Efficient translation of poly(A)-deficient mRNAs in *Saccharomyces cerevisiae*

Aaron Proweller and Scott Butler

Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642 USA

The polyadenylate tail of eukaryotic mRNAs is thought to influence various metabolic phenomena including mRNA stability, translation initiation, and nucleo-cytoplasmic transport. We have analyzed the fate of mRNAs following inactivation of poly(A) polymerase in *Saccharomyces cerevisiae* containing a temperature-sensitive, lethal mutation (pap1-1) in the gene for poly(A) polymerase (PAP1). Inactivation of poly(A) polymerase (Pap1) by shifting cells to the nonpermissive temperature resulted in the loss of at least 80% of measurable poly(A) within 60 min. Northern blot analysis revealed the disappearance of some mRNAs (CYH2 and HIS4) consistent with a role for poly(A) tails in mRNA stability. However, other mRNAs (TCM1, PAB1, ACT1, and HTB2) accumulate as poly(A)-deficient (A<~25) transcripts as defined by an inability to bind oligo(dT)-cellulose. Sucrose density gradient analysis of polyribosomes revealed a twofold reduction in the amount of each size class of polyribosomes in shifted cells and a commensurate increase in free ribosomes. However, poly(A)-deficient mRNAs in shifted cells remain associated with the same size polyribosomes as poly(A)^ mRNA in unshifted cells, indicating normal initiation of translation. RNase mapping of transcripts from pap1-1 cells revealed PAB1 mRNA to be poly(A)^ whereas TCM1 exists as equal amounts of poly(A)^ and poly(A)^ mRNA 60 min after shift. Interestingly, both of these classes of TCM1 mRNA appear in similar amounts in each polyribosome fraction indicating that ribosomes may not distinguish between them. These findings suggest that under conditions of excess translational capacity, poly(A)^ and poly(A)^ mRNAs may initiate translation with comparable efficiencies.

[Key Words: Polyadenylation; protein synthesis; mRNA stability; polyribosomes]

Received June 28, 1994; revised version accepted September 18, 1994.

Almost 20 years after the physical characterization of the poly(A) tail at the 3' end of mature eukaryotic mRNAs, the function or functions imparted by this feature remain unclear. Much of the debate concerns the possible role of poly(A) in mRNA stability, protein synthesis, and nuclear export (Brawerman 1981). In particular, posttranscriptional modifications of most eukaryotic mRNA impart structural features thought to operate as translational enhancers and include the addition of a 5'-m''GG cap and a 3'-poly(A) tail [Jackson and Standart 1990; Munroe and Jacobson 1990; Gallie 1991; Jackson 1993]. However, a mechanistic understanding of these observations remains elusive. With the advent of in vitro-synthesized mRNA it became possible to engineer RNA with poly(A) tails of defined length, and the subsequent injection of these mRNAs into amphibian or mammalian oocytes created a unique opportunity to study both the chemical (i.e., stability) and functional (i.e., synthesis of translation product) half-lives of the mRNAs. Deadenylated RNA microinjected into *Xenopus* oocytes had similar chemical half-lives compared to their poly(A)^ forms, and only after 24 hr did the poly(A)^ but not poly(A)^ forms mRNA rapidly degrade [Galili et al. 1988]. However, the poly(A) tail appeared to enhance protein synthesis independent of effects on mRNA stability, although deadenylated mRNA was translated with 50% efficiency relative to poly(A)^ mRNA in these experiments, suggesting that a pathway exists for the translation of poly(A)^ mRNAs [Drummond et al. 1985; Galili et al. 1988; Gallie 1991].

Developmental studies of clam, frog, and mouse oocytes perhaps best exemplify the effect of poly(A) tails on translation by revealing a dependence on poly(A) tails for recruitment of translationally dormant mRNAs into polyribosomes (Richter 1991; Bachyrova 1992; Wickens 1992). Furthermore, heat shock effects in *Drosophila melanogaster* embryos include alterations in poly(A) tail lengths that correlate with translational efficiency [Storti et al. 1980]. In contrast, deadenylation of specific RNAs can result in an increase in translatability as shown during early *Xenopus* embryogenesis, when deadenylation of histone mRNA actually facilitates translation activation of core histone messages [Ballantine and Woodland 1985].

Evidence for a role for poly(A) in translation comes from recent studies implicating poly(A)-binding protein

1 Corresponding author.
Proweller and Butler

[PABP] in translation initiation. Specifically, a yeast strain depleted of PABP resulted in a polyribosome profile suggestive of an initiation defect, and a mutation in ribosomal protein L46 (rpl46) could suppress the abnormality [Sachs and Davis 1989]. Because PABP binds to poly[A], as well as other homoribonucleotide polymers, it was proposed that PABP exerted its function in protein synthesis through binding to the poly[A] tail [Bernstein et al. 1989; Sachs and Davis 1989; Burd et al. 1991]. Support for this role came from studies showing that addition of PABP to a translation system in vitro could relieve the inhibition of translation of poly[A] RNAs by exogenous poly[A] [Jacobson and Favreau 1983; Lemay and Milward 1986; Grossi da Sa et al. 1988]. In this respect, the poly[A] tail would presumably mediate PABP function.

We reported previously that protein synthesis continues after inactivation of a temperature-sensitive poly[A] polymerase despite significant loss in poly[A] content [Patel and Butler 1992]. Specifically, the rate of [35S]methionine incorporation into protein remained nearly unchanged long after poly[A] levels had fallen to undetectable levels. This finding suggested that translation may not require poly[A] tails and prompted further investigation. We designed the experiments presented here to determine whether translation in poly[A]-deficient cells results from initiation by a subset of polyadenylated mRNAs, or whether poly[A]− mRNAs may be translated normally. Our findings, based in part on the similarity of polyribosome profiles and mRNA distribution, support the idea that poly[A]− mRNAs may indeed initiate translation as efficiently as poly[A] RNAs. Finally, we discuss the significance of these findings in light of evidence that poly[A] tail length plays a critical role in the efficiency of translation initiation.

Results

Stability and poly(A) status of mRNAs after inactivation of poly(A) polymerase in shifted pap1-1 cells

Previous experiments from our laboratory showing that thermal inactivation of poly[A] polymerase in vivo in a pap1-1 strain led to diminished levels of poly[A] were based on a poly[A] content assay involving protection of poly[A] from RNase A digestion [Patel and Butler 1992]. Although informative for quantitation of poly[A] tail loss, there remained several questions including the relative fate of global and specific mRNAs as well as their poly[A] tail status. To address these concerns, we determined the average poly[A] tail length of total RNA present in cells by labeling the 3' ends of total RNA with [5'32P]pCp, followed by RNase T1 and A hydrolysis, and subsequent separation of nuclease resistant poly[A] by polyacrylamide electrophoresis. Figure 1 illustrates the result of such an experiment and shows a significant reduction in the amount and overall size of poly[A] tracts (>10 nucleotides) after shifting a pap1-1 strain to 35°C for 60 min. Laser scanning densitometry revealed that poly[A] disappears with kinetics similar to that of total mRNA [t1/2 = 20 min; Hynes and Phillips 1976] suggesting rapid inactivation of poly[A] polymerase after temperature shift. Moreover, this experiment reveals loss of >80% of cellular poly[A] by 60 min after shift to the nonpermissive temperature. Of the poly[A]+ RNA remaining at this time, about one-third carries poly[A] tracts in the range of 50–60 residues, possibly resulting from residual poly[A] polymerase activity.

Poly[A] tails apparently play a crucial role in controlling mRNA chemical stability [Muhlrad and Parker 1992; Decker and Parker 1993; Lowell et al. 1993]. Accordingly, we determined by Northern blot analysis the amounts of several mRNAs after inactivation of poly[A] polymerase. Consistent with this function, polyadenylation shut off immediately results in a decrease in the amount of all species monitored [Fig. 2A], whereas shift of normal cells to 35°C has little if any effect on mRNA levels [Patel and Butler 1992; data not shown]. Although
Translation of poly(A)-deficient mRNA in vivo

Time (Min.) 0 15 30 60 120 240 360
Temp. °C 23 23 35 23 35 23 35 23 35 23 35 23 35

HIS4
18S
YCL31C
TCM1
CYH2
ACT1
HTB2
PGK1
PAB1

shift when the cells stop growing (Patel and Butler 1992). This pattern of mRNA levels coincides with an increase in the rate of RNA synthesis under these conditions [data not shown], suggesting that an increase in transcription may, in some cases, compensate for the instability of poly(A)-deficient mRNAs causing them to accumulate. Alternatively, the mRNAs detected at 60 min after inactivation of poly(A) polymerase may persist as, or become, poly(A)* by some unanticipated mechanism. The results shown in Figure 2B, however, indicate that the majority of TCM1, CYH2, PAB1, and PGK1 transcripts detected 60 min after shift do not bind to oligo(dT)-cellulose and so have very short, or no poly(A) tails (Groner et al. 1974; Patel and Butler 1992).

Protein synthesis in poly(A)-deficient cells

We previously measured the rate of translation in pap1-1 cells by a pulse-labeling procedure and found that the rate of translation in pap1-1 cells, although slower than in PAPl cells, did not change significantly for up to 2 hr after shift to 35°C, despite a dramatic loss of cellular poly(A) (Patel and Butler 1992). This finding contrasted with many observations indicating an important role for poly(A) in the initiation of protein synthesis and prompted us to determine the effect of polyadenylation shutoff on protein synthesis in growing cells by an alternative, continuous labeling procedure. The result (Fig. 3) shows nearly linear incorporation of [35S]methionine [normalized to the −30 min values] into protein in a

Figure 2. Amounts and polyadenylation status of specific mRNAs after inactivation of Pap1. [A] Accumulation of various mRNAs in a pap1-1 strain as a function of time at 23°C or 35°C. Cells were grown at 23°C, and at time zero, the culture was split and half remained at 23°C while the other half was incubated at 35°C. Equal numbers of cells were harvested at the times indicated and total RNA was prepared (Patel and Butler 1992). mRNA levels were determined by Northern blot analysis of equal amounts of total RNA. [B] Polyadenylation status of TCM1, CYH2, PAB1, and PGK1 mRNAs as a function of time after shift of a pap1-1 strain to 35°C. Total RNA was separated into poly(A)* and poly(A)-deficient RNA by oligo(dT)-cellulose chromatography and the amounts of each fraction determined by Northern blot analysis (Patel and Butler 1992).

some mRNAs (CYH2 and HIS4) disappear, others continue to accumulate (TCM1, PAB1, HTB2, YCL31C, and ACT1) or reaccumulate until between 2 and 4 hr after

Figure 3. Protein synthesis in a PAPl and a pap1-1 strain before and after shift to 35°C. Cells were grown in YPD at 25°C to a density of 1 x 10^6 to 2 x 10^6 cell/ml and labeled at −60 min with 0.1 mCi of [35S]methionine [1140 Ci/mmole] in 10 ml of the same media. Aliquots of 0.5 ml were withdrawn at the indicated times, and incorporation into protein was determined by the hot TCA assay. The data shown represent the average of two determinations and have been normalized to the −30 min before shift value (5 x 10^3 to 7 x 10^3 cpm for each strain).
PAPI strain for up to 4 hr after shift to 35°C. A pap1-1 strain, on the other hand, shows linear incorporation at a rate about one-half that of PAPI, until 60–90 min after shift, followed by a decrease in the amount of protein made over the next 90 min. This experiment indicates that although the amount of protein made after inactivation of poly[A] polymerase accumulates more slowly than that made in a normal PAPI cell, a significant amount of protein synthesis occurs during the time (0–60 min after shift) when 80% of the poly[A] disappears from pap1-1 cells. We considered two alternative explanations for these results: [1] The slowdown in protein synthesis reflects a reduced rate of translation initiation due to the loss of poly[A] tails from mRNAs; or [2] the decrease in protein synthesis results from a reduction in the amount of mRNA available for protein synthesis as suggested from the results in Figure 2A.

Polyribosome profiles from pap1-1 cells reveal reduced protein synthesis without a defect in translation initiation

A decrease in the rate of translation initiation should (1) reduce the number of large polyribosomes more than small ones and (2) cause poly[A]-deficient mRNAs to shift to smaller polyribosomes. To test this prediction, we prepared whole-cell lysates after inhibiting translation elongation with cycloheximide and analyzed polyribosome profiles by sedimentation on low salt sucrose gradients. Figure 4A shows typical polyribosome profiles from shifted and unshifted PAPI and pap1-1 cells. PAPI cells shifted to 35°C undergo a 17±10% [n = 4] decrease in the number of polyribosomes, perhaps because of a heat shock effect in this strain. However, pap1-1 cells show a 56±6% [n = 4] reduction in the number of polyribosomes and a concomitant increase in the 80S peak (Fig. 4A). The increased number of 80S subunits consisted of inactive [nontranslating] 80S subunits because inclusion of high salt in the lysate and sucrose gradient just prior to sedimentation resulted in the resolution of the 80S ribosomes into 60S and 40S subunits [Martin 1973; data not shown]. The pap1-1 profile at 35°C does not reveal a shift from large to smaller size polyribosomes typical of translation initiation defects. Instead, the small and intermediate-size polyribosomes (fractions 2–5) decrease proportionately when compared with unshifted and normal cells, whereas the largest polyribosomes (fractions 1–2) decrease far less.

We characterized further the fate of TCM1, PAB1, and PGK1 poly[A]-deficient mRNAs along the pap1-1 polyribosome gradient by determining the quantity of these mRNAs in each gradient fraction before and after temperature shift. We specifically chose TCM1 and PAB1 mRNAs because of [1] their relative abundance at 60 min after shift to 35°C, [2] their short half-lives (<11 min), which allows the detection of messages largely synthesized after the inactivation of poly[A] polymerase, and [3] their length (>1200 nucleotides), which upon an initiation defect, should allow them to move from large to small polyribosomes. Figure 4B shows the distribution of each mRNA among the different sized polyribosomes. As expected, shifting a PAPI strain from 25°C to 35°C resulted in no change in the number of ribosomes translating each mRNA [data not shown]. Translation of poly[A]-deficient mRNAs in pap1-1 cells shifted to 35°C for 1 hr does not occur on smaller polyribosomes as expected for an initiation defect. Instead, these mRNAs appear associated with more ribosomes, on average, than in unshifted cells. These findings suggest that poly[A]-deficient mRNAs may initiate translation as well, or somewhat better than poly[A]+ mRNAs in normal, and unshifted pap1-1 cells. Part of this surprising effect may result from an increase in the ratio of free ribosomes to mRNA caused by the loss of many mRNA species after polyadenylation shutoff. These findings suggest that although the amount of protein synthesis in poly[A]-deficient cells falls twofold because of a decrease in the quantity of mRNA, the rate of translation of poly[A]-deficient mRNAs remains similar to that of poly[A]+ mRNAs in unshifted and normal cells.

Pap1 inactivation produces poly(A)~ PAB1 and TCM1 mRNAs

We designated the mRNAs studied here as poly[A]-deficient on the basis of their inability to bind to oligo(dT)-cellulose. This matrix does not retain mRNAs with poly[A] tails of less than ~15 residues [Groner et al. 1974; Fig. 2B]. Because poly[A]-binding protein may interact with poly[A] tracts as small as 12 nucleotides and because this protein appears to mediate the role of poly[A] in translation initiation, we sought a method to determine whether these mRNAs might have short poly[A] tails that enhance their translation. We designed an RNase protection assay to determine the amount of a specific mRNA as well as its poly[A] tail length. First, we mapped the polyadenylation sites of PAB1 and TCM1 mRNAs by use of a reverse transcriptase–PCR (RT–PCR)-based approach that produces specific cDNAs carrying the first 17 adenosine residues of the poly[A] tail [Sadahle et al. 1991]. For PAB1 this produces a major cDNA of ~300 nucleotides from normal and unshifted pap1-1 cells, which disappears from shifted pap1-1 cells (Fig. 5A). After cloning into transcription vectors these cDNAs can produce RNA probes capable of distinguishing poly[A]+ mRNAs from those with very short tails. DNA sequence analysis of 16 independent cDNA clones of PAB1 revealed a major poly[A] site [10 of 16 clones] and three minor poly[A] sites [Fig. 5B]. None of these sites corresponds to that previously mapped for PAB1 [Sachs et al. 1986], however, the two minor poly[A] sites 3’ distal to the major poly[A] site appeared twice and only once, respectively, in the 16 clones, suggesting that sequencing more clones might reveal additional minor sites, including that mapped previously. Because we have shown previously the RT–PCR method to be more reliable than S1 nuclease analysis of mRNA 3’ ends [Sadahle et al. 1990], and because RNase protection analysis...
Translation of poly(A)-deficient mRNA in vivo

Figure 4. (A) Polyribosome distribution of mRNAs in a PAP1 and a papl-1 strain before and after shift to 35°C. Cells were grown in YPD at 25°C to 2×10^7 to 3×10^7 cells/ml, samples were taken and then the cells were shifted to 35°C for 60 min and samples were taken again. Polyribosomes were prepared by treating the cells with cycloheximide and breaking with glass beads. Each 15%-50% sucrose gradient was loaded with 25 A254 units of lysate and the ribosomes, subunits and polyribosomes separated by ultracentrifugation. The direction of sedimentation is from right to left, the position of the ribosomes is indicated and the position of the gradient fractions collected are indicated.

(B) Position of various mRNAs along the polyribosome gradients from papl-1 cells incubated at 25°C or 35°C. RNA was isolated from the gradient fractions indicated in Fig. 4A, and the amounts of the indicated mRNAs were determined by Northern analysis.

[see below] confirms the usage of the two most prevalent sites [M and m1, Fig. 5B], we consider them correct. RNase protection analysis with the major poly(A) site M cDNA probe and RNA from normal and unshifted papl-1 cells yielded 3 products (Fig. 5C, lanes 3–5). The largest (~190-nucleotide) diffuse product results from protection by mRNAs polyadenylated at the major poly(A) site, M, whereas the smallest product corresponds to protection by mRNAs ending at the 5' minor site, m1. The ~170-nucleotide product results from protection of the probe up to the major poly(A) site, M, by mRNAs ending at this site and, or those extending past it. Messages producing this product are the only ones present after inactivation of Pap1, suggesting that they may be major site poly(A)-mRNAs (Fig. 5C, lane 6). If this product results from protection by the major site poly(A)-mRNA rather than by mRNAs extending past this site, then it should remain in protection experiments featuring a probe extending to a 3' distal poly(A) site, whereas messages polyadenylated at or ending at 3' distal sites should yield longer protection products with such a probe. Protection of a probe derived from the 3'
Figure 5. Determination of PAB1 poly(A) tail lengths. (A) RT-PCR synthesis of cDNAs spanning the poly(A) site of PAB1 mRNA from Lane M) 123-bp molecular size markers (GIBCO-BRL; (lane 1) PAPI cells at 25°C; (lane 2) pap1-1 cells at 25°C; (lane 3) pap1-1 cells at 25°C for 60 min. (B) DNA sequence of the sense strand in mRNA from pap1-1 cells at 25°C for 60 min. (C) Ribonuclease protection assay of PAB1 mRNAs. (Lane M) DNA molecular size markers (32P-labeled Mspl fragments of pBR322) with lengths indicated at left. RNase protection of the major poly(A) site probe (lanes 1–6) and the 3’ distal poly(A) site probe (lanes 7–12). No RNase treatment (lanes 1,7). RNase protection with 10 μg of E. coli tRNA (lanes 2,8), 10 μg of PAPI RNA (25°C) (lanes 3,9), 10 μg of PAPI RNA (35°C, 60 min) (lanes 4,10), 10 μg of pap1-1 RNA (25°C) (lanes 5,11), 10 μg of pap1-1 RNA (35°C, 60 min) (lanes 6,12). The positions of protection products corresponding to the PAB1 poly(A) sites are indicated at right. The major site M probe used in lanes 1–6 contains 162 nucleotides from the DdeI site in PAB1 (position 2582) to the m3 poly(A) site, 17 U residues and 42 nucleotides of vector sequence. The 3’ distal m3 probe used in lanes 7–9 contains 199 nucleotides of sequence from the DdeI in PAB1 to the m3 poly(A) site, 17 U residues, and 42 nucleotides of vector sequence. (D) Effect of RNase dilution on RNase protection products. RNase stock (0.28 mg/ml of RNase A, 20 μg/ml of RNase T1) was diluted as indicated, and the protection experiment was carried out on RNA from pap1-1 cells grown at 25°C (−), or shifted to 35°C (+) as indicated. (Left) DNA molecular size markers and DNA sequencing lanes from an unrelated template for length determinations. Experiments in C and Figs. 6C and 7 were carried out using the 1:200 RNase stock dilution.

distal poly(A) site m3 cDNA (Fig. 5B) by RNA from shifted pap1-1 cells yields only the ~170-nucleotide product, indicating that the majority of PAB1 mRNAs end at the major poly(A) site M, 60 min after Pap1 inactivation (Fig. 5C, lane 12). Because none of these mRNAs protect the poly(A) derived portion of the major site probe to yield the ~190-nucleotide product, they must be poly(A)^− (Fig. 5C, cf. lanes 6 and 12).

The unexpected production of the ~170-nucleotide product by protection of the 3’ distal probe with mRNA from normal and unshifted pap1-1 cells suggested that poly(A)^− PAB1 mRNAs might exist under these conditions (Fig. 5C, lanes 9–11). However, close inspection of these products revealed that they are slightly longer than the 170-nucleotide products in lanes 3–6 and lane 12 of Figure 5C. Moreover, we found that increasing the RNase concentration shortened these slightly longer products to the same size as those from shifted cells, whereas the m1 and the 170-nucleotide products from shifted cells remain the same size (Fig. 5D). We suggest that this behavior results from the fact that the probe contains two U residues [nucleotides 2747–2748] capable of protecting the poly(A) tail added at site M and three U residues [nucleotides 2729–2731] capable of protecting the poly(A) tail added at site m1 (Fig. 5A). The ends of the hybrids thus formed at m1 will be more stable [more resistant to RNase] by virtue of two adjacent G-C base pairs compared to hybrids formed at M, which lie adjacent to 5 A–U base pairs. The specificities of RNases A and T1 dictate that they will not cleave the probe in the run of 5 As preceding the poly(A) site M, causing hybrids to poly(A)^− mRNAs ending at M to appear stable at higher concentrations of these enzymes. Thus we conclude that the RNase-sensitive products from protection of the 3’ distal poly(A) site probe result from protection by major site M poly(A)^+ mRNAs and that little, if any, poly(A)^− PAB1 mRNA exists in normal or unshifted pap1-1 cells. More importantly, the results in Figure 5 show that the majority of PAB1 transcripts present and translated in pap1-1 cells after shift to 35°C for 60 min do
not carry poly(A) tails, consistent with their production after Pap1 inactivation.

Amplification of TCM1 mRNA with a TCM1-specific primer and an oligo(dT) primer produced a single major product from normal, unshifted pap1-1 and shifted pap1-1 cells indicating the presence of a single polyadenylated transcript in these cells (Fig. 6A). We synthesized from a transcription vector containing this cDNA, an RNA probe that has the potential to hybridize to 166 nucleotides of TCM1 mRNA 5’ to its poly(A) site and to the first 17 adenosine residues of the poly(A) tail. RNase protection experiments revealed protected fragments of about 180 nucleotides in normal and unshifted pap1-1 cells indicating the presence of a single polyadenylated transcript in these cells (TCM1-A17, Fig. 6C). The relative absence of protected products from transcripts with less than about 17 adenosine residues agrees with experiments indicating that short oligoadenylate (A~12 nucleotides) species apparently constitute the last detectable intermediates in the mRNA turnover pathway for some mRNAs (Decker and Parker 1993). RNase protection of TCM1 mRNA from pap1-1 cells shifted to 35°C for 60 min revealed the appearance of a product of the length expected from protection of poly(A)^− TCM1 mRNA (TCM1-A0). Quantitation of these products by storage PhosphorImager analysis indicates that pap1-1 cells contain similar amounts of TCM1-A17 and TCM1-A0 species ([A17/A0 = 1.2±0.3, n = 5]) 60 min after inactivation of Pap1. This finding, and the fact that neither of these species bind to oligo(dT)-cellulose, supports a scenario in which inactivation of Pap1 results in the synthesis of poly(A)^− transcripts and the accumulation of transcripts whose poly(A) tails have been shortened to less than ~25 adenosine residues.

We investigated the possibility that the TCM1-A0 protection product might represent an artifact of the RNase protection procedure: If polyadenylation of TCM1 mRNA in shifted pap1-1 cells occurs at a different site

**Figure 6.** Determination of TCM1 poly(A) tail lengths. (A) RT–PCR synthesis of cDNAs spanning the poly(A) site of TCM1 mRNA from: (Lane 1) pap1-1 cells at 25°C; (lane 2) pap1-1 at 35°C for 60 min; (lane 3) PAP1 at 25°C. (Lane M) 123-bp molecular size markers (GIBCO-BRL). (B) Diagram of the sequence of the TCM1 cDNA product at the polyadenylation site (nontemplate encoded nucleotides in italics), the TCM1 mRNA, the probe and expected products from the ribonuclease protection assay. (C) Ribonuclease protection assay: (lane M) molecular size markers [32P-labeled MspI fragments of pBR322]; (lane 1) untreated probe; (lane 2) probe hybridized to 10 μg of E. coli tRNA and digested with RNase; (lane 3) probe hybridized to 10 μg of pap1-1 RNA (25°C) and digested with RNase; (lane 4) probe hybridized to 10 μg of pap1-1 RNA (35°C, 60 min) and digested with RNase; (lane 5) probe hybridized to 20 μg of pap1-1 RNA (35°C, 60 min) and digested with RNase; (lane 6) probe hybridized to 10 μg of pap1-1 RNA (35°C, 60 min, independent sample) and digested with RNase; (lane 7) probe hybridized to 10 μg of PAPI RNA (35°C, 60 min) and digested with RNase; (lane 8) probe hybridized to 20 μg of PAPI RNA (35°C, 60 min) and digested with RNase.
than in PAPI cells, then the riboprobe derived from TCM1 mRNA in PAPI cells will hybridize incompletely to pap1-1 mRNA leaving internal, RNase-sensitive, mismatched bases. Indeed, DNA sequence analysis of TCM1 cDNA clones from shifted pap1-1 cells revealed that whereas 5 of 11 independent clones have the normal CCA$_{17}$ 3’ end, 6 of 11 have the end CCA$_{17}$ or CA$_{17}$.

This polyadenylation site heterogeneity will yield hybrids during RNase protection with G–A mismatches potentially sensitive to RNase T1, but not to RNase A. Omission of RNase T1 from the RNase protection procedure, however, had no effect on the relative yield of the TCM1-A$_{17}$ and TCM1-A$_0$ species, indicating that the mismatches are not sensitive to the levels of RNase T1 employed in our procedure (data not shown).

Ribosomes translate poly(A)$^+$ and poly(A)$^-$ TCM1 mRNA with similar efficiency

The presence of similar amounts of poly(A)$^-$ and poly(A)$^+$ TCM1 mRNA provided a convenient test of the model that predicts that poly(A)$^+$ mRNAs should initiate translation more efficiently than poly(A)$^-$ mRNAs. If TCM1-A$_{17}$ mRNAs enjoy an advantage during translation initiation then they should appear on larger polyribosomes than TCM1-A$_0$ mRNAs. Instead, the two mRNA species appear in the same proportions across the polyribosome gradient, suggesting that they initiate translation at similar rates [Fig. 7A]. Polyribosomes from unshifted pap1-1 cells do not contain significant amounts of TCM1-A$_0$, mRNA, indicating that production of this species requires inactivation of Pap1 [Fig. 7B]. Moreover, comparison of the profile from unshifted pap1-1 cells containing predominantly poly(A)$^+$ TCM1 mRNA [Fig. 7B], with that from shifted pap1-1 cells [Fig. 7A] reveals that TCM1-A$_{17}$ mRNAs translate as well, or perhaps somewhat better than poly(A)$^+$ TCM1 mRNA.

Discussion

Our previous characterization of a thermosensitive defect in Saccharomyces cerevisiae poly(A)$^+$ polymerase provided us with a unique opportunity to address directly the role of poly(A) tails in vivo. Here we show that thermal inactivation of poly(A)$^+$ polymerase results in the production of poly(A)$^-$ mRNAs and that these mRNAs may initiate translation as well as poly(A)$^+$ mRNAs. We characterized poly(A) tails after inactivation of poly(A) polymerase and found [1] that the quantity of poly(A) disappears with a half-life similar to mRNA, suggesting rapid shut-off of poly(A) polymerase activity in pap1-1 after shift to 35°C, and [2] that only ~20% of cellular poly(A) remains after a 60-min shift to the nonpermissive temperature [Fig. 1]. Northern blot analysis of total RNA from pap1-1 cells revealed that inactivation of poly(A) polymerase results in the disappearance or reduction in the amount of various mRNAs [Fig. 2] consistent with a role for poly(A) tails in conferring mRNA stability. The reduction in the mRNA pool correlates with the polyribosome profiles obtained from shifted pap1-1 cells showing that these cells have approximately twofold less translating polyribosomes than unshifted cells [Fig. 4A]. Significantly, all polyribosome sizes decrease propor-
tionately and poly[A]-deficient mRNAs remain associated with large polyribosomes implying that translation initiation remains unchanged, whereas the amount of translation falls twofold. These findings contrast with polyribosome profiles from cells defective for translation initiation (Foiani et al. 1991; Yoon et al. 1992; Yoon and Donahue 1992; Zhong and Arndt 1993): In these cases the amounts of large polyribosomes decrease far more than the amounts of smaller ones. Thus, the defect in poly[A] tail synthesis results in a decrease in the quantity of mRNA and hence the amount of bulk translation falls. Finally, RNase mapping showed that PAB1 mRNA falls. Interestingly, this poly(A)~ mRNA is translated efficiently and poly(A)~ mRNA leaves. These phenotypes and our preliminary data indicating an increase in transcription after Pap1 inactivation support a role for poly(A) tails in translational activation and between the presence of a poly(A) tail and the translational efficiency of a mRNA. A compelling case for a role for poly[A] in translation emerges from studies showing that changes in polyadenylation status control the recruitment of translationally dormant mRNAs into polyribosomes during development of clam, frog, and mouse oocytes (Wickens 1992; Richter 1991). Observations in these systems demonstrated correlations between developmentally controlled addition of poly(A) tails to dormant mRNAs and translational activation, and between controlled poly[A] tail loss and translational inactivation (Rosenthal et al. 1983; McGrew et al. 1989; Vassalli et al. 1989; Varum and Wormington 1990; Sheets et al. 1994). These experimental systems all measure the translational potential of poly(A)+ mRNAs in the presence of an excess of poly(A)+ message, under conditions where components of the translation system that associate with poly[A], such as PABP, may be saturated with mRNA (Zelus et al. 1989). If a poly[A] tail lends a small competitive advantage in protein synthesis relative to an identical coding message lacking poly[A], then the effect of poly[A] may be prominent in a system where components required for translation initiation, rather than mRNA, are limiting (Laskey et al. 1977). In contrast, our studies indicate that polyadenylation shutoff causes a decrease in mRNA levels that results in a significant increase in the ratio of free ribosomes to mRNA. Thus, the lack of apparent discrimination between poly[A]+ and poly[A]− mRNAs in our system may result from the lack of competition for a normally limiting component of the translation initiation system such as initiation or elongation factors. This line of reasoning suggests that poly[A]+ and poly[A]− mRNAs may compete for some limiting component of the translation system in the presence of normal mRNA levels in S. cerevisiae and implies that our findings may not necessarily contradict the conclusions from other systems where such competition prevails. Stevens and colleagues recently provided evidence that translation occurs at near normal rates in an XRN1 deletion mutant containing significant quantities of uncapped, poly[A]-deficient mRNAs (Larimer et al. 1992; Hsu and Stevens 1993). Although their experiments do not distinguish between the relative efficiency of translation of poly[A]+ and poly[A]− mRNA, the apparently normal ratio of nontranslating to translating ribosomes in xrn1 cells and the lack of an apparent defect in the initiation of translation lends support to the idea that efficient translation initiation does not require findings show that inhibition of translation with drugs, or by cis-acting mutations inhibiting translation, result in no change, or an increase in mRNA stability (Baim and Sherman 1988; Herrick et al. 1990, Wisdom and Lee 1991; Beelman and Parker 1994).
polylA tails in vivo.

Translation systems in vitro have generated mixed conclusions regarding polylA tail function because of intrinsic differences (both qualitative and quantitative) in translational efficiencies that occur, for example, in wheat germ versus reticulocyte lysates (Bergman and Lodish 1979a). Hence, extrapolation of specific conclusions may not allow a more general understanding of polylA physiology. The enhancement of protein synthesis due to polylA is less striking in systems in vitro than in vivo, which may not allow a more general understanding of polylA.

Hence, extrapolation of specific conclusions in translational efficiencies that occur, for example, in systems in vitro. Interestingly, poly(A) tails with about 20 A residues competed with poly(A)− mRNA only about half as well as poly(A)− mRNA with 68 A residues. This system, depleted of endogenous mRNA, may be comparable to our cells after inactivation of polylA polymerase in that neither situation saturates the translation system with mRNA. The small effects seen in the reticulocyte lysates, especially for transcripts with short poly(A) tails, and the apparent lack of discrimination in our cells may result from the excess translational capacity in these systems.

PABP found in association with the polyadenylate region of eukaryotic mRNAs appears to mediate the effect of poly(A) tails on protein synthesis and mRNA stability (Sachs et al. 1987; Bernstein et al. 1989; Munroe and Jacobson 1990; Burd et al. 1991; Decker and Parker 1993; Lowell et al. 1993). Depletion of PABP in S. cerevisiae inhibits translation as determined by polyribosome profile analysis, and a mutation in the 6OS ribosomal subunit protein L46 (rpL46) suppresses the lethality of a PABP-deficient strain implying a role for PABP in translation initiation (Sachs and Davis 1989). Consistent with this interpretation, the addition of PABP relieved the inhibition of translation of poly(A)− mRNA by exogenous poly(A) in vitro (Jacobson and Favreau 1983; Lemay and Milward 1986; Grossi da Sa 1988). These results provided evidence for a role for PABP in translation initiation and implied that PABP mediates the effect of poly(A) tails on mRNA. The efficient translation of poly(A)− mRNA demonstrated in our experiments suggests that if translation initiation requires PABP bound to mRNA then it must bind to sequences within these transcripts. In this regard, the affinity of yeast PABP for sequences other than poly(A) appears potentially significant (Burd et al. 1991). It may be possible, under conditions of low poly(A) concentration, that excess PABP could bind to sequences internal to poly(A)− transcripts in a manner functionally similar to the interaction of PABP with short [A<25 nucleotides] poly(A) tails. Long poly(A) tails, however, might have the capacity to bind more molecules of PABP, possibly conferring an advantage during translation initiation. Alternatively, the decrease in mRNA levels we observe may result in an increase in the ratio of initiation factors to mRNA thus compensating for a decrease in PABP binding.

In summary, inactivation of poly(A) polymerase in vivo results in the synthesis of poly(A)− mRNAs that accumulate to varying degrees and appear to initiate translation with surprising efficiency. These findings support a role for poly(A) tails in influencing the stability of mRNA and suggest that poly(A) tails may function to enhance translation initiation only when the concentration of some translation factor becomes limiting.

Materials and methods

Yeast strains and media

The experiments reported here were carried out in the following strains: A364A (MATa, ade1, ade2, ura1, his7, lys2, tyr1, gal1), UR31481B (MATa, ade1/ade2, lys2, gal11, ura3-52, pap1-1). Strains were grown in YEPD media.

Poly(A) tail length determination

Total RNA [1 µg] was heated for 10 min at 65°C and added to 3 µl of ligation reaction mix [1.7 U/µl RNA ligase (NEB), 6.7 U/µl RNasin (Promega), 1.7 µCi/µl cytidine, 3′,5′-[S-32P] bisphosphate (3000 Ci/mmol, Amersham), 16.7 mM HEPES (pH 8.3), 3 mM MgCl2, 6% glycerol, 0.017 mM rATP, 2% DMSO] and incubated at 4°C for 16 hr. The reactions were diluted with 20 µl of 0.3M NaCl, 0.01 M Tris-CI (pH 8), 0.01 M Na2EDTA, and hydrolyzed with 80 µl of RNase digestion mix [0.5 mM NaCl, 20 mM Tris-CI at pH 8, 1 mM MgCl2, 1 mg/ml of E. coli tRNA, 0.125 mg/ml of RNase A, 1200 U/ml RNase T1] at 37°C for 3 hr. The reactions were stopped by the addition of 10 µl of 0.12M Na2EDTA, 2% SDS, and 4 mg/ml of proteinase K and incubated for 15 min at 37°C, followed by extraction with phenol/chloroform/isoamyl alcohol (PCI, 40:1:1) and precipitation with 10 µg of E. coli carrier RNA and 2 volumes of ethanol at −20°C for 30 min. Samples were separated by electrophoresis on a 12% polyacrylamide–urea, 0.5 × TBE gel at 2000 V until the bromophenol blue tracking dye migrated 30 cm from the well and visualized by autoradiography with Kodak X–Omab film.

Measurement of protein accumulation in vivo

Cells were grown at 25°C in 10 ml of YPED to a density of 1×10^6 to 2×10^6 cells/ml and labeled at −60 min (prior to shift to 35°C) by the addition of 100 µCi of [35S]methionine (1140 Ci/mmol, NEN). Aliquots of 0.5 ml were withdrawn every 30 min (until 240 min postshift) and added to 0.5 ml 10% TCA on ice. The mixture was boiled for 10 min and filtered through Whatman GF/C filters and washed twice with 5 ml of 5% TCA followed by 5 ml of 90% ethanol. The filters were dried and the 35S incorporation was measured by scintillation counting.

Preparation and analysis of yeast polysomes

Cells were grown in 500 ml of YEPD at 35°C to an A500 of 2.0±0.3. Approximately 6 × 10^8 cells/ml were harvested by centrifugation at 5000g for 5 min at room temperature, and the remaining culture shifted to 35°C for 1 hr followed by centrifugation as before. Pellets were resuspended in 5 ml of sterile water at room temperature and mixed with 50 µl of cycloheximide [10 mg/ml], incubated on ice for 1 min and pelleted at 5000g for 5 min at 4°C. Pellets were resuspended on ice in 0.5 ml of 1× breaking buffer (20 mM HEPES–KOH at pH 7.4, 2 mM Mg(OAc)2, 0.1 M KCl, 14.4 mM 2-mercaptoethanol, 0.1 mg/ml of...
cycloheximide). The cells were disrupted by eight cycles of vortexing for 30 sec and 20 sec chilling on ice in the presence of 400 μl of sterile glass beads. The debris was pelleted by centrifugation at 5000g for 8 min at 4°C, and the supernatants were stored at −70°C. For polysome analysis, 25 A254 units were layered on top of 15–50% sucrose gradients prepared with 1× (low salt) polysome buffer [10 mM Tris-Cl at pH 7.4, 70 mM NH₄OAc, 4 mM MgOAc₂]. Polysomes were separated by ultracentrifugation at 40,000 rpm in an SW40Ti rotor (Beckman) for 2.5 hr at 4°C. Profiles were obtained by continuous A254 measurement from the top of 15–50% sucrose gradients prepared with 1× (low salt) polysome buffer [10 mM Tris-Cl at pH 7.4, 70 mM NH₄OAc, 4 mM MgOAc₂]. Polysomes were separated by ultracentrifugation at 40,000 rpm in an SW40Ti rotor (Beckman) for 2.5 hr at 4°C. Profiles were obtained by continuous A254 measurement through an ISCO model UA-5 Absorbence/Fluorescence Monitor at a flow rate of 1 ml/min, chart speed 60 cm/hr, and a 0.5 sensitivity level. Gradient fractions [1 ml] were collected and stored in 3 volumes 95% ethanol at −20°C. The total amount of ribosomes sedimenting as polysomes was determined by cutting out the polysome portion of the gradient profiles and weighing them on an analytical balance.

*Extraction of RNA from polysome gradient fractions and RNA hybridization (Northern analysis)*

Gradient fractions precipitated in ethanol for at least 24 hr were centrifuged for 20 min at 10,000 rpm in a benchtop clinical centrifuge, supernatants discarded and pellets air dried at room temperature for 30 min. The pellets were resuspended in 100 μl of 10 mM Tris-Cl (pH 8.0), and 0.1 mM Na₂EDTA, transferred to new 1.5-ml Eppendorf tubes and digested in 0.02 ml Na₂EDTA, 0.2% SDS, and 0.4 mg/ml of proteinase K for 15 min at 37°C. The reactions were diluted to 400 μl with 10 ml Tris-Cl (pH 8.0) and 0.1 mM Na₂EDTA, extracted twice with PCI, and precipitated on dry ice by the addition of 40 μl of 3 M KAc and 800 μl of 95% ethanol.

Northern hybridization analyses were carried out as described previously (Patel and Butler 1992) with 30 μl of polysome-extracted RNA in each lane. Antisense DNA probes were prepared by the random hexamer method according to manufacturer’s instructions [Boehringer Mannheim] with the following gene-specific templates: a 2.95-kb HindIII–HindIII fragment containing the PGK1 gene from pDH8 [Mellor et al. 1983; kindly provided by A. Jacobson, University of Massachusetts Medical School, Boston], a 3.4-kb Sal–HindIII fragment containing the PAB1 gene from pYPA [Sachs et al. 1986; kindly provided by A. Jacobson] and a 2-kb fragment containing the TCM1 gene from p17–TCM1 (kindly provided by J. Warner, Albert Einstein College of Medicine, The Bronx, NY). Conditions for hybridization were described previously (Patel and Butler 1992).

*Ribonuclease protection analysis*

Ribonuclease protection experiments were carried out with antisense riboprobes prepared in vitro from transcription templates constructed as follows: RT–PCR with a downstream oligo(dT)₁₀ containing a BamHI restriction site (3’-TTT-TTT-TTT-TTT-TTT-TTT-CCTAGTTT-TT-5’) and an upstream mRNA-specific primer containing an EcoRI restriction sequence (5’-GGAGAATTCGCGGTCGGTTGGTCGTCGACGTACGCTGG-3’ for TCM1, and 5’-GGAGAATTCCGCGGTCGGTTGGTCGTCGACGTACGCTGG-3’ for PAB1) was carried out as described [Sathaye et al. 1991] except that the PCR annealing temperature was 55°C. PCR products were hydrolyzed with EcoRI and BamHI restriction enzymes and cloned into the corresponding polylinker sites of pGEM4Z [Promega]. Riboprobes with specific activities of 1×10⁶ to 2×10⁸ cpm/μg were synthesized from pAP3 linearized with PvuII for TCM1 and from pAPPB3 and pAPPB32 linearized with Ddel for the PAB1 major site and 3’ distal site probes, respectively.

For determination of poly[A] tail length, ribonuclease digestion experiments were carried out with total yeast RNA (10 μg) or polysome-associated RNA (5 μl per fraction) in 30 μl of 1× hybridization buffer [40 mM PIPES at pH 6.5, 0.4 mM NaCl, 1 mM EDTA, 20% deionized formamide] containing 5×10⁶ cpm of antisense riboprobes. The mixture was boiled for 4 min and transferred immediately to a 45°C heating block for 13–15 hr hybridization. Following hybridization, 350 μl of ribonuclease digestion buffer [10 mM Tris-Cl at pH 7.5, 300 mM NaCl, 5 mM EDTA] containing ribonuclease A [1.4 μg/ml, Sigma] and ribonuclease T1 (0.1 μg/ml, U.S.Biochemical) were added and the mixture incubated for 45 min at 30°C, PCI extracted, and ethanol precipitated. The samples were separated by electrophoresis on an 8% polyacrylamide/7 M urea gel at 600 V (17–25 mA) for 6 hr.

**Acknowledgments**

We thank Allan Jacobson, Alan Sachs, and John Warner for providing plasmids and technical advice, Allan Jacobson, Roy Parker, and Alan Sachs for stimulating discussions and Mark Dumont, Jay Greenberg, Allan Jacobson, Lasse Lindahl, Terry Platt, Jan Zengel, and the members of our laboratory for comments on the manuscript. This work was supported by a U.S. Public Health Service predoctoral training grant in microbial pathogenesis [5-T32-AI070362] to A.P. and National Science Foundation grants [DMB-9005602 and MCB-931664] awarded to J.S.B.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

**References**

Bachmova, R.F. 1992. A maternal tail of poly[A]: The long and the short of it. *Cell* 69: 895–897.

Bairn, S.B. and F. Sherman. 1988. mRNA structures influencing translation in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 8: 1591–1601.

Ballantine, I.E.M. and H.R. Woodland. 1985. Polyadenylation of histone mRNA in Xenopus oocytes and embryos. *FEBS Lett.* 180: 224–228.

Beclman, C.A. and R. Parker. 1994. Differential effects of translational inhibition in cis and in trans on the decay of the unstable yeast *MFA2* mRNA. *J. Biol. Chem.* 269: 9687–9692.

Bergmann, J.E. and H.F. Lodish. 1979a. Translation of capped and uncapped vesicular stomatitis virus and retrovirus mRNAs. *J. Biol. Chem.* 254: 459–468.

——. 1979b. A kinetic model of protein synthesis: Application to hemoglobin synthesis and translational control. *J. Biol. Chem.* 254: 11927–11937.

Bernstein, F., S.W. Peitz, and J. Ross. 1989. The poly(A)-poly(A)-binding protein complex is a major determinant of mRNA stability in vitro. *Mol. Cell. Biol.* 9: 659–670.

Brawerman, G. 1981. The role of the poly(A) sequence in mammalian messenger RNA. *CRC Crit. Rev. Biochem.* 10: 1–39.

Burd, C.G., E.L. Matunis, and G. Dreyfuss. 1991. The multiple binding protein complex is a major determinant of mRNA stability in vitro. *Mol. Cell. Biol.* 11: 3419–3424.

Decker, C.J. and R. Parker. 1993. A turnover pathway for both stable and unstable mRNAs in yeast: Evidence for a requirement for deadenylation. *Genes & Dev.* 7: 1632–1643.

Drummond, D.R., J. Armstrong, and A. Colman. 1985. The effect of capping and polyadenylation on the stability, movement and translation of synthetic mRNAs in X. laevis. *Nu...
Proweller and Butler

cleic Acids Res. 13: 7375–7394.
Foiani, M., A.M. Cigan, C.J. Paddon, S. Harashima, and A.G. Hinnebusch. 1991. GCD2, a translational repressor of the GCN4 gene, has a general function in the initiation of protein synthesis in Saccharomyces cerevisiae. Mol. Cell. Biol. 11: 3203–3216.
Galili, A., E.E. Kawata, L.D. Smith, and B.A. Larkin. 1988. Role of 3’-pol(A) sequence in translational regulation of mRNAs in X. laevis oocytes. J. Biol. Chem. 263: 5764–5770.
Gallie, D.R. 1991. The cap and pol(A) function synergistically to regulate mRNA translational efficiency. Genes & Dev. 5: 2108–2116.
Groner, B., N. Hynes, and S. Phillips. 1974. Length heterogeneity in the polyadenylic acid region of yeast mRNA. Biochemistry 13: 5378–5383.
Grossi da Sa, M-F., M. Standart, C. Martins de Sa, O. Akhayat, M. Huesca, and K. Scherrer. 1988. The poly(A)-binding protein facilitates in vitro translation of poly(A)-rich mRNA. Eur. J. Biochem. 176: 521–526.
Herrick, D., R. Parker, and A. Jacobson. 1990. Identification and comparison of stable and unstable mRNAs in Saccharomyces cerevisiae. Mol. Cell. Biol. 10: 2269–2284.
Hsu, C.L. and A. Stevens. 1993. Yeast cells lacking 5’-3’ exonibonuclease 1 contain mRNA species that are poly(A) deficient and partially lacking the 5’ cap structure. Mol. Cell. Biol. 13: 4826–4835.
Hynes, N.E. and S.L. Phillips. 1976. Turnover of polyadenylate-containing sequences in Saccharomyces cerevisiae. J. Bacteriol. 125: 595–600.
Jackson, R.J. 1993. Cytoplasmic regulation of mRNA function: The importance of the 3’ untranslated region. Cell 74: 9–14.
Jackson, R.J. and N. Standart. 1990. Do the poly(A) tail and 3’ untranslated region control mRNA translation? Cell 62: 15–24.
Jacobson, A. and M. Favreau. 1983. Possible involvement poly(A) in protein synthesis. Nucleic Acids Res. 11: 6353–6368.
Larimer, F.W., C.L. Hsu, M.K. Maupin, and A. Stevens. 1992. Characterization of the XRN1 gene encoding a 5’-3’ exonibonuclease: Sequence data and analysis of disparate protein and mRNA levels of gene disrupted yeast cells. Genes Dev. 120: 51–57.
Laskey, R.A., A.D. Mills, J.B. Gurdon, and G.A. Partridge. 1977. Protein synthesis in oocytes of Xenopus laevis is not regulated by the supply of messenger RNA. Cell 11: 345–351.
Lemay, G. and S. Milward. 1986. Inhibition of translation by free polyadenylic acid: Differences in sensitivity among different mRNAs and possible involvement of an initiation factor. Arch. Biochem. Biophys. 249: 191–198.
Lowell, J.E., D.Z. Rudner, and A.B. Sachs. 1993. 3’ UTR–dependent deadenylation by the yeast poly(A) nuclease. Genes & Dev. 6: 2088–2099.
Martin, T.E. 1973. A simple general method to determine the proportion of active ribosomes in eukaryotic cells. Exp. Cell Res. 80: 496–498.
McGrew, L.L., E. Dworkin–Rast, M.B. Dworkin, and J.D. Richter. 1989. Poly(A) elongation during Xenopus oocyte maturation is required for translational recruitment and is mediated by a short sequence element. Genes & Dev. 3: 803–815.
Mellor, J., M.J. Dobson, N.A. Roberts, M.F. Tuite, J.S. Emtage, S. White, P.A. Lowe, T. Patel, A.J. Kingsman, and S.M. Kingsman. 1983. Efficient synthesis of enzymatically active calf chymosin in Saccharomyces cerevisiae. Genes Dev. 24: 1–14.
Muhlrad, D. and R. Parker. 1992. Mutations affecting stability and deadenylation of the yeast MFA2 transcript. Genes & Dev. 6: 2100–2111.
Munroe, D. and A. Jacobson. 1990. mRNA poly(A) tail: A 3’ enhancer of translation initiation. Mol. Cell. Biol. 10: 3441–3455.
Patel, D. and J.S. Butler. 1992. Conditional defect in mRNA 3’ end processing caused by a mutation in the gene for poly(A) polymerase. Mol. Cell. Biol. 12: 3297–3304.
Richter, J. 1991. Translational control during early development. BioEssays 13: 179–183.
Rosenthal, E.T., T.R. Tansey, and J.V. Ruderman. 1983. Sequence-specific adenylations and deadenylations accompany changes in the translation of maternal messenger RNA after fertilization of pisicula oocytes. J. Mol. Biol. 166: 309–327.
Sachs, A.B. and R.W. Davis. 1989. The poly(A) binding protein is required for poly(A) shortening and 60S ribosomal subunit dependent translation initiation. Cell 58: 857–867.
Sachs, A.B., M.W. Bond, and R.D. Kornberg. 1986. A single gene from yeast for both nuclear and cytoplasmic polyadenylate-binding proteins: Domain structure and expression. Cell 45: 827–835.
Sachs, A.B., R.W. Davis, and R.D. Kornberg. 1987. A single domain of yeast poly(A)-binding protein is necessary and sufficient for RNA binding and cell viability. Mol. Cell. Biol. 7: 3268–3276.
Sadhale, P.P., R. Sapolsky, J.S. Butler, R.W. Davis, and T. Platt. 1991. Polymerase chain reaction mapping of yeast GAL7 mRNA polyadenylation sites demonstrates that 3’ end processing in vitro faithfully reproduces the 3’ ends observed in vivo. Nucleic Acids Res. 19: 3683–3688.
Sheets, M.D., C.A. Fox, T. Hunt, G. Van de Woude, and M. Wickens. 1994. The 3’ untranslated regions of c-mos and cyclin mRNAs stimulate translation by regulating cytoplasmic polyadenylation. Genes & Dev. 8: 926–938.
Storti, R.V., M.P. Scott, A. Rich, and M.L. Pardue. 1980. Translational control of protein synthesis in response to heat shock in D. Melanogaster cells. Cell 22: 825–834.
Varum, S.M. and W.M. Wormington. 1990. Deadenylation of maternal mRNAs during Xenopus oocyte maturation does not require specific cis-acting sequences: A default mechanism for translational control. Genes & Dev. 4: 2270–2286.
Vassalli, J.-D., J. Huarte, D. Belin, P. Gubler, A. Vassalli, M.L. O’Connell, L.A. Parton, R.J. Rickles, and S. Strickland. 1989. Regulated polyadenylation controls mRNA translation during meiotic maturation of mouse oocytes. Genes & Dev. 3: 2163–2171.
Wickens, M. 1992. Forward, backward, how much, when: Mechanisms of poly(A) addition and removal and their role in early development. Sem. Dev. Biol. 3: 399–412.
Wisdom, R. and W. Lee. 1991. The protein coding region of c-myc mRNA contains a sequence that specifies rapid mRNA turnover and induction by protein synthesis inhibitors. Genes & Dev. 5: 233–243.
Yoon, H. and T.F. Donahue. 1992. The sul1 suppressor locus in Saccharomyces cerevisiae encodes a translation factor that functions during tRNA24 recognition of the start codon. Mol. Cell. Biol. 12: 248–260.
Yoon, H., S.P. Miller, E.K. Pabich, and T.F. Donahue. 1992. SSI1, a suppressor of HIS4 5’-UTR stem-loop mutation, is essential for translation initiation and affects UV resistance in yeast. Genes & Dev. 6: 2463–2477.
Zelus, B.D., D.H. Giebelhaus, D.W. Eib, K.A. Kenner, and R.T. Moon. 1989. Expression of the poly(A)-binding protein during development of X. laevis. Mol. Cell. Biol. 9: 2756–2760.
Zhong, T. and K.T. Arndt. 1993. The yeast SIS1 protein, a DnaJ homolog, is required for the initiation of translation. Cell 73: 1175–1186.
Efficient translation of poly(A)-deficient mRNAs in Saccharomyces cerevisiae.

A Proweller and S Butler

*Genes Dev.* 1994, 8:
Access the most recent version at doi:10.1101/gad.8.21.2629