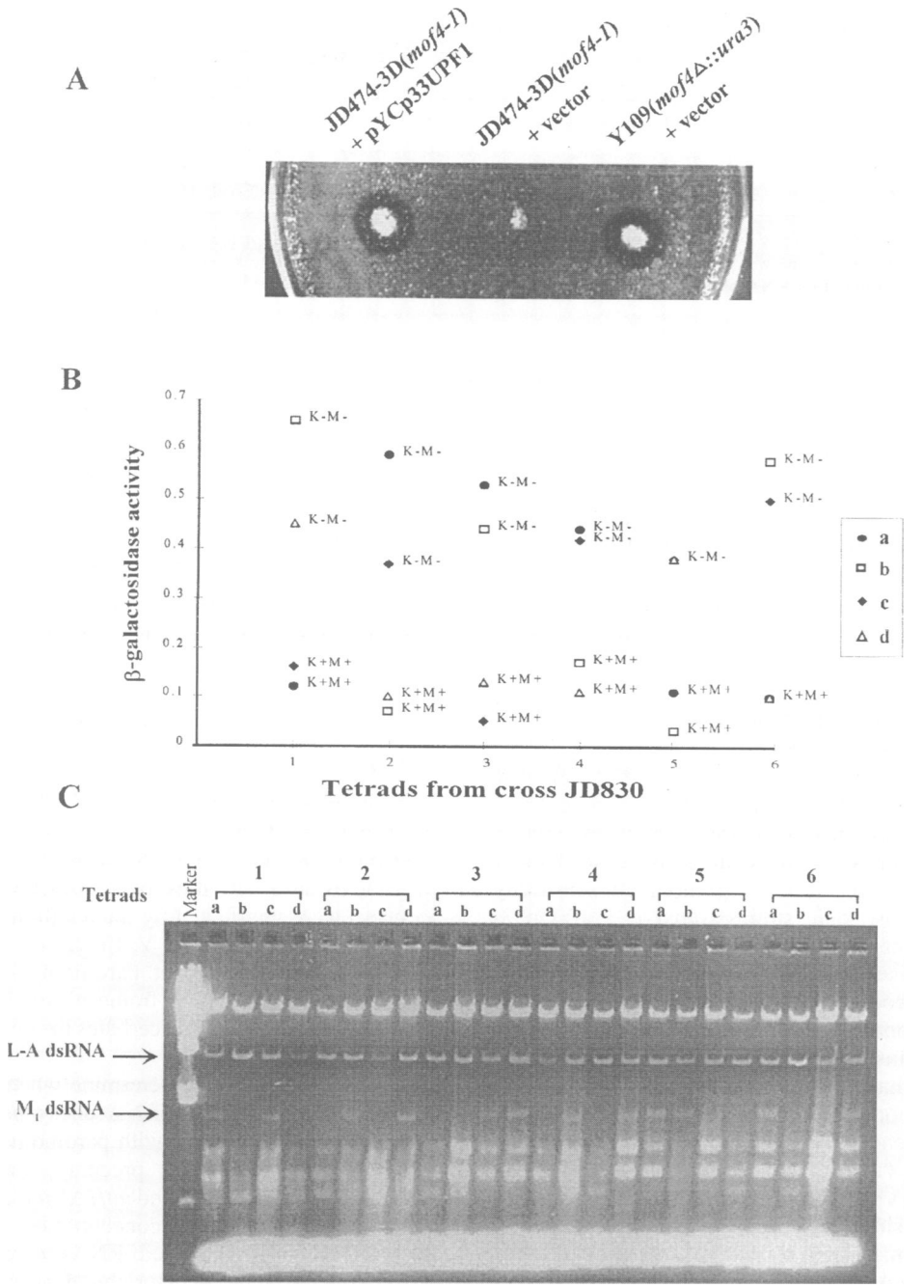

Fig. 3. The β-galactosidase activity, killer phenotype and maintenance of M_1 virus co-segregate with the *mof4-1* allele. (**A**) Killer assay in the *mof4-1* strain containing either the single copy wild-type *UPF1* gene (JD474-3D + pYCp33UPF1) or the vector alone (JD474-3D + vector), or in which the *mof4-1* allele of the *UPF1* gene was deleted from the genome [Y109 (*mof4Δ::ura3*) + vector]. Colonies of these strains were grown on the lawn of cells which are sensitive to the killer toxin secreted by the M_1 virus. The growth inhibition was seen around the strains harboring the M_1 virus. (**B**) Tetrad analysis was performed from a cross (cross JD830) between a *mof4-1* strain (JD474-5A) that does not maintain either the killer phenotype (K^-) or double-stranded M_1 RNA (M^-) with a wild-type strain (JD742-2D; M^+K^+). Both parental strains contained the chromosomally integrated *lacZ* frameshift construct (*leu2-1::pJD85*; Dinman and Wickner, 1994). The spore clones from each tetrad were assayed for their β-galactosidase activity, their killer phenotype and their ability to propagate the double-stranded M_1 RNA as described in Materials and methods. The β-galactosidase activity (*y* axis) for each set of tetrads (*x* axis) is shown as well as the ability of each of the spores to maintain either the killer phenotype (K+/K–) or the double-stranded M_1 RNA (M+/M–). (**C**) Total RNAs were isolated from the spore colonies described in (B) and run into a 1.5% agarose gel. The L-A and M_1 viral dsRNA were shown as 2.4 and 1.8 kb bands respectively.

activity. There was a 2:2 segregation of killer$^+$ and killer$^-$ phenotype, and high levels of β-galactosidase activity always co-segregated with the killer$^-$ phenotype (Figure 3B). Total nucleic acids from these spore clones were isolated and the RNA of M_1 and L-A viruses was monitored in each of the spore clones from the tetrads on an agarose gel (Figure 3C). The results demonstrate that the 1.8 kb M_1 dsRNA band is present in the *MOF*$^+$ killer$^+$ spore clones and is absent in the *mof4-1* killer$^-$ spore clones (Figure 3C). These experiments suggest that *mof4-1* is a specific allele of the *UPF1* gene that alters both mRNA decay and the efficiency of –1 ribosomal frameshifting.

Table III. Characterization of the killer phenotype of *mof4-1*, *ifs* and *upf* alleles

#	Strain	M_1 killer phenotype[a]	#	Strain	M_1 killer phenotype
1	JD61 (wild-type)	+	5	YGC112 (*upf2Δ*)	+
2	JD474–3D (*mof4-1*)	–	6	PLY136 (*upf2-1*)	+
3	Y52$^-$ (*upf1Δ*)	+	7	ifs1 (*ifs1-1*)	+
4	PLY36 (*upf1–2*)	+	8	ifs2 (*ifs2-1*)	+

[a]L-A and M_1 were introduced into cells by cytoduction with either strain 3164 or 3165 as donors for the *upf* and *ifs* alleles, or strain 1074 as donor for *mof4* alleles. The killer phenotype was analyzed by the killer plate assay (use strain 5X47 as the killer indicator), and the ability to maintain the M_1 dsRNA virus was monitored by RNA blot analysis.

Table IV. Paromomycin sensitivity and killer phenotype of *mof4-1* strains

#	Strain	M_1 killer phenotype	Paromomycin sensitivity (cm)[a]
1	JD474-3D (*mof4-1*) + pYCp33UPF1	+	1.6
2	JD474-3D (*mof4-1*) + vector	–	2.4
3	Y109 (*mof4Δ::ura3*) + vector	+	1.7
4	Y109 (*mof4Δ::ura3*) + pmof4AB	+	1.6
5	Y109 (*mof4Δ::ura3*) + pmof4XAE	–	2.3

[a]Cells were grown in –Ura –Leu liquid media and subsequently plated onto –Ura –Leu plates. A 0.625cm diameter disc containing 1.0 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 4 days. The numbers were the average of at least three independent experiments with error no more than ±10%. Y109 was derived from JD474-3D in which the *mof4-1* allele of the *UPF1* gene was deleted from the genome (*mof4Δ::ura3*).

Cells harboring the mof4-1 allele are more sensitive to paromomycin

Strains harboring mutations that lower translational fidelity have been shown to be hypersensitive to the aminoglycoside antibiotic paromomycin, a drug that is thought to increase the frequency of misreading in yeast (Palmer *et al.*, 1979; Singh *et al.*, 1979). Paromomycin sensitivity was monitored in isogenic strains (*mof4-1* + pYCp33-UPF1, *mof4-1* + vector and *mof4Δ::ura3* + vector; Figure 4A) by placing discs containing 1 mg of paromomycin onto the plate. By comparing the zone of growth inhibition around the disc containing the drug, the antibiotic sensitivity of these strains can be assessed. The results demonstrate that strains harboring the *mof4-1* allele were more sensitive to paromomycin than cells harboring either the wild-type *UPF1* gene or a *mof4Δ/upf1Δ* allele (Figure 4A, compare #1 with #2 and #4). Unlike the *mof4-1* strain, the isogenic *mof4Δ/upf1Δ* strain (*mof4Δ::ura3* + *vector*) was no more sensitive to paromomycin than the wild-type *UPF1*$^+$ strain, consistent with the results reported previously (Figure 4A, compare #2 with #4; Leeds *et al.*, 1992; Cui *et al.*, 1995). In addition, a paromomycin-resistant colony isolated from a parental *mof4-1* strain maintained M_1 and had wild-type –1 ribosomal frameshifting efficiency (data not shown). The co-reversion of these three phenotypes indicates that they are all linked to the *mof4-1* allele of the *UPF1* gene.

The effect of paromomycin on –1 ribosomal frameshifting was analyzed further by β-galactosidase assay using plasmids pJD107 (–1 frameshift reporter construct) or pJD108 (zero frame control) in isogenic strains harboring the wild-type *UPF1* gene on a single copy plasmid (*mof4-1* + pYCp22UPF1), the vector alone (*mof4-1* + vector) and a strain in which the *mof4-1* allele of the *UPF1* gene was deleted (*mof4Δ::ura3*). Cells were grown in liquid media in the presence of different concentrations of the drug and the β-galactosidase activities were determined, normalized to the number of cells used in the assay. The β-galactosidase activities from pJD107 (harboring the –1 frameshift reporter gene) in the *mof4-1* strain (*mof4-1* + vector) climbed steadily with increasing concentrations of paromomycin, while there was no change in β-galactosidase activity in *mof4-1* cells containing pYCp22UPF1 or in *mof4Δ::ura3* cells (Table V). The β-galactosidase activities from pJD108 (the zero frame control) were unaffected by the addition of paromomycin in all three strains (Table V; Figure 4B). Taken together, these results suggest that paromomycin can affect the efficiency of –1 ribosomal frameshifting in a *mof4-1* strain, and that paromomycin exacerbates the defect of the *mof4-1* allele of the *UPF1* gene.

We further wanted to determine whether substrates for the nonsense-mediated mRNA decay pathway increased in cells that were treated with paromomycin. The mRNA abundance of the CYH2 precursor was determined in *UPF1*$^+$*/MOF4*$^+$, *mof4-1* and *upf1Δ/mof4Δ* strains grown in the presence of increasing concentrations of paromomycin. Cell aliquots were collected, RNAs were isolated and the CYH2 mRNA and precursor abundances were determined by RNA blotting analysis as described above. The results demonstrate that the ratio of the CYH2 precursor to mRNA was not altered by the presence of paromomycin in any strains tested (Figure 4B). This result indicates that paromomycin treatment does not affect the nonsense-mediated mRNA decay pathway.

Discussion

mof4-1 was identified originally as a recessive mutation that increased the efficiency of programed –1 ribosomal frameshifting at the L-A frameshift site (Dinman and Wickner, 1994). Here we show that *MOF4* is allelic to the *UPF1* gene and *mof4-1* mutation increases the abundance of the nonsense-containing mRNAs, suggesting that this mutation completely abrogates the activity of the nonsense-mediated mRNA decay pathway (Figure 1).

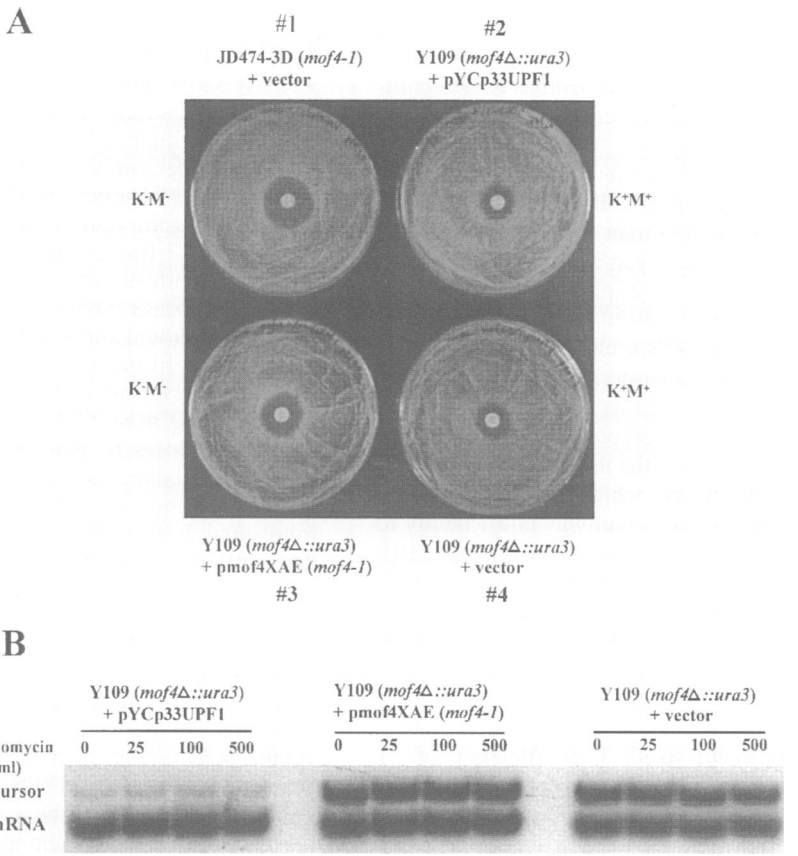

Fig. 4. Effects of paromomycin on the defect of *mof4-1* mutation. (**A**) Paromomycin sensitivity was determined in cells harboring the *mof4-1* allele on the chromosome or on a single copy plasmid (plates #1 and #3), or in the isogenic *MOF4/UPF1* deletion strains harboring either the wild-type *UPF1* gene on a single copy plasmid or the vector alone (plates #2 and #4). Cells were grown in –Ura –Leu liquid media and subsequently plated onto –Ura –Leu plates. A disc containing 1.0 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 4 days. K–M–, killer^{-}M$_1^{-}$; K+M+, killer^{+}M$_1^{+}$. (**B**) The steady-state levels of CYH2 precursor and mRNA in the isogenic *MOF4/UPF1* deletion strain harboring various plasmids in the presence of different concentrations of paromomycin.

Table V. Paromomycin effect on –1 ribosomal frameshifting efficiency in *mof4-1* strains

Paromomycin (μg/ml)	Percentage of –1 ribosomal frameshifting[a]		
	JD474-5A (*mof4-1*) + pYCp22UPF1	JD474-5A (*mof4-1*) + vector	Y109 (*mof4Δ::ura3*)
0	2.0	9.4	2.4
5	2.2	9.2	1.8
10	3.0	9.4	1.5
25	2.9	13.5	1.4
50	2.0	14.7	1.4
100	2.6	15.3	1.4
250	2.2	17.8	1.4
500	2.2	22.2	1.4

[a]Strain JD474-5A, harboring either pYCp22UPF1 or vector, and strain Y109 were transformed with either the high copy plasmid pJD107 (–1 ribosomal frameshift tester) or pJD108 (zero frame control). Paromomycin was added to cells inoculated at 0.1 OD_{595}/ml and grown in –Trp –Leu liquid media at 30°C for 4 h. The β-galactosidase activities were determined by subtracting the β-galactosidase activity of cells lacking these plasmids (harboring a single copy integrated –1 frameshift reporter construct) from β-galactosidase activity observed in cells harboring the reporter constructs on 2μ plasmids (pJD107, pJD108) and the percentage of –1 ribosomal frameshifting was calculated by: (pJD107/pJD108)×100%. The average β-galactosidase activities of cells with pJD108 in *mof4-1* + pYCp22UPF1, *mof4-1* + vector or *mof4Δ::ura3* strains were 50.1 ± 7.5, 48.9 ± 4.6 and 54.4 ± 9.1, respectively.

Although strains containing the *mof4-1* and *upf1Δ* alleles both increase the abundance of nonsense-containing mRNAs to equal degrees, strains harboring these alleles have significantly different phenotypes. Compared with the *upf1Δ* strain, the *mof4-1* strain: (i) increases the efficiency of –1 ribosomal frameshifting; (ii) is more sensitive to the aminoglycoside paromomycin than a *upf1Δ* strain; and (iii) unlike an isogenic *upf1Δ* strain, the *mof4-1* strain cannot propagate the M_1 killer dsRNA virus (Figure 3A; Table III). At present, only one *mof4* allele with these phenotypes has been identified (Dinman and Wickner, 1994; this study), although the mutagenesis analysis for frameshift mutants was not saturated.

The 39 nm L-A-encoded viral particle has icosahedral

symmetry and is composed of 59 Gag dimers and one dimer of Gag–pol (Cheng *et al.*, 1994). The 1.9% of –1 ribosomal frameshifting efficiency determines the stoichiometry of Gag to Gag–pol protein. Increasing the efficiency of –1 ribosomal frameshifting >2- to -3-fold affects the ability of cells to propagate M_1, presumably because the ratio of Gag to Gag–pol available for viral particle formation is inappropriate (Dinman and Wickner, 1992, 1994). Two arguments can be given as to why the loss of M_1 in *mof4-1* strains cannot be explained by stabilizing the frameshift-containing L-A mRNA: (i) overexpression of the L-A mRNA from a cDNA clone confers a Super killer (Ski^-) phenotype, or increased M_1 titers, upon yeast cells (Wickner *et al.*, 1991; Masison *et al.*, 1995), the opposite of the loss of M_1 phenotype (i.e. a Mak^- phenotype), and (ii) strains containing the *upf1Δ*, *upf1-2*, *upf2-1*, *upf2Δ*, *ifs1-1* or *ifs2-1* alleles, which all inactivate the nonsense-mediated mRNA decay pathway equivalently to the *mof4-1* allele, do not promote loss of M_1 (Table III). Thus, simply stabilizing the L-A mRNA does not in itself alter the ratio of Gag to Gag–pol and does not promote the loss of the M_1 dsRNA virus. That the *mof4-1* allele of the *UPF1* gene cannot maintain M_1 suggests that this mutation specifically affects programed –1 ribosomal frameshifting efficiency, changing the ratio of the Gag to Gag–pol products synthesized. The efficiency of –1 ribosomal frameshifting, as measured by the β-galactosidase assays, further supports this conclusion. Both the *mof4-1* and *upf1Δ* alleles stabilized nonsense-containing RNAs (CYH2 precursor, mini-PGK1 mRNA; Figure 1A and B) as well as the frameshift LacZ reporter mRNA to the same level (Figure 1C), yet *mof4-1* cells had 2-fold more –1 ribosomal frameshifting efficiency than *upf1Δ* or *ifs2-1* cells. Taken together, these results suggest that *mof4-1* is a unique allele of the *UPF1* gene that elevates the abundance of nonsense-containing mRNAs, and increases the efficiency of programed –1 ribosomal frameshifting.

Strains harboring *upf1Δ*, *upf1–2*, *upf2–1*, *upf2Δ*, *ifs1-1* and *ifs2-1* alleles may be identified as mutations that appear to increase programed –1 frameshifting in screens using frameshift reporter constructs. However, these alleles do not affect the maintenance of the M_1 dsRNA virus (Table III). The ability to propagate the M_1 dsRNA killer virus serves as a second independent assay to distinguish between mutations that affect ribosomal frameshifting efficiencies from those mutations that only affect mRNA turnover. Clearly, without such a secondary assay, use of only frameshift reporter constructs, which monitor only the level of the end-product expressed, will often identify mutations that apparently affect the level of frameshifting without actually altering this process. The isolation of *ifs* mutants is an example of this problem. *ifs1-1* and *ifs2-1* are alleles of *UPF2* and *UPF1*, respectively (see above; Lee *et al.*, 1995), which increased the apparent frameshifting efficiency 2-fold, similar to that observed in a *upf1Δ* strain. However, strains harboring these mutations did not promote loss of the M_1 virus (Table III). By using both the frameshift reporter system and monitoring the ability to propagate the M_1 virus as measures of alterations in frameshifting, the *ifs* mutants do not alter the efficiency of programed –1 frameshifting to promote loss of the M_1 virus. Similarly, the previously reported *mof3-1, mof7-1* and *mof8-1* lesions, which increase –1 frameshifting as monitored by the *lacZ* frameshift reporter but do not lose the M_1 virus (Dinman and Wickner, 1994), also cannot be classified strictly as mutations that effectively alter –1 ribosomal frameshifting efficiencies. At present, it is difficult to determine whether the increased β-galactosidase activities observed in these strains are consequences of stabilizing the frameshift reporter mRNA or changes in programed –1 frameshifting. Currently, we are developing new frameshift reporter constructs that will be insensitive to the nonsense-mediated mRNA decay pathway and, thus, these should allow us to separate the effects of mRNA stabilization from alterations in frameshifting efficiencies.

The observation that the efficiency of –1 ribosomal frameshifting in *mof4-1* cells was elevated in response to increasing doses of paromomycin is an important result since it demonstrates that a drug can modulate programed –1 frameshifting efficiencies. This supports the notion that ribosomal frameshifting may serve as a potential target for antiviral compounds, altering the Gag to Gag–pol ratio. It is anticipated that the identification and characterization of gene products involved in this process and of drugs that modulate it will lead to therapeutics to combat viral diseases.

The *UPF1* gene has been cloned and sequenced (Altamura *et al.*, 1992; Leeds *et al.*, 1992). The deduced amino acid sequence of the *UPF1* gene indicates that it encodes a 109 kDa protein with zinc finger motifs near its N-terminus and harbors the appropriate motifs for it to be classified as a member of the ATP binding RNA–DNA helicase superfamily group I (Altamura *et al.*, 1992; Koonin, 1992). Purification of the Upf1p demonstrated that it is an RNA binding protein with ATPase and helicase activities (Czaplinski *et al.*, 1995). A *UPF1* gene disruption results in stabilization of nonsense-containing mRNAs and also yields a nonsense suppression phenotype (Leeds *et al.*, 1991; Cui *et al.*, 1995). A single Cys→Tyr change at codon 62 in the N-terminal cysteine/histidine-rich region of the *UPF1* gene accounts for the *mof4-1* allele of the *UPF1* gene and $Mof4^-$ phenotypes (Figure 4). Interestingly, other mutations in the cysteine/histidine-rich region of the *UPF1* gene have been identified that were able efficiently to degrade nonsense-containing transcripts but inactivated its translation termination activity at a nonsense codon, thus allowing for suppression of nonsense alleles (Weng *et al.*, 1996b). These results indicate, but do not prove, that the nonsense-mediated mRNA decay properties of the Upf1 protein can be separated from its function in modulating translation termination at a nonsense codon. Furthermore, the results suggest that the cysteine/histidine-rich region may be required for modulating certain aspects of translation termination at nonsense codons. The *mof4-1* allele is unique because this lesion inactivates both the nonsense-mediated mRNA decay activity and alters programed translational frameshifting. Taken together, these results pose a very interesting question concerning how the processes of translation termination at a nonsense codon, programed –1 frameshifting and mRNA decay are related. Future work will be required to determine whether these are directly or indirectly related processes.

Materials and methods

Strain and media

The strains of *S.cerevisiae* used are listed in Table II. YPAD, YPG, SD, synthetic complete medium and 4.7 MB plates for testing the killer

phenotype were as previously reported (Dinman and Wickner, 1994). Strains harboring *mof4-1* alleles were isolated as described (Dinman and Wickner, 1994). Strain Y109 was constructed by deleting the protein-coding region of the *mof4-1* allele of the *UPF1* gene and replacing it with the *URA3* gene. This strain subsequently was plated on media containing 5-fluoro-orotic acid, and a *ura*$^-$ strain was isolated.

Genetic methods

Transformation of yeast and *Escherichia coli* was performed as described previously (Cui *et al.*, 1995; Hagan *et al.*, 1995; Zhang *et al.*, 1995). Cells were cured of L-A virus by streaking for single colonies at 39°C, and loss of L-A was confirmed by agarose gel analysis. Generation of rho° cells, cytoductions and the killer test were performed as previously described (Dinman and Wickner, 1992). Genetic crosses, sporulation and tetrad analysis, β-galactosidase assays and the killer test were performed as described (Dinman and Wickner, 1994). Testing for paromomycin sensitivity of the various strains was performed as described by Cui *et al.* (1995).

Analysis of RNA abundance and decay rates

dsRNA of L-A and M_1 viruses was prepared as described (Fried and Fink, 1978) and was analyzed by electrophoresis through 1.2% agarose gels. RNA abundance of CYH2, PGK1 and LacZ mRNAs were analyzed by Northern blotting, probing with DNA fragments that are complementary to these RNAs (Cui *et al.*, 1995; Hagan *et al.*, 1995; Zhang *et al.*, 1995). The LacZ mRNA was hybridized with a [^{32}P]dCTP-labeled 3.1 kb DNA fragment encoding the *lacZ* gene. The RNA blots were quantitated using either a Bio-Rad model G-250 Molecular Imager or model G-670 Imaging Densitometer (Cui *et al.*, 1995; Zhang *et al.*, 1995). The abundances of the CYH2 precursor, nonsense-containing mini-PGK1 and the frameshift LacZ reporter transcript were normalized to the abundance of the wild-type CYH2 or PGK1 mRNAs. Experiments to quantitate the abundances of these RNAs were performed at least three times and did not vary by >15%.

The mRNA decay rates were determined by transforming the plasmid harboring the *lacZ* reporter gene into a strain harboring a *upf1*Δ and the temperature-sensitive allele of the RNA polymerase II (*rpb1-1*). This strain was transformed with a centromere plasmid containing either the wild-type *UPF1* gene, the *mof4-1* allele of the *UPF1* or the vector, and the mRNA decay rates were determined as described previously (Cui *et al.*, 1995; Hagan *et al.*, 1995; Zhang *et al.*, 1995). The results of these experiments were quantitated using a Bio-Rad model G-250 Molecular Imager or a Bio-Rad model GS-670 Imaging Densitometer. These measurements were performed at least three times and did not vary by >15%.

Plasmid constructions

The plasmids pJD107 and pJD108 used for β-galactosidase assay were derived from pF8 and pT125 respectively (Dinman *et al.*, 1991). In pJD107, the 4.9 kb *Hin*dIII fragment from pF8 was ligated into *Hin*dIII-digested pRS426 (Christianson *et al.*, 1992) and contains the PGK1 promoter, a translational start site, followed by a 218 bp cDNA fragment of L-A containing the −1 ribosomal frameshift signal. This is followed by the *lacZ* gene, which is in the −1 frame with respect to the start site. pJD108 contains the 4.7 kb *Hin*dIII fragment of pT125 cloned into the *Hin*dIII site of pRS426, and the *lacZ* gene is in the zero frame without any intervening sequence. p315-JD85-ter, p315-JD86-ter, p314-JD85-ter and p314-JD86-ter were constructed and used for the measurement of LacZ mRNA abundance. *Hin*dIII fragments from pJD85 (*lacZ* in −1 frame; Dinman and Wickner, 1994) and pJD86 (*lacZ* in zero frame; Dinman and Wickner, 1994) were ligated into pRS315 or pRS314, and a 300 bp *Bgl*II–*Hin*dIII fragment containing the *PGK1* transcription termination signal was inserted downstream of the *lacZ* gene. The constructions of pYCp33UPF1, pYCp22UPF1 and pYCp33UPF2 were as described before (Cui *et al.*, 1995). The plasmids pmof4AE, pmof4AB and pmof4BE, used to clone the *mof4-1* allele, were constructed as follows: the 1.47 kb *Asp*718–*Eco*RI fragment or the 2.6 kb *Asp*718–*Bam*HI fragment from pYCp33UPF1, containing the *UPF1* gene, was deleted and replaced with the corresponding fragments of the *mof4-1* allele that were isolated by PCR (see below). pmof4BE was cloned by inserting the 4.2 kb *Eco*RI–*Bam*HI PCR DNA fragment from the *mof4-1* strain into pYCplac33. Since the pYCp33UPF1 contains more than one *Bst*XI site, pmof4XAE and pmof4XBE were constructed by two steps. A 978 bp *Bst*XI–*Asp*718 DNA fragment from pPUC-UPF1 (Cui *et al.*, 1995) was replaced with a *Bst*XI–*Asp*718 DNA fragment from pmof4AE and pmof4BE, forming pPUCmof4XAE and pPUCmof4XBE respectively. The 4.2 kb *Bam*HI–*Eco*RI fragments from these two plasmids were cloned into pYCplac33 vector.

Identification of the mof4-1 mutation

A PCR strategy was used to identify the *mof4-1* allele. The primers used for PCR DNA fragments from the *UPF1* gene were: primer-1, 5′-CCGGAATTCATGAACGGGAAA-3′; primer-2, 5′-GACCGGCCG TA-ACGGACGTTGTAATACAT-3′; primer-3, 5′-ATCCCCGCGGGAGTT-GAAAGTTGC CATC-3′; primer-4, 5′-GACGGATCCAAAGTATAT-TGGAC-3′. Genomic DNA (50–100 ng) was prepared (Rose *et al.*, 1990) from the *mof4-1* strain and used as the template in PCR. Primer pairs, primer-1 and primer-2, were used to synthesize the DNA fragment to construct pmof4AE (Figure 2), primer-3 and primer-4 were used to synthesize the DNA fragment to construct pmof4AB (Figure 2) and primer-1 and primer-4 were used to construct pmof4BE (Figure 2), respectively. Two PCR products from two different PCRs were used in the cloning reaction to minimize artifacts from PCR. The PCR conditions used were as follows: 95°C, 5 min; 94°C, 1 min; 45 or 50°C, 1 min; 72°C, 1.5 min for 25 cycles. The DNA fragments from PCR were purified from 1% agarose gel and used for swapping the corresponding DNA fragment of the wild-type *UPF1* gene which was on a YCplac33 vector as described above.

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