Disruption of Ribosomal Scanning on the 5′-Untranslated Region, and Not Restriction of Translational Initiation per se, Modulates the Stability of Nonaberrant mRNAs in the Yeast Saccharomyces cerevisiae*

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Translation and mRNA decay constitute key players in the post-transcriptional control of gene expression. We examine the mechanisms by which the 5′-untranslated region (UTR) of nonaberrant mRNAs acts to modulate both these processes in Saccharomyces cerevisiae. Two classes of functional relationship between ribosome-5′-UTR interactions and mRNA decay are identifiable. In the first of these, elements in the main open reading frame (ORF) dictate how the decay process acts to inhibitory structures in the 5′-UTR. The same types of stability modulation can be elicited by trans-regulation of translation via inducible binding of the iron-regulatory protein to an iron-responsive element located 9 nucleotides from the 5′ cap. A eukaryotic translational repressor can therefore modulate mRNA decay via the 5′-UTR. In contrast, translational regulation mediated via changes in the activity of the cap-binding eukaryotic translation initiation factor eIF-4E bypasses translation-dependent pathways of mRNA degradation. Thus modulation of mRNA stability via the 5′-UTR depends on disruption of the scanning process, rather than changes in translational initiation efficiency per se. In the second class of pathway, an upstream ORF (uORF) functions as a powerful destabilizing element, inducing termination-dependent degradation that is apparently independent of any main ORF determinants but influenced by the efficiencies of ribosomal recognition of the uORF start and stop codons. This latter mechanism provides a regulatable means to modulate the stability of nonaberrant mRNAs via a UPF-dependent pathway.

The steady-state abundance of mRNA in the eukaryotic cell is determined by the relative rates of its transcription and degradation. mRNA decay rates are not uniform, but rather vary over at least a 100-fold range (1–6), thus influencing significantly the rates of expression of individual genes. Moreover, modulation of mRNA decay constitutes an important means of regulating gene expression (3, 7–10). The mechanism of mRNA degradation has accordingly been the subject of intensive research activity and has been found to be a complex process, following a number of pathways (2, 11–13).

Any model of mRNA decay has to take into account that the same molecules that are turned over by the action of degradative enzymes also serve as templates for translation (see e.g. 14). Indeed, a number of investigations have indicated that translation influences the decay process. First, the rapidly degraded mRNAs of yeast MATα1 (15) and mammalian early response genes (16) contain translation-dependent destabilizing elements within their respective coding regions. Second, the presence of nonsense codons in the reading frames of at least some yeast mRNAs accelerates their degradation (17–19). In mammalian systems, nonsense codons destabilize nuclear, rather than cytoplasmic, mRNA (20, 21). In yeast, the pathway of accelerated decay triggered by nonsense codons, referred to as nonsense-mediated decay, is dependent on trans-acting factors encoded by the URF genes (22, 23). One of these, Upf1p, seems to be associated with ribosomes (4, 24). Third, an upstream open reading frame (uORF) in the 5′-UTR of a bacterial cat gene expressed in yeast destabilizes the whole mRNA (25). Fourth, translational inhibition of the yeast PGK1 mRNA by a stem-loop or a poly(G) sequence in its 5′-UTR leads to destabilization (26). However, the influence of such inhibitory structures was already shown earlier not to destabilize every mRNA (27–29), thus demonstrating that this is no straightforward relationship. Finally, two means of inhibiting translation lead to mRNA stabilization. They involve the inhibition of elongation using cycloheximide (2, 5, 29–31) and the use of a mutation in tRNA nucleotidyltransferase (31). Clearly, both of these experimental strategies impose a general block on translational elongation.

Up to now, the main model system for the study of translational influences on mRNA degradation in yeast has been the relatively stable mRNA encoded by PGK1 (11, 23, 26). Following the original observation that premature translational termination in URA3 destabilizes the mRNA (17), much of the work in this area has focused on the question of how nonsense codons in the reading frame of aberrant forms of PGK1 can accelerate the decay process (23). A current model specifies that nonsense-dependent decay requires a termination codon within the amino-terminal two-thirds of the PGK1 reading frame followed by specific sequence elements that include a site for reinitiation (24, 32). Other data suggest that similar principles apply to the nonsense-induced decay of HIS4 mRNA (32, 33).

Characterization of the principles governing the relationship between translation and mRNA stability is essential to achiev-

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MATERIALS AND METHODS

Strains and Media—We used the following yeast strains: YPM156B (a ade2-1 leu2-3 ade2-1 ura3-52 rpb1-1), a segregant product from a cross involving RY262 (38); SWP154 (a trp1-D1 upf1::URA3 leu2-1 his4-38 ura3-52 rpb1-1; 4); SWP154a (+) (a trp1-D1 upf1::URA3 leu2-1 his4-38 ura3-52 rpb1-1 <UPF1 TRP1 CEN>; 19); JMC 1/2 (a leu2 ura3-52 cdc33::LEU2 trp1-1 <cdc33A196 URA3 CEN>; 39). The bacterial strain used was Escherichia coli TG2 (supE hsdS5 5thi Δ(lac-proAB) Δ(srl-recA306::Tn10 tetr) F’ trpD36 proAB lacI(lacZM15)).

Plasmid Constructs—The plasmids were constructed according to standard methods (42) and verified by means of DNA sequencing. The basic expression plasmids used were YCpSUP131 (GFP promoter; 43) and YCp22FL (TEF1 promoter; 44). The leader sequences inserted are schematically represented in Fig. 1 and provided in detail in Table I. Each of these leader sequences was synthesized in the form of an oligodeoxyribonucleotide pair using an Applied Biosystems DNA synthesizer. The paired ends were BamHI and NdeI, whereby the NdeI site includes the translational start codon of the main reading frame. The sequences shown in Fig. 1 correspond to the mRNA leaders as they are synthesized in the cell. We have shown previously that transcription from the GFP and TEF1 promoters initiates upstream and downstream of the 5′ BamHI site, respectively (43, 44).

Enzyme Assays—Extracts were prepared from cells grown in YNB medium to OD₆₀₀ = 0.8–0.9. 2-ml samples from the cultures were pelleted by centrifugation; the pellets were washed twice using 50 mM Tris-Cl, pH 7.5, and frozen for subsequent analysis. The cells were broken by vortexing together with glass beads. Luciferase activities in the lysates were determined using standard procedures (45, 46), aided by a luminometer (Lumat-LB9501, Berthold). Chloramphenicol acetyltransferase activities were determined using either a radioactive assay (47) or an enzyme immunoassay (Boehringer chloramphenicol acetyltransferase enzyme-linked immunosorbent assay kit).

Polysomal Gradient Analysis and Half-life Determinations—The glassware and plasticware used in the following procedures were treated with diethyl pyrocarbonate and autoclaved before use. Total RNA was isolated using a modified version of the hot phenol procedure (48). Half-lives were determined using the lithium acetate method (41).

RESULTS AND DISCUSSION

The Influence of Structural Properties of the 5′-UTR on mRNA Stability Is Gene-dependent—Translational control on eukaryotic genes is imposed by a variety of different mechanisms involving the 5′-UTR. These mechanisms are linked to a number of structural properties of the leader region. We wanted to assess whether these distinct types of translational control mechanism determine common or independent effects on mRNA decay. Since previous work has indicated that the decay of distinct mRNAs can respond differently to translational modulation via the 5′-UTR (26–31), we chose three genes for a comparative study. As the results in this paper show, the use of only one reading frame would have generated results that are not representative of the range of translation-stability relationships that can occur in yeast. Moreover, as we shall see, the use of alternative reading frames helps distinguish two classes of stability modulation mediated via the 5′-UTR. Two of the reading frames used here are reporter genes whose products are detected readily using sensitive and quantitative assays, thus facilitating the assessment of overall expression rates. We initiated this investigation by examining the effects on translation and mRNA decay of inserting two types of structural element into the 5′-UTR, both of which are known to inhibit translation (Fig. 1). The first type of element was a stem-loop structure with a predicted stability sufficient to inhibit translation by more than 80%. Secondary structures...
These sequences correspond to the mRNA leaders initiated at the respective major transcriptional initiation sites of the GPF promoter (FL, B3, X3) and the TEF1 promoter (the remaining constructs). The transcriptional initiation site of the GPF promoter is located 5′ of the BamHI site, whereas that of the TEF1 promoter lies immediately 3′ of the BamHI site. Insertion of stem-loop structures (indicated by arrows) into the control leader FL generated the extended 5′-UTRs of B3 and X3, respectively. Alternatively, 18 G residues were introduced, creating the poly(G) leader. Two further leaders contained two versions of a “minimal” form (44) of the IRE (the stem-loop arms are indicated by arrows), one with the wild-type sequence (wt), the other with a mutant loop sequence lacking a C residue (ΔC). The remaining leaders were used in studies of the influence of uORFs (compare Fig. 7). A single base change at position +7 of the control leader B10 generated an uORF (B1A). In BIAS the distance of the uORF from the 5′ end was increased, whereas in OL the uORF overlaps with the main ORF. Finally, both wild-type and mutant forms of the natural PPR1 leader were investigated. The uORF sequences are shown in capitals.

| Leader | 5′-UTR sequences used in this work |
|--------|-----------------------------------|
| FL     | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| B3     | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| X3     | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| Poly(G)| cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| IRE    | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| IREΔC  | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| B10    | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| B1A    | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| B1AS   | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| OL     | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| PRPF   | cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |
| PPR1mut| cccttctacgaggagtaaccctttagagtacataaaacatacgaag aag aag att aac at |

FIG. 2. Stem-loops in the 5′-UTR (Fig. 1A) stabilize the LUC mRNA. A hairpin loop (see Table I for sequence) inserted either 3 nucleotides (B3) or 33 nucleotides (X3) from the cap stabilized the mRNA by approximately 70%. Samples were taken from cultures at the given time points subsequent to inhibition of transcription on the specified vectors brought about via galactose-glucose shift. Northern blot hybridization was performed using radioactive probes for LUC and PGK1 mRNAs, the latter being chromosomally encoded.
Overall, the above data show that any assessment of the influence of 5'-UTR structure on mRNA decay must take into account the role played by stability determinants present in the body of the mRNA. Three different types of relationship between translation and decay are possible. Although not the main theme of the present study, understanding this essential principle was of immediate relevance to the subsequent analysis of the effects of mRNA-binding proteins on mRNA decay.

Modulation of mRNA Stability via the Binding of a Translational Repressor Protein—It has been established that an important principle of specific gene regulation in eukaryotes involves translational inhibition mediated by the binding of a repressor protein to a site in the 5'-UTR of the target mRNA (35–37). For example, previous work has shown that the higher eukaryotic iron-regulatory protein (IRP) can be targeted to the 5'-UTR of an mRNA in yeast, where it interferes with translation of the reporter mRNA (44). Transcription of the gene encoding this cytoplasmic repressor can be placed under the control of an inducible promoter (44; Fig. 1C). Using this system, we could determine whether a 5'-UTR-specific RNA-binding protein can regulate gene expression via modulation of mRNA decay. Insertion of an iron-responsive element (IRE), which is tightly bound by IRP, into the 5'-UTRs of cat, LUC, and PGK1Δ allowed us to achieve inducible repression of the translation of the corresponding mRNAs (see e.g. Fig. 4B). As controls, we used a 5'-UTR containing a mutant derivative of

![Image](https://example.com/image1.png)

Fig. 3. Insertion of an element containing 18 successive G residues (Fig. 1B) stabilizes the LUC mRNA (panel A), destabilizes the PGK1 mRNA (panel C), and leaves the stability of the cat mRNA barely affected (panel B). Degradation of the cat mRNA, as in Fig. 4C, followed the same kinetics as observed with a 5'-UTR bearing little secondary structure (25, 28).

![Image](https://example.com/image2.png)

Fig. 4. Binding of the IRP to an IRE in the 5'-UTR (Fig. 1C) not only inhibits translational initiation (panel B), but also elicits modulatory effects on the stability of the LUC (panel A), cat (panel C), and PGK1 (panel D) mRNAs that are equivalent to those seen with strongly inhibitory intramolecular structures (Figs. 2 and 3). The ΔC mutant form of IRE is taken here as a reference. However, this mutant IRE structure, which is effectively inactive in mammalian cells, still results in some inhibition of translation and modulation of mRNA decay (compare Fig. 10). wt, wild-type.
IRE that is missing a C in the loop (ΔC; 44, 52), and a 5′-UTR lacking an IRE sequence (FL; Fig. 5). The effects of IRP binding to an IRE in the 5′-UTR of PGK1 and LUC were investigated by means of polysomal gradient analysis (as illustrated in Fig. 5). Once IRP had reached its maximal abundance in the cell, the major part of both mRNAs was localized in the 40S/43S fraction, consistent with strong inhibition of translational initiation. The equivalent IRE-FLUC (Fig. 4) and IRE-cat (data not shown) constructs directed the synthesis of greatly reduced enzyme activities. For the sake of simplicity, the luciferase activity obtained with the FLUC-I RE construct was normalized to 100% in Fig. 4 but was in fact 10% lower than that of the FL control. IRP binding to the wild-type IRE induced accelerated decay of the PGK1Δ mRNA (Fig. 4A), stabilization of the LUC mRNA (Fig. 4B), and no change in the stability of the cat mRNA (Fig. 4C). In other words, translational inhibition by a cytoplasmic repressor protein targeted to the 5′-UTR brought about effects that are equivalent to those observed when translation of these three mRNAs was inhibited by a stable stem-loop structure alone. This means that the binding energy of an RNA-binding protein can substitute for intrinsic structural elements of the mRNA.

Effects on mRNA Stability of Changes in the Binding Activity of eIF-4E—IRP functions as a translational repressor protein that is specifically targeted to only those mRNAs bearing one or more IREs. We next addressed the question whether an mRNA-binding protein essential to the translational initiation process, and involved in global translational regulation, could also influence mRNA stability. eIF-4E mediates binding of the cytoplasmic cap-binding complex (eIF-4F) to the mRNA cap. Given the potential significance of eIF-4E-cap interactions in terms of both translation and mRNA decapping, we assessed the ability of these mutants to bind capped, as opposed to uncapped, mRNA. This was achieved by means of an in vitro assay that uses biotinylated mRNAs synthesized in vitro (57). Both types of mutation reduce this protein’s absolute cap binding affinity and selectivity for capped mRNAs (see e.g. the comparison between wild-type eIF-4E and Δ196 in Fig. 6A). This mimics the effects expected when the wild-type activity of eIF-4E is regulated by means of interactions with regulatory proteins (54, 55) or via changes in its phosphorylation status (53–56). Expression of these mutant forms of eIF-4E from the relatively weak TRP1 promoter in S. cerevisiae supports reduced rates of translation in vivo (Fig. 6B). On the other hand, overexpression of the mutant CDC33 mutants using the GPA1 promoter leads to a dosage compensation effect, so that translation is either par-
tially (Δ196) or completely (Δ19/206) restored. Overexpression of wild-type eIF-4E partially depresses translation (compare Ref. 57). Analysis of the decay rates of the LUC mRNA in these respective strains revealed that translational attenuation mediated via mutations in eIF-4E does not influence the stability of this mRNA (Fig. 6B). Moreover, the PGK1Δ mRNA was only minimally destabilized, showing a reduction in half-life of maximally 10%. The generally shorter half-life estimated for PGK1Δ mRNA in these experiments is attributable to the fact that we used a galactose-glucose shift (as opposed to heat inactivation of polII) in the experimental procedure (compare e.g. Ref. 26). The minimal change in stability caused by reduced eIF-4E activity is in stark contrast to the effects of translational repression via IRP or of inhibitory structures introduced into the 5' UTR (see above). We conclude that eIF-4E activity, which is known to be regulated in eukaryotic cells, exerts a modulating effect on translation without interfering with the mRNA decay process, irrespective of the type of mRNA species involved.

An Upstream ORF Can Destabilize a Range of mRNAs—The translational modulation mechanisms considered above all involve inhibition of an early stage of interaction between the 40 S ribosomal subunit and/or the blockage of the process of scanning toward the first AUG in the mRNA. However, these are not the only mechanisms available to the cell for the attenuation and regulation of translational initiation. A considerable number of mRNAs in yeast (58–62) and higher cells (34) have uORFs in their 5' UTRs. Previous work showed that a short uORF not only inhibits the translation of cat mRNA in yeast, but also destabilizes it (25). We made use of the same uORF-containing leader to investigate whether this destabilizing effect also applies to other mRNAs (Fig. 7). Both the LUC and PGK1Δ mRNAs were indeed destabilized in the presence of the uORF (Fig. 8 and Table II), whereby the effect on the already very unstable LUC mRNA was comparatively small. The efficiency with which ribosomes recognize the start codon of an uORF can be expected to be determined by a number of factors, one of which is its distance from the 5' end of the mRNA (compare Refs. 63 and 64). We found that the degree of destabilization was dependent on the position of the uORF relative to the 5' end of the mRNA. In one type of leader (B1A), the uORF was only 6 nucleotides from the 5' end, in which position it inhibited translation of the main ORF less effec-
An uORF destabilizes mRNA in yeast to a degree related to the efficiency with which it is translated. Recognition of the uORF start codon by scanning ribosomes in the 5′-UTR is limited because of its proximity to the 5′ end of the mRNA, so that inhibition of translation of the main ORF is less than when the uORF distance is increased (B1AS; Table II). Accordingly, the B1AS leader has a considerably stronger destabilizing effect (compare panels A and B). In each case, the destabilization is partially reversed in a upf1 host. Extending the uORF of B1A into the main reading frame (OL) also leads to enhanced destabilization, despite the short cap-uORF distance (compare Tables I and II).

An example of a natural yeast mRNA bearing an uORF is that of PPR1, which is one of the least stable yeast mRNAs known (65). The upstream uORF of PPR1 is in the +1 reading frame with respect to the main ORF and terminates within the tetranucleotide AUGA, which also includes the start codon of the main ORF. Insertion of this leader upstream of PGK1Δ necessitated modification of the second codon of the PGK1Δ reading frame (to ACU; Table I). Again, the presence of an uORF rendered the half-life of the PGK1 mRNA immeasurably short (data not shown). Mutagenesis of the two consecutive start codons of the PPR1 uORF to AAGAAG nullified the destabilization effect. Thus both natural and synthetic uORFs act as destabilizing elements via a mechanism that is independent of other elements in the body of the mRNA.

Destabilization caused by nonsense codons within the main reading frame is dependent on a number of gene products encoded by the UPF genes. We therefore investigated the influence of the UPF1 gene product on the uORF-dependent changes in stability. Comparison of the stabilities of the uORF-containing mRNAs in the presence or absence of a functional UPF1 gene in otherwise isogenic strains revealed at least a partial dependence on this gene (Table II). This contrasted with the results obtained with the PGK1Δ reading frame preceded by a leader containing a poly(G) sequence; in this case, UPF1 had no effect on the half-life.

Mechanisms Underlying the Control of mRNA Stability via the 5′-UTR—In this paper we have analyzed how events on the 5′-UTR normally associated with the regulation of eukaryotic translation can also exert modulatory effects on gene expression by influencing mRNA decay. Our data therefore reflect cellular regulatory mechanisms operating at the post-transcriptional level. These principles could only be recognized using an experimental strategy that compares alternative modes of translational control, taking into account the important observation that the influence of translation on the fate of different mRNAs can be a function of their internal structure. Three of the inhibitory strategies used in our experiments, i.e. those involving the implementation of hairpin-loops, poly(G), or the IRE-IRP interaction in the 5′-UTR, block access of ribosomes to the start codon via mRNA-specific mechanisms. The poly(G) element used in this work was positioned 15 nucleotides from the cap and therefore interferes with scanning on the 5′-UTR (compare Ref. 50). This is also likely to be the main effect of stem-loops, especially when these are not situated close to the cap (Fig. 9B). It is as yet unclear whether a stem-loop structure at a cap-proximal site (as in B3) can significantly inhibit the initial interactions of the yeast 40 S subunit with the mRNA which precede scanning (66). On the other hand, in vitro experiments have indicated that the IRP-IRE interaction directly or indirectly blocks this early docking interaction (Fig. 9C and Ref. 67). Despite the differences in their modes of action, all three inhibitory mechanisms destabilize PGK1Δ mRNA and seem likely to achieve this destabilization by virtue of their influence on events in the 3′-UTR involved in the translational initiation pathway. An indirect analysis of the quantitative relationship between translation and mRNA stability demonstrates a negative correlation between translation rate and PGK1 mRNA decay rate, whereby the activity of the PGK1 destabilization mechanism can be varied over a wide range, apparently as a continuous function of the translation rate (Fig. 10).

At first sight, the mechanism of translational inhibition responsible for IRP-IRE-mediated repression seems closely related to that imposed by the cdc33 mutations. Both are thought...
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Fig. 9. There are several possible mechanisms of post-transcriptional control mediated by the 5'-UTR in the eukaryotic cell. The schematic representation of eukaryotic translation illustrates that there are a number of alternative steps at which control can be imposed (panel A). The present work focuses on modulatory effects involving translational initiation and termination. Structural elements (a hairpin loop or poly(G)) in the 5'-UTR are most likely to interfere with the overall rate ($k_\text{overall}$) of the scanning process (panel B). In contrast, binding of IRP to an IRE located near the 5' end interferes with a marked shift toward monosomes. In particular, the reduced eIF-4E activity in these strains allows accumulation of non-translating 80 S pairs (Ref. 39 and Fig. 9D). Binding of IRP to an IRE in the 5'-UTR of an mRNA in yeast, on the other hand, results in a redistribution of the target mRNA toward monomeric 40 S and 60 S subunits (Figs. 5 and 9C). Given that IRP is a cytoplasmic repressor, it is evident that cytoplasmic blocking of the 5'-UTR suffices to trigger the observed changes in mRNA stability. By analogy, secondary structures in the 5'-UTR are also likely to modulate post-transcriptional gene expression via their influence on cytoplasmic, rather than nuclear, events. eIF-4E may be one of the first cytoplasmic proteins to interact with mRNA in a eukaryotic cell. Indeed, the fact that this protein may be partially nuclear (57, 68) may even allow it to interact with mRNA before the latter is exported from the nucleus (69). Most importantly, reductions in eIF-4E activity will influence the frequency of ribosomal binding to the 5' end of the mRNA without interfering with the progress of the 40 S subunit along the leader. In contrast, the common denominator of the inhibitory elements in the 5'-UTR is the disruption of the normal function of this region, which will be accompanied by restructuring of the mRNP-ribosome complex.

Although the mechanisms underlying the differences in response of the overall decay rate to interference with ribosomal scanning are not the main theme of this study, we suggest that they relate to the respective degradation pathways of the individual mRNAs. As illustrated by the comparison of a stem-loop and a poly(G) element as stabilizers for the LUC mRNA, this mRNA is highly sensitive to the introduction of structures into the 5'-UTR known to attenuate 5' → 3' exonucleolytic degradation (26, 51). Since these elements do not trigger destabilization events in the LUC mRNA of the type reported for the PGK1 mRNA, stabilization via the blockage of exonucleolytic activity is the dominant observable effect. Degradation of the cat mRNA, on the other hand, remains unaffected because rate control on the decay pathway is determined by a step involving the main ORF. This could, for example, take the form of an endonucleolytic cleavage process within the reading frame (28), translation via eIF-4E would not be expected to interfere directly with any of the individual steps of translation indicated but rather to limit the availability of preinitiation complexes containing the 40 S subunit (panel D). Other studies have shown that the eIF-4E mutants allow accumulation of translationally inactive 80 S pairs, thus reducing the pool of active subunits available for initiation. Functional interactions between the 5'-UTR and the body of the mRNA determine the influence of structures in the 5'-UTR on mRNA stability. None of the inhibitory mechanisms is likely to affect the rate of elongation ($k_\text{cat}$). In contrast, the disruptive effects of an uORF in the 5'-UTR act via an independent pathway that involves the products of the UPF genes. The destabilizing effect of an uORF is dependent on the termination event ($k_\text{termination}$) and not determined by reinitiation downstream of the stop codon (panel E). On the other hand, ribosomal subunits that resume scanning ($k_\text{rescan}$) might contribute to the maintenance of structure and function typical of normal polysomes. This model predicts that the degree of destabilization associated with a stop codon will be linked to the efficiency of termination and/or ribosomal release directed by it. The effect of premature termination within the 5'-UTR will be redirection of the mRNA to the Upf-dependent decay pathway (panel F). This will involve rapid decapping of at least some mRNAs (compare Ref. 74), whereas the kinetics of deadenylation will be a function of the nature of the mRNA. 5' → 3' exonucleolytic activity will degrade the mRNA further, including fragments generated by endonucleolytic cleavage. O at the 5' end represents the cap.
as has been proposed for decay of the cat mRNA in E. coli (70). This suggests that there are translation-dependent destabilization elements in the PGK1 mRNA which are not present in the cat or LUC mRNAs.

Why should the destabilization induced by an uORF be capable of affecting all mRNAs, irrespective of the differences seen in their respective responses to the presence of upstream inhibitory structural elements? We propose that it is the termination process that causes the common destabilization response (Fig. 9E). As we have shown, increasing the efficiency of recognition of the uORF start codon results in more marked upf1-dependent destabilization. However, since previous work with this uORF-bearing leader revealed that blocking subsequent (re-)initiation (Fig. 9E) using a stem-loop structure does not prevent destabilization (25), it is evidently the release of terminating ribosomal subunits which triggers the nonsense-dependent decay process. Thus changing the efficiency of uORF recognition will influence the termination rate on the 5' -UTR and thereby modulate mRNA decay. The termination of translation by 80 S ribosomes changes the fate of the affected mRNAs, redirecting them into a decay pathway involving the UPF gene products (Fig. 9F). This is probably the same pathway that is followed by aberrant mRNAs whose translation is terminated by nonsense codons in the first two-thirds of the reading frame (23).

The mRNAs we have so far found to be destabilized by a short uORF in yeast are PGK1, cat, and LUC. Both synthetic and natural uORF-containing leaders induce this form of destabilization. Yun and Sherman (71) found that the CYC1 mRNA is also less stable in the presence of an uORF, which indicates that this mRNA is also destabilized by the same mechanism. The demonstration that uORFs can strongly influence mRNA stability reveals a new dimension to the role of the 5'-UTR in post-transcriptional control. Fine regulation of both the translation and the stability of any given mRNA should be possible via manipulation of the properties of individual uORFs. Yet a number of known 5'-UTRs contain more than one uORF. The most intensively studied example is that of the yeast gene GCN4 (62). This 5'-UTR manifests no obvious destabilizing function (25), and the stability of the GCN4 mRNA is not affected by inactivation of UPF1 (23). This lack of destabilization is likely to be due to specific properties of the GCN4 leader. The first uORF in this 5'-UTR directs only inefficient release of terminating ribosomes (62, 72), meaning that the downstream regions are amply populated by scanning ribosomes (compare Fig. 9). On the other hand, a GCN4-PGK1 hybrid mRNA can be destabilized by introducing a destabilizing element derived from PGK1 downstream of uORF1 in the GCN4 5'-UTR (73). Finally, our work highlights a new feature of nonsense-dependent destabilization, showing that it is not merely responsible for ridding the cell of mRNAs that have been incorrectly spliced or whose translation is prematurely terminated by nonsense codons. It also provides a mechanism for the post-transcriptional control of nonaberrant mRNAs. The degree to which this second type of destabilization mechanism influences the decay of an uORF-bearing mRNA depends on the efficiencies of initiation and termination (ribosomal release) on the uORF.

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