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 (maintenance of frame) 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 M1, 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 M1 virus, mutations in other UPF genes involved in nonsense-mediated mRNA decay maintain the M1 virus. The mof4-1 strain is more sensitive to the aminoglycoside antibiotic paromomycin than a upf1A 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, progranulated frameshifting has been utilized by a number of bacterial transposons, as well as in a few bacterial cellular genes and the ornithine decarboxylase anti-enzyme 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 frameshifting events 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. M1, 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 M1 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 frameshifting 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 frameshifting efficiencies in yeast cells identified chromosomal mutations that are called mof (for maintenance of frame; Dinman and Wickner, 1992, 1994; Dinman, 1995) and ifs (increased frameshifting; 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 frameshifting 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 M1 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 *mof4Δupf1Δ* 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 frameshifting *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-JD5-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 frameshifting reporter plasmid pJD107 (the *lacZ* gene in –1 reading frame) to the zero frame.
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 upf1Δ or upf2Δ 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 mofo-1 are in the same complementation group as determined by the β-galactosidase assay (data not shown). By assaying the programed -1 frameshifting

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**Table 1.** The mRNA decay rates in isogenic upf1Δ, mofo-1 and wild-type UPF1 strains

| mRNA            | t1/2 (min) upf1Δ | t1/2 (min) mofo-1 | t1/2 (min) 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            |

*The temperature shift experiments were performed in strain Y46 (Table II) which harbors rpb1-1 and upf1Δ alleles. The single copy plasmids containing either the wild-type UPF1 gene, the mofo-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%.*
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 an *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, pmo4F4AE1-2), or in cells containing plasmid pmo4F4BE1-2, which encodes the complete *mof4-1* allele of the *UPF1* gene. The abundance of CYH2 precursor was low in cells harboring plasmid pmo4F4AB1-2, 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, pmo4F4AB1-2). 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 pmo4F4AE1 and pmo4F4BE1 (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 pmo4F4AE1 and pmo4F4BE2 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 *Bsal*-Asp718 DNA fragment from the wild-type *UPF1* gene was replaced with an analogous DNA fragment from either plasmid pmo4F4AE1 or pmo4F4BE, harboring the *mof4-1* mutation (Figure 2B, pmo4XAE and pmo4XBE).

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 M1 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 *upf1* or *ifs* alleles, the *mof4-1* allele of *UPF1* increases ~1 ribosomal frameshifting efficiency and causes loss of the *M1* 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

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**Table II.** Strains used in this study

| Strain         | Genotype                      | Reference       |
|----------------|-------------------------------|-----------------|
| YGC106         | MATα ade2-3 ura3 leu2 his7 can1 sap3 upfΔ::hisG | this study      |
| Y109           | MATα leu2-1::pJD85 ura3 his4 mof4Δ::ura3 K+ | this study      |
| JD742-2D       | MATα leu2-1::pJD85 ura3 his3,4 LAHN M1 K+   | this study      |
| cross JD830    | MATα rph1-1 his4-192 ura3-25 upfΔ::hisG      |                |
| Y52−           | MATα rph1-1 his4-192 ura3-25 trp1-Δ1 upfΔ::hisG | this study      |
| Y46            | MATα his4-38 SUFI-1 ura3-25 met14 upf1-2     | this study      |
| PLY36          | MATα his4-38 SUFI-1 ura3-25 upf2-1           | this study      |
| PLY136         | MATα his4-38 SUFI-1 ura3-25 upf2-1           | this study      |
| JD61           | MATα his4-644 leu2-1::pJD85 K+             | this study      |
| JD747-3D       | MATα leu2-1::pJD85 ura3 his4 mof4-1 K+     |                |
| JD747-5A       | MATα leu2-1::pJD85 ura3 his4 trpl1 ade2 mof4-1 K+ |                |
| 1074           | MATα kar1-1 leu1 LAHN M1 K+               | this study      |
| 3164           | MATα kar1-1 arg1 LAHN M1 K+               |                |
| 3165           | MATα kar1-1 arg1 thr1(x) LAHN M1 K+       |                |
| SX47           | MATαMATα hisI+ trpl1+ ura3+/ K+             | this study      |
| ifs1           | MATα cup15::ura3 ura3-32 his3-Δ200 ade2 lys2 trpl1 leu2 ifs1-2 K+ | this study      |
| ifs2           | MATα cup15::ura3 ura3-32 his3-Δ200 ade2 lys2 trpl1 leu2 ifs2-1 K+ | this study      |

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*mo4-1* in mRNA turnover and ribosomal frameshifting
with cells harboring a ufp1Δ mutation must result from something other than stabilization of the reporter mRNA, suggesting that the mof4-1 allele increases the efficiency of programmed –1 ribosomal frameshifting.

The contribution of frameshift suppression independent of the programmed –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 (pTT24; Dinman et al., 1991) was determined with regard to pTT25, the zero frame control. We found that a mof4-1 strain was able to enhance frameshift suppression from the lacZ fusion transcript synthesized from pTT24 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 programmed 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 programmed –1 frameshift signal. Therefore, frameshift suppression at the termination codon does not account for the apparent increase in the efficiency of programmed –1 ribosomal frameshifting in mof4-1 cells.

The results above have demonstrated that the recessive mof4-1 allele increased the efficiency of programmed –1 frameshifting. The changes in ribosomal frameshifting efficiencies have been shown to have major effects on the ability of cells to propagate the M1 killer virus (Dinman and Wickner, 1992, 1994). Cells harboring the mof4-1 allele lose the ability to propagate the M1 dsRNA virus. Therefore, we next examined whether mutations that inactivate the nonsense-mediated mRNA decay pathway affect the maintenance of the M1 dsRNA virus. L-A and M1 were introduced by cytoduction into strains harboring the mof4-1, ufp1Δ, upf1-2, upf2-1, upf2Δ, 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 M1 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 mof4Δ/upf1Δ strains were both able to propagate the M1 dsRNA virus, as demonstrated by the zone of growth inhibition around these colonies (Figure 2A). Similarly, cells harboring the ufp1, upf2, ifs1 and ifs2 alleles maintained M1 (Table III). Consistent with the loss of killer phenotype, the 1.8 kb M1 dsRNA was absent in the mof4-1 cells but present in all other ufp 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 M1 dsRNA virus are the consequence of the mof4-1 allele rather than a secondary mutation within the cell. First, a single copy UFP1 gene introduced into mof4-1 cells on a centromere plasmid rescued the ability of mof4-1 cells to maintain M1, while the vector-transformed cells had no effect (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 UFP1 gene restored the ability of these cells to propagate M1 (see Figure 3A; Table IV #3). Third, tetrad analysis of cells harboring the mof4-1 allele crossed with a MOF+ L-A+ M1+ strain was performed to determine whether the loss of the killer phenotype co-segregated with the increased β-galactosidase.

Fig. 2. Identification and characterization of the lesion in the mof4-1 allele. Hybrid genes between the wild-type UFP1 and the mof4-1 alleles schematically represented in (A) were constructed, transformed into a ufp1Δ 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 UFP1 gene while the hatched rectangle represents sequences from the mof4-1 allele. The cysteine/histidine-rich region of the UFP1 gene is represented by a gray rectangle in the wild-type UFP1 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 (EcoRI), Bst (BsiXI), Asp (Asp718), B1 (BamHI).
Fig. 3. The β-galactosidase activity, killer phenotype and maintenance of M1 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 + PYCp3UPF1) 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 M1 virus. The growth inhibition was seen around the strains harboring the M1 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 M1 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 M1 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 M1 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 M1 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 M1 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 M1 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.
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 M1 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).
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 M1 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

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

| Paromomycin (µg/ml) | Percentage of -1 ribosomal frameshifting | 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                      |

*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<sub>600</sub>/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.*
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 M1, 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 M1 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 (Sk+) phenotype, or increased M1 titers, upon yeast cells (Wickner et al., 1991; Masison et al., 1995), the opposite of the loss of M1 phenotype (i.e. a Mak- phenotype), and (ii) strains containing the upf1Δ, upf1-2, upf2-1, upf2Δ, ifs1-1 or ifs2-1 alleles, which inactivate the nonsense-mediated mRNA decay pathway equivalently to the mof4-1 allele, do not promote loss of M1 (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 M1 dsRNA virus. That the mof4-1 allele of the UPF1 gene cannot maintain M1 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 M1 dsRNA virus (Table III). The ability to propagate the M1 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 M1 virus (Table III). By using both the frameshift reporter system and monitoring the ability to propagate the M1 virus as measures of alterations in frameshifting, the ifs mutants do not alter the efficiency of programed −1 frameshifting to promote loss of the M1 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 M1 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 ratio of Gag to Gag–pol. 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; Koontz et al., 1992). Purification of the UPF1 protein demonstrated that it is an RNA binding protein with ATPase and helicase activities (Czapinski 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 their 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
The plasmids pJD107 and pJD108 used for f-galactosidase assay 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 LacZ mRNA was purified with a [32P]CTP-labeled 3.1 kb DNA fragment encoding the lacZ gene. The RNA blots were quantitated times and did not vary by >15%.

**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 the solubilized golgiassidase assay for the killer was performed as described (Dinman and Wickner, 1994). Testing for paromycin 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 M1 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 [32P]CTP-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 mRNA 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 tetAΔ and the temperature-sensitive allele of the RNA polymerase II (rho−). The 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 G-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 HindIII fragment from pF8 was ligated into HindIII-digested pRS426 (Christianson et al., 1992) and contains the PGK1 promoter, a translational start site, followed by a 218 bp DNA 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 HindIII fragment of pT125 cloned into the HindIII 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 RNA abundance. HindIII fragments from pJD107 were digested to 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 BglII–HindIII 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 pmo4AE, pmo4AB, used to clone the mof4-1 allele, were constructed as follows: the 1.47 kb Asp718–EcoRI fragment or the 2.6 kb Asp718–BamHI 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). pmo4BE was cloned by inserting the 4.2 kb EcoRI–BamHI PCR DNA fragment from the mof4-1 strain into pYCP33. Since the pYCP33UPF1 contains more than one BsuXI site, pmo4XAE and pmo4XBE were constructed by two steps. A 978 bp BsaXI–Asp718 DNA fragment from pUCP-UPF1 (Cui et al., 1995) was replaced with a BsaXI–Asp718 DNA fragment from pmo4AE and pmo4BE, forming pUCPmo4XAE and pUCPmo4XBE respectively. The 4.2 kb BamHI–EcoRI fragments from these two plasmids were cloned into pYCp33 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’-CCGGTACCTGATTACGAC-3’; primer-2, 5’-GACGCGCTGAACGGACGTTGATC-3’; primer-3, 5’-ATCCGGCCGAGTTGAAAGTGC CATC-3’; primer-4, 5’-GACGGATCCAAGATATTTGAC-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 pmo4AE (Figure 2); primer-3 and primer-4 were used to synthesize the DNA fragment to construct pmo4AB (Figure 2) and primer-1 and primer-4 were used to construct pmo4BE (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 YCp33 vector as described above.

**Acknowledgements**

We thank Terri Kinzy, Kevin Czapinski, Kevin Hagan, Reed Wickner, Maria Ruiz-Echevarria, Thomas Thisted and Shuang Zhang for useful discussions and for critical reading of the manuscript. This work was supported by a grant from the National Institutes of Health (GM48631-01) and by an American Heart Association Established Investigator Award given to S.W.P. Y.C. is supported by the NIH training grant (AI07403-05) of ‘Virus–Host Interactions in Eukaryotic Cells’.

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Received on April 10, 1996; revised on July 1, 1996