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Mechanisms and Control of mRNA Turnover in *Saccharomyces cerevisiae*

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INTRODUCTION

It has been clear for over 30 years that mRNA turnover contributes to the control and regulation of gene expression. The steady-state level of an mRNA is established by the cumulative effects of its rates of synthesis and decay. Moreover, the time required for an mRNA to reach a new steady-state level following a change in its rate of synthesis is directly proportional to its half-life (40). Therefore, the induction and repression of genes encoding unstable mRNAs are more rapid than those of genes encoding stable mRNAs. In addition, the half-lives of many eukaryotic transcripts are altered in response to environmental cues, indicating that mRNA decay rate not only determines the steady-state level of an mRNA but also is an important regulatory point in gene expression (for a review, see reference 9).

By using a combination of molecular, genetic, and biochem-

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TABLE 1. Methods for measuring mRNA half-lives in *S. cerevisiae*

Method	Advantages	Disadvantages
Approach to steady-state labeling	Minimal cellular perturbation Special strains and constructs not required Half-lives of many transcripts can be measured simultaneously	Large amounts of radioactive material Possible poor signal-to-noise ratio
Thiolutin and 1,10-phenanthroline	Special strains and constructs not required Half-lives of many transcripts can be measured simultaneously	Potential loss of labile factors May affect decay of certain transcripts May induce transcription of certain genes
rpb1-1 mutation	Special constructs not required Half-lives of many transcripts can be measured simultaneously	Potential loss of labile factors Heat shock Requirement for a special strain Not useful with other conditional mutants
Regulated *GAL1* promoter	Minimal cellular perturbation Transcriptional pulse-chase possible	Requirement for a special construct May not be useful for carbon source-regulated transcripts

ical approaches, significant progress has been made toward understanding the process of mRNA degradation in the simple eukaryote *Saccharomyces cerevisiae*. In this review, we cover what is currently known about mRNA decay in *S. cerevisiae* and relate it to mRNA turnover in more complex eukaryotes where appropriate. (For a more comprehensive review of mRNA degradation in mammalian cells, see reference 98.)

METHODS FOR STUDYING mRNA TURNOVER IN *S. CEREVISIAE*

The study of mRNA turnover in *S. cerevisiae* has been aided by a number of simple methods for measuring mRNA half-lives. These include in vivo labeling of mRNAs and direct measurement of mRNA decay rates following transcription inhibition (Table 1). In addition, a number of convenient techniques for trapping intermediates in mRNA degradation have been developed. These methods, as well as their relative advantages and disadvantages, are detailed below.

Approach to Steady-State Labeling

One method for determining mRNA half-lives is through approach to steady-state labeling of total yeast RNA. In this method, yeast cells are incubated with radioactive nucleotides, typically tritiated adenine, and RNA samples are isolated at various times following the addition of label. Then, either total mRNA or poly(A)$^+$ mRNA is hybridized with immobilized DNA probes complementary to the transcript of interest. Half-lives of individual transcripts are then approximated through the kinetics of their initial labeling (5, 35, 47). One cautionary note is that the half-lives of mRNAs that are stable as poly(A)$^-$ species (see below) will be underestimated by this technique if poly(A)$^+$ rather than total mRNA is used (see, e.g., reference 47). This method has the advantage of not requiring the creation of either special strains or plasmids and causes minimal cellular perturbations. However, it does require large amounts of radioactive material, and a poor signal-to-noise ratio may be obtained for mRNAs that are transcribed at low levels (see, e.g., reference 47).

Inhibition of Transcription by Using Drugs

Two drugs, thiolutin, an inhibitor of all three yeast RNA polymerases, and 1,10-phenanthroline, a Zn^{2+}-chelating agent, have been used to repress transcription in order to measure mRNA half-lives (39, 47, 48, 103, 104). Since these drugs inhibit the transcription of most genes, half-lives of many mRNAs can be measured simultaneously by determining the amount of any transcript present as a function of time after drug addition by either Northern (RNA) blotting or RNase protection assays. This approach has several advantages over other methods used for inhibiting transcription. First, there is no requirement for the synthesis of special constructs or strains (see below). Second, since these drugs can be employed under a variety of experimental circumstances, they can be used in conjunction with conditional mutations, unlike when a temperature-sensitive mutation in the gene encoding RNA polymerase II is used to repress transcription (see below). However, both thiolutin and 1,10-phenanthroline induce transcription of certain heat shock genes (1). They may also affect the decay of some yeast transcripts, as demonstrated by the existence of a lag prior to the decay of some mRNAs following treatment with thiolutin and the stabilization of several mRNAs in the presence of 1,10-phenanthroline (1, 47, 48). In addition, since individual yeast strains can have different degrees of sensitivity to thiolutin, the amount of thiolutin required to repress transcription in the particular strain under study must be determined.

Inhibition of Transcription by Using a Conditional Allele of RNA Polymerase II

An alternative method for inhibiting mRNA synthesis uses a temperature-sensitive allele of the *RPB1* gene (*rpb1-1*), which encodes the large subunit of RNA polymerase II. In *rpb1-1* mutants, RNA polymerase II is rapidly inactivated following a shift from 24 to 36°C (86). Use of the *rpb1-1* mutation to inhibit transcription again allows the half-lives of many mRNAs to be measured simultaneously without a requirement for the creation of specific plasmids. However, this technique does require the construction of yeast strains that contain the *rpb1-1* mutation. Since transcription repression by *rpb1-1* also requires that cells be shifted to 36°C, this approach is not useful for measuring half-lives in strains harboring other conditional mutations, such as cold-sensitive lesions or temperature-sensitive mutations that require long incubations at their restrictive temperatures. Moreover, not all promoters may be repressed by inactivation of *rpb1-1* following a shift to 36°C (1). Use of this approach also necessitates heat shocking cells, which may affect the decay of some mRNAs (48, 74). However, for most

yeast mRNAs examined, heat shock does not affect transcript half-lives (47). Despite these various drawbacks, the lack of pleiotropic effects associated with this approach has made it a widely and successfully employed strategy for measuring mRNA half-lives.

Inhibition of Transcription by Using Regulated Promoters

A more specific method for measuring mRNA half-lives is to use a promoter that can be regulated to conditionally produce the transcript of interest. The promoter most commonly used is the *GAL1* promoter (44, 54, 115), although other regulated promoters should also prove useful for this purpose (e.g., the *MET3* promoter [23]). Genes under the control of the *GAL1* promoter are expressed at high levels in the presence of galactose, and rapid transcriptional repression of these genes is accomplished through the addition of glucose to the medium. In contrast to other methods of transcription repression, only a subset of genes are repressed by glucose. Therefore, it is unlikely that decay rates of mRNAs will be altered by depletion of labile turnover factors as a result of using this technique, as they may be when global inhibitors of transcription are employed. In addition, the *GAL1* promoter can be used to perform transcriptional pulse-chase experiments (30; see below). However, the *GAL1* promoter may not be useful for the study of mRNAs whose stability is influenced by the carbon source (21, 65, 74, 78, 115).

Transcriptional Pulse-Chase: a Method for Examining Pathways of Decay

Measurement of the steady-state half-life of an mRNA provides little information about the mechanisms by which that transcript is degraded. To overcome this limitation, methods for examining the pathways through which individual transcripts are degraded have been devised for both yeast and mammalian systems. These methods exploit regulated promoters to rapidly induce and then repress the transcription of a given gene, thereby creating a synchronously produced pool of newly synthesized mRNA whose decay can be monitored in the subsequent chase (30, 108, 122). This general approach has been termed a transcriptional pulse-chase.

Transcriptional pulse-chase experiments have been performed with *S. cerevisiae* by using the *GAL1* promoter (30). In this case, cells are grown in medium containing a neutral carbon source, such as raffinose or a mixture of raffinose and sucrose, in which expression from the *GAL1* promoter is neither induced nor repressed (54). Addition of galactose to these cultures causes a rapid induction of transcription from the *GAL1* promoter. After a brief induction, typically 10 to 15 min, transcription is quickly repressed by the addition of glucose. Most mRNA is synthesized between 6 and 10 min following transcription induction (29a). Thus, this technique enables the production of a population of mRNA transcripts that are all recently synthesized and therefore have uniformly long poly(A) tails (Fig. 1). Following transcription repression, the absolute levels of the mRNA, the status of the poly(A) tail before and during mRNA degradation, and the appearance and/or disappearance of decay intermediates can all be monitored (see below). This protocol has proven useful in establishing temporal correlations between events in mRNA degradation such as poly(A) tail loss and mRNA decay and precursor-product relationships between a full-length mRNA and its decay intermediates (Fig. 1; see below).

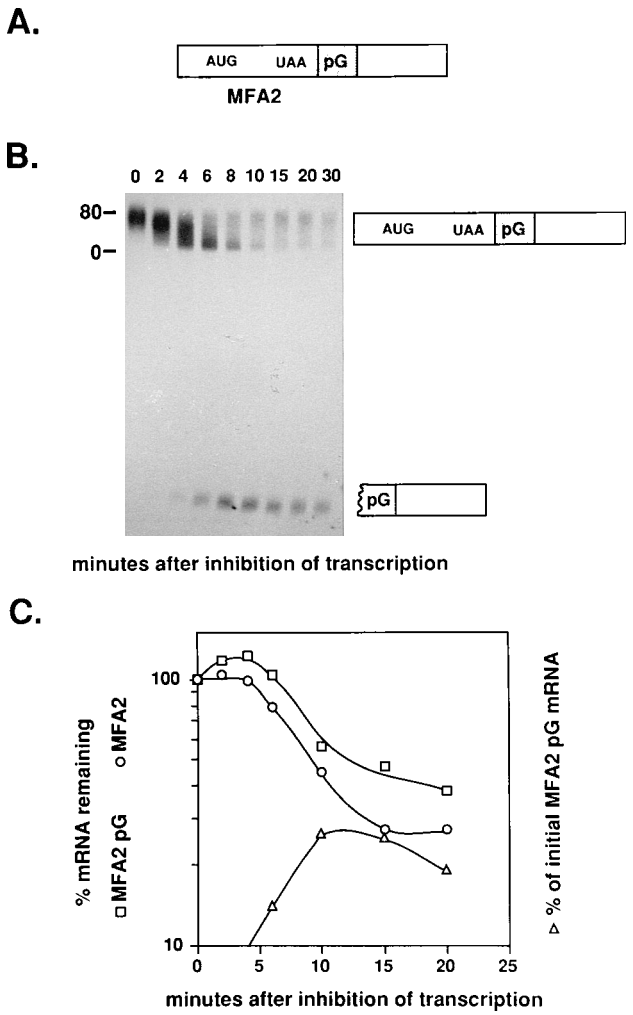

FIG. 1. Transcriptional pulse-chase experiment examining the decay of the *MFA2pG* mRNA. (A) Schematic representation of the *MFA2pG* mRNA. The shaded region depicts the site of insertion in the 3′ UTR of this mRNA of a poly(G) tract (pG), which forms a stable secondary structure. (B) Polyacrylamide Northern analysis of the *MFA2pG* mRNA from a transcriptional pulse-chase experiment. The upper band is full-length *MFA2pG* mRNA, and the reduction in size of this mRNA with time is due to progressive shortening of the poly(A) tail [determined by treatment of the time zero sample RNA with RNase H and oligo(dT) (data not shown)]. The lower band corresponds to a 5′-to-3′ decay product trimmed to the 5′ side of the poly(G) tract. The full-length mRNA and decay product are shown schematically on the right, and poly(A) tail lengths on the full-length mRNA are shown on the left. The time following transcription repression for each sample is shown above the figure. (C) Comparison of the decay rates of the *MFA2* and *MFA2pG* mRNAs during transcriptional pulse-chase experiments. Also shown are levels of the poly(G) decay product as a percentage of the initial amount of *MFA2pG* mRNA. The *MFA2pG* mRNA points represent the means of three separate experiments. Reprinted from reference 30 with permission of the publisher.

Identification of Intermediates in mRNA Decay

To understand mRNA decay, the nucleolytic events that lead to the degradation of individual transcripts must be determined. However, intermediates in mRNA decay are only rarely observed. This result strongly suggests that following an initial nucleolytic event, the body of the transcript is degraded extremely rapidly. To prevent or slow this rapid degradation, strategies that block exonucleases, thereby allowing detection of decay intermediates, have been developed. These approaches include the insertion of thermodynamically stable

secondary structures into mRNAs to block nucleases and the use of mutations in genes encoding the nucleases. From the analysis of decay intermediates trapped by these methods, the pathways by which several mRNAs are degraded have been inferred.

Several different sequences capable of forming stable stem-loops have been used to trap decay intermediates that arise from yeast mRNAs (8, 30, 80, 120). The most efficient of these is a poly(G) tract, which forms a very stable secondary structure in vitro (121, 125). It was first demonstrated that poly(G) tracts could be used to trap decay intermediates in vivo when an 18-nucleotide poly(G) tract was inserted into the 3′ untranslated region (UTR) of the *PGK1* mRNA (120). This structure has proven effective in trapping both 5′-to-3′ and 3′-to-5′ decay intermediates (30, 79, 80, 82, 120). Insertion of poly(G) tracts into the 3′ UTRs of the *PGK1* and *MFA2* transcripts does not appear to alter the rates or the mechanisms of decay. This suggests that insertion of poly(G) tracts into 3′ UTRs will in general not activate novel pathways of mRNA degradation (30) (Fig. 1C). In contrast, when poly(G) tracts are inserted into the 5′ UTR, this structure inhibits translation (8, 119), which can alter the rates of mRNA deadenylation and decapping (see, e.g., reference 80). This caveat must be considered when interpreting the mechanisms of decay based on such 5′ UTR insertions, and, whenever possible, supporting information should be obtained by analyzing the transcript's turnover in nuclease mutants (see below). In addition, the effect of this structure on either translation elongation or mRNA decay when inserted into the coding region is not yet known.

A second method for trapping decay intermediates is to utilize mutations in genes encoding the nucleases that degrade mRNAs. One such gene is *XRN1*, which encodes a major cytoplasmic 5′-to-3′ exonuclease (60). In vitro, this nuclease degrades mRNAs that lack a 5′ cap (111), and consistent with this activity, full-length decapped intermediates in mRNA decay are stabilized in *xrn1*Δ mutants (51, 79, 80, 82). Such decapped intermediates are undetectable in wild-type cells (51, 79, 80). The *xrn1*Δ mutation may also prove useful in the study of endonucleolytic cleavage of yeast mRNAs, since this mutation might stabilize 3′ decay products that result from such cleavages.

The analysis of degradation intermediates has allowed a discrete set of mRNA decay pathways to be defined (30, 51, 79, 80, 82). An extension of this work is that specific decay intermediates can now be diagnostic for a particular pathway of mRNA decay. For instance, poly(G) tracts trimmed to their 5′ side are diagnostic of 5′-to-3′ mRNA decay (see below). Similarly, features of the mechanism of degradation of a given transcript can be inferred by studying the effects of mutations in genes required for specific pathways of decay. For example, if an mRNA is stabilized in cells lacking either the *XRN1* 5′-to-3′ exoribonuclease or the decapping enzyme (encoded by the *DCP1* gene [see below]), the transcript can be inferred to be degraded by decapping and 5′-to-3′ digestion. Similarly, transcripts whose decay is dependent on the *UPF1*, *UPF2*, or *UPF3* gene, required for rapid decay of mRNAs with early nonsense codons, can be inferred to have properties of this decay pathway in their turnover (see below).

DETERMINANTS OF mRNA STABILITY IN *S. CEREVISIAE*

The half-lives of individual yeast mRNAs vary over a wide range, from as little as 1 min to as long as 60 min (47, 66). Determining the basis for these differences is central to understanding how mRNA half-lives are controlled. The available information suggests that mRNA half-lives are determined in large part by specific sequences within each mRNA. It is also clear that less well defined features of an mRNA, such as specifically positioned rare codons and translation rate, can influence the decay of individual transcripts.

Specific Sequences Influence mRNA Half-Lives

Elements that affect mRNA decay rates have been identified through the analysis of chimeric mRNAs that are composed of portions of transcripts which degrade with different half-lives and through the study of mRNAs that contain deletions and point mutations that alter their decay. One general class of elements to be revealed by these methods consists of sequences that destabilize mRNAs. These instability elements have been identified throughout the lengths of transcripts, indicating that they need not be localized to any general region of mRNAs.

Instability elements have been identified in the 5′ UTRs of two yeast mRNAs. The 5′ UTR of the highly unstable *PPR1* transcript ($t_{1/2}$ = 1 min) is capable of destabilizing the moderately unstable *URA3* mRNA ($t_{1/2}$ = 10 min) (5, 66, 94). Similarly, the 5′ UTR of the *SDH* Ip mRNA is responsible for the rapid degradation of this transcript that occurs in the presence of glucose (21). How either of these 5′ UTR sequences promote mRNA decay is not known. However, one speculation, given the proximity of the 5′ UTR to both the 5′ cap and the translation start site, is that these 5′ UTRs may affect mRNA decapping (see below) either directly or indirectly through the regulation of translation initiation. Consistent with this model is the observation that like other mRNAs whose decay is initiated by mRNA decapping, the *SDH* Ip transcript is not rapidly degraded in *xrn1*Δ cells (21, 51, 79, 80, 82; also see below).

Instability elements have also been identified in the coding regions of several yeast mRNAs including the unstable *MATα1*, *HIS3*, *STE3*, and *SPO13* transcripts (18, 44, 46, 87, 115). The best-defined of these is a 65-nucleotide region from the unstable *MATα1* transcript ($t_{1/2}$ = 3.5 min). This 65-nucleotide element is composed of two parts, one located in the 5′ 33 nucleotides and the other located in the 3′ 32 nucleotides. The 3′ 32 nucleotides of this element contain the information specifying rapid decay, while the 5′ 33 nucleotides cause a roughly twofold stimulation of turnover mediated by the downstream region. This instability element serves to promote mRNA turnover by increasing the rates at which mRNAs are both deadenylated and decapped (19; also see below). Thus, coding region elements can both be relatively small and affect steps in decay that occur at distal sites on the mRNA.

The mechanisms by which this and other coding-region instability elements function are unknown. However, because these elements are located in the coding regions of mRNAs, they may interact with the translation machinery to influence decay. This possibility is strongly supported by two observations. First, translation up to, or through, the *MATα1* instability element is required for it to promote rapid decay (87). Second, the important feature of the upstream 33-nucleotide region of this element is the presence of rare codons rather than any specific RNA sequence or structure (18). These results suggest that decay mediated by this element requires both that the ribosome (or, alternatively, a ribosome-associated factor) be present and that the ribosome be paused at a particular site on the mRNA.

A coding-region element has been identified within the unstable mammalian c-*fos* mRNA that is similar to the *MATα1* instability element for two reasons (108, 109). Like the *MATα1* instability element, the c-*fos* instability element stimulates the

rate at which mRNAs are deadenylated (108, 109). In addition, the c-*fos* coding region element appears to require translation to promote mRNA decay since it does not destabilize a reporter mRNA that is untranslated because of the presence of a stem-loop structure in its 5′ UTR (105). Thus, discrete sequence elements within the coding regions of mRNAs that stimulate turnover in a manner that is linked to mRNA translation are likely to be common features of eukaryotic mRNAs.

Instability elements are present within the 3′ UTRs of the unstable *HTB1*, *MFA2*, and *STE3* mRNAs (44, 81, 123). The most extensively analyzed of these 3′ UTRs in terms of specific sequences that promote mRNA degradation is the *MFA2* 3′ UTR. This analysis strongly suggests that the elements within this 3′ UTR that promote rapid mRNA decay are redundant, since various deletions and point mutations can be tolerated with only small effects on decay rate whereas double mutations or larger deletions have a greater effect on slowing mRNA decay (81). The exact sequences that serve to promote rapid mRNA decay are not known. However, one possibility is that the sequence motif Y_{6-8}CAU (where Y = C or U) is a feature that promotes mRNA turnover, since there are six copies of this motif within the *MFA2* 3′ UTR and a point mutation in one of these motifs can contribute to slowing mRNA decay. Through analogy to several labile mammalian transcripts that contain AU-rich sequences capable of promoting decay (see below), a second motif within the *MFA2* 3′ UTR that may stimulate rapid mRNA decay is the sequence U(A/C)AUUU AUU, of which two copies are present (81).

The 3′ UTR of *MFA2* mRNA promotes rapid mRNA turnover by stimulating the rates at which this mRNA is both deadenylated and decapped (81). Instability elements similar to those found in the *MFA2* 3′ UTR have also been identified in several unstable mammalian transcripts such as the c-*fos*, c-*myc*, and granulocyte-macrophage colony-stimulating factor transcripts (55, 56, 107–109, 122). For example, the c-*fos* mRNA contains within its 3′ UTR a repeated motif, UUAUU UA(U/A)(U/A), that stimulates the rates of both deadenylation and subsequent degradation of the body of the transcript (58, 108, 122, 126). How these regions within mRNA 3′ UTRs increase the rates of deadenylation and subsequent degradation of the body of transcripts is not known. However, the fact that these instability elements within 3′ UTRs are often redundant and are situated close to the poly(A) tail suggests that they may provide multiple binding sites for factors that stimulate mRNA deadenylation. Along these lines, a factor has been identified that binds to U-rich sequences that contain AUUUA sequence motifs within the c-*myc* mRNA. This factor also stimulates turnover of this mRNA in vitro (14). Perhaps this protein and analogous factors in *S. cerevisiae* serve to directly influence the activity of the deadenylation machinery (see below).

Two observations raise the possibility that there are also sequence elements that stabilize mRNAs. First, the 3′ UTR of the *STE3* mRNA contains an instability element capable of destabilizing the *PGK1* mRNA only if 82% of the *PGK1* coding region has been deleted. This result suggests that sequences capable of overriding the instability element within the 3′ UTR of the *STE3* mRNA are present within the *PGK1* coding region (44). Second, the presence of a premature translation termination codon destabilizes the *PGK1* mRNA (see below). However, insertion of a specific region from the 3′ portion of the *PGK1* coding sequence upstream of the early nonsense codon partially stabilizes the mRNA (89). This result provides further support for the existence of sequences that can stabilize mRNAs.

The identification of specific sequence elements within mRNAs that affect decay rates is often difficult. The most commonly used approach is to construct chimeric genes between genes encoding stable and unstable mRNAs and then to determine the half-lives of the resulting mRNAs. The ability of a region from an unstable mRNA to destabilize a stable transcript when it is either fused to or inserted into the recipient mRNA is taken as evidence for an instability element. However, the interpretation of chimeric mRNA half-lives may be complicated for two reasons. First, construction of chimeric mRNAs may involve the removal of portions of the stable mRNA that contain stability elements (see, e.g., reference 44). Second, chimeric mRNAs contain novel combinations of mRNA sequence at junctions between the different transcripts which could potentially form instability elements. It should be noted, however, that this possibility is unlikely, since chimeric mRNAs composed of various portions of the stable *PGK1* and *ACT1* mRNAs are themselves stable (44).

Several other possibilities must also be considered during the analysis of sequences that affect mRNA decay. Identification of elements that affect mRNA stability may be obscured by the presence of additional antagonistic or redundant elements within an mRNA. For instance, the coding region and 3′ UTR of the *STE3* mRNA both contain instability elements. Because of the presence of the two functionally redundant elements, deletion of a single element has little or no effect on the half-life of this mRNA (44). Through analogy to nonsense-mediated mRNA decay (see below), the deletion or insertion of sequences into an mRNA may also cause novel pathways of degradation to be activated. Despite these caveats, the identification of a large number of elements that affect mRNA decay illustrates that transcript half-lives are determined in large part by specific sequences within mRNAs. These elements should be useful tools for understanding how different mRNAs are degraded and for identification of *trans*-acting factors that influence mRNA-specific rates of decay (see below).

Nonspecific Features of mRNAs Generally Do Not Influence mRNA Half-Lives

Most evidence suggests that nonspecific features of transcripts, such as mRNA length, translational efficiency, and percentage of rare codons, do not contribute in any consistent way to mRNA stability. However, while there may not be any general relationship between these features of an mRNA and its half-life, these features can contribute to the half-lives of individual transcripts.

There is no correlation between mRNA length and stability. It has been proposed that mRNA length is inversely proportional to mRNA half-life (104). The original rationale behind this model was that longer mRNAs would present a larger target area for endonucleolytic attack than would shorter ones. This possibility seems less likely now, given that the major pathway of mRNA decay in *S. cerevisiae* appears to be 5′ to 3′ and not endonucleolytic (see below). However, a correlation between length and decay rate has been reported for several yeast mRNAs (104), although a more extensive comparison of length and stability for a large number of yeast transcripts suggests that no such general relationship exists (47). Furthermore, in direct contrast to this model, large deletions within the stable *PGK1* and *ACT1* mRNAs slightly destabilize these mRNAs. Thus, there is no general rule correlating mRNA half-life and length.

Ribosome protection cannot account for mRNA half-lives. It has been proposed that ribosomes protect an mRNA from nucleolytic attack; therefore, less efficiently translated or un-

translated mRNAs should be more unstable (50). This model stems largely from the facts that early nonsense codons, which create a "ribosome-free" area 3′ of the early stop codon, destabilize mRNAs (67, 89) whereas the translation inhibitor cycloheximide, which stalls ribosomes on an mRNA, stabilizes most transcripts (47). However, several observations indicate that in general, ribosome-mediated mRNA protection is unlikely to influence mRNA half-lives. First, ribosome packing densities do not differ between stable and unstable transcripts (103). Second, at least in some cases, treatment of cells with cycloheximide stabilizes untranslated as well as translated transcripts, indicating that stabilization by this drug is not due to the presence of elongating ribosomes on an mRNA that directly prevent nucleolytic attack (8). Third, mRNA destabilization by early nonsense codons is dependent on specific sequences 3′ of the early termination codon and not simply on the presence of ribosome-free regions 3′ of premature stop codons (89). Thus, ribosome-mediated protection of an mRNA does not account for differential mRNA half-lives.

While there is no simple relationship between mRNA decay and translation, it should be noted that translation is an important determinant of stability for some mRNAs. For example, inhibition of translation of the *PGK1* mRNA through the insertion of a stable secondary structure into its 5′ UTR destabilizes this transcript. This destabilization is due to an increase in the rates of deadenylation and decapping of this mRNA (80; also see below). In contrast, translation up to, or through, an instability element within the coding region of the *MATα1* transcript is required for this element to promote rapid mRNA decay (87). Thus, translation is likely to affect the stability of mRNAs in an mRNA-specific manner.

Rare codons are not general determinants of mRNA stability. A third model is that the presence of a high percentage of rare codons in an mRNA increases the rate of mRNA degradation (50). Evidence in support of this hypothesis comes from the observation that unstable mRNAs contain a higher percentage of rare codons than do stable mRNAs, although this correlation may also reflect independently evolved mechanisms of maintaining low levels of gene expression (47). Also, replacement of the first 164 codons (39%) of the *PGK1* transcript with their rare counterparts reduced steady-state levels of this transcript approximately threefold (50). However, these manipulations also caused changes in the primary sequence within a region of the *PGK1* gene that may contain an mRNA-stabilizing sequence (44) and/or a transcriptional enhancer (73). In addition, inserting rare codons into the *PGK1* transcript or fusing an mRNA containing a high percentage of rare codons to the *PGK1* transcript is not sufficient to destabilize this mRNA (18). Thus, it is unlikely that rare codons per se are general determinants of mRNA stability. Rare codons can, however, affect mRNA stability in an mRNA-specific manner, as demonstrated by the observation that decay mediated by a 32-nucleotide region of the *MATα1* transcript is significantly enhanced when a cluster of rare codons is positioned 5′ of this region (18; also see above).

A COMMON PATHWAY OF mRNA DECAY

To understand the basis for differential mRNA half-lives, the various pathways of mRNA decay must be determined. Once these pathways have been established, they will provide a framework for understanding how both *cis*-acting sequences and *trans*-acting factors serve to influence the stabilities of specific mRNAs. One model consistent with the available data is that many mRNAs in *S. cerevisiae* are degraded at different rates through a common pathway. The initial nucleolytic event in this pathway is shortening of the poly(A) tail to an oligo(A) length of 10 to 15 nucleotides. Following this shortening, the transcript is decapped by the product of the *DCP1* gene, rendering it susceptible to 5′-to-3′ exonucleolytic digestion performed by the product of the *XRN1* gene (30, 51, 79, 80, 86, 120) (Fig. 2).

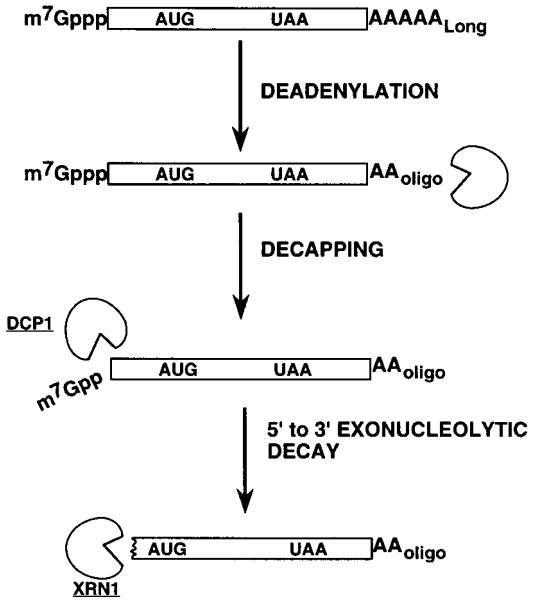

FIG. 2. Common pathway of mRNA decay in *S. cerevisiae*. Shown is a schematic representation of the deadenylation-dependent decapping pathway of mRNA decay. The initial step in this pathway is shortening of the poly(A) tail of an mRNA to an oligo(A) length (~10 to 15 residues). Following deadenylation, the mRNA is decapped by the *DCP1* gene product and subsequently degraded in a 5′-to-3′ direction primarily by the product of the *XRN1* gene.

Deadenylation Precedes the Decay of Some Yeast mRNAs

Several lines of evidence strongly suggest a role for deadenylation in the decay of yeast mRNAs. In one case, point mutations in the 3′ UTR of the unstable *MFA2* mRNA ($t_{1/2}$ = 3.5 min) that stabilize this transcript up to fourfold have been identified. These mutations change the rate of deadenylation of the *MFA2* mRNA to an extent that either entirely or at least partially accounts for the observed changes in decay rate (81). In addition, results of transcriptional pulse-chase experiments examining the degradation of the stable *PGK1* ($t_{1/2}$ = 35 min) and unstable *MFA2* and *STE3* ($t_{1/2}$ = 3 min) transcripts indicate that degradation of the body of these mRNAs occurs only following shortening of their poly(A) tails to an oligo(A) length of ~10 to 15 residues (30) (Fig. 1). Thus, deadenylation precedes the decay of these mRNAs. This temporal correlation between shortening of the poly(A) tail and degradation of the transcript body is significant because transfer of the *MFA2* 3′ UTR, which is capable of stimulating rapid deadenylation, to the stable *PGK1* mRNA decreases the time required to deadenylate this chimeric mRNA and correspondingly alters the time at which the transcript body begins to decay (30).

Examination of decay intermediates that arise from 5′-to-3′ degradation of the *PGK1* and *MFA2* transcripts provides further evidence that deadenylation precedes the decay of these mRNAs (30, 120). First, in transcriptional pulse-chase experiments, these decay products accumulate only after the poly(A) tails of the full-length mRNAs have shortened to an oligo(A) length (Fig. 1). Importantly, these intermediates also have only

short poly(A) tails even though the full-length mRNA consists of a mixture of short and intermediate-length poly(A)-tailed species (30). Similar results are seen with decay intermediates trapped by deletion of the *XRN1* gene, when only deadenylated transcripts are observed to be decapped (51, 79). These results indicate that decay products are generated only from full-length mRNAs that have been deadenylated (30).

Following deadenylation, mRNAs are degraded at different rates. For instance, decay of oligoadenylated *PGK1* mRNA is relatively slow whereas oligoadenylated *MFA2* mRNA is highly unstable (30). This observation provides an alternative explanation for several results that have been previously interpreted to argue that deadenylation does not contribute to mRNA decay (47, 92). First, the steady-state populations of several stable yeast mRNAs, including the *PGK1* transcript, have predominantly short (or no) poly(A) tails (47, 103). Second, the polyadenylated forms of the *CYH2* and *PGK1* mRNAs chase into a stable poly(A)-deficient form too rapidly to account for their overall stability (47, 120). This accumulation of poly(A)-deficient mRNA species is now understood to result from the fact that although deadenylation precedes decay of these transcripts, degradation of the oligoadenylated mRNA species is slower than deadenylation (30, 80).

Decapping and 5′-to-3′ Exonucleolytic Digestion Follow Deadenylation of Some Yeast mRNAs

Following deadenylation, the body of the *MFA2* and *PGK1* transcripts are degraded by 5′-to-3′ exonucleolytic digestion. This degradation is initiated at or near the 5′ cap. The evidence for this mechanism of decay has come from the analysis of decay products trapped by various strategies. For example, the presence of a poly(G) tract in the 3′ UTRs of the *PGK1* and *MFA2* mRNAs results in the accumulation of decay products trimmed to the 5′ side of the poly(G) insertion (30, 120). Movement of the poly(G) tracts close to the 5′ ends of these mRNAs again results in the accumulation of decay products trimmed to the 5′ side of the poly(G) insertion. Moreover, in mRNAs containing multiple poly(G) insertions, decay products trimmed to the 5′ side of more distal poly(G) tracts are less abundant than those trimmed to the 5′ side of the proximal poly(G) tract (79, 80, 120).

Examining the decay of the *MFA2* and *PGK1* mRNAs in cells lacking the *XRN1* gene product indicates that the initial nucleolytic event following deadenylation is mRNA decapping. The *XRN1* gene encodes a 5′-to-3′ exonuclease that in vitro degrades RNA lacking a 5′ cap (60, 111). In *S. cerevisiae* strains harboring a *xrn1*Δ mutation, 5′-to-3′ decay intermediates trapped by the insertion of a poly(G) tract into the *MFA2* 3′ UTR cannot be detected (79). Levels of a similar decay product arising from the *PGK1* transcript with a poly(G) in its 3′ UTR are also greatly reduced in these mutants (80). These results indicate that Xrn1p is required for most of the 5′-to-3′ digestion of these transcripts. In the absence of 5′-to-3′ digestion by Xrn1p, *MFA2* and *PGK1* mRNAs accumulate with time as decapped species. As judged by primer extension analysis, decapped *MFA2* transcripts are full length and decapped *PGK1* mRNAs are shortened by 2 nucleotides from their 5′ ends relative to the capped *PGK1* mRNAs (79, 80). It should be noted that whether the *PGK1* transcript is initially decapped in the cap linkage and then undergoes an Xrn1p-independent removal of two bases or is cleaved initially at the +2 position is not yet clear. Thus, following deadenylation, these two transcripts are decapped by cleavage at or very near their 5′ ends. In both cases, the enzyme responsible for the decapping reaction appears to be encoded by the *DCP1* gene (8a; also see below).

Following decapping, the *MFA2* and *PGK1* mRNAs are degraded primarily by the product of the *XRN1* gene (60, 79, 80). However, two observations suggest that there is a second 5′-to-3′ exonuclease capable of performing 5′-to-3′ digestion of at least some decapped transcripts. First, low levels of *PGK1* 5′-to-3′ decay product are observed in cells lacking Xrn1p (79, 80). Second, *PGK1* mRNA is stabilized to a greater extent by inhibition of decapping by cycloheximide than by inhibition of 5′-to-3′ degradation by the *xrn1*Δ mutation, suggesting the presence of a second 5′-to-3′ exonuclease that is blocked by a 5′ cap (8, 80). One candidate for this second 5′-to-3′ exonuclease is the product of the *RAT1*/*HKE1* gene (3, 57). Localization studies suggest that Rat1p is primarily nuclear (57). This observation, coupled with the fact that *RAT1* mutant strains have defects in both nuclear-cytoplasmic RNA transport and 5.8S rRNA processing, strongly suggests that this exonuclease functions primarily in the nucleus (3, 45). However, the similar activities of Xrn1p and Rat1p (57) and the fact that they functionally overlap in the processing of 5.8S rRNA (45) suggest that Rat1p may also degrade decapped mRNAs.

Deadenylation-Dependent Decapping Is a Common Pathway of mRNA Decay

Only the *PGK1* and *MFA2* transcripts have been rigorously demonstrated to degrade through the deadenylation-dependent decapping pathway. However, for several reasons, this pathway is likely to be common to most yeast mRNAs. First, deadenylation precedes the decay of the *STE3* and *MATα1* mRNAs (19, 30). Most other yeast transcripts are also deadenylated, since bulk poly(A) tails are initially long and undergo progressive shortening with time (36, 93). Moreover, bulk poly(A) is probably deadenylated by the same factors that shorten the poly(A) tails of mRNAs that are degraded through this pathway. This possibility is suggested by the observation that mutations in the poly(A)-binding protein that slow deadenylation of the *PGK1* and *MFA2* mRNAs also result in an increase in the average length of total cellular poly(A) (20, 101; also see below).

Evidence that decapping and 5′-to-3′ digestion are common to many yeast transcripts also comes from an examination of mRNA decay in strains lacking either the Dcp1p decapping enzyme, or the Xrn1p exoribonuclease. In *dcp1*Δ strains, several yeast mRNAs, including the unstable *MATα1*, *MFA2*, *HIS3*, and *GAL10* mRNAs as well as the stable *ACT1*, *PGK1*, and CYH2 transcripts, are stabilized (8a). Similarly, in cells lacking Xrn1p, full-length deadenylated forms of the *Rp51A*, *CYC1*, *ACT1*, *MFα1*, and *MATα1* transcripts accumulate (19, 51). The deadenylated forms of several of these mRNAs (*ACT1*, *Rp51A*, and *CYC1*) are degradable by purified Xrn1p, suggesting that they lack a 5′ cap (51). Lastly, Xrn1p is very abundant and primarily cytoplasmic (49). It is therefore possible that this protein degrades the majority of yeast transcripts.

The deadenylation-dependent decapping pathway of mRNA decay may also occur in more complex eukaryotes. The mammalian c-*myc* and c-*fos* mRNAs are deadenylated prior to their decay (15, 59, 108, 117, 122). In addition, activities that could catalyze both the decapping reaction and 5′-to-3′ mRNA digestion have been identified in extracts from mammalian cells (24) and potential Xrn1p homologs have been identified in both *Drosophila melanogaster* and *Xenopus laevis* (49). It will be of great interest in future experiments to determine the mechanisms by which transcripts are degraded in more complex eukaryotes.

Control of mRNA Half-Lives through the Deadenylation-Dependent Decapping Pathway

The decay rates of individual transcripts degraded by deadenylation-dependent decapping are determined by their specific rates of deadenylation and decapping. By combining a variety of different rates of deadenylation and decapping, a wide spectrum of mRNA decay rates is possible. For instance, the *MFA2* transcript is degraded rapidly because of fast deadenylation and fast decapping whereas the stable *PGK1* transcript exhibits slow deadenylation and decapping. In the following sections, we discuss the available information about the processes of deadenylation and decapping and how different rates of these key steps may be specified.

Control of mRNA deadenylation. Since the rate at which an mRNA is deadenylated contributes to its overall stability, it is critical to determine how differential rates of deadenylation are specified. It is clear that some *cis*-acting sequences that stimulate mRNA turnover, such as the *MFA2* 3′ UTR, do so in part by accelerating mRNA deadenylation (30, 81). An important issue concerns how these sequences exert control over mRNA deadenylation and which nucleases are the targets of this regulation.

(i) Poly(A)-binding protein influences deadenylation. One protein that influences deadenylation in *S. cerevisiae* is the poly(A)-binding protein, Pab1p, encoded by the essential *PAB1* gene (100). In *pab1* mutants, there is an increase in the length of bulk cellular poly(A) (101). In addition, the *PGK1* and *MFA2* mRNAs are deadenylated three- and sixfold more slowly, respectively, in *pab1*Δ mutants than in *PAB1* strains (20).

Two general types of models can explain the reduced rates of deadenylation observed in *pab1*Δ strains. In one view, proteins bound to the poly(A) tail in the absence of Pab1p could inhibit poly(A) shortening. Alternatively, Pab1p could itself influence deadenylation (101). Although neither model can be ruled out, some support for the latter hypothesis comes from the partial purification of a Pab1p-dependent poly(A) nuclease (PAN [see below]).

(ii) Poly(A)-binding protein-dependent nuclease activity from *S. cerevisiae*. A largely Pab1p-dependent poly(A) nuclease activity, termed PAN, has been identified in yeast extracts. This nuclease exhibits little or no activity in the absence of Pab1p in crude extracts or following further purification (68, 99). This nuclease is a good candidate for performing deadenylation in vivo, since versions of Pab1p that fail to activate PAN in vitro also cause defects in deadenylation in vivo (99, 101). However, since the cloned *PAN1* gene (99) does not encode a required catalytic subunit of the PAN activity (99), a direct test of the role of this enzyme in mRNA decay awaits cloning and mutational analysis of the actual gene(s) that encodes the PAN enzyme.

(iii) Other proteins possibly involved in deadenylation. Several lines of evidence indicate either that there are poly(A) nucleases in addition to PAN or that PAN retains some activity in the absence of Pab1p. First, in *pab1*Δ strains, the *MFA2* and *PGK1* mRNAs are deadenylated slowly and shortened poly(A) species are present in the total cellular poly(A) population (20, 99). Second, partially purified PAN preparations contain two Pab1p-independent nuclease activities in addition to the Pab1p-dependent activity. The first activity slowly degrades poly(A) tracts of fewer than 25 nucleotides, and the second degrades longer poly(A) tracts in a spermidine-dependent manner. These two activities have been ascribed to PAN; however, the possibility that they are due to additional poly(A) nucleases cannot be ruled out (68).

Two other proteins, the products of the *RNA14* and *RNA15* genes, have been proposed to negatively regulate mRNA deadenylation. This proposal is based on the observation that there is a decrease in bulk poly(A) tail lengths following inactivation of these two gene products in vivo (69, 76). However, several results indicate that the *RNA14* and *RNA15* gene products are involved in adenylation rather than deadenylation. Transcriptional pulse-chase analysis of the *PGK1* mRNA in temperature-sensitive *rna14* and *rna15* mutants indicates that inactivation of these two gene products does not affect the rate at which this mRNA is deadenylated (70). Furthermore, in vitro, Rna14p and Rna15p have been demonstrated to be components of the polyadenylation machinery, specifically the cleavage factor 1 complex (75), which is involved in both cleavage and polyadenylation (22). Consistent with this observation, inactivation of these two proteins in vivo leads to defects in polyadenylation cleavage site selection of the *ACT1* mRNA (70). Thus, the *RNA14* and *RNA15* gene products and probably their homologs in *D. melanogaster* (77) and mammals (118) do not appear to be involved in the control of deadenylation.

(iv) Models of poly(A) shortening. An important issue concerns how mRNA-specific rates of deadenylation are achieved. One model is that modulation of PAN activity is responsible for differential rates of deadenylation. Support for this model comes from the observation that in *pab1*Δ mutants the difference in the rates of deadenylation between the *MFA2* and *PGK1* mRNAs is mostly lost (20). This result suggests that PAN is largely responsible for differential rates of deadenylation in vivo, although the possibility that this loss is due to other factors, such as different proteins bound to poly(A) tails in the absence of Pab1p, cannot be ruled out.

How might sequence elements that affect deadenylation modulate PAN activity? One possibility is that they stimulate PAN either directly or through Pab1p without the involvement of additional proteins (68) (Fig. 3A). Evidence in favor of this model comes from the observation that in purified PAN preparations, deadenylation of the *MFA2* 3′ UTR is slightly faster than deadenylation of the *HIS3*, *ACT1*, *CYH2*, and *RP29* 3′ UTRs (68). Since *MFA2* mRNA is also deadenylated rapidly in vivo, this result raises the possibility that mRNA-specific stimulation of PAN accounts for differences in deadenylation measured in vivo. However, the rate at which the other mRNAs examined are deadenylated in vivo is not known, making the significance of this result difficult to establish. Furthermore, since these preparations of PAN activity also contain additional proteins, these differential rates of deadenylation cannot be attributed only to PAN and Pab1p. A likely alternative possibility for the way specific sequences influence deadenylation is that they are recognition sites for additional factors that modulate PAN activity (Fig. 3B). There is no direct evidence for such factors in *S. cerevisiae*; however, a protein, termed the AU-rich element-binding factor, which binds to a U-rich sequence containing the AUUUA motif in the c-*myc* mRNA and stimulates decay of this mRNA in vitro, has been identified (14). This mRNA is deadenylated prior to decay (15, 59, 117), and one speculation is that this factor may stimulate deadenylation.

There are several interesting possibilities that can explain how rates of deadenylation might be modulated by specific sequences within mRNAs. In one model, sequences within mRNAs which stimulate deadenylation may do so by recruiting poly(A) nucleases in addition to PAN. Alternatively, it may be that the poly(A) tail is available for deadenylation only during a certain phase of the translation cycle. For instance, the poly(A) tail may be able to be shortened during 40S scanning but not after 60S joining at the initiation codon. In this view,

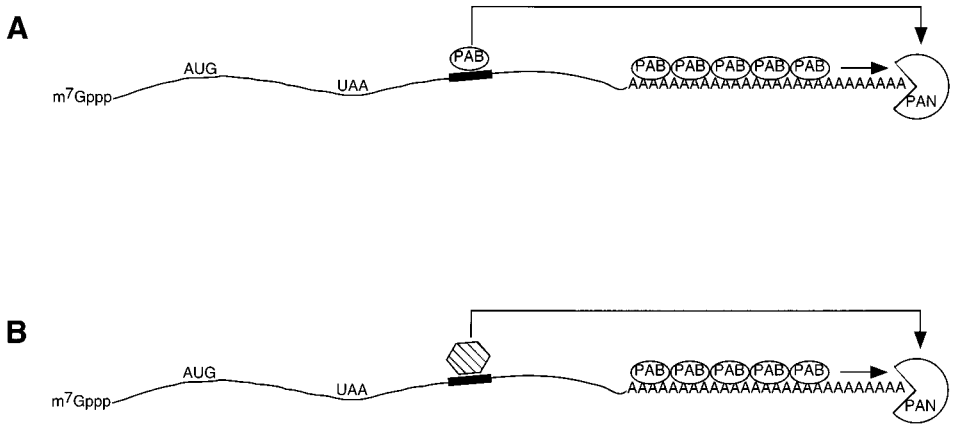

FIG. 3. Two models for the control of mRNA deadenylation. (A) PAN and Pab1p are sufficient for differences in rates of deadenylation. In this model, Pab1p-dependent PAN activity is primarily responsible for deadenylation in vivo. Specific sequence elements within mRNAs that influence rates of deadenylation (shown as a black box on the mRNA) do so by either directly stimulating PAN or providing binding sites for Pab1p, which in turn modulates PAN activity. (B) Additional factors alter rates of poly(A) shortening performed by PAN. In this model, the bulk of deadenylation is again performed by the Pab1p-dependent PAN activity. Specific sequences within mRNAs are binding sites for additional factors (shown as a hexagon). These factors serve to influence PAN activity either directly, through Pab1p, or by affecting mRNP structure in general.

sequences that promote deadenylation might do so by altering the relative rates of the steps in translation initiation.

A simpler model for how sequences may influence rates of deadenylation is that they do so by affecting the activity of PAN through alterations in the k_{cat} or k_d of this enzyme. Support for this model comes from the observation that in vivo the poly(A) tails of initially homogeneous populations of the rapidly deadenylated *MFA2* and *STE3* mRNAs are shortened nonuniformly whereas initially homogeneous populations of the slowly deadenylated *PGK1* mRNA are shortened uniformly (30). One interpretation of these results is that deadenylation of the *MFA2* and *STE3* mRNAs is more processive than deadenylation of *PGK1* mRNA. A change in the processivity of PAN has been explicitly proposed on the basis of results of in vitro experiments. The key observation is that the *MFA2* poly(A) tail is deadenylated less uniformly than other substrate mRNAs and that not all *MFA2* precursor substrate is utilized prior to the completion of deadenylation of other molecules in the total population (68). However, for several reasons, this model remains speculative. First, it is unclear whether the in vitro reactions were performed in substrate excess, thus making evaluation of the processivity of PAN activity difficult to assess. Second, a determination of the enzymatic mode of activity was further complicated by the fact that in most reactions a great deal of substrate mRNA was never hydrolyzed (68). Moreover, the differences in deadenylation between the *MFA2* and *STE3* transcripts and the *PGK1* transcripts observed in vivo can be accounted for in several alternative models. The future analysis of mutations in PAN and the refinement of an in vitro deadenylation system that recapitulates in vivo differences in deadenylation rates should provide insight into how deadenylation rates are controlled.

(v) Terminal deadenylation is not a rate-determining step for 5′-to-3′ decay. mRNAs degrade at different rates following shortening of their poly(A) tail to an oligo(A) length. The basis for these differences is not yet clear (see below), but one proposal is that differential rates of oligo(A) tail removal, termed terminal deadenylation, may determine the stability of oligoadenylated transcripts (68, 81). However, several observations indicate that terminal deadenylation does not contribute to the rate at which an mRNA is degraded following deadenylation. First, 5′-to-3′ decay products from both the *PGK1* and *MFA2* mRNAs have oligo(A)-length poly(A) tails, indicating that these transcripts are decapped independently of complete removal of the poly(A) tail (20, 30, 80, 120). In addition, increasing the rate of terminal deadenylation of the *PGK1* mRNA does not decrease the stability of the oligo(A) form of this mRNA (30). From these observations, it can be concluded that terminal deadenylation does not contribute in a significant way to subsequent events in the decapping and 5′-to-3′ pathway of mRNA decay. However, it should be noted that the *PGK1* mRNA is also degraded in a 3′-to-5′ manner (80, 82). It is possible that complete removal of the poly(A) tail is required for degradation of an mRNA through this 3′-to-5′ pathway (see below).

Control of mRNA decapping. The rates at which an mRNA is both deadenylated and decapped contribute to its stability. However, unlike deadenylation, mRNA decapping effectively terminates the cytoplasmic functionality of an mRNA, since degradation of the body of an mRNA is extremely rapid following decapping. For this reason, decapping is likely to be the key control point in the regulation of mRNA decay. Support for this hypothesis comes from the fact that decapping is the site of numerous controls. First, in most instances, the poly(A) tail is an inhibitor of mRNA decapping (see below). Second, decapping of oligoadenylated mRNAs occurs at different rates as a result of specific sequences within mRNAs (80, 81). Lastly, rapid mRNA decay triggered by premature translation termination codons occurs by deadenylation-independent decapping (see below).

(i) The Pab1p-poly(A) tail complex inhibits mRNA decapping. That decapping does not normally occur until the poly(A) tail is shortened to an oligo(A) length strongly suggests that the poly(A) tail inhibits this process. Poly(A) tail-mediated inhibition of decapping is dependent on the poly(A)-binding protein, since mRNAs in *pab1* mutants are decapped with long poly(A) tails (20). Thus, mRNA decapping is controlled in part by the poly(A) tail-Pab1p complex.

In vitro, Pab1p is capable of high-affinity binding to poly(A) tracts containing as few as 12 residues but binds poorly to shorter substrates (102). This observation may explain why mRNA decapping requires only shortening of the poly(A) tail to an oligo(A) length rather than complete deadenylation. In this view, mRNAs would be decapped following dissociation of

the last bound Pab1p molecule, which would occur once an mRNA had a short, rather than no, poly(A) tail. Importantly, by inhibiting decapping until shortening of the poly(A) tail is completed, Pab1p enables the rate of deadenylation to contribute to the overall decay rate of an mRNA.

Inhibition of decapping by the Pab1p-poly(A) complex suggests that Pab1p may be involved in either the establishment or maintenance of an interaction between the 5′ and 3′ ends of an mRNA. This possibility is also suggested by the observations that Pab1p, in conjunction with a poly(A) tail, can enhance translation initiation in vitro (37, 83). Similarly, depletion of Pab1p in vivo causes a reduction in overall translation and the lethality of a *pab1* deletion can be suppressed by mutations that affect levels of the 60S ribosomal subunit (101). This putative 5′-to-3′ interaction is likely to be important for the control of multiple cytoplasmic events in mRNA metabolism, including mRNA translation (for reviews, see references 27 and 52) and decay.

(ii) Control of decapping after deadenylation. mRNAs are decapped at different rates following deadenylation. These differences are due, at least in part, to specific sequences within mRNAs that can influence rates of mRNA decapping (19, 80, 81). How might these sequences affect the rate of mRNA decapping? Since the Pab1p-poly(A) tail complex inhibits mRNA decapping, binding of Pab1p to other sites within an mRNA might also be expected to influence mRNA decapping following deadenylation. In this light, these sequences may be internal binding sites for Pab1p or, alternatively, may antagonize binding of Pab1p to other sites within an mRNA. However, Pab1p probably does not affect rates of decapping following loss of the poly(A) tail. The key observation is that decapping of mRNAs in *pab1* mutant strains occurs at similar rates to those of decapping following poly(A) removal in *PAB1* strains, although decapping in a *pab1* mutant strain occurs independently from deadenylation (20). Thus, control of decapping by specific sequences is likely to be due to factors other than Pab1p.

(iii) Decapping activities from *S. cerevisiae*. The factors that perform the decapping reaction are likely to be the targets of regulation of this process. Identification and characterization of the decapping activities are therefore of great interest. Recent experiments suggest that most if not all of mRNA decapping is carried out by a single decapping enzyme encoded by the *DCP1* gene. This gene was isolated in a screen for mutants with deficiencies in mRNA decapping (8a, 41). Following cloning and complementation, three observations indicate that this gene encodes a decapping enzyme. First, the predicted amino acid sequence of the *DCP1* gene product matches the N-terminal sequence of a highly purified decapping activity. This decapping activity was initially detected in high-salt washes of yeast ribosomal fractions as an activity that cleaves capped RNA substrates between the second and third phosphate bonds, generating m^7GDP and an RNA chain with a monophosphate end (57, 112, 113). Second, *dcp1*Δ strains yield cell extracts that contain no detectable levels of this decapping activity. Finally, decapping activity is increased roughly fivefold in strains overexpressing the *DCP1* gene from a 2μm plasmid (8a).

Importantly, by several criteria, Dcp1p is responsible for decapping in vivo. First, in *dcp1*Δ strains (which are viable but growth impaired), several transcripts including both stable and unstable mRNAs are stabilized (8a). Second, for at least one of these mRNAs, the *MFA2* transcript, stabilization occurs following deadenylation but prior to decapping. Moreover, transcripts that undergo deadenylation-independent decapping are also stabilized in *dcp1*Δ strains. Thus, Dcp1p is responsible for both deadenylation-dependent and -independent decapping of unstable and stable mRNAs. Determining how Dcp1p is controlled in an mRNA-specific manner is therefore central to the understanding of how mRNA half-lives are specified.

Two general models may explain how Dcp1p activity is controlled in an mRNA-specific manner. In one model, control of Dcp1p could occur indirectly through the regulation of cap accessibility. Cap accessibility would in turn be determined by how often translation initiation factors were bound to the cap. In this view, *cis*-acting elements and *trans*-acting factors that influence rates of mRNA decapping would do so by either increasing or decreasing the efficiency of cap recognition by translation factors. Alternatively, Dcp1p may be directly controlled, serving as a target of regulatory input. In this view, *cis*- and *trans*-acting factors that affect mRNA-specific rates of decapping would do so by directly altering Dcp1p activity in some way.

A second protein capable of decapping transcripts is the product of the *gag* gene from the double-stranded L-A virus, which is found in some yeast strains. In vitro, this protein cleaves mRNAs between the first and second phosphate bonds, generating m^7GMP and an RNA chain with a diphosphate end (12, 13). This protein is also capable of performing this reaction in vivo (72). However, the GAG protein does not detectably contribute to mRNA decay in vivo, since 5′-to-3′ decay products from the *MFA2* mRNA are generated with similar kinetics in isogenic yeast strains that either lack or contain the L-A virus (83).

(iv) Translation and mRNA decapping. A possible link between translation and decapping of an mRNA is suggested by the observation that various manipulations of the *PGK1* mRNA that alter its translation also change its rate of decapping. For example, insertion of a stable secondary structure that blocks translation into the 5′ end of this mRNA increases its rate of decapping (80). In addition, the presence of an early termination codon in the *PGK1* mRNA causes rapid deadenylation-independent decapping of this mRNA (79; also see below). One speculation is that the rate of decapping of some mRNAs may be influenced by the efficiency of either their translation initiation or their elongation.

Reductions in the rate of total cellular translation elongation may also affect mRNA decapping. Treatment of *S. cerevisiae* with the translation elongation inhibitor cycloheximide stabilizes many yeast mRNAs (47). For the *PGK1* and *MFA2* transcripts, this stabilization is due to an inhibition of mRNA decapping (8, 80). Furthermore, cycloheximide stabilizes both translated and untranslated *MFA2* transcripts, indicating that stabilization is independent of inhibition of translation elongation of this mRNA in *cis* (8). Thus, stabilization of the *MFA2* mRNA and probably of additional transcripts by cycloheximide is the result of a general inhibition of mRNA decapping.

A second observation suggesting that reductions in the rate of total cellular translation elongation may affect mRNA decapping comes from studies of a temperature-sensitive allele of the gene encoding the tRNA nucleotidyl transferase enzyme (2). Shifting these mutant yeast cells to the restrictive temperature results in many phenotypes similar to those observed following cycloheximide addition, including a rapid cessation of protein synthesis, a general increase in the number of ribosomes per mRNA, and stabilization of several mRNAs (90). Why mRNAs are stabilized in this mutant is not known. However, given the similarities of its effects to those of cycloheximide, this stabilization could arise from a general inhibition of mRNA decapping.

On the basis of the above results, an interesting possibility is that mRNA decapping is regulated in response to reduced or

abolished protein production. This model is appealing since increases in mRNA stability would be expected to compensate for decreases in translation. Such coordinated regulation might be part of a general system of controls that maintain proper levels of gene expression.

ADDITIONAL PATHWAYS OF mRNA DECAY IN *S. CEREVISIAE*

There are mechanisms of mRNA decay in addition to the deadenylation-dependent decapping and 5′-to-3′ digestion pathway. These pathways include 3′-to-5′ digestion, deadenylation-independent decapping, and endonucleolytic cleavage of mRNAs (80, 96, 120). These mechanisms of mRNA decay potentially provide additional means through which transcript half-lives are controlled.

3′-to-5′ mRNA Decay

The *PGK1* transcript can be degraded in a 3′-to-5′ direction, although this pathway normally appears to be slower than 5′-to-3′ decay for this transcript. Thus, decapping and 5′-to-3′ digestion is not the only mechanism of decay for this mRNA. Evidence for this 3′-to-5′ pathway of mRNA turnover comes from the observation that when 5′-to-3′ decay is inhibited, mRNA species with truncated 3′ ends arise from the *PGK1* transcript (80, 82). These species are also detected at low levels in the absence of a block to 5′-to-3′ decay, indicating that 3′-to-5′ mRNA degradation is not simply activated by a block to 5′-to-3′ decay (80).

MFA2 transcripts that completely lack a poly(A) tail are stable in the presence of a strong block to 5′-to-3′ decay, and 3′-to-5′ decay products are detected at only very low levels (7a). Thus, unlike the *PGK1* mRNA, degradation of the *MFA2* transcript through the 3′-to-5′ pathway either does not normally occur or is very slow. The observation that 3′-to-5′ mRNA degradation may be somewhat limited to a subset of total yeast mRNAs suggests that 3′-to-5′ exonucleases are not simply scavenger nucleases that degrade any deadenylated transcripts that fail to decay through other specific pathways of mRNA turnover. Furthermore, this observation raises the interesting possibility that 3′-to-5′ degradation is the primary mode of decay for particular yeast mRNAs.

The factors that catalyze this 3′-to-5′ reaction are not known. However, it has recently been demonstrated that mutations in the *SKI2* and *SKI3* genes are lethal in combination with a temperature-sensitive *xrn1* allele (53). One intriguing possibility is that the *SKI2* and *SKI3* gene products are involved in 3′-to-5′ mRNA decay. In this view, synthetic lethality in *ski2* or *ski3*/*xrn1* double mutants would result from the simultaneous loss of both 5′-to-3′ and 3′-to-5′ mRNA decay. This model is analogous to the synthetic lethality exhibited by *Escherichia coli* that harbor mutations in both the *rnb* and *pnp* genes, which encode two major 3′-to-5′ exonucleases (31).

Endonucleolytic Cleavage of mRNAs

Because endonucleolytic cleavage of vertebrate mRNAs has been observed (16, 17, 85, 114), it is likely that the decay of some yeast transcripts is initiated by site-specific endonucleolytic cleavage. The best example of endonucleolytic cleavage of a yeast mRNA comes from studies of the *L2A* mRNA. This mRNA is subject to posttranscriptional-feedback control when levels of L2Ap are elevated (95). Several observations suggest that this control is due to endonucleolytic cleavage within a 360-nucleotide region within the *L2A* coding region. First, there is a greater abundance of 5′ than 3′ ends of this mRNA under conditions when the level of L2Ap is elevated (95). Second, insertion of a poly(G) tract 3′ of the potential cleavage site leads to the accumulation of a poly(A)$^+$ decay product trimmed to the 5′ side of the poly(G) insertion and to lower levels of a 5′ decay intermediate (96). Interestingly, this cleavage is likely to occur in the nucleus, since this 360-nucleotide sequence confers feedback control upon an mRNA when present within an intron (96).

A second possible example of endonucleolytic cleavage of yeast mRNAs comes from studies of the *PGK1* mRNA. It has been suggested that this mRNA can be cleaved within its coding region (120). Subsequent analysis of this mRNA has indicated that it is degraded primarily via 5′-to-3′ and 3′-to-5′ pathways of mRNA decay (80). However, it should be noted that the endonucleolytic cleavage products were detected in strains wherein the *PGK1* transcript was greatly overproduced. Perhaps the endonucleolytic cleavage of this mRNA does occur but is slow relative to other modes of degradation and can therefore be detected only when there is a very large pool of substrate mRNA. Examples of endonucleolytic cleavage in vertebrate systems often involve transcripts with regulated half-lives (11, 17). It is probable, therefore, that analogous to the *L2A* mRNA, other yeast mRNAs with regulated half-lives will contain specific endonuclease cleavage sites (see below).

mRNA Surveillance: Rapid Deadenylation-Independent Decapping

Another pathway of mRNA decay in *S. cerevisiae* is deadenylation-independent mRNA decapping (see below). This pathway of mRNA decay has been determined for the decay of an mRNA that contains a premature translation termination codon (82). In all eukaryotes, mRNAs containing premature translation termination codons (UAA, UAG, and UGA) are highly unstable (6, 28, 33, 39, 64, 67, 71, 89, 97). This phenomenon is termed nonsense-mediated decay in *S. cerevisiae* (92). In mutant yeast strains which are unable to activate rapid decay of transcripts containing early nonsense codons, mRNAs which are incorrectly processed are stabilized and are also associated with ribosomes (43). Therefore, one hypothesis is that nonsense-mediated decay serves as an mRNA surveillance system which rapidly removes aberrant mRNAs to prevent the formation of truncated and potentially detrimental polypeptides (97). Some support for this theory comes from the observation that in mutant strains of *Caenorhabditis elegans* which are unable to activate rapid decay of aberrant mRNAs, some recessive alleles of the myosin heavy-chain gene are converted to dominant negative alleles (97). However, it should be noted that in both *C. elegans* and *S. cerevisiae*, this "mRNA surveillance system" is dispensable for growth, indicating that stabilization of mRNAs that are aberrantly produced by the inherent rate of error in the various RNA-processing events is not sufficient to kill cells (25, 42, 61, 62, 97). Lastly, recent experiments suggest that some normal (no early nonsense codons) transcripts are also degraded by this mechanism of decay. This conclusion rests on the observation that the transcripts from the *PPR1* and *CTF13* genes are stabilized in strains lacking Upf1p, a protein that is required for this type of mRNA degradation (25a, 62). Thus, the process of deadenylation-independent decay may be exploited to control the turnover rates of a subset of cellular mRNAs.

Early nonsense codons trigger mRNA decapping. The stable *PGK1* mRNA is destabilized up to 12-fold by the presence of an early nonsense codon (89). Several lines of evidence suggest that this destabilization is due to rapid deadenylation-independent decapping and subsequent 5′-to-3′ mRNA decay (Fig. 4).

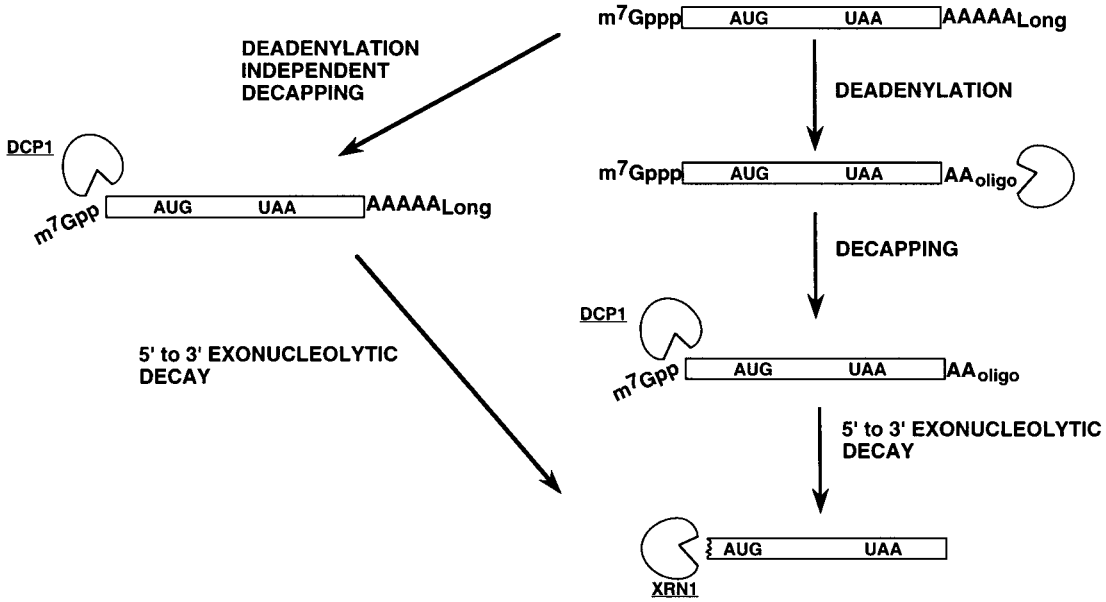

FIG. 4. A pathway of decay for mRNAs containing premature translation termination codons. Shown is a schematic representation of the common deadenylation-dependent pathway of mRNA decay and the deadenylation-independent pathway through which early nonsense codon-containing transcripts are degraded. In this latter pathway, mRNAs are decapped by the *DCP1* gene product while they still contain long poly(A) tails and are subsequently degraded in a 5′-to-3′ direction, primarily by the product of the *XRN1* gene.

In transcriptional pulse-chase experiments, *PGK1* transcripts that contain premature stop codons are degraded immediately following their synthesis and with long poly(A) tails. In addition, 5′-to-3′ decay intermediates that arise from this mRNA have long poly(A) tails. Finally, full-length *PGK1* transcripts containing nonsense codons are stabilized in *xrn1*Δ strains (82).

Two models can explain how deadenylation-independent decapping is triggered by an early nonsense codon. In one view, a premature stop codon may activate degradation machinery unique to the nonsense-mediated pathway. Alternatively, decapping triggered by an early nonsense codon could result from a stimulation of the decay machinery that normally decaps mRNAs. Support for the latter hypothesis comes from several observations. First, decapping of both early nonsense codon-containing *PGK1* mRNA and wild-type *PGK1* mRNA is performed by the *DCP1* gene product (8a). Second, both nonsense codon-containing *PGK1* mRNA and wild-type *PGK1* mRNA are also primarily degraded by Xrn1p following decapping (80, 82). Third, the *CYH2* precursor mRNA, which is degraded by nonsense codon-mediated decay, is both stabilized and shortened by several nucleotides at the 5′ end in an *xrn1*Δ strain and stabilized in a *dcp1*Δ strain, indicating that it is both decapped and degraded in a 5′-to-3′ direction (39). Thus, it is likely that rapid nonsense codon-mediated mRNA decay results from a variation of the basic deadenylation-dependent decapping pathway.

Recognition of early nonsense codons. There are two issues central to understanding nonsense-mediated decay. The first issue concerns how early nonsense codons are recognized as premature, and the second concerns how premature stop codons trigger mRNA decapping following their recognition (see Fig. 5). One observation that pertains to how an early stop codon may be recognized is that premature termination codons do not destabilize mRNAs as efficiently, or at all, with increasing distance from the 5′ end of an mRNA (39, 67, 89). This distance effect occurs because specific sequences, both upstream and downstream of early nonsense codons, influence their ability to trigger rapid decay.

(i) Specific sequences are required 3′ of early nonsense codons. One reason for the effect of distance on nonsense codon-mediated decay is a requirement for specific sequences 3′ of the early stop codon. Evidence for such sequences has come from the examination of a *PGK1* mRNA, termed mini-*PGK1*, which contains an early stop codon but is deleted for the majority of the coding region downstream of the early stop codon. This deletion abolishes nonsense codon-mediated decay of this transcript. Insertion of specific portions of the *PGK1* coding region downstream of the early stop codon in the mini-*PGK1* transcript restores rapid nonsense-mediated decay. Thus, specific sequences, termed downstream elements, are required 3′ of a termination codon for it to trigger rapid mRNA decay (89).

The specific sequence that is recognized within these downstream elements has not yet been completely defined. However, deletion analysis of two downstream elements suggests that a repeated motif, TGYYGATGYYYYY (where Y = C or U), is a necessary feature of this element (89, 124). Further support for the notion that this motif is an important component of downstream elements comes from the following observations. Portions of the *ADE3* and *HIS4* transcripts, each containing two copies of this motif, and a sequence that contains four copies of this motif also destabilize the mini-*PGK1* transcript (124). In addition, this sequence is found in many transcripts, as shown by a computer search which indicates that 75% of the total yeast sequences contain at least one imperfect copy (two mismatches) of this motif (124). This finding is important since every yeast transcript so far examined is subject to destabilization by an early nonsense codon (62, 63, 67, 88, 89). It should be noted, however, that the percentage of these motifs that reside within the coding regions of their respective mRNAs is not clear (124).

There are likely to be components of the downstream element in addition to this motif. Deletion analysis indicates that this motif is not sufficient for nonsense codon-mediated decay (89, 124). Furthermore, in at least one instance, partial mRNA destabilization by an early stop codon occurred in the absence

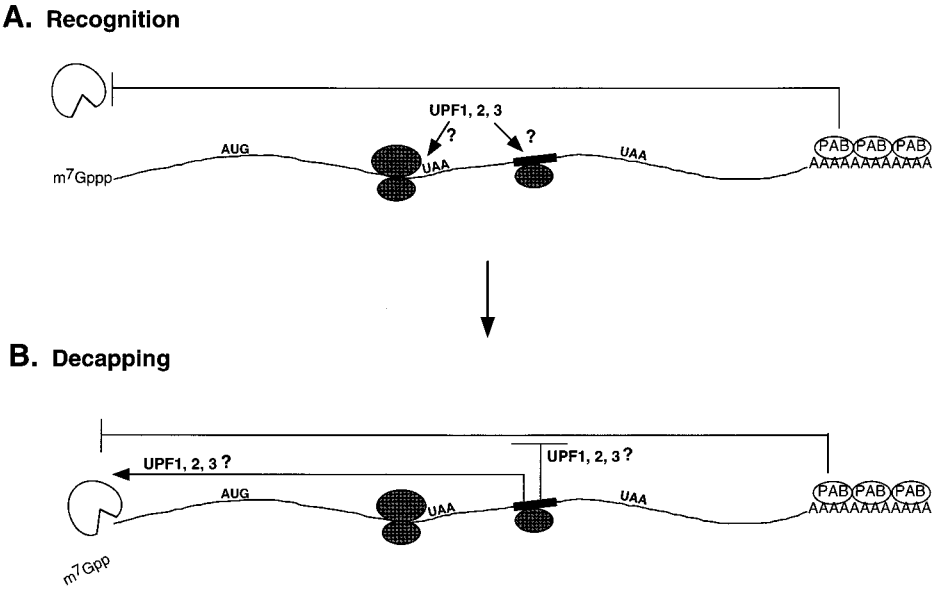

FIG. 5. Models for both the recognition of early nonsense codons and the mechanisms through which they may trigger rapid mRNA decapping. (A) Recognition of early nonsense codons. In this model, recognition occurs cotranslationally and is therefore likely to involve the ribosome or possibly a scanning 40S ribosomal subunit (shaded regions). This recognition could take place either at the termination codon itself or at a downstream destabilizing element (shown as a black box on the mRNA). The *UPF1*, *UPF2*, and *UPF3* gene products could act during any or all of these steps. (B) Rapid mRNA decapping. Once an early nonsense codon is recognized, mRNAs are rapidly decapped. This decapping must overcome the normal controls imposed on this process, shown here as an inhibition of decapping by Pab1p (PAB). Recognition could activate the decapping nuclease either directly, or indirectly by antagonizing the normal inhibitions to mRNA decapping. Again, the *UPF1*, *UPF2*, and *UPF3* gene products are likely to function in this process.

of this motif (89). These results suggest either that this motif can be very degenerate or that other sequences may also contribute to nonsense-mediated decay. Thus, the precise nature of the downstream element is not yet understood.

What is the role of the downstream element in nonsense-mediated decay? The TGYYGATGYYYYY motif is potentially capable of base pairing to nucleotides 879 to 887 of the 18S rRNA, although these nucleotides are predicted to fall within a region of intramolecular secondary structure (29, 92, 124). One model that has been proposed is that this sequence may mediate an important interaction between a scanning ribosome or 40S ribosomal subunit, a consequence of which would be rapid mRNA decapping (89, 124) (Fig. 5). Although intriguing, this model awaits rigorous experimental investigation.

(ii) Specific upstream elements partially block nonsense codon-mediated decay. Nonsense codon-mediated decay not only requires specific downstream elements but also is influenced by stabilizing sequences that reside upstream of a nonsense codon. For example, a 3′ region of the *PGK1* transcript partially stabilizes an mRNA when inserted upstream of an early nonsense codon whereas an equivalent-sized 5′ portion of the *PGK1* transcript does not. This stabilizing portion of *PGK1* spans a region of this mRNA after which nonsense codons no longer trigger rapid decay, suggesting that specific sequences within this region render mRNAs insensitive to this pathway. The requirement for a downstream element combined with the presence of stabilizing sequences provides an explanation of why the destabilizing effects of early nonsense codons diminish with increasing distance from the 5′ end of the mRNA.

How might these sequences serve to partially reduce the effects of early nonsense codons on mRNA decay? At least one of the proteins required for nonsense-mediated decay, the product of the *UPF1* gene (62, 63; also see below), is ribosome associated (4). One possibility is that stabilizing sequences inhibit nonsense codon-mediated decay by promoting the dissociation of the nonsense decay machinery from the ribosome, perhaps by inducing changes in ribosomal conformation (38, 106, 110).

***trans*-Acting factors in nonsense codon-mediated mRNA decay.** Three factors involved specifically in nonsense codon-mediated decay have been identified. They are the products of the *UPF1*, *UPF2*/*NMD2*, and *UPF3* genes (25, 26, 42, 61–63). These gene products are dispensable for growth and have been found to affect only the decay of transcripts that contain premature termination codons (25, 42, 62, 63). Whether these proteins function during the recognition of an early stop codon or in the subsequent stimulation of mRNA decapping is not known (Fig. 5; also see below).

The *UPF1*, *UPF2*, and *UPF3* genes have been cloned. How these proteins function in nonsense-mediated decay is not understood; however, several observations suggest that at least Upf1p and Upf2p are part of a ribosome-associated complex. Upf1p may be an RNA-binding protein, since it contains possible Zn^{2+} finger and RNA helicase domains (63). In addition, this protein is both cytoplasmic and polysome associated (4, 91). The localization of Upf2p is not known, but as judged by the two-hybrid technique (32), Upf1p and Upf2p interact in vivo via the carboxy terminus of Upf2p. Thus, Upf2p is likely to be polysome associated, along with Upf1p. Interestingly, when the carboxy terminus of Upf2p is both overexpressed and cytoplasmically localized, it causes a dominant negative phenotype with respect to nonsense-mediated decay (42). The interaction between these two proteins is therefore likely to be of functional significance to nonsense codon-mediated decay. Finally, yeast strains harboring mutations in either *UPF1* or *UPF2* also display a slight cycloheximide sensitivity phenotype (25, 63). These proteins may therefore play some role in translation in addition to functioning in nonsense codon-mediated

decay. Conversely, the *UPF3* gene product may not be ribosome associated, since it sediments during low-speed centrifugation experiments under conditions in which both monosomes and polyribosomes remain soluble (61). Future work on the *UPF1*, *UPF2*, and *UPF3* gene products should lead to an understanding of whether these proteins function in the recognition of an early stop codon, in a signal transduction pathway that triggers mRNA decapping, or in a combination of the two (Fig. 5).

Where in the cell does recognition of an early nonsense codon occur? A question with implications about how early nonsense codons trigger rapid decapping concerns where recognition of a premature termination codon occurs. Two observations suggest that recognition of early stop codons occurs in the cytoplasm in *S. cerevisiae*. Upf1p is cytoplasmic, and since Upf1p and Upf2p interact, Upf2p may also be cytoplasmic (4, 42). In addition, tRNA suppressors partially prevent nonsense codon-mediated decay (67). However, in mammalian systems, nonsense codons may be recognized while a transcript is either nuclear or in transit between the nucleus and the cytoplasm, since transcripts containing early nonsense codons are unstable while fully spliced but nuclear (7, 10).

These results may be indicative of fundamentally different mechanisms of nonsense codon-mediated decay in *S. cerevisiae* and more complex eukaryotes. Alternatively, early nonsense codon recognition may occur during the initial round of translation of an mRNA, perhaps coincident with nuclear export. Such a model is attractive, since it may explain how normal controls of mRNA decapping are overcome by the presence of an early nonsense codon. In this view, recognition of an early nonsense codon would occur during or before the establishment of a normal cytoplasmic messenger ribonuclear protein (mRNP) structure. This model may also explain why early nonsense codons that are either downstream of a stabilizing sequence or upstream of a poor destabilizing sequence only partially destabilize mRNAs (89). In this hypothesis, a certain percentage of mRNAs containing these inefficiently recognized early nonsense codons would escape detection during the initial round of translation and therefore be degraded through the normal decay pathway of that particular mRNA. The mixture of rapidly decapped and normally degraded transcripts would together give rise to an overall intermediate half-life. In light of this model, determining where early nonsense codons are recognized may aid in understanding how nonsense-mediated decay is coordinated with additional pathways of mRNA decay.

REGULATED mRNA TURNOVER IN *S. CEREVISIAE*

There are a few instances in which the half-lives of yeast mRNAs are altered in response to environmental cues. The most dramatic of these is the *SDH* Ip mRNA, which is destabilized up to 12-fold following a shift from glycerol- to glucose-containing medium (21, 65). In another example, the *SPO11* and *SPO13* transcripts are twofold less stable when grown in glycerol-containing medium as opposed to acetate-containing medium (115), and these differences in mRNA half-lives are dependent on the products of the *UME2* and *UME5* genes (116). Other, less well defined examples include the *L25* and *S10* mRNAs, which may be destabilized in response to heat shock (48); the *PCK1* and *FBP1* mRNAs, which are slightly destabilized in response to carbon source and/or heat shock (74); and the *L2A* transcript, whose levels are autoregulated at a posttranscriptional level (95).

The pathways through which these regulated yeast transcripts decay under both destabilizing and stabilizing conditions have not been completely defined. However, changes in the rate of deadenylation do not account for the decreased stability of the *SPO13* transcript (116). In addition, autoregulation of levels of the *L2A* mRNA is due to endonucleolytic cleavage within this transcript (95, 96; also see above). One likely possibility is that the multiple pathways of mRNA decay in *S. cerevisiae* will all be exploited to regulate the half-lives of individual transcripts.

CONCLUSIONS

There is now a large body of knowledge concerning how mRNA degradation occurs in *S. cerevisiae*. Many *cis*-acting determinants of mRNA stability have been identified, and the various underlying mechanisms of mRNA degradation are being defined. From these studies, a more complete picture of how cellular mRNA levels are controlled has started to emerge.

Currently, the major challenge in this field is the identification of both the general and specific *trans*-acting factors that function in mRNA decay. Modulation of the activities of the general factors involved in common pathways of mRNA decay is likely to be the major mechanism through which mRNA-specific rates of mRNA decay are determined. Identification of these general factors is therefore of great importance. In addition, since modulation of the activities of general factors is likely to be accomplished through the actions of mRNA-specific *trans*-acting factors, identification of these specific factors is also of great significance.

A second challenge is to understand how events that occur before and during mRNA degradation influence this process. Such events include mRNA transport, localization, and translation. Strong evidence for links between mRNA translation and decay already exists (18, 34, 80, 89). Additional events, such as the subcellular localization and possibly the "history" of an mRNA (i.e., the specific factors that associate with an mRNA before or during mRNA transport), are also likely to affect mRNA degradation. Determining the relationships between these different processes will lead to a fully integrated picture of how the various events in mRNA biogenesis converge in the regulation of gene expression.

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