Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 8895–8900, August 1996
Biochemistry

Degradation of *CYC1* mRNA in the yeast *Saccharomyces cerevisiae* does not require translation

(cytochrome *c*/mRNA stability)

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Contributed by Fred Sherman, May 28, 1996

ABSTRACT **Several studies have indicated that degradation of certain mRNAs is tightly coupled to their translation, whereas, in contrast, other observations suggested that translation can be inhibited without changing the stability of the mRNA. We have addressed this question with the use of altered *CYC1* alleles, which encode iso-1-cytochrome *c* in the yeast *Saccharomyces cerevisiae*. The *cyc1-1249* mRNA, which lacks all in-frame and out-of-frame AUG triplets, was as stable as the normal mRNA. This finding established that translation is not required for the degradation of *CYC1* mRNAs. Furthermore, poly(G)$_{18}$ tracks were introduced within the *CYC1* mRNA translated regions to block exonuclease degradation. The recovery of 3′ fragments revealed that the translatable and the AUG-deficient mRNAs are both degraded 5′ → 3′. Also, the increased stability of *CYC1* mRNAs in *xrn1-Δ* strains lacking Xrn1p, the major 5′ → 3′ exonuclease, established that the normal and AUG-deficient mRNAs are degraded by the same pathway. In addition, deadenylylation, which activates the action of Xrn1p, occurred at equivalent rates in both normal and AUG-deficient mRNAs. We conclude that translation is not required for the normal degradation of *CYC1* mRNAs, and that translatable and untranslated mRNAs are degraded by the same pathway.**

mRNA turnover is important for determining the levels and regulation of gene expression. In general, the rate at which a particular protein is made is proportional to the cytoplasmic level of the corresponding mRNA. Thus, the use of mRNA requires the proper coordination of transcription, translation, and mRNA turnover. The degradation of mRNA in eukaryotic cells is initiated by endonucleolytic cleavage (1, 2) or by shortening of the poly(A) tails (3–6) that, for some mRNAs, activates a deadenylylation-dependent decapping reaction (7) and subsequently is followed by 5′ → 3′ exonucleolytic degradation of the whole transcript (for reviews, see refs. 8 and 9). The major cytoplasmic 5′ → 3′ exonuclease, encoded by *XRN1* in the yeast *Saccharomyces cerevisiae*, is responsible for the 5′ → 3′ exonucleolytic degradation of decapped transcripts (10–13).

mRNA half-life and translation are linked in ways that are not completely understood (for reviews, see refs. 14 and 15). It has been reported that the degradation of certain mRNAs is tightly coupled to their translation; examples of these reports follow. (*i*) Pachter *et al.* (16) and Yen *et al.* (17) reported that the level of unpolymerized tubulin subunits directly affects the cytoplasmic stability of α and β tubulin mRNAs cotranslationally by recognition of the nascent amino-terminus of the tubulin polypeptides. (*ii*) Parker and Jacobson (18) reported that in *MATα1* mRNA, blocking translation of the instability element, a 65-nt region containing several rare codons, by insertion of an upstream translation termination codon, inhibits the rapid decay induced by the instability element. (*iii*) Savant-Bhonsale and Cleveland (19) reported that blocking translation by a mutation of the initiator AUG codon results in an increase the stability of the mRNA containing the granulocyte/macrophage colony-stimulating factor AU-rich element. (*iv*) Aharon and Schneider (20) reported that translation restoration by inserting an internal ribosomal entry site into the mRNA 5′ untranslated region (UTR) led to specific destabilization of the corresponding mRNA that harbors a copy of the granulocyte/macrophage colony-stimulating factor AU-rich element in its 3′ UTR. Also a relationship between translation and degradation was revealed by the introduction of premature termination codons, which decrease the stability of the mRNAs, and by restoration of the instability to almost that of the wild-type mRNA by introduction of nonsense suppressors (for reviews, see refs. 21–23). Furthermore, Ross (24, 25) reported at least some mRNases appear to be associated with ribosomes. Therefore, it appears as if mRNA is degraded as the mRNA is being translated.

In contrast, it has been reported that a more than 95% reduction in translation rate, by inserting a stable stem-loop structure in the 5′ UTR, does not affect the stability of some mRNAs (26–30). These results indicate that wild-type rates of translation initiation are not required for inducing mRNA decay. However, the stability of untranslatable mRNAs was not addressed in these studies. Because translation initiation was not completely inhibited by the stable stem-loop structure in the 5′ UTR, ≈5% of initiation events can still occur per mRNA. It is possible that a requirement for translation in decay is actually a requirement for recruiting translation factors and these factors are themselves associated with the degradative enzymes, so that a minimum level of translation is required for efficient mRNA decay.

In this study, we have further investigated the role of the translational process in the decay of mRNA with the use of altered *CYC1* alleles, which encode iso-1-cytochrome *c* in the yeast *S. cerevisiae*. Untranslatable *CYC1* mRNAs were constructed by removing all the in-frame and out-of-frame AUG triplets in the mRNA. The *cyc1-1249* mRNA, which lacks all in-frame and out-of-frame AUG triplets, was shown to be approximately as stable as the normal mRNA. We also have examined *CYC1* mRNAs with poly(G)$_{18}$ tracks that blocked exonuclease degradation, and demonstrated the occurrence of 3′ fragments, indicative of 5′ → 3′degradation in both the normal and AUG-deficient mRNAs. The examination of *CYC1* mRNA in *xrn1-Δ* strains, lacking Xrn1p, the major 5′ → 3′ exonuclease, revealed increased stability of normal and AUG-deficient mRNAs, establishing that both mRNAs are degraded by the same pathway. Thus, this study establishes that translation is not required for the normal degradation of *CYC1* mRNAs, and that translatable and untranslatable mRNAs are degraded by the same pathway.

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Abbreviation: UTR, untranslated region.

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Table 1. Yeast strains

Strain	Oligonucleotide	Plasmid	Genotype
B-7996	—	—	*MATα cyc1-944 CYC7⁺ ura3-52 leu2-3 leu2-112 cyh2ᴿ*
B-8883	—	—	*MATα cyc1-1249 CYC7⁺ ura3-52 leu2-3 leu2-112 cyh2ᴿ*
B-8242	—	—	*MATα cyc1::CYH2ˢ CYC7⁺ ura3-52 leu2-3 leu2-112 cyh2ᴿ*
B-9859	OL95-105	pAB1662	*MATα cyc1-1381 CYC7⁺ ura3-52 leu2-3 leu2-112 cyh2ᴿ*
B-9860	OL95-106	pAB1663	*MATα cyc1-1382 CYC7⁺ ura3-52 leu2-3 leu2-112 cyh2ᴿ*
B-9861	OL95-105	pAB1662	*MATα cyc1-1381 CYC7⁺ ura3-52 leu2-3 leu2-112 cyh2ᴿ xrn1-Δ*
B-9862	OL95-106	pAB1663	*MATα cyc1-1382 CYC7⁺ ura3-52 leu2-3 leu2-112 cyh2ᴿ xrn1-Δ*

MATERIALS AND METHODS

Genetic Nomenclature and Yeast Strains. The genetic *CYC1⁺* denotes the wild-type allele encoding iso-1-cytochrome *c* in the yeast *S. cerevisiae*, whereas *CYC1* denotes the locus and is the general genetic symbol of any allele. Mutant alleles that produce either normal or decreased levels of iso-1-cytochrome *c* are designated *cyc1* followed by the allele number, e.g., *cyc1-944*, *cyc1-1249*. *CYC1* mRNA denotes normal or altered mRNAs transcribed from any *CYC1* or *cyc1* allele. The *cyc1::CYH2ˢ* denotes disruption of *CYC1* by the *CYH2ˢ* gene; *CYC7⁺* denotes the wild-type gene encoding iso-2-cytochrome *c*.

Strain *cyc1-944* (B-7996, Table 1) contains only one TATA element, resulting in initiation of transcription of the *cyc1* gene within a restricted region (31). The *cyc1-944* strain and the corresponding *cyc1-944* mRNA are considered normal in this study. *cyc1-1249* (B-8883, Table 1) was derived from *cyc1-944* by altering the normal ATG initiator codon and all six internal in-frame and out-of-frame ATG triplets (32). *cyc1-1381* and *cyc1-1382* were derived from *cyc1-944* and *cyc1-1249*, respectively, by inserting tracks of 18 continuous G residues [poly(G)$_{18}$] at the *Kpn*I site, corresponding to nucleotide position 247, by oligonucleotide-directed mutagenesis with oligonucleotides OL95-105 and OL95-106 (Table 2), and plasmid pAB458 and pAB1105, respectively, using the methods of Kunkel *et al.* (33). B-8242 was transformed with the resulting plasmids, pAB1662, containing *cyc1-1381*, and pAB1663, containing *cyc1-1382*. Subsequently, strains *cyc1-1381* (B-9859 and B-9861) and *cyc1-1382* (B-9860 and B-9862), having single copies of the altered alleles, were constructed by the methods of Li and Sherman (31) and Yun and Sherman (32).

Nucleic Acid Manipulations. The oligonucleotides, listed in Table 2, were synthesized on an Applied Biosystems model 380A DNA synthesizer, and these included OL95-105 and OL95-106 for oligonucleotide-directed mutagenesis *in vitro*, OL93-210 and OL88-68 for Northern blot analysis of *CYC1* mRNA and *ACT1* mRNA, respectively, OL95-39 and OL95-40 for PCR amplifying a *XRN1* fragment, OL88-193 for primer extension analysis of *CYC1* mRNA, and OL34 for sequencing a portion of the *CYC1* gene. Oligonucleotide-directed mutagenesis was performed by the method of Kunkel *et al.* (33). Northern blot analysis of yeast total RNA was performed as described by Li and Sherman (31) and Yun and Sherman (32), using 20 μg of total RNA for each sample. Primer extension analysis of *CYC1* mRNA was performed with 50 μg of total RNA for each sample as described by Li and Sherman (31), except that the OL88-193 was labeled by ^{32}P. Analysis of poly(A) tail length was performed as described by Decker and Parker (4) using oligonucleotide OL93-209 as a *CYC1* mRNA specific oligonucleotide in the RNase H reaction and oligonucleotide OL93-210 as the probe for *CYC1* mRNA in Northern blot analysis; 25 μg of total RNA was used for each sample in the RNase H reaction.

Disruption of the *XRN1* Gene. The yeast chromosomal *XRN1* gene was disrupted by transforming yeast cells with the ≈4.2-kb DNA fragment that was prepared by digesting the plasmid pFL306 with *Xho*I and *Sal*I. pFL306 was a derivative of pFL304 containing the *xrn1-Δ* allele, which was constructed by deleting the *Bgl*II fragment of pFL304, and subsequently inserting a 1.1-kb *Hin*dIII fragment carrying the *URA3* gene (10). The transformants were selected on uracil omission plates, and the correct disruption was confirmed by the size of the appropriate PCR-amplified fragment. The PCR product of the wild-type *XRN1⁺* gene was a 2,2-kb fragment, whereas the PCR product of the gene containing the correct *xrn1-Δ* disruption was a 1.4-kb fragment (data not shown).

Half-Life Determination. Cells were harvested at various times after addition of the antifungal agent thiolutin (3 μg/ml), which blocks transcription, and total RNA was isolated as described by Herrick *et al.* (34). The relative amount of the *CYC1* mRNAs were determined by standard Northern blot analysis using the procedures previously described (31, 32).

RESULTS AND DISCUSSION

Untranslatable mRNAs, Lacking AUG Triplets, Are as Stable as Fully Translatable Normal mRNAs. We previously reported that the untranslatable *cyc1-1249* mRNA, lacking all AUG triplets, was normally transcribed and exported to the cytoplasm, where it formed a polyribosomal complex essentially distributed across the entire sucrose gradient (32). Although the reason for this unusual distribution was not established, we suggested that the *cyc1-1249* mRNA was associated with 40S ribosomal subunits and RNP proteins, resulting in protection against degradation. There is no iso-1-cytochrome *c* in the *cyc1-1249* strain. On the other hand, the polysomal distribution indicated that the normal *cyc1-944* mRNA was

Table 2. List of oligonucleotides

Oligonucleotide no.	Sequence
OL95-105	AAGAAATATATTCCTGGTTAGGAATTTGGGGGGGGGGGGGGGGGGAATTCCTACCAAGATGGCCTTTGGT
OL95-106	AAGAAATATATTCCTGGTTAGGAATTTGGGGGGGGGGGGGGGGGGAATTCCTACCAAGATCGCCTTTGGT
OL88-68	(+426) TCTTGGTCTACCGACGATAGATGGGAAGACAGCA (+393)
OL88-193	(+48) GAAAAGTGTAGCACCTTTCTTAGCAGAACCGGCC (+15)
OL93-209	(+396) ACAAAGGAAAAGGGGCCTGTTTACTCACAGGCTT (+362)
OL93-210	(+396) GGGAGGGCGTGAATGTAAGCGTGACATAACTAAT (+363)
OL95-39	GCGATAAACTTCGATGAGAC
OL95-40	GAAGCATGAGTATTATCACG
OL34	(−40) TCTATAGACACGCAAACAC (−23)

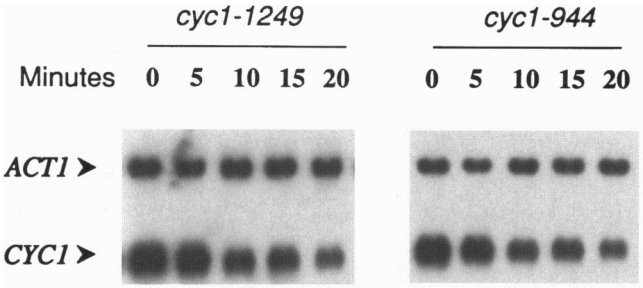

FIG. 1. Northern blot analysis of *cyc1-1249* and *cyc1-944* mRNAs after various times of inhibition of transcription with 3 μg/ml of thiolutin. A total of 20 μg of RNA was isolated from cells grown in yeast extract/peptone/dextrose (YPD) medium, and the RNA was denatured, separated by electrophoresis in agarose, transferred to a nitrocellulose membrane, and cohybridized to the ^{32}P-labeled probes OL93-210 for the detection of *CYC1* mRNA and OL88-68 for the detection of *ACT1* mRNA. The relative amount of *CYC1* mRNAs was quantified by densitometry using *ACT1* mRNA as an internal standard. Quantitative estimates of half-lives are presented in Table 3.

primarily associated with two to six 80S ribosomes (32) and the *cyc1-944* strains contain a normal amount of iso-1-cytochrome *c*.

The half-lives of both the *cyc1-1249* and *cyc1-944* mRNAs, determined with the procedure described by Herrick *et al.* (34), were ≈13 min (Fig. 1, Table 3). Because AUG is the only codon capable of initiating translation at appreciable levels in yeast (35–37) and because all the AUG triplets were altered in the *cyc1-1249* mRNA, we can rule out the formal possibility that translation initiation can occur on *cyc1-1249* mRNA. Thus, we conclude that both translation initiation and elongation are not required for the degradation of *CYC1* mRNA.

***CYC1* mRNAs Are Degraded from 5′ → 3′ Independent of Translation.** To elucidate decay pathways for *CYC1* mRNAs in *cyc1-944* and *cyc1-1249* strains, poly(G)$_{18}$ tracts were inserted at a *Kpn*I site corresponding to nucleotide position 247 in the *CYC1* coding region by oligonucleotide-directed mutagenesis, resulting in the *cyc1-1381* and *cyc1-1382* alleles (Table 3). The poly(G)$_{18}$ tract forms an extremely stable RNA secondary structure that is an efficient structural block to yeast 5′ → 3′ exonuclease (4, 7, 38). If the *CYC1* mRNAs are degraded 5′ → 3′ or degraded by endonucleolytic cleavage, we would expect to observe the 3′ fragment by Northern blot analysis. However, if the *CYC1* mRNAs are degraded 3′ → 5′, the 5′ fragment would not be observed because the probe, OL93-210, hybridizes only to the 3′ untranslated region of *CYC1* mRNA. The detection of the 3′ fragment, shown in Fig. 2, suggested that the translatable *cyc1-1381* mRNA and the AUG-deficient *cyc1-1382* mRNA are both probably degraded 5′ → 3′ or degraded by endonucleolytic cleavage.

Further studies to investigate the pathway of degradation made use of *xrn1*-Δ strains that lack Xrn1p, the major 5′ → 3′ exonuclease in yeast (10–13). The levels and half-lives of the *CYC1* mRNAs in the various *XRN1*$^+$ and *xrn1*-Δ strains containing or lacking poly(G)$_{18}$ tracts are summarized in Table 3.

Two *CYC1* mRNA bands were observed in *XRN1*$^+$ strains: the longer one corresponded to the full-length *CYC1* mRNA

Table 3. *CYC1* mRNA level and half-life

Allele	AUG triplets	poly(G)$_{18}$ track	% normal mRNA		Half-life, min*	
			XRN1$^+$	*xrn1*-Δ	*XRN1*$^+$	*xrn1*-Δ
cyc1-944	+	0	100	190	13	—
cyc1-1249	0	0	100	—	13	—
cyc1-1381	+	+	110	187	13.5	30
cyc1-1382	0	+	96	200	12.5	33

*The disappearance of the full-length molecules of *CYC1* mRNA.

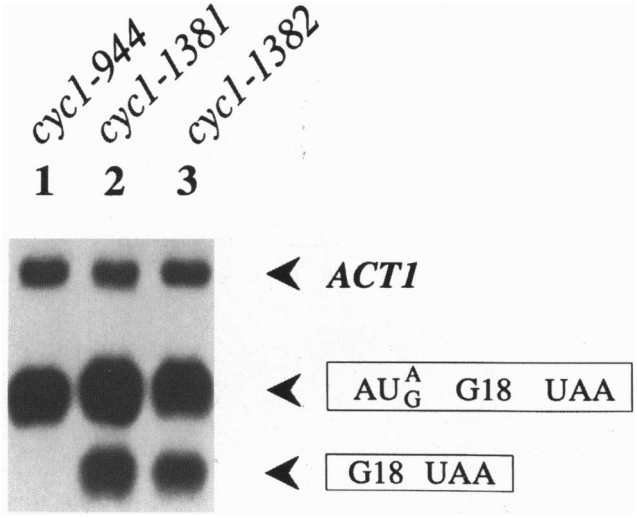

FIG. 2. Northern blot analysis of *cyc1-944*, *cyc1-1381* and *cyc1-1382* mRNAs from strains grown in yeast extract/peptone/ethanol (YPE) medium. A total of 20 μg of total RNA was used for each sample. The probes are described in the legend of Fig. 1. The longer bands (AU^A_G G18 UAA) represent the full-length *CYC1* mRNAs, whereas the shorter bands (G18 UAA) represent the 3′ fragment of the *CYC1* mRNA, which was only observed after extended exposure of the autoradiograph.

and the shorter one corresponded to the 3′ fragment (Fig. 2). Quantitative estimates (see Table 3) showed that the steady-state levels of the full-length *CYC1* mRNAs in *cyc1-1381* and *cyc1-1382* strains were approximately the same as the amount in the *cyc1-944* strain. However, only the full-length *CYC1* mRNAs were normally observed in the *xrn1*-Δ cells (Fig. 3), and these mRNAs were considerably more stable than those in the *XRN1*$^+$ strains (Table 3). Nonetheless, a trace of the 3′ fragment could be detected in the *xrn1*-Δ strains when the autoradiographs were overexposed (data not shown).

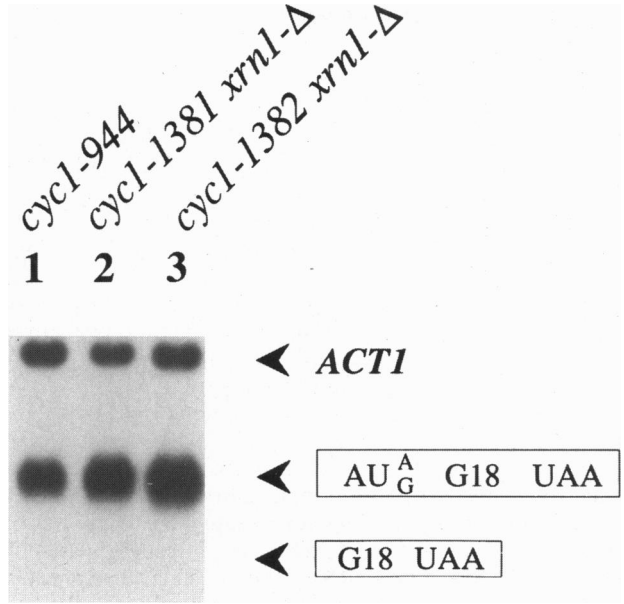

FIG. 3. Northern blot analysis of *cyc1-944*, *cyc1-1381*, and *cyc1-1382* mRNAs from *xrn1*-Δ strains grown in YPE medium. A total of 20 μg of total RNA was used for each sample. The probes are described in the legend of Fig. 1. The longer bands (AU^A_G G18 UAA) represent the full-length *CYC1* mRNAs molecular, whereas the shorter bands (G18 UAA) represent the 3′ fragment of the *CYC1* mRNA.

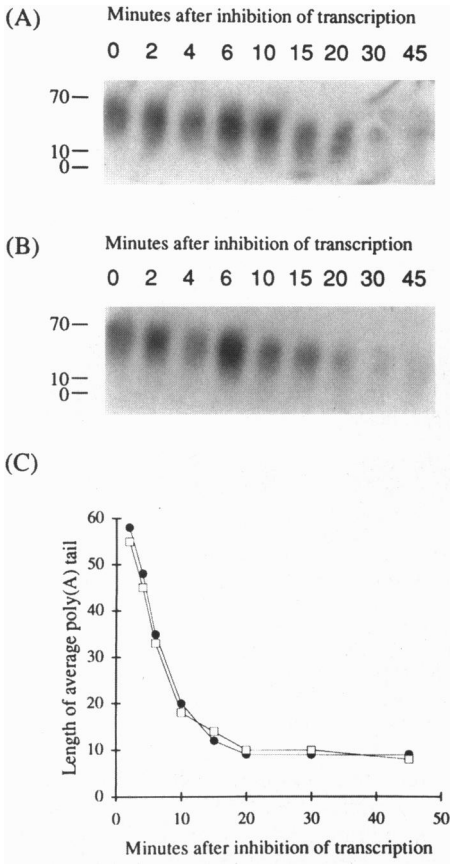

FIG. 4. Polyacrylamide Northern blot analysis of (*A*) *cyc1-1381* mRNA, and (*B*) *cyc1-1382* mRNA. Samples were taken at the indicated times after inhibition of transcription. A total of 25 μg of total RNA for each sample was treated with RNase H in the presence of the oligonucleotide OL93-209. The hybridization probe was oligonucleotide OL93-210. The numbers at left represent the approximate migration of the fragment with the indicated number of adenylylate residues. (*C*) The deadenylylation curve of the *cyc1-1381* mRNA (□) and *cyc1-1382* mRNA (●). The average length of poly(A) tail for each sample was the mean of the longest and the shortest the poly(A) tails at each time point.

The accumulation of 3′ fragments in *XRN1*$^+$ strains and its almost absence in *xrn1*-Δ strains indicates that Xrn1p is responsible for the 5′ → 3′ degradation of full-length *CYC1* mRNA. We conclude that both translatable and untranslatable *CYC1* mRNAs are degraded by the same pathway.

Deadenylylation Rates Are the Same for *cyc1-1381* and *cyc1-1382* mRNAs. The first step in the 5′ → 3′ degradation by Xrn1p is initiated by the cytoplasmic shortening of poly(A) tails (4). To determine if translation is coupled to deadenylylation, we examined the rate of deadenylylation of the untranslatable *cyc1-1382* and translatable *cyc1-1381* mRNAs by using thiolutin as an inhibitor of transcription (34) (Fig. 4). The poly(A) tails of *cyc1-1381* and *cyc1-1382* mRNAs shortened from an initial length of 55 adenylylate residues to tails of about 10 residues, with a maximum shorting rate of approximately six adenylylate residues per minute. Since there was no significant difference between the rates of deadenylylation of *cyc1-1381* and *cyc1-1382* mRNAs, we conclude that untranslatable mRNA can be deadenylylated, as well as fully translated mRNA.

Full-Length *CYC1* mRNAs Accumulate in Both *cyc1-1381 xrn1*-Δ and in *cyc1-1382 xrn1*-Δ Strains. Because Xrn1p is responsible for the 5′ → 3′ degradation of the bulk of yeast mRNA and because the disruption of the *XRN1* gene allows the accumulation of decapped molecules (7, 10–13), it was possible to determine the site of the 5′ cleavage by mapping the 5′ ends of these decapped mRNAs. This was performed by primer extension analysis of the *CYC1* mRNAs 5′ ends at an early time, when the *CYC1* mRNAs are still capped, and at a later time, when the majority of the *CYC1* mRNAs are decapped. The results of the primer extension products, shown in Fig. 5, indicate that the 5′ ends of the *cyc1-1381* and *cyc1-1382* mRNAs from *xrn1*-Δ strains are identical at both early and late times point and correspond to the 5′ ends of *cyc1-1381* and *cyc1-1382* mRNAs from *XRN1*$^+$ strains and also correspond to the 5′ ends of *cyc1-944* mRNA (31). We conclude that the 5′ cleavage event that removes the cap structure does not change the length of the *CYC1* mRNAs in the presence or absence of translation.

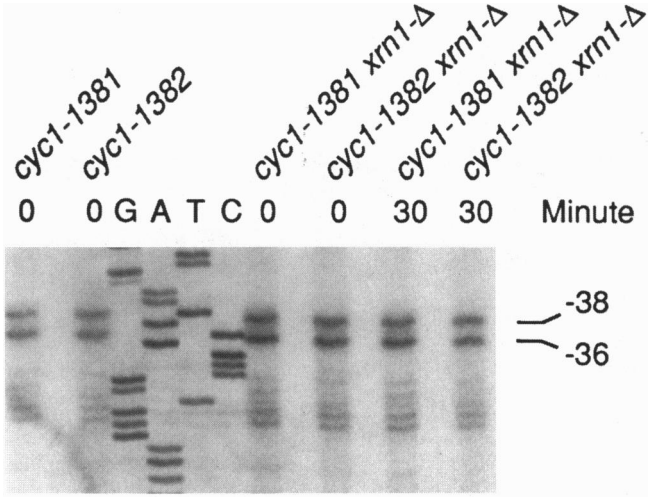

FIG. 5. Primer extension analysis of *CYC1* mRNAs containing poly(G)$_{18}$ tracts inserted at *Kpn*I sites corresponding to nucleotide position 247 site of mRNAs from *XRN1*$^+$ and *xrn1*-Δ strains. The times after inhibition of transcription are indicated at the top of the lanes. DNA size markers are shown in the lanes denoted G, A, T, and C. The two major extension products correspond to the major 5′ ends at nucleotide positions −38 and −36. A total of 50 μg of total RNA was used for each sample.

Translation Does not Affect the Rate or Pathway of the 5′ → 3′ Degradation of *CYC1* mRNA. The experiments reported above establish that the decay pathway of *CYC1* mRNA was not altered in *cyc1-1382* strains. To determine whether the decay rate was altered in *cyc1-1382* strains, we measured the half-lives of *CYC1* mRNAs in *cyc1-1381* and *cyc1-1382* strains containing either *XRN1*$^+$ or *xrn1*-Δ alleles. In this experiment, cells were grown in YPD medium and transcription was inhibited by the addition of thiolutin. Total RNA was isolated at various times, and the relative amounts of *CYC1* mRNA for each time point were determined by Northern blot analysis (Figs. 6 and 7); the quantitative results are summarized in Table 3. There was no significant difference in the stability of *CYC1* mRNAs in *cyc1-1381* and *cyc1-1382 XRN1*$^+$ strains, as indicated by the half-lives of 13.5 and 12.5 min, respectively. There was also no significant difference in the stability of *CYC1* mRNA in *cyc1-1381* and *cyc1-1382 xrn1*-Δ strains, which exhibited half-lives of 30 and 33 min, respectively. In addition, 3′ fragments accumulated but were eventually degraded in both the *cyc1-1381 XRN1*$^+$ and *cyc1-1382 XRN1*$^+$ strains. It should be noted that because the short 3′ fragment did not accumulate proportionally to the diminution of the full-length *CYC1* mRNA, the block in 5′ → 3′ degradation either was not absolute or there were other degradation events occurring less frequently. Since there is no significant difference in the stability of *CYC1* mRNA and in the accumulation or diminution of 3′ fragments in *cyc1-1381* and *cyc1-1382* strains, we conclude that translation does not alter the decay rate of *CYC1* mRNA.

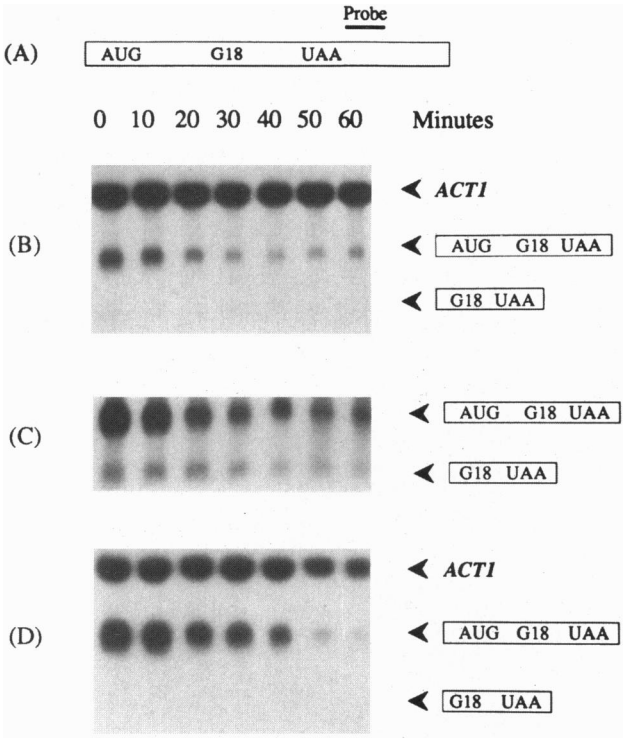

FIG. 6. Northern blot analysis of *CYC1* mRNAs from the *cyc1-1381* strain grown in YPD medium after various time of inhibition of transcription with 3 μg/ml of the thiolutin. A total of 20 μg of total RNA was used for each sample. The numbers above each lane denote minutes after inhibition of transcription. (*A*) Schematic representation of the *cyc1-1381* mRNA showing the AUG initiator codon, the UAA terminator codon, the poly(G)$_{18}$ tract indicated by G18, and the probe OL93-2110, which hybridizes only to the 3′ UTR of the *CYC1* mRNA. (*B*) Total RNA was isolated from the *cyc1-1381 XRN1*$^+$ strain, and the autoradiogram was exposed for 10 hr. (*C*) The same autoradiogram shown in *B* was exposed for 36 hr, but only the two bands corresponding to the *CYC1* mRNAs are presented. (*D*) Total RNA was isolated from the *cyc1-1381 xrn1*-Δ strain, and the autoradiogram was exposed for 10 hr.

Normal Degradation of *CYC1* mRNAs Does not Require Translation. In this study, we have examined the relationship between translation and degradation *CYC1* mRNAs. The lack of translation was achieved by using a *CYC1* mRNA deficient in AUG triplets. The direction of degradation was determined by using poly(G)$_{18}$ tracks to block exonuclease degradation, and examining the presence of 3′ fragments. The pathway of degradation was investigated with *xrn1*-Δ strains lacking Xrn1p, the major 5′ → 3′ exonuclease.

Our results indicated that *CYC1* mRNAs were degraded by the Xrn1p "default" pathway that is initiated by shorting of poly(A) tail, followed by decapping, and 5′ → 3′ exonucleolytic degradation of the *CYC1* mRNA (8). Our results also indicated that the lack of translation did not affect the half life, deadenylylation rate, and appearance of specific decay intermediates of *CYC1* mRNAs, which establishes that untranslatable mRNAs were degraded by the same mechanism and rate as translatable normal mRNAs.

Although translation is not required for degradation of the normal types of *CYC1* mRNAs described in this paper, translation may be required for the degradation of specialized mRNAs with specific motifs as discussed.

We thank Linda Comfort for synthesizing the oligonucleotides and Dr. A. Stevens (Martin Marietta Energy Systems, Oak Ridge, TN) for providing plasmid pFL306. This investigation was supported by the U.S. Public Health Service Research Grant R01 GM12702 from the National Institutes of Health.

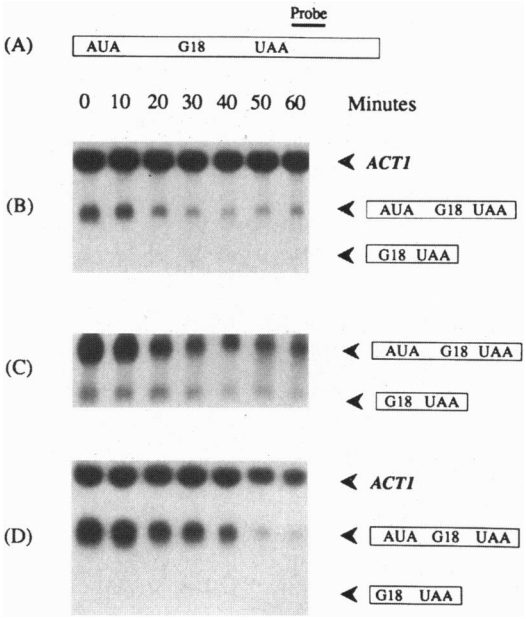

FIG. 7. Northern blot analysis of *CYC1* mRNAs from the *cyc1-1382* strain grown in YPD medium after various time of inhibition of transcription with 3 μg/ml of the thiolutin. A total of 20 μg of total RNA was used for each sample. The numbers above each lane denote minutes after inhibition of transcription. (*A*) Schematic representation of the *cyc1-1382* mRNA as described in the legend of Fig. 6. (*B*) Total RNA was isolated from *cyc1-1382 XRNI* mutant cells, and the autoradiographic film was exposed for 10 hr. (*C*) The same radiogram as shown in *B* except that autoradiographic film was exposed for 36 hr and only two bands corresponding to *CYC1* mRNA are presented. (*D*) Total RNA was isolated from *cyc1-1382 xrn1*-Δ mutant cells, and autoradiographic film was exposed for 10 hr.

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