

Promoter Elements Regulate Cytoplasmic mRNA Decay

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SUMMARY

Promoters are DNA elements that enable transcription and its regulation by *trans*-acting factors. Here, we demonstrate that yeast promoters can also regulate mRNA decay after the mRNA leaves the nucleus. A conventional yeast promoter consists of a core element and an upstream activating sequence (UAS). We find that changing UASs of a reporter gene without altering the transcript sequence affects the transcript's decay kinetics. A short *cis* element, comprising two Rap1p-binding sites, and Rap1p itself, are necessary and sufficient to induce enhanced decay of the reporter mRNA. Furthermore, Rap1p stimulates both the synthesis and the decay of a specific population of endogenous mRNAs. We propose that Rap1p association with target promoter in the nucleus affects the composition of the exported mRNP, which in turn regulates mRNA decay in the cytoplasm. Thus, promoters can play key roles in determining mRNA levels and have the capacity to coordinate rates of mRNA synthesis and decay.

INTRODUCTION

Promoters were originally defined as DNA *cis*-acting elements that direct the initiation of transcription. The collective efforts of numerous scientists have revealed that one key function of these elements is to promote the assembly of RNA polymerases in the correct location of the transcription units, at the right time, in a manner compatible with productive transcription (Kornberg, 2007). More recent work has demonstrated that promoters can affect the entire process of transcription, including capping, splicing, and polyadenylation (see, for example, Komili and Silver, 2008; Orphanides and Reinberg, 2002). In higher eukaryotes, promoters that drive RNA polymerase II (Pol II) transcription are highly complex. The yeast promoters are less complex and can be divided into two basic elements: the core promoter and the "upstream activating sequence" (UAS) (Guarente, 1988). The core promoter encompasses the transcription start site and recruits Pol II and the basal transcription apparatus. UASs are analogous to enhancers in higher eukaryotes and basically enhance or repress assembly of competent basal transcription apparatus, thereby regulating transcription (Harbison et al.,

2004 and references therein). Usually, UASs contain several *cis*-acting elements capable of binding *trans*-acting factors (e.g., chromatin remodelers, transcription activators or repressors, adapters) (Harbison et al., 2004).

For historical reasons, mRNA decay has been studied less intensively than transcription (Coller and Parker, 2004; Liu and Kiledjian, 2006; Parker and Song, 2004; Wilusz and Wilusz, 2004), although RNA turnover is no less complex or important than RNA synthesis. Several mRNA decay mechanisms have been characterized, both in the nucleus (mainly for quality control) and in the cytoplasm (Coller and Parker, 2004; Garneau et al., 2007). Our understanding of mRNA decay in the yeast cytoplasm is based on a few model mRNAs (mainly *MFA2* and *PGK1* mRNAs). Two major cytoplasmic decay pathways exist. Both are initiated by shortening of the mRNA poly(A) tail. The mRNA can then be exonucleolytically degraded by the exosome from 3' to 5' or by the Xrn1p exonuclease from 5' to 3'. The latter pathway involves removal of the mRNA 5' cap (m(7)GpppN) (Decker and Parker, 1993), which is a prerequisite stage for Xrn1p activity (Coller and Parker, 2004; Larimer et al., 1992; Parker and Song, 2004). Although most yeast mRNAs are degraded by either or both of these pathways, the half-lives of specific mRNAs vary widely, ranging from 3 min to more than 90 min (Wang et al., 2002). What determines the half-life of a specific mRNA? Conventional wisdom holds that mRNAs carry all of the necessary information for this, both in their sequence and structure. This premise is supported by a wealth of data (Clark et al., 2009; LaGrandeur and Parker, 1999; Leipuviene and Theil, 2007; Parker and Jacobson, 1990). However, this notion was challenged by our previous discovery that Pol II can control the fate of its transcripts in the cytoplasm (Goler-Baron et al., 2008; Harel-Sharvit et al., 2010). Provoked by these discoveries, we further tested the notion that other components of the transcription apparatus can affect the decay machineries.

Here, we show that the mRNA half-life can be controlled by a UAS within the promoter. The studied promoters affect the major decay pathway, executed by Xrn1p, and seem to affect also an Xrn1p-independent pathway. In the cases presented here, the promoters seem to be the major elements that determine the decay rates of their transcripts. Moreover, a small *cis*-acting element consisting of two Rap1p-binding sites is required and sufficient to destabilize the transcript. Rap1p is a well-known transcription activator of highly transcribed genes (~5% of the yeast genes). We found that depletion of Rap1p leads to the stabilization of mRNAs whose synthesis is activated by this protein. Thus, Rap1p plays a dual role in maintaining the level

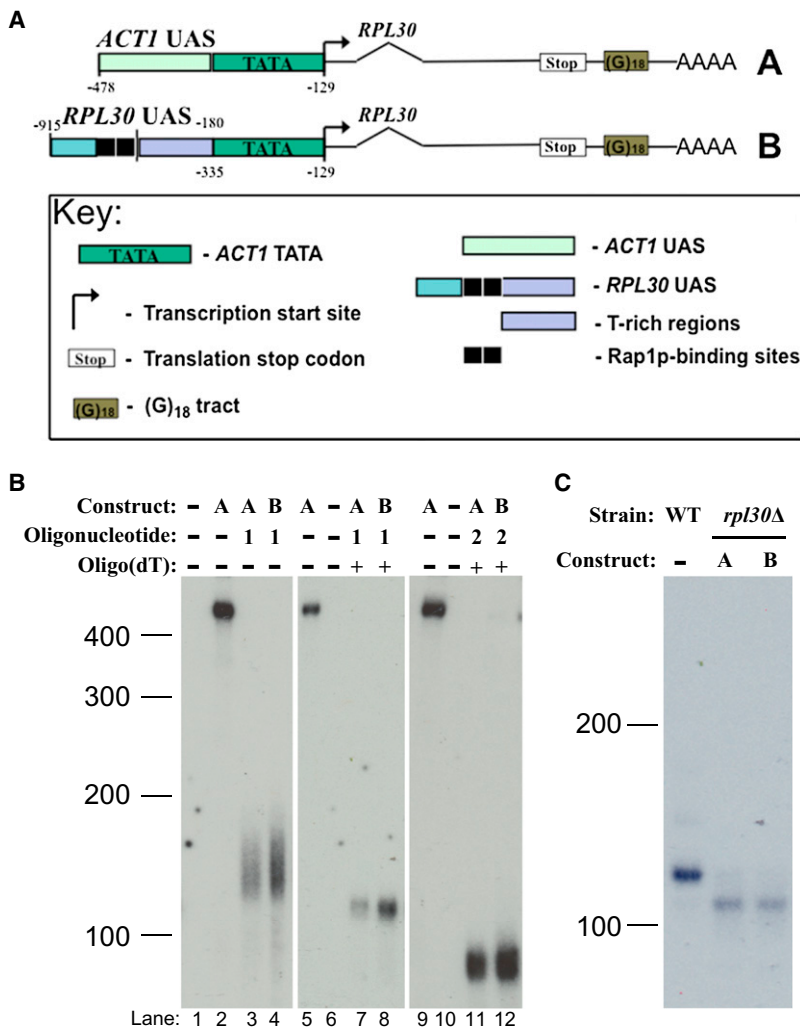


Figure 1. Reporter Genes Encode Identical mRNA

(A) Reporter constructs. The constructs are derivatives of those reported by Li et al. (1999). We inserted an oligo(G)₁₈ tract 30 bases downstream of the stop codon and 70 bases upstream of the 3' end (excluding the poly(A) tail). The constructs are identical except for the nature of their upstream activating sequence (UAS), located upstream of the ACT1 core promoter that includes the TATA box (designated "TATA"). The nucleotide boundaries of the RPL30 sequences are depicted above the constructs, and those of the ACT1 sequences are depicted below the constructs. The numbering is in reference to the translation start codon.

(B) mRNA A and mRNA B have identical 3' ends. RNA purified from cells carrying construct A or construct B, as indicated, was hybridized with the indicated oligonucleotide or with both oligonucleotide and oligo(dT) and digested with RNase H, as described in Experimental Procedures. The digests were resolved by polyacrylamide gel electrophoresis, followed by electrotransfer and hybridization with RPL30pG probe at 75°C (see Figure S1). Note that the distance between the position of the 5' ends of oligonucleotide 1 and 2 along RPL30pG mRNA is 21 bases. (C) mRNA A and mRNA B have identical 5' ends. RNA purified from WT cells lacking any plasmid or from rpl30Δ cells carrying either construct A or construct B (providing the essential RPL30), as indicated, was hybridized with oligonucleotide oMC1299 and digested with RNase H. The digests were analyzed as in (B), except that the probe was designed to detect the 5' fragment. See also Figure S1.

of specific mRNAs. We propose that Rap1p represents a class of factors, synthegradases, whose recruitment to promoters stimulates (or represses) both mRNA synthesis and decay. We conclude that promoters and their *trans*-acting factors can play more complex roles in gene expression than previously appreciated.

RESULTS

Identical mRNAs, Whose Synthesis Is Governed by Different UASs, Are Differentially Degraded in the Cytoplasm

Promoters promote mRNA synthesis. To determine whether they can also affect the decay kinetics of their transcripts, we compared half-lives of mRNAs derived from two similar plasmids that were constructed previously (Li et al., 1999). Each construct contains the RPL30 transcription unit, including the 3' noncoding region. Transcription from both constructs is governed by the ACT1 TATA box (Figure 1A). One of our constructs ("construct A") contains the ACT1 UAS, and the other ("con-

struct B") contains the RPL30 UAS (see Figure 1A). These two UASs were selected because the natural ACT1 and RPL30 mRNAs are degraded with different kinetics (Wang et al., 2002). The endogenous natural RPL30 was not deleted and served as an internal control. To differentiate between the plasmid-borne and the endogenous RPL30 transcripts, we introduced a tract of oligo(G)₁₈ into the 3' noncoding sequence of the plasmid-derived RPL30 genes. Because a (G)₁₈ tract serves as a barrier to exonuclease activity (Decker and Parker, 1993; Vreken and Raué, 1992), 5'-to-3' mRNA degradation generates a degradation intermediate fragment that stretches from the oligo(G)₁₈ tract to the 3' end (called "Fragment"). Thus, insertion of the oligo(G)₁₈ allowed us to differentiate between the plasmid-borne and the endogenous transcripts, as well as to evaluate the degradation efficiency and the decay pathway involved (Cao and Parker, 2001). Utilizing the oligo(G)₁₈ as a tag, we used two specific probes and two hybridization programs to detect either the plasmid-derived transcript or the endogenous transcript (Figure S1 available online).

In order to verify that the two constructs transcribe identical transcripts, we mapped the 5' and 3' ends of their mRNAs. To map the 3' end, we cleaved RPL30pG mRNA around the stop codon by hybridizing it with a specific oligonucleotide followed by digestion with RNase H (Muhlrad and Parker, 1992). The poly(A) tail was similarly removed by including oligo(dT) in the reaction (Cao and Parker, 2001; Lotan et al., 2005, 2007).

The digests were analyzed by PAGE northern, using “*RPL30*pG probe” (see Figure S1) to light up the 3′ end of the cleaved mRNAs. As shown in Figure 1B, lanes 3 and 4, the 3′ ends of the studied mRNAs ran as smears due to the heterogeneity of their tails. After removing the poly(A) tail, the 3′ fragments comigrated as bands, indicating that the 3′ ends are identical (Figure 1B, lanes 7 and 8). We used an additional oligonucleotide that hybridizes downstream of the stop codon, immediately upstream of the (G)₁₈ tract. This resulted in shorter 3′ fragments that also comigrated (Figure 1B, lanes 11 and 12). Moreover, polyacrylamide gel electrophoretic mobility of their Fragment at 120 min posttranscriptional arrest, when the poly(A) tail was naturally removed (Cao and Parker, 2001; Lotan et al., 2005, 2007), was identical (results not shown). Importantly, the poly(A)-containing smears were similar (Figure 1B, lanes 3 and 4), suggesting that the length of their tails is identical. To map the 5′ end, we deleted the endogenous *RPL30*, leaving the plasmid as the only source of the essential Rpl30p (normal proliferation of this strain demonstrated that the plasmid-borne transcript encodes a functional protein). mRNA A and mRNA B were cleaved near the 5′ ends and analyzed by northern blotting. As shown in Figure 1C, the 5′ ends of the studied *RPL30*pG mRNAs are identical. These ends are shorter than that of the endogenous *RPL30* mRNA (Figure 1C), indicating that the 5′UTRs of the endogenous and plasmid-borne mRNAs are different. This difference is probably due to the *ACT1* TATA box in the core promoter region of the constructs that is absent in the promoter of the endogenous gene. Collectively, the plasmid-borne transcripts have identical 5′ and 3′ ends, most probably because the reporter genes contain identical TATA boxes, ORFs, and 3′ noncoding regions. Hence, the two plasmid-borne mRNAs are identical.

We monitored mRNA decay after blocking transcription using two different drugs, 1,10-phenanthroline or thiolutin. The former drug is a metal chelator that most likely inhibits Pol II by sequestering Mg⁺². Thiolutin is not a chelator (it can act in the presence of 1 mM Cu⁺²; see Figures 5 and S5), and it acts by interacting with Pol II (Tipper, 1973). Remarkably, the decay of the two identical transcripts exhibited different kinetics (Figures 2A, 2B, 2E, and S2A–S2C). Consistently, accumulation of Fragment—indicative of the decay efficiency (Cao and Parker, 2001; Decker and Parker, 1993; Lotan et al., 2007; Vreken and Raué, 1992)—was different for the two mRNAs (Figure 2A; see Fragment panel). The accumulation kinetics of Fragment is complex, as it is generated by Xrn1p-mediated 5′-to-3′ degradation and is then further degraded by the exosome from 3′ to 5′ (Anderson and Parker, 1998) (for mathematical simulation of Fragment accumulation, see Cao and Parker [2001]). Nevertheless, the different accumulation of Fragment in the two cases clearly illustrates the difference in the rate of mRNA decay. The level of Fragment relative to the full-length mRNA was determined at steady state. The relative level of Fragment was higher in the case of mRNA encoded by construct B (designated herein “mRNA B”) as compared to mRNA encoded by construct A (designated herein “mRNA A”) (Figure 2F). This can also be observed in the “time 0” lanes shown in Figure 2A and S2D. Thus, even in optimally proliferating cells whose transcription proceeds normally, the effect of the studied UASs on mRNA decay is evident.

The northern blot membrane was deprobed and then probed with “*RPL30* (endogenous)” probe (see Figure S1). Evidently, the endogenous *RPL30* mRNA was degraded identically in the cells expressing either of the two plasmids (Figures 2A and 2C). We normalized the decay kinetics of the plasmid-borne mRNA to that of the endogenous mRNA. The results, shown in Figure 2D, clearly show that mRNA A (whose synthesis is driven by *ACT1* UAS) was degraded more slowly than endogenous *RPL30* mRNA (hence the gradual increase in the ratio between the plasmid-borne and endogenous mRNA). In contrast, mRNA B (whose synthesis is driven by *RPL30* UAS) was degraded similarly, albeit not identically, to endogenous *RPL30* mRNA. This difference might be due to the different 5′UTRs in mRNA B and endogenous *RPL30* mRNA (see Figure 1C) and/or the different context of their promoters. The *RPL30* core promoter, which construct B lacks, might also affect the decay of the endogenous *RPL30* mRNA. Be that as it may, these results indicate that the *RPL30* UAS serves as a major, albeit not necessarily the sole, element that determines *RPL30* mRNA degradation. mRNA levels were also normalized to the endogenous *ACT1* mRNA, whose decay is slower than that of *RPL30* mRNA (Wang et al., 2002). As shown in Figure 2E, mRNA A was degraded similarly, albeit not identically, to that of *ACT1* mRNA, whereas mRNA B was degraded faster.

The *RPL30* primary transcript contains an intron (see Figure 1). As shown in Figures S2D–S2F, the stability of the intron-containing transcript is also dependent on the UAS. Specifically, following transcription arrest, transcript B disappears faster than transcript A. Disappearance of the intron-containing transcript might be due to splicing or, as was shown for the *ACT1* intron, degradation in the cytoplasm (Hilleren and Parker, 2003), or a combination of the two. In the case that the disappearance is due to splicing, faster splicing that characterizes transcript B should negatively affect the apparent disappearance of the mature mRNA B (i.e., the actual decay of the mature mRNA is faster than observed). Hence, there are two options; each supports our model whereby the UAS regulates mRNA decay. Either the promoter affects splicing, in which case the difference in the decay rates between mRNA A and B is in fact larger than observed; or the decay of the primary transcript is controlled by the same UAS-dependent mechanism that degrades the mature mRNA. Since the pre-mRNA disappearance is slower in *xrn1Δ* (results not shown), the pre-mRNA is probably degraded in the cytoplasm; we therefore suspect that the latter possibility is more likely.

In yeast, cytoplasmic mRNA decay is executed mainly by two major pathways, one mediated by the 5′-to-3′ exonuclease Xrn1p and the other by 3′-to-5′ exonuclease—the exosome (see Introduction). To determine which of the two major cytoplasmic decay pathways is responsible for the decay of the examined mRNAs, we deleted *XRN1* or *SKI7*, thereby compromising Xrn1p-mediated 5′-to-3′ degradation or exosome-mediated 3′-to-5′ degradation, respectively. Deletion of *SKI7*, the adaptor that links the SKI complex with the exosome and is required for the exosome activity (Araki et al., 2001; van Hoof et al., 2000), did not lead to detectable stabilization of either mRNA A or mRNA B (data not shown). In contrast, deletion of *XRN1* compromised the decay of both mRNAs (Figure S3),

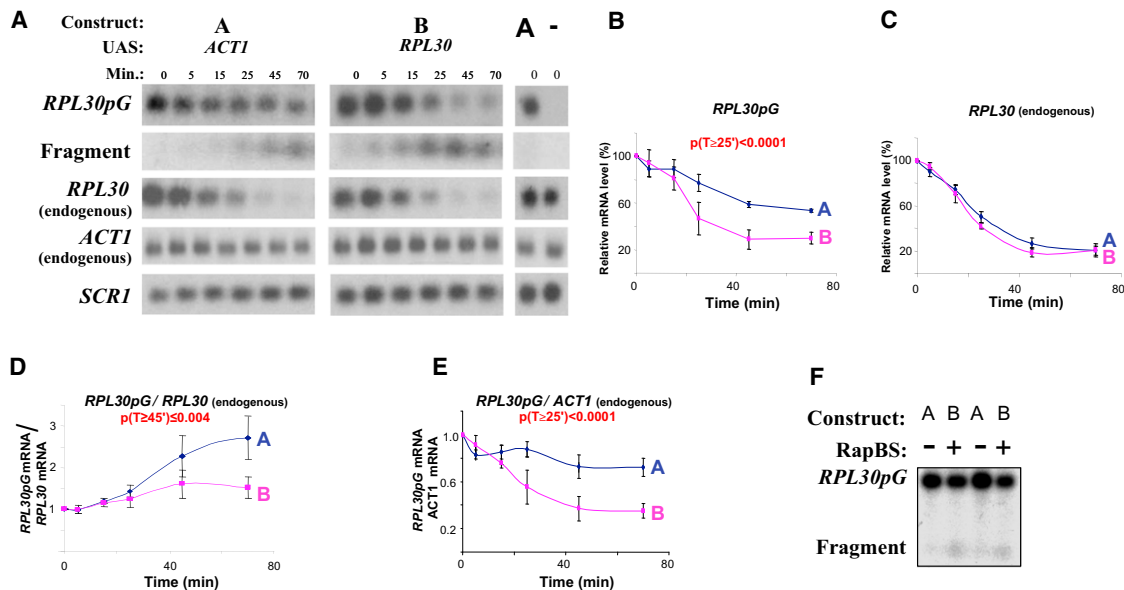


Figure 2. Upstream Activating Sequence Can Affect the Stability of the Resulting mRNA

(A) Decay of mRNAs derived from the two constructs exhibit different kinetics. Cells were harvested in midlog phase at the indicated time points following transcriptional arrest by 1,10-phenanthroline. Decay kinetics was determined, as reported previously (Lotan et al., 2005), by monitoring mRNA levels at the indicated time points postdrug addition using northern analysis. The same membrane was reacted sequentially with the probes that are indicated at the left. *RPL30pG* transcript and its Fragment were detected using an oligo(C)-containing probe (see Figure S1). The membrane carrying construct A was exposed to X-ray film longer than the other membrane. Pol III transcript *SCR1* is shown to demonstrate equal loading; its intensity was used for normalization in (B). The right panel is shown to demonstrate the probe specificity; it contains RNA taken from cells expressing construct A or cells carrying no plasmid (-). See also Figure S1B. (B and C) Band intensities were quantified by PhosphorImager. The intensity at time 0 (before adding the drug) was defined as 100%, and the intensities at the other time points were calculated relative to time 0. Results were plotted as a function of time postdrug addition. Error bars represent standard error of three assays. Statistical analysis demonstrates significant differences in decay kinetics. The most significant differences were detected at 25 min and later time points, $p(T \geq 25') < 0.0001$ (B) (see Experimental Procedures). No significant differences were observed between endogenous *RPL30* mRNAs obtained from the different strains (C).

(D) Results normalized to the endogenous *RPL30* mRNA. The ratio at time 0 was arbitrarily defined as 1. Error bars represent standard error of three assays. Statistical analysis demonstrates significant differences for these ratios at or following 45', $p(T \geq 45') < 0.004$.

(E) Results normalized to the endogenous *ACT1* mRNA, as in (D).

(F) Steady-state level of Fragment illustrates the impact of the studied UASs on mRNA degradation during optimal proliferation (in the absence of any drug). RNA samples extracted from optimally proliferating cells (in midlogarithmic phase) were loaded such that the intensity of the full-length mRNAs (designated "*RPL30pG*") would be comparable (left lanes) or that the intensity of mRNA A would be higher than that of mRNA B (right lanes).

See also Figure S2.

indicating that both mRNAs are substrates of Xrn1p. Nevertheless, deletion of *XRN1* affected more substantially the decay of mRNA A than B. Consequently, mRNA B was not completely stable in *xrn1Δ* cells (Figures 3D, S3A, and S3B), indicating that an Xrn1p-independent mechanism also contributes to its degradation. This suggests that UASs can also affect the 3'-to-5' decay pathway (or as yet undefined mechanism). It seems that the exosome effect on the decay of these mRNAs is either too weak to be observed or that it does not require Ski7p. Because Xrn1p is mainly a cytoplasmic protein (Johnson, 1997; Sheth and Parker, 2003), we conclude that the degradation of both mRNAs occurs in the cytoplasm. Indeed, these mRNAs are mainly cytoplasmic, as most of them are associated with polysomes (data not shown). Collectively, our results indicate that UASs can regulate the Xrn1p-dependent and independent cytoplasmic mRNA decay pathways. Consistently, differential decay is observed in both *ski7Δ* and *xrn1Δ* cells (Figure 3). Note that, although the error bars in Figure 3D are large,

statistical analysis indicated that the decay kinetics of the two mRNAs is nevertheless significantly different ($p < 0.0004$). The differential decay kinetics observed in both *xrn1Δ* and *ski7Δ* strains indicate that no single pathway is fully responsible for these differences.

As shown in Figure S3C, lanes 1–6, accumulation of Fragment in *ski7Δ* cells was correlated with the progression of mRNA B degradation. This result is interpreted to indicate that the degradation of every full-length mRNA gave rise to a relatively stable Fragment, designated herein Fragment B. Fragment stability was probably due to defective exosome activity. In contrast, the Fragment derived from mRNA F (whose transcription is governed by RapBS-lacking promoter), designated Fragment F, accumulated to a lesser degree during the time course of the experiment (Figure S3C, lanes 7–12). This result is consistent with the poor decay of mRNA F. Alternatively, the relatively small accumulation of Fragment F is due to its faster degradation relative to Fragment B. Both of these possibilities support our

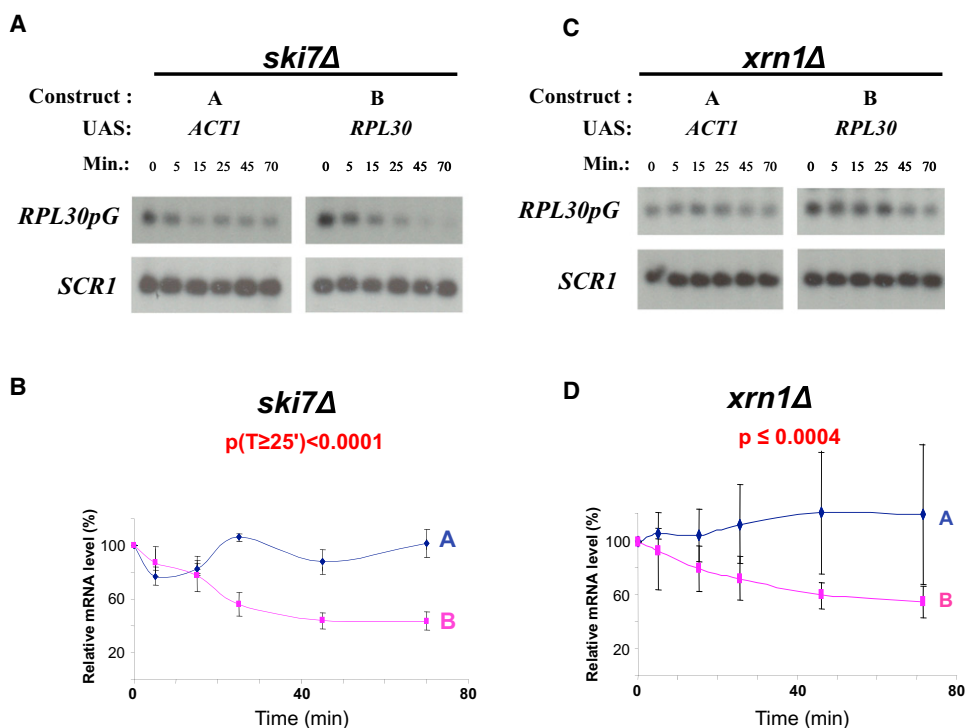


Figure 3. Differential Decay of mRNA A and mRNA B Is Maintained in Both *ski7Δ* and *xrn1Δ* Cells

(A and B) Decay kinetics of mRNAs encoded by construct A or B, expressed in *ski7Δ* strain, isogenic to the strain used in Figures 2, 3, and 4, was determined as described in Figures 2A and 2B.

(C and D) Decay kinetics of mRNAs encoded by construct A or B, expressed in *xrn1Δ* strain, isogenic to the strain used in Figures 2, 3, and 4, was determined as described in Figures 2A and 2B.

See also Figure S3.

premise that mRNA B and mRNA F, which are identical (data not shown), are degraded at a different pace or by different mechanisms.

Rap1p-Binding Sites Are Necessary and Sufficient to Confer Enhanced mRNA Decay

Like other promoters of genes encoding ribosomal proteins, the *RPL30* promoter contains several *cis*-acting elements (Li et al., 1999). To determine which UAS element is responsible for the mRNA decay, we analyzed the stability of transcripts whose transcription is controlled by truncated promoters lacking different portions of the *RPL30* UAS. As shown in Figures 4B–4D, deleting most of the *RPL30* UAS but leaving the two Rap1-binding sites (RapBS) intact had little effect on the transcript stability, as both the decay kinetics of the full-length mRNA and the relative level of Fragment were very similar. These results raise the possibility that RapBS is responsible for the transcript's rapid decay.

RapBS is a well-studied element found in ~90% of ribosomal protein (RP) promoters (see Discussion). To determine whether RapBS is both required and sufficient to confer the characteristic short half-life, we deleted it from *RPL30* UAS in “construct B” and inserted it into the *ACT1* UAS of “construct A,” thus creating constructs F and E, respectively (Figure 4E), which encode mRNAs that are identical to mRNA A and B (data not shown).

Remarkably, surgical removal of RapBS from the *RPL30* UAS stabilized the transcript, whereas its insertion into the *ACT1* UAS destabilized the transcript (Figures 4F and 4G, respectively). Thus, in the context of the *RPL30* and *ACT1* UASs, RapBS has a dominant effect on mRNA decay.

To examine the effect of RapBS on the degradation status of the studied mRNAs in optimally proliferating cells, we determined accumulation of Fragment at steady-state conditions. As shown in Figure 4H, the presence of RapBS is associated with a relatively high Fragment level. This result demonstrates the capacity of RapBS to modulate mRNA decay in the cytosol under optimal conditions. Note that this assay does not involve any drug or any particular cell treatment.

To examine the effect of the promoter context on the capacity of RapBS to affect mRNA decay, mRNA levels transcribed by constructs A, B, E, and F were analyzed, using three-way ANOVA statistical analysis (see statistical analysis in Experimental Procedures). As shown in Figure S4, mRNAs derived from constructs A and F, lacking RapBS, had similar decay kinetics, and both were relatively stable. Moreover, mRNAs derived from constructs B and E, which harbor RapBS, had similar decay kinetics, exhibiting faster decay. Thus, the presence of RapBS is correlated with enhanced mRNA decay, irrespective of the other promoter elements. We then asked whether other promoter elements contribute to mRNA decay.

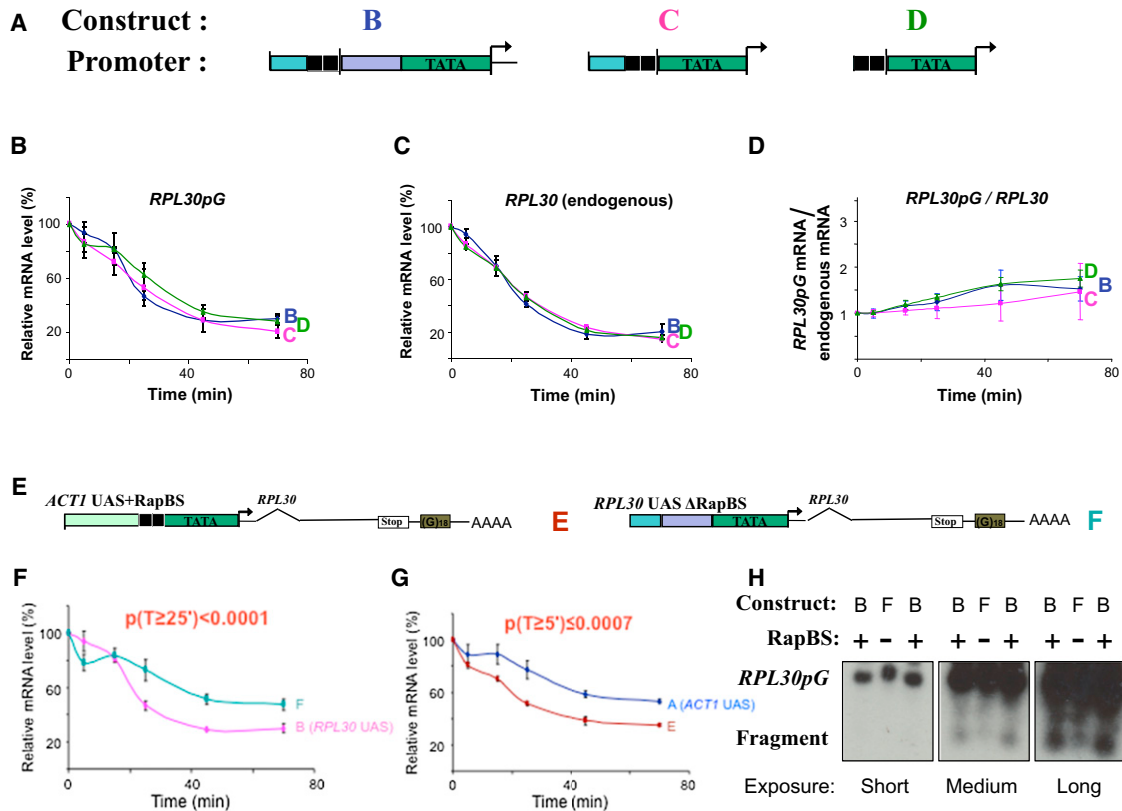


Figure 4. RPL30 RapBS Is Required and Sufficient for Maintaining the Characteristic Enhanced Decay Rate of RPL30pG mRNA

(A) Constructs used in (B)–(D). Construct B is described in Figure 1A. Construct C is identical to B except that the T-rich domain was deleted. Construct D lacks the entire UAS except for the RapBS (Li et al., 1999). Symbol key is as in Figure 1A.

(B) Decay kinetics of the indicated mRNAs was performed and quantified as in Figure 2B.

(C) Levels of the endogenous RPL30 as a function of time posttranscriptional arrest.

(D) Results of RPL30pG mRNA levels normalized to the endogenous mRNA were calculated as in Figure 2D. Error bars in all panels represent standard error of three assays. No significant differences were found between the behaviors of the three samples in any of the assays.

(E–H) Removal of RapBS from RPL30 UAS stabilizes the RPL30pG mRNA, whereas its insertion in ACT1 UAS destabilizes the transcript.

(E) Constructs E and F are derivatives of constructs A and B (see Figure 1), created by site-directed mutagenesis (see Experimental Procedures). The symbols key is as in Figure 1A.

(F and G) Decay kinetics of the indicated mRNAs was determined as described in Figure 2B.

(H) The impact of RapBS on the relative level of Fragment. Assay was performed as in Figure 2F. Different exposure durations of the same membrane were used to demonstrate the relative intensities of the full-length mRNAs (short) and that of Fragment (medium and long).

See also Figure S4.

Statistical analysis indicated that the interaction between RapBS and other promoter sequences (i.e., all other sequences except for the RapBS) was not significant. Therefore, we analyzed each factor independently. By so doing, we revealed that the presence or absence of RapBS in any of the two promoters was sufficient to confer significant differences on mRNA decay kinetics, $p(T \geq 15') < 0.0001$ (see statistical analysis in Experimental Procedures). The same statistical analysis also indicated that the two promoter sequences (P_{ACT1}/P_{RPL30}), independently of RapBS, have significant differences, though smaller, on mRNA decay kinetics, $p(T \geq 25') < 0.002$. Thus, the three-way ANOVA analysis demonstrates that the effect of RapBS on mRNA decay is independent of the context of the two studied promoters. Nevertheless, UAS elements other than RapBS have additional effect, albeit a more modest one.

Rap1p Is Both mRNA Synthetic and a Decay Factor

The effect of RapBS on mRNA decay suggests that Rap1p is involved in determining the mRNA decay pathway and/or kinetics. Rap1p is an essential protein. Therefore, we transiently depleted it without affecting cell viability. To this end, we utilized a Cu^{2+} -modulated expression shutoff system (“Copper-degron”) (Moqtaderi et al., 1996). A control strain and Cu^{2+} -depletable Rap1p strain (Pardo and Marcand, 2005) were treated with $CuSO_4$ for 3 hr (see efficacy and time course of Rap1p depletion in Figure S5A) and then with Thiolutin to block transcription. As expected, decay kinetics of mRNAs of control (WT) cells revealed differential decay of mRNA A and mRNA B (Figure 5B, cf left and right panel). Remarkably, this differential decay was diminished upon depletion of Rap1p due to increased mRNA B stability (Figure 5B, right). Depletion of Rap1p did not abolish the effect of RapBS completely, suggesting that either the

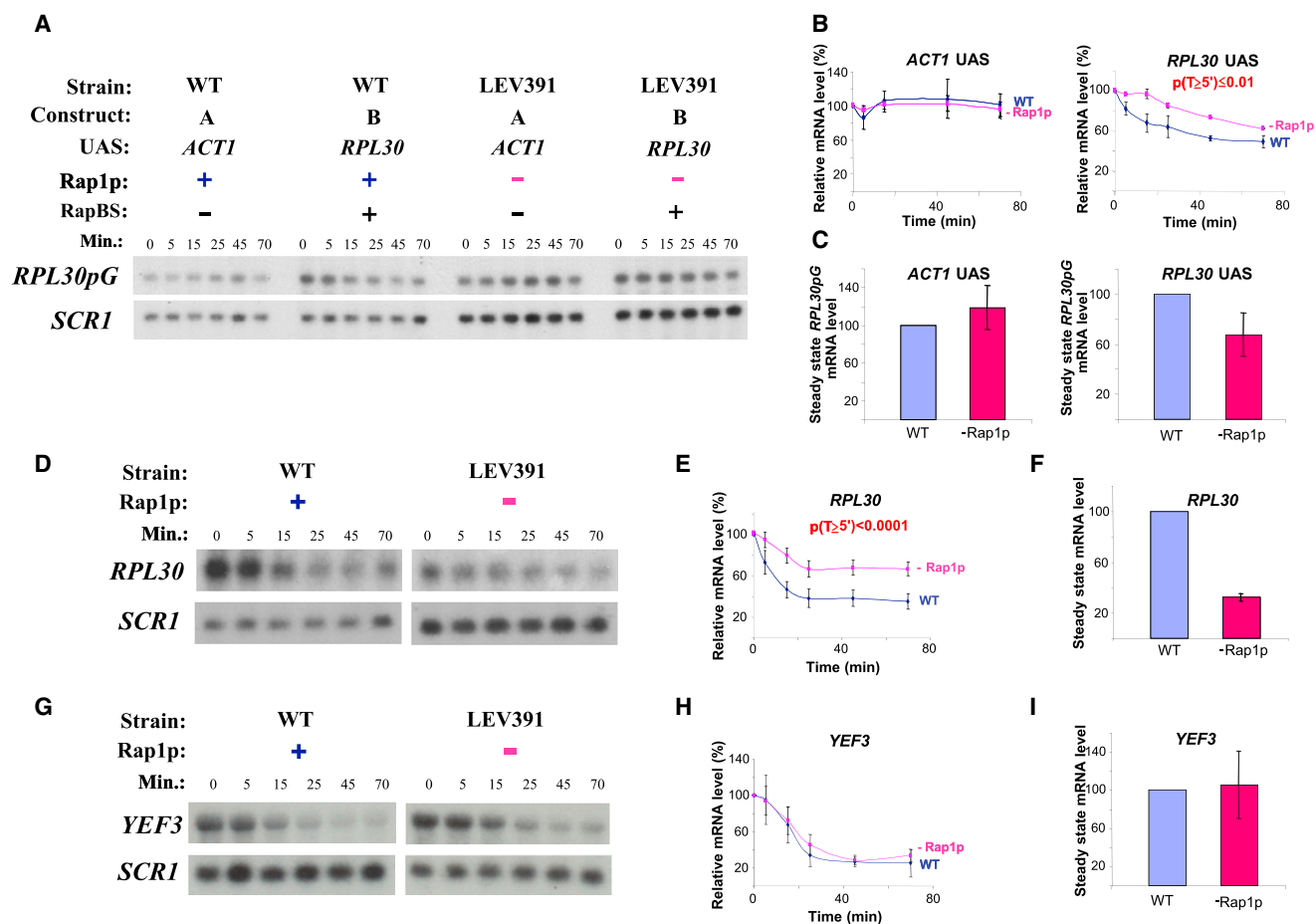


Figure 5. Depletion of Rap1p Specifically Compromises Degradation of mRNAs Whose Synthesis Is Activated by Rap1p

(A) Depletion of Rap1p affects degradation kinetics of mRNA B. Cells were treated with CuSO_4 , as described in Figure S5A, for 3 hr. Thiolotin ($3 \mu\text{M}$) was then added and decay kinetics determined as in Figure 2A.

(B) Band intensities were quantified as in Figure 2B (normalized to *SCR1* transcript). Error bars represent standard error of three assays. p value was calculated as in Figure 2B. “-Rap1p” denotes Rap1p depletion.

(C) Steady-state level of the indicated mRNA. The level in WT strain was arbitrarily defined as 100%. Error bars represent standard error of six assays. The p value for *ACT1* UAS panel was insignificant (0.3). The p value for *RPL30* UAS panel was marginal (0.08); however, because mRNA B becomes stable as a result of Rap1p depletion, we conclude that transcription was downregulated after Rap1p depletion.

(D–I) Decay kinetics of the indicated endogenous mRNAs. Assays were performed as in (A), (B), and (C). The p value for (F) is < 0.0001 and for (I) is 0.72. See also Figure S5.

residual Rap1p was still effective or that RapBS affects mRNA decay by an additional Rap1p-independent mechanism. Significantly, Rap1p depletion affected the decay of the endogenous *RPL30* mRNA (Figures 5D and 5E), as well as that of *RPL5* mRNA (Figures S5B and S5C) and *NSR1* (data not shown), but not that of *YEF3* mRNA (Figures 5G and 5H). As indicated by steady-state mRNA levels and taking into account the mRNA stability, Rap1p stimulated transcription of *RPL30* (Figure 5F), *RPL5* (Figure S5D), and *NSR1* (data not shown) but did not affect transcription of *YEF3* (Figure 5I). Rap1p-stimulated mRNA synthesis and decay is correlated with the presence of RapBS in the affected genes (Lieb et al., 2001). Thus, Rap1p is required for enhanced degradation of mRNA B as well as efficient decay of mRNAs whose transcription is regulated by Rap1p. Collectively, the capacity of Rap1p to stimulate mRNA synthesis and decay is

dependent on the presence of RapBS in the UAS, a feature that reflects natural genes whose promoters contain RapBS.

Summarily, our results indicate that Rap1p is both an mRNA synthetic and degradation factor. The capacity of Rap1p to activate mRNA synthesis and decay is dependent on the presence of RapBS in the promoters.

DISCUSSION

Promoters Can Regulate mRNA Decay in the Cytoplasm

Although recent studies have revealed that consecutive stages of gene expression are coupled (Komili and Silver, 2008), conventional wisdom holds that, after its release from the Pol II, the fate of the mRNA in the cytoplasm is unaffected by the promoter. In the present study, we demonstrate that this view

is oversimplified and that promoter elements can affect mRNA decay in the yeast cytoplasm. Specifically, *RPL30* UAS conferred a short half-life on the reporter mRNA, similar to the half-life of the endogenous *RPL30* mRNA. In contrast, *ACT1* UAS conferred a longer half-life on an identical reporter mRNA, similar to that of the endogenous *ACT1* mRNA (Figure 2). An early study revealed that the steady-state level of a premature stop codon-containing β -globin is influenced by the nature of its promoter, raising the possibility that the promoter can regulate mRNA decay in humans as well (Enssle et al., 1993).

We note that some of our results might be consistent with the possibility that the drugs we used here repress transcription in a differential manner, depending on the presence or absence of RapBS. Several observations argue against it. First, experiments performed with two different drugs that repress Pol II by different mechanisms (one is metal chelator and the other acts by interacting with Pol II) yielded similar results. Second, the relative Fragment level, which is indicative of the decay status (Cao and Parker, 2001), was affected by RapBS (Figures 2F, 4H, S2D, and S3C). Third, *XRN1* deletion affected differentially the decay of mRNA A and B (Figures S3A and S3B). Fourth, accumulation of Fragment, which became relatively stable due to *SKI7* deletion, was inconsistent with identical decay of mRNA B and F and their Fragments (see discussion of Figure S3C in Results).

The role of the promoter in mRNA decay broadens our view of the crosstalk between the synthetic and decay machineries that operate in different compartments. We propose that the transcripts are not exported to the cytoplasm as “independent” entities. They are marked, or imprinted, with some tags that later control their fate (Choder, 2011). The nature of this tag can vary. Thus, the transcript can be imprinted by tagging it with an additional RNA or RNP molecule (e.g., through base pairing). Imprinting can also involve a protein, or a complex of proteins such as the exon-exon junction complex, or certain components of the RNA cleavage and polyadenylation complex. Alternatively, the promoter can recruit a factor that acts catalytically without leaving the promoter. This factor can then modify some specific transcript bases or the transcript structure, thereby affecting the transcript function and/or its fate at later stages (Choder, 2011). The length of the poly(A) tail or its associated factors (e.g., the number of Pab1p molecules) can also serve as tags. In the case presented here, however, the poly(A) tails of the studied mRNAs were very similar; hence, the tail length does not seem to play an important role. A documented case of tagging is the cotranscriptional binding of Rpb4/7, an mRNA coordinator (for definition of mRNA coordinator, see Harel-Sharvit et al., 2010), with the emerging Pol II transcripts (Goler-Baron et al., 2008; Harel-Sharvit et al., 2010). The extent of mRNA imprinting by Rpb4/7 can be subject to regulation, thereby regulating mRNA decay after the mRNA leaves the nucleus (Shalem et al., 2011). We hypothesize that promoters can contribute to the cotranscriptional imprinting of mRNAs, similar to the contribution of Pol II-mediated imprinting (Choder, 2011). The nature of this tagging remains to be determined.

Rap1-Binding Sites Impact the Cytoplasmic mRNA Decay

Systematic dissection of the *RPL30* UAS uncovered RapBS as an element that is required and sufficient to confer short half-

life. Furthermore, RapBS can dominantly destabilize the reporter mRNA if placed in *ACT1* UAS (Figure 4). RapBS is found in telomeres as well as in ~5% of Pol II promoters. Approximately 90% of ribosomal protein (RP) promoters contain predicted RapBSs (Lascaris et al., 1999; Warner, 1999). Rap1p is bound to essentially all such RP promoters in vivo (Lieb et al., 2001; Schawalter et al., 2004) and is involved in their transcription activation (Lieb et al., 2001 and references therein).

RapBS is highly variable. The only indisputable feature for all RapBSs is that they harbor an extended sequence of 12–14 bp (Piña et al., 2003). Interestingly, Rap1p function is modulated by the precise architecture of its binding site and its surroundings. It was therefore proposed that Rap1p alters its structure to bind to different versions of its DNA binding sequence (Piña et al., 2003). Evidently, Rap1 is a rather unusual factor whose activity is dependent on context. It can function as an activator or repressor, can affect chromatin architecture, and can enhance Pol II pausing (Pelechano et al., 2009). This latter function is also context specific, as it characterizes only RP genes. It seems that Rap1p plays unique roles in RP promoters. We propose that the Rap1p-RapBS complex affects imprinting of mRNAs encoding RP by recruiting (a) certain protein(s) to the promoter. The recruited protein(s) bind(s) the transcript cotranscriptionally and later regulate(s) its demise, similar to the function assigned to Rpb4/7 (Goler-Baron et al., 2008; Harel-Sharvit et al., 2010).

The observation that promoter elements can have such a strong impact on mRNA decay was unexpected. At least in the case of *RPL30*, the two RapBSs were responsible, to a great extent, for the characteristic decay kinetics of the endogenous mRNAs (Figures 2D and 4D). This observation suggests that, in some cases, the most important decisions regarding the transcript stability are already made during transcription in the nucleus. This model challenges a common view that the cytoplasmic decay factors regulate mRNA decay in the cytoplasm (Coller and Parker, 2004; Liu and Kiledjian, 2006; Parker and Song, 2004; Wilusz and Wilusz, 2004). On the other end of the spectrum, our results also call for re-evaluating previous results that relied on mRNA levels as a means to study the transcriptional activities of promoters and promoter elements.

Importantly, the promoter does not seem to play a major role in the decay of all mRNAs. For example, replacing *PGK1* 3'UTR with *MFA2* 3'UTR led to enhanced decay of the chimeric mRNA relative to that of *PGK1* mRNA (LaGrandeur and Parker, 1999). It is possible that *MFA2* 3'UTR is an independent *cis*-acting RNA element with the capacity to stimulate mRNA decay (although the effect of changing RNA *cis* elements was determined by manipulating the DNA, which encodes the element, and the possible impact of the DNA was not ruled out). We suspect that mRNA decay rates are regulated by a combination of nuclear and cytoplasmic processes and that the relative contribution of each compartment varies between the genes and/or between environmental conditions.

Synthegradaes: Factors that Act on Both mRNA Synthetic and Decay Machineries

A common theme is now emerging whereby some transcription activators (e.g., Rap1p, Rpb4/7, Ccr4p) enhance mRNA decay.

We propose to name these factors “synthegradases” to emphasize their dual role. Recent studies demonstrated that environmentally induced genes are subject to transcriptional induction that is accompanied by an increase in decay rate of their transcripts (Elkon et al., 2010; Molin et al., 2009; Rabani et al., 2011; Shalem et al., 2008). This counterintuitive “counteraction” characterizes mainly mRNAs whose levels are shaped by a sharp “peaked” behavior (Rabani et al., 2011; Shalem et al., 2008). The capacity of the synthegradases, like Rap1p, to enhance both mRNA synthesis and decay might serve as a mechanistic basis for this phenomenon. As proposed previously, the combination of enhanced synthesis and decay permits rapid acquisition of a new steady-state level (Shalem et al., 2008). We suspect that the two-arms mechanism of the synthegradases is more responsive to regulatory signals. Specifically, signaling pathways can modulate either the synthetic or the decay function of the synthegradases, thereby fine-tuning the desired steady-state levels, as well as the kinetics with which they are achieved.

Recent comparison between mRNA decay kinetics in two related *Saccharomyces* species revealed a significant difference in ~11% of the orthologous mRNAs. In half of these cases, the different decay was coupled to a difference in transcription. Coupling almost always involves enhancement of both mRNA synthesis and decay or, conversely, repression of both mRNA synthesis and decay (Dori-Bachash et al., 2011). Moreover, some yeast factors (most notably Rpb4p and Ccr4p) seem to have evolved in a manner that either enhances both mRNA synthesis and decay or represses both activities simultaneously. At least 5% of the 3,000 yeast genes examined in this study (that excludes genes encoding ribosomal proteins) is likely to be regulated by synthegradases during optimal proliferation conditions (Dori-Bachash et al., 2011). We suspect that this number is likely to increase upon shifts from optimal to stress conditions and after including the Rap1p-regulated genes. A corollary of the double roles of promoters and synthegradases has evolutionary implications, whereby a single mutation in either a promoter or a synthegradase can affect both mRNA synthesis and decay, which otherwise would require two independent mutations (see also Dahan et al., 2011).

Concluding Remarks

Previous work has uncovered intricate linkages between the various stages of the mRNA life cycle (recently reviewed in Dahan et al., 2011). Such linkages can better regulate the ratio between signal and noise; they can play pivotal roles during the adaptation to a new environmental condition by regulating the proper dynamics in obtaining new mRNA levels and their proper translation (Dahan et al., 2011). Our results show that promoters can contribute to the interplay between mRNA synthesis and decay. We propose that some promoters can coordinate between the two processes that determine the steady-state mRNA level and play a key role in shaping the kinetic of obtaining new levels in response to the environment. It would be very interesting to examine whether the dialog between the synthetic and decay machineries is bilateral, whereby mRNA decay machineries affect the transcriptional function of promoters.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth

The BY4741 yeast strain (Euroscarf) (*MATa*, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*) and its *xrn1Δ* or *ski7Δ* derivatives were grown at 30°C in batch cultures with shaking at 200 rpm, using selective synthetic medium. ZMY60 (*MATa*, *ura3-52*, *trp1-Δ1*, *ade2-101* p*ACE1-UBR1*, p*ACE1-ROX1*) (Moqtaderi et al., 1996) and lev391 (*MATa*, *ura3-52*, *trp1-Δ1*, *ade2-101*, p*ACE1-UBR1*, p*ACE1-ROX1* rap1-(Δ)::KAN R (KANR-ANB-UBI-R-lacI-4HA-RAP1) (Pardo and Marcand, 2005) were transformed with construct A or construct B and were grown in a selective medium at 30°C as above. When the culture reached 5×10^6 cells/ml, CuSO₄ (1 mM) was added and the cultures were shaken for an additional 3 hr before Thiolutin was added. yBA57–yBA60 are *rp130 Δ* haploid derivatives of BY4743 (*Mat a/α*; *his3Δ1/his3Δ1*; *leu2Δ0/leu2Δ0*; *lys2Δ0/LYS2*; *MET15/met15Δ0*; *ura3Δ0/ura3Δ0*; *rp130Δ::kanMX4/RPL30*) that express the various constructs as the only source of *RPL30*.

Site-Directed Mutagenesis

Insertion of (G)₁₈ tract, as well as insertion or deletion of RapBS, was performed using the Muta-Gene M13 in vitro Mutagenesis kit (Bio-Rad).

RNA Cleavage by RNase H

Site-specific cleavage of mRNA A and B was performed basically as described previously (Muhrad and Parker, 1992). In brief, deoxyoligonucleotides were designed as follows. oMC1296 (designated in Figure 1B as “1”) is 5′-TTACCTTATTTAAGCCAAGG-3′; oMC1297 (designated in Figure 1B as “2”) is 5′-CTTCCAACAAATCG-3′; oMC1299 (used in Figure 1C) is 5′-CCTAAGGTGACTTACC-3′. RNA (12 μg) was mixed with 300 ng of the respective oligonucleotide and dried in a speed vac, and the pellet was resuspended in 10 μl of 25 mM Tris (pH 7.5), 1 mM EDTA, and 50 mM NaCl. The mixture was heated at 68°C for 10 min and cooled slowly to 30°C at room temperature. RNase H digestion was performed as described (Muhrad and Parker, 1992). The tubes were incubated at 30°C for 50 min and 15 min at 37°C. RNA was extracted by phenol/chloroform followed by ethanol precipitation. The pellet was dissolved in 80% formamide and 10 mM EDTA and dyes. The cleaved RNA (5 μg) was electrophoresed in 6% PAGE for ~2.5 hr at 300 V in TBE buffer followed by electrotransfer onto GeneScreen plus membrane.

Determining mRNA Levels and mRNA Degradation Profile

A cell aliquot from the culture was taken for time 0 and then treated with 100 μM of 1,10-phenanthroline (Merk) or 3 μM of Thiolutin (Pfizer). Cell harvesting, RNA extraction, and northern analysis were performed as described previously (Lotan et al., 2005). Polyacrylamide northern analysis (Sachs and Davis, 1989) was performed as described previously (Lotan et al., 2005).

Statistical Analysis

In most cases, two-way analysis of variance (ANOVA) was performed, using “Fit Model” of JMP7 statistical program to analyze the mRNA levels. The factors that were used in the model were: (1) yeast strains that differ in the construct they contain (Construct), (2) time points of cell harvest (Time), and (3) the interaction between these factors (Construct-Time). Factor with $p(F) < 0.05$ was considered significant. When interaction was found to be significant, mRNA levels were compared in each time point using *F* test in the slice option of the software (equivalent to *t* test). We concluded that there are significant differences in the decay kinetics when the $p(F)$ for a given time point was significant, and the significance was larger at the later time points. In the figures, we indicated the $p(F)$ for the first time point that exhibits a highly significant value. Note that, in all of our experiments, the significance was larger at the later time points. In Figure S4, the levels of *RPL30pG* mRNAs derived from constructs A, B, E, and F were divided into two factors: (1) Rap1p-binding sites (presence/absence) and (2) all promoter sequences other than Rap1p-binding site (*P_{ACT1}*/*P_{RPL30}*), leading to three-way ANOVA. In the cases of Figures 5 (C, F, and I) and S5D, the differences in the steady-state levels were analyzed by *t* test.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and can be found with this article online at doi:10.1016/j.cell.2011.12.005.

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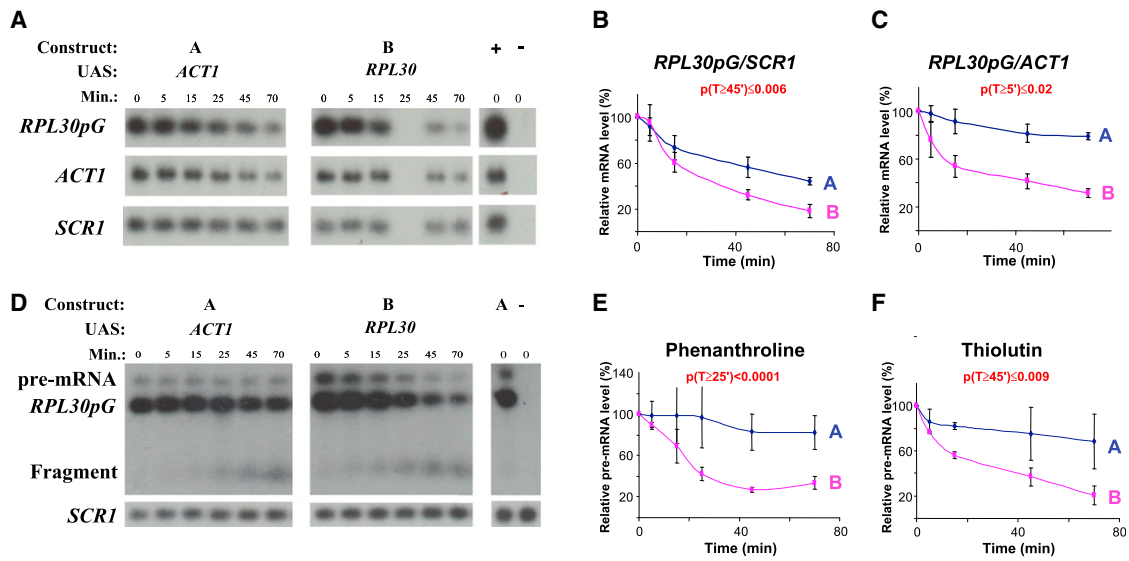


Figure S2. Related to Figure 2

(A–C) Differential decay kinetics of mRNA A and mRNA B is detected after blocking transcription by Thiolutin. Decay kinetics was performed as in Fig. 2A, B, and E except that Thiolutin (3 μ M) was used instead of 1, 10-phenanthroline.

(D–F) Disappearance of pre-mRNA following transcription arrest by either 1, 10-phenanthroline (D and E) or Thiolutin (F). Kinetics and statistical analysis of pre-mRNA decline was obtained as described in Figures 2A and 2B.

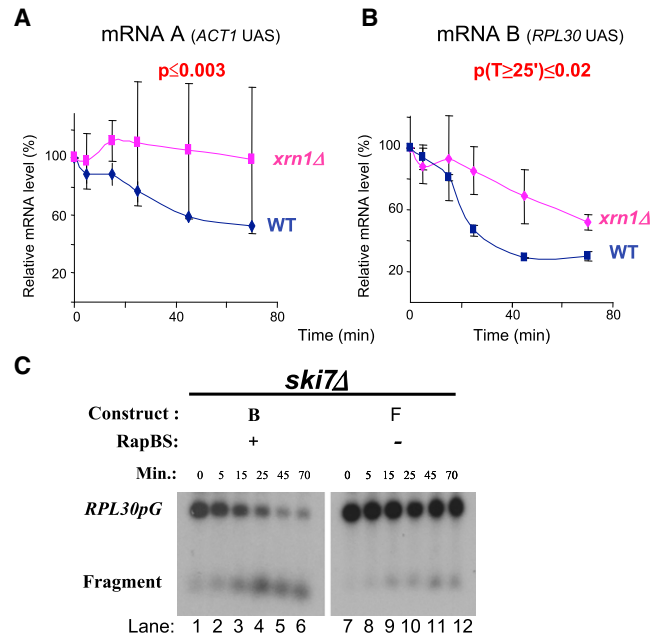


Figure S3. Effects of Deleting *XRN1* on the Decay of mRNA A and mRNA B, Related to Figure 3

(A and B) mRNA decay kinetics of the indicated mRNAs in WT and *xrn1Δ* strains was determined as in Figure 2B.

(C) mRNA decay kinetics of the indicated mRNAs in *ski7Δ* strain was determined as in Figure 3A, except that here both full length *RPL30pG* and its degradation intermediate (Fragment) are shown. As expected, no Fragment was detected in *xrn1Δ* cells (data not shown).

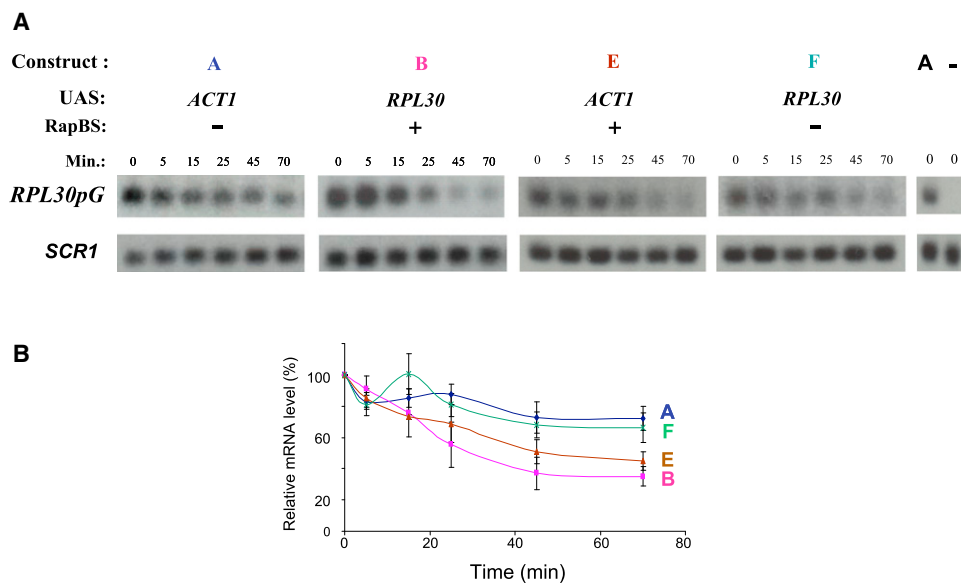


Figure S4. The Impact of RapBS and Other Promoter Elements on mRNA Stability, Related to Figure 4

(A and B) Decay kinetics of the indicated mRNAs was determined as described in Figures 2A, 2E, 4F, and 4G. *SCR1* mRNA is shown to demonstrate equal loading. Three-way ANOVA was used here (see [Experimental Procedures](#)).

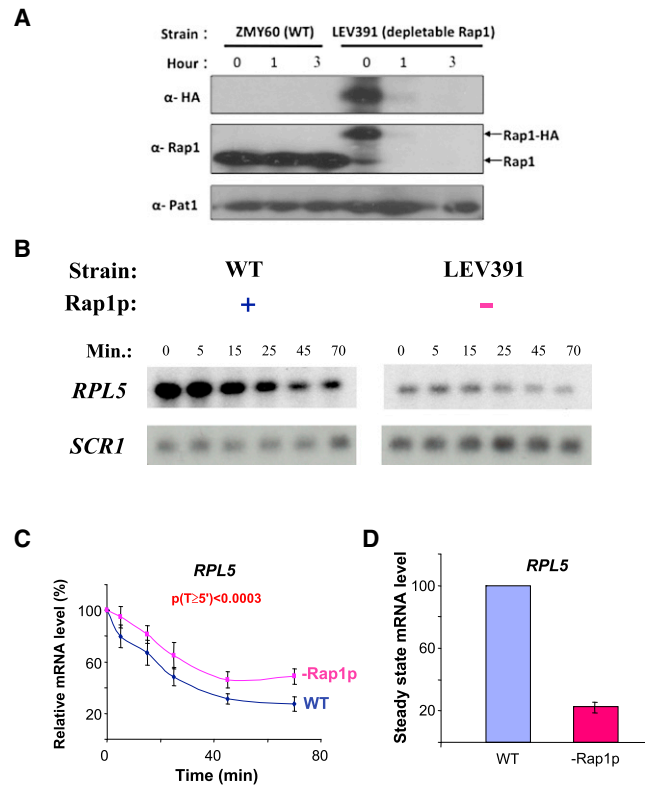


Figure S5. Related to Figure 5

(A) Efficacy and time course of Rap1p depletion. The indicated cells were allowed to proliferate till 5×10^6 cells/ml. CuSO_4 (1 mM) was then added and cell harvested at the indicated time thereafter. Equal amount of proteins were examined by Western analysis using the indicated antibodies. Pat1p is shown to demonstrate equal loading. See also Moqtaderi et al., 1996.

(B–D) Rap1p is involved in the synthesis and decay of *RPL5* mRNA. Assays were performed as in Figures 5A, 5B and 5C. The p value for D is < 0.0001 .