Differential Effects of Translational Inhibition in cis and in trans on the Decay of the Unstable Yeast MFA2 mRNA*

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Clare A. Beelman and Roy Parker†

From the Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721

Several observations in eukaryotic cells suggest that the processes of translation and mRNA turnover are interrelated. To understand this relationship, we examined the effects of translational inhibition on the decay of the unstable yeast MFA2 mRNA, which is degraded in a 5' to 3' direction following deadenylation (1). Although inhibition of translation in cis stabilizes several unstable mammalian transcripts, inhibiting translation of the MFA2 mRNA in cis, by the insertion of a large stem-loop structure in the 5'-untranslated region (UTR), did not affect the half-life, deadenylation rate, or appearance of specific decay intermediates. Therefore, efficient translational elongation on the MFA2 mRNA is not a requirement for the normal rate, or mechanism, of degradation of this transcript. In contrast, inhibition of translation in trans, by the addition of cycloheximide, stabilized the deadenylated form of MFA2 mRNA. Furthermore, the MFA2 transcripts that were not translated due to a stem-loop in the 5'-UTR were also stabilized in the presence of cycloheximide, suggesting that cycloheximide is likely to affect mRNA stability indirectly. These results suggest possible relationships between the mechanisms of mRNA decay and the translational process.

The utilization of mRNA in the cytoplasm of eukaryotic cells requires the proper coordination of translation and mRNA turnover. Diverse experimental evidence suggests that the degradation of eukaryotic transcripts is often coupled to the translation process (for reviews, see Refs. 2 and 3). For example, the inhibition of protein synthesis, through the use of antibiotics or by mutation of the translational machinery, stabilizes many mRNAs (4-7). Similarly, alterations to mRNAs that block translation initiation or introduce premature stop codons can dramatically change mRNA decay rates (8-12). In order to understand how mRNA degradation rates are determined, it will be important to ascertain the mechanisms by which translation and turnover are related.

Determination of the relationship between translation and mRNA degradation will be aided by a description of the role of the translation process in the turnover of an individual mRNA for which the mechanism of degradation is known. We recently described the decay pathway for the unstable mRNA encoded by the yeast MFA2 gene. In this decay pathway, MFA2 mRNA undergoes rapid poly(A) shortening as a prerequisite for a decapping reaction that exposes the mRNA to degradation in a 5' to 3' direction (1). The rapid decay of this mRNA is, in part, due to specific sequences within the 3'-untranslated region (UTR) of the MFA2 transcript that stimulate the rates of both deadenylation and subsequent decay (13). These MFA2 3'-UTR sequences can be considered analogous to the AU-rich sequences found in the 3'-UTRs of many unstable mammalian mRNAs, such as c-fos and c-myc, that also stimulate both poly(A) shortening and subsequent degradation events (14-16).

Several studies indicate that these mammalian AU-rich instability elements require translation of the mRNA in order to stimulate mRNA degradation. For example, mutation of the translational start codon either of a chimeric β-globin-GM-CSF transcript (destabilized through insertion of the AU-rich element from the GM-CSF 3'-UTR) or of the c-myc mRNA significantly increases the half-life of these transcripts (7, 9, 11). Similarly, the insertion of a stem-loop into the 5'-UTR to block translation initiation can also inhibit the rapid decay induced by the AU-rich element from the GM-CSF transcript (17). Conversely, it has been reported that a 95% reduction in translation rate does not affect the rapid decay induced by the AU-rich element from the c-fos mRNA (18). This apparent translational independence of the c-fos AU-rich element could be due to the possibility that only a low level of translation is needed to activate the decay induced by the AU-rich elements or, alternatively, that individual AU-rich elements may have different requirements for translation. However, given that the bulk of data indicates that the stimulation of mRNA decay by elements within the 3'-UTR requires translation of the mRNA, we hypothesized that translation would also be required for the rapid decay of the yeast MFA2 transcript.

In order to determine the role of the translation process in the decay of MFA2 mRNA, we examined the effects of translational inhibition on the degradation of the transcript. Surprisingly, inhibition of translation in cis did not affect the half-life, deadenylation rate, or accumulation of observed decay intermediates. This result indicated that efficient translational elongation of the MFA2 mRNA is not a requirement for the normal degradation of the transcript. In contrast, inhibition of translation in trans, by the addition of cycloheximide, stabilized the deadenylated form of MFA2 mRNA, by inhibiting the decapping reaction. Furthermore, the MFA2 transcripts that were not translated due to a stem-loop in the 5'-UTR were also stabilized in the presence of cycloheximide, suggesting that cycloheximide is likely to affect mRNA stability indirectly. These results suggest possible relationships between the mechanisms of mRNA decay and the translational process.

**EXPERIMENTAL PROCEDURES**

**Plasmids**

The plasmid expressing the MFA2(B28) transcript, pRP455, was constructed by inserting an 2.3-kilobase pair ScaI/NdeI restriction frag-

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†To whom correspondence should be addressed. Tel.: 602-621-9347; Fax: 621-3799.

1. Muhlrad, D., Decker, C., and Parker, R. (1994) Genes & Dev., in press.

2. The abbreviations used are: UTR, untranslated region; UAS, upstream activating sequence; ZNF, ribonucleoprotein.
ment, containing the GAL1 UAS and a portion of the MFA2 gene of pRP410 (1), into the Scal/XcmI sites of pRP270 (13) in which the autonomic replicating sequence BglII site was filled, creating pRP493. Thus, pRP493 contains an M13 replication origin, centromere and autonomic replicating sequence markers, and the TRP1 gene, as well as the MFA2 transcript under GAL1 control. A BglII site (B28), located 10 nucleotides upstream of the MFA2 start codon, was created on this plasmid by using site-directed mutagenesis with the oligonucleotide, cRP151, 5'-AGGTTTGGATCTTGGTTGGTAT-3', creating the plasmid pRP455. The two underlined nucleotides introduce base changes that create the new BglII site.

The plasmid expressing the MFA2(5'stem) transcript, pRP456, was derived from pRP455 by inserting 5'-GATCCCGCGGTTCGCCGCGG-3', as well as the corresponding anti-sense strand, into B28 of pRP455. This insertion is predicted to form a stable 11-base pair C-Hg stem having a UUCG tetraloop structure within the mRNA with a calculated thermal stability of 11.4 kcal/mol (19).

Plasmids with the 3'poly(G) insertion were made by replacing the 400-nucleotide BamHI/BamHI piece of the MFA2 3'-UTR in both pRP456 and pRP456 with a BamHI/BamHI piece from the plasmid pRP486 (1) that contains a poly(G) insertion in its MFA2 3'-UTR.

MFA2/LACZ fusions were made by fusing the MFA2 coding region to the LACZ coding region at the BamHI site in the coding region of MFA2. The LACZ sequences came from a plasmid, pRP186, which contains a LACZ coding region with a Xhol linker at the 5' end of LACZ sequences and transcriptional terminator and poly(A) addition site from the PGK1 gene of S. cerevisiae.

When relevant, constructs were verified by DNA sequencing of oligonucleotide insertions and translational reading frame junctions.

**Yeast Strains**

Two yeast strains were used during this study. The first strain, yRP713 (MATa, ura3-52, rpl1-1, trp1-1, his3-200, mfa2::URA3, mfa1::LEU2) was derived from a cross of yRP582 (MATa, ade2, ura3-52, lys2-201) and yRP384 (MATa, trp1-A1, his3-MO0, rpbl-I, his4, ete1, and transformants were selected and maintained on synthetic media. Both yRP713 and yRP835 were transformed using lithium acetoacetate. The plasmid expressing the MFA2(5'stem) transcript, pRP264 (13). This strain was used for halo assays, mating assays, in vivo labeling of a-factor, β-galactosidase assays, and for some RNA half-life experiments. The second strain, yRP835 (MATa, rpl1-1, leu2, trp1-1, his3-200, mfa2::URA3), was derived from crossing yRP582 and a MATa, leu2, his, trp1-1 strain. yRP835 was used for the transcriptional pulse-chase experiments and for RNA half-life determinations. Both yRP713 and yRP835 were transformed using lithium acetate, and transformants were selected and maintained on synthetic medium lacking tryptophan.

**Procedures to Determine Translation of MFA2 Constructs**

*Halo Assays*—yRP713 cells transformed with pRP456 and pRP456 were assayed for their ability to form a-factor halos when plated on synthetic media. Cells were grown in a 75-ml volume of medium having a UUCG tetraloop structure within the mRNA with a calculated thermal stability of 11.4 kcal/mol (19).

Immunoprecipitation of a-Factor—Levels of intracellular a-factor produced from pRP455 and pRP456 constructs were ascertained in yRP713 cells by immunoprecipitation of 35S-cysteine-labeled a-factor, essentially as previously described (20). A-factor antibody was kindly provided by Susan Michaelis. Cells were grown in a 75-ml volume of minimal synthetic medium containing only histidine, adenine, lysine, and uracil, and were harvested at 30 °C to a mid-log phase. Cultures were shifted in mid-log phase, and then immediately assayed at 420 nm.

**RNA Procedures**

*Half-life Determination*—Harvesting of cells during time courses and isolation of total RNA for each time point was performed as described by Caponigro et al. (22). Levels of MFA2 mRNAs were probed using standard methods and quantitated using a Betascope (Betagen, Framingham, MA).

Levels of MFA2 mRNAs were standardized by stripping and reprobing the blots with probes specific for scr1, a stable RNA transcribed by RNA polymerase III (23).

Transcriptional Pulse-Chase Experiments—Transcriptional pulse-chases were performed essentially as described by Decker and Parker (1) except that medium containing raffinose was adjusted to pH 6.5 with ammonium hydroxide to prevent acid hydrolysis of raffinose and thus inducing the GAL1 UAS prior to the addition of galactose. Cultures were shifted in mid-log phase, and then run on 6% polyacrylamide denaturing gels. MFA2 mRNA was analyzed by probing specifically for this mRNA.

**RESULTS**

*Strategy*—The first strategy used to determine the effects of translation on the decay of the MFA2 mRNA was to specifically inhibit translational elongation on the MFA2 mRNA. In this experiment, we utilized the approach of blocking translational initiation in cis by the insertion of a strong stem-loop structure into the 5'-UTR. This strategy has been used previously to inhibit the translation of specific transcripts in yeast (10, 24, 25) and in more complex eukaryotes (26). In order to insert sequences into the MFA2 5'-UTR, we first created a new BglII site 10 bases 5' of the translational start codon by the introduction of two base changes. These point mutations do not affect the decay of the MFA2 mRNA (data not shown; see below). Into this BglII site (MFA2/B28) we inserted oligonucleotides that created a secondary structure with an 11-base stem stabilized by a UUCG tetraloop (27). Based on the predicted thermal stability of this structure (∼−17.4 kcal/mol; Ref. 19) and its proximity to the translational start codon, this stem would be predicted to strongly inhibit translational initiation in yeast (24, 25, 26).

The MFA2 transcript arising from this construct is referred to as MFA2(5'stem).

Translation of MFA2(5'stem) mRNA Is Severely Inhibited—To evaluate whether the MFA2(5'stem) transcript was being translated, we introduced plasmids expressing the MFA2(5'stem) transcript under the GAL1 promoter into the yeast strain yRP713 and examined the production of the MFA2 gene product, a-factor. Since the MFA2/B28 transcript behaves identically to the wild-type MFA2 transcript by all assays (data not shown) and is the direct precursor to the MFA2(5'stem) transcript, we included this transcript in each experiment as a wild-type control. The yeast strain yRP713 used in these experiments is disrupted for the chromosomal copy of both the MFA1 and MFA2 genes, thus the only source of a-factor in these strains is from the introduced plasmid.

Three different methods were used to assay the relative translation rate of the MFA2(5'stem). First, we utilized a simple plate assay taking advantage of the ability of a-factor to inhibit the growth of a lawn of α cells in a "halo" around a colony of MATa cells (e.g. see Ref. 29). Using a MATa yeast strain supersensitive to a-factor, we observed that while strains expressing the MFA2/B28 mRNA produced large halos, strains expressing the MFA2(5'stem) produced no detectable halo (Table 1). Although this assay is only semiquantitative, expression of 5% of the level derived from the endogenous MFA2 gene is sufficient to produce a halo.3 Moreover, since these transcripts are overexpressed from the GAL1 promoter at least 10-fold relative to the MFA2 promoter (data not shown),

3 S. Michaelis, personal communication.
these results suggest that the MFA2(5' stem) mRNA is transcribed extremely poorly, if at all.

In order to obtain a more accurate measurement of the relative translation of the MFA2(5’stem) mRNA, we quantitated the amount of immunoprecipitated intracellular radioactive α-factor produced in a brief labeling with [35S]-Cys (see “Experimental Procedures”). As shown in Fig. 1, although a-factor is easily detectable from yeast expressing the MFA2(B28) mRNA, we were unable to detect any labeled a-factor from strains expressing the MFA2(5’stem) mRNA. Based on quantitation of the a-factor bands by directly counting the gel (see “Experimental Procedures”), and from long overexposures of the autoradiogram, we estimated that we could detect 1% of the wild-type level. This result indicated that translation must be inhibited at least 99% percent by the insertion of the 5’ stem-loop structure.

Since the assays described above were not able to detect very low levels of translation of the MFA2(5’stem) mRNA, we replaced the 3’ portion of the MFA2 coding region with a lacZ reporter gene and assayed β-galactosidase activity (see “Experimental Procedures”). This spectrophotometric assay is extremely sensitive, and translational efficiencies less than 1% can be observed (e.g. Ref. 30). In comparison to the unaltered construct, the insertion of the 5’ stem-loop insertion reduced β-galactosidase activity to less than 1% (Table 1). Although this assay has been carried out on a MFA2 mRNA altered by the introduction of the lacZ sequences, these results are consistent with the halo assays and immunoprecipitation of α-factor from the unaltered MFA2 transcripts. Based on these observations, we concluded that the 5’ stem-loop structure inserted into the 5’-UTR of MFA2 mRNA severely inhibited translation.

Inhibition of Translation in cis on the MFA2 mRNA Does Not Alter the Rate of mRNA Decay—Since translation of the MFA2(5’stem) mRNA was severely reduced, we measured the decay rate of this mRNA to determine if efficient ribosome elongation on the MFA2 transcript was required for its rapid decay. In this experiment cells were grown in media containing galactose at 24 °C and transcription was inhibited by thermal inactivation of a temperature-sensitive allele of RNA polymerase II present in the strain and the simultaneous addition of glucose to additionally repress the GAL1 UAS (see “Experimental Procedures”). As shown in Fig. 2, there is no significant difference in the stability of MFA2 constructs with or without the secondary structure insertions (MFA2(5’stem), t1/2 = 6.8 ± 1.0 min; MFA2(B28), t1/2 = 6.2 ± 0.2 min). It should be noted that the decay of the wild-type MFA2 transcript (t1/2 = 6.0 min; data not shown) is slightly slower than in cells grown in glucose medium (t1/2 = 4 min; Ref. 13). The basis for this difference is not known but may be due to the slightly slower growth rate of cells growing with galactose as a carbon source. Since there is no difference between the MFA2(5’stem) mRNA and the essentially wild-type mRNA, we conclude that inhibition of translation in cis on MFA2 mRNA does not affect the stability of this message.

Mechanism of MFA2 mRNA Decay Is Not Affected by Inhibition of Translation in cis—The above experiment indicated that the decay rate of the MFA2 mRNA was not altered by severe inhibition of translation in cis. In order to verify that the mechanism of mRNA decay was not altered, we examined the decay pathway of the MFA2(5’stem) mRNA. Using a procedure to analyze the decay of a synchronously produced population of MFA2 transcripts, referred to as a transcriptional pulse-chase, we recently described a pathway by which the MFA2 mRNA is degraded (1). Features of this pathway include a rapid deadenylation phase preceding 5’ to 3’ decay of the body of the transcript. A useful marker for this decay pathway is an mRNA fragment that accumulates following deadenylation if strong RNA secondary structures, such as a poly(G) tract, are inserted into the 3’-UTR to block 5’ to 3’ exonucleases (1).

In order to determine if the MFA2(5’stem) transcript was degraded by the mechanism described above, we constructed a MFA2(5’stem) transcript containing a poly(G) tract in the 3’-UTR and we examined the decay of the transcript by the transcriptional pulse-chase procedure in comparison to the translatable MFA2(B28) mRNA. As shown in Fig. 3, the MFA2(5’stem) decayed by the same mechanism as the translated MFA2 transcript. In both cases, the transcripts present after the pulse have long, relatively homogeneous poly(A) tails (as judged by comparison to a sample in which the poly(A) tails have been removed by treatment with RNase H and oligo(dT); see lane odG). The poly(A) tails then shorten at the same rate, in a somewhat heterogeneous manner, to produce transcripts with short oligo(A) tails. In addition, for both MFA2(B28) and MFA2(5’stem) transcripts we observed that, following poly(A) shortening, a mRNA fragment corresponding to the 3’ portion of the mRNA accumulated as oligoadenylated mRNA levels decreased. Based on these observations, we conclude that effi-
Fig. 3. MFA2(5'stem) and MFA2(5'stem) mRNAs are degraded by the same mechanism. The figure shows an acrylamide gel of a transcriptional pulse-chase analysis of the decay of the MFA2(5'stem) and MFA2(5'stem) transcripts. The numbers above each lane denote minutes after transcriptional inhibition following a 10-min induction of the GAL1 UAS. The last lanes for both gels show RNA from the 0-min time point that was incubated with oligo(dT) and then digested with RNase H to remove poly(A) tails. Both MFA2(5'stem) and MFA2(5'stem) RNAs were probed with a poly(C) sequence complementary to the poly(G) insert in the 3'-UTR. A, decay of MFA2(5'stem) mRNA; B, decay of MFA2(5'stem) mRNA.

sient translational elongation on MFA2 mRNA is not a requirement for the normal rate, or pathway, of decay for the MFA2 transcript.

Cycloheximide Stabilizes the MFA2 mRNA by Blocking Decapping—One piece of evidence suggesting that translation and mRNA degradation are coupled is the observation that, in several types of eukaryotic cells, the addition of protein synthesis inhibitors prevents the decay of many different transcripts. Since our results suggested that translation of MFA2 mRNA was not required for its decay, we wanted to determine whether or not the turnover of the MFA2 mRNA would be affected by the addition of the protein synthesis inhibitor cycloheximide, which stabilizes many yeast mRNAs (5). To determine if cycloheximide affects MFA2 mRNA decay, cells expressing the MFA2(5'stem) transcript were grown continuously in media containing galactose and then cycloheximide was added at the time of transcriptional inhibition. Since the half-life of the MFA2(5'stem) mRNA was greatly increased in the presence of cycloheximide (Table II), these experiments indicated that the decay of the MFA2 transcript can be inhibited by cycloheximide.

In principle, cycloheximide could slow degradation of the MFA2 transcript by inhibiting deadenylation and/or subsequent decay steps. In order to determine the step(s) in the decay pathway affected by cycloheximide addition we examined the decay of the MFA2(5'stem) mRNA in a transcriptional pulse-chase experiment in which cycloheximide was added at the time of transcriptional inhibition. As shown in Fig. 4A, MFA2(5'stem) transcripts were deadenylated with the same kinetics as in the absence of cycloheximide (see Fig. 3A) but persisted as a 5' to 3' exonuclease caused by a ribosome stalled at the translation initiation codon. These results are consistent with previous work indicating that a ribosome positioned at the AUG codon will protect ~12 adenosine residues. From these data we concluded that the stabilizing effect of cycloheximide on MFA2 mRNA is to greatly reduce the rate of mRNA decay following deadenylation. It should be noted that cycloheximide does not completely block decay after deadenylation, as levels of the deadenylated species decrease with time.

The two nucleolytic events that occur following deadenylation in the decay of the MFA2 mRNA are decapping and 5' to 3' degradation of the transcript body. In order to determine which of these steps is inhibited by cycloheximide, we immunoprecipitated the MFA2 transcripts at early and late time points in the transcriptional pulse chase with antibodies directed against the 5' cap structure. If cycloheximide blocks decapping, we would predict that MFA2 transcripts present at the late time point would still be immunoprecipitated. In contrast, if cycloheximide blocks the 5' to 3' exonucleolytic process following cap removal, then MFA2 transcripts present at late time points would be predicted to not be precipitated. As shown in Fig. 5, at both early and late time points the majority of the MFA2 transcripts are immunoprecipitable in the presence of cycloheximide. Based on this observation, we concluded that cycloheximide inhibits the decapping reaction that follows deadenylation.

A Decay Fragment Corresponding to Full-length MFA2 mRNA Trimmed at the 5' End Accumulates in Cells Treated with Cycloheximide—On overexposures of the autoradiogram shown in Fig. 4A, we noticed that small amounts of mRNA fragments, which are shortened by approximately 20-25 nucleotides, accumulated after deadenylation (Fig. 4B). By primer extension analysis these smaller fragments are shown to be shortened at the 5' end by approximately 24 nucleotides (Fig. 4C). In addition, RNase protection assays indicated that these fragments have the same 3' end as the full-length mRNA (data not shown). These results are consistent with observations that following deadenylation, MFA2 mRNA is degraded in a 5' to 3' direction following deadenylation. Since cycloheximide inhibits translational elongation, the accumulation of these fragments could be explained by a slow rate of decay in the presence of cycloheximide accompanied by a steric block to a 5' to 3' exonuclease caused by a ribosome stalled at the translation initiation codon. These results are consistent with previous work indicating that a ribosome positioned at the AUG codon will protect ~12-13 nucleotides 5' of the AUG codon (31).

Cycloheximide Stabilizes MFA2(5'stem) mRNA—Several types of models have been proposed to explain how protein synthesis inhibitors affect mRNA decay (e.g. Ref. 3). Cycloheximide could have a direct effect on the stability of the message by "freezing" elongating ribosomes onto the RNA and thus physically blocking the degradation machinery from accessing the mRNA. Alternatively, cycloheximide could have an indirect effect on mRNA decay, perhaps by reducing the levels of a labile turnover factor (e.g. Refs. 16 and 18). The MFA2(5'stem) transcript provided a means to distinguish between these hypotheses. Since very few, if any, MFA2(5'stem) transcripts will have elongating ribosomes on them, these mRNAs should be unaffected by cycloheximide addition if cycloheximide effects on mRNA decay are due to a block to translational elongation in cis. Alternatively, if cycloheximide acts indirectly on mRNA
decay, then the untranslatable MFA2(5'stem) transcript would still be stabilized by the addition of cycloheximide. As shown in Table II, the MFA2(5'stem) transcripts are stabilized in the presence of cycloheximide to the same extent as MFA2(B28) mRNA (11/2 > 30 min). Moreover, the step in decay blocked by cycloheximide addition for the MFA2(5'stem) transcript is after deadenylation (data not shown), identical to the block seen for the translatable MFA2(B28) transcript (Fig. 4A). Since both untranslated and efficiently translated MFA2 messages are stabilized by cycloheximide, we conclude that the stabilization of the deadenylated form of MFA2 mRNA by cycloheximide is not due to the accumulation of ribosomes in the coding region (see “Discussion”).

**DISCUSSION**

In this work we have examined the effects of translational inhibition on the degradation of the yeast MFA2 transcript. The mechanism of degradation for the MFA2 mRNA initiates with rapid deadenylation, followed by a decapping reaction that exposes the mRNA to degradation in a 5' to 3' direction (1). Our experiments demonstrated that cycloheximide slows decay of the MFA2 mRNA by inhibiting the decapping reaction. The key observation is that in the presence of cycloheximide, the rate of deadenylation is not significantly affected but deadenylated full-length MFA2 transcripts persist (Fig. 4) and are immunoprecipitable with antibodies against the cap structure even 30 min following transcriptional repression (Fig. 5). Our data suggest that the decapping reaction can occur at a slow rate in the presence of cycloheximide, since the transcript is eventually degraded, and we observed that low levels of mRNA fragments, trimmed approximately 24 nucleotides from the 5' end of the message (Fig. 4C), accumulated after deadenylation. It is interesting to note that the size of this mRNA fragment is consistent with a steric block to the 5' to 3' exonuclease due to a ribosome stalled at the translational initiation codon.

How might cycloheximide inhibit the decapping reaction? Since both poorly (MFA2(5'stem)) and efficiently translated transcripts (MFA2(B28)) are stabilized in the presence of cycloheximide, it is unlikely that the effect of cycloheximide on the decapping reaction is from physically blocking access of degradation factors to the mRNA by driving the transcript into the polysome state. These data could be explained by the proposal that cycloheximide affects the stability of mRNA by blocking the synthesis of a very labile protein required for degradation (16, 18). However, since the effect of cycloheximide on mRNA degradation in yeast is essentially immediate (Ref. 5 and data not shown), such a protein would have to be extremely unstable.

The observation that the degradative event inhibited by cycloheximide in decapping suggests an alternative model for how cycloheximide might affect mRNA decay. In this model, the accessibility of the cap structure to the enzyme responsible for the decapping reaction would be dependent on the mRNP structure of the 5'-UTR. Since the 5'-UTR is the site of a dynamic process of initiation factor assembly, ribosome loading, and translational initiation, there are likely to be many discrete mRNP forms of this region of the transcript. Cycloheximide, by indirectly affecting the levels of translational initiation factors...
and ribosomal subunits, or their post-translational modifications (e.g. Ref. 32), could lead to transcripts accumulating as an mRNP form that is particularly resistant to decapping. An extension of this model is that the poly(A) tail might prevent the decapping reaction before deadenylation by stabilizing a similar mRNP structure (see below).

Our results also indicated that elongating ribosomes on the MFA2 transcript are not required for the proper degradation of the transcript. The critical observation was that, although translation of the MFA2(5' stem) mRNA was severely inhibited, the decay rate of this mRNA was not affected. The half-lives, kinetics of deadenylation, requirement of deadenylation before decay, and the precursor-product relationship of the full-length mRNA to a decay intermediate were identical for both the poorly translated and wild-type MFA2 transcripts. These observations indicated that the MFA2(5' stem) mRNA was degraded by the same mechanism as the MFA2 mRNA. We cannot rule out the formal possibility that an extremely low residual level of translation is required, and sufficient, for this degradative mechanism. However, given that translation of the MFA2(5' stem) mRNA is likely to be less than 1% of the parental MFA2 mRNA, our data strongly favor the conclusion that none of the steps in the degradation of MFA2 mRNA: deadenylation, decapping, or 5' to 3' exonucleolytic digestion, require translocating ribosomes. However, since transcripts with stem-loops in the 5'-UTR could be required for proper mRNA degradation.

The observation that the rapid decay of the MFA2 mRNA does not require elongating ribosomes is surprising, given the number of studies that have shown a translational dependence for the rapid decay of some unstable mammalian mRNAs (7, 9, 11, 17). Whether or not these differences are due to inherent differences between yeast and mammals, different mRNA decay mechanisms, or differences in how individual mRNAs interact with the degradative machinery is currently unclear. Nevertheless, our results indicate that ribosomal elongation will not be a universal requirement for rapid mRNA decay in eukaryotes.

How might the MFA2 mRNA be rapidly degraded independently of elongating ribosomes? In the simplest model, the features of the MFA2 transcript that stimulate degradation interact sufficiently with the degradative machinery to promote decay independently of translation. In this view, the translational dependence of some AU-rich instability elements in mammalian cells could be explained by the presence of ribosomes enhancing the interaction between degradative factors and the sequence elements (e.g. Ref. 9).

An alternative model is suggested by interactions between the 5' and 3' termini of eukaryotic mRNAs. An interaction between the mRNA termini is suggested by the observations that poly(A) tails can stimulate translational initiation (e.g. Refs. 33-35), as well as inhibit cleavage of the cap linkage during mRNA degradation.1 In this view, different forms of this 5'3'-interaction may function to stimulate translation initiation, prevent decapping, and possibly slow deadenylation. Therefore, the function of sequences in the 3'-UTR that stimulate decay, such as those in the 3'-UTR of the MFA2 mRNA, may be to disrupt, or alter, the 5'-3' interaction and thus affect the rates of deadenylation and decapping. Interestingly, the AU-rich instability elements from mammalian cells that stimulate deadenylation have been shown to affect translational efficiency independently (36-38). In future experiments discerning the relationship between translation and mRNA decay, it will be important to determine the nature of the interaction between the transcript termini.