Nucleic Acids Research, Vol. 20, No. 20 **5365–5373**

Analysis of chimeric mRNAs derived from the *STE3* mRNA identifies multiple regions within yeast mRNAs that modulate mRNA decay

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Received July 15, 1992; Revised and Accepted September 17, 1992

ABSTRACT

In the yeast *Saccharomyces cerevisiae* unstable mRNAs decay 10–20 fold more rapidly than stable mRNAs. In order to examine the basis for the differences in decay rate of the unstable *STE3* mRNA and the stable *PGK1* and *ACT1* mRNAs we have constructed and measured the decay rates of numerous chimeric mRNAs. These experiments indicate that multiple regions within yeast mRNAs are involved in modulating mRNA decay rates. Our results suggest that at least two regions within the *STE3* mRNA are involved in stimulating rapid decay. One region is located within the coding region and requires sequences between codons 13 and 179. In addition, the *STE3* 3′ UT can also function to stimulate decay. Surprisingly, the *STE3* 3′ UT is not sufficient to accelerate the turnover of the stable *PGK1* transcript unless portions of the *PGK1* coding region are first deleted. These results not only identify sequences that function within yeast to stimulate mRNA turnover but also have important implications for an understanding of the basis of differences in eukaryotic mRNA decay rates.

INTRODUCTION

Differences in mRNA decay rates can be a significant factor in determining both the level and the regulation of eukaryotic gene expression (for reviews see 10,24). To understand cytoplasmic mRNA turnover it will be necessary to identify the features of individual mRNAs that dictate their inherent decay rates and understand how these features affect mRNA degradation. By analogy to prokaryotic systems (see 1,10 for reviews), eukaryotic mRNA decay rates could be specified both by the presence of sequences that stimulate either general or specific turnover pathways, and/or by the inclusion of features that inhibit general mRNA degradation pathways. Evidence demonstrating the existance of sequences that stimulate mRNA decay pathways has come from the construction of chimeric mRNAs derived from stable and unstable mRNAs. These experiments have led to the identification of a small number of specific sequence elements that can be demonstrated to confer rapid mRNA turnover when transferred to stable mRNAs. These 'instability elements' can be found within the 3′ untranslated (UT) regions (2,3,6,19,25) and in coding regions (5,12,26). Whether or not there are also features that function to stabilize mRNAs either directly or indirectly, by antagonizing 'instability elements', is at present unclear.

The genetic approaches possible in the yeast *Saccharomyces cerevisiae* make this organism an ideal system for the analysis of eukaryotic mRNA decay. We have previously reported the decay rates of a large number of yeast mRNAs, including both stable and unstable mRNAs (8). The next step in our analysis was to examine a subset of these mRNAs for sequences that determine their decay rates. Utilizing the general strategy of constructing chimeric genes encoding portions of both stable and unstable mRNAs, introducing these genes into yeast, and measuring the decay rates of the resulting hybrid transcripts we have identified sequences within the coding regions of the mRNAs encoded by the *MATα1* and *HIS3* genes that are involved in stimulating rapid mRNA decay (9,21). In this paper we report the analysis of the unstable yeast mRNA encoded by the *STE3* gene wherein we identify two regions, one within the 3′ UT and one within the coding region, that are involved in promoting mRNA decay. Surprisingly, the *STE3* 3′ UT is not sufficient to accelerate the turnover of the stable *PGK1* transcript unless portions of the *PGK1* coding region are first deleted. These results not only identify sequences which function within yeast to stimulate mRNA turnover but raise the interesting possibility that there may be sequences or features of yeast mRNAs that function to increase the stability of a transcript.

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RESULTS

Experimental strategy

We have previously measured the decay rates of a large number of yeast mRNAs by quantitating the levels of individual RNAs remaining after thermal inactivation of RNA polymerase II in a temperature-sensitive *rpb1-1* mutant (8). In that study, the mRNA encoded by the *STE3* gene was one of the most unstable mRNAs examined ($t_{1/2}$=4.2 min), decaying 8−10 fold faster than stable mRNAs such as those encoded by the *ACT1* ($t_{1/2}$=34 min) or the *PGK1* ($t_{1/2}$=45 min) genes. For comparison, northern blots showing the decay of these three mRNAs following the inhibition of transcription in a temperature-sensitive RNA polymerase II mutant are shown in Fig. 1.

To evaluate the possibility that specific sequences within the *STE3* mRNA dictate its rapid decay rate we have constructed chimeric mRNAs comprised of segments of the *STE3* mRNA fused to segments of the stable mRNAs encoded by the *ACT1* and *PGK1* genes and then measured the decay rates of these chimeric transcripts. Application of this strategy required that we rearrange and/or delete sequences from stable mRNAs. Since previous experiments have suggested that some aberrant mRNAs can be recognized by the cell and rapidly degraded (11,13,16), it was necessary to consider the possibility that rapid degradation of chimeric transcripts might result from their recognition by the cell as aberrant, either as a result of changes to mRNA or mRNP structure, or perhaps due to alterations in nascent peptide folding. To examine this possibility in yeast we have constructed several altered transcripts where all the sequences are derived from stable mRNAs and examined their decay rates. These include internal deletions of both the *PGK1* and *ACT1* mRNAs as well as chimeric mRNAs between the two. If altered transcripts are recognized as aberrant we would expect some, or all of these hybrids to exhibit increased rates of decay. Alternatively, if rapid decay requires specific sequences found on unstable mRNAs then these hybrids, being derived from stable mRNAs, should be devoid of such sequences, and would be stable mRNAs.

Deletions and chimeras of stable mRNAs are themselves stable

The first set of altered transcripts we examined consisted of internal deletions within the *PGK1* and *ACT1* coding sequence. In the case of the *PGK1* mRNA we have constructed a set of deletions which remove different portions of the coding region of this gene (shown in Figure 2, see Materials and Methods for details). In each case, the deletion initiates at the BglII site near the translational termination codon and extends in a 5′ direction. (In these, and all other rearrangements between translated regions, the reading frame is maintained across the hybrid junction as confirmed by DNA sequencing; see Materials and Methods). This set of six deletions remove between 16 and 82% of the *PGK1* coding region. Each deletion is named by the gene, (e.g., *PGK1*), and the amount of the coding region deleted (e.g., PGK1Δ82 is a construct in which 82% of the coding region is removed). In order to distinguish the deletions from the endogenous *PGK1* transcripts the 3′ UT of these constructs was marked by the insertion of a 32 base oligonucleotide into the ClaI site. This insertion was previously determined to have no effect on the decay of these mRNAs (36 and Figure 2A). This set of *PGK1* derivatives contains deletions of 202 (Δ16), 469 (Δ38), 619 (Δ48), 665 (Δ54), 798 (Δ64), and 1024 nucleotides (Δ82). Each construct was placed under the control of the *GAL1* UAS and the decay of the transcripts was assayed by inhibition of transcription utilizing the temperature sensitive RNA pol II mutant (8,22). As shown in Figure 2A, all of the mRNAs containing the deletions are still quite stable ($t_{1/2}$'s range from 27−45 min.), although the largest deletion (PGK1Δ82) does show a modest increase (roughly 1.6 fold) in its rate of decay as compared to wild-type *PGK1* mRNA. In repeated experiments decay rates measured in this manner have a variation of 10−20% (e.g., $t_{1/2}$ of PGK1Δ82 = 27+/−4 min. [n=3]). Essentially identical rates of mRNA decay were obtained following inhibition of transcription by glucose repression of the *GAL1* UAS (data not shown).

Similar results are also seen with a deletion which removes 73% of the *ACT1* coding region, termed ACT1Δ73 (see Figure 2B). Note that although still quite stable ($t_{1/2}$=23 min.), the shortened *ACT1* mRNA does decay slightly faster than the endogenous *ACT1* mRNA ($t_{1/2}$=32 min. in this experiment). Since steady state mRNA levels are a function of the rates of mRNA synthesis and decay we can also estimate the decay rate of the ACT1Δ73 mRNA by comparing the levels of this transcript at steady state (t=0) to the endogenous *ACT1* mRNA which is under the control of the same promoter. The steady state levels of the ACT1Δ73 transcript are approximately the same as the endogenous *ACT1* mRNA (compare relative amounts at t=0 in Fig. 2B), consistent with their similar decay rates.

We have also constructed a pair of chimeric genes containing portions of the *ACT1* and *PGK1* genes and measured the decay rates of the respective chimeric mRNAs. One of these hybrids, designated PA, encodes 1194 nucleotides of the 1465 nucleotide *PGK1* mRNA, including the 5′ UT, fused to the 3′ 600 nucleotides of the 1335 nucleotide *ACT1* mRNA. A northern blot of RNA isolated from a strain transformed with a plasmid containing the PA hybrid gene is shown in Fig. 2C. Using a probe for the *PGK1* mRNA, the steady-state level of the hybrid PA mRNA is approximately two-thirds that of the *PGK1* mRNA (compare relative amounts at t=0 in Fig. 2C), suggesting that the hybrid mRNA is similar in stability to the *PGK1* mRNA. This conclusion is confirmed by measuring the decay rate directly as shown in Fig. 2C. Quantitation of the blot in Fig. 2C, and other independent experiments, gives a $t_{1/2}$ of 45 min for the PA mRNA, essentially identical to that obtained for the *PGK1* mRNA in the same experiment (Fig. 2C).

The second chimeric gene encoding portions of two stable mRNAs, designated AP, produces a transcript containing the 5′ 365 nucleotides of the *ACT1* mRNA fused to the 3′ 230

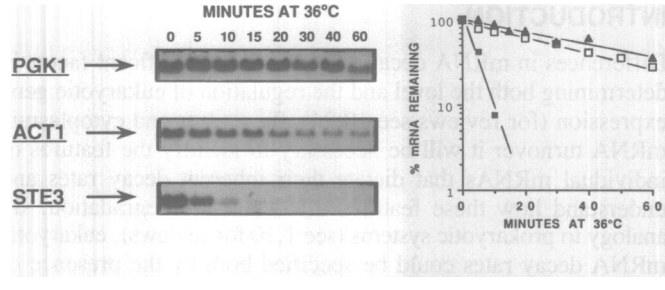

Figure 1. Comparison of stable and unstable mRNAs in yeast. The figure shows the decay of two stable mRNAs, those encoded by the *PGK1* and *ACT1* genes, and one unstable mRNA, encoded by the *STE3* gene. Time points are minutes after a shift to 36°C in a temperature-sensitive RNA polymerase mutant (Y262). The decay of each mRNA is plotted in the graph to the right; filled squares, *STE3*; triangles, *PGK1*; open squares, *ACT1*.

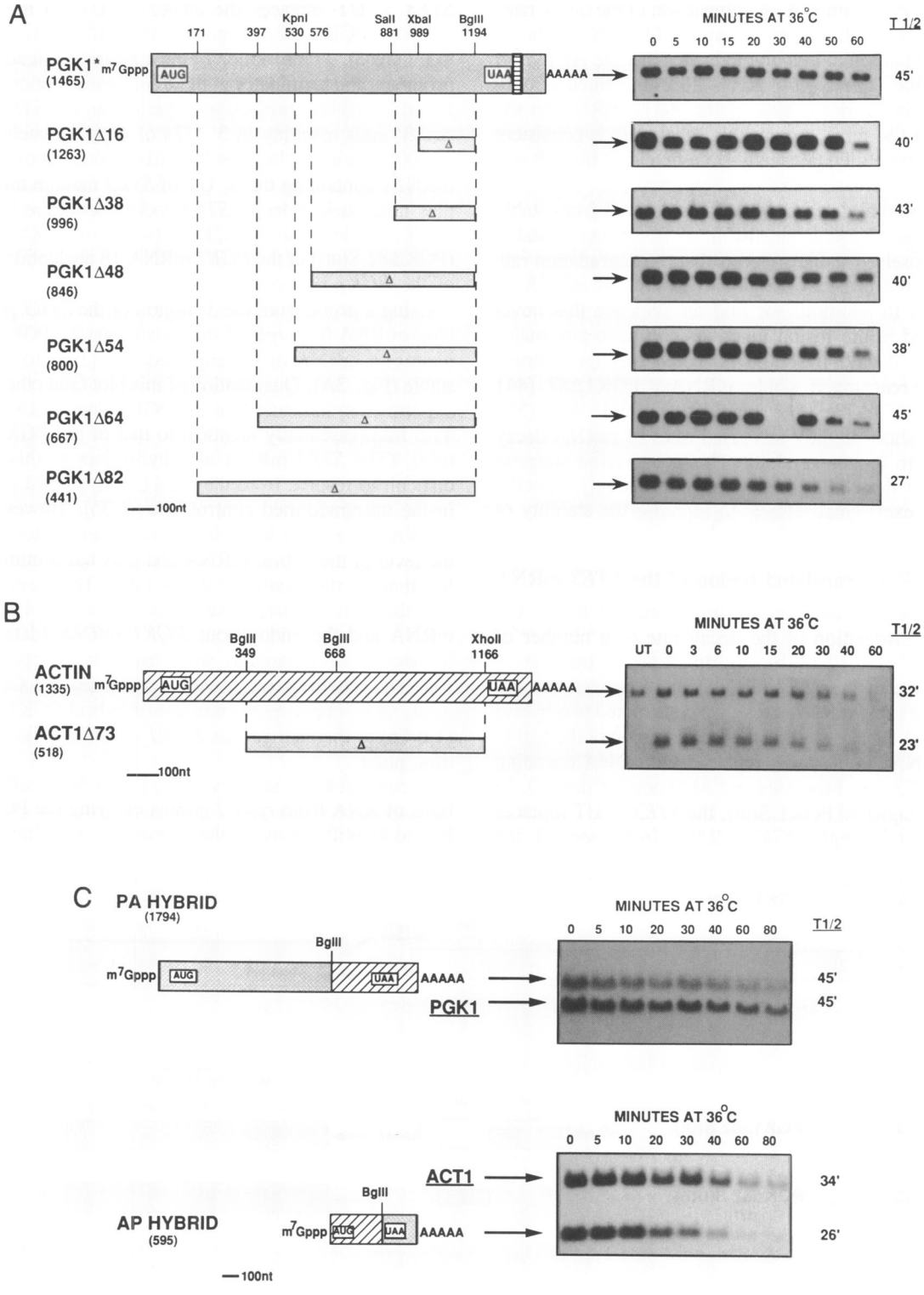

Figure 2. Decay of altered transcripts derived from stable mRNAs. (A) Northern blot analysis of the decay of the deletions within the PGK1 gene. In the diagram to the left, *PGK1* sequences are shown as gray boxes. Regions deleted in each construct are shown as open boxes below the main schematic. The probe for these experiments is a 32 base oligonucleotide which was inserted into the *PGK1* 3′ untranslated region (illustrated as the ladder of parallel lines) as a tag to distinguish these constructs from the endogenous wild-type *PGK1* transcript. The asterisk on the full length *PGK1* transcript denotes that this RNA contains the oligonucleotide insertion. The 30 minute time point in the experiment shown for the PGK1Δ64 construct was lost during RNA isolation. (B) A northern blot showing the decay of the ACT1Δ73 transcript. *ACT1* sequences are shown as slashed boxes. In this and all other figures, UT indicates a lane which includes RNA from an untransformed strain. The probe in this experiment is a random primed BamHI-XhoI restriction fragment which contains sequences encoded within the first *ACT1* exon. (C) Northern blot analysis of the decay of the PA and AP hybrids. The probe for the PA experiment is a random primed DraI-HindIII restriction fragment containing the entire *PGK1* gene. The probe for the AP half-life measurement is a random primed BamHI-XhoI restriction fragment which contains sequences encoded within the first *ACT1* exon.

nucleotides of the *PGK1* mRNA. A comparison of the decay rates of this hybrid mRNA with the endogenous *ACT1* mRNA is shown in Fig. 2C. Quantitation of this blot indicates that the AP hybrid is also quite stable. The $t_{1/2}$ for AP is 26 min, which is only slightly faster than the decay rate of the *ACT1* mRNA in the same experiment (34 min). Again, this decay rate is consistent with the approximately equal steady-state levels of the AP and *ACT1* mRNAs.

The observation that deletion transcripts derived from stable mRNAs as well as chimeras consisting of fragments of stable mRNAs are themselves stable suggests that the degradation rate of an mRNA is not sensitive to global features of mRNA or mRNP structure. In addition, we find no evidence that novel sequences created at the fusion junctions can have any major effect on mRNA decay. It should be noted that the shortest versions of the rearranged stable mRNAs, PGK1Δ82 (441 nucleotides), ACT1Δ73 (518 nucleotides),and AP (595 nucleotides), all show slightly increased rates of mRNA decay when compared to longer versions. This observation suggests that the length of the coding region or, alternatively, specific sequences, may exert small effects to *increase* the stability of an mRNA.

Analysis of the 3′ untranslated region of the *STE3* mRNA

Several studies have focused on the importance of the 3′ UT region in the determination of the decay rate of a number of different mRNAs (3,6,12,14,19,25). In order to test the 3′ untranslated region of the *STE3* mRNA for sequences that are sufficient to cause rapid mRNA decay we constructed two hybrid genes in which the DNA encoding the 3′ UT region of the highly stable yeast mRNA, *PGK1*, was replaced with DNA encoding the 3′ UT of *STE3* (see Materials and Methods for details). In one construct, designated PGK1.S(ut), the *STE3* 3′ UT replaces the 3′ UT of the full length *PGK1* mRNA. In the second, the *STE3* 3′ UT replaces the *PGK1* 3′ UT on the large *PGK1* deletion, PGK1Δ82, generating PGKΔ82.S(ut) (see schematic to Figure 3). Transcription of these hybrids initiates at the *PGK1* promoter and terminates at the *STE3* transcriptional terminator. Based on RNase protection experiments the *STE3* mRNA has two 3′ ends resulting in 3′ UTs of approximately 230 (major) or 200 (minor) nucleotides long (data not shown). The chimeric mRNAs containing the 3′ UT of *STE3* include these sequences plus nine nucleotides of *STE3* coding sequence fused to either 1309 nucleotides (PGK1.S(ut)) or 285 nucleotides (PGKΔ82.S(ut)) of the *PGK1* mRNA 18 nucleotides downstream of the *PGK1* stop codon.

Using a probe from the 3′ region of the *STE3* gene, northern blots of RNA from *rpb1-1* strains carrying the PGK1.S(ut) hybrid identify a mRNA of the expected size (1.6 kb) which is quite stable (Fig. 3A). Quantitation of this blot (and other independent experiments) indicates that the PGK1.S(ut) mRNA has a $t_{1/2}$ of 48.5 min, essentially identical to that of the *PGK1* mRNA (45 min). (The *STE3* mRNA also hybridizes to this probe and is difficult to resolve from the PGK1.S(ut) hybrid [see faint band in the untransformed control lane (UT)]. However, the *STE3* mRNA has a short half-life and is present at less than 11% of the level of the hybrid mRNA and thus has a minimal effect on lengthening the measured decay rate). This result agrees well with the approximately equal steady-state levels of the PGK1.S(ut) mRNA and the endogenous *PGK1* mRNA (data not shown). Steady-state comparisons were made by stripping the blot in Fig. 3A, rehybridizing it with a probe derived from the *PGK1* gene, and comparing the amount of hybrid PGK1.S(ut) mRNA (1.6 kb) to the endogenous *PGK1* mRNA (1.4kb) in the 0 min time point.

In contrast to the stability of the PGK1.S(ut) mRNA, northern blots of RNA from *rpb1-1* strains carrying the PGK1Δ82.S(ut) hybrid identify a band of the appropriate size that exhibits rapid

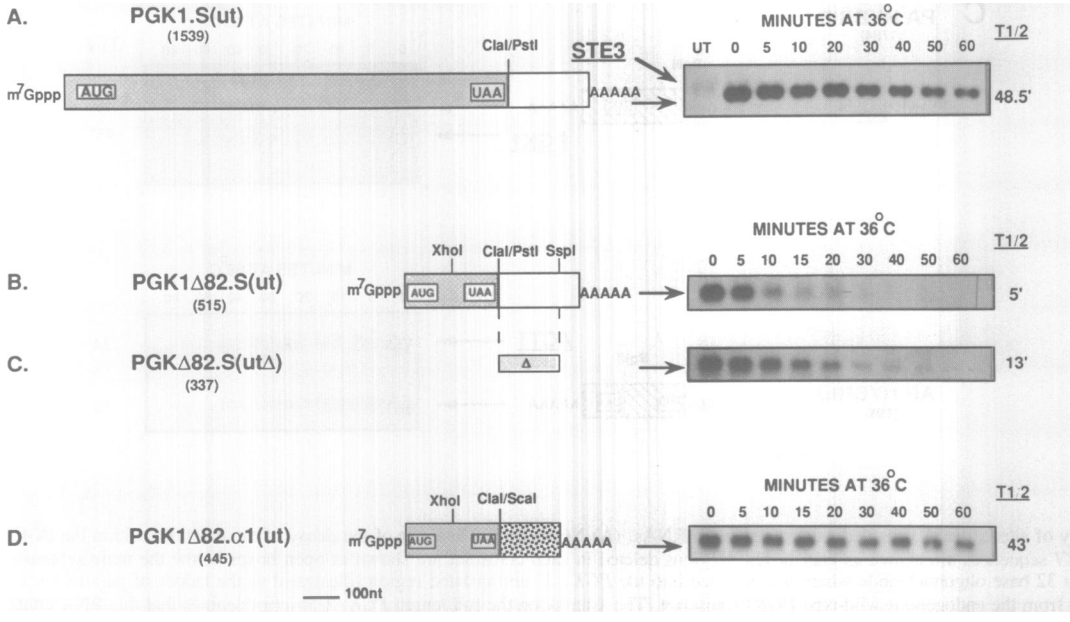

Figure 3. Effects of 3′ untranslated regions on mRNA decay. The figure shows northern blots of the decay of chimeric mRNAs containing the *STE3* or *MATα1* 3′ UTs. A) PGK1.S(ut); B) PGK1Δ82.S(ut); C) PGK1Δ82.S(utΔ); D) PGK1Δ82.α1(ut). The *STE3* mRNA detected in the UT lane (untransformed) in panel A is present just above the PGK1.S(ut) hybrid, but is of insufficient quantity to affect the $t_{1/2}$ determination. In the schematic *STE3* sequences are shown as open boxes, *PGK1* sequences as shaded boxes, and *MATα1* sequences as speckled boxes. The probe for panel A was a random primed fragment (HindIII-HindIII) from the 3′ end of the *STE3* gene. In the other panels the probe is a random primed fragment from the 5′ 171 nucleotides of the *PGK1* mRNA.

decay (Figure 3B). Quantitation of this blot and others reveals a half-life of 5+/−0.7 minutes [n=3]. This result agrees well with the steady state levels of this transcript which are roughly 8-fold lower than the *PGK1* mRNA itself (data not shown). For comparison we also constructed a second chimera, termed PGK1Δ82.α1(ut), utilizing the 3'UT from the unstable mRNA encoded by the *MATα1* gene ($t_{1/2}$=4', 34). Transcription of this hybrid initiates at the *PGK1* promoter and terminates at the *MATα1* transcriptional terminator. Northern blots of RNA from *rpb1-1* strains carrying the PGK1Δ82.α1(ut) hybrid identify a mRNA of the expected size (~445 nt.) thath decays slowly following a shift to high temperature (Fig. 3D). Quantitation of this blot (and other independent experiments) indicates that the PGK1Δ82.α1(ut) mRNA has a $t_{1/2}$ of 43+/−4 min [n=3].

The results with the *STE3* 3' UT raise an important question. Why is it that on the construct containing the entire *PGK1* coding region the *STE3* 3' UT has no effect on mRNA decay whereas on the transcripts containing deletions of *PGK1* coding sequences the *STE3* 3' UT stimulates mRNA decay at a rate 5−6 fold faster than either the *PGK1* or the *MATα1* 3' UTs? There are two general models to explain these results. In one view, deletion of *PGK1* sequences leads to the stimulation of decay by an unknown mechanism. In this model, the differences in stability of the three PGKΔ82 transcripts with different 3' UT sequences would be due to sequences present in the *PGK1* 3' UT (and the *MATα1* 3' UT), but not the *STE3* 3' UT, that inhibit the stimulation of decay caused by the *PGK1* internal deletion. An alternative possibility is that only the 3' UT derived from the *STE3* gene can act to stimulate mRNA decay. In this model, the inability of the *STE3* 3' UT to stimulate decay when placed on the full length transcript would be attributable to features of the *PGK1* transcript that act in a dominant manner to prevent the *STE3* 3' UT from accelerating decay.

If the rapid decay is due to destabilizing sequences within the *STE3* 3' UT region then deletion of such sequences should lead to a stabilization of the PGK1Δ82.S(ut) transcript. Alternatively, if the rapid decay of the PGK1Δ82.S(ut) transcript is due to the absence of stabilizing sequences in the *STE3* 3' UT, then deletion of such sequences should have no effect on mRNA decay. In this light we determined the decay rate of a transcript identical to PGK1Δ82.S(ut), but with a deletion of 180 nucleotides from the *STE3* 3'UT (see Materials and Methods for details). As shown in Figure 3C, this transcript, termed PGK1Δ82.S(utΔ), is roughly 2.5−3 fold more stable than PGK1Δ82.S(ut) ($t_{1/2}$=13 +/−1 minutes [n=2]). Since the rapid decay of the PGK1Δ82.S(ut) transcript can be slowed significantly by deletion of 180 nucleotides within the *STE3* 3' UT we argue that sequences within this region play a role in stimulating mRNA decay. Moreover, since the PGK1.S(ut) transcript is quite stable, this conclusion implies that the ability of these sequences to affect mRNA decay can be influenced greatly by either the length of the coding region or the presence of particular sequences within the *PGK1* coding region (see Discussion).

Sequences within the *STE3* coding region can also stimulate rapid mRNA decay

A second experiment we have performed to determine if the *STE3* 3' UT can stimulate mRNA decay is to remove the 3' UT from the *STE3* mRNA itself and determine if there is any increase in the half-life. To do this experiment we removed the 3' UT from the *STE3* gene and replaced it with the 3' end of the *ACT1* gene. This chimeric transcript encodes the 5' UT and the coding region of *STE3* fused in frame to the same fragment of the *ACT1* gene used in the construction of the stable ($t_{1/2}$=45 min) chimeric mRNA, PA (see Fig. 2C). We have included a portion of the *ACT1* coding sequence in this construct (184 codons) to allow for subsequent deletion analysis (see below). This hybrid, designated SA.Δ1, encodes all of the *STE3* mRNA (1450 nucleotides) with the exception of the last 3 codons and the 3' UT region. A northern blot showing the decay of the mRNA encoded by this hybrid, in comparison to the *STE3* mRNA, is shown in Fig. 4A. From this and other experiments we calculate the $t_{1/2}$ of the SA.Δ1 transcript to be 8+/−0.5 min. The rate of decay of the SA.Δ1 mRNA is roughly twofold slower than

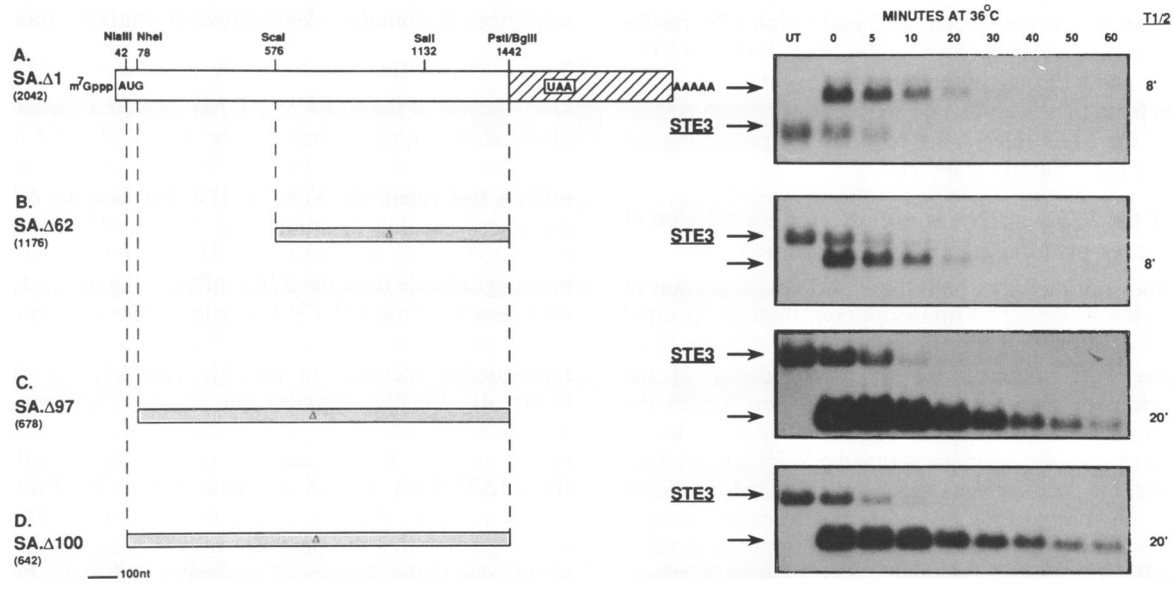

Figure 4. Northern analysis of chimeric mRNAs containing *STE3* coding sequences. The figure shows a northern blot analysis of the decay of hybrids between the *STE3* and *ACT1* mRNAs. (A) SA.Δ1; (B) SA.Δ62; (C) SA.Δ97; (D) SA.Δ100. The probe for these blots is the same oligonucleotide, RP11, used in Fig. 6.

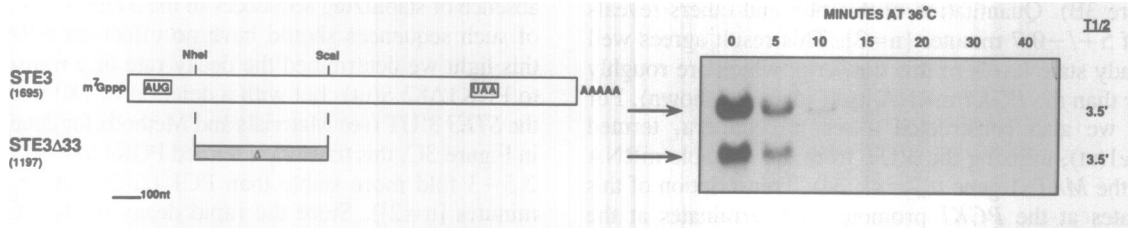

Figure 5. Northern analysis of *STE3* deletion transcripts. The figure shows a northern blot analysis of the decay of the STE3Δ33 transcript. The probe for these blots is a 0.9 kb EcoRI fragment from STE3Δ33.

the decay of the whole *STE3* mRNA. This difference is reflected in the twofold higher steady-state levels of the hybrid transcript as compared to the endogenous *STE3* mRNA (compare relative amounts at t=0 in Fig. 4A). These results are consistent with the hypothesis that the *STE3* 3′ UT contributes to the rapid decay of the *STE3* mRNA. However, given that the SA.Δ1 transcript is still relatively unstable, these results suggest that other regions of the *STE3* transcript are also capable of promoting rapid decay.

In order to determine which other regions of the *STE3* transcript might be responsible for this effect we constructed a series of hybrids containing the 5′ UT and progressively less of the *STE3* coding sequence. These hybrids, designated SA.Δ23, SA.Δ62, SA.Δ97, and SA.Δ100, respectively encode 1170 (5′ UT + 364 codons), 550 (5′ UT + 179 codons), 125 (5′ UT + 13 codons), and 42 nucleotides (5′ UT alone) of *STE3* mRNA sequences fused to the same 600 nucleotide region of the actin mRNA used in SA.Δ1. In each case the deletion is named after the percentage of the *STE3* coding region deleted. Northern blots showing the decay of SA.Δ62, SA.Δ97, and SA.Δ100 mRNAs are shown in Figs. 4B–D. Since the SA.Δ23 hybrid is similar in size to the endogenous *STE3* mRNA, we used an RNase protection assay to determine the half-life of this hybrid and found it to be 8 minutes (data not shown). Hybrids containing 550 nucleotides or more of the *STE3* mRNA (SA.Δ1, SA.Δ23, and SA.Δ62) all have similar steady-state mRNA levels (roughly twofold the level of *STE3* mRNA) and similar decay rates ($t_{1/2}$ ~ 8 min, Fig. 4). In contrast, in hybrids SA.Δ97 and SA.Δ100 (Figs. 4C and D), deletion of sequences between codon 13 and codon 179, results in higher steady-state levels of hybrid transcripts (4–5-fold *STE3* mRNA levels) and a change in the rate of decay ($t_{1/2}$=20 min). We conclude from these results that sequences between codons 13 and 179 of the *STE3* mRNA are required for promoting the rapid decay of the chimeric transcript.

The decay of the *STE3* mRNA is not affected by deletion of coding sequences promoting mRNA degradation

The results above argued that both the 3′ UT and a portion of the coding region of the *STE3* transcript contribute to its rapid decay. The observation that specific sequences within the *STE3* coding region were required for the rapid decay of the *STE3-ACT1* chimeric transcripts allowed us to test further the hypothesis that the 3′ UT was capable of stimulating rapid decay. To do this, we constructed deletions within the *STE3* mRNA itself which remove the nucleotides which we defined as being important for the ability of the coding region to promote rapid decay. If the *STE3* 3′ UT region is sufficient to promote rapid decay these mRNAs should decay rapidly. One of these deletions, STE3Δ33, removes 33% of the *STE3* coding region (498 nucleotides) corresponding to the nucleotides encoding codons 13 to 179. As shown in Figure 5, the STE3Δ33 mRNA decays at a rate indistinguishable from the *STE3* mRNA itself. Similarly a larger deletion of the majority (75%) of the *STE3* coding region (1056 nucleotides corresponding to codons 13–365), STE3Δ75, also decays at the same rate as the *STE3* mRNA (Parker, Peltz, Brown, and Jacobson, unpublished observation). These results are consistent with the ability of the *STE3* 3′ UT to promote rapid decay of both the *STE3* mRNA and the chimeric mRNAs.

DISCUSSION

In eukaryotic cells, experiments with several metazoan mRNAs have identified specific structural determinants which appear to dictate rapid mRNA decay (2,3,12,14,19,25). To examine yeast mRNAs for features which influence mRNA decay rates we have constructed a number of chimeric genes which encode: a) portions of the unstable mRNA, *STE3*, fused to stable reporter mRNAs, b) sequences derived solely from stable mRNAs, and c) sequences derived solely from *STE3*. Two observations suggest this is a valid approach to identifying sequences that dictate mRNA decay rates. First, the stability of novel transcripts constructed by deletion or rearrangement of stable mRNAs implies that when hybrids which consist of stable and unstable mRNAs are unstable, the instability is likely to be a consequence of sequences derived from the unstable mRNA. More importantly, deletions or mutations that inactivate instability elements within the *HIS3* (9), *MFA2* and *MATα1* (Muhlrad, Caponigro, and Parker, manuscript in prep.) mRNAs correspondingly inactivate the ability of these sequences to stimulate degradation of chimeric molecules.

The 3′ UT of the *STE3* mRNA stimulates mRNA decay

Our analysis of the *STE3* 3′ UT has created a paradox. Several observations argue togther that the 3′ UT of *STE3* functions to stimulate mRNA degradation. Deletion mutants of the *STE3* mRNA that retain the *STE3* 3′ UT, but lack the *STE3* coding sequences capable of stimulating rapid mRNA turnover, i.e. STE3Δ33 and STE3Δ75, still exhibit decay at rates indistinguishable from the *STE3* mRNA (Figure 5). Conversely, replacement of the *STE3* 3′ UT with similar sequences from the *ACT1* mRNA results in a small (approximately 2 fold) but reproducible increase in half-life (SA.Δ1; $t_{1/2}$=8 minutes; Figure 4). Finally, replacement of the 3′UT on the stable PGK1Δ82 transcript ($t_{1/2}$=27 minutes) with the *STE3* 3′ UT results in a 5–6 fold increase in the rate of mRNA decay (PGK1Δ82.S(ut), $t_{1/2}$=5 minutes; Figure 3). Two pieces of evidence argue that the rapid decay of the PGK1Δ82.S(ut) transcript requires the presence of *STE3* sequences and is not simply due to the absence of sequences within the *PGK1* 3′ UT that protects the analogous PGK1Δ82 transcript from degradation. First, a construct comparable to PGK1Δ82.S(ut), but containing the 3′ UT of the unstable *MATα1* mRNA, PGK1Δ82.α1(ut), is

itself stable (Figure 3). More importantly, deletion of 180 nucleotides of *STE3* sequences from PGK1Δ82.S(ut) results in a slower decay rate (PGK1Δ82.S(utΔ), $t_{1/2}$=13 minutes; Figure 3).

Why then, if the *STE3* 3′ UT contains sequences that can stimulate mRNA decay, is the PGK1.S(ut) transcript so stable ($t_{1/2}$=45 min, Figure 3)? These results suggest that the presence of sequences that can stimulate decay is not sufficient to cause rapid decay, i.e. that the activity of such sequences can be antagonized or otherwise regulated. A corollary of this conclusion is that differential decay rates are not solely due to the presence or absence of sequences that stimulate decay, i.e. mRNAs are not inherrently stable. We consider it likely that there may be a basal decay mechanism by which any mRNA could be degraded at a default rate. In this view, differences in mRNA decay rates could arise both by the inclusion of sequences that stimulate decay, of either the basal or other pathways, but also by features that inhibit the basal decay pathway. A mRNA turnover system that can be modulated in both directions would be analogous to that seen in some prokaryotic systems (see 1,10 for reviews). In this model, the differences between the PGK1.S(ut) and PGK1Δ82.S(ut) transcripts would be the presence of such a stabilizing feature within the *PGK1* coding region that can negate the effect of the *STE3* 3′ UT.

The possibility that there are features of mRNAs that may function to stabilize transcripts is also raised by the observation that the large deletion within the *STE3* 3′ UT, PGK1Δ82.S(utΔ) ($t_{1/2}$=13 minutes, Figure 3), fails to increase the stability of this transcript to the stability of PGK1Δ82 ($t_{1/2}$=27 minutes, Figure 2). While we cannot rule that we have simply failed to delete the entire instability element within the *STE3* 3′ UT, an alternative possibility is that this intermediate value represents the default rate and that to increase the half life beyond this point may require additional 'stabilizer' sequences. Support for this view comes from comparable experiments with the unstable *MFA2* and *MATα1* mRNAs where mutation of instability sequences within these mRNAs also increases the half-life to approximately 12−15 minutes (ref & Muhlrad, Caponigro, and Parker, manuscript in prep.).

What are the features of the *STE3* 3′ UT and how might they function to stimulate decay? We have recently identified regions within the 3′ UT of the unstable transcript encoded by the yeast *MFA2* gene that stimulate mRNA decay by accelerating the loss of the poly(A) tail (18). Comparison of the 3′ UTs of the *STE3* and *MFA2* transcripts identifies a similar sequence found in both 3′ UTs. In *STE3* this sequence, UCAUCAGCU, is found 114 nucleotides 3′ of the stop codon; in *MFA2*, a similar sequence, UCAUUAGCU, is found 88 3′ of the stop codon. However, our analysis of the *MFA2* transcript suggests these sequences are not required for stimulating mRNA decay (18). Additional experiments will be needed to determine if this homology has any signifigance and the mechanism by which the *STE3* 3′ UT stimulates mRNA decay.

The coding region of the *STE3* mRNA can stimulate mRNA decay

Chimeric transcripts containing the *STE3* coding region (SA.Δ1, SA.Δ23, SA.Δ62; Fig. 4) exhibit relatively rapid mRNA decay ($t_{1/2}$=8 min). The absence of the *STE3* 3′ UT from these transcripts argues that the *STE3* coding region can stimulate mRNA degradation independently. Evidence that a discrete portion of the coding region of the *STE3* mRNA is sufficient for this rapid decay is provided by: i) deletions that remove approximately one kb of *STE3* sequence without affecting mRNA decay rates (SA.Δ1 to SA.Δ62; Fig. 4) and ii) a deletion of a portion of the coding region that stabilizes the SA mRNAs (SA.Δ62 to SA.Δ97, Fig. 4). These results argue that the accelerated decay of hybrid mRNAs containing the *STE3* coding sequence is not due to the loss of sequences within stable mRNAs which protect or stabilize those mRNAs, but is due primarily to *STE3* sequences, between codons 13 and 179, that promote mRNA decay. It should be noted that, while these results indicate that sequences 3′ of codon 179 are not required for the accelerated decay of the SA mRNAs, we cannot rule out that other portions of the *STE3* coding region can also stimulate decay. It is possible that the features of this mRNA that influence turnover are quite complex and that the *STE3* coding region contains several features capable of stimulating turnover, one of which is localized to codons 13−179. It is interesting to note that there is a partial overlap between a potential signal sequence for translocation into the ER of the *STE3* protein (codons 5 to 22, (7)) and the coding region destabilizing element identified in this study (codons 13−179). Since subcellular location can have an effect on mRNA turnover rate (17,28), it will be important to determine if the specific sequences responsible for rapid turnover of the *STE3* mRNA overlap with this putative signal sequence.

Sequences modulating mRNA turnover in yeast are complex

Our results indicate that the sequences that influence eukaryotic mRNA decay rate are not simple. As discussed above, at least two regions within the *STE3* mRNA can stimulate mRNA turnover. An additional layer of complexity comes from our observations suggesting that the ability of the *STE3* 3′ UT to stimulate decay can be modulated by features of the stable *PGK1* transcript. These observations together suggest that an mRNA's decay rate could be result of several distinct features and the interplay between those features and the cellular mRNA turnover machinery. This complexity is not unique to yeast transcripts. Examination of the unstable mRNAs encoded by the c-fos and c-myc mRNAs indicates that these mRNAs also contain multiple regions that can independently contribute to their rapid decay (12,26,27). Interestingly, the presence of multiple decay elements within a single mRNA allows the modulation of the decay rate of the mRNA in multiple manners (see 26). In this way the cell can differentially regulate the turnover of many mRNAs by controlling a limited number of different decay pathways. Such multiple arrangements of regulatory elements is reminiscent of many eukaryotic promoter regions and the flexibility that they give the cell in the transcriptional regulation of such genes.

MATERIALS AND METHODS

Strains

The yeast strain used for these studies was Y262 (genotype = *MATa his4-519, ura3-52, rpb1-1*; kindly provided by Michael Nonet and Richard Young, 20).

Plasmid constructions

The yeast vector for these experiments was a derivative of pUN50 (4), in which the HindIII site flanking the *URA3* gene, the BglII site and the XhoI site were sequentially filled in with Klenow enzyme to yield the plasmid pRP10. Deletions within the *PGK1* coding sequence were constructed as follows. First, a 144 base EcoRI fragment containing the *GAL1* UAS (from plasmid

BM1164, obtained from Mark Johnston) was inserted into the EcoRI site of pRP10 to yield pRP22. Then the *PGK1* gene, from the PvuI site (overhang filled in with Klenow enzyme) 252 nucleotides 5′ of the transcriptional start site to the 3′ HindIII site was inserted into the BamHI (filled in, but recreated by fusion to the PvuI site) and HindIII sites of pRP22 yielding the plasmid, pRP129. The 3′ end of this gene was marked in the plasmid pRP170 by swapping the XbaI-HindIII fragment containing the 3′ UT with the same XbaI-HIndIII site from a PGK1 gene (pRP160) in which a 32 nucleotide insertion of the oligonucleotide, 5′CGATGGTGCGAGAGCGTCAGTATTAAGCGGAT3′, had previously been made (23). Some internal deletions were constructed by generating restriction fragments extending from the 5′ flanking BamHI site to internal sites (KpnI (Δ54, pRP229), SalI (Δ38, pRP231), XbaI (Δ16, pRP232)) and then inserting these fragments into the BamHI and BglII sites of the starting plasmid pRP170. Three internal deletions (Δ82, pRP227; Δ64, pRP228; Δ48, pRP230) were made by Bal31 nuclease treatment from the BglII site in a 5′ direction prior to subcloning the fragments back into the BamHI-BglII cut pRP170. In all these deletions within *PGK1*, except Δ38, a XhoI linker was added at the site of the fusion between the 5′ deletion endpoint and the BglII site. The XhoI linker (NEB # 1072) was added to the 3′ BglII site (filled in). The linkers added at the 5′ endpoint of each deletion were: Δ54, NEB # 1072, Δ16, NEB # 1006, and to the ends of the Bal31 digestion products, NEB # 1006.

The ACTΔ73 deletion was constructed by first inserting the 3.25 kb BamHI-EcoRI fragment containing the yeast *ACT1* gene into the BamHI and EcoRI sites of pRP10 to give the plasmid pRP62. Subsequently, the 350 nucleotide XhoII-NdeI(filled in) fragment containing the 3′ portion of the *ACT1* coding region and the transcriptional terminator was inserted into the 5′ most BglII site and the EcoRI site (filled in) of pRP62 to yield pRP253.

Hybrids between the *PGK1* and *ACT1* mRNAs were constructed as follows: For the PA hybrid, a 1.76 kb BamHI (from the polylinker)-BglII fragment containing the 5′ portion of the *PGK1* gene from pRIP4 (22) was inserted into the BamHI-BglII (3′ most) sites of pR62 giving the plasmid pRP73. The AP hybrid was constructed by inserting the 0.37 kb BglII-HindIII (filled in) fragment containing the 3′ portion of the *PGK1* gene from pRIP4 into the BglII(5′ most)-EcoRI (filled in) sites of pRP62 yielding the plasmid pRP72.

Construction of fusions with the 3′ UTs of *STE3* and *MATα1* was as follows. First, for the PGK1.S(ut) hybrid (replacement of the *PGK1* 3′ untranslated region on the full length *PGK1* gene with that of *STE3*), the BamHI-HindIII fragment containing the *PGK1* gene from pRIP4 (BamHI site from the polylinker) was subcloned into the BamHI-HindIII sites of pRP10 to yield pRP65. The 3′ untranslated region of *STE3* (PstI-HindIII; PstI overhang removed with Klenow) was placed just downstream of the *PGK1* stop codon by insertion into the ClaI-HindIII sites of pRIP5 (after filling in of the ClaI site), giving the plasmid pRP80. The 3′ UTs of *STE3* and *MATα1* were then transferred to the Δ82 form of *PGK1* by utilizing in vivo recombination (15) to repair a gapped plasmid. For this recombination, the plasmids, pRP80 and pRIP.PGK(ut)*MATα1* (21) were gapped by cleavage with the BglII and MscI sites within the *PGK1* coding sequence. This gap was then repaired by recombination with the BamHI-ClaI fragment from the PGK1Δ82 plasmid pRP227. The resulting recombinants were confirmed to be of the expected structure by restriction analysis and sequencing of plasmids isolated from *E.coli*. The PGK1Δ82.S(utΔ) gene was constructed by inserting the SspI-HindIII fragment containing the last portion of the *STE3* 3′ UT and the transcriptional terminator into the ClaI (filled in)-HindIII sites of pRP227 to yield pRP311.

The SA.Δ1, SA.Δ23, SA.Δ62, and SA.Δ97 hybrids were constructed by inserting DNA from the HindIII (filled in) to PstI (SA.Δ1), SalI (SA.Δ23), ScaI (SA.Δ62), or NheI (SA.Δ97) sites in *STE3* into the XbaI (filled in)-BglII (3′ most) sites of pRP62. In each case the filled in HindIII site was ligated to the filled in XbaI site and the various sites were fused in-frame to the BglII site in *ACT1* by filling in their ends with Klenow enzyme and adding the appropriate linker to maintain the reading frame. For SA.Δ1, SA.Δ23 and SA.Δ62 the linker was # 1051 (5′ GAAGATCTTC 3′) from New England Biolabs. For SA.Δ97 the linker was # 1052 (5′ GGAAGATCTTCC 3′). SA.Δ100 was constructed by inserting the XbaI-HindIII (filled in) fragment of *STE3* from pRP49 into the XbaI-BglII sites of pRP62. In this case the reading frame was maintained by the addition of New England Biolabs linker # 1001 (5′CAGATCTG 3′). Plasmids carrying the respective chimeric genes were designated pRP85, pRP86, pRP87, pRP88, and pRP89.

Plasmids containing deletions within the *STE3* coding region were constructed by first inserting the SalI-HindIII fragment of pRP88, which contains the 5′ portion of the *STE3* gene with a BglII site at codon 13 into the SalI-HindIII sites of pRP10 to yield pRP10.5′STE3. Subsequently, 3′ portions of the *STE3* gene from either the ScaI (STE3Δ33) or SalI (STE3Δ75) to the EcoRV site beyond the 3′ end of the gene in CY809 (obtained from George Sprague) were inserted into the BglII and HindIII (filled in) sites of pRP10.5′STE3. The reading frame was maintained in each case by incorporation of BglII linkers.

RNA analysis

mRNA decay rates were measured as previously described (8,22).

ACKNOWLEDGEMENTS

We thank Agneta Brown, Giordi Caponigro, and Stuart Peltz for helpful discussions and assistance. This work was supported by a grants to R.P. from the Chicago Community Trust and the National Institutes of Health (GM45443), a grant to A.J. (GM27757) from the National Institutes of Health, and a fellowship (DRG-1141) from the Damon Runyon-Walter Winchell Cancer Research Fund to C.D.

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