Characterization of a General Stabilizer Element That Blocks Deadenylation-dependent mRNA Decay*

Maria J. Ruiz-Echevarria‡§§, Raj Munshi¶¶, Julie Tomback†, Terri Goss Kinzy**‡‡, and Stuart W. Peltz***§§

Received for publication, November 30, 2000, and in revised form, June 8, 2001
Published, JBC Papers in Press, June 22, 2001, DOI 10.1074/jbc.M010833200

The Journal of Biological Chemistry, Vol. 276, No. 33, Issue of August 17, pp. 30995–31003, 2001

mRNA degradation is a regulated process that can play an important role in determining the level of expression of specific genes. The rate at which a specific mRNA is degraded depends largely on specific cis-acting sequences located throughout the transcript. cis-Acting destabilizer sequences that promote increased rates of decay have been identified in several short-lived mRNAs. However, little is known about elements that promote stability, known as stabilizer elements (STEs), and how they function. The work presented here describes the characterization of a STE in the PGK1 transcript. The PGK1 stabilizer element (P-STE) has been delineated to a 64-nucleotide sequence from the coding region that can stabilize a chimeric transcript containing the instability elements from the 3′-untranslated region of the MFA2 transcript. The P-STE is located within the PGK1 coding region and functions when located in the translated portion of the transcript and at a minimum distance from the 3′-untranslated region. These results further support the link between translation and mRNA degradation. A conserved sequence in the TEF1/2 transcript has been identified that also functions as a STE, suggesting that this sequence element maybe a general stability determinant found in other yeast mRNAs.

Differences in the decay rates of individual mRNAs can have profound effects on the overall levels of expression of specific genes. A key to understanding how a specific mRNA is degraded requires characterizing the cis-acting elements and trans-acting factors that regulate its turnover. Studies in the yeast Saccharomyces cerevisiae have revealed several mRNA degradation pathways that can provide insights into how this process can be regulated. mRNA degradation might initiate by endonucleolytic cleavage (1), loss of the cap and subsequent 5′ → 3′ exonucleolytic digestion (2), or deadenylation, which can be followed either by 3′ → 5′ exonucleolytic degradation or decapping and 5′ → 3′ exonucleolytic degradation (3–6). However, many wild-type yeast mRNAs that have been investigated decay through the pathway that initiates with the shortening of the poly(A) tail to an oligo(A) form. Removal of the poly(A) tail triggers rapid decapping of the transcript by the Dcp1p and subsequent degradation of the body of the transcript by a 5′ → 3′ exoribonuclease, Xrn1p (reviewed in Ref. 7).

cis-Acting sequences that modulate the decay rate of an mRNA have revealed several types of destabilizer elements (1, 8–16). These instability elements have been identified by their ability to promote rapid decay when transferred to an appropriate location within inherently stable mRNAs or by mutational analysis. For example, the MFA2 mRNA is very unstable, with a half-life of 3 min (15). Previous studies have determined that the sequences responsible for the destabilization of MFA2 were localized to the 3′-UTR and that they function by promoting increased rates of deadenylation, which is followed by decapping and 5′ → 3′ decay (2). In the case of the MAta1 transcript, a 65-nt instability element (MIE) is located in the protein coding region of the transcript and requires translation in order to be functional (17). Interestingly, a given transcript can have multiple destabilizer sequences, suggesting that the process of targeting an mRNA for degradation can be redundant (reviewed in Ref. 18).

In addition to instability elements, a few examples of stabilizer elements that block rapid mRNA decay have been identified (19–22). In mammals, a sequence in the 3′-UTR of the α-globin transcript slows down poly(A) shortening and rapid decay of the mRNA (23). In yeast, two types of sequences that promote specific stabilization of nonsense-containing transcripts have been described. Transcripts containing premature nonsense codons are rapidly degraded via the nonsense-mediated mRNA decay (NMD) pathway. Using the PGK1 and HIS4 transcripts, it was shown that rapid decay takes place only if the nonsense mutation occurs approximately within the first two thirds of the transcript and that 3′-proximal nonsense mutations are resistant to the NMD pathway (20, 24). These results indicate that specific sequences in the last third of the PGK1 transcript confer resistance to NMD when translated. A second sequence that promotes stabilization of nonsense-containing transcripts was identified in the leader region of the

* This work was supported in part by National Institutes of Health Grants GM55226 (to S. W. P.) and GM57483 (to T. G. K.), by an American Heart Association Established Investigator award (to S. W. P.), and by an American Heart Association grant-in-aid (to M. J. R.-E.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby advertised by the payment of page charges. This article must therefore be hereby

† Supported by a Howard Hughes undergraduate fellowship during part of this research.
‡ To whom correspondence may be addressed: Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Ln., Piscataway, NJ 08854. Tel.: 732-235-5223; E-mail: kinzyt@umdnj.edu.
§ To whom correspondence may be addressed: Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Ln., Piscataway, NJ 08854. Tel.: 732-235-5223; E-mail: peltz@umdnj.edu.

§§ To whom correspondence may be addressed: Curagen Corp., New Haven, CT 06511.
¶¶ These authors contributed equally to this work.
** Supported by a Howard Hughes undergraduate fellowship during part of this research.
†† To whom correspondence may be addressed: Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Ln., Piscataway, NJ 08854. Tel.: 732-235-5223; E-mail: peltz@umdnj.edu.

1 The abbreviations used are: UTR, untranslated region; nt, nucleotide(s); NMD, nonsense-mediated mRNA decay; STE, stabilizer element; MIE, MAta1 instability element; P-STE, PGK1 stabilizer element.

This paper is available on line at http://www.jbc.org
that can modulate the stability of mRNAs. A stabilizer sequence that inactivates the deadenylation-dependent decay pathway has been found in the stable PGK1 mRNA (19). The experimental approach involved constructing chimeric genes harboring the PGK1 gene fused with portions of an unstable mRNA. Studies with chimeric genes containing the PGK1 coding region and the MFA2 3′-UTR demonstrated that the MFA2 3′-UTR could not destabilize an intact PGK1 mRNA (19). Interestingly, the PGK1-MFA2 chimeric transcript was stabilized as an oligoadenylated form, suggesting that the stabilizer element protects the transcript from being rapidly degraded and/or from 3′ → 5′ exonucleolytic attack (19). Similar results were obtained using the STE3 3′-UTR, also known to contain an instability determinant (11). Thus, it seems very likely that stabilizer elements occur in other stable transcripts.

In this work, we have mapped and characterized the stability element in the PGK1 transcript (P-STE). The results presented here indicate that a 65-nucleotide sequence from the PGK1 coding region promotes stabilization of a PGK1-MFA2 chimeric transcript. Furthermore, a larger region that contains this element also stabilizes a PGK1-STE3 chimera. The P-STE functions not only when located in the translated portion of the transcript and not immediately proximal to the MFA2 3′-UTR. In addition, we have identified a sequence similar with the P-STE in the TEI1/2 transcript that promotes stabilization of a PGK1-MFA2 chimeric transcript. Taken together, these results indicate that we have identified a general stability determinant that can modulate the stability of mRNAs.

MATERIALS AND METHODS

Yeast Strains, Growth Conditions, and Transformation Procedures—The S. cerevisiae upf1 deletion strain used in this study were: RY262/MATA his4–519 ura3–52 upf1·hisG rpb1–1; Ref. 23) and RY262-a/MATA his4–519 ura3–52 upf1·hisG rpb1–1) obtained by mating type switch of RY262– cells as described (26). Cells harboring the rpb1–1 allele were grown at 24 °C. Yeast media were prepared as described (26). Cells were cultured on defined minimal synthetic dextrose medium, lacking uracil to select and maintain the plasmids used in these studies. Cells lacking plasmids were grown non-selectively in YPD medium (28). Yeast transformations were performed using the lithium acetate protocol (29). 

A General mRNA Stabilizer Element

The various hybrid PGK1-MFA2 and PGK1-STE3 alleles were transformed into strains harboring the temperature-sensitive allele of the RNA polymerase gene, and the mRNA decay rates were determined by Northern analyses as described previously (20, 24). All blots in Figs. 1, 2, 4, and 5 were probed with a 32P-labeled fragment containing the entire MFA2 gene, and all blots in Fig. 3 were probed with a 32P-labeled fragment containing the 3′-UTR of STE3. The results of these experiments were quantitated using a Bio-Rad model G-250 molecular imager and the appropriate oligonucleotides as primers and total yeast DNA as template.

RESULTS

Deletion Analysis Demonstrates the Presence of a Stability Element within the First Two Thirds of the PGK1 Transcript—The MFA2 gene encodes an inherently unstable transcript with a half-life of 3 min (15). The sequences responsible for the destabilization of the MFA2 mRNA have been localized to the 3′-UTR (33). Surprisingly, a chimeric PGK1-MFA2 mRNA, in which the PGK1 3′-UTR was replaced with that from MFA2 transcript, encodes a stable mRNA that lacks the poly(A) tail (19). This result is consistent with previous observations demonstrating that the poly(A)-deficient form of the PGK1 transcript is stable (34). These and other observations suggested that the coding region of the PGK1 gene contains a stabilizer element (P-STE) that blocks decapping of the mRNA when translated. Our goal was to identify and characterize the P-STE in the PGK1 transcript.

The boundaries of the P-STE were initially defined by deletion analysis of the stable PGK1-MFA2 transcript. The deletions were constructed by using long terminal repeats that were cotransformed into the PGK1 protein coding region and deleting the 3′ region of the termination codon. The MFA2 3′-UTR that includes the region required for rapid mRNA decay, as well as the cleavage and polyadenylation sites, was inserted immediately 3′ of the termination codon (Fig. 1A). These alleles were transferred to yeast centromere plasmids and transformed into a upf1Δ strain...
harboring the rpb1–1 temperature-sensitive allele of the RNA polymerase II. mRNA decay rates were determined by RNA blotting analyses (see “Materials and Methods”). Half-life measurements were performed in a upf1/H9004 strain to eliminate any effect of the NMD pathway that could result from the introduction of premature termination codons. The results of these experiments indicated that transcripts harboring the amino-terminal 55% of the PGK1 coding sequences (up to nt position 979) were rapidly degraded, with half-lives of 3 to 6 min (Fig. 1A, constructs 1A–1C). A hybrid transcript harboring 67.7% of the PGK1 coding sequences encoded a moderately stable mRNA with a half-life of 18 min, indicating the presence of a STE (Fig. 1A, construct 1D). Replacing the MFA2 3’-UTR with the PGK1 3’-UTR resulted in stabilization of all these transcripts, indicating that the instability observed is due to the MFA2 3’-UTR (20; data not shown). Taken together, these results indicate that there is a sequence within the first two thirds (67.7%) of the PGK1 transcript that blocks rapid degradation of the PGK1-MFA2 chimeric transcript.

**Insertion of Early Termination Codons into Full-length PGK1 Indicates That the P-STE Requires Translation up to 55% of the PGK1 Transcript**—The function of several cis-acting instability elements requires ribosome translocation up to or through the element (Ref. 35; reviewed in Refs. 7, 36, and 37). Based on these observations, we explored whether translation is required for the P-STE to be functional. As a first test, translation was disrupted by inserting in-frame nonsense codons at four different locations within the PGK1 protein coding region and the decay rate of the PGK1-MFA2 mRNA was determined in a upf1/H9004 strain (Fig. 1B, constructs 1E–1H). A PGK1-MFA2 chimeric gene was constructed by inserting the MFA2 3’-UTR into a unique BglII site located at 92.6% of the PGK1 coding region. The Northern blots for these alleles are shown below the schematics.
PGK1 coding region. A premature stop codon was inserted at 5.6%, 39%, 55%, and 67.7% of the PGK1 coding region (Fig. 1B, constructs 1E–1H, respectively). Therefore, although the size of the transcripts is equivalent, the amount of the PGK1 protein coding region that is translated is different in each allele. mRNA decay rates were determined in a upf1/H9004 strain harboring the rpb1–1ts allele of RNA polymerase II. The results demonstrated that, relative to translation of the entire PGK1 mRNA, translation termination after 5.6% or 39% of the PGK1 coding region, respectively, promoted destabilization of the PGK1-MFA2 transcript (half-life of 6 and 8 min, respectively; Fig. 1B, constructs 1E and 1F). However, location of premature nonsense mutations following 55% or greater of the PGK1 coding region remained stable with a half-life of greater than 25 min (Fig. 1B, constructs 1G and 1H). Therefore, translation of up to 55% of the coding region is sufficient to promote stabilization of the PGK1-MFA2 mRNA when the C-terminal part of the transcript is also present.

The Distance between the P-STE and the 3′-UTR Affects the Decay Rate of the mRNA—The results obtained above indicated that the MFA2 3′-UTR does not promote rapid decay if 55% (nt 979) or greater of the PGK1 protein coding region is translated (Fig. 1B). Interestingly, these results seem in conflict with the results shown in Fig. 1A, in which insertion of the MFA2 3′-UTR at 55% of the protein coding region promoted accelerated mRNA decay (Fig. 1A, construct 1C compared with B; construct 1G). This raises the possibility that the P-STE is upstream of nt 979 (55%) and that either: (i) it requires a specific sequence 3′ of a translation termination codon, or (ii) there needs to be a certain amount of sequence between the P-STE and the instability element in the 3′-UTR. To test which of these hypotheses is valid, the following constructs were prepared. In these constructs, a nonsense mutation at position 979 (at 55% of the PGK1 protein coding region) was inserted, and the amount of sequence immediately downstream of the termination codon was varied. In construct 2A (identical to construct 1C in Fig. 1A), the nonsense codon is immediately followed by the MFA2 3′-UTR. In construct 2B, the MFA2 3′-UTR was inserted at position 1138 of the PGK1 coding sequence and therefore it contains the 159 nt naturally located downstream of the nonsense mutation. Therefore, translation of up to 55% of the coding region is sufficient to promote stabilization of the PGK1-MFA2 mRNA when the C-terminal part of the transcript is also present.

The Distance between the P-STE and the 3′-UTR Affects the Decay Rate of the mRNA—The results obtained above indicated that the MFA2 3′-UTR does not promote rapid decay if 55% (nt 979) or greater of the PGK1 protein coding region is translated (Fig. 1B). Interestingly, these results seem in conflict with the results shown in Fig. 1A, in which insertion of the MFA2 3′-UTR at 55% of the protein coding region promoted accelerated mRNA decay (Fig. 1A, construct 1C compared with B; construct 1G). This raises the possibility that the P-STE is upstream of nt 979 (55%) and that either: (i) it requires a specific sequence 3′ of a translation termination codon, or (ii) there needs to be a certain amount of sequence between the P-STE and the instability element in the 3′-UTR. To test which of these hypotheses is valid, the following constructs were prepared. In these constructs, a nonsense mutation at position 979 (at 55% of the PGK1 protein coding region) was inserted, and the amount of sequence immediately downstream of the termination codon was varied. In construct 2A (identical to construct 1C in Fig. 1A), the nonsense codon is immediately followed by the MFA2 3′-UTR. In construct 2B, the MFA2 3′-UTR was inserted at position 1138 of the PGK1 coding sequence and therefore it contains the 159 nt naturally located downstream of the nonsense mutation.
The P-STE functions to promote stabilization of a PGK1-STE3 chimeric transcript. Schematic representation of the PGK1-STE3 chimeric transcripts indicate the PGK1 coding sequence with a dotted rectangle and the position of the stop codon is indicated with the nucleotide number. The number at the top of the PGK1 schematic indicates the percentage of the PGK1 coding region that is translated. Non-translated PGK1 sequences are shown as white rectangles. The STE3 3′-UTR is shown as a small patterned square. Half-lives were determined in the upf1Δ strain and are shown at the right of the figure. The RNA blots are shown below the schematics.

The P-STE Needs to Be Translated in Order to Be Active—The results described above suggested that the P-STE was located between positions 789 and 979 and that it required translation in order to be active. However, since the distance between the stop codon and the 3′-UTR is critical for the stabilization effect, it was possible that translation of the P-STE per se is not required for its activity. One possibility is that transcripts that contain the P-STE but harbor nonsense codons at positions less than 55% (Fig. 1B, constructs 1E and 1F) are not stabilized because the stop codon is too far away from the 3′-UTR. Two constructs were prepared to test this possibility. In construct 2E, a nonsense mutation was inserted at position 789 to avoid translation of the coding region between nt 789 and 979 (Fig. 2B, construct 2E). The MFA2 3′-UTR was inserted at a HisCII site at nt 1138 of the PGK1 coding region. Therefore, all the sequences between the stop codon and the 3′-UTR were derived from PGK1. In construct 2E, the distance between the stop codon and the 3′-UTR is sufficient to allow the P-STE to function (compare with the distance between the stop codon and the 3′-UTR in construct 2D in Fig. 2A and construct 1G in Fig. 1B). As a control, a construct was used which is identical to construct 2E except that the 159 nt located immediately upstream of the MFA2 3′-UTR were replaced with a 159-nt region from the GCN4 leader sequence (Fig. 2B, construct 2F) known to have no effect on mRNA stability. The decay rates of the mRNAs encoded by these alleles were determined in an upf1Δ strain as described above. The results indicated that both transcripts were unstable with a half-life of 6 min. As shown above, changing the position of the stop codon to nt position 979 in otherwise identical constructions (Fig. 2A, constructs 2B and 2C) resulted in stable transcripts. Taken together, these results indicated that the P-STE is located between positions 789 and 979 of the PGK1 coding region and that translation over this sequence is required for its activity.

A Region of PGK1 Containing the P-STE Functions to Promote Stabilization of a PGK1-STE3 Chimeric Transcript—The results presented above indicated that the P-STE promotes stabilization of the MFA2 mRNA, a transcript known to decay through the deadenylation-dependent decay pathway. Previous studies have shown that the 3′-UTR from the STE3 mRNA, encoding the a-mating factor receptor, promotes rapid deadenylation-dependent mRNA decay (11). Furthermore, the STE3 3′-UTR does not promote rapid decay when inserted after the complete PGK1 protein coding region (11). Thus, we hypothesized that the P-STE would also stabilize a PGK1-STE3 hybrid transcript. To test this possibility, the MFA2 3′-UTR from two of the PGK1-MFA2 chimeric transcripts was replaced with the 3′-UTR of the STE3 transcript and the half-lives of the corresponding mRNAs were determined (Fig. 3). Both constructs contain 92.6% (starting at the N terminus) of the PGK1 coding region followed by the STE3 3′-UTR. Construct 3A contains a stop codon at position 5.6% (nt 363) of the PGK1 protein coding region so that the P-STE is not functional. Construct 3B harbors a stop codon at position 67.7% (nt 1138) of the coding region; therefore, the P-STE is translated and functional. The decay rates of these transcripts were determined as described previously. The results indicated that insertion of a nonsense mutation, which terminates translation at 5.6% of the PGK1 coding region promotes destabilization of the transcript (Fig. 3, construct 3A). By contrast, a nonsense mutation that allows translation of up to 67.7% of the PGK1 coding region results in a stable mRNA (Fig. 3, construct 3B). These results parallel the results obtained with the PGK1-MFA2 transcripts (compare with Fig. 1B, constructs 1E and 1F) and suggest that the region containing the P-STE can...
A General mRNA Stabilizer Element

The P-STE Functions when Inserted within the MFA2 Transcript—The results described above indicate that we have identified a sequence in the PGK1 coding region that, when translated, promotes stabilization of a PGK1-MFA2 chimeric transcript. We next tested whether the P-STE could function in other heterologous transcripts. To test this possibility, we analyzed whether the P-STE was functional when positioned within the unstable MFA2 transcript. We inserted different DNA fragments spanning the P-STE into the MFA2 transcript, and the mRNA half-life of the resulting mRNAs was determined. The relevant features of the constructs used are shown in Fig. 4. A unique BamHI site located at the 3′ end of the MFA2 coding region was used to insert the sequences from the PGK1 mRNA (constructs 4A–4D). Construct 4A contains the PGK1 coding region from nt 789 to 1138, which harbors the putative P-STE plus an additional 159 nucleotides inserted in frame in the MFA2 transcript. Construct 4B is essentially identical to construct 4A, except that it contains a stop codon at position 979, immediately downstream of the sequences corresponding to the P-STE. In construct 4C, the stop codon was inserted at position 789, precluding translation of the P-STE. As a control, a 350-nt fragment from the N-terminal region of the PGK1 transcript (nt 302 to 652) was inserted in frame with MFA2 sequences. In construct 4D, part of the PGK1 sequences were inserted in frame and therefore are translated (dotted rectangle) but the presence of a stop codon at position 979 prevents translation of the distal part of the inserted PGK1 sequences (white rectangle). In construct 4C, PGK1 sequences were inserted downstream of a stop codon and therefore are not translated (white rectangle). In construct 4D, an N-terminal PGK1 fragment (rectangle with vertical lines) from nt 302 to 652 was inserted in frame with the MFA2 sequences. Half-lives were determined in the upf1 strain as well as in a wild-type strain (Fig. 4A). Interestingly, the conservation in most sequences identified was restricted to a 60–65-nt region in the central portion of the 190-nt P-STE (Fig. 5A and B). Additional searches utilizing this conserved region revealed several shorter conserved sequences. Use of the 5′ and 3′ regions of the P-STE identified only one sequence from the CHC1 transcript with 78% identity in 40-nt overlap to the 3′ region of the P-STE.

Since the TEF1/2 transcript shows the greatest homology with the P-STE, we tested whether it contains a stabilizer element. For this purpose, we inserted different TEF1 fragments spanning the P-STE homology region in frame into the MFA2 transcript, and the mRNA half-life of the resulting mRNAs was determined. The relevant features of the constructs used are shown in Fig. 5C. The unique BamHI site at the 3′ end of the MFA2 coding region was used to insert the sequences from the TEF1 mRNA (constructs 5A and 5B). Construct 5A contains 330 nt from the TEF1 coding region inserted in frame in the MFA2 transcript. This fragment (nt 1080–1409, ending 32 nt downstream of the stop codon) harbors the region of homology to the P-STE plus additional 5′- and 3′-flanking sequences. The 3′ sequences provide the minimum distance defined from the PGK1 mRNA stabilizer element to the 3′-UTR. As shown above, this distance allowed the P-STE to be active. Construct 5B is identical to construct 4B and 4C (Fig. 4), except that the 190 nt corresponding to the P-STE were replaced with 65 nt from the TEF1 transcript harboring the function to override the instability caused by different instability determinants.

The P-STE Functions when Inserted within the MFA2 Transcript—We next determined whether stabilizer elements similar to the P-STE could be identified in other yeast transcripts. We carried out a computer search in which the 190-nt P-STE was compared against the complete S. cerevisiae nucleotide data base. The TEF1/2 transcripts, which encode identical forms of eEF1A, revealed the highest conservation, showing 70% identity in a 63-nt region (Fig. 5A). Interestingly, the conservation in most sequences identified was restricted to a 60–65-nt region in the central portion of the 190-nt P-STE (Fig. 5A and B). Additional searches utilizing this conserved region revealed several shorter conserved sequences. Use of the 5′ and 3′ regions of the P-STE identified only one sequence from the CHC1 transcript with 78% identity in 40-nt overlap to the 3′ region of the P-STE.

Since the TEF1/2 transcript shows the greatest homology with the P-STE, we tested whether it contains a stabilizer element. For this purpose, we inserted different TEF1 fragments spanning the P-STE homology region in frame into the MFA2 transcript, and the mRNA half-life of the resulting mRNAs was determined. The relevant features of the constructs used are shown in Fig. 5C. The unique BamHI site at the 3′ end of the MFA2 coding region was used to insert the sequences from the TEF1 mRNA (constructs 5A and 5B). Construct 5A contains 330 nt from the TEF1 coding region inserted in frame in the MFA2 transcript. This fragment (nt 1080–1409, ending 32 nt downstream of the stop codon) harbors the region of homology to the P-STE plus additional 5′- and 3′-flanking sequences. The 3′ sequences provide the minimum distance defined from the PGK1 mRNA stabilizer element to the 3′-UTR. As shown above, this distance allowed the P-STE to be active. Construct 5B is identical to construct 4B and 4C (Fig. 4), except that the 190 nt corresponding to the P-STE were replaced with 65 nt from the TEF1 transcript harboring the
Fig. 5. The TEF1/2 transcript contains a functional stabilizer element homologous to the P-STE. A, schematic representation of the P-STE. The central region of the P-STE, showing 70% identity to a region of the TEF1/2 transcript is hatched (nt 850–912). The percentage of identity as well as the nucleotide region spanning the conservation for TEF1/2 and other transcripts identified in the computer search with the core element (hatched) are indicated. B, comparison of the central region of the P-STE (nt 850–912) with the corresponding region in the TEF1 transcript. C, schematic representation of the MFA2-TEF1 chimeric transcripts used. MFA2 sequences are shown in black. The MFA2 3’-UTR is shown as a black square. Coordinates correspond to the TEF1 coding sequence. In construct 5A, TEF1 sequences (grey and hatched rectangle) are inserted in frame with MFA2 sequences. In construct 5B, only 65 nt from the TEF1 sequences conserved with the P-STE (hatched square) are inserted in frame with MFA2 sequences. Half-lives were determined in the upf1Δ strain and are shown at the right of the figure. The Northern blots are shown below the schematics.

**DISCUSSION**

Differences in decay rates of mRNAs were initially thought to be attributed to the presence or absence of specific destabilizer elements within the transcript. However, the identification of mRNA stabilizer elements in the yeast PGK1 and human α-globin mRNAs suggests that mRNA stabilization can also be an active process that requires the presence of specific sequences. In this scenario, the rate of decay of a specific mRNA would be mainly determined by the presence of specific sequences that function by increasing (instability elements) or decreasing (stability elements) the rates of poly(A) shortening and/or decapping and/or any of the steps in the decay pathway.

We have characterized a sequence in the coding region of the PGK1 transcript that functions as an mRNA stabilizer element (P-STE). The results demonstrated that the P-STE: (i) is contained within a 65-nt sequence; (ii) promotes stabilization of a PGK1-MFA2 or a PGK1-STE3 chimeric transcript; (iii) must be translated; (iv) must be located at least a minimal distance from the 3′-UTR to function; and (v) is conserved in other transcripts, i.e. the TEF1/2 mRNA.

The PGK1 Transcript Contains at Least Two Different Stabilizer Elements—Studies of yeast mRNAs have revealed a certain degree of diversity in the structural determinants that dictate rapid mRNA decay of a specific transcript (Refs. 1, 3, 9–12, 14, and 33; reviewed in Ref. 18). For example, at least two different regions within the STE3 transcript can stimulate rapid mRNA degradation (11). Presumably, the presence of multiple decay elements within a single mRNA allows for flexibility in how decay rates can be modulated. A similar picture is beginning to emerge from the studies of mRNA stability elements. Previous studies have identified a stabilizer element in the PGK1 protein coding region that inactivates the NMD pathway (18). The results presented here identify a second element in the PGK1 coding region (P-STE) that stabilizes mRNAs that degrade via 3′-UTR elements and the deadenylation-dependent decay pathway. Both of these elements need to be translated in order to be functional. However, they are not functionally redundant; the P-STE does not prevent the NMD pathway from functioning. For example, the presence of a premature termination codon at position 55% of the PGK1 protein coding region still promotes rapid mRNA decay through the NMD pathway (18, 24). However, translation of this region fully activates the ability of the P-STE to stabilize mRNAs containing the MFA2 or STE3 3′-UTR. This result suggests that these stability elements block the activity of a different factor(s) in their respective decay pathways (see below).

Three other stabilizer elements have been characterized in addition to those identified within the PGK1 transcript. One stabilizer element has been identified in the yeast GCN4 mRNA (GCN4-STE; Ref. 21) and is functionally equivalent to a STE found in the YAP1 mRNA (Y-STE; Ref. 25). The third one was isolated from the human α-globin mRNA (38). The GCN4-STE and Y-STE are located in the 5′-untranslated region of the GCN4 and YAP1 transcripts, respectively, and function to prevent degradation of the transcript through the NMD pathway. These two STEs can also function when inserted into the
untranslated region of heterologous transcripts 3′ of a non-sense codon. However, we have shown previously that the GCN4-STE does not stabilize wild-type transcripts containing the MFA2 3′-UTR (21). The α-globin mRNA stability element is a pyrimidine-rich sequence located within the 3′-UTR of the transcript. Several highly stable mRNAs contain a similar sequence motif in the 3′-UTRs, suggesting that it is a general determinant for stabilization of eukaryotic mRNAs (39–41). The GCN4-STE, the Y-STE, and the α-globin stabilizer differ from the P-STE in that they are located in untranslated portions of the transcripts. Therefore, the identification of the P-STE is a novel type of stabilizer element that uses a different mechanism to stabilize wild-type transcripts. Sequence analysis of the P-STE demonstrates that it is U-rich but has no sequence conservation with the α-globin stabilizer element. Comparison of the P-STE sequence to the yeast data base at the nucleotide level identified regions of sequence identity in several genes (Fig. 5A). The most highly conserved of these regions is located in the TEF1/2 transcript, with 70% identity over a 65-nt region. Interestingly, the results presented here indicated that a fragment spanning the highly conserved 65-nt region from TEF1/2 region can function to prevent rapid degradation of transcripts containing the MFA2 3′-UTR. Therefore, the conservation at the nucleotide level corresponds with functional conservation, indicating that the P-STE is a general stabilizer element. Further investigations will determine whether other regions identified during the data base comparison, and which have less homology to the P-STE, can also function as stabilizer elements. These analyses will aid in the determination of what are the important nucleotides for the function of the P-STE.

The P-STE Functions through the 3′-UTR and Requires Translation—Several lines of evidence suggest that the P-STE functions through the 3′-UTR: (i) it requires a minimal distance from the 3′-UTR in order to function; (ii) it stabilizes transcripts harboring the MFA2 3′-UTR instability element, which is degraded by the poly(A) shortening-dependent pathway; and (iii) it can stabilize a chimeric transcript as part of residues 363–1138 of PGK1 with the STE3 3′-UTR. The results described for PGK1 above indicate that the P-STE requires translation elongation in order to promote stabilization of the mRNA. Preventing translation of the P-STE leads to a mRNA that is nearly 3-fold less stable when compared with an almost identical mRNA that allows translation of the P-STE. This is consistent with numerous reports that indicate the existence of a link between translation and mRNA decay (reviewed in Refs. 7, 36, and 37). The question remains as to what step(s) in the translation process is required for the instability/stability effect. Furthermore, the P-STE of PGK1 and TEF1/2 encode peptides that are 50% identical and 60% similar. Compared with the 27% identity and 31% similarity of an end-weighted gap analysis of both proteins, it may be possible that the protein sequence has an effect on P-STE function.

Several reports demonstrate a link between ribosomal scanning and mRNA stabilization (13, 42–44). In one case the authors demonstrate that insertion of inhibitory structures in the 5′-UTR can modulate the activity of stability determinants present in the mRNA (13). This led to the conclusion that disruption of ribosomal scanning in the 5′-UTR rather than changes in translation initiation efficiencies per se modulate mRNA stability. However, the strategies used to block ribosomal scanning could also block translation initiation making the interpretation of these results difficult. Another approach utilized to investigate the relationship between translation initiation and mRNA decay involved using mutants in translation factors. The results of these experiments demonstrated that the mRNA decay rate of a subset of mRNAs can be modulated by mutations in several translation initiation factors (42, 44–46). More recent results using chimeric MFA2-PGK1 mRNAs demonstrated that both the PGK1 translation start codon context and the coding region act together to increase the stability of this mRNA (43). These results also demonstrate that the initiation and coding sequences are required for efficient translation of the mRNA. These results led to the interesting proposition that the nature of the translation initiation complex may modulate the rate of decapping and decay (43). Studies of c-fos instability elements further support these links, and additionally the requirement for spacing between an element and the 3′-UTR (16).

Our results indicated that the 65-nt P-STE is sufficient to promote stabilization of the MFA2 transcript when inserted in the translated portion of the transcript. If, as suggested, the nature of the initiation codon is important for the stabilization effect, this result would indicate that the MFA2 initiation codon could act in combination with the P-STE to promote stabilization of the chimeric transcript. The apparent disagreement of this conclusion with the results reported previously could be due to the nature of the chimeric mRNAs utilized in each case. The constructs used in this study harbor all the sequences from the MFA2 transcript, whereas the previously reported constructs only contained the 5′-UTR and the first three codons of the MFA2 transcript followed by the PGK1 coding region. As for the PGK1 transcript, it is possible that efficient translation initiation at the MFA2 AUG requires sequences downstream that are not present in the PGK1 transcript (specific combination of sequences). Additionally, whereas the presence of the P-STE in the context of PGK1 stabilizes an mRNA with the STE3 3′-UTR (Fig. 3), prior work has shown elements 5′ of the P-STE can affect mRNA stability (11). Further work is necessary to delineate which elements of PGK1 are fully responsible for effects on the STE3 3′-UTR chimeras and if stability elements can differentially affect different destabilizing 3′-UTRs.

Previous results suggested that the P-STE does not block the activity of instability determinants located in the translated portion of the transcript. A PGK1-MATα1 hybrid transcript in which the MIE was inserted downstream and in-frame with the P-STE was still rapidly degraded (8, 17). The 65-nt MIE is located in the protein coding region and is composed of two domains: a 5′-33–nt domain, which has a high content of rare codons; and the 3′-32–nt domain, which is predominantly AU-rich. The function of the MIE is dependent on ribosomal translation up to the last rare codon, which coincides with the 3′ end of a 15-nt sequence complementary to the 18 S rRNA (35). It has been proposed that the rare codons together with a rRNA-mRNA interaction induces a translational pause that allows recognition of the downstream AU-rich portion of the MIE promoting rapid turnover (8, 18). The MIE promotes rapid mRNA decay by increasing the rates of deadenylation and/or decapping (13). An attractive possibility to explain why the P-STE does not prevent MIE-promoted degradation is that translation termination, but not translational pausing, is required for the activity of the P-STE. This model suggests that the effect of the P-STE would not be manifested until the translation termination cycle is repeated. Since the MIE promotes rapid mRNA decay during the translation elongation cycle, the P-STE will not affect how this sequence element functions.

A Model for the Function of the P-STE on mRNA Stability—Based on the observations described above, one possible mode to explain the P-STE function is based on observations that suggest that the poly(A) tail-Pab1p interaction with the 5′ end
of the mRNA is an inhibitor of decapping (reviewed in Refs. 7 and 47), suggesting that the P-STE may protect the transcript from being rapidly decapped and/or from 3′→5′ exonucleolytic attack (19). During translation of the P-STE, a change on the translating ribosome allows the modification of a factor that binds to the ribosome during the initiation process of translation (or de novo binding of a factor). After translation terminates, the putative factor is delivered to the 3′-UTR and/or associated factor(s). As a consequence, these factors re-establish or maintain the interaction between the 5′ and 3′ ends, preventing rapid decay. The distance required between the P-STE and the 3′-UTR would provide certain flexibility in the mRNP to allow rapid decay. The distance required between the P-STE and the 3′-UTR promotes decapping. However, the fact that the P-STE is competing for the same factor that binds to the 5′-UTR and/or associated factor(s). As a consequence, these factors re-establish or maintain the interaction between the 5′ and 3′ ends, preventing rapid decay. The distance required between the P-STE and the 3′-UTR suggests some kind of steric hindrance, suggesting the activity of the P-STE requires some distance from the 3′-UTR and promotes decapping. However, the fact that the activity of the P-STE requires some distance from the 3′-UTR suggests some kind of steric hindrance, suggesting the interplay of more than one factor. Alternatively, if the P-STE functions as a protein sequence, such as in MATα1 (8, 17), this may directly affect translocation by the ribosome, a question that requires further analysis. P-STE function may require trans-acting factors, interactions between the stability and instability elements, and effects on interactions between the proteins associated with the 5′ and 3′ ends of an mRNA. This system may provide a novel means for a genetic approach to dissect both stability and instability elements, and the interactions between the two.

Acknowledgments—We thank the members of the Peltz and Kinzy laboratories for critical reading of the manuscript.

REFERENCES

1. Presutti, C., Villa, T., Hall, D., Pertica, C., and Bozzone, I. (1995) EMBO J. 14, 4922–4930
2. Muhlrad, D., and Parker, R. (1994) Nature 370, 578–581
3. Caponigro, G., and Parker, R. (1996) Nucleic Acids Res. 24, 4304–4312
4. Hsu, C.-L., and Stevens, A. (1993) Mol. Cell. Biol. 13, 4826–4835
5. Jacobs Anderson, J. S., and Parker, R. (1994) EMBO J. 17, 1497–1506
6. Muhlrad, D., Decker, C. J., and Parker, R. (1994) Genes Dev. 8, 855–866
7. Jacobson, A., and Peltz, S. W. (1996) Annu. Rev. Biochem. 65, 683–739
8. Caponigro, G., Muhlrad, D., and Parker, R. (1993) Mol. Cell. Biol. 13, 5141–5148
9. Cerginho, G. P., Atencio, D. P., Saghnini, M., Reiner, J., and Scheffler, I. E. (1995) Mol. Biol. Cell 6, 1125–1143
10. González, C. I., and Martin, C. E. (1996) J. Biol. Chem. 271, 25801–25809
11. Heaton, B., Decker, C., Muhlrad, D., Donahue, J., Jacobson, A., and Parker, R. (1992) Nucleic Acids Res. 20, 5365–5373
12. Herrick, D., and Jacobson, A. (1992) Gene 135, 35–41
13. Licciardi, A. L., Kolotova, N., Vasiliescu, S., and McCarthy, J. E. G. (1997) J. Biol. Chem. 272, 9131–9140
14. Suorsky, R. T., and Esposito, R. E. (1992) Mol. Cell. Biol. 12, 3948–3958
15. Herrick, D., Parker, R., and Jacobson, A. (1990) Mol. Cell. Biol. 10, 2269–2284
16. Gorues, C., Chen, C., Xu, N., Sonenberg, N., Jacquemin-Sablon, H., and Shyu, A. (2000) Cell 103, 29–40
17. Parker, R., and Jacobson, A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2780–2784
18. Peltz, S. W., and Jacobson, A. (1993) in Control of mRNA Stability (Brawerman, G., and Belasco, J., eds) pp. 291–328, Academic Press, New York
19. Decker, C. J., and Parker, R. (1993) Genes Dev. 7, 1632–1643
20. Peltz, S. W., Brown, A. H., and Jacobson, A. (1995) Genes Dev. 7, 1737–1754
21. Ruiz-Echevarría, M. J., González, C. I., and Peltz, S. W. (1996) EMBO J. 17, 575–589
22. Wang, X., Kiledjian, M., Weiss, I. M., and Liebhäber, S. A. (1995) Mol. Cell. Biol. 15, 1769–1777
23. Wang, Z., Day, N., Trifilis, P., and Kiledjian, M. (1999) Mol. Cell. Biol. 19, 4552–4560
24. Hagan, K. W., Ruiz-Echevarría, M. J., Quan, Y., and Peltz, S. W. (1995) Mol. Cell. Biol. 15, 869–883
25. Ruiz-Echevarría, M. J., and Peltz, S. W. (2000) Cell 101, 741–751
26. Herskowitz, I., and Jensen, R. E. (1991) Methods Enzymol. 194, 132–160
27. Rose, M. D., Winston, F., and Hieter, P. (1990) Methods in Yeast Genetics, pp. 97–112, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
28. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1992) Current Protocols in Molecular Biology, Vol. 2, pp. 11.12.1–11.13.3
29. Schiestl, R. H., and Getz, R. D. (1989) Curr. Genet. 16, 339–346
30. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
31. Myszinski, E., Segoull, V., and Branlant, C. (1990) Science 247, 1213–1216
32. Beelman, C. A., and Parker, R. (1994) J. Biol. Chem. 269, 9687–9692
33. Muhlrad, D., and Parker, R. (1992) Genes Dev. 6, 2109–2111
34. Verklen, P., and Rau, H. A. (1993) Mol. Cell. Biol. 12, 2986–2996
35. Hennigan, A. N., and Jacobson, A. (1996) Mol. Cell. Biol. 16, 3833–3843
36. Ross, J. (1995) Microbiol. Rev. 59, 423–450
37. Theodorakis, N. G., and Cleveland, D. W. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds) pp. 631–652, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
38. Weiss, I. M., and Liebhäber, S. A. (1994) Mol. Cell. Biol. 14, 8123–8132
39. Holcik, M., and Liebhäber, S. A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2410–2414
40. Paulding, W. R., and Czyzyk-Krzeska, M. F. (1999) J. Biol. Chem. 274, 2523–2538
41. Stefanovic, B., Hellerbrand, C., Holcik, M., Briendl, M., Liebhäber, S., and Brenner, D. A. (1997) Mol. Cell. Biol. 17, 5201–5209
42. Barnes, C. A. (1998) Nucleic Acids Res. 26, 2433–2441
43. LaGranderie, T., and Parker, R. (1999) RNA 5, 420–433
44. Zuk, D., and Jacobson, A. (1998) EMBO J. 17, 2914–2925
45. Barnes, C. A., MacKenzie, M. M., Johnston, G. C., and Singer, R. A. (1995) Mol. Gen. Genet. 246, 619–627
46. Barnes, C. A., Singer, R. A., and Johnston, G. C. (1993) EMBO J. 12, 3323–3332
47. Beelman, C. A., and Parker, R. (1995) Cell 81, 179–183

Downloaded from http://www.jbc.org/ by guest on July 25, 2018
Characterization of a General Stabilizer Element That Blocks Deadenylation-dependent mRNA Decay

Maria J. Ruiz-Echevarria, Raj Munshi, Julie Tomback, Terri Goss Kinzy and Stuart W. Peltz

*J. Biol. Chem.* 2001, 276:30995-31003.
doi: 10.1074/jbc.M010833200 originally published online June 22, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M010833200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 43 references, 28 of which can be accessed free at http://www.jbc.org/content/276/33/30995.full.html#ref-list-1