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Mutations in the *MOF2/SUI1* gene affect both translation and nonsense-mediated mRNA decay

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ABSTRACT

Recent studies have demonstrated that cells have evolved elaborate mechanisms to rid themselves of aberrant proteins and transcripts. The nonsense-mediated mRNA decay pathway (NMD) is an example of a pathway that eliminates aberrant mRNAs. In yeast, a transcript is recognized as aberrant and is rapidly degraded if a specific sequence, called the DSE, is present 3′ of a premature termination codon. Results presented here show that strains harboring the *mof2-1*, *mof4-1*, *mof5-1*, and *mof8-1* alleles, previously demonstrated to increase the efficiency of programmed –1 ribosomal frameshifting, decrease the activity of the NMD pathway. The effect of the *mof2-1* allele on NMD was characterized in more detail. Previous results demonstrated that the wild-type *MOF2* gene is identical to the *SUI1* gene. Studies on the *mof2-1* allele of the *SUI1* gene indicate that in addition to its role in recognition of the AUG codon during translation initiation and maintenance of the appropriate reading frame during translation elongation, the Mof2 protein plays a role in the NMD pathway. The Mof2p/Sui1p is conserved throughout nature and the human homolog of the Mof2p/Sui1p functions in yeast cells to activate NMD. These results suggest that factors involved in NMD are general modulators that act in several aspects of translation and mRNA turnover.

Keywords: frameshifting; nonsense mutation; ribosome

INTRODUCTION

Cells have evolved elaborate mechanisms to rid themselves of aberrant proteins and transcripts that can dominantly interfere with the normal functioning of cellular complexes (He et al., 1993; Pulak & Anderson, 1993; Jacobson & Peltz, 1996; Ruiz-Echevarria et al., 1996; reviewed in Gottesman et al., 1997; Suzuki et al., 1997; Weng et al., 1997). Such pathways can be viewed as regulators of gene expression or as sensors for inappropriate polypeptide synthesis. An example of a cellular process that eliminates aberrant mRNAs that produce such polypeptides is the nonsense-mediated mRNA decay pathway (NMD; Maquat, 1995; Caponigro & Parker, 1996; Jacobson & Peltz, 1996; Ruiz-Echevarria et al., 1996; reviewed in Weng et al., 1997). Nonsense mutations within the protein coding region of a gene can enhance the decay rate of the mRNA transcribed from that gene at least 20-fold. The NMD pathway appears to have evolved to ensure that transcripts that terminate at the inappropriate codon within the mRNA are eliminated and has been observed to function in all eucaryotic systems examined. Transcripts containing premature nonsense codons are rapidly degraded, thus preventing synthesis of incomplete and potentially deleterious proteins.

The NMD pathway has been extensively investigated in the yeast *Saccharomyces cerevisiae* (Zhang et al., 1995; Caponigro & Parker, 1996; Jacobson & Peltz, 1996; Ruiz-Echevarria et al., 1996; reviewed in Weng et al., 1997; see Czaplinski et al., 1998). Based on these studies, a model for the mechanism of how the NMD pathway functions has been proposed (Ruiz-Echevarria et al., 1996; Weng et al., 1997; Czaplinski et al., 1998). Either concurrently or immediately after export of the mRNA to the cytoplasm, cytoplasmic ribosomes become associated with the transcript and begin translation. The presence of a nonsense mutation causes the ribosome to prematurely terminate translation. Following termination, we hypothesize that a "surveillance complex" scans 3′ of the nonsense codon and interacts with specific sequences and/or their

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associated factors, which have been defined as downstream elements (DSEs). DSEs are required for destabilization of nonsense-containing mRNAs (Peltz et al., 1993; Hagan et al., 1995; Zhang et al., 1995; Ruiz-Echevarria & Peltz, 1996; Ruiz-Echevarria et al., 1998b). The sequence requirements of the DSE in the yeast *S. cerevisiae* have been investigated (Peltz et al., 1993; Hagan et al., 1995; Zhang et al., 1995; Ruiz-Echevarria & Peltz, 1996; Ruiz-Echevarria et al., 1998b). A major determinant of a DSE consists of two copies of a sequence motif UGYYGAUGYYYYY, in which Y can be U or C (Zhang et al., 1995). We suggest that interaction of the surveillance complex at the DSE and with factors associated with the DSE promotes an aberrant RNP structure and renders the mRNA susceptible to rapid decapping (Muhlrad & Parker, 1994; Hagan et al., 1995; Czaplinski et al., 1998; Ruiz-Echevarria et al., 1998a). The uncapped nonsense-containing mRNA is then rapidly degraded by the 5′ → 3′ Xrn1p exoribonuclease (Muhlrad & Parker, 1994; Hagan et al., 1995).

We have been characterizing the *trans*-acting factors implicated to function as part of the surveillance complex and that are essential for NMD. Mutations in the *UPF1*, *UPF2*, and *UPF3* genes were shown to selectively stabilize mRNAs containing early nonsense mutations without affecting the decay rate of most wild-type mRNAs (Leeds et al., 1991, 1992; Cui et al., 1995; He & Jacobson, 1995; Lee & Culbertson, 1995). Studies using the two-hybrid system demonstrated that the Upf1p, Upf2p, and Upf3p interact and form a complex (He & Jacobson, 1995; Weng et al., 1996a; He et al., 1997).

The *UPF1* gene and its protein product have been most extensively investigated (Altamura et al., 1992; Koonin, 1992; Leeds et al., 1992; Czaplinski et al., 1995; Cui et al., 1996; Weng et al., 1996b, 1997). The Upf1 protein (Upf1p) demonstrated RNA binding, ATPase, and RNA helicase activities (Czaplinski et al., 1995; Weng et al., 1996a, 1996b, 1998). Recently, the Upf1p was demonstrated to interact with the translation termination release factors and is thought to enhance their translation termination efficiency (Weng et al., 1996b, 1998; Czaplinski et al., 1998). The recently identified human homolog of the *UPF1* gene, RENT1 or HUPF1, can function in preventing nonsense and frameshift suppression in yeast cells, indicating that this is an evolutionarily conserved pathway (Perlick et al., 1996; Applequist et al., 1997; Czaplinski et al., 1998).

Recent studies have linked the Upfps and programmed −1 ribosomal frameshifting (Cui et al. 1996; Ruiz-Echevarria et al., 1998b). Programmed −1 ribosomal frameshifting is used predominantly by viruses to induce elongating ribosomes to shift reading frame in response to specific mRNA signals (for reviews see Chandler & Fayet, 1993; Dinman, 1995; Farabaugh, 1995; Hayashi & Murakami, 1995). Further, it can also be used as an assay to monitor the ability of cells to accurately maintain a translational reading frame. A screen for mutations that increased the programmed −1 ribosomal frameshift efficiencies in yeast cells identified nine chromosomal *mof* mutants (for maintenance of frame; Dinman & Wickner, 1992, 1994). The *mof4-1* allele was demonstrated to be allelic to *UPF1* (Cui et al. 1996). Subsequently, strains in which the *UPF3* gene was deleted (*upf3*Δ), but not strains harboring a *UPF2* deletion (*upf2*Δ), also increased programmed −1 ribosomal frameshifting efficiencies (Ruiz-Echevarria et al., 1998b). These results suggest that the complex containing the Upf1p and Upf3p may be part of a general surveillance pathway involved in modulating the fidelity of several aspects of translation and mRNA turnover (Cui et al. 1996; Ruiz-Echevarria et al., 1998b).

Based on the results obtained with the *mof4-1* and *upf3*Δ strains, we investigated whether other *mof* alleles affected the activity of the NMD pathway. In this report, we investigated the effects of the *mof* mutations on the accumulation of nonsense-containing mRNAs. The results demonstrated that strains harboring the *mof2-1*, *mof5-1*, and *mof8-1* alleles also accumulated a subset of nonsense-containing mRNAs. The wild-type *MOF2* gene was previously shown to be allelic to the *SUI1* gene (Cui et al. 1998). Mutations in the *SUI1* gene were shown to affect start site selection, allowing translation to initiate at a non-AUG codon, and to increase programmed −1 ribosomal frameshifting (Yoon & Donahue, 1992; Cui et al. 1998). The results presented here demonstrate that a mutation in the Mof2/Sui1 protein (Mof2p/Sui1p) also stabilizes a subset of nonsense-containing mRNAs. Taken together, these results suggest that the Mof2p/Sui1p may function as a general regulator for RNA recognition in the processes of translation and mRNA decay.

RESULTS

A subset of *mof* mutants affected the accumulation of nonsense-containing mRNAs

Previous results demonstrated that strains harboring the *mof4-1* allele of the *UPF1* gene accumulated nonsense-containing mRNAs and increased programmed −1 ribosomal frameshifting (Cui et al., 1996). Based on these observations, we determined whether additional *mof* mutants also affected the nonsense-mediated mRNA turnover pathway. To accomplish this, the abundance of the nonsense-containing mini-PGK1 mRNAs was determined. The mini-*PGK1* alleles harbored an amino-terminal UAA, UAG, or UGA nonsense mutation in which most of the protein coding region was deleted and a DSE from the *PGK1* gene inserted 3′ of the premature stop codon (Fig. 1A; Peltz et al., 1993; Hagan et al., 1995; Zhang et al., 1995). Consequently, these nonsense-containing mini-PGK1 mRNAs

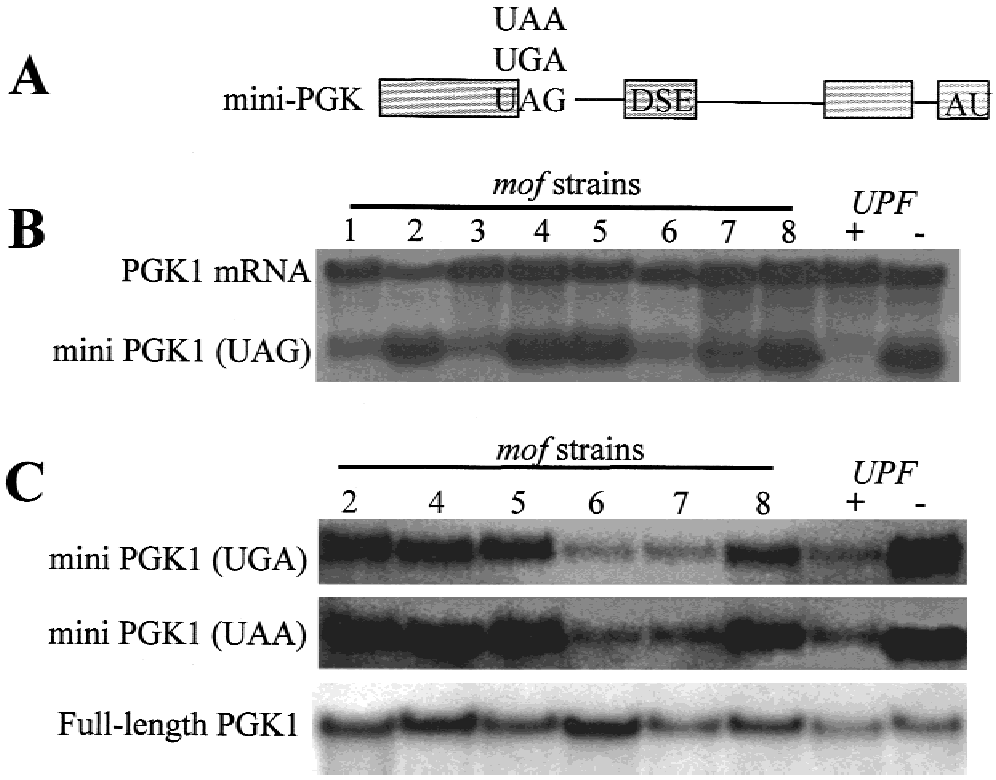

FIGURE 1. A subset of *mof* mutants affect nonsense-mediated mRNA decay. **A**: The schematic drawings for the corresponding transcripts are shown. **B**: The steady-state levels of the PGK1-mRNA and the UAG nonsense-containing mini-PGK1-mRNA were determined by Northern blot analysis in *mof* mutant and control cells grown at 24 °C. **C**: The steady-state levels of the UGA and UAA nonsense-containing mini-PGK1-mRNA were determined by Northern blot analysis in *mof* mutant and control cells grown at 24 °C. The steady-state level of full-length PGK1-mRNA is also shown.

are rapidly degraded in wild-type cells (Peltz et al., 1993; Hagan et al., 1995; Zhang et al., 1995). The susceptibility of these mRNAs to NMD is absolutely dependent on the presence of a DSE; a nonsense-containing mini-PGK1 mRNA lacking a DSE is a stable transcript (Peltz et al., 1993; Hagan et al., 1995; Zhang et al., 1995). Centromere-based plasmids harboring nonsense-containing mini-*PGK1* alleles were transformed into the eight *mof* strains. The steady-state levels of the mini-PGK1 mRNAs were determined by Northern blot analysis. The results demonstrated that, in addition to *mof4-1*, strains harboring *mof2-1*, *mof5-1*, and *mof8-1* alleles demonstrated a fivefold increase in the abundance of the nonsense-containing mini-PGK1 mRNAs regardless of which stop codon was used (Fig. 1B,C). *mof1-1*, *mof3-1*, and *mof6-1* had no significant effect on the accumulation of these mRNAs (Fig. 1B). Interestingly, although strains harboring the *mof7-1* allele demonstrated an increased abundance of the UAG-containing mini-PGK1 mRNA (Fig. 1B), the abundances of the UAA- and UGA-containing mini-PGK1 transcripts did not increase in this strain and were equivalent to their abundances in wild-type cells (Fig. 1C). This may be a consequence of translational read-through at the UAG termination codon in the *mof7-1* strain or due to a mutation in a specific factor involved in the degradation of UAG-containing transcripts.

The effect of *mof* mutants on the accumulation of other substrates of the NMD pathway was also examined. The abundance of the precursor and mature CYH2 mRNA, which encodes a ribosomal protein, was monitored. The inefficiently spliced CYH2 precursor, which contains an intron near the 5′ end, is a naturally occurring substrate for the nonsense-mediated mRNA decay pathway (He et al., 1993). In addition, the abundance of both the full-length wild-type and a nonsense-containing PGK1 transcript harboring a 5′-proximal nonsense mutation was determined in the *mof* strains. The results demonstrated that cells harboring the *mof4-1* allele of the *UPF1* gene increased the steady-state levels of both the CYH2 precursor and full-length nonsense-containing PGK1 mRNA tenfold (Fig. 2A,B) and their mRNA abundances were equivalent to that found in a *upf1*Δ strain (Fig. 2A and data not shown). However, the *mof2-1*, *mof5-1*, and *mof8-1* mutants accumulated the CYH2 precursor to less than twofold more compared to wild-type cells (Fig. 2A). The full-length nonsense-containing PGK1 mRNA also accumulated to less than twofold in the *mof5-1* and *mof8-1* mutants (Fig. 2B). The abundances of the wild-type CYH2 and

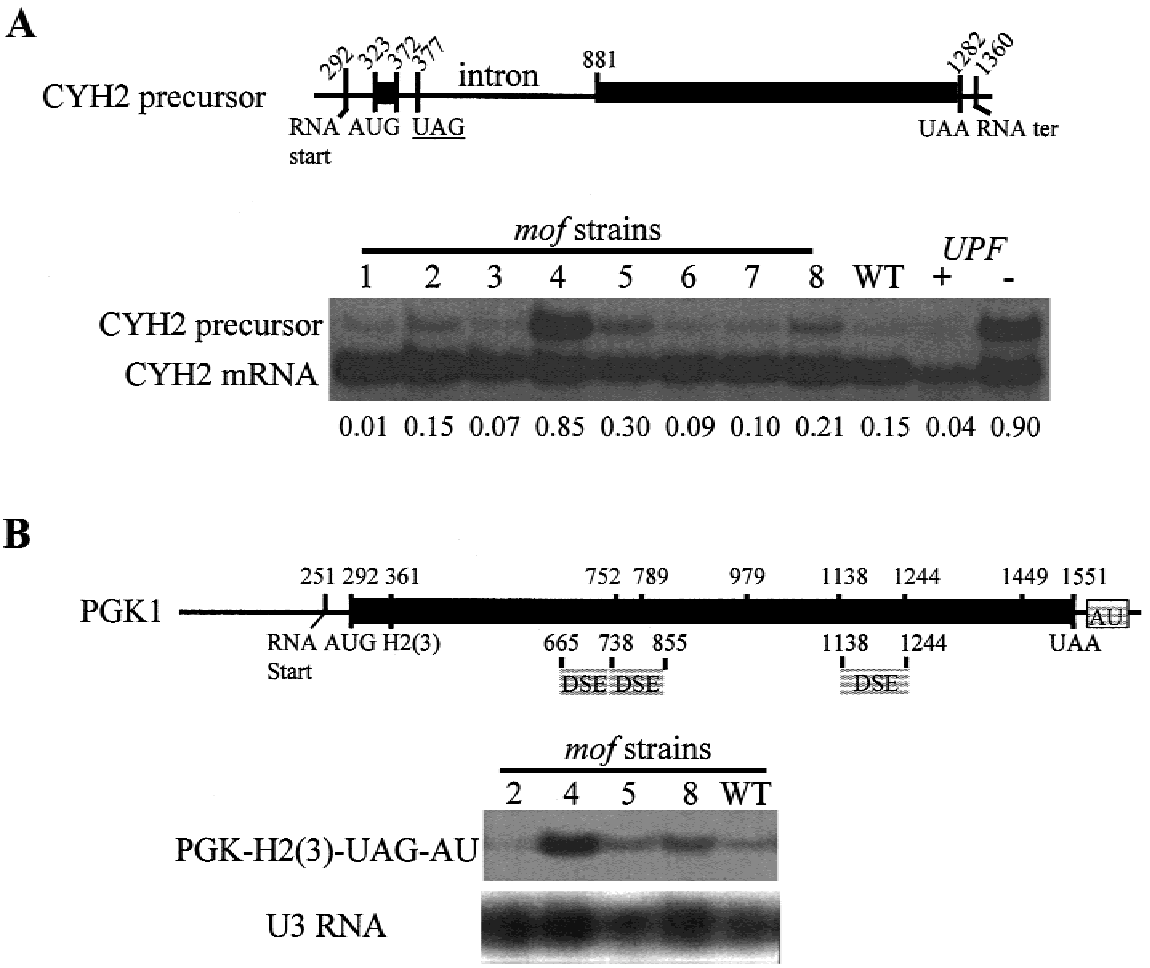

FIGURE 2. The effect of *mof* mutants on steady-state abundance of two nonsense-containing mRNAs. **A**: The abundance of CYH2 precursor and mRNA. **B**: The PGK1 mRNA abundance. All membranes were probed with ^{32}P-radiolabeled CYH2 or PGK1 DNA fragments. The abundance of nonsense-containing mRNAs was quantitated by Bio-Rad model G-250 Molecular Imager and normalized to the wild-type mRNAs and total amount of RNAs loaded.

PGK1 transcripts, which are not substrates of the NMD pathway, were equivalent in both *mof* mutants and wild-type cells (Fig. 2 and data not shown). These results indicate that the effect of *mof2*, *mof4*, *mof5*, and *mof8* alleles on the abundance of the mini-PGK1 transcript is identical to what is observed in a *upf1*Δ strain (Fig. 1). In fact, the abundance of the mini-PGK1 transcript in *mof2-1*, *mof5-1*, and *mof8-1* strains is equivalent to what is observed in a strain harboring a *mof4* allele, which is an allele of the *UPF1* gene (Cui et al., 1996).

The observation that the *mof2-1* allele affects the activity of the NMD pathway is very interesting in light of previous observations demonstrating that mutations in this gene affect translation start site selection and programmed −1 ribosomal frameshifting (Castilho-Valavicius et al., 1990; Yoon & Donahue, 1992; Cui et al., 1998). Therefore, since Sui1p/Mof2p appears to be an important factor that modulates the fidelity of numerous processes in translation and decay, the NMD properties of the *sui1/mof2* alleles were characterized further.

The nonsense-containing mini-PGK1 transcript accumulated in *mof2-1* cells at both the permissive and nonpermissive temperatures

An experiment was performed to determine whether the accumulation of nonsense-containing mRNAs is linked to the temperature-sensitive growth phenotype of the *mof2-1* allele. The accumulation of nonsense-containing mRNAs in this strain was monitored at the nonpermissive temperature. Cells were grown at 24 °C to mid-log, shifted to 37 °C, and cell aliquots were collected at various times after the temperature shift. The abundances of nonsense-containing mini-PGK1 mRNA and the CYH2 precursor were determined by Northern blot analysis. The results demonstrated that the abundance of the mini-PGK1 mRNA and the CYH2 precursor in a *mof2-1* strain was equivalent at both 24 °C and 37 °C (Fig. 3), indicating that the NMD pathway was not further inactivated at 37 °C. Similar results were observed in a *mof4-1* strain, which is not temperature sensitive for growth (Fig. 3).

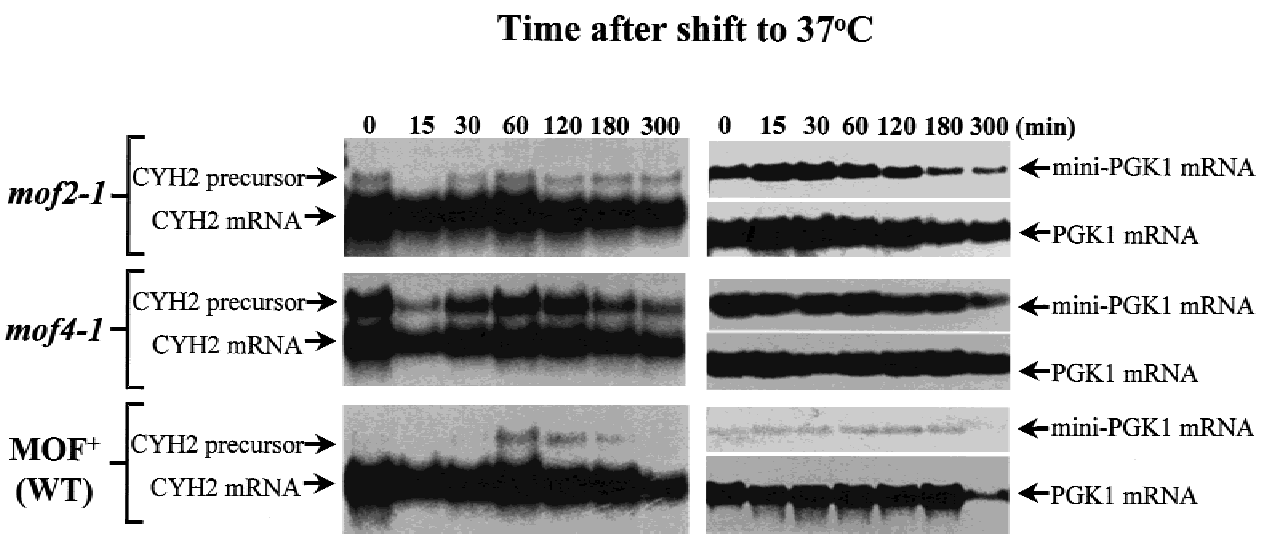

FIGURE 3. Effect of temperature on the abundance of nonsense-containing mRNAs in wild-type, *mof2-1*, and *mof4-1* strains. Cells harboring the mini-*PGK1* allele were grown at 24 °C and shifted to 37 °C. Cell aliquots were collected at different time points after the shift to 37 °C, and total RNA was isolated and subjected to Northern blot analysis. The left panel shows the abundance of CYH2 precursor and mRNA. The right panel shows the mini-PGK1 mRNA abundance. All membranes were probed with ^{32}P-radiolabeled CYH2 or PGK1 DNA fragments. The abundance of nonsense-containing mRNAs were quantitated by Bio-Rad model G-250 Molecular Imager and normalized to the wild-type mRNAs and total amount of RNAs loaded.

Strains harboring the *mof2-1* allele do not efficiently recognize the DSE in the mini-PGK1 transcript

We investigated the reason why a *mof2-1* strain accumulated the nonsense-containing mini-PGK1 transcript whereas the abundance of either the full-length nonsense-containing PGK1 mRNA or the CYH2 precursor were not significantly affected. Previous results have shown that the turnover of the nonsense-containing mini-PGK1 transcript is absolutely dependent on the presence of the DSE 3′ of the nonsense codon (Hagan et al., 1995). The DSE is a 100-bp DNA fragment isolated from the *PGK1* gene (Peltz et al., 1993; Zhang et al., 1995). It was further demonstrated that multiple copies of a DSE function more effectively in NMD (Zhang et al., 1995). We hypothesized that this DSE is a suboptimal element that can function in wild-type cells but less effectively if the decay apparatus is made less efficient by a *mof2-1* mutation. This possibility was tested by introducing an extra copy of the DSE from the *PGK1* gene into the mini-*PGK1* allele 3′ of the original DSE sequence (Fig. 4, see construct p4072). The abundance of the mini-PGK1 transcripts containing either one or two DSEs was determined in isogenic wild-type *MOF2*$^+$ and *mof2-1* strains (strains Y218 and Y219, respectively) by Northern blot analysis. The results of these experiments demonstrated that, when normalized to the wild-type PGK1 transcript, the level of mini-PGK1 mRNA harboring two DSEs in the wild-type strain was equivalent to the level of mini-PGK1 mRNA harboring a single DSE (Fig. 4B, compare p3279 to p4072). In contrast, the mini-PGK1 mRNA harboring two DSEs exhibited a fivefold reduction in abundance in the *mof2-1* strain when compared to the abundance of the mini-PGK1 mRNA containing a single DSE (Fig. 4B, compare p3279 to p4072). The abundance of the mini-PGK1 mRNA harboring two DSEs accumulated in a *upf1*Δ strain (Fig. 4B, compare lane 7 to lane 8), indicating that this mRNA is a substrate for the NMD pathway.

A potential explanation for the results shown in Figure 4 is that the sequence motif in the DSE, which was shown to be essential for it to promote NMD, is too close to the termination codon to be active in a *mof2-1* strain. Previous results have shown that distance can affect the ability of the DSE to function (Ruiz-Echevarria et al., 1998a). To test this possibility, 71 nt from the DSE lacking the sequence motif were inserted 5′ of the DSE containing the sequence motif in the mini-*PGK1* allele (Fig. 4A, p4073). This makes the distance between the stop codon and the first sequence motif in this construct (plasmid p4073) equivalent to the distance between the stop codon and the second sequence motif in the mini-*PGK1* allele harboring two DSEs (Fig. 4A, compare p4072 to p4073). The abundance of these mRNAs was determined in wild-type *MOF2* and *mof2-1* strains as described above. The results demonstrated that the 71 nt inserted 5′ of the sequence motif did not destabilize the mini-PGK1 mRNA in *mof2-1* cells (Fig. 4, p4073). These results also indicate that a single DSE in the mini-PGK1 mRNA is poorly recognized in *mof2-1* cells. The decreased abundance of the nonsense containing mini-PGK1 alleles was a consequence

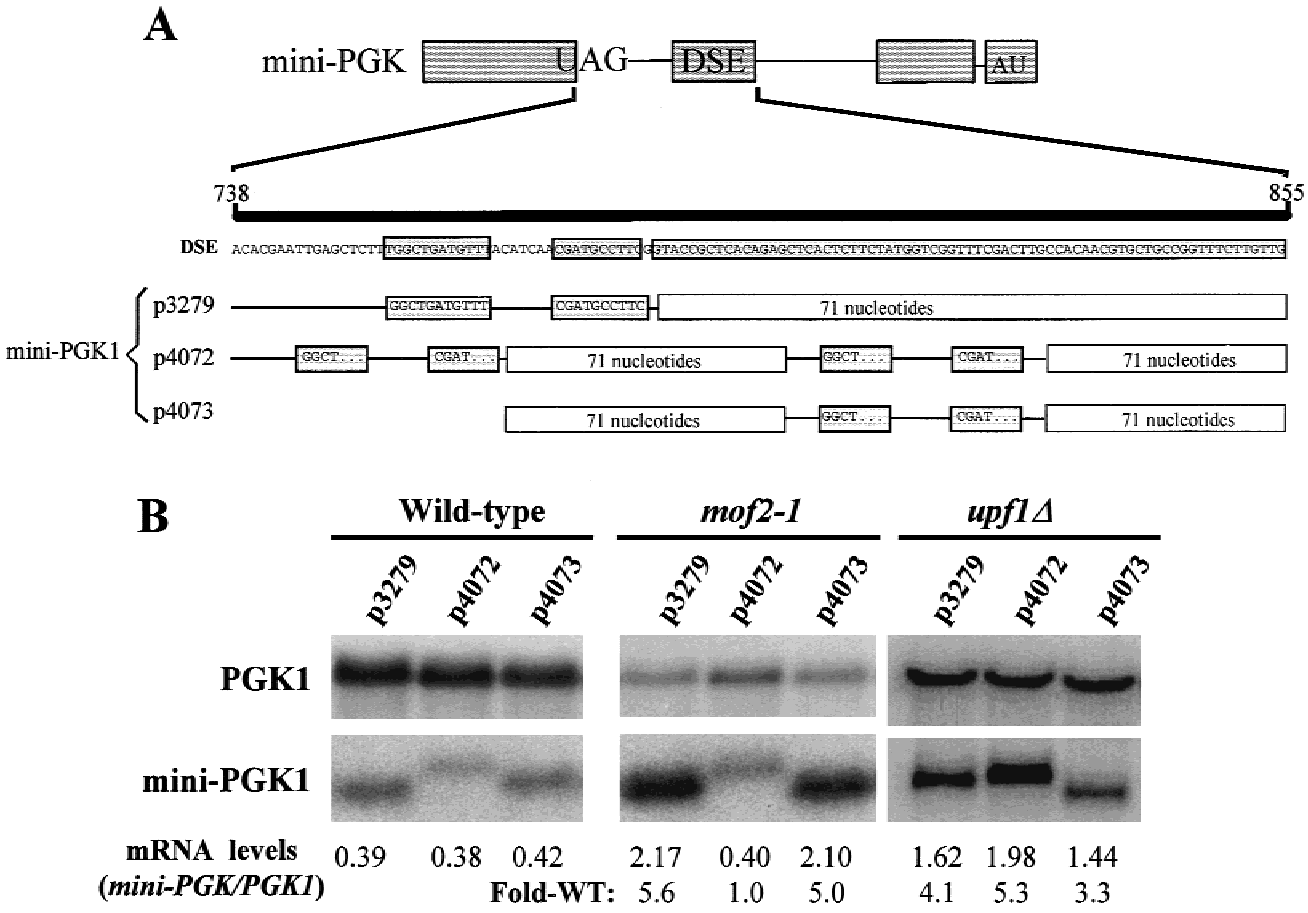

FIGURE 4. Multiple DSEs are required to promote nonsense-mediated mRNA decay in *mof2-1* cells. **A**: Schematic representation of the mini-*PGK1* alleles in which the distances between the DSEs and premature stop codon were varied, or multiple downstream DSEs were inserted 3′ of the stop codon. The sequence of the DSE is shown beneath the thick black line. The shaded rectangles depict the sequence motif and the open rectangles enclose the 71 nt 3′ of the sequence motif. **B**: The abundance of mRNAs corresponding to the constructs above were measured by Northern blot analysis in the isogenic *MOF2/SUI1* cells. The Northern blots were quantitated by GS-670 Densitometer and are presented by the ratios of mini-PGK1 mRNA to PGK1 mRNA. The accumulation of mini-PGK1 mRNAs in *mof2-1* cells is presented by dividing the ratio of mini-PGK1/PGK1 mRNAs from *mof2-1* cells with values from wild-type cells (fold WT). As controls, the abundance of the mini-PGK1 mRNA harboring the double DSE was monitored in both a *upf1*Δ and isogenic wild-type strain.

of NMD as evidenced by an increased abundance of these mRNAs in an isogenic *upf1*Δ strain (Fig. 4B, *upf1*Δ). Taken together, these results suggest that the *mof2-1* allele reduces the ability of the putative surveillance complex to recognize a suboptimal DSE. However, the mutant complex can recognizes a "good" DSE.

The *mof2-1* allele of *SUI1*, but not the *sui1-1* allele nor the *sui2-1* or *SUI3-3* alleles, affect nonsense-mediated mRNA decay

Since mutations in *the MOF2/SUI1* gene affect both −1 ribosomal frameshifting and NMD, we investigated the possibility that these effects are solely attributable to translation initiation defects. Strains harboring the *sui2-1* and *SUI3-3* alleles show similar levels of translational suppression at a UUG codon as *sui1* alleles as monitored by the *his4-UUG-lacZ* reporter construct (Castilho-Valavicius et al., 1990). Therefore, we asked whether the strains harboring the *sui* alleles affected NMD. The NMD phenotypes of strains containing the *mof2-1*, *sui1-1*, *sui2-1*, and *SUI3-3* alleles were determined by monitoring the steady-state levels of the mini-PGK1 mRNA in these strains by Northern blot analysis as described above. The results demonstrated that strains harboring the *mof2-1* allele showed a fivefold increased abundance of the mini-PGK1 mRNA compared to the wild-type *MOF2/SUI1* strain (Fig. 5A). Similar to the results observed using a his4-UUG-lacZ reporter mRNA (Castilho-Valavicius et al., 1990), the mini-PGK1 mRNA abundance in *sui1-1* cells was comparable to wild-type cells, suggesting that the *sui1-1* defect did not affect general mRNA turnover. The differences seen in the *mof2-1* and *sui1-1* strains for the accumulation of nonsense-containing mRNAs was not specific to the strain used in the experiment, as the

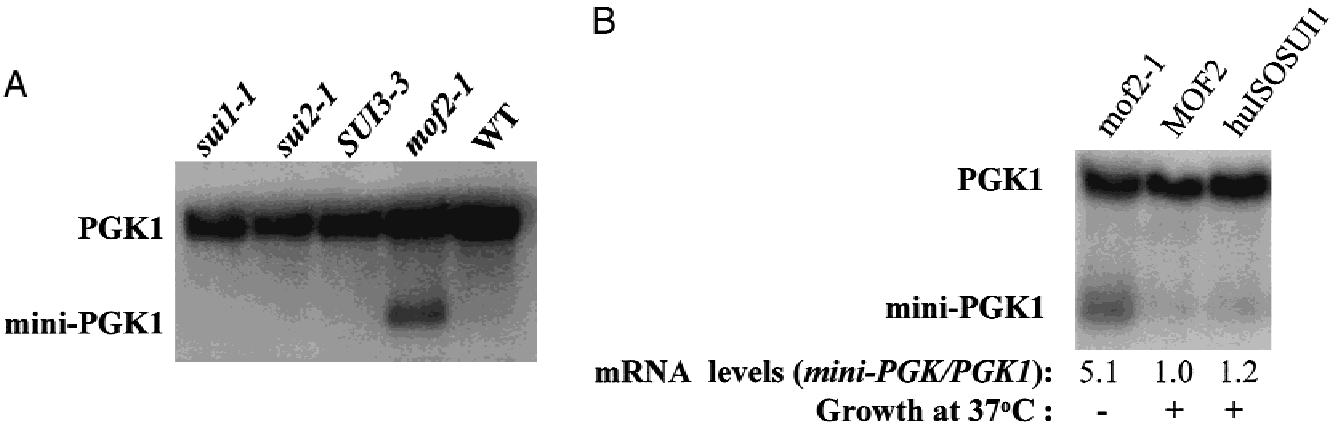

FIGURE 5. A: Nonsense-containing mRNA abundance in *mof2* or *sui* mutant cells. The steady-state levels of nonsense-containing mini-PGK1 mRNA were determined by Northern blot analysis in isogenic wild-type, *mof2-1*, and *sui* suppressor strains (Yoon & Donahue, 1992). The wild-type PGK1 mRNA was probed as a control. **B**: The human *SUI1* gene complements the NMD defect in yeast cells. Either the human homolog of the *MOF2/SUI1* gene (huISOSUI1), yeast *MOF2/SUI1*, or the *mof2-1* allele was expressed in isogenic yeast cells (yeast strains Y221, Y218, and Y219, respectively). The abundance of mRNAs corresponding to the constructs above are measured by Northern blot analysis in the isogenic *MOF2/SUI1* cells. The Northern blots were quantitated by GS-670 Densitometer and the ratios of mini-PGK1 mRNA to PGK1 mRNA were determined. The accumulation of mini-PGK1 mRNAs in *mof2-1* cells is presented by dividing the ratio of mini-PGK1/PGK1 mRNAs from *mof2-1* cells with values from wild-type cells (fold WT). The ability of these plasmids to allow the cells to grow at the nonpermissive temperature was also monitored by replica plating the cells and monitoring their growth at 37 °C. The ability to grow at 37 °C is represented as + while the inability to grow is represented as a −.

same result was obtained when the isogenic *mof2-1* and *sui1-1* strains were utilized (strains Y219 and Y220, data not shown). The mini-PGK1 mRNA abundances in *sui2-1* and *SUI3-3* cells were equivalent to the abundance of this transcript in a wild-type strain (Fig. 5A). The fact that the *sui* mutants did not accumulate the nonsense-containing mini-PGK1 mRNA reflects a different spectrum of defects in a strain harboring a *mof2-1* allele when compared to the other *sui* alleles.

Expression of the yeast or human *MOF2/SUI1* gene reduces the abundance of nonsense-containing mRNAs

A human homolog of the *MOF2/SUI1* gene product (huISOSUI1) has recently been identified by assembling expressed sequence tags (Fields & Adams, 1994). The yeast Sui1p and huISOSUI1 share 60% identity and 80% similarity (Fields & Adams, 1994). To test whether mammalian eIF-1 can substitute for its yeast counterpart Sui1p/Mof2p in vivo to affect NMD, we cloned the human homolog of the *MOF2/SUI1* gene from a human cDNA library by PCR and expressed it from a single copy plasmid containing the yeast glyceraldehyde-3-phosphate dehydrogenase promoter (*GAPDH*) and *PGK1* terminator (Cui et al., 1998). This plasmid was transformed into the *mof2*Δ*/sui1*Δ strain and the yeast *SUI1* gene was lost by plasmid shuffling (see Materials and Methods). The results from these experiments are shown in Figure 5B. The huISOSUI1 supported the growth of *mof2*Δ*/sui1*Δ cells at both permissive and nonpermissive temperatures and reduced the abundance of the nonsense-containing mini-PGK1 mRNA to levels observed in cells harboring the yeast wild-type *MOF2/SUI1* gene (Fig. 5B). These results indicated that the mammalian homolog of the Mof2p/Sui1p can function in yeast cells.

DISCUSSION

We have been investigating the mechanism of how nonsense-containing mRNAs are recognized and degraded in the yeast *S. cerevisiae*. To this end we have been characterizing the *trans*-acting factors involved in the NMD pathway. The results presented here have shown that a subset of the *mof* alleles, which were isolated initially as mutations that affected programmed −1 ribosomal frameshifting, reduced the activity of the nonsense-mediated mRNA decay pathway.

The abundance of the mini-PGK1 transcript in *mof2-1*, *mof4-1*, *mof5-1*, or *mof8-1* alleles was equivalent to the abundance observed in a *upf1*Δ strain and was much higher than the level observed in wild-type strains (Fig. 1). These nonsense-containing mRNAs were not abundant in strains harboring the *mof1-1* and *mof6-1* alleles. Strains harboring either the *mof1-1* or *mof6-1* alleles promote either equivalent or higher efficiencies of programmed −1 ribosomal frameshifting than *mof4-1*, *mof5-1*, and *mof8-1* (Dinman & Wickner, 1994). This suggests that the argument that simply increasing frameshifting efficiencies will result in a general increase in the abundance of nonsense-containing mRNAs cannot be correct. Thus, these results support the hypothesis that the *mof2-1*, *mof4-1*, *mof5-1*, and *mof8-1* alleles

have a specific effect on the process of NMD rather than having a nonspecific effect as a consequence of increased programmed −1 ribosomal frameshifting or generally reduced translational fidelity.

Interestingly, strains harboring these alleles also demonstrated substrate specificity in the accumulation of different nonsense-containing transcripts, and for *mof2-1*, the nature of the DSE. The initial observation that the nonsense-containing mini-PGK1 mRNA accumulated in *mof2-1* to equivalent levels as observed in a *upf1*Δ *s*train whereas the abundance of either a full-length nonsense-containing PGK1 mRNA or the CYH2 precursor was not dramatically altered led us to hypothesize that the *mof2-1* mutant impaired recognition of the suboptimal DSE by the surveillance complex. This appeared to be correct, as inserting a second copy of the DSE reduced the abundance of the nonsense-containing mini-PGK1 transcript. This result may indicate that specialized factors are involved in the NMD pathway in addition to the classic factors such as Upf1p, Upf2p, and Upf3p.

The results presented here demonstrate the involvement of the Sui1p in NMD. In the absence of a functional in vitro NMD assay system, we cannot definitively determine whether the effect of the Mof2-1p on NMD is direct or indirect. However, the notion that this protein plays a direct role in NMD is supported by the following results: (1) The *sui2* and *SUI3* mutants, which allow an equivalent level of translation initiation at a UUG codon as did the *sui1-1* and *mof2-1* mutants, did not accumulate the nonsense-containing mini-PGK1 mRNA (Fig. 5A); (2) four mutant alleles in the *GCD10* gene, encoding another protein that may be associated with the eIF-3 complex (Garcia-Barrio et al., 1995), did not alter the abundance of the nonsense-containing mini-PGK1 mRNA (data not shown), indicating that defects in factors involved in translation suppression do not generally affect mRNA turnover; and (3) the *mof2-1* mutation does not affect the efficiency of translation initiation at a normal AUG start codon (Cui et al., 1998). Taken together, these results support the view that Sui1p/Mof2p is directly involved in the NMD pathway.

A human homolog of Sui1p/Mof2p has recently been identified, HuISO1SUI1 (eIF-1). HuISO1SUI1 shares 60% identity and 80% similarity with the Mof2p/Sui1p (Fields & Adams, 1994). We have shown that *mof2-1* strain harboring the HuISOSUI1 gene was viable at 37 °C, and no longer accumulated the nonsense-containing mini-PGK1 mRNA. Previous results demonstrated that programmed −1 frameshifting efficiency was reduced to wild-type levels and the M_1 killer virus was maintained when the human homolog was expressed in *mof2-1* cells (Cui et al., 1998). These results suggested that the function of the *SUI1/MOF2* gene is conserved between mammalian and yeast cells. Interestingly, a human homolog of the *UPF1* gene, called RENT1 or hUPF1, has also recently been identified (Perlick et al., 1996; Applequist et al., 1997). The human *UPF1* homolog can also function in yeast cells (Applequist et al., 1997; Czaplinski et al., 1998). Thus, at least two factors involved in this pathway are conserved throughout evolution and affect both mRNA turnover and various aspects of translation.

The *mof2-1* allele is unique in that it resulted in increased accumulation of the nonsense-containing mini-PGK1 mRNA (Figs. 1 and 5A), elevated −1 ribosomal frameshifting efficiency and loss of M_1 killer virus (Cui et al., 1998), and altered translation initiation codon selection (Yoon & Donahue, 1992). Previous results have demonstrated that 30% of the Sui1p is associated with the eIF-3 complex (Naranda et al., 1996). eIF-3 is thought to stabilize the Met-tRNA$_i^{met}$ to the 40S ribosomal subunit and prevent 60S ribosomal subunit joining (Merrick, 1992). We hypothesize that the 70% of the Sui1p that is not associated with this complex may function independently of its role in translation initiation. At present, we do not know whether the eIF3 complex functions in the NMD pathway or if the Mof2p/Sui1p acts independently.

It is important to consider how the Mof2p/Sui1p may be a general regulator of both translation and mRNA turnover. Recent studies examining the role of the mammalian eIF1 (h*SUI1/hMOF2*) in translation initiation have demonstrated that this protein is required for enabling 43S preinitiation complexes to promote formation of 48S subunits at the correct translation initiation codon while destabilizing incorrectly assembled complexes (Pestova et al., 1998). These results suggest that eIF1 monitors fidelity of the translation initiation process by ensuring proper assembly of the initiation complex and promotes disassembly of incorrect or inappropriately bound translation initiation complexes. Based on these results, it is interesting to speculate that the eIF1/Sui1/Mof2 functions as a key regulator in translation (both initiation and elongation) and mRNA turnover by scrutinizing the assembly of complexes that control these processes. We hypothesize that mutations in eIF1 that reduce its ability to promote remodeling of these complexes leads to reduced fidelity. The consequence of these mutations would be initiation at a non-AUG codon, increased programmed frameshifting, and, as described here, stabilization of nonsense-containing mRNAs.

How does the Sui1p regulate the assembly/disassembly process? Although the answer is not known, the paradigm for fidelity in translation initiation and elongation may give us a clue to this problem (see Fig. 6). Nucleotide triphosphate (NTP) hydrolysis is an activity that is common to factors that play central roles in translation initiation and elongation. For example, eIF2, eEF1-α, and eEF-2 utilize GTP hydrolysis as a key component to regulate the accuracy of these processes (reviewed in Kozak, 1992; Merrick, 1992; Burgess & Guthrie, 1993; Rodnina et al., 1995), and in NMD, ATP

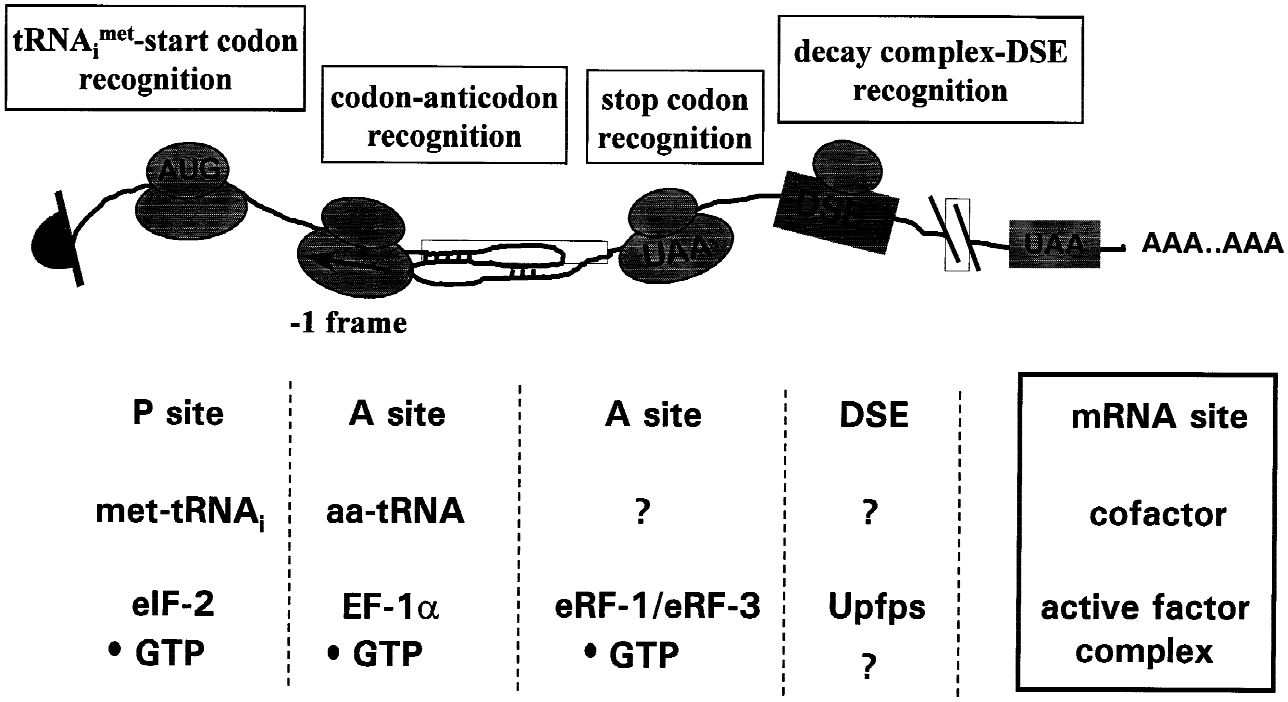

FIGURE 6. Model for how the Mof2p/sui1p can affect multiple steps in translation and mRNA decay. Step 1: Mutations in Mof2p/Sui1p alter recognition of the start codon, potentially via modulating the GTPase activity of eIF-2. Step 2: During elongation, Mof2p/Sui1p functions in proofreading of cognate aa-tRNA in the A-site and may help sense a productive ribosome-eEF-1α interaction required for stimulation of the GTPase activity of eEF-1α. Step 3: Stop codon recognition occurs at the A-site and is modulated by unknown mechanisms, although at least one RF protein is a GTP binding protein and potential Mof2p/Sui1p target. Step 4: Mof2p/Sui1p assists in recognition of the DSE, potentially via an interaction with the Upf protein complex and modulation of nucleotide hydrolysis.

hydrolysis is an activity of the Upf1p (Czaplinski et al., 1995). The *MOF2/SUI1* gene does not demonstrate any similarity with other known genes harboring NTP-binding motifs, suggesting that this protein is not an NTP hydrolyzing protein. It is possible, however, that the Sui1p/Mof2p may be a factor that regulates NTP hydrolysis rates. Thus, the Mof2p/Sui1p may regulate GTP hydrolysis by functioning as a GTPase activating protein (GAP), or as a modulator of the GAP, to enhance GTP hydrolysis by other proteins such as eIF-2, EF-1α, and eEF-2. Additionally, although the mechanism of how DSE recognition occurs has not been established, we suggest that its recognition is also governed by NTP hydrolysis, and that the Mof2p/Sui1p is also involved in regulating this process as well (Fig. 6). Our observations are consistent with the hypothesis that mutant forms of this protein may lead to reduced fidelity of these processes by altering NTP hydrolysis rates and reducing RNA recognition (Fig. 6). Thus, this hypothesis suggests that the remodeling role of the eIF1/Sui1p would be regulated by GTP hydrolysis.

Multiple lines of evidence are beginning to lead to the notion that the factors in the NMD pathway have expanded roles in the cell rather than only degrading aberrant mRNAs (Cui et al., 1995, 1996, 1998; Weng et al., 1996a, 1996b, 1997; Czaplinski et al., 1998; Ruiz-Echevarria et al., 1998b). The fact that mutations in the *UPF* or *MOF* genes reduce the accuracy of multiple posttranscriptional processes including translation elongation, translation termination, and mRNA stability suggests that cells harbor a surveillance complex that contains these factors and that this complex functions as a "translational checkpoint" to ensure the accuracy of these processes. The goal of future experiments will be to determine how these factors function in translation and mRNA turnover.

MATERIALS AND METHODS

Strains, media, and genetic methods

The strains of *S. cerevisiae* used are listed in Table 1. YPAD, YPG, SD, synthetic complete medium, and 4.7-MB plates for testing the killer phenotype were as previously reported (Dinman & Wickner, 1992). Strains Y218–221 were constructed by plasmid shuffling (Rose et al., 1990). Briefly, strain JD272 was transformed with plasmids carrying different alleles of *MOF2/SUI1* gene, including wild-type *MOF2*, *mof2-1*, *sui1-1*, and huISOSUI1. The chromosomal copy of the *MOF2/SUI1* gene was then deleted and replaced with the hisG-*URA3*-hisG cassette (Alani et al., 1987). These strains were subsequently plated on media containing 5-FOA and *ura*$^-$ strains (*mof2::*hisG) were isolated. Transformation of yeast and *Escherichia coli* were performed as described previously (Cui et al., 1995).

TABLE 1. Strains used in this study.

Strain	Genotype	Reference
YGC106	*MATa ade2,3 ura3 leu2 his7 can1 sap3 upf1::*hisG	Cui et al., 1996
JD75-1A	*MATa leu2-1::pJD85 his4-644 ura3 ade2 mof1-1*	Dinman & Wickner, 1994
742-9B	*MATa leu2-1::pJD85 his3 and/or his4 ura3 mof2-1*	Dinman & Wickner, 1994
JD65-5C	*MATα leu2-1::pJD85 his3,his4 ura3 ade2 trp1 mof3-1*	Dinman & Wickner, 1994
JD474-2C	*MATa leu2-1::pJD85 ura3 ade2 mof4-1*	Dinman & Wickner, 1994
744-3C	*MATα leu2-1::pJD85 his3,his4 ura3 ade2 trp1 mof5-1*	Dinman & Wickner, 1994
469-2C	*MATα leu2-1::pJD85 ura3 ade2 trp1 mof6-1*	Dinman & Wickner, 1994
471-1A	*MATα leu2-1::pJD85 ura3 mof7-1*	Dinman & Wickner, 1994
472-1A	*MATa leu2-1::pJD85 ura3 mof8-1*	Dinman & Wickner, 1994
JD272	*MATα leu2 lys11 ura3-52 trp1Δ* K^-	This study
JD758	*MATa kar1-1 leu1 L-AHN* M_1 K^+	Dinman & Wickner, 1992
5X47	*MATa/MATα his1/+ trp1/+ ura3/+* K^- R^-	Dinman & Wickner, 1992
Y218	*MATα leu2 lys11 ura3-52 trp1Δ mof2::*hisG *[pYcp22MOF2]* K^-	JD272 derivative, this study
Y219	*MATα leu2 lys11 ura3-52 trp1Δ mof2::*hisG *[pYcp22mof2-1]* K^-	JD272 derivative, this study
Y220	*MATα leu2 lys11 ura3-52 trp1Δ mof2::*hisG *[pYcp22sui1-1]* K^-	JD272 derivative, this study
Y221	*MATα leu2 lys11 ura3-52 trp1Δ mof2::*hisG *[pGhuISOSUI1]* K^-	JD272 derivative, this study
sui1	*MATα leu2 his4-303(AUU) ura3-52 sui1-1*	Yoon & Donahue, 1992
sui2	*MATa leu2 his4-303(AUU) ura3-52 sui2-1*	Yoon & Donahue, 1992
SUI3	*MATα leu2 his4-303(AUU) ura3-52 ino1-13 SUI3-3*	Yoon & Donahue, 1992

Analysis of RNA abundance

RNA abundance of CYH2, U3, and PGK1 mRNAs were analyzed by Northern blot analysis using radiolabeled DNA fragments as probes that are complementary to these RNAs (Cui et al., 1996). 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 abundance of the CYH2 precursor and nonsense-containing mini-PGK1 mRNAs 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 more than 15%.

Plasmid constructions

The plasmids pYCp33sui1-1 or pYCp22sui1-1 and strains harboring these plasmids were constructed as described (Cui et al., 1998). To delete the *MOF2/SUI1* gene from the yeast chromosome plasmid, pKOM2 was prepared by first inserting the 1.2-kb *Bam*HI-*Hin*dIII DNA fragment harboring the whole *MOF2/SUI1* gene from YCpBH1.2 into a pUC19 vector and then replacing a 0.75-kb fragment of the *MOF2* gene between *Pst*I and *Bam*HI sites (containing the *MOF2* transcription initiation site and part of the *MOF2* coding region) with a 3.0-kb DNA fragment harboring the hisG-*URA3*-hisG cassette. To make the *mof2*Δ strain, the *Pvu*II-*Pvu*II fragment from pKOM2 was used for transformation.

The plasmid p3279 containing the mini-*PGK1* allele was described previously (Zhang et al., 1995). p4072 harboring multiple DSEs was constructed by inserting an extra copy of full-length functional DSE (containing the sequence motif and downstream flanking sequence of 71 nt) into 3′ terminal *Bgl*II site of plasmid p3279, so that this plasmid contains two exact copies of the DSE. p4073 was constructed by inserting only the 71 nt (lacking the sequence motif) from p3279 into *Nco*I site right after the premature stop codon of p3279, so that this 71 nt provide only the distance between the stop codon and the DSE. The human homolog of the *MOF2/SUI1* gene (huISOSUI1) was isolated and expressed in yeast as previously described (Cui et al., 1998).

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