Ribosome Concentration Contributes to Discrimination against Poly(A)$^-$ mRNA during Translation Initiation in Saccharomyces cerevisiae

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Aaron Proweller and J. Scott Butler‡
From the Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Inactivation of Saccharomyces cerevisiae poly(A) polymerase in a strain bearing the temperature-sensitive lethal pap1-1 mutation results in the synthesis of poly(A)$^-$ mRNAs that initiate translation with surprising efficiency. Translation of poly(A)$^-$ mRNAs after polyadenylation shut-off might result from an increase in the ratio of ribosomes and associated translation factors to mRNA, caused by the inability of poly(A)$^-$ mRNAs to accumulate to normal levels. To test this hypothesis, we used ribosomal subunit protein gene mutations to decrease either 40 or 60 S ribosomal subunit concentration in strains carrying the pap1-1 mutation. Polyadenylation shut-off in such cells results in a nearly normal ratio of ribosomes to mRNA as revealed by polyribosome sedimentation analysis. Ribonuclease protection and Northern blot analyses showed that a significant percentage of poly(A)-deficient and poly(A)$^-$ mRNA associate with smaller polyribosomes compared with cells with normal ribosome levels. Analysis of the ratio of poly(A)-deficient and poly(A)$^-$ forms of a specific mRNA showed relatively more poly(A)$^-$ mRNA sedimenting with 20–60 S complexes than do poly(A)$^+$ forms, suggesting a block in an early step of the translation initiation of the poly(A)$^-$ transcripts. These findings support models featuring the poly(A) tail as an enhancer of translation and suggest that the full effect of a poly(A) tail on the initiation strength of a mRNA may require competition for a limited number of free ribosomes or translation factors.

Virtually all known eukaryotic precursor mRNAs undergo processing to mature forms by a series of intranucleolar posttranscriptional modifications including intron removal (splicing), 5′-end capping, 3′-end cleavage and polyadenylation, and in some cases, coding sequence editing. Although normal splicing and editing ensure appropriate coding information, both end modifications have a particular impact on regulating expression levels of mature mRNA. Numerous studies have shown that cap structures serve as recognition motifs for translation initiation factors required for protein synthesis (reviewed in Ref. 1). The consistent finding of a poly(A) tract generated after site-specific cleavage in the mRNA 3′-untranslated region has led to numerous investigations whose results suggest functions for poly(A) tails in mRNA stability and translation (2). Recently, evidence for a mRNA turnover pathway in Saccharomyces cerevisiae and mammalian cells specifically implicates poly(A) tail trimming (deadenylation) as the first and sometimes rate-determining step in the degradation of some mRNAs (3–7).

The debate about the importance of poly(A) tails for mRNA translation reflects an attempt to combine experimental observations in systems in vitro including wheat germ extracts, rabbit reticulocyte lysates, and S. cerevisiae extracts (8–11) and in living cells such as Xenopus laevis oocytes, HeLa cells, and S. cerevisiae (12–17). In general, the in vitro studies demonstrated discrimination against the translation of poly(A)-deficient or poly(A)$^-$ input mRNAs; however, the extent of reduced translatability was modest, possibly reflecting an extract-dependent capacity for multiple rounds of translation initiation (i.e. reinitiation) as well as the degree of saturation of the translational machinery by endogenous mRNAs. In contrast, some in vivo studies showed dramatic enhancement of the translation of an mRNA after poly(A) tail addition. In these cases it is unclear whether the injection of engineered mRNAs into the cell cytoplasm might lead to mislocalization of the mRNA and perhaps bypass critical associations with factors normally participating in translational regulation. Indeed, mRNA expressed from a plasmid injected into the nucleus of a X. laevis oocyte enters the cytoplasm but is translationally inactive, whereas the same mRNA directly injected into the cytoplasm is translationally competent (18).

Perhaps the most striking evidence for a translational function for poly(A) tails comes from developmental studies in frog, mouse, and clam oocytes (19–24). Translational activation of preexisting maternal mRNAs carrying, at most, short poly(A) tails constitutes a common feature during stages of oocyte maturation and postfertilization. Competing protein interactions or possibly the absence of various protein factors required to facilitate translation may mask these mRNAs from the translational apparatus (reviewed in Ref. 25). Regulated extension of their poly(A) tails causes these mRNAs to become translationally active. In some cases, the mere presence of a long 3′ poly(A) tract signals recruitment, but more recent evidence suggests that the catalytic act of polyadenylation may trigger cap methylation, which, in turn, activates translation of some maternal mRNAs (26). Additional postfertilization studies in slime molds and sea urchins also revealed an increased translational capacity of many mRNAs subsequent to the poly(A) addition (27, 28). These polyadenylated mRNAs displayed a competitive advantage over their poly(A)-deficient counterparts for assembly into polyribosomes, suggesting that poly(A)
tails might increase the probability of translation initiation and, more specifically, ribosomal subunit reinitiation.

Current mechanistic models of poly(A) tail function feature enhancement of an early step in translation initiation. For example, Sachs and Davis (29) observed that a yeast strain depleted of essential poly(A) binding protein (Pab1p) exhibits a larger pool of free 80 S ribosomes as well as an increase in the free 60 S to 40 S subunit ratio, suggesting a block in the 60 S subjoining step during translation initiation (29). Suppressor mutations (spb2) that restored viability of these cells provided further evidence for a role for Pab1p in translation (29). The spb6 mutations decrease the ratio of 60 S to 40 S subunits, and alter 60 S assembly as in the case of spb2, a mutation in the single copy, large subunit ribosomal protein gene, L46 (RPL46). These observations provided a genetic link between poly(A) metabolism and the ribosome and supported a role for Pab1p as a mediator of a poly(A) tail function.

Experiments in rabbit reticulocyte lysates revealed a 2–3-fold greater translational activity of poly(A)→ to poly(A)− mRNA, a difference that apparently resulted from decreased ability of poly(A)− mRNAs to bind 60 S ribosomes during initiation (30). These findings provided part of the basis for the “closed loop model” for poly(A) function in translation, which features enhancement of 60 S ribosome subunit joining by the poly(A)-Pab1p complex (2). Tarun and Sachs (11) recently presented evidence that Pab1p enhances the interaction of poly(A)−, but not poly(A)− mRNAs, with the 40 S ribosomal subunit. Although these experiments could not assess the role of 60 S subunit joining, they do provide a rationalization for the observed increase in 40 S to 60 S ratios in all spb6 mutants, since an increase in 40 S levels might bypass the need for poly(A)-Pab1p at this step.

Our laboratory recently showed that yeast cells with a conditional mutation (pap1–1) in the poly(A) polymerase gene accumulated poly(A)−-deficient and poly(A)− mRNA following inactivation of the enzyme by cell growth at a nonpermissive temperature (35 °C) (31). A 1-h incubation at 35 °C reduced the total amount of mRNA approximately 2-fold, consistent with an important role for poly(A) tails in mRNA stability. Surprisingly, we found that some poly(A)− mRNAs associated with at least the same number of ribosomes as in wild-type cells, suggesting that poly(A)− mRNAs initiate translation efficiently under these conditions. The polyribosome profiles from these cells revealed that the reduction in mRNA levels generated a relatively large excess of free ribosomes, which could possibly compensate for the low efficiency of translation of poly(A)− mRNAs. Here we report experiments designed to test the hypothesis that discrimination against poly(A)− mRNAs during translation initiation requires competition for a limiting number of ribosomes. We effectively reduced the number of ribosomes in pap1–1 cells by deleting selected nonessential ribosomal protein genes. Inactivation of polyadenylation in these ribosome-deficient cells produced poly(A)− mRNAs that translate less efficiently than in cells with normal ribosome levels. Moreover, discrimination against poly(A)− mRNAs occurred regardless of whether we limited 40 or 60 S subunits, consistent with a poly(A)-dependent component to the binding of each ribosomal subunit.

EXPERIMENTAL PROCEDURES

Yeast Strains, Ribosomal Protein Deletion Derivatives, and Media—UR3148–1B (MATα, ade1/ade2, lys2, gal11, ura3-52, pap1–1) cells were transformed with a BglII-EcoRI fragment from the plasmid pAS195 containing the RPL46 gene sequence partially replaced by the URA3 gene to generate an RPL46 deletion strain, pap1–1, rpl46Δ (YAP201). UR3148–6A (MATα, leu2-3, gal11, ura3-52, pap1–1) cells were used to construct strains bearing deletions in ribosomal protein genes RPL16B or RPS51A. A HindIII-SalI fragment from plasmid pGOBLEU2 (kindly provided by M. Rosbash, Brandeis University; Ref. 32) containing a partial deletion of RPS51A was incorporated into the UR3148–6A genome by homologous recombination to generate the strain pap1–1, rps51AΔ (YAP301). Likewise, a HindIII-BamHI fragment from plasmid pL16BALK2 (kindly provided by J. Woolford, Carnegie-Mellon University; Ref. 33) was used to replace the chromosomal RPL16B gene with a deleted allele following electrot transformation of UR3148–6A cells to generate the strain pap1–1, rpl16BΔ (YAP401). Conversion to leucine prototrophy by accurate homologous gene replacements in pap1–1, rps51AΔ and pap1–1, rpl16BΔ strains was confirmed by Southern blotting (data not shown). All strains were propagated in YEPD (2% dextrose) media.

Polyribosome and RNA Analysis—Yeast polyribosome lysates were prepared by glass bead lysis and 25 A260 units layered onto 12-mL low salt sucrose (15–50%) gradients as described previously (34). Following 4°C centrifugation at 40,000 rpm (Beckman SW40Ti rotor) for 2.5 h, gradient fractions (20 × 0.6 ml) were collected while recording an A260 polyribosome profile by continuous flow measurement using an ISCO model UA-5 absorbance/fluorescence monitor. Isolation of total RNA from polyribosome gradient fractions and subsequent ribonuclease protection and Northern analyses were carried out as described previously (31). Note that for each gradient RNA fractions 18, 19, and 20 were pooled and are proportionally represented in fraction 18 in the experiments illustrated here. All quantitation of the sedimentation pattern of specific mRNAs was done by storage PhosphorImager (Molecular Dynamics) analysis of the ribonuclease protection gels and Northern blots.

RESULTS

Rationale—To test the hypothesis that the ratio of ribosomes to mRNA might play an important role in discrimination against poly(A)− mRNAs during translation initiation, we sought to reduce ribosome levels in pap1–1 cells to parallel the loss of mRNA resulting from inactivation of poly(A) polymerase (Pap1p). Normal ribosome subunit assembly requires certain ribosomal proteins whose loss reduces the levels of the mature subunits (Refs. 33–37; reviewed in Ref. 38). This effect is usually specific for the ribosomal subunit, thus allowing alteration of the levels of one subunit without significantly affecting the production of the other (39). Accordingly, deletion of certain nonessential ribosomal protein genes or one copy of a duplicated ribosomal protein gene results in a decrease in the amount of one of the two ribosomal subunits and, in effect, the number of 80 S ribosomes available for translation. The ability to specifically decrease 60 or 40 S levels allowed us to monitor the impact of limiting subunit concentrations on translation of poly(A)− mRNA.

Limiting 60 S Ribosome Levels Causes Translational Discrimination against Poly(A)− mRNAs—Our initial attempt at reducing the effective concentration of ribosomes was to delete pap1–1 cells of the nonessential ribosomal protein L46 (Rpl46p) in order to lower the number of free 60 S ribosomal subunits (40). Analysis of total RNA from a pap1–1, rpl46Δ strain showed a 30–40% reduction in the 25 S to 18 S RNA ratio (data not shown) consistent with a requirement for Rpl46p in normal processing of 28 S rRNA. The deletion of the single copy RPL46 gene leads to the production of lower than normal amounts of ribosomes that lack ribosomal protein L46. Moreover, RPL46 mutations (spb2) bypass poly(A)-binding protein (PAB1) mutations, suggesting a role for Rpl46p in the translational function of poly(A) tails (29). Therefore, we constructed a second pap1–1 derivative by deleting RPL16B, one of the duplicated genes encoding Rpl16p (38). Deletion of only one of two copies of RPL16 allowed us to lower the 60 S subunit levels while producing ribosomes with a normal complement of ribosomal proteins (35, 36, 39).

We assessed the impact of a RPL46 or RPL16B deletion on overall mRNA translation in pap1–1 cells by polyribosome profile analyses. In poly(A)+ cells (pap1–1, 25 °C) we observed (i) a decrease in the height of the largest polyribosome peaks and an increase in the smallest polyribosome peaks suggestive of a decrease in the number of ribosomes per mRNA, (ii) the pres-
Ribosome Concentration Affects Poly(A)-deficient mRNA Translation

The discrimination against poly(A)− mRNA during translation initiation implies that it competes with other poly(A)+ mRNAs. Indeed, the normal shapes of the polyribosome profiles in pap1−1; pap1−1,rpl46Δ; and pap1−1,rpl16BΔ cells shifted to 35°C suggests that some mRNAs translate efficiently despite the ribosome deficiencies. We reasoned that polyadenylation shut-off should have a more modest impact on mRNAs with long half-lives than on those with short half-lives, since a smaller fraction of the former will have been synthesized as poly(A)− transcripts in the hour between inactivation of poly(A) polymerase and harvest of the cells for polyribosome analysis. Accordingly, we monitored the polyribosome density of PGK1 (t½ = 45 min) and PAB1 (t½ = 11 min), which have lengths similar to TCM1 (t½ = 11 min; Ref. 41). We showed previously that, at 35°C in a pap1−1 background, PAB1 mRNA exists without detectable poly(A) tails, while PGK1 mRNA exists as a mixture of mostly poly(A)− and poly(A)+ species as determined by oligo(dT) cellulose selection (31). FIG. 4, A and B, shows the percentage distribution of PAB1 mRNA (upper panels) and PGK1 mRNA (lower panels) in pap1−1,rpl46Δ (35°C) and pap1−1,rpl16BΔ (35°C) strains, respectively. The results indicate that both mRNAs exhibit a reproducible shift toward smaller polyribosomes in ribosome-deficient cells (Fig. 4, A and B, lower panels). The impact of ribosome reduction on PAB1 mRNA translation is similar to TCM1 mRNA, which also has a short half-life (10−15 min) and competes less well for ribosomes (compare Figs. 2, A and B, and Fig. 4, A and B). Taken together, these results suggest that the translational apparatus discriminates against poly(A)− mRNA when the ratio of mRNA to ribosomes is normalized in pap1−1 cells. In addition, the presence of large polyribosome complexes in the deletion strains most probably reflects the population of
poly(A)$^+$ mRNA produced before or soon after inactivation of poly(A) polymerase.

Deletion of the Small Subunit Ribosomal Protein Gene, RPS51A, Reduces 80 S Ribosome Levels and Causes Translational Discrimination against poly(A)$^-$ mRNAs—The decrease in translation efficiency of poly(A)$^-$ mRNAs resulting from a reduction in the number of 60 S ribosomal subunits supports the idea that discrimination between poly(A)$^+$ and poly(A)$^-$ mRNAs occurs at the 60 S joining step during translation initiation (2, 30). On the other hand, Tarun and Sachs (11) presented the results of experiments that suggested a role for poly(A) tails in enhancing mRNA interaction with 40 S ribosomes. If poly(A)$^+$ mRNAs compete more effectively than poly(A)$^-$ mRNAs for 40 S ribosome binding, then limitation of 40 S levels should decrease the rate of initiation of poly(A)$^-$ mRNAs. We tested this hypothesis by deleting one of the du-
plicated copies of the gene (RPS51A) encoding the small ribosomal subunit protein, Rps51p (32, 34). Polyribosome profile analyses of a RPS51A deletion strain reveals a similar decrease in polyribosome density observed for the larger ribosomal subunit deletions, but with a large decrease in the number of free 40 S subunits and a parallel increase in the number of 60 S subunits (Fig. 1G). Inactivation of Pap1p causes an increase in average polyribosome size consistent with an increase in the ratio of ribosomes to mRNA. We monitored the translational efficiency of TCM1, PAB1, and PGK1 mRNAs as for the 60 S subunit deletion strains. Limitation of 40 S ribosomes causes a fraction of TCM1 mRNA to associate with the smallest of polyribosome complexes (Fig. 2C, upper panel) and increases the ratio of poly(A)− to poly(A)-deficient TCM1 in fractions containing 48 S, 43 S, and mRNP complexes (Fig. 3C). Comparison of Northern blots of polyribosome gradient fractions from poly(A)-deficient cells with normal 40 S ribosome levels (pap1−1) and those with decreased 40 S levels (pap1−1,rps51AΔ) reveals a decrease in the amount of PAB1 mRNA in large polyribosomes, while PGK1 mRNA remains associated with heavy polyribosomes (Fig. 4C). The impact of the RPS51A deletion on these mRNAs is not as dramatic as seen in pap1−1,rpl46Δ or pap1−1,rpl16BΔ strains. This may reflect the degree of reduction of ribosome levels in the various strains, or it may suggest that 40 S ribosomes do not play as important a role in enhancing translation of poly(A)− mRNAs as 60 S subunits.

DISCUSSION

We utilized a yeast strain harboring a temperature-sensitive mutation (pap1−1) in the poly(A) polymerase gene to monitor the chemical and functional properties of poly(A)-deficient and poly(A)− mRNAs. We demonstrated previously that inactivation of Pap1p resulted in the inability of cells to accumulate poly(A)− mRNAs (34). Many mRNAs also fail to accumulate as poly(A)− species, consistent with a role for poly(A) tails in the synthesis and maintenance of stable mRNA (4, 7). The poly(A)-deficient and poly(A)− mRNA that accumulate in pap1−1 (35°C) cells associate with polyribosomes of at least the same size as those observed in wild-type (PAP1) cells, suggesting that poly(A) tails are not required for efficient translation. However, our results also indicated that mRNA loss in poly(A)-deficient cells resulted in a significant increase in the ratio of ribosomes to mRNA. We suggested that the excess of translational components such as ribosomes and their associated factors might overcome any mechanism for translational discrimination against poly(A)-deficient or poly(A)− mRNA. Hence, we tested whether discrimination against translation of poly(A)− mRNA would occur in poly(A)-deficient cells with a normal ratio of mRNA to ribosomes. Our results show that deletions of ribosomal protein genes RPL46, RPL16B, or RPS51A in pap1−1 cells results in a nearly normal ratio of ribosomes to mRNA after polyadenylation shut-off. Under these conditions, poly(A)− TCM1 and PAB1 mRNAs form smaller polyribosomes, suggesting that they may not compete for ribosomes as well as...
existing polyadenylated mRNAs. Candidates for the latter mRNAs include relatively stable, polyadenylated messages (half-life > 40 min), the bulk of which remain after 1 h at 35 °C. We monitored the polyribosome sedimentation pattern of PGK1 mRNA (t½ ~ 45 min) and found that a large portion of it exists in polyribosomes comparable in size with those found in cells with normal ribosome levels. Finally, our ability to distinguish between poly(A)+ and poly(A)- forms of TCMI mRNA allowed us to show that the poly(A)- forms appear to exhibit a defect in entering polyribosomes.

Taken together, the results from both large and small ribosomal protein-deleted pap1–1 strains indicate that, in vivo, poly(A) tails may enhance the translation of mRNA most effectively when the ratio of total mRNA to ribosomes approximates the levels observed in normal cells at steady state. Under these conditions, we observe discrimination against the translation of poly(A)- TCMI, and PAB1 mRNAs and a competitive advantage favoring the translation of poly(A)+ TCMI and PGK1 mRNAs. The fractions in the nonpolyribosomal portions sedimenting lighter than 60 S, but heavier than approximately 20 S, display the most significant difference in the A-/A+ TCMI mRNA ratio. This region encompasses free 40 S ribosomal subunits and associated preinitiation complexes (i.e., 43 and 48 S), suggesting inefficient formation of 80 S ribosomes (i.e., 60 S subunit joining) by poly(A) TCMI. Since we do not observe significant differences in the A-/A+ TCMI mRNA ratio in smaller polyribosome fractions, it might be that the partial block in 60 S subunit joining occurs during the loading of the very first ribosome, while subsequent ribosomes (and subunits) escape the inhibition, allowing for the formation of relatively large polyribosome complexes. Alternatively, TCMI mRNA bearing short poly(A) tails (A < 20) may have only a modest competitive advantage in translation compared with poly(A)+ mRNA. We could compare the polyribosome distribution of poly(A)- TCMI mRNA with its full-length poly(A) tail (A = 60–70) counterpart, the A-/A+ ratio might be significantly lower in the polyribosome fractions. A link between poly(A) tails and the translation machinery was proposed more than 2 decades ago, but the biochemical nature of the interaction remains obscure. Evidence that poly(A) tails enhance the translational efficiency (i.e. functional half-life) of a mRNA came from several in vitro and in vivo experiments comparing the rate or amount of polyribosome formation of poly(A)+ mRNA with its poly(A)- form in programmed rabbit reticulocyte lysates or following injection of synthetic mRNAs into X. laevis oocytes. For instance, Gallati et al. (14) provided evidence that in stage VI Xenopus oocytes, injected poly(A)+ and poly(A)- zein mRNAs did not compete for the binding of the first ribosome, yet only poly(A)+ mRNA associated with large polyribosome complexes after further incubation. In this system, the poly(A)- mRNA was as stable as its poly(A)+ form, leading to the suggestion that poly(A) tails enhance, in cis, the reinitiation of ribosome loading at the 5′-end of mRNA. One important feature of this experiment was that stage VI Xenopus oocytes have a saturated translational capacity, and it remains unclear whether discrimination against poly(A)-deficient mRNAs would be found at other stages of development where the translational machinery is in excess of substrate.

In experiments employing rabbit reticulocyte lysates, Munroe and Jacobson (30) found that only 50% of input poly(A)+ mRNA associated with polyribosomes compared with its poly(A)- counterpart and provided evidence that the decreased efficiency of poly(A)- mRNA translation resulted from a reduced ability to couple 60 S ribosomal subunits to 48 S preinitiation complexes (30). In this system, endogenous mRNA re-moved by nuclease treatment yielded an excess of ribosomes and translation factors compared with the levels of input mRNAs. Although translational discrimination was observed, the competitive advantage of poly(A)+ mRNA might have been greater if the translational apparatus was saturated with mRNA. Indeed, Gallié showed that a poly(A) tail increased translation of a reporter mRNA 40-fold in yeast protoplasts, which presumably translate a full complement of cellular mRNAs (15).

The extent to which the translational machinery in normal S. cerevisiae is saturated by mRNA is unclear. In Escherichia coli, induction of mRNA synthesis from multicopy plasmids decreases the translation of preexisting mRNAs by competition for limiting translation components (42). A similar effect is observed in oocytes of X. laevis, where the injection of exogenous mRNA decreased translation of cellular mRNAs at all concentrations of injected mRNA (43). Furthermore, when polyribosome complexes were introduced into these same oocytes, no effect on endogenous protein synthesis was observed, suggesting that a limiting component for translation is polyribosome-associated (43). In our system, 35 °C-shifted pap1–1 cells carrying deletions in ribosomal proteins appear to have a normal ratio of ribosomes to mRNA. The existing ribosomes and associated translation factors participate in discrimination against poly(A)-deficient mRNAs unlike in a pap1–1 (35 °C) strain, where ribosome levels are in excess of substrate mRNAs. Since reducing the numbers of 60 or 40 S subunits effectively lowers ribosome concentration and in turn promotes translational discrimination, one might speculate that the levels of intact ribosomes alter the abundance or function of an associated discriminatory factor.

The ability of poly(A) tails to enhance translation might be mediated by the capacity of poly(A) to recruit poly(A)-binding protein and in turn engage the translational apparatus in some undetermined manner. Poly(A)-binding protein reverses the inhibitory effect of excess poly(A) added to reticulocyte lysates, suggesting that poly(A)-binding protein is a titratable factor that may have a role in translation (14). Sachs and Davis (29) provided evidence that yeast cells harboring a temperature-sensitive lethal allele of PAB1 (pab1-1/pab1-3641) have defects in poly(A) tail shortening and translation initiation (29). Interestingly, this mutation also gives rise to an increased ratio of free 60 S to 40 S ribosomal subunits. Several cold-sensitive suppressors of the pab1-1/pab1-3641 mutant, including one (spb2) bearing a mutation in ribosomal protein L46, display an inversion of the free subunit ratio but do not restore normal poly(A) tail lengths (29). Improved growth in spb2 cells is therefore linked to better translation of existing mRNAs by alteration of 60 S subunit levels. These observations led to the suggestion that Pab1p is intimately linked to the ribosome via the 60 S subunit, a notion consistent with recent work from our laboratory demonstrating a Pab1p association with polyribosomes translating poly(A)-deficient or poly(A)- mRNAs (40).

Recently, Tarun and Sachs (11) used a S. cerevisiae in vitro translation system to monitor the translational potential of synthetic poly(A)+ versus poly(A)- mRNA in the presence or absence of poly(A)-binding protein (Pab1p). The results of their experiments suggested that Pab1p enhances the binding of poly(A)+ mRNA to 40 S ribosomal subunits, implying that the Pab1p-poly(A) tail complex serves a role in the translational recruitment of mRNA from mRNPs to active polyribosome complexes. However, these experiments, which employed an inhibitor of 60 S subunit joining could not assess the relative impact of Pab1p on 60 and 40 S subunit binding.

The results presented here indicate that poly(A) tails may confer a small competitive advantage to a mRNA at both the 60
and 40 S binding steps; limitation of either subunit appears to reduce the efficiency of translation of poly(A)− mRNAs more than poly(A)+ mRNAs. Similarly, some, but not all, mutations that reduce ribosomal subunit levels reduce the expression of poly(A)− yeast viral mRNAs (44). In both cases, 60 S subunit changes produced a more pronounced effect, possibly reflecting a more critical role of the 60 S subunit joining step in enhancing translation of poly(A)+ mRNAs. Interestingly, while these studies suggest that limitation of 60 S ribosomal subunit levels inhibits the translation of poly(A)− mRNAs, such changes bypass the need for Pab1p in translation (29, 45). It remains unclear whether this paradox reflects fundamental aspects of Pab1p-poly(A) complex function or whether specific ribosomal alterations may suppress or antagonize different defects in mRNA 3′-end structure. Finally, the Pab1p-poly(A) complex plays a critical role in determining mRNA levels, since Pab1p alterations may suppress or antagonize different defects in Pab1p-poly(A) complex function or whether specific ribosomal alterations may suppress or antagonize different defects in mRNA function before we reach a full understanding of how mRNA structure affects gene expression.

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