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A Small Segment of the *MATα1* Transcript Promotes mRNA Decay in *Saccharomyces cerevisiae*: a Stimulatory Role for Rare Codons

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Differences in decay rates of eukaryotic transcripts can be determined by discrete sequence elements within mRNAs. Through the analysis of chimeric transcripts and internal deletions, we have identified a 65-nucleotide segment of the *MATα1* mRNA coding region, termed the *MATα1* instability element, that is sufficient to confer instability to a stable *PGK1* reporter transcript and that accelerates turnover of the unstable *MATα1* mRNA. This 65-nucleotide element is composed of two parts, one located within the 5′ 33 nucleotides and the second located in the 3′ 32 nucleotides. The first part, which can be functionally replaced by sequences containing rare codons, is unable to promote rapid decay by itself but can enhance the action of the 3′ 32 nucleotides (positions 234 to 266 in the *MATα1* mRNA) in accelerating turnover. A second portion of the *MATα1* mRNA (nucleotides 265 to 290) is also sufficient to destabilize the *PGK1* reporter transcript when positioned 3′ of rare codons, suggesting that the 3′ half of the *MATα1* instability element is functionally reiterated within the *MATα1* mRNA. The observation that rare codons are part of the 65-nucleotide *MATα1* instability element suggests possible mechanisms through which translation and mRNA decay may be linked.

The regulation of eukaryotic gene expression can be significantly affected by differences in mRNA decay rates. Critical to the understanding of mRNA turnover is a detailed knowledge of the mRNA features that specify differences in decay rates. Recent experiments, examining the decay rates of chimeric and/or mutant transcripts, suggest that at least in some cases, there are discrete sequence elements that affect mRNA turnover rates (for reviews, see references 19 and 28). These instability elements have been described in the protein coding and/or 3′ untranslated regions of a small number of mRNAs.

Determinants of mRNA stability have been identified in the protein coding regions of mammalian (14, 32, 41) and yeast (16, 17, 27, 36) transcripts. These observations suggest that the coding region will be a common location for sequences that affect mRNA half-lives ($t_{1/2}$s). However, the critical features of such coding-region stability determinants, and how these elements function in the presence of translocating ribosomes, are largely unknown. Recent results suggest that recognition of information in the coding region specifying mRNA decay rates may occur by two distinctly different mechanisms. In the case of the mammalian β-tubulin mRNA, recognition occurs at the level of the nascent peptide, demonstrating how translation and decay can be linked (44). Alternatively, coding-region determinants in c-*fos* and c-*myc* mRNAs may be recognized by proteins that bind directly to the RNA (6, 10), although the relationship between protein binding, mRNA decay, and translocating ribosomes is unclear.

The genetic approaches possible in the yeast *Saccharomyces cerevisiae* make this organism a useful system for the analysis of eukaryotic mRNA decay. Previously, we reported that a 363-nucleotide region from the unstable *MATα1* mRNA ($t_{1/2}$ = 5 min) was capable of destabilizing the otherwise stable *ACT1* and *PGK1* ($t_{1/2}$ = 30 and 45 min, respectively) mRNAs (27). Furthermore, the rapid turnover mediated by *MATα1* appeared to require ribosomal translocation through *MATα1* coding sequences, as the placement of a stop codon immediately upstream of the *MATα1* portion of a *PGK1-MATα1* hybrid mRNA abolished the accelerated turnover (27).

In this work, we have undertaken a detailed analysis of the *MATα1* instability element (MIE) in order to determine the limits of the element and the mechanistic requirement for translation in stimulating mRNA decay. We have found that a minimal 65-nucleotide segment of the coding region, the MIE, is sufficient to destabilize a stable reporter mRNA. Additional analysis has revealed that the 65-nucleotide element is bipartite. The first part of the element (composed of the first 33 of 65 nucleotides) contains several rare codons. The role of these rare codons appears to be to increase the effectiveness of sequences located in the 3′ 32 nucleotides of the element in promoting rapid mRNA decay. Additionally, sequences within the latter half of the MIE that are necessary to accelerate decay appear to be functionally reiterated within the following 25 nucleotides of *MATα1*. However, the presence of only one of these two similar regions is required downstream of rare codons in order to increase turnover of the *PGK1* mRNA. The observation that rare codons are a part of the 65-nucleotide MIE suggests a means through which coding-region determinants of mRNA stability and translocating ribosomes may interact to stimulate decay.

MATERIALS AND METHODS

Strains and media. The yeast strains used were RY262 (*MATα rpb1-1 ura3-52 his4-519*) (27) and yRP673 (*MATα rpb1-1 ura3-52 leu2*). Synthetic medium lacking uracil was used to select and maintain plasmids introduced by transformation.

Plasmid construction. The DNA sequences at all junction points within the coding regions of constructed plasmids were sequenced by the dideoxy method (30).

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3′ deletions within *PGK1-MATα1* hybrids. Plasmid pRIP1H.pα1Δ52 was erroneously reported previously (27) to lack only the 5′ untranslated region (UTR) and first 192 nucleotides of the coding region of *MATα1*. The transcript produced from this chimeric gene actually lacks the first 200 nucleotides of the coding region and for simplicity has been renamed Pα1.201-725. To make 3′ deletions, Pα1.201-725 was cleaved with *Hin*dIII and treated with BAL 31 nuclease; single-stranded overhangs were filled and ligated to linkers that contain a stop codon as well as *Xho*I and *Cla*I restriction sites (CTCGAGTAGATCG ATCTACTCGAG). The DNA was digested with *Cla*I and *Bam*HI, and DNA fragments containing the *PGK1* and *MATα1* regions were ligated into *Cla*I-*Bam*HI-cut pRIP4 (27). Plasmids containing these deletions are referred to as PαP.201-x, where x is the 3′-most nucleotide of *MATα1* remaining in the fusion. In these and all subsequently described constructs, nucleotide numbers are based on the A residue in the start codon of *MATα1* (26). Only deletions that terminate translation at the UAG stop codon in the linker were considered for analysis. The reading frame of Pα1.201-266 was restored such that it would terminate at the UAG by digestion with *Xho*I and fill-in of the overhanging ends.

***MATα1* internal deletions.** pSL599 (5) was cleaved with *Dra*I, *Bgl*II linkers (New England Biolabs no. 1051) were added, the plasmid was digested with *Bgl*II and *Eco*RI, and a 965-nucleotide fragment carrying the entire *GAL1* upstream activation sequence (UAS) and 5′ *MATα1* sequences was isolated. This 965-nucleotide fragment was then ligated to *Eco*RI-*Bgl*II fragments containing the vector and *MATα1* sequences from plasmids pRIP.Pα1Δ188 and pRIP.Pα1Δ35 (a member of the 5′ deletion series described previously [27] that deletes *MATα1* sequences to nucleotide 265), creating pGAL-MATα1.$\Delta^{182\text{-}339}$ and pGAL-MATα1.$\Delta^{182\text{-}265}$, respectively. The reading frame of MATα1.$\Delta^{182\text{-}265}$ was restored by cleaving with *Bgl*II, filling the overhanging ends, adding *Bgl*II linkers (New England Biolabs no. 1051), and religating. To create pGAL.MATα1, the entire *GAL1* UAS and *MATα1* sequences from pRP46 were isolated on an *Eco*RI-*Hin*dIII fragment and ligated into pRIP1H (27) digested with *Eco*RI and *Hin*dIII.

Oligonucleotide insertions. The parent vector for all of these constructs is one of the 5′ BAL 31 deletions, termed Pα1.234-725 (27). Pα1.234-725 is identical to Pα1.201-725 except that an additional 33 nucleotides have been deleted from *MATα1* (see Fig. 3A). Pα1.AGG_4 234-725, Pα1.AGA_4 234-725, Pα1.UCG_4 234-725, and Pα1.NRC 234-725 were all created by cleaving Pα1.234-725 with *Bgl*II and ligating the following double-stranded oligonucleotides into the *Bgl*II site: for Pα1.AGG_4 234-725, 5′-GATCAGGAGGA GGAGGAA-3′ and 5′-GATCTTCCTCCTCCTCCT-3′; for Pα1.AGA_4, 5′-GATCAGAAGAAGAAGAAA-3′ and 5′-GATCTTTCTTCTTCTTCT-3′; for Pα1.UCG_4 234-725, 5′-GATCAATTCGTCGTCGTCGAATAAAGAAGACAA-3′ and 5′-GATCTTGTCTTCTTTATTCGACGACGACGAA TT-3′; and for Pα1.NRC 234-725 5′-GATCTTGTCTTCTT TATTCGACGACGACGAATT-3′ and 5′-GATCAATTCGT CGTCGTCGAATAAAGAAGACAA-3′. To create the Pα1.UCG_4 and Pα1.AGG_4 291-725, 339-725, and 396-725 vectors, Pα1.UCG_4 or Pα1.AGG_4 234-725 was digested with *Bgl*II and *Hin*dIII, and the fragment containing vector and *PGK1* sequences was purified and then ligated to *Bgl*II-*Hin*dIII fragments from pRIP.Pα1Δ140, pRIP.Pα1Δ188, and pRIP.Pα1Δ247 (27) that contain *MATα1* sequences, creating Pα1.UCG_4 (AGG_4) 291-725, Pα1.UCG_4 (AGG_4) 339-725, and Pα1.UCG_4 (AGG_4) 396-725, respectively. Pα1.UCG_4 265-725 was created by ligating a 1,391-nucleotide *Bgl*II-*Hin*dIII fragment from pGAL-MATα1.$\Delta^{182\text{-}265}$ with a *Bgl*II-*Hin*dIII fragment from Pα1.UCG_4 234-725 that contains *PGK1* and vector sequences. The reading frame of Pα1.UCG_4 265-725 was restored by cutting with *Bgl*II, filling the overhangs with Klenow enzyme, and religating the plasmid. Pα1.AGG_4 265-725 was created by ligating a *Bgl*II-*Hin*dIII fragment containing *MATα1* sequences from pRIP.Pα1Δ35 with a *Bgl*II-*Hin*dIII fragment from Pα1.AGG_4 234-725 that contains *PGK1* and vector sequences. The reading frame was restored by inserting a double-stranded oligonucleotide with *Bgl*II overhanging ends (5′-GATCTGTCGAC-3′ and 5′-GATCGTCGACA-3′) into the *Bgl*II site.

Site-directed mutagenesis. Site-specific oligonucleotide-directed mutagenesis was carried out on Pα1.201-725 by a modified version of the standard method (21), using an oligonucleotide complementary to the region of Pα1.201-725 spanning from nucleotide 1153 in *PGK1* to nucleotide 239 in *MATα1*, except for the positions specified in Fig. 3A.

***GAL* constructions.** pRP22 was created by inserting a 144-nucleotide *Eco*RI fragment containing a portion of the *GAL1* UAS from nucleotides 365 to 509 (a kind gift from Mark Johnston) into *Eco*RI-cut pRP10 (16). Plasmid pRIP1H.Pα1 (27) was linearized by digestion with *Pvu*I, and overhanging ends were filled. The plasmid was then digested with *Hin*dIII, and the fragment containing *PGK1* and *MATα1* sequences was purified, ligated into pRP22 which had previously been digested with *Bam*HI, filled with Klenow enzyme, and then cleaved with *Hin*dIII, creating pGAL.Pα1. pGAL.Pα1 was digested with *Xba*I and *Hin*dIII, and the fragment containing *PGK1* and vector sequences was purified. All plasmids used in this report that are described above except the *MATα1* internal deletions were cut with *Xba*I and *Hin*dIII, and the fragments containing 3′ *PGK1* and *MATα1* sequences were purified. These fragments were ligated to the large *Xba*I-*Hin*dIII fragment from pGAL.Pα1, thereby placing all of the aforementioned constructs under the transcriptional control of the *GAL1* UAS.

mRNA decay measurements, RNA isolation, and RNA analysis. In all cases, thermal repression of transcription was accomplished by first growing cells until early to mid-log phase at 24°C, pelleting the cells, and then resuspending them in medium prewarmed to 36°C. Simultaneous glucose repression was carried out by growing cells either in medium containing 2% galactose and 2% sucrose (all experiments except the *MATα1* internal deletion experiments) or in medium containing only 2% galactose as a carbon source (*MATα1* internal deletion experiments) at 24°C until early to mid-log phase. Cells were then pelleted and resuspended in 2% glucose containing medium prewarmed to 36°C. RNA was isolated as follows. Cells were resuspended in 150 μl of LET (25 mM Tris [pH 8], 100 mM LiCl, 20 mM EDTA) and 150 μl of phenol equilibrated with LET in 2-ml Eppendorf tubes. Tubes were then vortexed for 5 min with acid-washed glass beads, after which 250 μl of phenol-chloroform equilibrated with LET and 250 μl of diethyl pyrocarbonate-treated water were added. After vortexing, the aqueous phase was extracted with phenol-chloroform, chloroform extracted, and then ethanol precipitated. RNA pellets were washed with 70% ethanol, dried, and resuspended in diethyl pyrocarbonate-treated water. For each Northern (RNA) gel, 8 μg of total RNA was loaded into each lane. mRNA levels were determined by Northern blot analysis (37), using ^{32}P-labeled random-primed DNA probes (12) and directly counting β decays occurring in each band with a Betascope (18). Differences in RNA loading for each time point were ad-

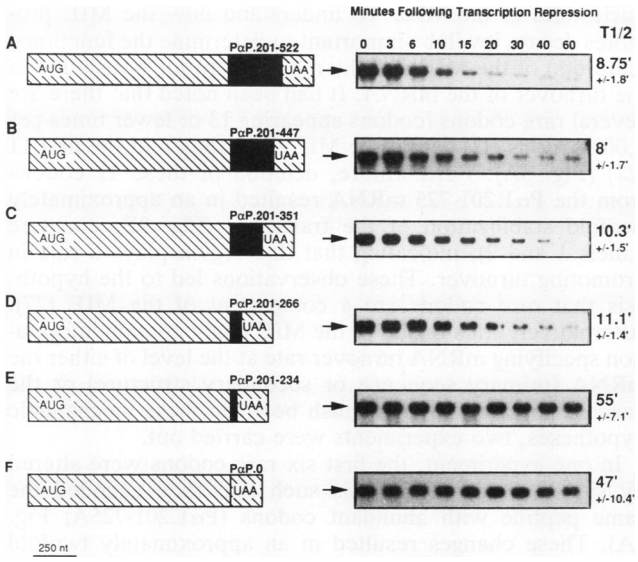

FIG. 1. A 65-nucleotide segment of the *MATα1* mRNA is sufficient to destabilize the *PGK1* mRNA. Shown are Northern blot analyses of *PGK1-MATα1* fusion mRNAs containing 3′ deletions of *MATα1* sequences. To the right of each blot is shown the half-life of the mRNA, determined from the average of multiple experiments (at least three for each mRNA). A schematic representation of each mRNA is shown to the left of each Northern blot. In the schematics, hatched areas represent *PGK1* sequences and *MATα1* portions of the fusions are filled. Nucleotides (nt) of *MATα1* that remain in each of the constructs are shown in the names. Here and in all subsequent figures, nucleotide numbers were determined with the A residue of the start codon of *MATα1* being considered position 1. The probe used in this and all subsequent figures, except PαP.0, is a random-primed DNA fragment containing the entire *MATα1* coding region and 3′ UTR. The probe used to detect the PαP.0 mRNA was a 3′-end-labeled oligonucleotide that spanned the junction between the *PGK1* coding and 3′ UTR (5′-TCTATCGATCTACTCGAGG ATCT-3′). Transcription shutoff was achieved through thermal repression.

justed for by stripping and reprobing blots with an oligonucleotide complementary to the 7S small cytoplasmic mRNA, a polymerase III transcript not subject to glucose or the temperature-sensitive polymerase II repression.

RESULTS

A 65-nucleotide region of the *MATα1* mRNA is sufficient to destabilize a stable transcript. We wished to determine the minimal portion of *MATα1* that was sufficient to destabilize a stable mRNA. To do so, derivatives of the unstable *PGK1-MATα1* hybrid mRNA Pα1.201-725 ($t_{1/2}$ = 9 min) that had successively larger deletions from the 3′ end of the *MATα1* portion of the transcript were generated, and the half-lives of these mRNAs were determined. Pα1.201-725 is composed of *MATα1* nucleotides 201 to 725 fused in frame 3′ of the *PGK1* 5′ UTR and first 1,157 nucleotides of the coding region (Fig. 1). Nucleotide 201 of *MATα1* has previously been shown to be the 5′ border of sequences within *MATα1* required to destabilize this *PGK1* mRNA (27). Deletion of 3′ *MATα1* sequences from the Pα1.201-725 mRNA should therefore reveal the minimal portion of *MATα1* required to destabilize the *PGK1* mRNA. Because deletion of the 3′ end of *MATα1* sequences required that the 3′ UTR and translational stop codon be removed, each member of the deletion series was fused to a linker containing a stop codon and to the *PGK1* 3′ UTR. All deletions examined terminate translation at the stop codon within the linker and are termed PαP.201-x, where x is the 3′ most nucleotide of *MATα1* remaining in the construct (see Materials and Methods and Fig. 1).

Measurement of the decay rates of transcripts from the PαP.201-x constructs demonstrates that the first 65, 150, 246, and 321 nucleotides of the *MATα1* mRNA, beyond nucleotide 201, are sufficient to destabilize the *PGK1* transcript (Fig. 1A to D). Shortening of the *MATα1* sequences to 33 nucleotides (Fig. 1E) resulted in an approximately fivefold increase in the half-life of the mRNA from 11 to 55 min, a half-life similar to that of the PαP.0 mRNA (47 ± 10.4 min; Fig. 1F), which contains no *MATα1* sequences. It should be noted that in all of these constructs, a translational stop codon is located immediately 3′ of the sequences that promote decay. To ensure that the presence of this stop codon was not affecting the ability of the *MATα1* sequences to affect the mRNA decay rate, we also examined the decay rates of a series of transcripts in which the translational stop codon and the *PGK1* 3′ UTR were replaced with the entire coding region (183 nucleotides) and 3′ UTR of the *CUP1* mRNA such that translation would continue beyond *MATα1* sequences through the *CUP1* transcript. In these constructs, the presence of 65 nucleotides of the *MATα1* transcript was again sufficient to destabilize the chimeric mRNA ($t_{1/2}$ = 10.7 ± 1.2 min, compared with 23.6 ± 4.2 min). Similarly, the presence of 33 nucleotides of the *MATα1* transcript had no effect on the half-life of the *PGK1-CUP1* fusion mRNA ($t_{1/2}$ = 26.5 ± 2.1 min; data not shown). Thus, the proximity of the 3′ UTR and the stop codon to *MATα1* sequences in the mRNAs depicted in Fig. 1 did not appear to influence their decay rates.

The deletion experiments, in conjunction with the previously reported 5′ deletion analysis (27), have therefore defined a 65-nucleotide segment, nucleotides 201 to 266 of the *MATα1* coding region, which we term the MIE, that is sufficient to stimulate the degradation of this stable reporter mRNA. It is not yet clear whether these sequences are sufficient to destabilize any stable yeast mRNA. Since the MIE does not completely destabilize the *PGK1* transcript to the same extent as the parental *MATα1* transcript, there may be additional features of these mRNAs that influence their decay rates (see Discussion).

Deletion of the instability element from the *MATα1* mRNA results in a two- to threefold increase in the half-life of the transcript. To test whether the same 65 bases of *MATα1* that can accelerate turnover of the stable *PGK1* reporter transcript also accelerate turnover of the *MATα1* mRNA, two in-frame internal deletions of the *MATα1* gene were created (Materials and Methods; Fig. 2). The first, termed MATα1.$\Delta^{182\text{-}265}$ (Fig. 2B), deletes nucleotides 182 to 265 from the coding region of *MATα1*. These 83 nucleotides include the first 64 of 65 nucleotides of the MIE and an additional 19 nucleotides 5′ of the MIE. The 19 additional nucleotides removed 5′ of the MIE are from a region of the mRNA shown by chimeric transcript analysis to be dispensable for rapid decay (27). For reasons discussed below, we constructed a larger deletion, termed MATα1.$\Delta^{182\text{-}339}$ (Fig. 2C), that eliminates an additional 74 bases 3′ of the element to nucleotide 339 of *MATα1*. The two deletions, as well as the full-length *MATα1* transcript, were placed under the transcriptional regulation of the *GAL1* UAS, and the half-lives of the respective mRNAs were determined. In these and all subsequent experiments, differences in mRNA levels

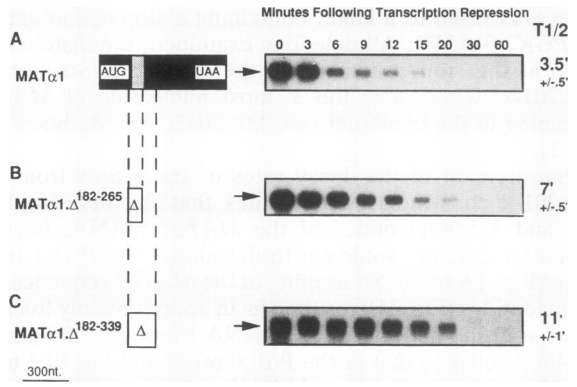

FIG. 2. The 65-nucleotide MIE stimulates decay of the *MATα1* transcript. Decay rates of the wild-type *MATα1* transcript and *MATα1* mRNAs containing internal deletions are shown to the right of each Northern blot. A schematic of the *MATα1* mRNA is shown to the left of the Northern blot in panel A. Filled regions represent *MATα1* sequences, and the lightly shaded region indicates the position of the MIE. Regions deleted from *MATα1* are shown as open boxes below the schematic in panel A, and names of the mRNAs are shown to the left of the schematics. Nucleotides (nt) deleted from the constructs depicted in panels B and C are shown in superscript next to the Δ symbol in the names. Both deletions were in frame, resulting in translation to the normal *MATα1* termination site (see Materials and Methods). Differences in RNA loading were corrected for here and in all other experiments by monitoring levels of the 7S RNA on the blot (see Materials and Methods). Transcription shutoff was achieved by simultaneous glucose and thermal repression.

due to loading and/or sample handling for each time point were normalized by quantitating levels of the 7S transcript (see Materials and Methods). The levels of this transcript are not affected by either glucose or thermal repression (data not shown). The *MATα1* mRNA decays rapidly with a $t_{1/2}$ of 3.5 min (Fig. 2A), slightly faster than previously described (5 min [18, 27]). This small difference presumably reflects the more complete transcriptional shutoff achieved through simultaneous thermal and glucose repression as opposed to simply the thermal repression used previously. Deletion of the MIE resulted in an approximately twofold increase in the half-life of the mRNA, to 7 ± 0.5 min (Fig. 2B). Interestingly, removal of additional sequences 3′ of the 65-nucleotide element further stabilized the transcript ($t_{1/2}$ of MATα1.$\Delta^{182\text{-}339}$ = 11 ± 1 min; Fig. 2C).

We conclude that the 65-nucleotide MIE accounts, in part, for the rapid turnover of the *MATα1* mRNA. Moreover, the observation that deletion of sequences 3′ of the 65-nucleotide MIE resulted in a further stabilization of the transcript to 11 min suggests that there may be additional sequences, residing between nucleotides 265 and 339, that can function in promoting decay of *MATα1* (see below). It should also be noted that the most stable deletion construct of *MATα1* had a half-life of only 11 min, a decay rate lower than those observed for stable or even moderately stable mRNAs ($t_{1/2}$ = 20 to 50 min [18]). This result suggests that in addition to the MIE, there could be other instability elements within the *MATα1* mRNA that contribute to its rapid decay. Alternatively, the intermediate decay rate of the MATα1.$\Delta^{182\text{-}339}$ transcript could be due to the absence of sequences that act to stabilize an mRNA present within stable transcripts (16) (see Discussion).

Rare codons are the important feature of the first 33 nucleotides of the MIE. To understand how the MIE promotes decay, it will be important to determine the functional feature(s) of the MIE and the requirement for translation in the turnover of the mRNA. It had been noted that there are several rare codons (codons appearing 13 or fewer times per 1,000 codons [1]) within the MIE, including 6 of the first 11 (27) (Fig. 3A). Furthermore, deletion of these 11 codons from the Pα1.201-725 mRNA resulted in an approximately twofold stabilization of the transcript (Fig. 3B; compare panels 1 and 2), indicating that this region plays a role in promoting turnover. These observations led to the hypothesis that rare codons are a component of the MIE (27). Alternatively, this region of the MIE might contain information specifying mRNA turnover rate at the level of either the mRNA (primary sequence or secondary structure) or the nascent peptide. To distinguish between these mechanistic hypotheses, two experiments were carried out.

In one experiment, the first six rare codons were altered by site-directed mutagenesis such that they encoded the same peptide with abundant codons (Pα1.201-725A; Fig. 3A). These changes resulted in an approximately twofold increase in the half-life of the mRNA, from 9 to 16.5 min (Fig. 3B; compare panels 1 and 3). The 16.5-min half-life obtained for the Pα1.201-725A mRNA is virtually identical to the half-life observed for an mRNA with a complete deletion of this region of the mRNA (Pα1.234-725; Fig. 3B, panel 2).

In the second experiment, the first 33 nucleotides of the MIE were replaced with four different sequences, two containing and two lacking rare codons. Replacement of this region of the mRNA with sequences containing a run of either four rare arginine codons (AGG) or four rare serine codons (UCG) yielded unstable mRNAs (Fig. 4A and C). In contrast, replacement of this region with nucleotide sequences that contained either a run of four abundant arginine codons (AGA) or a sequence devoid of rare codons (NRC) resulted in a transcript with a half-life similar to that of the Pα1.234-725 mRNA (compare Fig. 4B and D with Fig. 3B, panel 1). These results, in combination with the results from the previous experiment, argue that neither the peptide sequence encoded by this portion of the mRNA nor the actual mRNA sequence itself is critical for turnover and suggest that rare codons are the important feature of the first 33 nucleotides of the MIE (see Discussion).

Specific sequences 3′ of the rare codons are also required for rapid decay. Analysis of the 3′ deletion series suggested that rare codons do not function to stimulate mRNA decay on their own (Fig. 1E). Furthermore, deletion of the rare codons, or their replacement with abundant codons, only partially stabilized the transcripts from which they were removed (Fig. 3B and 4). These observations suggested that rare codons, while unable to accelerate turnover themselves, serve to stimulate the ability of sequences, located 3′ of them, to mediate rapid mRNA decay.

To test this hypothesis, a series of deletions were made 3′ of the rare codons starting with the Pα1.UCG_4 234-725 and Pα1.AGG_4 234-725 constructs. These deletions removed 31, 57, 108, and 168 nucleotides, starting with nucleotide 234, and are termed Pα1.UCG_4 and Pα1.AGG_4 x-725, where x is the 5′-most nucleotide of *MATα1* present in the construct and UCG_4 or AGG_4 denotes the type of rare codons used to replace nucleotides 201 to 233.

Deletion of 57, 108, and 168 nucleotides from the *MATα1* coding region 3′ of both the rare serine and rare arginine codons results in a three- to fourfold increase in the half-lives of these transcripts (Fig. 5A and B, panels 3 through 5).

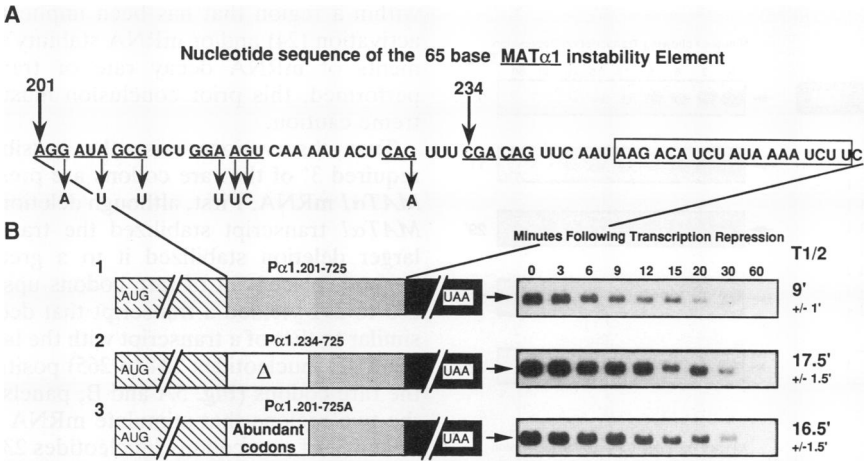

FIG. 3. (A) Nucleotide sequence of the 65-nucleotide MIE. Nucleotide 201 of *MATα1* is the 5′ boundary of the MIE and the first nucleotide of *MATα1* in the Pα1.201-725 fusion mRNA. Nucleotide 234 is the first nucleotide of *MATα1* present in the Pα1.234-725 mRNA. Small arrows followed by a nucleotide represent the mutations made in this region of the mRNA that change the rare codons (which are underlined) to abundant ones and give rise to the Pα1.201-725A mRNA. The potential 19-nucleotide instability sequence located in the 3′ portion of the MIE is boxed (see Discussion). (B) Deletion of the first 33 nucleotides of the MIE, or alteration of its rare codons to abundant ones, stabilizes *PGK1-MATα1* fusion mRNAs. Hatched portions of the schematics represent *PGK1* sequences, lightly shaded regions represent the 65 (or 32 in the case of Pα1.234-725) nucleotides of the MIE, and filled regions represent the rest of *MATα1*. The open box containing the Δ symbol in the schematic in panel 2 represents the deleted portion of the MIE (nucleotides 201 to 233), and the open region in the schematic in panel 3 represents the region containing abundant codons. The nucleotides given in the name of each construct represent the nucleotides of *MATα1* present in the fusion mRNAs. Schematics are not drawn to scale. Transcription shutoff was achieved by simultaneous glucose and thermal repression.

These results indicate that specific sequences, located between nucleotides 234 and 291, rather than simply nonspecific translated sequences are required 3′ of the rare codons for rapid decay. Furthermore, these results reinforce the conclusion that rare codons alone are incapable of accelerating mRNA turnover.

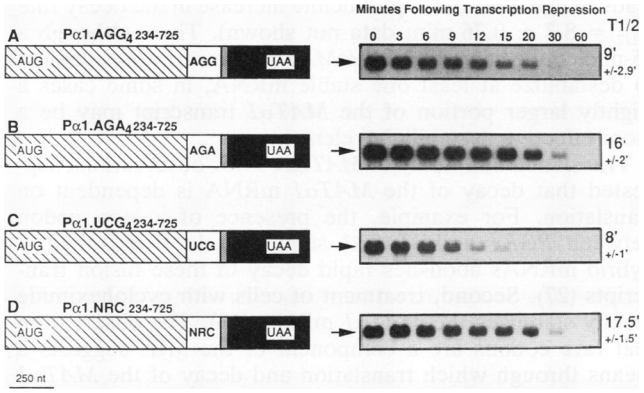

FIG. 4. mRNA sequences containing rare codons can functionally replace the first 33 nucleotides of the MIE. Half-lives are shown to the right of each blot, and schematics of the mRNAs are shown to the left. Shading schemes are the same as those in Fig. 1 to 3 except that the lightly shaded regions represent only the last 32 nucleotides (nt) of the MIE and open regions represent the sequences used to replace the first 33 nucleotides of the MIE (nucleotides 201 to 234 of *MATα1*; see Fig. 3A). The sequences are as follows: Pα1.AGG_4 234-725 (A), AGG AGG AGG AGG AAG ATC; Pα1.AGA_4 234-725 (B), AGA AGA AGA AGA AAG ATC; Pα1.UCG_4 234-725 (C), AAT TCG TCG TCG TCG AAT AAA GAA GAC AAG ATC; and Pα1.NRC 234-725 (D), TTG TCT TCT TTT TTC GAC GAC GAC GAA TTG ATC. Transcription shutoff was achieved by simultaneous glucose and thermal repression.

Nucleotides 234 to 266 were previously shown to be sufficient to promote decay when combined with the region of rare codons (Fig. 1D). However, these nucleotides can be deleted from constructs with little effect on mRNA decay rate provided that nucleotides 265 to 291 are then positioned 3′ of the rare codons (Fig. 5A and B; compare panels 1 and 2). These results suggest that nucleotides 265 to 291, which are not required for rapid decay (compare Fig. 1C and D), can functionally replace nucleotides 234 to 265.

Taken together, the results presented in Fig. 1 and 5 indicate that specific sequences, located between nucleotides 234 and 291, are required to accelerate rapid mRNA decay. Furthermore, there appear to be two regions within these 57 bases, one located between nucleotides 234 and 265 and the second located between nucleotides 265 and 291, either of which can promote turnover when positioned 3′ of rare codons. It remains to be determined exactly what features of these two regions stimulate mRNA decay; however, it is interesting to note that these two regions contain similar nucleotide sequences (see Discussion).

DISCUSSION

We have defined a 65-nucleotide segment from the coding region of the *MATα1* mRNA that contains sequences that can promote mRNA decay in yeast cells. The critical observations are that insertion of the MIE into an otherwise stable mRNA was sufficient to accelerate decay of that transcript (Fig. 1D) and that deletions which removed the MIE from the *MATα1* mRNA stabilized the transcript (Fig. 2B and C). Analysis of the MIE presented in this report argues that it is bipartite. The first part of the element, located in the initial 33 nucleotides, is a cluster of rare codons. Although rare codons are not sufficient to promote decay, they appear to stimulate decay mediated by the second part of the element which is located in the latter 32 nucleotides.

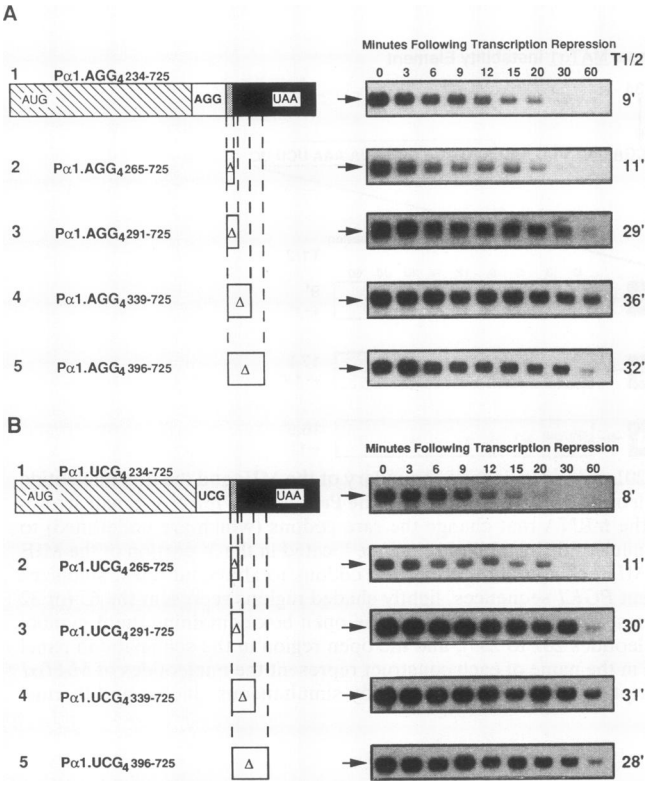

FIG. 5. (A) Deletion of sequences 3′ of the rare arginine codons stabilizes the mRNA. Shading schemes are the same as in Fig. 4. The open boxes shown below the main schematic in panel 1 represent the sequences deleted from the Pα1.AGG$_4$ 234-725 that gave rise to the various deletion mRNAs. (B) Deletion of sequences 3′ of the rare serine codons stabilizes the mRNA. Representations are the same as in panel A. Transcription shutoff was achieved by simultaneous glucose and thermal repression.

Two observations strongly suggest that rare codons are the important feature of the first 33 nucleotides of this element. First, the initial 33 nucleotides can be functionally replaced by other sequences that contain rare codons (Fig. 4A and C). In addition, substituting abundant for rare codons in this region has the same effect on the half-life of the mRNA as does deleting these 33 nucleotides (Fig. 3B, panels 1 and 3). Alternate possibilities, such as that the key feature within these 33 nucleotides is a specific RNA sequence or secondary structure or that the nascent peptide sequence is important, are inconsistent with these observations.

Several lines of evidence indicate that specific sequences are required 3′ of the rare codons for rapid decay. First, insertion of the region of the MIE containing the rare codons into the stable *PGK1* reporter mRNA was not sufficient to accelerate turnover of the transcript (Fig. 1E), whereas insertion of the rare codons and the following 32 nucleotides was sufficient (Fig. 1D). In addition, transcripts with runs of rare codons are stable ($t_{1/2}$ = ≥28 min) unless specific portions of the *MATα1* mRNA are positioned 3′ of them (Fig. 5A and B, panels 1 and 2). Our data are in contrast to those of a previous report which concluded that the replacement of the first 164 codons of the *PGK1* mRNA with all rare codons was sufficient to destabilize this transcript (20). However, since these codon changes made numerous alterations in the primary sequence of the *PGK1* coding sequence within a region that has been implicated in transcriptional activation (24) and/or mRNA stability (16) and no measurements of mRNA decay rate or transcription rate were performed, this prior conclusion must be treated with extreme caution.

Two observations raise the possibility that sequences required 3′ of the rare codons are present twice within the *MATα1* mRNA. First, although deletion of the MIE from the *MATα1* transcript stabilized the transcript by twofold, a larger deletion stabilized it to a greater extent (Fig. 2). Second, placement of rare codons upstream of nucleotides 265 to 291 yielded a transcript that decayed with a half-life similar to that of a transcript with the latter 32 nucleotides of the MIE (nucleotides 234 to 265) positioned downstream of the rare codons (Fig. 5A and B, panels 1 and 2). Strikingly, the two regions that stimulate mRNA turnover when positioned 3′ of rare codons (nucleotides 234 to 265 and 265-291) both contain 19-nucleotide sequences that are identical to one another at 14 positions (see below and the boxed region in Fig. 3A):

```
246                  264
 AAGACAUCUAUAAAAUCUU
 |||| || | |||| || |
 AAGAAAUAUUUAAACUCAU
267                  285
```

One speculation is that this 19-nucleotide sequence serves as a recognition site for some factor involved in the turnover pathway. Moreover, the close proximity of these two regions in the *MATα1* mRNA suggests that the functional element within the *MATα1* mRNA may be composed of the 65-nucleotide MIE and the next 3′ 25 nucleotides. This model is supported by the observation that while insertion of the 65-nucleotide MIE into a *PGK-CUP1* hybrid transcript destabilizes these transcripts approximately 2- to 2.5-fold (23.6 ± 4.2 min versus 10.7 ± 1.2 min), insertion of a larger portion of *MATα1*, including the next 3′ 25 nucleotides, leads to a modest but reproducible increase in the decay rate ($t_{1/2}$ = 8.2 ± 0.76 min; data not shown). Thus, although a 65-nucleotide segment of the *MATα1* transcript is sufficient to destabilize at least one stable mRNA, in some cases a slightly larger portion of the *MATα1* transcript may be a more effective destabilizing element.

Translation and decay of *MATα1*. Prior observations suggested that decay of the *MATα1* mRNA is dependent on translation. For example, the presence of a stop codon between *PGK1* and *MATα1* sequences in *PGK1-MATα1* hybrid mRNAs abolishes rapid decay of these fusion transcripts (27). Second, treatment of cells with cycloheximide greatly stabilizes the *MATα1* mRNA (18). The observation that rare codons are a component of the MIE suggests a means through which translation and decay of the *MATα1* mRNA may be coupled. It has been shown that in some cases rare codons can slow elongation rates along an mRNA and cause ribosomal pausing (4, 8, 33, 38, 42). Moreover, nonuniform translation rates along individual mRNAs have been implicated as being important in a number of cellular processes, including protein secretion, frameshifting, transcriptional attenuation, and protein folding (2, 4, 11, 39, 43). Indeed, it has been postulated that ribosomal pausing may be involved in the decay of other mRNAs (6, 13).

Although we have no direct evidence that a ribosomal pause is involved in the decay of the *MATα1* mRNA, the simplest interpretation of the requirement for rare codons for accelerated decay mediated by the MIE is that they cause

ribosomes to stall at a particular site on the mRNA. There are numerous possible mechanisms by which a putative ribosomal pause could stimulate mRNA turnover mediated by the MIE. For instance, a ribosome stalled at a particular site might affect RNA folding (43) and promote formation of a structure that is recognized as a signal for decay. Alternatively, a pause in translational elongation might permit sequences 3′ of the pause to be relatively ribosome free. This could allow sufficient time for the interaction of soluble, or ribosome-bound, mRNA turnover factors (3, 6, 10, 34, 35) with specific sequences 3′ of the stall. Similarly, the pausing of ribosomes may create a particular polysome structure, such as a ribosome "hole" (29) which triggers mRNA decay.

The link between translation and decay for the MIE appears to be different from other paradigms of decay and translation such as for the β-tubulin mRNA and early nonsense codon-containing mRNAs (23, 44). This suggests that while decay of many mRNAs appears to be in some way coupled to translation (7, 15, 22, 36, 40), the manner in which the two processes are linked may differ greatly between mRNAs.

What determines the decay rates of different mRNAs? Our results defining the MIE are consistent with the general view that specific sequences within mRNAs control their half-lives (9). However, several of our results reported here and elsewhere (16, 25) suggest that the observed decay rates of different mRNAs may depend on the combined effects of multiple elements within each mRNA. For example, although the MIE can increase the rate of decay of the *PGK1* transcript by fivefold, this chimeric transcript is still approximately two- to threefold more stable than the parental *MATα1* mRNA. Similarly, deletion of the MIE from the *MATα1* transcript maximally increases the half-life by three- to fourfold to 11 min, still less stable than the most stable mRNAs in yeast cells.

There are two general types of models to explain these observations. In one view, each mRNA contains a number of sequences that influence the rate at which mRNA turnover factors for different pathways of decay act on the mRNA. The observed half-life of the transcript would result from the summation of the individual rates conferred by the various elements. Thus, the addition or deletion of any one decay element would not completely alter the observed decay rate to the extremes of stability except in rare cases where the differences between the contributions of individual elements was large (e.g., reference 31). An alternative explanation is suggested by recent experiments that indicate that several yeast mRNAs are degraded in a deadenylation-dependent manner with at least two distinct steps that can be rate limiting in the decay of different mRNAs (11a). In this case, an mRNA's half-life is a function of the rates of individual steps in the decay pathway. Elements that affect mRNA decay do so by altering the rate of a specific step, e.g., deadenylation, in the decay pathway. In such a pathway, the half-life of the mRNA will be predominated by the rate of the slowest step in the pathway. Thus, although addition (or deletion) of sequences that stimulate the rate of an individual step in the reaction will change the decay rate, the change in decay rate will not be complete since not every step in the pathway has been affected. To distinguish among these possibilities and to clearly understand differences in mRNA decay rates, it will be critical to determine both what different mRNA decay pathways exist and through which pathway(s) different mRNAs are degraded.

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