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Identification and Comparison of Stable and Unstable mRNAs in *Saccharomyces cerevisiae*

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We developed a procedure to measure mRNA decay rates in the yeast *Saccharomyces cerevisiae* and applied it to the determination of half-lives for 20 mRNAs encoded by well-characterized genes. The procedure utilizes Northern (RNA) or dot blotting to quantitate the levels of individual mRNAs after thermal inactivation of RNA polymerase II in an *rpb1-1* temperature-sensitive mutant. We compared the results of this procedure with results obtained by two other procedures (approach to steady-state labeling and inhibition of transcription with Thiolutin) and also evaluated whether heat shock alters mRNA decay rates. We found that there are no significant differences in the mRNA decay rates measured in heat-shocked and non-heat-shocked cells and that, for most mRNAs, different procedures yield comparable relative decay rates. Of the 20 mRNAs studied, 11, including those encoded by *HIS3*, *STE2*, *STE3*, and *MATα1*, were unstable ($t_{1/2} < 7$ min) and 4, including those encoded by *ACT1* and *PGK1*, were stable ($t_{1/2} > 25$ min). We have begun to assess the basis and significance of such differences in the decay rates of these two classes of mRNA. Our results indicate that (i) stable and unstable mRNAs do not differ significantly in their poly(A) metabolism; (ii) deadenylation does not destabilize stable mRNAs; (iii) there is no correlation between mRNA decay rate and mRNA size; (iv) the degradation of both stable and unstable mRNAs depends on concomitant translational elongation; and (v) the percentage of rare codons present in most unstable mRNAs is significantly higher than in stable mRNAs.

Differences in the decay rates of individual mRNAs can have profound effects on the overall levels of expression of specific genes (80, 93). Although the potential importance of mRNA stability as a mechanism for regulating gene expression has been recognized (7, 86), the structures and mechanisms involved in the determination of individual mRNA decay rates have yet to be elucidated. As an approach to understanding the determinants of mRNA stability, we have begun to compare the properties of mRNAs in *Dictyostelium discoideum* which differ significantly in their respective decay rates (94). In this report, we describe our initial efforts to perform a similar analysis of mRNAs in the yeast *Saccharomyces cerevisiae*. Our objective was the identification of both stable and unstable yeast mRNAs that were encoded by genes which had already been well characterized. Success in such an endeavor would make it possible to explore the structural determinants of mRNA stability, for example, by analyzing the decay rates of mRNAs transcribed from chimeric genes (25, 42, 43, 81, 96, 97).

Decay rates for both the poly(A)$^+$ RNA population and for individual yeast mRNAs have been measured previously by several different functional or chemical assays. Half-lives ranging from 16 to 23 min have been measured for the average turnover rate of the poly(A)$^+$ RNA population, whereas half-lives of individual mRNAs span a broader range from 1 to over 100 min (3, 18, 19, 35, 36, 46, 47, 50, 52, 54, 72, 90, 104, 109). However, the majority of the assays used in these previous studies were (i) not potentially useful for the characterization of chimeric mRNAs, (ii) not amenable to the simultaneous assessment of the decay rates of large numbers of individual mRNAs, or (iii) a source of possible artifacts in the measurements (e.g., by the inhibition of transcription and other cellular metabolic events [15, 17, 41, 48]). Therefore, we sought to establish an alternative procedure for the reliable evaluation of the decay rates of individual yeast mRNAs.

Nonet et al. (70) have constructed and characterized a conditionally lethal mutant (*rpb1-1*) with a temperature-sensitive (ts) lesion in the largest subunit of RNA polymerase II. In strains harboring the *rpb1-1* allele, a shift to 36°C leads to the rapid and selective cessation of mRNA synthesis and to a reduction in the steady-state levels of preexisting mRNAs (70). The latter reduction is a reflection of ongoing mRNA turnover in the absence of new mRNA synthesis and serves as the basis for the procedure used here. Cells growing at 24°C were abruptly shifted to 36°C by the addition of medium prewarmed to 48°C, and culture aliquots were removed at different times after the temperature shift. RNA was isolated from each aliquot, and the relative amounts of individual mRNAs were quantitated by RNA-blotting methods. Using this procedure, half-lives were determined for 20 different mRNAs and found to range from 2.5 to 45 min. mRNA decay rates measured in this manner were compared with those obtained by two additional, independent procedures (approach to steady-state labeling and inhibition of transcription with Thiolutin) and with those determined in the presence and absence of a heat shock.

Having identified mRNAs with large differences in their respective decay rates, we then compared them with respect to several physiological or structural parameters which might contribute to the determination of their inherent stability or instability: (i) relative decay rates of poly(A)$^+$ and poly(A)$^-$ forms, (ii) mRNA size, (iii) the dependence of decay on ribosomal translocation, (iv) relative content of rare codons, and (v) specific sequences or structures within 3′ untranslated (UT) regions. These comparisons showed

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that unstable mRNAs have a higher content of rare codons than stable mRNAs, but otherwise showed no significant differences between stable and unstable mRNAs with respect to poly(A) metabolism, size, sensitivity to treatment with cycloheximide, or 3′ UT sequences.

MATERIALS AND METHODS

Yeast strains. Four strains of the yeast *S. cerevisiae* were used for these experiments. Strain DBY747 (*MAT***a** *his3Δ leu2-3 leu2-112 ura3-52*) was used to analyze mRNA decay in approach to steady-state labeling experiments as well as in cells treated with Thiolutin. Strains Y260 (*MAT***a** *ura3-52 rpb1-1*) and Y262 (*MAT*α *ura3-52 his4-539 rpb1-1* [70]; kindly provided by Richard Young, Massachusetts Institute of Technology) were utilized in temperature shift experiments in which their thermolabile RNA polymerases were inactivated. Strain N222 (*MAT*α *ura3-52 leu2-3 leu2-112 his3-Δ200 lys2-Δ201 ade2*; kindly provided by Michael Nonet) is a derivative of Y262 with a wild-type RNA polymerase that was used for several control experiments.

Analysis of mRNA decay in ts RNA polymerase mutants. mRNA decay rates were measured in cultures (100 to 200 ml) of Y260 or Y262 (0.5×10^7 to 1×10^7 cells per ml) in which the temperature of the culture was abruptly adjusted from 24 to 36°C by adding an equal volume of YEPD medium (66) at 48°C and then transferring the culture flask to a shaker bath at 36°C. Aliquots of the culture were removed after 0 to 100 min at 36°C, cells were harvested by centrifugation, and the cell pellets were frozen on dry ice. Total cellular RNA was extracted from the frozen cells, suspended in sterile H_2O, and stored at −80°C. Integrity of the extracted RNA was monitored by electrophoresis and staining in a denaturing gel. The relative levels of individual mRNAs present at different times after the temperature shift were determined by Northern (RNA) blotting or RNA dot blotting. To analyze the decay rate of total poly(A)$^+$ RNA, cells (strain Y260) were grown in YEPD medium at 24°C to a density of 5×10^6 cells per ml and labeled for one generation (3.5 h) before the temperature shift with 20 μCi of carrier-free [^{32}P]phosphoric acid per ml. The fraction of poly(A)$^+$ RNA in each sample was monitored by hybridization to poly(U) filters (73).

Treatment of cells with Thiolutin. The antifungal agent Thiolutin (39) (kindly provided by Donald Tipper, University of Massachusetts Medical School) was dissolved in dimethyl sulfoxide at 2 mg/ml. To analyze the effects of Thiolutin on total poly(A)$^+$ RNA, cells (strain DBY747) grown in YEPD medium at 30°C to 5×10^6 cells per ml were labeled with 20 μCi of [^{3}H]uridine per ml for 1 h and then exposed to 0, 3, or 6 μg of Thiolutin per ml. Culture samples (10 ml) were removed and frozen in dry ice after 0 to 60 min of Thiolutin treatment, and total RNA was extracted. Radioactivity in poly(A)$^+$ RNA was determined by hybridizing equal volumes of total RNA to poly(U) filters (73). To measure the effects of the drug on the decay rates of individual mRNAs, Thiolutin (3 μg/ml) was added to cells grown at 30°C to 5×10^6 cells per ml in YEPD medium, and culture samples (40 ml) were removed, centrifuged, and frozen after 0 to 100 min of drug exposure. Total RNA was extracted, and 4 μg of RNA from each sample was fractionated in 1% agarose–2.2 M formaldehyde gels (53). Individual mRNAs were subsequently quantitated by Northern blotting.

Approach to steady-state labeling. Cells (strain DBY747) were grown in phosphate-depleted YEPD medium (87) at 30°C to a density of approximately 4×10^6 cells per ml. Carrier-free [^{32}P]phosphoric acid was added at 100 μCi/ml, and samples (47 ml) of the culture were removed after 5 to 70 min of labeling. Total RNA was extracted from frozen cell pellets, and poly(A)$^+$ RNAs were purified by an oligo(dT)-cellulose batch procedure (see below). To measure decay rates of individual mRNAs, we hybridized 1 μg of poly(A)$^+$ RNA from each sample to filters containing dots of specific plasmid DNAs (see below). RNA decay curves were generated by plotting $1 - A/A^\infty$ versus the time of $^{32}PO_4$ labeling (where A is the specific activity of an RNA at time t and A^∞ is the specific activity at time ∞ [29, 46]). For total poly(A)$^+$ RNA, the specific activity of the 70-min sample was used as A^∞, and for individual mRNAs, the specific activity of the 30-min samples was used as A^∞.

Heat shock. To measure mRNA decay rates in the presence or absence of heat shock, we grew 125-ml cultures of N222 at 36 or 24°C in YEPD to the mid-log phase. Decay rates in the absence of heat shock were measured after inhibition of transcription by the addition of 125 ml of 36°C YEPD medium containing Thiolutin at 6 μg/ml (final concentration, 3 μg/ml) to the 36°C culture. Decay rates in the presence of heat shock were measured by the addition of 125 ml of 48°C YEPD medium containing Thiolutin to the 24°C culture. In both cases, RNA was prepared at various times after continued incubation at 36°C and the decay of individual mRNAs was determined by Northern blotting, autoradiography, and densitometry.

RNA extraction. RNA was extracted by modifications of the methods of Zitomer et al. (109) and Domdey et al. (22). The former procedure included a step to remove contaminating DNA by two successive washes with ice-cold 3.0 M sodium acetate (pH 6.0). No differences in mRNA decay rates attributable to the method of RNA isolation were observed. RNA samples were suspended in sterile water, and RNA concentration was determined by A_{260} after incubation at 65°C for 5 min. The integrity of each RNA preparation was monitored by electrophoresis in a 1% agarose–2.2 M formaldehyde denaturing gel and subsequent staining with ethidium bromide.

Purification of poly(A)$^+$ RNA. Poly(A)$^+$ RNA was purified by an oligo(dT)-cellulose batch procedure. To remove fine particles, oligo(dT)-cellulose (type III; Collaborative Research, Inc., Waltham, Mass.) was suspended and allowed to settle in elution buffer (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.5], 1 mM EDTA, and 0.05% sodium dodecyl sulfate [SDS]) four times. Samples of the resin (20 mg) were transferred to 1.5-ml microcentrifuge tubes and equilibrated with binding buffer (20 mM HEPES [pH 7.6], 0.5 M NaCl, 1 mM EDTA, 0.1% SDS) by two cycles of gentle shaking and subsequent centrifugation at top speed in a clinical centrifuge. Total cellular RNA (0.1 to 0.5 mg) in sterile H_2O was heated to 65°C for 5 min, added to an equal volume of 2× binding buffer, mixed with the oligo(dT)-cellulose on a rotating mixer for 15 min at room temperature, and centrifuged as before. The oligo(dT)-cellulose was washed twice with binding buffer (800 μl) followed by two washes (900 μl) with binding buffer containing 0.1 M NaCl. Poly(A)$^+$ RNAs were eluted by the addition of 450 μl of sterile H_2O at 37°C. After centrifugation for 2 min, 400 μl of each eluate was removed and added to 45 μl of 3 M sodium acetate (pH 5.2) and 890 μl of 95% ethanol. The elution procedure was repeated [yielding approximately 10 to 20% more poly(A)$^+$ RNA], and the two eluates were combined. RNA was precipitated either overnight at −20°C or for 1 h at −80°C. Using labeled synthetic mRNAs, these procedures yield >95% recovery of nonadenylated RNAs in the unbound fraction and >95%

TABLE 1. Cloned DNAs used to evaluate mRNA decay rates

Plasmid or bacteriophage	Gene	Description	Reference(s)
pDH6	*ACT1* (actin)	3.8-kilobase (kb) *Eco*RI fragment in pUC9	27, 69
pDH8	*PGK1* (phosphoglycerate kinase)	2.95-kb *Hin*dIII fragment in pUC9	63
pAB518	*STE2* (α-factor receptor)	3.2-kb *Sal*I-*Bam*HI fragment in pRC3	10, 68
pYPA	*PAB* [poly(A)-binding protein]	3.4-kb *Eco*RI-*Hin*dIII fragment in pUC9	88
pDH1	*HTB1* (histone H2B1)	195-base-pair (bp) *Pvu*II-*Hin*dIII fragment in pUC9	106
pDH2	*HTB2* (histone H2B2)	195-bp *Hin*dIII-*Pvu*II fragment in pUC9	106
cDNAs 39, 74, 90	cDNA clones encoding unknown genes	C-tailed inserts of 900 bp average length in G-tailed pBR322	90
TCM	*TCM1* (encodes ribosomal protein L3; trichodermin resistance)	0.8-kb *Sal*I-*Hpa*I 3′ fragment in M13mp9	91
CYH	*CYH2* (encodes ribosomal protein L29; cycloheximide resistance	506-bp *Acc*I-*Eco*RI fragment in M13mp9	44
RP51	*RP51A* (ribosomal protein)	235-bp *Hin*cII 3′-exon fragment in M13mp8	101
A13	*RP29* (ribosomal protein)	1.0-kb *Eco*RI-*Hin*dIII fragment in M13mp9	26
YEp24	*URA3* (orotidine 5′-monophosphate decarboxylase)	1.1-kb *Hin*dIII fragment in pBR322-2μm derivative	84
pUC8-Sc2676	*HIS3* (imidazoleglycerol phosphate dehydratase) *DED1* (essential gene)	1.7-kb *Bam*HI fragment in pUC8	100
MFα1	*MFα1* (α factor)	1.9-kb *Eco*RI fragment in pUC9	51
pSM39	*MFa1* (a factor)	1.6-kb *Eco*RI-*Xba*I fragment in pUC18	65
pSM29	*MFA2* (a factor)	1.75-kb *Hin*dIII fragment in pRM3-1	65
pSL7	*STE3* (a factor receptor)	3.2-kb *Hin*dIII-*Sal*I fragment in YIp5	31
MATα1	*MATα1* (mating-type regulator)	545-bp *Eco*RV-*Sca*I fragment	2
FUS1	*FUS1* (required for fusion during mating)	2.0-kb *Eco*RI-*Pst*I fragment from pSL324	61

recovery of mRNAs containing poly(A) tails 68 nucleotides long in the bound fraction (D. Munroe and A. Jacobson, submitted for publication).

RNA blotting. Equal amounts of RNA from each time point of experiments in which transcription was inhibited were analyzed by Northern blotting or RNA dot blotting (40, 58, 94, 102). DNA probes were labeled to high specific activity as described previously (24), and blots were quantitated by autoradiography (−80°C, with an intensifying screen) and densitometry or by direct count of the beta decays present in each band by the use of a Betascope Blot Analyzer (Betagen, Waltham, Mass.). The latter procedure is especially useful for mRNAs whose decay rates are so rapid ($t_{1/2}$ < 5 min) that the initial time points in a decay curve exceed the limited linear response range of X-ray film to beta emissions. For these rapidly decaying mRNAs, direct counting of blots with the Betagen Blot Analyzer gives decay rates which appear to be up to twofold faster than those obtained by autoradiography and densitometry but which are significantly more accurate.

DNA dot blots. Plasmid DNA was denatured by incubation in 0.4 N NaOH for 15 min at 37°C, followed by quick cooling. Samples (5 and 10 μg) of each DNA were dot blotted in duplicate to a Zeta-Probe membrane in 450 μl of ice-cold 0.4 N NaOH. After application, DNA dots were rinsed with 500 μl of ice-cold 0.4 N NaOH, air dried, and subsequently dried in vacuo for 2 h at 80°C. Filters were washed in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)—0.5% SDS for 1 h at 65°C and immediately prehybridized at 45°C for a minimum of 10 min in PHB (50% formamide [deionized with Bio-Rad AG 501-X8 resin], 4× SSPE [1× SSPE is 0.15 M NaCl–0.01 M $NaH_2PO_4 \cdot H_2O$–0.001 M EDTA, adjusted to pH 7.4], 1% SDS). ^{32}P-labeled poly(A)$^+$ RNA was heated to 65°C for 5 min, and an equal mass (1 to 3 μg) was added to each hybridization reaction. Hybridizations were done in PHB for 24 to 48 h at 45°C. After hybridization, the filters were washed with 2× SSC–0.1% SDS (15 min at room temperature), 0.1× SSC–0.1% SDS (15 min at room temperature, twice), and 0.1× SSC–0.1% SDS (15 min at 55°C). The filters were exposed to Kodak X-Omat AR film at −80°C. For relatively weak hybridization signals requiring long exposures, a Cronex Hi-Plus intensifying screen was used. Care was taken to ensure that the use of intensifying screens did not exaggerate the signal from highly radioactive dots beyond the linear range of the film relative to the weaker signals.

Manipulation of DNA. Enzymes were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.), New England BioLabs, Inc. (Beverly, Mass.), or Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) and were used according to the instructions of the manufacturers. Restriction analysis, nick translation, DNA ligations, and electrophoresis of DNA were performed by standard methods (57), except that the DNA ligase buffer used was 20 mM Tris (pH 7.5)–10 mM $MgCl_2$–10 mM dithiothreitol–1 mM ATP–15 mM NaCl. Transformation of *Escherichia coli* JM83 was done essentially as described previously (79). The alkaline SDS lysis technique (6) was used for all plasmid DNA preparations. DNA fragments were purified from agarose gels with the Geneclean kit (Bio 101), which uses NaI to solubilize agarose and a silica matrix to bind DNA (105). Labeling of DNA in low-melting-temperature agarose with random oligonucleotides as primers was done as described previously (24). Table 1 is a list of the cloned DNAs which were used in this study. With the exception of pUC8-Sc2676, each DNA fragment used as a hybridization probe encodes a single species of mRNA. pUC8-Sc2676 encodes three mRNAs (*HIS3*, *DED1*, and *PET56*), all of which are readily separated from each other by Northern blot analysis if the entire cloned fragment is used as a probe (100).

Rare-codon screening. Codon usage is measured as the frequency with which each coding triplet appears per thousand codons. Using codon frequencies compiled from the

DNA sequences of 153 yeast genes (1), we arbitrarily defined a rare codon as one which appears 15 or fewer times per 1,000 codons. Rare-codon frequencies were calculated for 15 mRNAs whose half-lives were measured with the *rpb1-1* mutant.

RESULTS

Our initial objective was to establish a routine method for the reliable measurement of yeast mRNA half-lives. mRNA decay was analyzed after thermal inactivation of RNA polymerase II in cells harboring the *rpb1-1* ts allele (70) and compared with results obtained when transcription was inhibited by treatment of cells with the antifungal agent Thiolutin (39, 103) or with results of a procedure in which mRNA decay was measured as a function of its kinetics of labeling with $^{32}PO_4$. To compare the results of different procedures, we measured half-lives for both the total poly(A)$^+$ RNA population and selected individual mRNAs. The latter were measured either by Northern blot or dot-blot assays with RNAs isolated at different times after inhibition of transcription or by hybridizing $^{32}PO_4$-labeled mRNA (isolated at different time points) to dots of excess cloned DNA.

mRNA decay after thermal inactivation of RNA polymerase II. The decay of the poly(A)$^+$ RNA population after thermal inactivation of RNA polymerase II is depicted in Fig. 1A. In this experiment, cells (Y260) were labeled with $^{32}PO_4$ (20 μC/ml) for 3.5 h at 24°C and the relative amount of poly(A)$^+$ RNA in each RNA sample isolated after the shift to 36°C was determined by hybridization to immobilized poly(U). The decay kinetics for the poly(A)$^+$ RNA population were complex, with the fastest- and slowest-decaying components having half-lives of 5 to 6 and 42 to 43 min, respectively (Table 2). Extrapolation of the long-lived component suggested that stable mRNAs compose approximately 30% of newly synthesized mRNA. However, since the length of labeling (3.5 h) was significantly in excess of the half-life of the rapidly decaying component, it is likely that the relative percentage of stable mRNAs is overrepresented in Fig. 1A.

The relative amounts of individual mRNAs remaining at different times after a temperature shift were determined by RNA blotting assays. Figures 1B and D are examples of dot-blotting and Northern blotting experiments, respectively, in which samples of total cellular RNA extracted from cells at 36°C for 0 to 100 min were hybridized with ^{32}P-labeled DNA probes complementary to specific mRNAs. Data from such experiments were quantitated either directly (with a Betagen Blot Analyzer) or by autoradiography and scanning densitometry. Decay rates were determined from semilog plots of the percentage of hybridizing material remaining at different times after the inhibition of transcription. Figures 1C and E illustrate the quantitation of the blots in Fig. 1B and D and indicate that in these experiments, the mRNAs encoded by *ACT1*, cDNA 74, *RP29*, *TCM1*, and *STE3* decayed with first-order kinetics and had half-lives of 31, 18, 11, 11, and 3.5 min, respectively. Most (>90%) of the mRNA encoded by the *STE2* gene also decayed with first-order kinetics and had a half-life of 4.0 min, but a small fraction (approximately 5%) decayed more slowly (Fig. 1C). This biphasic decay was reproducible (e.g., see Fig. 2C), but its basis is unclear. It may reflect heterogeneity within the cell population (e.g., a small fraction of dying cells) or a fraction of *STE2* mRNA resistant to decay.

These procedures were applied to a total of 20 different mRNAs which were expected to identify the stable and unstable extremes of the mRNA population. Highly abundant mRNAs encoding housekeeping functions (e.g., actin and glycolytic enzymes) were expected to be stable (13, 27, 34, 69). In contrast, since mating-type switching requires the rapid transition to a new set of mating-type-specific functions, mRNAs encoding these functions were expected to be unstable. These predictions are largely borne out by the data of Table 3, which indicate that the half-lives of these mRNAs range from 2.5 to 45 min. Within this range, we arbitrarily defined three stability phenotypes: unstable (mRNAs with a $t_{1/2}$ of <7 min; e.g., *STE2*, *STE3*, *HIS3*, *MFα1*, and *FUS1*), moderately stable (mRNAs with a $t_{1/2}$ of 10 to 20 min; e.g., *TCM1*, *PAB*, and *RP29*), and stable (mRNAs with a $t_{1/2}$ of >25 min; e.g., *ACT1*, *PGK1*, and *CYH2*).

Northern blotting, which has the advantages of indicating the quality of the RNA sample and of resolving more than one mRNA per lane, was used to determine the majority of the decay rates indicated in Table 3. For a given mRNA, decay rates obtained by either Northern or dot blotting were consistent and, in independent experiments, varied by only ±15% (data not shown).

Comparison of mRNA decay rates measured in *rpb1-1* cells with decay rates measured by other procedures. (i) mRNA decay after inhibition of transcription with Thiolutin. The antifungal agent Thiolutin was previously shown to inhibit all three yeast RNA polymerases both in vivo and in vitro (39, 103). When used at 3 μg/ml, transcription is effectively inhibited, polysomes decay with a $t_{1/2}$ of 20 min, and cellular growth (measured as optical density at 650 nm) abruptly ceases (39; D. Herrick and A. Jacobson, unpublished observations). Decay curves for total poly(A)$^+$ RNA in cells exposed to 0, 3, or 6 μg of Thiolutin per ml at 30°C are shown in Fig. 2A. In this experiment, cells were labeled with [^{3}H]uridine for 1 h before Thiolutin treatment and the relative amounts of labeled poly(A)$^+$ RNA were determined by hybridization of total cellular RNA to poly(U) filters. The decay curves for poly(A)$^+$ RNA in cells treated with either 3 or 6 μg of Thiolutin per ml were essentially identical, and as in experiments with temperature-shifted *rpb1-1* cells, the decay kinetics were complex. In Thiolutin-treated cells, the most rapidly decaying component of poly(A)$^+$ RNA had a half-life of 4 to 5 min and the slowest-decaying component had a half-life of 60 to 61 min (Fig. 2A; Table 2). The latter decay rate was slightly slower than the comparable decay component in *rpb1-1* cells (Fig. 1A) and was reflected in the decay rates of individual mRNAs (see below).

The relative amounts of individual mRNAs remaining at different times after treating cells with Thiolutin at 30 or 36°C were determined by Northern blotting, and mRNA decay rates were calculated as described above (Fig. 2B and C; Table 3). These data indicate that (i) the relative decay rates of the majority of the mRNAs studied in Thiolutin-treated cells and in temperature-shifted *rpb1-1* cells were consistent (e.g., in Thiolutin-treated cells, the mRNAs encoded by *STE2* and *STE3* decayed rapidly, the mRNAs encoded by *ACT1*, *CYH2*, and *PGK1* decayed very slowly, and the mRNAs encoded by *TCM1* and *RP29* had intermediate decay rates); and (ii) mRNAs in Thiolutin-treated cells decay faster at 36°C than at 30°C and these faster rates more closely approximate the values obtained in *rpb1-1* cells.

(ii) Approach to steady-state labeling with $^{32}PO_4$. Cells grown in phosphate-depleted YEPD medium were labeled with $^{32}PO_4$, and total and poly(A)$^+$ RNAs were purified from culture samples after 5 to 70 min of labeling. RNA decay curves were generated by a semilog plot of $1 - A/A^\infty$ versus the time of $^{32}PO_4$ labeling (where A is the specific activity of

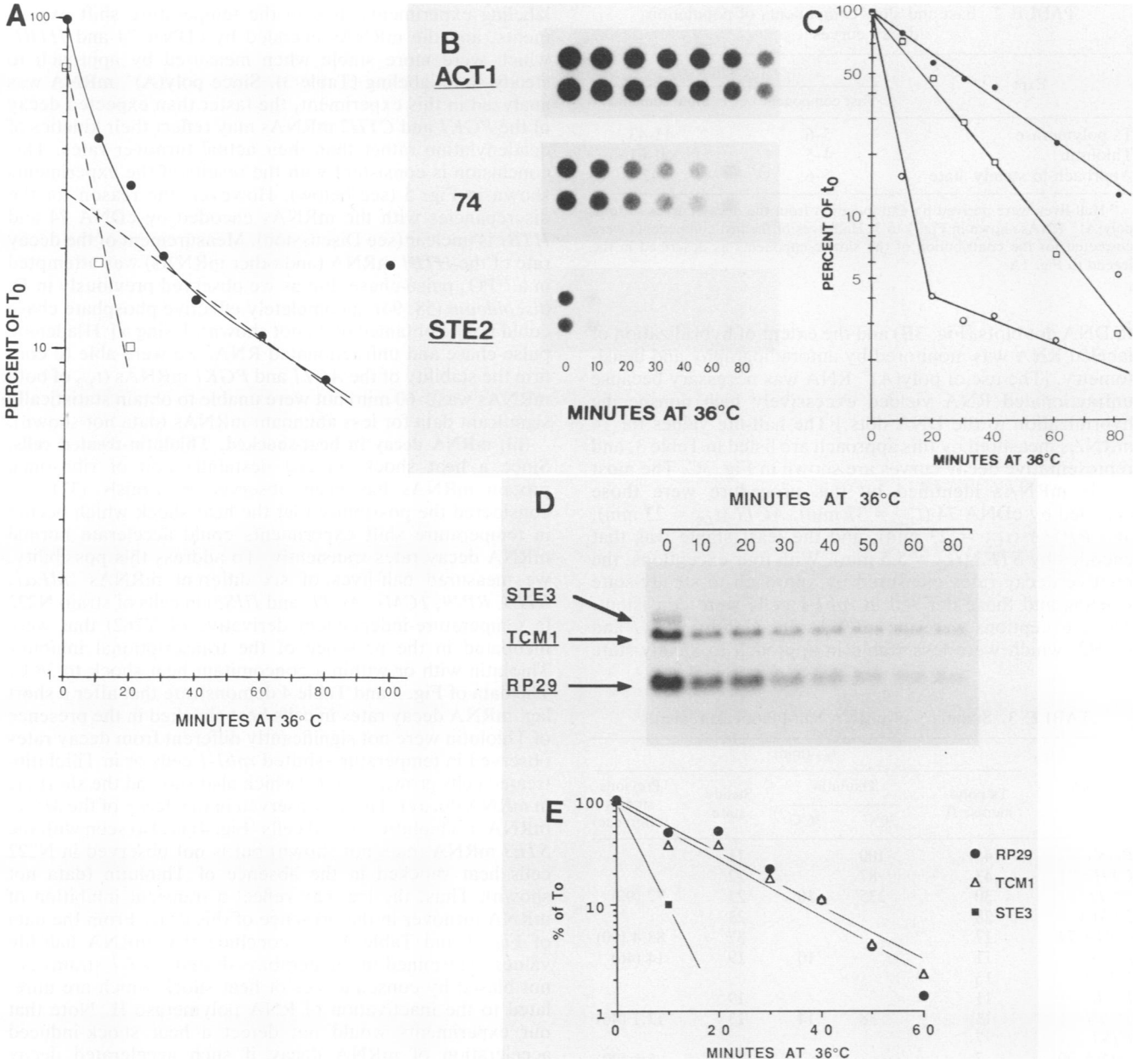


FIG. 1. mRNA decay after the inhibition of transcription in a ts RNA polymerase II mutant. (A) Decay of the poly(A)$^+$ RNA population. Temperature shifts (with Y260 cells) and RNA quantitation were as described in Materials and Methods. Data are plotted (●) as the percentage of poly(A)$^+$ RNA remaining compared with $t = 0$ versus minutes at 36°C. Dotted lines indicate the major kinetic components. The slow component is a simple extrapolation from the latter half (40 to 80 min) of the decay curve. To derive the rapid decay component (□----□), we subtracted the contribution of the extrapolated slow decay component at each time point from the measured value at that time. (B) Decay of individual mRNAs. RNA (3 μg) from each time point was denatured, applied in duplicate dots to Zeta-Probe filters, and probed with 5×10^6 cpm of ^{32}P-labeled insert DNA from pDH7 (*ACT1*), pAB515 (*STE2*), or cDNA clone 74 (gene unknown). The figure shows autoradiographs of the RNA dots (different exposures were used for *ACT1*, *STE2* and cDNA 74). (C) Decay rates of individual mRNAs. Autoradiographs of the dot blots shown in panel B were quantitated by densitometry, and the data were plotted as the percentage of each RNA remaining versus time at 36°C. Symbols: ●, *ACT1*; □, cDNA 74; and ○, *STE2*. (D) Northern analysis of mRNA decay. As in panel B except that individual mRNAs were assayed by Northern blotting. (E) Decay rates of individual mRNAs assayed by Northern blotting. Autoradiographs of Northern blots (D) were quantitated and blotted as in panel C. Symbols: ●, *RP29*; △, *TCM1*; ■, *STE3*.

an RNA at time t and A^∞ is the specific activity at time ∞ [29, 46]). The decay kinetics for the poly(A)$^+$ RNA population were complex; extrapolations of the decay curve indicated that the fastest-decaying component had a $t_{1/2}$ of 5 to 6 min and the slowest-decaying component had a $t_{1/2}$ of 42 min (Fig. 3A; Table 2).

To measure half-lives of individual mRNAs, 1 μg of ^{32}P-labeled poly(A)$^+$ RNA from each time point was hybridized

TABLE 2. Fast and slow components of population decay curves[a]

Expt	$t_{1/2}$ (min)	
	Fast component	Slow component
Ts polymerase	5–6	42–43
Thiolutin	4–5	60–61
Approach to steady state	5–6	42

[a] Half-lives were derived by extrapolation from the decay curves of total poly(A)$^+$ RNAs shown in Fig. 1 to 3. Half-lives of the fast components were corrected for the contribution of the slow components as described in the legend to Fig. 1A.

to DNA dot blots (Fig. 3B) and the extent of hybridization of labeled RNA was monitored by autoradiography and densitometry. [The use of poly(A)$^+$ RNA was necessary because unfractionated RNA yielded excessively high nonspecific hybridization to the DNA dots.] The half-life values for 14 mRNAs measured by this approach are listed in Table 3, and representative decay curves are shown in Fig. 3C. The most stable mRNAs identified by this procedure were those encoded by cDNA 74 ($t_{1/2}$ = 37 min), *ACT1* ($t_{1/2}$ = 23 min), and *RP51A* ($t_{1/2}$ = 23 min), and the least stable was that encoded by *STE2* ($t_{1/2}$ = 5.5 min). With four exceptions, the relative decay rates measured by approach to steady-state labeling and those derived in *rpb1-1* cells were consistent. These exceptions were the mRNAs encoded by *PGK1* and *CYH2*, which were less stable in approach to steady-state labeling experiments than in the temperature shift experiments, and the mRNAs encoded by cDNA 74 and *HTB1*, which were more stable when measured by approach to steady-state labeling (Table 3). Since poly(A)$^+$ mRNA was analyzed in this experiment, the faster than expected decay of the *PGK1* and *CYH2* mRNAs may reflect their kinetics of deadenylation rather than their actual turnover rates. This conclusion is consistent with the results of the experiments shown in Fig. 5 (see below). However, the reason for the discrepancies with the mRNAs encoded by cDNA 74 and *HTB1* is unclear (see Discussion). Measurement of the decay rate of the *HTB1* mRNA (and other mRNAs) was attempted in a $^{32}PO_4$ pulse-chase, but as we observed previously in *D. discoideum* (58, 94), a completely effective phosphate chase could not be obtained (data not shown). Using a [^{3}H]adenine pulse-chase and unfractionated RNA, we were able to confirm the stability of the *ACT1* and *PGK1* mRNAs ($t_{1/2}$ of both mRNAs was >60 min) but were unable to obtain statistically significant data for less abundant mRNAs (data not shown).

TABLE 3. Summary of mRNA half-life measurements[a]

mRNA	$t_{1/2}$ (min)				
	Ts polymerase II	Thiolutin		Steady state	Previous studies (reference)
		30°C	36°C		
PGK1	45	>100		11	
CYH2	43	87		12	
ACT1	30	135	30	23	77 (90)
RP51A	28			23	
cDNA 74	17			37	83.4 (90)
RP29	11		10	19	14 (46)
MFA1	13				
PAB1	11			19	
TCM1	11	38	14	13	13.1 (46)
HIS3	7				
cDNA 90	7			13	6.6 (90)
HTB1	<5			14	15 (74)
STE2	4.0	7		5.5	
DED1	4.0				
MATα1	5.0				
URA3	3.0				7.3 (46) 10.5 (3)
MFα1	5.0		5		
FUS1	<3				
STE3	3.5		5		
MFA2	2.5				
HTB2				15	
PHO5				10	
cDNA 39				19	18.3 (90)

[a] As described in Materials and Methods, mRNA half-lives were measured with total RNA in the ts polymerase II and Thiolutin experiments and with poly(A)$^+$ RNA in the approach to steady-state experiments. In multiple determinations values presented are accurate to ±15%. The experiments cited in reference 3 and 46 utilized approach to steady-state labeling to measure mRNA decay rates; in the experiments described in reference 72 and 90, mRNA half-lives were measured, respectively, by pulse-chase analysis or treatment of cells with 1,10-phenanthroline.

(iii) mRNA decay in heat-shocked, Thiolutin-treated cells. Since a heat shock-induced destabilization of ribosomal protein mRNAs has been observed previously (32), we considered the possibility that the heat shock which occurs in temperature shift experiments could accelerate normal mRNA decay rates transiently. To address this possibility, we measured half-lives of six different mRNAs (*MFα1*, *STE3*, *RP29*, *TCM1*, *ACT1*, and *HIS3*) in cells of strain N222 (a temperature-independent derivative of Y262) that were incubated in the presence of the transcriptional inhibitor Thiolutin with or within a concomitant heat shock to 36°C. The data of Fig. 4 and Table 4 demonstrate that after a short lag, mRNA decay rates in cells heat shocked in the presence of Thiolutin were not significantly different from decay rates observed in temperature-shifted *rpb1-1* cells or in Thiolutin-treated cells grown at 36°C (which also showed the short lag in mRNA decay). The lag observed in the decay of the *MFα1* mRNA in Thiolutin-treated cells (Fig. 4) is also seen with the *STE3* mRNA (data not shown) but is not observed in N222 cells heat shocked in the absence of Thiolutin (data not shown). Thus, the lag may reflect a transient inhibition of mRNA turnover in the presence of this drug. From the data of Fig. 4 and Table 4, we conclude that mRNA half-life values determined in temperature-shifted *rpb1-1* strains are not biased by consequences of heat shock which are unrelated to the inactivation of RNA polymerase II. Note that our experiments would not detect a heat shock-induced acceleration of mRNA decay if such accelerated decay depended on new mRNA synthesis.

Comparisons of stable and unstable mRNAs. Having identified mRNAs which differ significantly in their respective decay rates, we compared these mRNAs with respect to properties which might, in part, play a role in determining stability or instability. Unless otherwise specified, mRNA decay rates considered in the experiments which follow are those determined in temperature-shifted *rpb1-1* cells.

(i) Decay of poly(A)$^+$ and poly(A)$^-$ mRNAs. Since the experiments of Fig. 1 to 3 indicated differences in the decay rates of some mRNAs with total or poly(A)$^+$ RNA and since experiments in mammalian cells have suggested that the degradation of some mRNAs is initiated by deadenylation (8, 107), we examined the decay rates of poly(A)$^+$ and poly(A)$^-$ *CYH2*, *PGK1*, *STE2*, and *HIS3* mRNAs. Samples (100 μg) of total RNA isolated from Y260 cells after a shift to 36°C were fractionated on oligo(dT)-cellulose, and the bound [poly(A)$^+$] and unbound [poly(A)$^-$] fractions were analyzed by Northern blotting. The degree of stringency for the

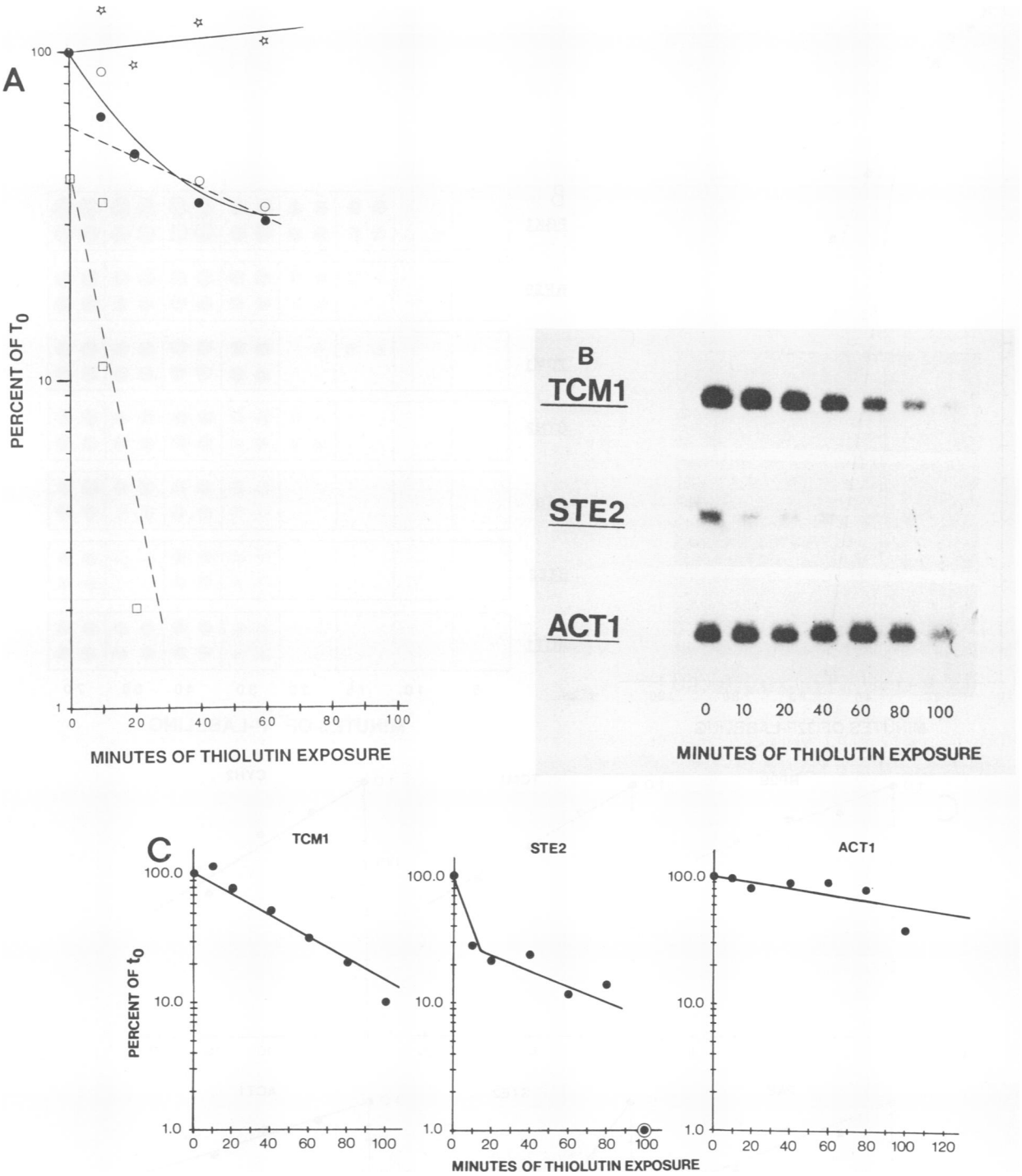
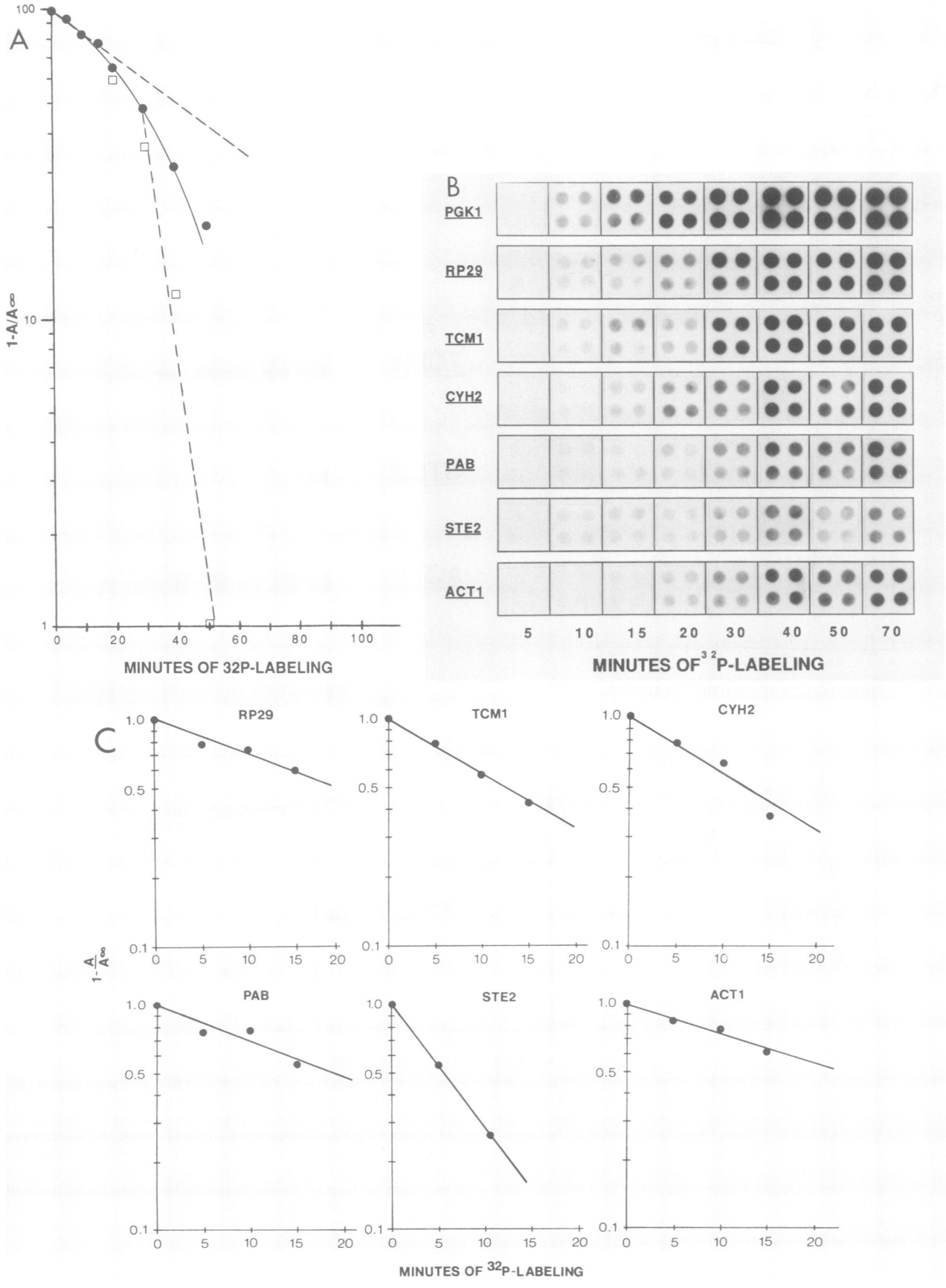


FIG. 2. mRNA decay in Thiolutin-treated cells. (A) Decay of the poly(A)$^+$ RNA population. Cells (strain DBY747) were grown (at 30°C) for 1 h in the presence of [^{3}H]uridine (20 μCi/ml) and then treated with Thiolutin (0, 3, or 6 μg/ml). RNA was extracted after 0, 10, 20, 30, 40, and 60 min of further incubation. ^{3}H counts per minute in poly(A)$^+$ RNA were measured by hybridizing an equal mass of total RNA from each time point to poly(U) filters. Data are plotted as the percentage of radioactivity remaining versus minutes of exposure to Thiolutin. Symbols: ☆, no Thiolutin; ●, plus Thiolutin (3 μg/ml); ○, plus Thiolutin (6 μg/ml). Dotted lines indicate the major kinetic components (the rapid decay component [□] has been corrected for the contribution of the slow decay component as in Fig. 1A). (B) Decay of individual mRNAs. RNA was extracted from cells (DBY747) 0 to 100 min after exposure to 3 μg of Thiolutin per ml and quantitated by Northern blotting. Autoradiographs for the Northern blots probed with *TCM1*, *STE2*, and *ACT1* are shown. (C) Decay rates of mRNAs in Thiolutin-treated cells. Autoradiographs of the Northern blots shown in panel B were quantitated by densitometry, and the data are plotted as the percentage of each RNA remaining versus time of incubation in the presence of Thiolutin.


A
100
10
1
1-A/A∞
0
20
40
60
80
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MINUTES OF 32P-LABELING
B
PGK1
RP29
TCM1
CYH2
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ACT1
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MINUTES OF 32P-LABELING
C
RP29
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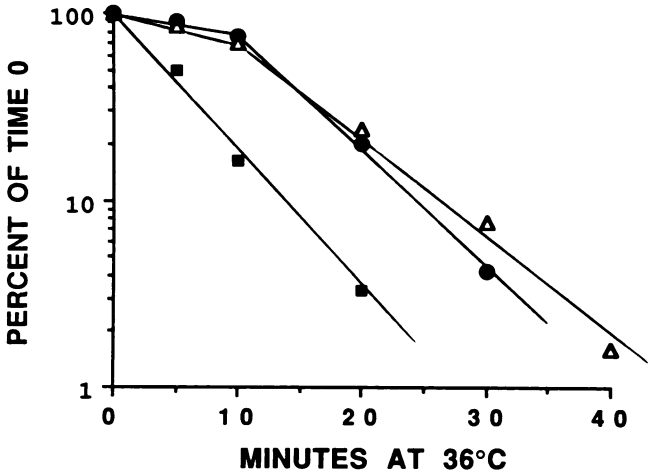


FIG. 4. Decay of *MFα1* mRNA with or without heat shock. The figure shows the decay of the *MFα1* mRNA under three conditions: inhibition of transcription in the ts RNA polymerase II mutant (■), inhibition of transcription with Thiolutin in the (*RPB1*) wild-type strain, N222, at 36°C (●), and inhibition of transcription by Thiolutin accompanied by a heat shock from 24 to 36°C in N222 (△) (see Materials and Methods). The initial lag seen in the presence of Thiolutin is reproducible and is seen with both the *MFα1* and *STE3* mRNAs.

oligo(dT) selection procedure used in these experiments resulted in no visibly detectable rRNA species (after ethidium bromide staining) on agarose-formaldeyde gels containing poly(A)$^+$ RNA isolated from the equivalent of 10 μg of total RNA (data not shown). For the purposes of discussion, the unbound fraction is referred to as poly(A)$^-$ mRNA. However, since the unbound fraction includes RNAs with short poly(A) tails (<15 adenylate residues [30]) which would be capable of binding to the poly(U) filters used in the experiments of Fig. 1A and 2A (74, 85), the absolute amount of poly(A)$^-$ mRNAs is overestimated by this assay. Figures 5A and B show that after a 10 to 15-min lag, poly(A)$^+$ *CYH2* mRNA decayed with a half-life of 10 min. Poly(A)$^-$ *CYH2* mRNA increased in relative abundance for the first 20 min after a shift to 36°C (reaching a maximum value of 143% compared with t_0) and then decayed with a half-life slightly longer than that of unfractionated *CYH2* mRNA ($t_{1/2} > 60$ min [Fig. 5B and C]). Similar changes in the relative abundance of the poly(A)$^+$ and poly(A)$^-$ fractions were observed for the stable *PGK1* mRNA, although the poly(A)$^+$ form of this mRNA showed no appreciable lag in its decay [poly(A)$^+$ $t_{1/2}$ = 7 min; poly(A)$^-$ $t_{1/2} > 60$ min (Fig. 5A, C, and D)]. Since at t_0 approximately 40% of the *PGK1* and *CYH2* mRNAs are poly(A)$^+$ (see the legend to Fig. 5) and since the decay curves for these two mRNAs measured with unfractionated RNA are linear and not biphasic (R. Parker and A. Jacobson, Proc. Natl. Acad. Sci. USA, in press; D. Herrick and R. Parker, unpublished data), the results shown in Fig. 5A to D indicate that the poly(A)$^+$ *PGK1* and *CYH2* mRNAs must be quantitatively chased into the poly(A)$^-$ fraction, i.e., the poly(A) tails of these mRNAs are rapidly shortened to a poly(A)$^-$ or poly(A)-deficient state at a rate three- to fourfold faster than the overall mRNA decay rates measured by analyzing total RNA. These results are consistent with a generalized shortening of mRNA poly(A) tracts of steady-state length (30 to 50 adenylate residues [78]) to a size below the minimum required for oligo(dT) binding (30). The half-life of poly(A) shortening in these cells must be approximately 7 to 10 min since the stable *CYH2* and *PGK1* mRNAs ultimately disappear from the poly(A)$^+$ fraction at that rate. The lag in the disappearance of poly(A)$^+$ *CYH2* mRNA suggests that all stable mRNAs do not share a uniform steady-state poly(A) tail length, a conclusion consistent with previous experiments (94). On the basis of these observations, we conclude that shortening or removal of the poly(A) tail from the *CYH2* and *PGK1* mRNAs is not the rate-limiting step in their decay. These experiments do not preclude the possibility that the loss of the poly(A) tail is a prerequisite for subsequent steps in the decay of these mRNAs.

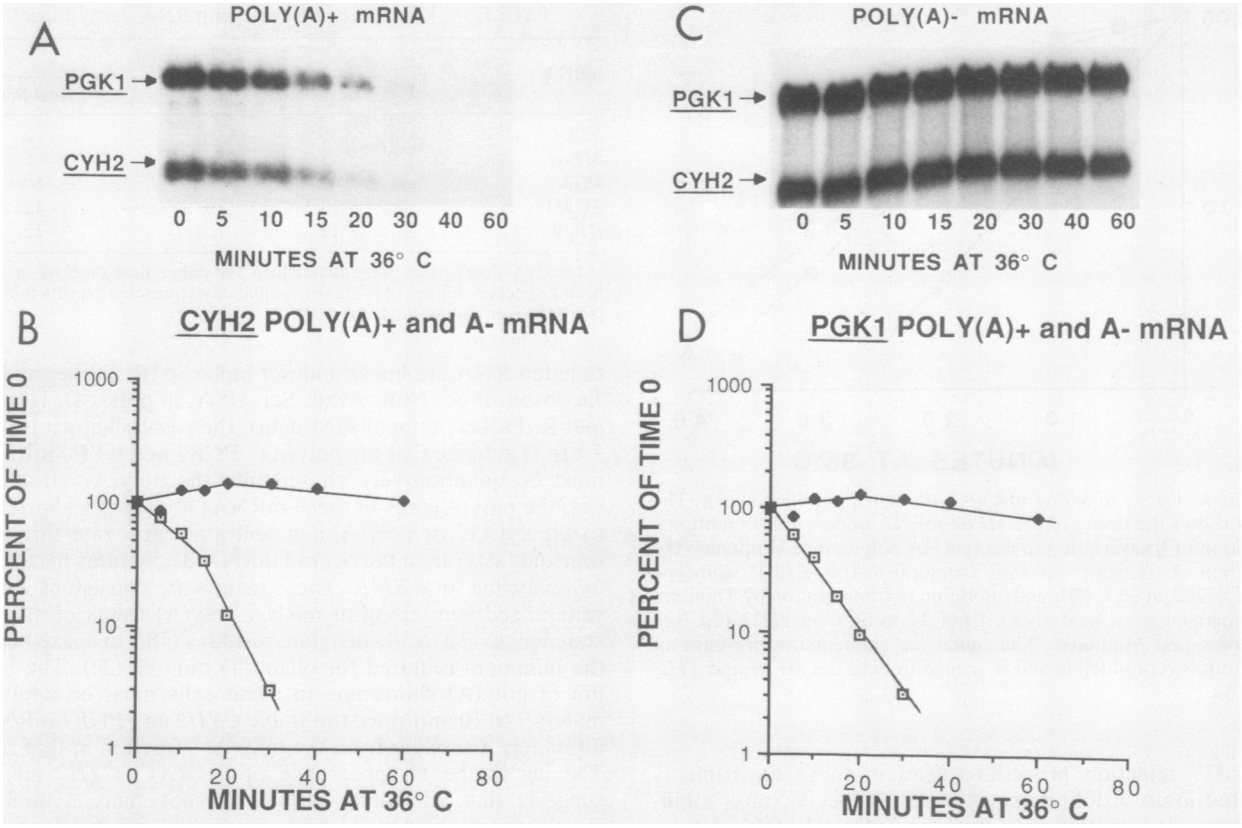
TABLE 4. Effect of heat shock on mRNA decay rates[a]

mRNA	$t_{1/2}$ (min)	
	+Heat Shock	−Heat Shock
STE3	5	3
MFα1	5	5
PGK1	60	>60
TCM1	13.5	12
RP29	10	12.5

[a] mRNA decay rates were determined for either heat-shocked or non-heated-shocked cultures of N222 after inhibition of transcription with thiolutin (see Materials and Methods for details).

In contrast to the results with stable mRNAs, the unstable *STE2* mRNA disappeared from both the poly(A)$^+$ and poly(A)$^-$ fractions rapidly (Fig. 5E). As was observed for total *STE2* mRNA (Fig. 1B and C), poly(A)$^+$ *STE2* mRNA decayed with a half-life of 3 to 5 min (Fig. 5E and F). Similar rapid decay kinetics were observed for the poly(A)$^+$ form of the unstable mRNA encoded by *HIS3* (data not shown). Poly(A)$^-$ *STE2* mRNA showed a lag in its decay (approximately 10 min) and then also disappeared with a half-life of 3 to 5 min (Fig. 5F). It is not clear from these results whether the loss of the poly(A) tract from unstable mRNAs is the rate-limiting step in their decay. However, several observations suggest that it is not. (i) The relative amounts of poly(A)$^+$ (55%) and poly(A)$^-$ (45%) *STE2* mRNA (see the legend to Fig. 5) at t_0 are consistent with those expected if

FIG. 3. Measurement of mRNA decay by approach to steady-state labeling. (A) Decay of poly(A)$^+$ RNA. $^{32}PO_4$ was added (100 μCi/ml) to cells (DBY747) growing in phosphate-depleted YEPD medium (87), and RNA was isolated after 5 to 70 min of labeling. (After a 5-min lag, the increase in RNA specific activity versus time was linear, indicating that $^{32}PO_4$ pools were of constant specific activity.) Poly(A)$^+$ RNA was purified by the oligo(dT) batch procedure, and specific activities were determined for each sample. The decay of poly(A)$^+$ RNA (●) was monitored by plotting $1 - A/A^\infty$ versus the length of labeling (A is the specific activity of a given RNA at time t; A^∞ was the specific activity after 70 min of labeling). Dotted lines indicate the major kinetic components (as in Fig. 1A and 2A, the rapid decay component [□] has been corrected for the contribution of the slow decay component). (B) Decay of individual mRNAs. An equal mass (1 μg) of poly(A)$^+$ RNA from each time point (1 μg) was hybridized to identical filters containing dots of excess plasmid DNA which encode single mRNAs. The figure shows the autoradiographs from individual dots for the *PGK1*, *RP29*, *TCM1*, *CYH2*, *PAB*, *STE2*, and *ACT1* genes. (C) Decay rates for individual mRNAs. Autoradiographs of the dots shown in Fig. 3B were quantitated by densitometry, and decay curves were generated as described for panel A except that A^∞ was the value from the 30-min time point.



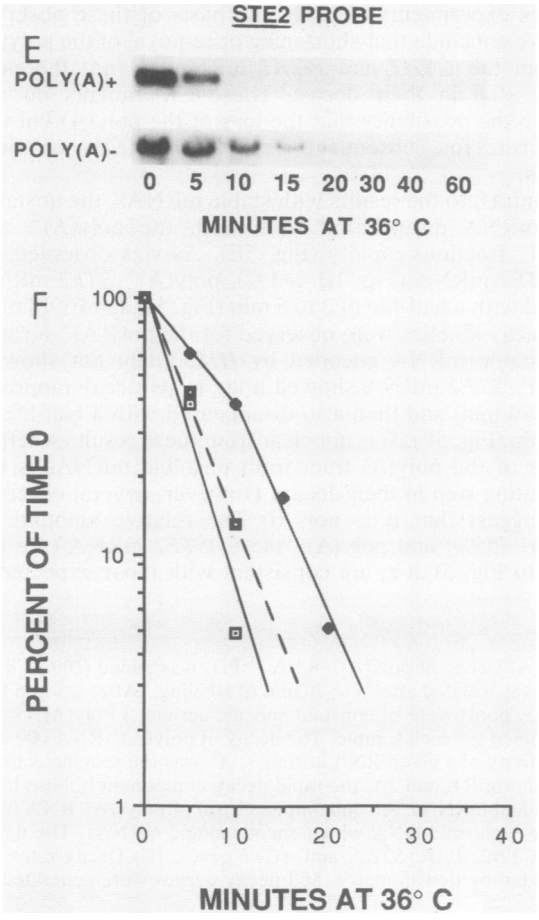


FIG. 5. Decay of poly(A)$^+$ versus poly(A)$^-$ mRNA. (A to D) Decay of *CYH2* and *PGK1* mRNAs. Samples (100 μg) of RNA isolated from Y260 cells 0 to 100 min after a shift from 24 to 36°C were fractionated on oligo(dT)-cellulose, and 10% of the total poly(A)$^+$ and poly(A)$^-$ RNA recovered from each sample was analyzed for the *CYH2* and *PGK1* mRNAs by Northern blotting. Autoradiographs of the Northern blots are shown in panels A and C. Quantitation of the decay rates for *CYH2* and *PGK1* poly(A)$^+$ (⊡) and poly(A)$^-$ (◆) mRNAs is shown in panels B and D. At t_0, 42 and 43.2%, respectively, of the *CYH2* and *PGK1* mRNAs are poly(A)$^+$. (E and F) Decay of the *STE2* mRNA. As in panels A to D except that 20% of the total poly(A)$^+$ and poly(A)$^-$ RNA was analyzed for the decay of the *STE2* mRNA. To determine the decay rate of poly(A)$^-$ *STE2* mRNA independent of the contribution of poly(A)$^+$ *STE2* mRNA chased into this fraction, we plotted the data for poly(A)$^-$ RNAs using either the t_0 or the t_{10} sample as the initial time point. Symbols: ⊡, *STE2* POLY(A)$^+$ RNA; ◆, *STE2* poly(A)$^-$ RNA; and (■), *STE2* poly(A)$^+$ RNA with the 10-min time point as t_0. At t_0, 55.3% of the *STE2* RNA is poly(A)$^+$.

the poly(A) tail of *STE2* was lost with the same 7- to 10-min half-life observed for poly(A) tail loss from the stable *CYH2* and *PGK1* mRNAs, i.e., the disappearance of *STE2* mRNA from the poly(A)$^+$ fraction would be more rapid than the inferred rate of poly(A) shortening; (ii) there is a transient accumulation of *STE2* mRNA in the poly(A)$^-$ fraction (i.e., there is a lag in its rate of disappearance); and (iii) poly(A)$^+$ and poly(A)$^-$ *STE2* mRNAs decay at the same rate.

(ii) mRNA decay rate versus mRNA size. Previous experiments in yeast suggested that an inverse correlation existed between the size of an mRNA and its stability (90). In these experiments, mRNA decay rates were measured in cells treated with 1,10-phenanthroline, a drug which affects a number of cellular pathways (17, 41, 48) and may exert its effects by chelating cellular zinc. If the nucleases involved in mRNA degradation include those which are zinc metalloen-

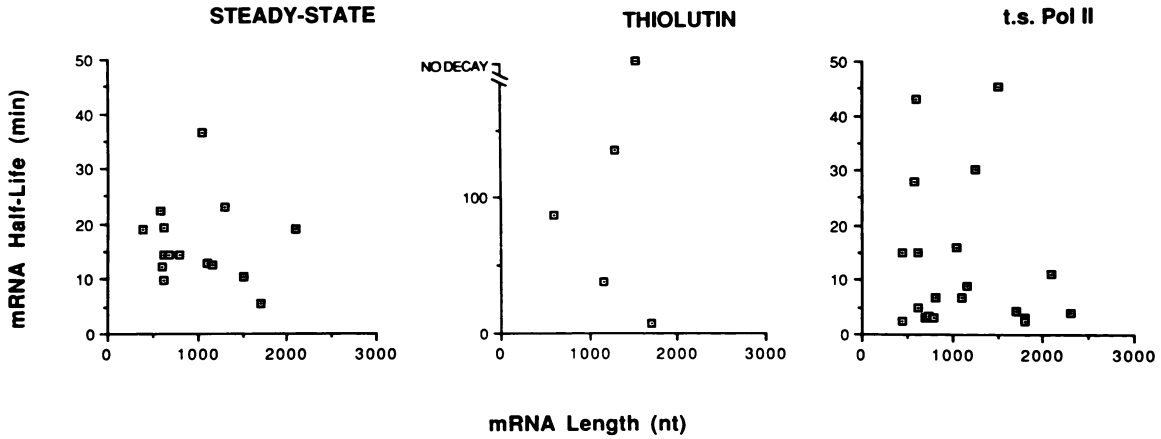


FIG. 6. mRNA size versus decay rate. mRNA sizes determined by Northern blotting are compared with mRNA decay rates determined by each of three different procedures. Simple regression analysis failed to indicate statistically significant R values ($P = 0.01$) for any of the data sets (steady-state $R = 0.10$; Thiolutin $R = 0.44$; and ts polymerase II $R = 0.14$). Sizes (in nucleotides [nt]) of the mRNAs shown in this figure are *URA3* (700), *RP29* (620), *RP51A* (580), *HIS3* (830), *HTB1* (610), cDNA 74 (1,050), *FUS1* (1,800), cDNA 90 (1,100), *TCM1* (1,170), *MATα1* (740), *ACT1* (1,250), *MFα1* (800), *PGK1* (1,500), *MFA1* (450), *STE2* (1,700), *STE3* (1,800), *PAB* (2,100), *MFA2* (450), *DED1* (2,300), *CYH2* (600), cDNA 39 (380), *PHO5* (620), and *HTB2* (800 and 680). t.s. Pol II, ts polymerase II.

zymes, they would presumably be inhibited by this drug. Experiments with other organisms and other experimental approaches to measuring mRNA decay rates failed to demonstrate a correlation between mRNA decay rate and mRNA size (28, 94) and led to the suggestion (94) that the previously observed correlation is a consequence of the measurement of mRNA decay rates in phenanthroline-treated cells. To address this discrepancy, we compared mRNA sizes with mRNA decay rates determined by each of the three procedures used here. The data of Fig. 6 indicate that no statistically significant correlation between mRNA size and stability was observed with any of the three procedures.

(iii) mRNA decay rates in the presence of cycloheximide. A large number of experiments in several different systems have demonstrated a reduction in mRNA decay rates in the presence of cycloheximide or other inhibitors of translational elongation (23, 25, 45, 82, 99). The significance of such observations is still unclear. It is unlikely that arrested ribosomes simply protect mRNA from nucleases since, in normally growing cells, the ribosome packing density does not differ for stable and unstable mRNAs (89, 94). The possibility exists that essential nucleases are metabolically unstable or that mRNA degradation depends on ribosomal translocation through or up to specific mRNA regions (9, 28). Since we identified mRNAs with large differences in their respective decay rates, we sought to evaluate whether inhibition of translational elongation had comparable effects on degradation pathways which were potentially different. The experiments summarized in Table 5 indicated that the decay rates of stable, moderately stable, and unstable mRNAs are all reduced significantly in the presence of cycloheximide, a result which implies that the cycloheximide-sensitive step is shared by the degradative pathways of all three classes of mRNA.

(iv) Correlation between percentage of rare codons and mRNA decay rates. Bennetzen and Hall (5) observed that highly expressed genes in yeasts are biased toward the use of only 25 of the 61 coding triplets. Similar observations have been made by Sharp et al. (95). Such restricted codon usage correlates with the levels of the corresponding isoaccepting tRNAs in both yeasts and *E. coli*, although the extent of codon bias is much higher in yeasts (37, 38). Hoekema et al. (33) replaced 164 high-frequency codons of the yeast *PGK1* gene with rare codons and observed a concomitant 10-fold reduction in PGK protein synthesis and a 3-fold reduction in PGK mRNA levels. Assuming comparable transcription rates of the two types of PGK mRNA, this result would suggest a relationship between the percentage of rare codons present in a given mRNA and its rate of decay. We determined codon usage for 15 of the mRNAs studied here and compared the relative content of rare codons (frequency, <15/1,000) with mRNA decay rates. Interestingly, we found that most unstable mRNAs contain a significantly higher percentage of rare codons than stable mRNAs (Fig. 7) (see Discussion).

DISCUSSION

Simple method for measuring mRNA decay rates in yeasts. mRNA decay rates have been measured in yeasts previously by several different functional or chemical assays (3, 18, 19, 35, 36, 46, 47, 50, 52, 54, 72, 90, 104, 109). Previous approaches have measured mRNA decay rates by assaying

TABLE 5. Cycloheximide inhibits mRNA decay[a]

mRNA	$t_{1/2}$ (min)	
	−Cycloheximide	+Cycloheximide
ACT1	35	>80
PGK1	45	>80
RP29	12	>40
TCM1	12	>40
STE3	4	>40
MATα1	4	>80
HIS3	8	>80

[a] The effect of cycloheximide on mRNA decay was determined as follows. A 250-ml culture of Y262 cells was grown to the mid-log phase (optical density of 0.5 at 600 nm) in YEPD at 24°C. At $t = 0$, 125-ml samples were added to 125 ml of 48°C YEPD, one of which contained cycloheximide at 100 μg/ml. RNA was prepared at various times (0 to 60 min), and the decay rates of individual mRNAs were quantitated by Northern blotting and densitometry.

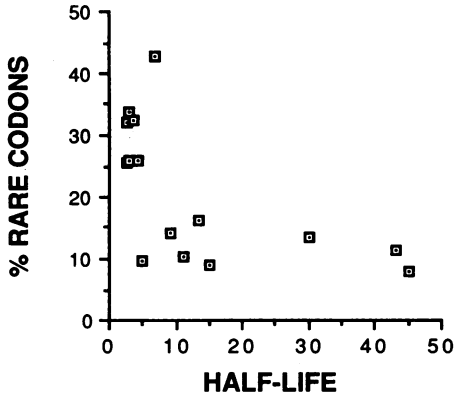


FIG. 7. Relative content of rare codons in stable and unstable mRNAs. Sequences of mRNAs for which half-lives were measured in temperature-shifted *rpb1-1* cells were scanned for the presence of rare codons, i.e., codons whose frequency of occurrence in a large sample of sequenced yeast genes (1) is <15/1,000. Percentages of rare codons were calculated and plotted versus mRNA decay rate. Simple regression analysis ($P = 0.01$) generated a significant R value of 0.60. Percentages of rare codons in specific mRNAs were as follows: *URA3*, 25.7; *RP29*, 9.0; *HIS3*, 42.7; *HTB1*, 9.8; *FUS1*, 33.7; *TCM1*, 14.2; *MATα1*, 32.4; *ACT1*, 13.6; *PGK1*, 7.9; *MFA1*, 16.2; *STE2*, 25.9; *STE3*, 32.1; *PAB*, 10.2; *MFA2*, 25.6; and *CYH2*, 11.3.

changes in mRNA labeling kinetics (3, 36, 46, 54, 72, 109) or by quantitating the disappearance of mRNA after the inhibition of transcription (50, 90, 104) or RNA processing (19, 35, 47). Those experiments which assayed changes in labeling kinetics (pulse-chase and approach to steady-state labeling) employed labeling with [^{3}H]nucleosides to monitor specific mRNAs and, therefore, precluded consideration of mRNAs with relatively low transcription rates. The general experimental protocol in these studies involved the hybridization of labeled RNAs to cloned DNAs immobilized on filters which were then quantitated by scintillation counting; unlike Northern blots, such filters were not readily reused for future experiments. Moreover, most of the experiments which monitored the disappearance of specific mRNAs did so by inhibiting RNA accumulation in a nonspecific manner; inhibition of transcription with daunomycin or ethidium bromide (104), lomofungin (11), or 1,10-phenanthroline (90) is accompanied by inhibition of many other biosynthetic pathways (16, 21). Inhibition of RNA processing in ts*136* inhibits the accumulation of all classes of RNA, not just mRNA (35). In contrast to the shortcomings of previous methods, the temperature shift procedure used here (with *rpb1-1* cells) affords several advantages. (i) RNA polymerase II is selectively and rapidly inactivated; (ii) there is no requirement for in vivo labeling or consideration of changes in pool sizes; (iii) mRNAs of any abundance class can be readily detected and their decay rates measured; and (iv) the use of Northern blots allows the monitoring of mRNA integrity in parallel with the quantitation of mRNA decay rates, the detection of several mRNAs simultaneously, the reusability of blots for future experiments with different probes, and the quantitation of chimeric and parental mRNAs in the same experiment (Parker and Jacobson, in press; R. Parker and A. Jacobson, submitted for publication; D. Herrick and A. Jacobson, manuscript in preparation).

The reliability of decay rate measurements in temperature-shifted *rpb1-1* cells was addressed in several ways. We showed that (in the absence of ongoing transcription) mRNA decay rates are not altered by the consequences of heat shock (Fig. 4; Table 4), that the relative stabilities of most of the mRNAs examined here are consistent regardless of the method used to determine half-lives (Table 3), that the range of half-lives observed for individual mRNAs is consistent with the complex decay kinetics observed for the entire population of cellular mRNAs, and that the decay components of the latter are comparable in all three procedures. Despite the shortcomings of earlier procedures alluded to above, mRNA decay rates measured here were generally in good agreement with rates measured previously by others (Table 3). The reliability of the decay rates measured by the temperature shift procedure is also addressed by a recent study in which we compared the decay rates and steady-state levels of 12 chimeric mRNAs with those of their parental mRNAs transcribed from the same promoter (Parker and Jacobson, submitted). For 11 of 12 chimeric mRNAs, there was good agreement between relative decay rates and relative steady-state mRNA levels. In the only case in which the steady-state mRNA level is inconsistent with measured decay rates, the discrepancy may be due to an alteration in nuclear turnover or processing of the chimeric mRNA (Parker and Jacobson, submitted for publication). Two other observations suggest that mRNA decay is proceeding normally in temperature-shifted *rpb1-1* cells. (i) Cycloheximide drastically reduced mRNA decay rates (Table 5) and (ii) 5′-proximal nonsense mutations (in the *HIS4* mRNA) accelerate mRNA decay rates (R. Parker, S. Peltz, and A. Jacobson, unpublished observations). Both types of phenomena have been observed previously in yeasts and other organisms (4, 20, 23, 25, 45, 55, 59, 77, 82, 99).

With regard to the few inconsistencies that were observed between different procedures for determining mRNA decay rates, it is important to note the following. (i) Cells with thermally inactivated RNA polymerase II and cells treated with Thiolutin have ceased to grow, whereas cells used for approach to steady-state labeling experiments are growing exponentially. (ii) At $t = 0$, the relative representation and poly(A) status of individual mRNAs in a pulse-labeled RNA sample (analyzed in the approach to steady-state labeling procedure) will differ from that in the steady-state mRNA population (analyzed in Thiolutin-treated cells and in temperature-shifted *rpb1-1* cells).

Identification of stable and unstable mRNAs. The half-lives of individual mRNAs examined in temperature-shifted *rpb1-1* cells ranged from 2.5 to 45 min (Table 3). These decay rates are in good agreement with the complex decay kinetics observed for the total mRNA population (Fig. 1A, 2A, and 3A) and indicate that the complexity of the decay curves for the mRNA population reflects the sum of the half-lives of individual mRNAs and that the collection of mRNAs analyzed here is a representative sample of the continuum of mRNA decay phenotypes in the cell. For simplicity, we arbitrarily divided this continuum into three mRNA stability classes: unstable mRNAs ($t_{1/2} < 7$ min), moderately stable mRNAs ($t_{1/2} = 10$ to 20 min), and stable mRNAs ($t_{1/2} > 25$ min). Clearly, the availability of a set of well-characterized genes encoding mRNAs representative of the different yeast mRNA decay phenotypes should facilitate an analysis of the molecular basis of such differences.

What is the basis of the 10- to 20-fold difference in the decay rates of stable and unstable mRNAs? Possible determinants of mRNA stability can be subdivided into those which are specific and those which are nonspecific. Specific determinants are mRNA sequences or structures which either interact directly with components of the cellular turnover

machinery or target that machinery to other specific sites. Nonspecific determinants are general mRNA features [such as the cap, poly(A) tail, or overall size] which could contribute to mRNA decay rates by promoting or hindering random interactions with nonspecific nucleases.

The possibility that the polyadenylation status of an mRNA influences its decay rate has been supported (8, 60, 64, 71, 107) and refuted (49, 62, 73, 92, 94) by numerous studies in several organisms. An earlier study in yeasts (89) found no correlation between mRNA decay rates and poly(A) lengths, an observation supported by the work described here. We found that (i) poly(A)-deficient *CYH2* and *PGK1* mRNAs are extremely stable (Fig. 5), (ii) rapid turnover of the *STE2* mRNA is not clearly linked to its deadenylation (Fig. 5), and (iii) the decay curves of mRNAs with half-lives longer than the 7- to 10-min half-life for poly(A) shortening are linear, not biphasic (e.g., Fig. 1C). From these and previous (73, 94) data, we conclude that poly(A) lengths alone do not determine mRNA decay rates and that, at least for the *CYH2* and *PGK1* mRNAs, deadenylation is not the rate-limiting event in mRNA degradation. This conclusion may not hold for all yeast mRNAs since, for example, the *RP51A* mRNA is relatively stable in both the ts polymerase assay (with unfractionated RNA) and the approach to steady-state labeling procedure [with poly(A)$^+$ RNA].

Santiago et al. (90) have suggested that an inverse relationship exists between the size and stability of yeast mRNAs. Half-lives in their study were measured after the inhibition of transcription with high concentrations of the drug 1,10-phenanthroline. To address the possibility that a size-stability correlation is apparent under certain physiological conditions but not others, we compared mRNA sizes with decay rates obtained by each of the three procedures used here (Fig. 6). Our results do not support a possible relationship between mRNA size and stability. This conclusion is supported by our previous study with *D. discoideum* mRNAs (94) and by recent experiments with chimeric yeast mRNAs (Parker and Jacobson, submitted). In the latter study, large deletions which caused two- to threefold differences in mRNA lengths had no effect on mRNA decay rates.

Bennetzen and Hall (5) observed that highly expressed genes in yeasts are biased toward the use of only 25 of the 61 coding triplets. The general trends of this correlation have withstood the analysis of a much larger number of yeast genes (1) and are consistent with the levels of the corresponding isoaccepting tRNAs (38). The possibility that codon bias (i.e., the percentage of rare codons) influences mRNA decay rates in yeasts is suggested by experiments in which a threefold reduction in the steady-state levels of *PGK1* mRNA was elicited by a systematic replacement of frequent codons with rare codons (33). An analysis of the mRNAs examined in this study revealed a correlation between mRNA instability and the content of rare codons (Fig. 7). This correlation may reveal an underlying mechanism for regulating mRNA decay rates or may simply reflect the fact that some highly expressed genes have independently evolved high rates of transcription and translation and slow rates of mRNA decay. A role for rare codons may be related to sequence context. When clustered together, rare codons may reduce translational elongation rates (76, 83, 98) to the point that a paused ribosome could trigger a nucleolytic event. In this regard, it is interesting to note that structural determinants of mRNA stability in the *MATα1* and *HIS3* genes overlap with regions containing clusters of rare codons (Parker and Jacobson, in press; Herrick and Jacobson, in preparation). The hypothetical role of rare codons and paused ribosomes in promoting decay is not inconsistent with the observation that cycloheximide reduces the decay rates of all three stability classes of mRNA (Table 5) since treatment of cells with this drug completely inhibits protein synthesis. Hence, in the presence of cycloheximide, ribosome progression up to pause sites would be blocked, as would the resynthesis of potentially unstable nucleases.

Experiments in mammalian cells have demonstrated that sequences within mRNA 3′ UT regions can dictate rapid decay rates (14, 25, 42, 43, 56, 67, 75, 81, 96, 97). To assess whether yeast mRNAs of a common stability phenotype share a common 3′ UT sequence element (such as the mammalian ARE [12, 96]), we used computer algorithms to compare the sequences of the 3′ UT regions of each of the unstable mRNAs with each other. Homologies which appeared in such searches were then compared with the sequences of the 3′ UT regions of the stable mRNAs. Likewise, the sequences of all the respective mRNAs were analyzed for dyad symmetries. UT regions of the respective mRNAs were as defined previously by published experiments or, in the absence of such data, arbitrarily set to consist of the 200 nucleotides 3′ to the translational terminator. These searches have, so far, failed to identify any sequence or structural elements which are unique to the unstable mRNAs or to a subset of the unstable mRNAs. These searches did, however, confirm the existence of 3′ UT homologies among most of the mRNAs which were analogous to those previously identified by Zaret and Sherman (108) and which have been postulated to play a role in transcriptional termination. The absence of significant class-specific 3′ UT homologies is consistent with recent experiments from this laboratory which show that replacement of the 3′ UTs of the stable *ACT1* and *PGK1* mRNAs with those of the *HIS3*, *STE3*, and *MATα1* mRNAs is not sufficient to increase the decay rates of the stable mRNAs (Parker and Jacobson, in press; Parker and Jacobson, submitted; Herrick and Jacobson, in preparation). Of interest in the same studies are experiments which demonstrate that sequences which dictate rapid decay can be localized to the coding regions of all three unstable mRNAs (Parker and Jacobson, in press; Parker and Jacobson, submitted; Herrick and Jacobson, in preparation).

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