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Translation and a 42-nucleotide segment within the coding region of the mRNA encoded by the *MATα1* gene are involved in promoting rapid mRNA decay in yeast

(cis-acting instability element/rare codons/ribosome)

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ABSTRACT **In yeast, the mRNA encoded by the *MATα1* gene is unstable ($t_{1/2}$ = 5 min) and the mRNAs encoded by the *ACT1* gene ($t_{1/2}$ = 30 min) and the *PGK1* gene ($t_{1/2}$ = 45 min) are stable. To understand the RNA structural features that dictate mRNA decay rates in yeast, we have constructed *PGK1/MATα1* and *ACT1/MATα1* gene fusions and analyzed the decay rates of the resultant chimeric transcripts. Fusion of a *MATα1* segment containing 73% of the coding region and the 3′ untranslated region to either of the stable genes is sufficient to cause rapid decay of the chimeric mRNAs ($t_{1/2}$ = 6–7.5 min). Sequences required for this rapid decay are not found in the *MATα1* 3′ untranslated region but are located within a 42-nucleotide segment of the coding region that has a high content (8 out of 14) of rare codons. Introduction of a translational stop codon upstream of this region stabilizes the hybrid mRNAs, indicating that the rapid decay promoted by these sequences is dependent on ribosomal translocation.**

Individual mRNAs are degraded at rates that vary by more than an order of magnitude. Although such differences can affect the intrinsic level of gene expression and serve as a site of posttranscriptional regulation, little is known about the features of mRNAs that dictate their respective decay rates. Initially, it was suggested that the stability of mRNAs is related to basic mRNA properties such as size (1, 2), poly(A) tail length (3), and translational efficiency (4, 5). However, several comparisons have failed to reveal any correlation between these variables and the rate of mRNA turnover, suggesting that general mRNA features are not the primary determinants of mRNA decay rate (6, 7). An alternative explanation for differences in decay rate is that unstable mRNAs contain specific sequences that promote their recognition by the cellular turnover machinery. This view is supported by recent experiments in which specific sequences derived from unstable metazoan mRNAs promoted rapid decay when transferred to stable mRNAs. To date, these "instability elements" have been found in the 3′ untranslated region (UTR) (8–11) and in coding regions (12–15). Although the mechanisms by which such elements promote mRNA turnover are unknown, there appears to be a close link between the translation process and mRNA decay (16, 17).

In this paper we describe experiments to identify specific sequences that promote mRNA decay in the yeast *Saccharomyces cerevisiae*. We recently reported the decay rates of a number of yeast mRNAs, including both stable and unstable species (18). In agreement with previous work, no correlation was found between mRNA decay rate and several general mRNA properties. Among the unstable mRNAs identified were those encoded by genes involved in mating (e.g., *MATα1*, *STE2*, *STE3*, *MFα1*, and *MFA2*), an observation consistent with the rapid transition to a new set of specific functions that occurs during mating-type switching (18). To understand the structural basis for the instability of the *MATα1* mRNA, we have constructed several chimeras between the *MATα1* gene and the *PGK1* and *ACT1* genes, both of which encode stable mRNAs. The decay rates of the hybrid mRNAs identify a 42-nucleotide (nt) coding-region segment that is required for rapid decay. The insertion of a nonsense codon immediately 5′ to this 42-nt segment prevents rapid mRNA turnover, suggesting that the decay of the *MATα1* mRNA is promoted by the translocation of ribosomes through a specific region of the coding sequence.

MATERIALS AND METHODS

Strain and Media. The yeast strain was Y262 (*MATα*, *his4-519*, *ura3-52*, *rpb1-1*; provided by M. Nonet and R. Young; ref. 19). Synthetic media lacking uracil (20) were used to select for and maintain plasmids introduced by transformation (21).

Plasmid Constructions. The DNA sequences of all junctions within the translated regions of gene fusions were determined by the dideoxy method (22), using oligonucleotides complementary to flanking sequences as primers. Plasmid constructions (23) were as follows.

Pα1. Plasmid pRIP4 (a pUC19 derivative containing the *PGK1* gene) was cut with *Bgl* II. Overhanging ends were filled in with Klenow enzyme and the DNA was recut with *Hin*dIII. The 1.6-kilobase (kb) *Eco*RV–*Hin*dIII fragment from p1.9, a subclone of *MATα1* in pBR322 (ref. 25; obtained from K. Tatchell), was inserted into these sites to yield pUC19.Pα1. The resulting hybrid gene was then subcloned into a yeast centromere vector, pRIP1H [a derivative of pUN50 (26) in which the *Hin*dIII site flanking the *URA3* gene had been filled in] to yield pRIP1H.Pα1.

Aα1. First, the *Cla* I–*Hin*dIII fragment of the *PGK1* gene in pRIP4 was replaced with the *Asu* II–*Hin*dIII fragment of the *MATα1* gene to yield pUC19.Pα1*. The *Bam*HI–*Hin*dIII fragment of this hybrid gene was then subcloned into the *Bam*HI and *Hin*dIII sites of pRIP1H to yield pRIP1H.Pα1*. This plasmid was then digested with *Bam*HI and partially digested with *Eco*RV (to liberate the remaining *PGK1* fragment and a small segment of the *MATα1* coding region) and the *Bam*HI–*Hin*dIII fragment of the actin gene from pYact1 (27) was inserted. The resulting plasmid was designated pRIP1H.Aα1.

P(ut)α1. pRIP4 (see above, Pα1) was cut with *Cla* I, filled in with Klenow enzyme, digested with *Hin*dIII, and ligated to the 1.1-kb *Sca* I–*Hin*dIII fragment of *MATα1* isolated from p1.9. This plasmid was designated pRIP1.P(ut)α1.

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Abbreviations: nt, nucleotide(s); UTR, untranslated region.

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Pα1P. The 0.26-kb *Cla* I (filled-in)–*Hin*dIII fragment of *PGK1* that contains the 3′ UTR was inserted into pRIP1H.-Pα1 that had been digested partially with *Sca* I (site just downstream of the *MATα1* translational stop) and completely with *Hin*dIII, yielding pRIP1H.Pα1P.

Deletions of Pα1. pUC19.Pα1.UAG (see below) was cut with *Bgl* II and then treated with BAL-31 nuclease for various times. Single-stranded overhangs were filled in with Klenow enzyme and then ligated to *Bgl* II linkers (no. 1001; New England Biolabs). The DNA was then cut with *Hin*dIII and the fragments containing the *MATα1* region were purified and inserted into *Bgl* II/*Hin*dIII-cut pRIP1H.Pα1.UAG. Plasmids containing these deletions are designated as pRIP1H.-Pα1Δ*x*, where *x* is the number of nucleotides deleted in the 3′ direction from the *Eco*RV site of the *MATα1* gene. For pRIP1H.Pα1Δ52, the reading frame was restored, following sequencing, by filling in the *Bgl* II site at the new junction between the *PGK1* and *MATα1* sequences.

Pα1.UAG. pUC19.Pα1* (see above) was cut with *Eco*RV and ligated to the linker 5′-CTAGATCTAG-3′. The DNA was then cut with *Bgl* II (cutting the linker and the *Bgl* II site in the *PGK1* sequences) and the plasmid was ligated to itself. This plasmid, pUC19.Pα1.UAG, was cut with *Bam*HI and *Hin*dIII, and the fragment containing the hybrid gene was inserted into the *Bam*HI and *Hin*dIII sites of pRIP1HB (a derivative of pRIP1H in which the *Bgl* II site in the backbone had been filled in with Klenow enzyme), yielding the plasmid pRIP1HB6.Pα1.UAG.

Aα1.UAG. The 1.4-kb *Bam*HI–*Hin*dIII (filled-in) fragment containing the 5′ portion of the *ACT1* gene was inserted into the *Bam*HI and *Bgl* II (overhang removed with mung bean nuclease) sites of pRIP1HB.Pα1.UAG to yield pRIP1HB6.-Aα1.UAG.

mRNA Decay Measurements, RNA Preparation, and RNA Analysis. mRNA decay rates were measured as described (18). In brief, transcription was inhibited by thermal inactivation of RNA polymerase II and mRNA levels were quantitated by Northern blotting (28) with DNA probes labeled to high specific activity (29). Northern blots were quantitated by densitometry or by directly counting the β decays occurring in each band with a Betascope (Betagen, Waltham, MA; ref. 18). Data are expressed as the $\log_{10}$ of the percentage of each RNA remaining vs. time at 36°C. In multiple determinations half-life ($t_{1/2}$) values varied by a maximum of ±23%.

RESULTS

Chimeric Transcripts Containing *MATα1* Sequences Decay Rapidly. Recently, we measured the decay rates of 20 yeast mRNAs by quantitating the levels of individual RNAs remaining after thermal inactivation of RNA polymerase II in a temperature-sensitive *rpb1-1* mutant (18). One of the least stable mRNAs examined in that study was the mRNA encoded by the *MATα1* gene. Its rate of decay ($t_{1/2}$ = 5.0 min) is 6- to 9-fold faster than that of stable mRNAs, such as those encoded by the *ACT1* (actin, $t_{1/2}$ = 30 min) or *PGK1* (phosphoglycerate kinase, $t_{1/2}$ = 45 min) genes. For comparison, Northern blots showing the decay of the *PGK1* and *MATα1* mRNAs following the inhibition of transcription in a temperature-sensitive RNA polymerase II mutant are shown in Fig. 1.

One explanation for the instability of the *MATα1* mRNA is the presence within this mRNA of a specific sequence element that promotes rapid degradation. Our approach to delineating such sequences is to identify segments of unstable mRNAs that will promote rapid mRNA decay when transferred to mRNAs that are normally stable (24). As a first step in using this approach for the analysis of the *MATα1* mRNA, we created a gene fusion, designated Pα1, that produces a transcript containing 1205 (of 1380) nt from the stable *PGK1* mRNA fused, in frame, to 580 (of 736) nt from the *MATα1*

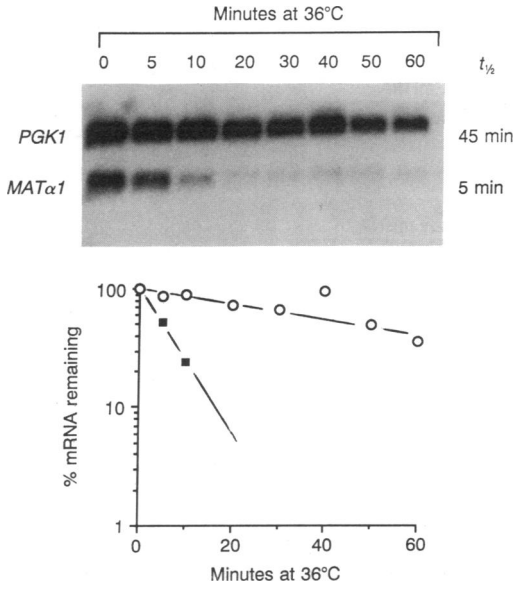

FIG. 1. Decay of stable and unstable mRNAs in yeast. Relative levels of the *PGK1* and *MATα1* mRNAs were measured at different times after a shift to 36°C in a temperature-sensitive RNA polymerase II mutant. (*Upper*) Northern analysis of mRNA levels at different times after the temperature shift. The probe for the *PGK1* mRNA was a random-primed *Dra* I–*Hin*dIII restriction fragment containing the entire *PGK1* coding sequence. The probe for the *MATα1* mRNA was the same as for Fig. 2. (*Lower*) Quantitation of the Northern blot. mRNA levels are normalized to the level at time zero. ■, *MATα1*; ○, *PGK1*. Half-lives (45 min and 5 min) calculated from the slopes are shown to the right of the autoradiogram.

mRNA (see schematic, Fig. 2). The only *MATα1* sequences absent from this hybrid are the 21-nt 5′ UTR and the initial 140 nt of coding sequence.

Decay of Pα1 mRNA was assayed by transferring the hybrid gene to a yeast centromere plasmid and introducing the resulting plasmid into a strain (RY262) containing the *rpb1-1* temperature-sensitive RNA polymerase II mutation. mRNA synthesis was inhibited by shifting these cells to the restrictive temperature (36°C) and mRNA decay rates were measured by Northern blot analysis of equal amounts of RNA isolated at various times after the temperature shift (Fig. 2*A*). Quantitation of this blot indicates that the decay rate of the hybrid Pα1 mRNA is quite rapid ($t_{1/2}$ = 6 ± 0.9 min) and is comparable to the decay rate of the *MATα1* mRNA ($t_{1/2}$ = 5 min). This rapid decay differs significantly from the turnover kinetics of both the endogenous *PGK1* mRNA ($t_{1/2}$ = 45 min) and a chimeric mRNA containing the same fragment of *PGK1* mRNA fused to the 3′ portion of the stable *ACT1* mRNA ($t_{1/2}$ = 45 min; unpublished work).

To rule out the possibility that the rapid decay of the Pα1 mRNA was a fortuitous consequence of the specific hybrid that was formed (e.g., that the junction sequence caused rapid mRNA decay), we created a second gene fusion, Aα1. In the transcripts derived from this construct, the same sequences of *MATα1* that were used in the Pα1 hybrid are fused downstream of a fragment of the stable *ACT1* mRNA. A Northern blot illustrating the decay of this mRNA in temperature-shifted Y262 cells is shown in Fig. 2*B*. This blot shows that fusion of *MATα1* sequences to a second stable mRNA also promotes rapid decay ($t_{1/2}$ = 7.5 min), although a small fraction (10%) of this mRNA is resistant to decay. These results indicate that sequences sufficient to promote rapid mRNA degradation are contained within the 580 nt of the *MATα1* mRNA present in the Pα1 and Aα1 hybrids.

The 3′ UTR of *MATα1* Is Not Sufficient or Required for Rapid mRNA Decay. Studies with several mammalian

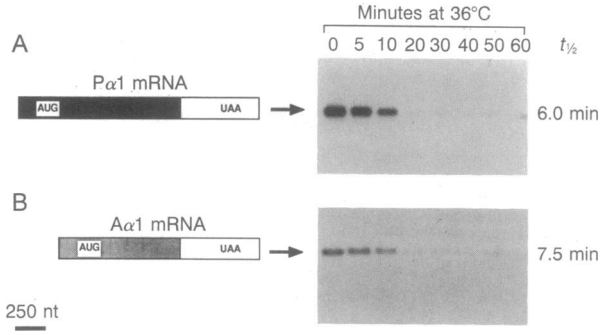

FIG. 2. Northern blot analysis of the decay of the Pα1 (*A*) and Aα1 (*B*) mRNAs. In the diagrams to the left (and in all other figures), *PGK1* sequences are shown as black boxes, *MATα1* sequences as open boxes, and *ACT1* sequences as gray boxes. The probe for both blots was an *Eco*RV–*Sca* I restriction fragment containing 400 nt of *MATα1* coding sequence.

mRNAs have shown that structural determinants that contribute to the rapid decay of those mRNAs can be localized to 3′ UTRs (8–11). In this light we sought to determine the role of the 3′ UTR in the rapid decay of the Pα1 and Aα1 mRNAs. To test whether the 3′ UTR of *MATα1* mRNA was sufficient to promote rapid decay, we constructed a hybrid gene, P(ut)α1, in which the majority of the *MATα1* 3′ UTR (170 of 200 nt) was fused 18 nt downstream of the *PGK1* translational termination codon. The chimeric mRNA transcribed from this hybrid gene contains 85 nt more of the *PGK1* mRNA, including the termination codon, than the Pα1 mRNA and has a half-life of 45 min (Fig. 3*A*). Since this decay rate is the same as that of the *PGK1* mRNA, we conclude that the majority of the 3′ UTR of the *MATα1* mRNA is not sufficient to promote rapid mRNA decay.

The failure of the *MATα1* 3′ UTR to destabilize the *PGK1* mRNA does not preclude the possibility that an "instability element" of the *MATα1* mRNA includes the 3′ UTR. To test whether the *MATα1* 3′ UTR was required for rapid mRNA decay, we created a hybrid gene in which we replaced the majority of the 3′ UTR of the Pα1 hybrid (170 of 200 nt) with the 3′ UTR from the *PGK1* gene (30). This hybrid, Pα1P, produces an mRNA that contains 1200 nt of the *PGK1* mRNA fused to 410 nt of the *MATα1* mRNA (including the translation stop codon and 30 nt of the 3′ UTR) fused to the 75-nt 3′ UTR of the *PGK1* mRNA. Following a brief lag, the Pα1P mRNA decays with a $t_{1/2}$ of 5.0 min (Fig. 3*B*); i.e., the Pα1P mRNA decays at essentially the same rate as the Pα1 mRNA. We conclude that the majority of the *MATα1* 3′ UTR is not sufficient or required for rapid decay. [The brief lag seen in the decay of the Pα1P mRNA is seen occasionally with other rapidly decaying mRNAs (e.g., Pα1, Aα1, and Pα1.Δ52; see below). This lag does not correlate with any particular stage of cellular growth, the degree of temperature shift, or the particular construct, but it is suggestive of a possible aging component in the decay of these mRNAs.]

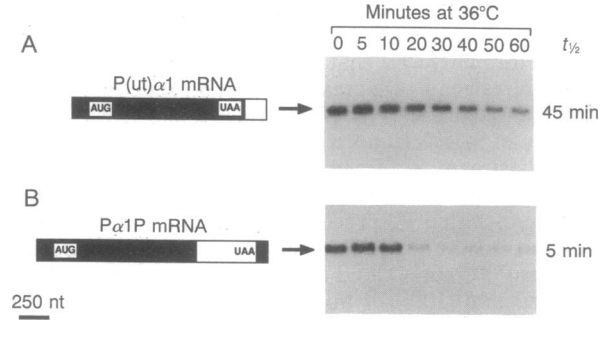

FIG. 3. Decay of P(ut)α1 (*A*) and Pα1P (*B*) mRNAs analyzed by Northern blotting. The probe for the blot in *A* was a 1.1-kb *Sca* I–*Hin*dIII restriction fragment from the 3′ end of the *MATα1* gene. The probe for the blot in *B* was the same as for Fig. 2.

Sequences Within the *MATα1* Coding Region Are Required for Rapid mRNA Decay. To test whether specific sequences from the *MATα1* coding region would promote rapid decay of the chimeric mRNAs, we constructed a series of deletion constructs in which *MATα1* coding sequences were progressively removed from the Pα1 fusion gene. These deletions, shown schematically in Fig. 4, commence at the *PGK1*/*MATα1* junction and remove 52–247 nt of *MATα1* sequence. All fusions are in the proper reading frame to translate the remaining *MATα1* coding sequence (confirmed by sequencing the hybrid genes). Each deletion gene was introduced into RY262 cells and the consequences of these deletions on the decay of the respective chimeric transcripts are shown in Fig. 4. Compared to the Pα1 mRNA ($t_{1/2}$ = 6.0 min), deletion of 52 nt has little or no effect on the mRNA decay rate (Pα1.Δ52; $t_{1/2}$ = 7.5 min, Fig. 4*A*). In contrast, deletion of 94 nt (Fig. 4*B*) or 140 nt (Fig. 4*C*) results in a 4- to 5-fold increase in the half-life of the corresponding mRNA (e.g., Pα1.Δ94, $t_{1/2}$ = 35 min). mRNAs with larger deletions, of 188 or 247 nt (Fig. 4 *D* and *E*), decay with kinetics similar to that of the *PGK1* mRNA ($t_{1/2}$ = >45 min). These results indicate that specific sequences within the 42 nt between the endpoints of Δ52 and Δ94 are required for the rapid decay of the hybrid mRNAs. The possibility that other specific sequences also contribute to the small difference (1.5-fold) in mRNA turnover rates observed upon deletion of >140 nt cannot be ruled out at present.

We have compared the 42-nt region between the endpoints of Δ52 and Δ94 to other unstable yeast mRNAs and have yet to find any striking homologies in either the RNA or the protein sequence. This may reflect the possibility that the 42-nt region is part of a larger element whose 3′ boundary has yet to be defined. However, we do note that the 42-nt region is rich in rare codons (8 out of 14), where a rare codon is defined by its occurrence fewer than 13 times per 1000 yeast codons (31). Including one codon at the deletion boundary, this region also contains a stretch of 6 out of 7 contiguous rare codons (Fig. 5). This concentration of rare codons suggests either that a conserved sequence occurs within this region or that there may be a specific requirement for a translational pause at this site (see *Discussion*).

Upstream Nonsense Codons Prevent Rapid Turnover Promoted by *MATα1* Coding Sequences. The results described above demonstrate that specific sequences within the *MATα1* coding region are required for rapid mRNA decay. The involvement of the coding region suggests that, as observed for several other mRNAs (32), there may be a requirement for translation in the degradation of the *MATα1* mRNA. To test whether translation is required for mRNA turnover, we constructed two hybrid genes in which ribosome translocation through the *MATα1* sequences was prevented by upstream stop codons. The first, Pα1.UAG, is identical to Pα1 except that an amber stop codon has been introduced at the junction between the *PGK1* and *MATα1* sequences. Similarly, Aα1.UAG is identical to Aα1 except for a stop codon at the junction of the *ACT1* and *MATα1* sequences. Fig. 6 shows that the decay of the mRNAs containing the stop codons is significantly slower than the decay of the corresponding mRNAs in which translation continues through the *MATα1* sequence. The Pα1.UAG mRNA ($t_{1/2}$ = 37 min) decays 5- to 6-fold slower than Pα1 mRNA ($t_{1/2}$ = 6 min), and the Aα1.UAG mRNA ($t_{1/2}$ = 17 min) decays 2- to 3-fold slower than Aα1 mRNA ($t_{1/2}$ = 7.5 min). Since an out-of-frame UAG normally exists within the 42-nt *MATα1* instability element, we consider it unlikely that the inserted UAG simply provides a site for direct binding of some cellular factor. Rather, since the reduction in decay rates is attribut-

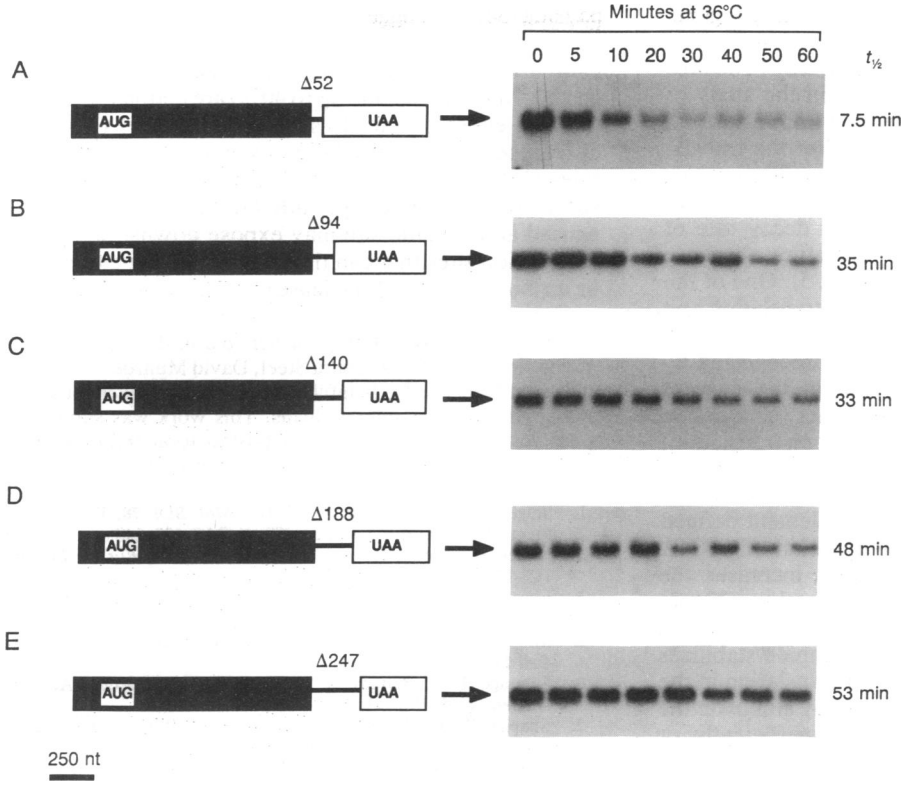

FIG. 4. Deletion analysis of *MATα1* sequences contributing to rapid decay. The effect of deleting *MATα1* sequences from the Pα1 mRNA was quantitated by Northern blotting. In the schematic, deleted sequences are shown as single lines with the number of nucleotides deleted shown above each deletion. Half-lives shown at the right are the average of multiple experiments (e.g., for Δ52, $t_{1/2}$ = 7.5 ± 0.25 min, n = 3; for Δ94, $t_{1/2}$ = 35 ± 8 min, n = 3).

able to the introduction of stop codons, we conclude that ribosome translocation through *MATα1* sequences is required for facilitating rapid mRNA decay. (Although we do not fully understand the faster decay rate of the Aα1.UAG hybrid mRNA, we note that the *ACT1* mRNA is normally less stable than the *PGK1* mRNA.)

DISCUSSION

Nucleotides Within the *MATα1* Coding Region Can Promote Rapid mRNA Decay. In eukaryotic cells, the relationship between mRNA structure and stability has yet to be determined. Experiments with several mammalian mRNAs have identified specific structural determinants, primarily confined to the 3′ UTR, that appear to dictate rapid decay (8–11, 33). We have begun an analysis of mRNA decay in yeast with the goal of identifying both cis-acting sequences and trans-acting components of the cellular mRNA turnover machinery (18, 24). In this paper we have described the decay rates of chimeric mRNAs composed of fragments of the unstable mRNA encoded by the *MATα1* gene and the stable mRNAs encoded by the *PGK1* and *ACT1* genes. We find that fusion of the majority of the unstable *MATα1* mRNA to either of these stable reporter mRNAs results in accelerated mRNA degradation. Formally, this accelerated decay could be due to the loss of specific sequences within the stable mRNAs that protect those mRNAs from decay. However, since either deletion (Fig. 4) or changes in the translation status (Fig. 6) of specific *MATα1* sequences convert the hybrid mRNA into a stable form, the rapid decay is clearly dependent on specific *MATα1* sequences.

Based on an analysis of chimeric mRNAs, virtually none of the *MATα1* 3′ UTR is either sufficient or required for rapid mRNA decay. In contrast, a 42-nt segment within the *MATα1* coding sequence is required for rapid decay. Further work will be necessary to test the sufficiency of this "element" and to define its 3′ limit. Fig. 3 indicates that sequences required for the function of this element cannot extend beyond the first 30 nt of the 3′ UTR. Along with other work (13, 15), these results add to the evidence that stability determinants will be

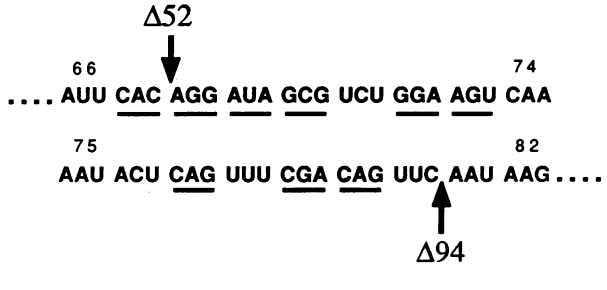

FIG. 5. The segment required for rapid decay is rich in rare codons. Shown is a portion of the *MATα1* sequence in which the 3′ endpoints of Δ52 and Δ94 are indicated. The small numbers identify codon positions within the *MATα1* mRNA. Codons with a frequency of occurrence <13/1000 are underlined.

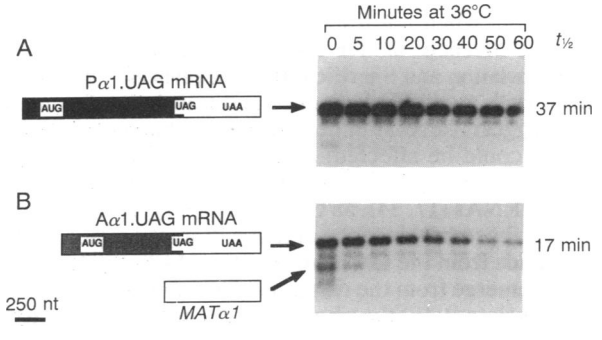

FIG. 6. Translation is required for rapid mRNA decay. The decay of hybrid mRNAs that contained an amber stop codon at the junction of the stable reporter mRNA and the *MATα1* sequences was measured by Northern blotting. The probe for these blots was the same as for Fig. 1. (*A*) Pα1.UAG. (*B*) Aα1.UAG. The RNAs shorter than intact hybrid mRNAs are apparent only when nonsense codons are introduced upstream of the *PGK1/MATα1* or *ACT1/MATα1* junctions. These RNAs lack sequences complementary to probes specific for the 3′ end of *MATα1* and thus may be decay products.

found not only in the 3′ UTR but also in the coding regions of unstable mRNAs.

Ribosome Translocation Stimulates Rapid Decay Promoted by the *MATα1* mRNA. An emerging theme in the study of mRNA turnover is a strong link between the translation and degradation of mRNA. Perturbations that alter the translation status of an mRNA often have dramatic effects on its decay rate. For example, treatment of cells with inhibitors of protein synthesis can drastically reduce the decay rate of many mRNAs (11, 17, 18). Similarly, premature nonsense codons can lead to an increase in the rate (4, 5). One of the clearest examples of a relationship between translation and mRNA decay comes from experiments with mammalian tubulin mRNAs, which show that the signal recognized by the cellular turnover machinery is found in the nascent polypeptide (17, 32, 34). To determine whether the function of the *MATα1* instability element is dependent on translation, we inserted stop codons upstream of these sequences in hybrids formed with both *PGK1* and *ACT1*. Ribosome translocation up to or through the 42-nt *MATα1* element defined by deletion must be involved in the stimulation of mRNA decay since insertion of these stop codons increases the half-lives of the Pα1 and Aα1 mRNAs from 6 min to 37 min and from 7.5 min to 17 min, respectively. Consistent with this conclusion, treatment of cells with cycloheximide stabilizes the *MATα1* mRNA (18). The exact role of translation in the turnover process is unknown. Translation may stimulate the activity of an element that normally functions inefficiently, or translation may be absolutely required for the *MATα1* sequences to function. In the latter case, the decay (albeit slow) of the Pα1.UAG and Aα1.UAG hybrids could be due to alternative decay elements.

Decay rates of the chimeric mRNAs analyzed in this study have been obtained by direct measurement, using a temperature-sensitive RNA polymerase II mutant. A second, but indirect, approach to measuring mRNA decay rates compares the steady-state levels of chimeric mRNAs to those of endogenous mRNAs that have a known half-life and utilize the same transcriptional promoter (24). Consistent with its rapid decay rate, the steady-state level of Pα1 mRNA is reduced relative to *PGK1* mRNA (data not shown). However, the steady-state level of the Pα1.UAG mRNA is similar to that of Pα1 (data not shown). Although the indirect nature of this assay makes it very difficult to interpret this result, it is tempting to speculate that the requirement for translation is more stringent under our assay conditions (higher temperature; inhibited transcription). This reasoning is most consistent with a model in which the role of translation is to stimulate the activity of an element that can function at a reduced rate when not translated.

Possible Mechanisms for the Linkage of mRNA Decay to Both Translation and Specific mRNA Sequences. The requirement of both a specific sequence and translation for mRNA decay suggests several nonexclusive mechanisms by which turnover could be affected: (*i*) the actual recognition event may occur, not at the RNA level, but as with mammalian tubulin mRNAs (17, 34), as the nascent polypeptide emerges from the ribosome (since the putative "target" region is ≈100 amino acids from the C terminus of the *MATα1* polypeptide, it would emerge from the ribosome while the mRNA was still being translated); (*ii*) the ribosome itself may be critical to the degradation process, either by delivering a nuclease (16, 34, 35) or by triggering a catalytic event itself; or (*iii*) the passage of a ribosome through this region may alter the secondary structure of the mRNA in such a way as to expose sequences containing nuclease-recognition sites that would normally not be available (16). The concentration of rare codons in the sequences required for rapid decay (Fig. 6), coupled with the prevalence of rare codons in unstable yeast mRNAs (18) and the known ability of rare codons to induce translational pausing (36, 37), suggests a model in which mRNA structural changes may be affected by the particular positioning of a paused ribosome. However, since the stable *PGK1* mRNA can be altered to include up to 40% rare codons with, at most, a 3-fold effect on steady-state mRNA level (38), and this difference may actually be due to a change in transcription rates (39), it seems unlikely that ribosome pausing *per se* is sufficient to promote rapid mRNA decay. Thus, a ribosome paused at a specific site may expose downstream nuclease-recognition sites that can then be cleaved by either a soluble or a ribosome-bound nuclease.

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