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Messenger RNA stability in *Saccharomyces cerevisiae*: the influence of translation and poly(A) tail length

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ABSTRACT

A comparison between the half-lives of 10 specific yeast mRNAs and their distribution within polysomes (fractionated on sucrose density gradients) was used to test the relationship between mRNA translation and degradation in the eukaryote Saccharomyces cerevisiae. Although the mRNAs vary in their distribution across the same polysome gradients, there is no obvious correlation between the stability of an mRNA and the number of ribosomes it carries in vivo. This suggests that ribosomal protection against nucleolytic attack is not a major factor in determining the stability of an mRNA in yeast. The relative lengths of the poly(A) tails of 9 yeast mRNAs were analysed using thermal elution from poly(U)-Sepharose. No dramatic differences in poly(A) tail length were observed amongst the mRNAs which could account for their wide ranging half-lives. Minor differences were consistent with shortening of the poly(A) tail as an mRNA ages.

INTRODUCTION

mRNA half-lives range from about 1 to over 100 minutes in Saccharomyces cerevisiae (1-4). An inverse relationship between mRNA length and half-life (relative to that of 18S ribosomal RNA) has been reported for 15 different yeast sequences (4), suggesting that the length of an mRNA influences its half-life. These 15 mRNA species are clearly divisible into two populations based upon their lengths and half-lives, indicating that at least one factor, in addition to mRNA length, affects mRNA stability in yeast (4). Most yeast mRNAs appear to have a 5'-cap (5,6) and a 3'-poly(A) tail of approximately 50 nucleotides (7,8) and therefore, the presence or absence of these structures is unlikely to account for the existence of two mRNA populations in yeast. Furthermore, the presence of one or other of the two 5'-cap structures found in yeast (5,6) does not appear to correlate with mRNA half-life (Peter Piper, personal communication). However, a significant difference in the length of the 3'-poly(A) tail might provide the basis for the two mRNA populations, since in at least one case, an mRNA carrying a short poly(A) tail seems to be relatively unstable (9). The possible influence of poly(A) tail length upon

mRNA stability in yeast has been investigated in this study.

There is evidence that mRNA translation and degradation are linked. In Escherichia coli the presence of a premature termination codon in the tryptophan operon causes destabilization of the mRNA sequences downstream from the mutation (10,11). Also, rates of translation and degradation of the beta-galactosidase mRNA appear to be linked in streptomycin-dependent mutants of E. coli (12). Furthermore, the addition of antibiotics which inhibit translational elongation seems to increase the stability of E. coli mRNAs (13-16), while inhibitors of initiation reduce their stability (14,16). These observations support the proposal that ribosomes may passively protect an mRNA from degradation during translation (11,16). Similar observations have been made in eukaryotic systems. For example, point mutations in the human beta-globin gene that convert the codons for $lysine_{17}$ or $glycine_{39}$ to a termination codon cause a reduction in the steady-state level of the beta-globin mRNA (17,18). Also, premature termination codons in the URA1 and URA3 genes of S. cerevisiae seem to destabilize their respective mRNAs (19,20). Furthermore, the degree of destabilization of the URA1 and URA3 mRNAs is dependent upon the position of the premature termination codon; the closer the termination codon to the 5'-end of the coding region, the less stable the mRNA (19,20). Therefore, a close relationship between mRNA translation and stability might exist in eukaryotes. This has been tested in this study by analysing the distribution 10 mRNAs on yeast polysomes fractionated by sucrose density gradient centrifugation. The distribution of an mRNA on these polysome gradients is a measure of the number of ribosomes loaded on the mRNA in vivo.

MATERIALS AND METHODS

Strains and Plasmids

The S. cerevisiae strain DBY746 (His3, Leu2, Trp1, Ura3) was used throughout. The cDNA plasmids used as hybridization probes for each of the unknown mRNAs have been described previously (4). The probe for the pyruvate kinase (PYK) mRNA was pSPK2 which contains the 511bp XbaI/BglII fragment from the coding region of PYK (21) cloned into SP64.

RNA Preparation

RNA was prepared using the procedures of Lindquist (22).

Thermal Elution from Poly(U)-Sepharose

Procedures for thermal elution from poly(U)-Sepharose-4B (Pharmacia) were adapted from those described previously (23,24) using the following modifications. 400ug of yeast RNA was loaded onto a 0.5g poly(U)-Sepharose

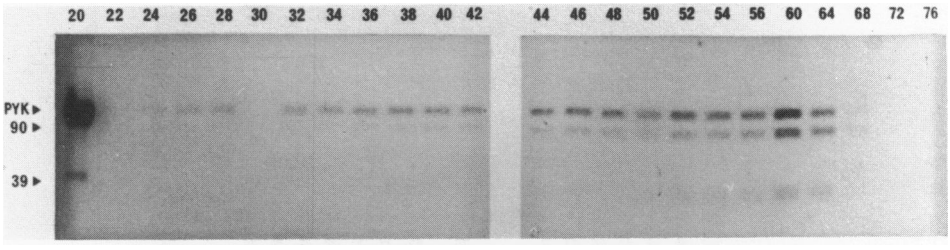

Figure 1: Northern Blot Of Yeast RNA Subjected To Thermal Elution From Poly(U)-Sepharose. RNA from approximately one-fiftieth of each fraction was subjected to northern blotting and probed for three mRNAs simultaneously. The positions of mRNA39, mRNA90 and the PYK mRNA are shown (4); the numbers on each lane indicate the temperature of elution (^{o}C). (There was an error in loading for the 30^{o}C fraction.)

column at 20^{o}C (24). RNA eluted during column loading and washing was pooled in the 20^{o}C fraction. The temperature of the column was then increased in 2^{o}C increments until reaching 56^{o}C, when the temperature increments were increased to 4^{o}C, until a final temperature of 76^{o}C was achieved. 1ml fractions were eluted at each temperature increment and then used to prepare dot blots for mRNA quantitation.

Preparation of Yeast Polysomes

Spheroplasts were prepared using a procedure adapted from Beggs (25), and polysomes then prepared using the methods of Hutchison and Hartwell (26). 500ul of a post-mitochondrial supernatant made from lysed spheroplasts was layered onto a 37ml 10-50% (w/w) sucrose gradient, and the gradient centrifuged at 25,500rpm using an SW28 Beckman rotor for 2.75 hours at 4^{o}C. The gradient was then drawn, from the bottom, using a spectrophotometer (LKB Ultraspec 4050) to monitor continuously the absorbance at 260nm. Fractions, usually of about 700ul, were dripped directly into eppendorf tubes containing 420ul of 20xSSC (4) and 280ul of 37% formaldehyde. Fractions were then heated at 60^{o}C for 15 minutes, and stored at -70^{o}C until used for dot blotting.

Blotting and Hybridization

For northern blotting, RNA was electrophoresed on formaldehyde gels (27), and blotted according to the methods of Thomas (28). 25S, 18S, 5.8S and 5S rRNAs were used as size standards. RNA dot blotting, and nick-translation of plasmid DNA for hybridization probes were performed as described previously (4).

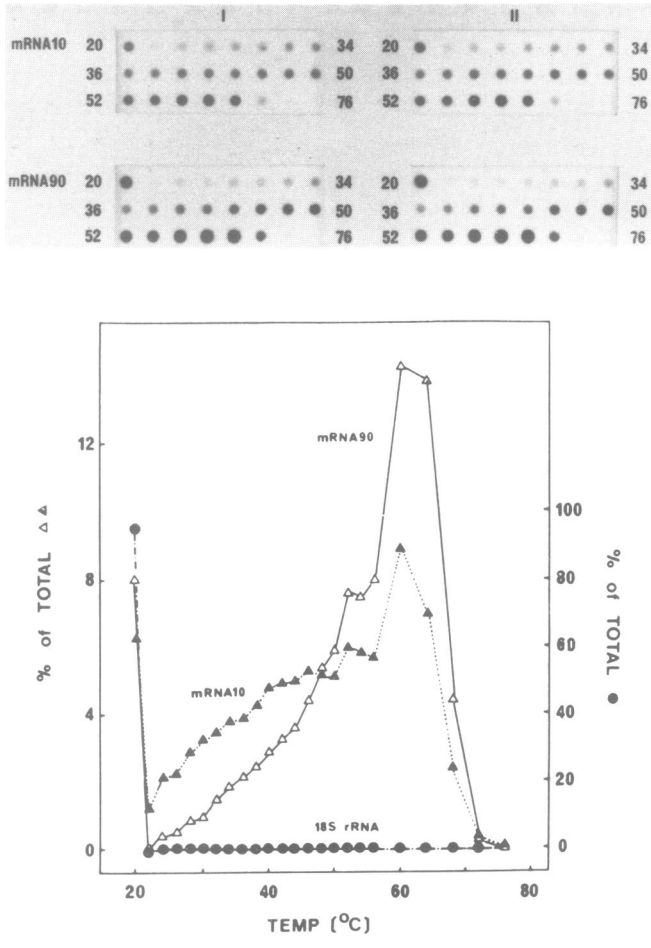

Figure 2: Thermal Elution Of mRNAs From Poly(U)-Sepharose.
(A) Dot blots made using RNA fractions obtained by thermal elution from poly(U)-Sepharose and probed for mRNA10 and mRNA90 are shown. 'I' and 'II' indicate duplicate filters for each mRNA, and the numbers at each side of a filter show the order of dotting (using the temperature of elution in °C). **(B)** The profile of elution for mRNAs 10 and 90, and 18S rRNA, obtained by scintillation counting of each dot (expressed as % of total cpm on each filter).

RESULTS

Poly(A) Tail Length

Thermal elution from poly(U)-Sepharose has been used previously for the analysis of poly(A) tails on eukaryotic mRNAs, since the temperature of elution of an RNA is dependent upon the length of its poly(A) tract (23,24;

Table 1: Thermal Elution Of mRNAs From Poly(U)-Sepharose

Data on mRNA half-lives and lengths taken from Ref. 4. NT = not tested.

mRNA	HALF-LIFE (Minutes)	mRNA LENGTH (Bases)	THERMAL ELUTION (% of total cpm)		
			20°C	30-50°C	55-70°C
90	6.6 +/- 0.67	1,100	8.0	34.3	40.4
100	10.4 +/- 1.1	500		NT	
13	12.1 +/- 1.0	740		NT	
19	15.3 +/- 2.7	550	8.2	31.9	40.7
9	16.5 +/- 1.5	440	6.6	47.9	26.8
11	18.0 +/- 2.2	700		NT	
39	18.3 +/- 1.5	380	4.0	34.6	39.8
82	22.3 +/- 3.1	370	9.6	26.9	44.9
10	56.9 +/- 6.0	1,250	6.2	49.1	24.0
PYK	59.9 +/- 7.8	1,600	6.6	47.9	26.8
74	83.4 +/- 9.2	1,050	21.3	33.8	29.8
46	>100	550	6.4	43.1	29.0
18S rRNA			94.9	2.3	1.3

results not shown). Therefore, the relative lengths of the poly(A) tails for different yeast mRNAs can be compared by analysing their thermal elution profiles from poly(U)-Sepharose. The integrity of the RNA eluted from poly(U)-Sepharose was tested by northern blotting, using probes for three mRNAs simultaneously (Figure 1). The PYK mRNA and mRNAs 39 and 90 appear to be intact across the temperature gradient. A significant proportion of all mRNAs examined was eluted in the 20°C fraction (Figures 1 and 2, Table 1). This could be due either to the complete absence of a poly(A) tail on a proportion of each mRNA, or the presence of a poly(A) tail too short to bind to poly(U)-Sepharose. There was no obvious relationship between the stability of an mRNA and the proportion eluted in this fraction (Table 1).

Fractions obtained by elution of yeast RNA from poly(U)-Sepharose were used to prepare dot blots to quantitate specific mRNAs across the temperature gradient (Figure 2). Each mRNA was analysed at least twice, and the elution of a poly(A)- RNA (18S rRNA) was also investigated. The vast majority of the 18S rRNA was eluted in the 20°C fraction. In contrast, all the mRNAs were eluted

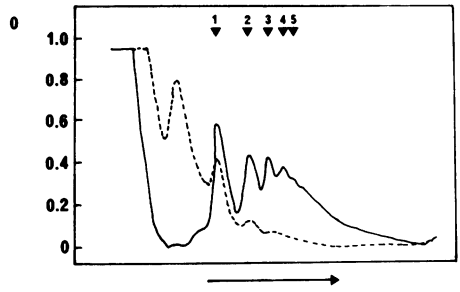

Figure 3: The Effect Of EDTA On Yeast Polysomes.
A post-mitochondrial supernatant from an exponentially-growing culture (see Materials and Methods) was split into two equal portions. The first was subjected to sucrose density gradient centrifugation with no prior treatment to obtain the control absorbance profile (continuous line), while EDTA was added to the second portion (final concentration of 25mM) before centrifugation (dotted line). The arrow below the diagram indicates the direction of sedimentation, and the arrows at the top show the positions of polysomes containing 1,2, 3, 4 and 5 ribosomes, respectively.

over a range of temperatures up to about 70°C, which is consistent with each being polyadenylated (4). A peak of elution was observed at about 60°C for each mRNA, but the size of this peak varied relative to a shoulder of elution between about 30 to 50°C (Figure 2, Table 1). In general, this shoulder of elution was larger for more stable mRNAs (Figure 2, Table 1). The reasons for the divergence of mRNA9 from the general pattern, and the high proportion of mRNA74 eluted in the 20°C fraction (Table 1) are not known. However, the observed pattern is consistent with the post-transcriptional addition of a poly(A) tail of about the same length to each mRNA, followed by shortening of the poly(A) tail with age. This phenomenon has been observed previously on HeLa cell and adenoviral mRNAs (29,30). The poly(A) tail is thought to protect an mRNA from 3'-exonucleolytic attack (29,31). Therefore, if the length of the poly(A) tail were to provide the basis for the division of yeast mRNAs into relatively stable and unstable populations (4), we would have expected to observe that unstable mRNAs be maximally eluted from poly(U)-Sepharose at a lower temperature than stable mRNAs. This was not the case (Figure 2).

Polysome Gradients

A number of control experiments were performed to demonstrate that under our conditions the A_{260} profile, obtained after density gradient centrifugation of post-mitochondrial supernatants from spheroplast lysates, was not due to random aggregation of ribosomes, but to the association of ribosomes in polysomes. Chelation of Mg^{2+} by EDTA is known to cause

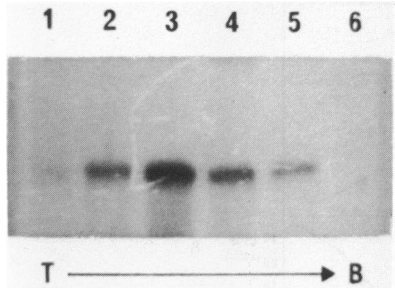

Figure 4: A Northern Blot Of RNA Isolated From Different Regions Of A Polysome Gradient. A polysome gradient was divided into 6 approximately equal fractions and RNA isolated from each fraction. A northern blot containing roughly equal portions of each fraction was probed for the PYK mRNA. The arrow indicates the direction of sedimentation, and 'T' and 'B' show the lanes from the top and bottom fractions, respectively.

dissociation of polysomes into ribosomal subunits and mRNP (32). Therefore, 25mM EDTA was added to one half of a post-mitochondrial supernatant immediately before density gradient centrifugation and the resulting A_{260} profile compared with that of the other half (Figure 3). The addition of EDTA caused the rapidly sedimenting material to sediment predominantly in the position expected for ribosomal subunits. The effects of cycloheximide and RNase treatment upon the A_{260} profile were analysed in further control experiments (results not shown). The addition of 5mM cycloheximide to spheroplasts for 10 minutes immediately before lysis led to a reduction in the size of the 'monosome peak' and a general increase in the rate of sedimentation of the 'polysomal' material. The treatment of the post-mitochondrial supernatant with 100ug/ml RNase A for 3 minutes at $4^{o}C$ prior to centrifugation led to a complete shift of rapidly sedimenting material into the 'monosome peak', as observed previously (33). Therefore, by these three criteria, the A_{260} profile represents yeast polysomes.

The integrity of mRNA across polysome gradients was tested by northern blotting. A gradient was divided into 6 fractions of similar size, and RNA isolated from each. Approximately equal portions of each fraction were subjected to northern blotting and probed for the PYK mRNA (Figure 4). The amount of hybridization obtained with the PYK probe across the northern blot is consistent with the distribution of the PYK mRNA across polysome gradients obtained by dot blotting (Figure 5). The northern blot also shows that the PYK mRNA is intact across the gradient, suggesting that differential degradation of mRNA has not taken place in different regions of the gradient.

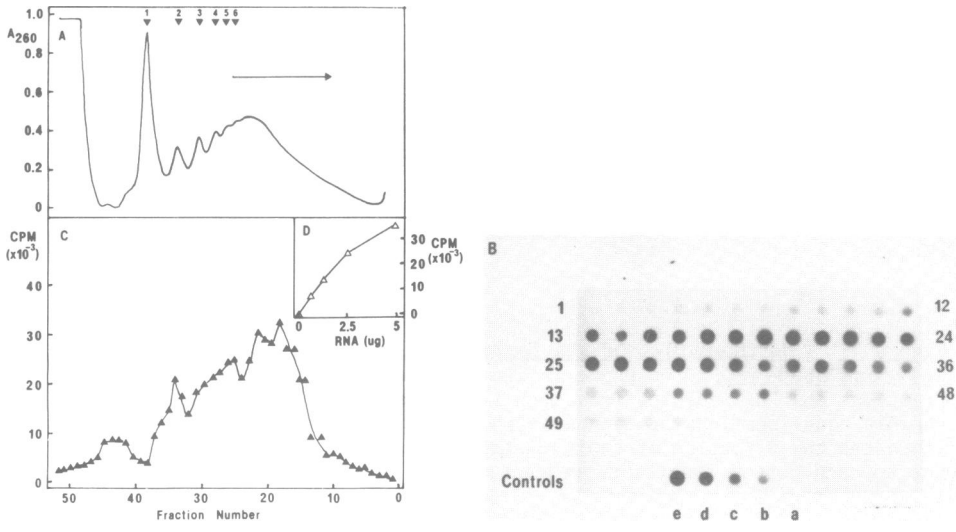

Figure 5: Procedure For Determining The Distribution Of An mRNA Across A Polysome Gradient. Yeast polysomes were prepared as described in Materials and Methods. **(A)** The absorbance profile of the polysome gradient. The large arrow shows the direction of sedimentation, and the numbered arrows indicate the positions of polysomes containing 1 to 6 ribosomes, respectively. **(B)** The gradient shown in (A) was divided into 52 fractions which were dotted onto a filter and probed for the PYK mRNA. The numbers at each side show the order in which the fractions were dotted. 'Controls' indicates the position of an RNA dilution series. **(C)** The amount of radioctivity bound to each dot in (B) was determined by scintillation counting. **(D)** The amount of RNA in the Controls, plotted against the amount of bound radioactivity obtained using the PYK probe.

mRNA Distribution on Polysome Gradients

The distribution of a specific mRNA across a polysome gradient was determined by quantifying the relative amount of the mRNA in fractions from the gradient using dot blotting techniques (Figure 5). The A_{260} profiles were used to establish, for each gradient, the positions of peaks corresponding to polysomes containing known numbers of ribosomes (Figure 5A). Usually gradients were divided into 50 to 60 fractions which were then used to prepare a number of identical filters. Hybridizations were performed under conditions which gave single bands on northern blotting (4), and filters were always autoradiographed to ensure that there was no significant background hybridization on the filters. Following autoradiography, individual dots were

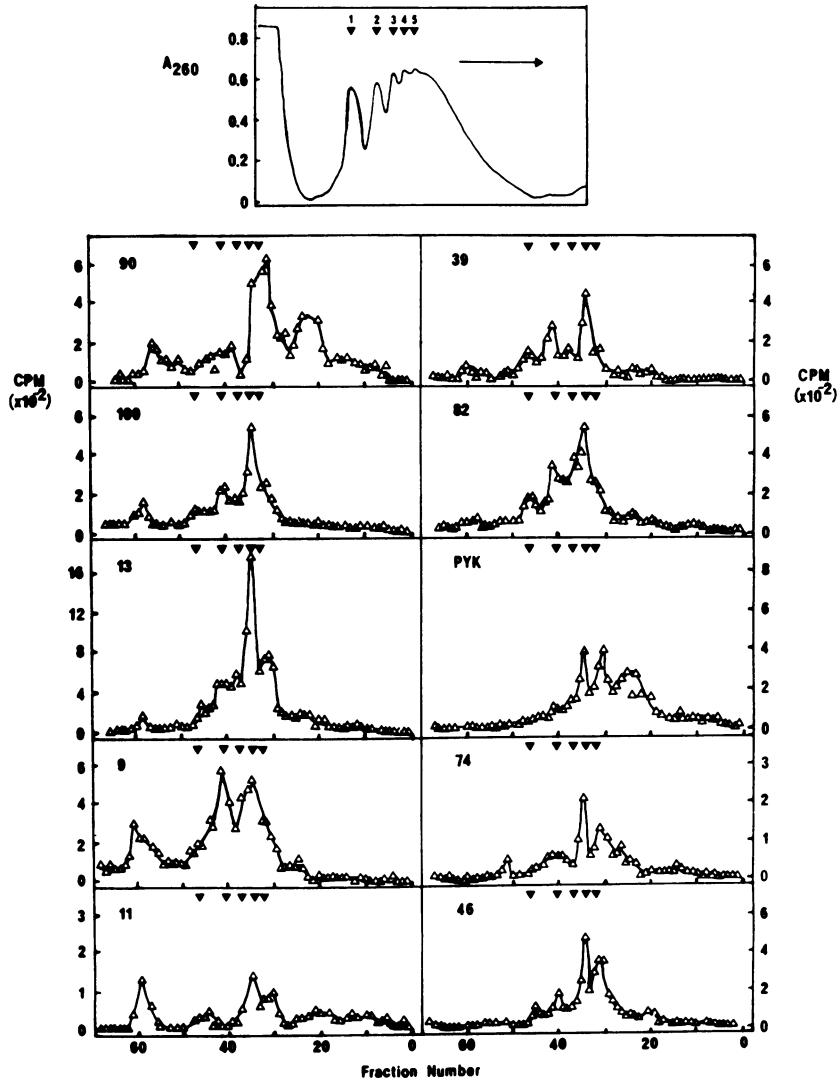

Figure 6: Comparison Of The Distribution Of Ten Different mRNAs On The Same Polysome Gradient. The polysome gradient shown at the top of the figure was divided into 68 fractions which were used to prepare 10 identical dot blots for analysis of the mRNAs shown. The small arrows at the top of each profile correspond to the numbered arrows in the absorbance profile which show the positions of polysomes containing 1 to 5 ribosomes, respectively. The lengths and half-lives of each mRNA are presented in Table 1.

cut out and used for scintillation counting (Figure 5C). To ensure that hybridizations were quantitative, an RNA dilution series was incorporated on each filter (Figure 5D). A consistent pattern of hybridization was obtained for a specific mRNA across the same gradient, but patterns differed for an mRNA on different gradients. There were two main reasons for this: firstly, polysome profiles are extremely susceptible to alterations in the physiological state of the cells used for the preparation, and therefore, despite the care taken to harvest cells at a particular stage of exponential growth, differences in the A_{260} profiles were apparent between preparations; secondly, slight differences also existed in the formation of the 10-50% sucrose gradient between preparations. Therefore, the distributions of different mRNAs were only compared using dot blots made from the same gradient, not between gradients.

Figure 6 shows the distribution of 10 specific mRNAs across a single polysome gradient (mRNA half-lives and lengths are summarized in Table 1). Whilst, for the reasons stated above, the distributions of these mRNAs were not identical on different gradients, the relative distributions of the different mRNAs were similar between gradients. For example, a significant proportion of mRNA9 was always observed in the presumed mRNP fractions (fractions 55 to 64 in Figure 6), whereas a large proportion of the PYK mRNA tended to be present on large polysomes (fractions 20 to 30 in Figure 6) compared with other mRNAs.

Significant differences are apparent in the distribution of the mRNAs across the same gradient. However, interpretation of Figure 6 is complicated by the necessity to account for the effect of variation in mRNA length upon ribosome loading. Nevertheless, differences are still apparent between mRNAs of similar length (e.g. mRNAs 9, 39 and 82). However, these differences do not correlate with mRNA half-life. For example, the half-life of mRNA74 is almost 12-fold that of mRNA90, yet a greater proportion of mRNA90 is present on large polysomes. Also, mRNAs 11 and 13 are of similar length, but a higher proportion of the more stable mRNA is present in the putative mRNP fraction. In contrast, mRNA46 is at least 10-fold more stable than mRNA100; a greater proportion of mRNA46 is present in polysomes containing more than 5 ribosomes. Therefore, there is no obvious correlation between ribosome loading and mRNA half-life.

DISCUSSION

In this study, the analysis of yeast mRNAs using thermal elution from poly(U)-Sepharose has revealed no dramatic differences in the relative lengths

of the poly(A) tails of mRNAs with widely differing half-lives. Differences in the elution patterns amongst mRNAs are consistent with the shortening of the poly(A) tail with age (29,30), and therefore, are more likely to have resulted from differences in mRNA half-life, rather than be the basis for the different half-lives.

The results of this study demonstrate that there is no obvious correlation between the degree of ribosome loading on an mRNA and its stability in yeast. Whilst ribosome loading may affect mRNA stability to a minor extent, it certainly cannot account for the dramatic difference in stability between the two mRNA populations (4). However, the sequences, and therefore the lengths of the coding regions, are not known for most of the mRNAs used in this study. Our conclusions are based upon the premise that the mRNAs of similar length have coding regions of roughly equivalent sizes. This is more tenable for longer mRNAs.

Our observations on ribosome loading and stability for specific mRNAs seem to contradict those of Lacroute's group concerning URA1 and URA3 mRNAs with nonsense mutations (19,20). However, this discrepancy may be due to the fact that we have analysed functional mRNAs, whereas they have analysed mutant mRNAs. They did not exclude the possibility that the aberrent URA mRNAs were degraded by an alternative mechanism (20). The analysis of a CYC1 mutation in S. cerevisiae has generated data that is consistent with our observations (34). The mutation of two codons close to the 5'-end of the coding region increases the potential for secondary structure formation on the CYC1 mRNA. Although the ribosome loading on the altered mRNA is dramatically reduced compared with the wild-type CYC1 mRNA, the steady-state levels of the altered and wild-type mRNAs are similar (suggesting that the mutation has not affected mRNA half-life significantly; ref. 34). Our results suggest that mRNA translation and degradation are not as intimately linked in eukaryotes as would appear to be the case in prokaryotes.

We have excluded both ribosome loading and poly(A) tail length as factors which account for the division of yeast mRNAs into relatively stable and unstable populations (4). The annalysis of 5 specific mRNAs in mouse erythroleukemia cells led Krowczynska and coworkers to draw similar conclusions (35).

The most obvious remaining candidate for the unknown factor which strongly influences mRNA half-life in yeast is the existence of internal structural signals which either cause stabilization or destabilization of yeast mRNAs. Considerable evidence is now emerging from a wide variety of

systems for the presence of specific sequences within mRNAs which strongly influence their stability (36-41). These sequences can act to stabilize mRNAs, as in the case of the REP sequences in *E. coli* (36), or to destabilize mRNAs, as is observed for the AU sequences present in the 3'-untranslated regions of some mammalian mRNAs (39).

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