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The relationship between mRNA stability and length in *Saccharomyces cerevisiae*

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ABSTRACT

A rapid and convenient procedure has been developed for the measurement of mRNA half-life in S.cerevisiae using the transcriptional inhibitor, 1,10-phenanthroline. A range of half-lives from 6.6 ± 0.67 minutes to over 100 minutes, relative to the stability of the 18S rRNA control, has been obtained for fifteen mRNAs. They include the pyruvate kinase and actin mRNAs, as well as 13 randomly picked mRNAs of unknown function. The mRNAs clearly fall into two populations when their lengths and half-lives are analysed; one population is considerably more stable than the other when mRNAs of similar length are compared. Also, within each population, there is an inverse relationship between mRNA length and half-life. These results suggest that mRNA length and at least one additional factor strongly influence mRNA stability in yeast.

INTRODUCTION

mRNA half-lives must play a major role in controlling the level of expression for many genes since the steady-state level of an mRNA species is determined both by its rate of synthesis and degradation. Clearly, mRNAs have different half-lives. In sea urchin embryos the abundance of several mRNAs correlates with their relative stability rather than their rates of synthesis (1). Also, the different levels of beta- and delta-globin mRNAs in human nucleated bone marrow cells are consistent with their different half-lives (2).

In some cases, changes in the rate of gene expression are partly mediated by altering mRNA half-life. For example, casein mRNA is stabilised in the rat mammary gland in response to prolactin (3), and the administration of oestrogen stabilises the vitellogenin mRNA in Xenopus (4). Furthermore, the stability of some mRNAs are regulated during differentiation both in mouse erythroleukemia cells (5) and in Dictyostelium (6). Yeast appears to compensate for extra copies of a ribosomal protein gene partly by reducing the half-life of its mRNA (7).

Despite the importance of mRNA half-life, almost nothing is known about the factors which influence mRNA stability or about the mechanisms of mRNA degradation in eukaryotes. In S. cerevisiae, mRNA half-lives range from about 1 to 70 minutes (8-10). Most yeast mRNAs have a 3'-poly(A) tail (11) and a 5'-cap (12), but it is unlikely that variations in these structures can account completely for the observed range of half-lives. The range of mRNA half-lives is likely to be based to a certain extent upon the internal features of individual mRNAs (13-16). We present evidence that stability is influenced by mRNA length and that yeast mRNAs are clearly divisible into two populations when their length and stability are compared. Possible reasons for the existence of the two mRNA populations are discussed.

MATERIALS AND METHODS

Strains and Plasmids

S.cerevisiae strains DBY746 (His3, Leu2, Trp1, Ura3) and DBY868 (Ade2, His4), and E.coli 1400 (SupE, SupF, Hsd5, Met$^-$, RecA, lambda L512) were used throughout. pPYK1E contains the 5'-1.9 kb EcoRI fragment of the yeast PYK gene (17) cloned into pAT153. Actin sequences were analysed using pYA301, a subclone of pYA208 (18). pMA700 contains the yeast HIS3 gene cloned into pBR325 (19). The 18S rRNA probe was generated using pSP65R which contains the 1.0 kb Xba1/Sst1 fragment from pYIrG12 (20) cloned into pSP65.

RNA Preparation

Total RNA was prepared from yeast as described by Lindquist (21) and where necessary, poly(A)+ RNA was fractionated using oligo(dT)-paper or oligo(dT)-cellulose columns (22).

cDNA Cloning

The methods used to synthesise and clone mRNA:cDNA hybrids were similar to those described previously (23,24). Yeast poly(A)+ RNA was reverse transcribed according to the procedures of Crabtree and Kant (25), except that RNasin was used at 1u/ul in addition to the other ingredients. The mRNA:cDNA hybrids were run on a Sephadex-G50 column in 100mM NaCl, 1mM EDTA, 10mM Tris, pH 7.5. Approximately 15 deoxycytosine residues were added to the 3'-end of the hybrids using terminal transferase (26), the C-tailed mRNA:cDNA hybrids were annealled with G-tailed, Pst1-cut, pBR322 (BRL), and the mixture used to transform E.coli (27).

Blotting and Hybridization

For Southern blotting, EcoR1-cut DNA from DBY746 was electrophoresed on 1% agarose gels. Size standards were pMA700 cut with Bg1II (6.4 and 0.7 kb),

pPYK1E cut with EcoR1 (3.6 and 1.9 kb), and pPYK1E cut with HindIII (5.5 kb). The gels were blotted (28), and filters baked, hybridised, washed and autoradiographed as described previously (29).

For northern blotting, yeast RNA was electrophoresed on formaldehyde gels (30). 25S, 18S, 5.8S, 5S and rRNAs were used as size standards. After blotting (31) filters were treated as for Southerns.

RNA dot-blotting was performed in a 'hybridot' apparatus (BRL) using the procedures of White and Bancroft (32). RNA dilutions were dotted onto every filter to determine whether each hybridisation was quantitative. Identical filters were made using the same RNA solutions to ensure that filters were directly comparable after hybridisation with different probes. After autoradiography, individual dots on the blots were cut out and the amount of bound radioactivity determined by scintillation counting.

DNA probes were made by nick-translation (33). Synthetic rRNA probes were made using the SP polymerase system (34).

mRNA Half-Life Measurements

1,10-Phenanthroline (1ml of a 100mg/ml solution in ethanol) was added to a litre culture of yeast (A_{650} =0.45). At various times after the addition of drug, 100ml portions of the culture were added to 200ml ethanol, mixed and stored at -20°C for up to one week. When convenient, RNA was prepared and the relative levels of specific mRNAs in each timepoint measured by dot blotting. Differences in yields amongst RNA preparations were corrected by probing identical filters with synthetic 18S rRNA (pSP65R). The half-life of each mRNA was then calculated statistically using the linear relationship between the logarithm of corrected mRNA levels and time. All mRNA half-lives were measured in DBY746, except the URA3 mRNA which was measured in DBY868.

Rates of RNA and Protein Synthesis

For rates of RNA synthesis, the amount of [^{3}H]-uracil incorporated into cold TCA precipitable material was assayed during 5 minute pulses, using 5 uCi/ml of [^{3}H]-uracil in mid-logarithmic cultures of DBY746. The midpoint of the labelling period was used as the time at which the rate of synthesis was measured. Phenanthroline was added in ethanol (1000 fold concentrated) to cultures at time zero; ethanol was added to control cultures. Results are expressed as a percentage of the control value at each appropriate point. Rates of protein synthesis were measured in the same basic manner except that incorporation of [^{35}S]-methionine into hot-TCA precipitable material was assayed.

To measure the relative rate of poly(A)+ RNA synthesis in DBY746, RNA

was pulse labelled with [^{3}H]-uracil (5 uCi/ml) at various times after the addition of phenanthroline (100 ug/ml). At the end of each labelling period, [^{3}H]-RNA was isolated and incubated with oligo (dT) paper. The percentage of [^{3}H]-RNA remaining bound to the paper after washing (22) was used as a measure of the relative rate of poly(A)+ RNA synthesis.

RESULTS

cDNA Cloning

A yeast cDNA library was constructed, and a set of clones selected from the library for use as hybridisation probes to investigate the metabolism of their corresponding mRNAs. Approximately 5ug of poly(A)+ mRNA from exponentially growing DBY746 was reverse transcribed; the efficiency of conversion was about 30%, and the average length of the cDNAs synthesised was 900 nucleotides as determined on alkaline agarose gels (results not shown). A library of 150,000 Tet^R cDNA clones was obtained when *E.coli* was transformed with 1ug of C-tailed mRNA:cDNA hybrids annealled with 2ug of G-tailed, Pst1-cut pBR322. 90% of the transformants contained plasmids with cDNA inserts.

Characterisation of cDNA clones

Thirteen cDNA clones from the yeast library (9, 10, 11, 13, 19, 22, 39,

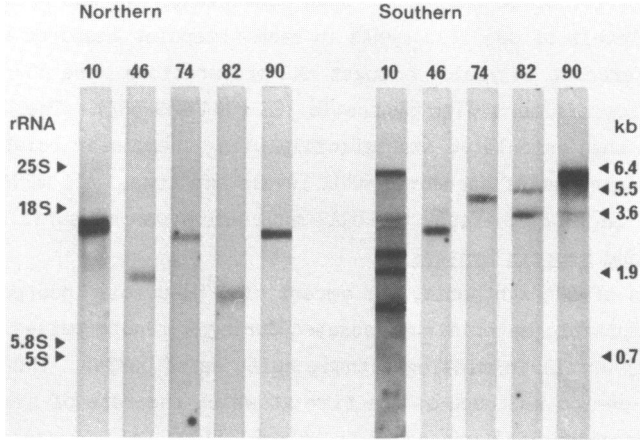

Figure 1 Northern Blots of Yeast RNA and Southern Blots of Yeast DNA probed with the cDNA plasmids.
Southern blots were performed using EcoR1-cut yeast DNA. The northern and Southern blots for a particular cDNA were hybridised together in the same bag, and also washed together. Numbers at the top of each lane indicate the cDNA plasmid used as a probe. Yeast ribosomal RNAs were used as size standards in northern blots. Specific DNA restriction fragments were used as size standards in Southern blots (see Materials and Methods).

46, 74, 82, 85, 90 and 100), and genomic clones of pyruvate kinase (pPYKIE), and actin (pYA301) were used in this study. Since these plasmids were to be used in dot blot analyses for mRNA half-lifedeterminations, it was crucial to establish that they would only hybridize with a unique mRNA. Therefore, all the plasmids were nick translated and used as probes in northern blots of total RNA, and in Southern blots of EcoR1-cut DNA from DBY746. Figure 1 shows the results for cDNAs 10, 46, 74, 82 and 90. All the cDNAs used in this study, except 10, appear to be derived from single copy genes and give single bands on northern blots. Clone 10 hybridised strongly with 4 EcoR1 fragments of yeast DNA, and produced a single band on northern blotting (Figure 1). Unless the gene for cDNA10 contains introns, it would appear that mRNA10 is derived from a family of genes. Therefore, it is possible that the half-life measurements obtained using cDNA10 are an average of more than one mRNA.

The results from the Southern and northern blots also demonstrate that all the cDNA clones are different. This was confirmed in an experiment in which all the cDNA inserts were purified, nick translated and used separately as probes against all the clones. No cross-reaction was observed between the cDNAs (results not shown).

Use of Phenanthroline as an Inhibitor of Transcription

Many methods have been used for the determination of mRNA half-life in yeast. Estimations of biological half-life have been made using a number of different transcriptional inhibitors (35-38), or strains which carry the conditional-lethal mutation in RNA transport ts136 (8,9). Chemical half-lives have been determined using incorporation or decay kinetics of radio-labelled precursors (39-43).

We have developed a method for measurement of chemical mRNA half-life using the transcriptional inhibitor, phenanthroline. The method, which is crudely analogous to the use of rifampicin in E.coli (44), is convenient and reproducible. Only modest amounts of isotope are required for probe synthesis, relative to the amounts required for labelling mRNAs in vivo (39-42). Also, the measurements can be done in rich growth media which has allowed us to compare our mRNA half-life measurements directly with in vivo translation measurements which are done in rich media (manuscript in preparation).

The disadvantage of using a transcriptional inhibitor is that it may affect other processes, for example translation. Therefore, control

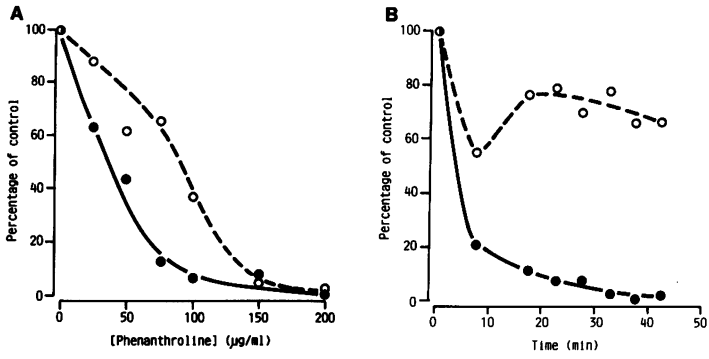

Figure 2 The Effect of Phenanthroline on RNA and Protein Synthesis
A) The rates of RNA (●) and protein synthesis (O) 7.5 minutes after the addition of various concentrations of phenanthroline. **B)** The rates of RNA (●) and protein synthesis (O) at various times after the addition of phenanthroline (100ug/ml). All the values are expressed as percentages of the control values obtained using identical cultures to which only ethanol was added.

experiments were performed to determine the concentration of phenanthroline which gives maximal inhibition of RNA synthesis, but minimal disruption of protein synthesis. The effects of a range of phenanthroline concentration on transcription and translation were analysed at a fixed timepoint, 7.5 minutes after the addition of drug to an exponential culture of DBY746 (Figure 2A). The methods for measuring rates of RNA and protein synthesis are described in 'Materials and Methods'. It is clear that phenanthroline affects both RNA and protein synthesis, but that at concentrations between 75 to 100 ug/ml, inhibition of RNA synthesis is about 5-fold that of protein synthesis. Further analysis revealed that the 60% inhibition of the rate of protein synthesis observed 7.5 minutes after the addition of phenanthroline at 100 ug/ml (Figure 2A) was misleading, since protein synthesis appears to recover after this time (Figure 2B). Twenty minutes after drug addition, the rate of protein synthesis had recovered to nearly 80% of the control. In contrast, the inhibition of RNA synthesis continued for the duration of the experiment. Therefore, phenanthroline was used at 100 ug/ml for mRNA half-life measurements. While the reason for the transient shock to protein synthesis remains unclear, a decrease in protein synthesis was expected as mRNAs were cleared from the cytoplasm following inhibition of transcription.

It is possible that phenanthroline inhibits the three RNA polymerases to

TABLE 1 The Effect of Phenanthroline on the Rate of Synthesis of Poly(A) ± RNA Relative to Total RNA

Time after addition of Phenanthroline (minutes)	[Phenanthroline] (ug/ml)	
	0	100
5	21%	14%
15	17%	16%

different extents. Therefore, the proportion of poly(A)+ RNA synthesized, relative to total RNA, was measured 5 and 15 minutes after the addition of phenanthroline (Table 1). The data show that the percentage of poly(A)+ RNA synthesised after drug addition, is less than normal and hence that polymerase II is inhibited to a slightly greater extent than the other RNA polymerases.

Finally, an experiment was done to confirm that mRNAs decay at different rates in the presence of phenanthroline. A northern blot was performed on approximately equal amounts of RNA prepared at various times after the

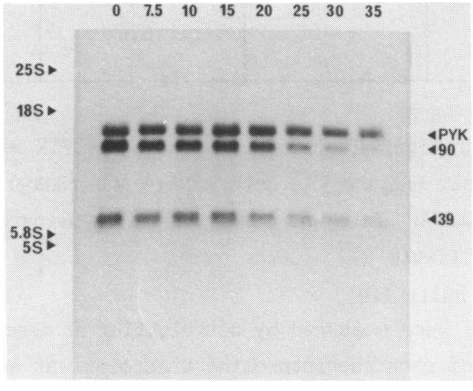

Figure 3 Decay of mRNAs Following Addition of Phenanthroline to Yeast Cultures.
A northern blot was performed on approximately equal amounts of RNA isolated at various times after the addition of phenanthroline (100ug/ml) to an exponentially growing yeast culture. The blot was hybridized simultaneously with probes for the PYK mRNA, mRNA39 and mRNA90. The numbers at the top of the figure indicate the times after the addition of phenanthroline (in minutes) at which the RNAs were prepared. Numbers on the left show the positions of ribosomal RNA size standards, and the positions of the three mRNAs analysed are shown to the right of the figure.

TABLE 2 **mRNA Length versus Half-Life: Comparison of Present Data with Previously Published Observations.**

cDNA CLONE	mRNA LENGTH (BASES)	HALF-LIFE (MINUTES)	REFERENCE
9	440	16.5 +/- 1.5	THIS STUDY
10	1,250	56.9 +/- 6.0	THIS STUDY
11	700	18.0 +/- 2.2	THIS STUDY
13	740	12.1 +/- 1.0	THIS STUDY
19	550	15.3 +/- 2.7	THIS STUDY
22	3,100	44.7 +/- 2.4	THIS STUDY
39	380	18.3 +/- 1.5	THIS STUDY
46	550	>100	THIS STUDY
74	1,050	83.4 +/- 9.2	THIS STUDY
82	370	22.3 +/- 3.1	THIS STUDY
85	550	>100	THIS STUDY
90	1,100	6.6 +/- 0.67	THIS STUDY
100	500	10.4 +/- 1.1	THIS STUDY
PYK	1,600	59.9 +/- 7.8	THIS STUDY
ACTIN	1,300	76.6 +/- 15	THIS STUDY
URA3	700	11	THIS STUDY
URA3	700	10.5	39,40
PPR1	2,900	2	10
URA1	1,500	15	41
Rp1	1,310	13.1	42
Rp29	620	14.0	42
Rp39	660	12.0	42
Rp73	480	16.6	42

addition of phenanthroline. The blot was probed for PYK mRNA, mRNA39 and mRNA90, simultaneously (Figure 3). Both mRNA39 and mRNA90 appear to be less stable than the PYK mRNA. This was confirmed later using quantitative dot-blotting techniques (Table 2).

Measurement of mRNA Half-Life

mRNA half-lives were measured by dot blotting as described in 'Materials and Methods'. RNA from each phenanthroline timepoint was dotted in triplicate onto a series of identical filters which were then probed, washed, autoradiographed, and counted (Fig 4). Meanwhile, identical filters were probed for 18S rRNA to correct for minor differences in RNA yields between timepoints. The half-life of the mRNA was calculated using linear regression analysis on this corrected data. Each mRNA was analysed at least three times (Table 2). A range of values was obtained from 6 minutes for mRNA90, to over 100 minutes for mRNA85 and mRNA46. The half-lives of mRNAs 85 and 46 were hard to measure accurately, presumably because they tended towards the

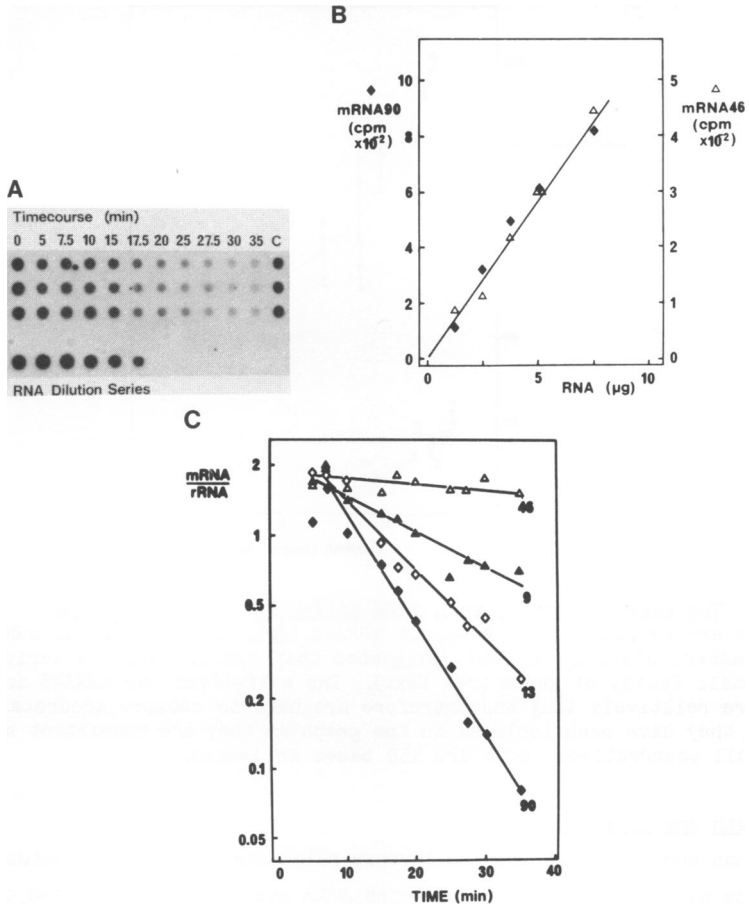

Figure 4 Measurement of mRNA Half-Life
A) An autoradiograph of a dot blot for mRNA90 following phenanthroline addition ('C' corresponds to dots of a control RNA preparation to which phenanthroline was not added). See Materials and Methods. **B)** RNA dilution series for mRNA90 and mRNA46. **C)** Semilogarithmic plot of the relative levels of mRNA9 (▲), mRNA13 (◇), mRNA46 (△) and mRNA90 (◆) versus time after inhibition of transcription using phenanthroline.

half-life of the 18S rRNA control. Also, since phenanthroline does not inhibit RNA synthesis completely under our conditions, these values may be slight overestimates of the true half-lives. However, using this method, we have estimated the half-life of the URA3 mRNA to be approximately 11 minutes in DBY868. This is consistent with previous estimates of about 10.5 minutes using both incorporation and decay kinetics (39,40).

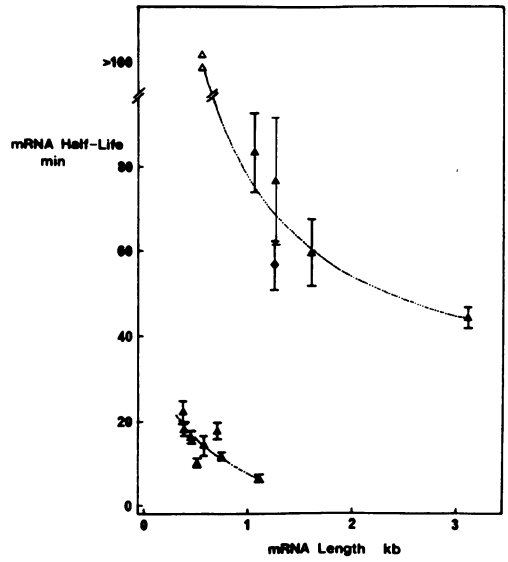

Figure 5 The Relationship between mRNA Half-Life and mRNA Length. All mRNAs are represented by ▲ except mRNA10 (◆), mRNA46 (△) and mRNA85 (△). Southern blotting analysis suggested that mRNA10 could be derived from a small family of genes (see Text). The half-lives for mRNA46 and mRNA85 are relatively long and therefore are hard to measure accurately. However, they have been included in the graph as they are consistent with the overall observation; both are 550 bases in length.

mRNA Length and Half-Life

It has been reported that an inverse relationship may exist between mRNA length and half-life. Analysis of gross mRNA populations on sucrose density gradients has revealed that long-lived mRNA tends to be shorter than newly-synthesised mRNAs in HeLa cells (45), human lymphocytes (46), mouse L-cells (47), and in insect cell lives (48,49). However, the relationship between mRNA length and stability has remained unclear since in these systems, some stable mRNAs are relatively long, and some unstable mRNAs are relatively short. We have compared mRNA length with half-life using our set of specific mRNAs (Figure 5). Even though random mRNAs have been analysed, they are clearly divisible into two distinct populations. Also a clear relationship between mRNA length and half-life exists within each population; longer mRNAs tend to be less stable than shorter mRNAs. The existence of two distinct mRNA populations of relatively stable and unstable species, explains why no clear correlation between mRNA length and stability was observed previously using gross mRNA populations (45-49).

DISCUSSION

Fifteen mRNAs have been analysed in DBY746; thirteen unknowns as well as the pyruvate kinase and the actin mRNAs. The main drawback of using randomly chosen mRNAs for analysis is that the function of most of the mRNAs are not known. However, this protects against bias in the selection of mRNAs to be analysed, and hence strengthens the generality of the conclusions.

The results suggest that mRNA length influences the rate of mRNA degradation, and are consistent with the previous observations (45-49). They suggest that the rate-limiting step in mRNA degradation is a random event, with longer mRNAs presumably presenting a larger target for an initial endonucleolytic cut. Exonucleolytic attack at the 3'-poly(A) tail, or removal of the 5'-cap is unlikely to result in an inverse relationship between mRNA length and stability. The results also suggest that mRNA sequences are degraded rapidly following the initial nucleolytic event. If mRNA sequences were not degraded rapidly, longer mRNAs would take more time to degrade than shorter mRNAs; the opposite is observed. Furthermore, we have not detected accumulation of degradation intermediates on northern blots (Figure 3). In E.coli, there appears to be at least two different mechanisms by which specific mRNAs are degraded (44). Initial cleavage of bla transcripts appears to be rate limiting and is followed by rapid degradation of all regions of the transcript. In contrast, initial cleavage and subsequent degradation of ompA transcripts appears to be slow, and degradation occurs in a 3'-to-5' direction (13).

The existence of two populations of yeast mRNAs (Figure 5) demonstrates clearly that at least one other major factor, apart from mRNA length, must influence the stability of mRNAs in yeast. A possible relationship between mRNA translation and stability was suggested as early as 1969 by Morse and Yanofsky (50). mRNAs containing premature stop codons are degraded more rapidly than wild-type mRNAs in E.coli (50), S. cerevisiae (40,41), and human reticulocytes (51,52). A simple explanation for this is that ribosomes may protect an mRNA from random endonucleolytic digestion during translation. However, results from this laboratory suggest strongly that differences in mRNA translation cannot account for the existence of two mRNA populations in yeast (manuscript in preparation). Similarly, the presence or absence of the 3'-poly(A) tail cannot account for the existence of 'stable' and 'unstable' mRNA populations, since all the cDNA clones were constructed using poly(A)+ mRNA. In contrast, a fundamental difference in the 5'-cap could account for the observation. Two cap structures have been identified on yeast mRNAs;

$m^7G(5')pppAp$ and $m^7G(5')pppGp$ (12). Therefore, it is possible that 'stable' and 'unstable' mRNAs have different types of 5'-cap. Similarly, specific sequence or structural signals could be an important factor. This has already been demonstrated for some prokaryotic (53,54) and bacteriophage mRNAs (55), and strong evidence for this is also emerging in eukaryotic systems. The most convincing evidence for this has been the identification (13) and analysis (56) of a conserved sequence in the 3'-untranslated regions of some mammalian mRNAs. Introducing the conserved A:U-rich sequence into the 3'-untranslated region dramatically reduces the stability of the relatively stable rabbit beta-globin mRNA (56). Also, a deletion at the 3'-end of the hsp70 mRNA in Drosophila increases its stability (14). Aberrant c-myc mRNAs which contain a deletion of the 5'-untranslated region have longer half-lives (15,16). It has been suggested that the 3'-untranslated regions of the human beta- and delta- globin mRNAs determine the difference in their half-lives (16.5 and 4.5 hours, respectively; ref 2). Furthermore, the 3'-untranslated region of the yeast CYC1 mRNA appears to affect its stability, since altering the 3'-terminus affects the levels of CYC1 mRNA (57). Interestingly, there is no correlation between the apparent stability of the mutant CYC1 mRNAs and their length (57). Hence the inverse relationship between length and stability may only hold for wild-type mRNAs. Further experiments are required to test the influence of sequence or cap-structure upon the stability of yeast mRNAs.

The half-lives of several yeast mRNAs have been measured previously and these fit our data reasonably well as does the URA3 mRNA from DBY868 (Table 1). There does not appear to be any obvious functional division between the stable and unstable mRNA populations. There is no correlation between the presence of introns in the gene and the mRNA being a member of the 'unstable' group (see ribosomal protein and actin mRNAs; Table 1). Also, mRNAs which encode metabolic enzymes can belong to either group (see PYK and URA mRNAs).

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REFERENCES

1. Cabrera, C V, Lee, J J, Ellison, J W, Britten, R J & Davidson, E H (1984) J. Mol. Biol. **174**, 85-111.
2. Ross, J & Pizarro, A (1983) J. Mol. Biol. **167**, 607-617
3. Guyette, W A, Matusik, R J & Rosen, J M (1979) Cell **17**, 1013-1023
4. Brock, M L & Shapiro, D J (1983) Cell **34**, 207-214
5. Krowczynska, A, Yenofsky, R & Brawerman, G (1985) J. Mol. Biol. **181**, 231-239
6. Mangiarotti, G, Giorda, R, Ceccarelli, A & Perlo, C (1985) Proc. Natl. Acad. Sci. USA **82**, 5786-5790
7. Pearson, N J, Fried, H M & Warner, J R (1982) Cell **29**, 347-355
8. Koch, H & Friesen, J D (1979) Mol. Gen. Genet. **170**, 129-135
9. Chia, L-L & McLaughlin, C (1979) Mol. Gen. Genet. **170**, 137-144
10. Fuchs, R P P & Lacroute, F (1983) EMBO J. **2**, 2179-2184
11. McLaughlin, C S, Warner, J R, Edmonds, M, Nakazato, H & Vaughan, M H (1973) J. Biol. Chem. **248**, 1466-1471
12. Sripati, C E, Groner, Y & Warner, J R (1976) J. Biol. Chem. **251**, 2898-2904
13. Caput, D, Beutler, B, Hartog, K, Thayer, R, Brown-Shimer, S & Cerami, A (1986) Proc Natl Acad Sci USA **83**, 1670-1674
14. Simcox, A A, Cheney, C M, Hoffman, E P & Shearn, A (1985) Mol. Cell. Biol. **5**, 3397-3402
15. Eich, D, Piechaczyk, M, Henglein, B, Blanchard, J-M, Traub, B, Kofler, E, Wiest, S, Lenoir, G M & Bornkamm, G W (1985) EMBO J. **4**, 3717-3725
16. Rabbitts, P H, Forster, A, Stinson, M A & Rabbitts, T H (1985) EMBO J. **4**, 3727-3733
17. Burke, R L, Tekamp-Olson, P & Najarian, R (1983) J. Biol. Chem. **258**, 2193-2201
18. Gallwitz, D & Sures, I (1980) Proc. Natl. Acad. Sci. USA **77**, 2546-2550
19. Monteil, J F, Norbury, C J, Tuite, M F, Dobson, M J, Mills, J S, Kingsman, A J & Kingsman, S M (1984) Nucleic Acids Res. **12**, 1049-1068
20. Petes, T D, Hereford, L M & Skryabin, K G (1978) J. Bacteriol. **134**, 295-305
21. Lindquist, S (1981) Nature **293**, 311-314
22. Aviv, H & Leder, P (1972) Proc. Natl. Acad. Sci. USA **69**, 1408-1412
23. Roninson, I B & Ingram, V M (1981) Proc. Natl. Acad. Sci. USA **78**, 4782-4785
24. Leibold, E A, Aziz, N, Brown, A J P & Munro, H N (1984) J. Biol. Chem. **259**, 4327-4334
25. Crabtree, G R & Kant, J A (1981) J. Biol. Chem. **256**, 9718-9723
26. Nelson, T & Brutlag, D (1979) Meth. Enzymol. **68**, 41-50
27. Dagert, M & Ehrlich, S D (1979) Gene **6**, 23-28
28. Southern, E M (1975) J. Mol. Biol. **98**, 503-517
29. Brown, A J P, Leibold, E A & Munro, H N (1983) Proc. Natl. Acad. Sci. USA **80**, 1265-1269
30. Lehrach, R H, Diamond, D, Wozney, J M & Boedtker, H (1977) Biochem. **16**, 4743-4751
31. Thomas, P S (1980) Proc. Natl. Acad. Sci. USA **77**, 5201-5205
32. White, B A & Bancroft, F C (1982) J. Biol. Chem. **257**, 8569-8572
33. Maniatis, T, Jeffrey, A & Kleid, D G (1975) Proc. Natl. Acad. Sci. USA **72** 1184-1188
34. Melton, D A, Kreig, P A, Rebagliati, M R, Maniatis, T, Zinn, K & Green, M R (1984) Nucleic Acids Res. **12**, 7035-7056
35. Tonnesen, T & Freisen, J D (1973) J. Bacteriol. **115**, 889-896
36. Elorza, M V, Lostau, C M, Villanueva, J R & Sentandreu, R (1976) Biochim. Biophys. Acta **454**, 263-272
37. Fraser, R S S & Creanor, J (1974) Eur. J. Biochem. **46**, 67-73

38. Johnston, G C & Singer, R A (1978) Cell **14**, 951-958
39. Bach, M-L, Lacroute, F & Botstein, D (1979) Proc. Natl. Acad. Sci. USA **76**, 386-390
40. Losson, R & Lacroute, F (1979) Proc. Natl. Acad. Sci. USA **76**, 5134-5137
41. Pelsey, F & Lacroute, F (1984) Curr. Genet. **8**, 277-282
42. Kim, C H & Warner, J R (1983) J. Mol. Biol. **165**, 79-89
43. Kraig, E & Haber, J E (1980) J. Bacteriol. **144**, 1098-1112
44. von Gabain, A, Belasco, J G, Schottel, J L, Chang, A C Y & Cohen, S N (1983) Proc. Natl. Acad. Sci. USA **80**, 653-657
45. Singer, R H & Penman, S (1973) J. Mol. Biol. **78**, 321-334
46. Berger, S L, & Cooper, H L (1975) Proc Natl Acad Sci USA **72**, 3873-3877
47. Meyuhas, O, & Perry, R P (1979) Cell **16**, 139-148
48. Spradling, A, Hui, H & Penman, S (1975) Cell **4**, 131-137
49. Levis, R & Penman, S (1977) Cell **11**, 105-113
50. Morse, D E & Yanofsky, C (1969) Nature **224**, 329-331
51. Maquat, L E, Kinniburgh, A J, Rachmilewitz, E A & Ross, J (1981) Cell **27**, 543-553
52. Gorski, J, Fiori, M & Mach, B (1982) J. Mol. Biol. **154**, 537-540
53. Stern, M J, Ames, G F-L, Smith, N H, Robinson, E C & Higgins, C F (1984) Cell **37**, 1015-1026
54. Wong, H C & Chang, S (1986) Proc Natl Acad Sci USA **83**, 3233-3237
55. Gorski, K, Roch, J M, Prentki, P & Krisch, H M (1985) Cell **43**, 461-469
56. Shaw, G & Kamen, R (1986) Cell, in press.
57. Zaret, K S & Sherman, F (1984) J. Mol. Biol. **176**, 107-135