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Saccharomyces cerevisiae mRNA populations of different intrinsic stability in unstressed and heat shocked cells display almost constant m^7GpppA:m^7GpppG 5′-cap structure ratios

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The half-lives of mRNAs in yeast vary from about 1 to over 100 min. While mRNA stabilities must strongly influence overall gene expression in this organism, very little is known about how they are determined. Labellings of yeast cells were conducted to investigate whether the 5′-cap structures of yeast mRNAs might influence their stability. Variation of the pulse-labelling period from 7.5 min to 120 min did not have any major influence on the relative labelling of m^7GpppA (A cap) and m^7GpppG (G cap) in total polyadenylated RNA. Whether an mRNA has the A cap or the G cap does not therefore have a marked effect on its stability. During the heat shock response the relative labelling of A caps to G caps in total polyadenylated RNA also does not fluctuate appreciably. This indicates that cap structure alone does not determine the destabilisation of non-heat shock mRNAs and stabilisation of heat shock mRNAs during this stress response.

mRNA stability; 5′-Cap; Heat shock; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

Interest in mRNA stability has increased recently with the discovery that cellular transformation by some oncogenes may involve mRNA stabilisation [1–3]. There is also a growing appreciation that a number of eukaryotic genes might be regulated partly at the level of mRNA degradation in response to hormones [4,5] or during differentiation [6,7]. Even simple eukaryotes have mRNAs of very different stabilities. In *Saccharomyces cerevisiae* mRNA half-lives range from about 1 min to over 100 min ([8], and references cited therein). Also yeast mRNAs appear to fall clearly into two populations, a rapidly turning over class with half-lives of 20 min or less and a much more stable class with half-lives of 40 min to more than 100 min [8]. Within both classes there appears to be an inverse correlation between length and stability. Although nonsense mutations destabilise the *S. cerevisiae* URA3 mRNA [9], ribosome occupancy of the less stable and the more stable mRNA classes does not appear to be significantly different [10]. It is thought therefore that the existence of the two stability classes of yeast mRNAs cannot be attributed solely to ribosome occupancy and the protection it affords against endonucleolytic attack.

All yeast mRNAs are believed to carry a 3′-poly(A) of 40–70 adenosines [11], but neither the presence or absence of poly(A) nor the length of poly(A) [10] can be the underlying reason behind the existence of stable and unstable mRNA populations. A difference in the 5′-cap could be the basis of such differential stability, especially since yeast mRNAs have two alternative cap struc-

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tures, $m^7G(5')ppp(5')A$ and $m^7G(5')ppp(5')G$ [12]. In this study we employed pulse labelling to label preferentially either the rapidly turning over mRNAs or the stable mRNAs. Labelling of the cap structures in these mRNA samples was then analysed, as was also the labelling of cap structures in the mRNA of heat shocked cells. The results show that the cap structure alone on yeast mRNAs is not the primary determinant of either of the two stability classes in unstressed cells, or the destabilisation and stabilisation of particular mRNA classes during heat shock.

2. MATERIALS AND METHODS

The haploid *S. cerevisiae* strain S2072D [13] was used for all experiments. Cultures were grown at 25°C or 30°C to exponential growth in shaking flasks on a low phosphate YEPD medium [13]. At a density of $0.5–1 \times 10^7$ cells per ml they were labelled with [^{32}P]orthophosphate (Amersham) at 0.1 mCi per ml. Total RNA was extracted from harvested cells by the glass bead vortex-mixing procedure as in [14]. Total cellular polyadenylated RNA was prepared from these RNA samples by two cycles of binding to, and elution from, oligo(dT) cellulose (PL Biochemicals) [15].

Ribonuclease T2 digestion was for 30 min at 37°C with 10 μl of 2 U/ml enzyme (Sigma) in a solution containing 0.05 M ammonium acetate, pH 4.5, 1 mg/ml RNA. Two-dimensional fingerprinting of digests used pH 3.5 electrophoresis on cellulose acetate in the first dimension (fig.1, 1) and ascending chromatography on a 20 × 20 cm PEI cellulose TLC plate (Macherey-Nagel) in the second dimension (fig.1, 2) using 0.8 M LiCl as solvent. The $m^7GpppAp$ and $m^7GpppGp$ spots were distinguished by sequentially performing: (i) a digestion with calf intestinal phosphatase (Boehringer) (10 U/ml in 10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 30 min at 37°C); (ii) heating for 2 min at 65°C to inactivate the phosphatase; (iii) a digestion with nucleotide pyrophosphatase (Sigma) (10 U/ml in 20 μM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 30 min at 37°C); and (iv) TLC on PEI cellulose alongside pm^7G, pG and pA standards using 1.0 M ammonium acetate, pH 3.5, as solvent. The cap spots of each fingerprint were cut out and quantitated by liquid scintillation counting.

3. RESULTS AND DISCUSSION

A recent study of 15 *S. cerevisiae* mRNAs showed that 8 had half-lives of less than about 20 min at 30°C, and 7 half-lives of 40 min to over 100 min [8]. Some of the most abundant mRNAs, such as those for pyruvate kinase and actin [8] are in the more stable category. A short pulse labelling will preferentially label rapidly turning over mRNAs, and conversely a long labelling period will greatly increase labelling of much more stable mRNAs. To determine whether a difference in 5'-cap structure might determine the two stability classes of yeast mRNA we pulse labelled 30 ml *S. cerevisiae* cultures with [^{32}P]orthophosphate for 7.5, 15, 45 and 120 min at 30°C (see section 2). RNA was prepared from each labelling and its polyadenylated fraction isolated by two sequential bindings to oligo(dT) cellulose. These polyadenylated RNA samples were then digested with ribonuclease T2 and the $m^7GpppAp$ and $m^7GpppGp$ cap structures of each digest resolved from each other and from labelled mononucleotides by two-dimensional fingerprinting as shown in fig.1. The label present as the two cap structures on each fingerprint is given in table 1a. These quantitations show that the ratio of the two caps was essentially unchanged in all four RNA samples and had therefore been unaffected by a considerable difference in time of pulse labelling. In agreement with Sripati et al. [12] the m^7GpppA cap was found to be the most abundant and comprised approximately 70% of the total 5'-caps in both rapidly labelled and more stable mRNA (table 1a). Chia and McLaughlin [16] cite a preliminary

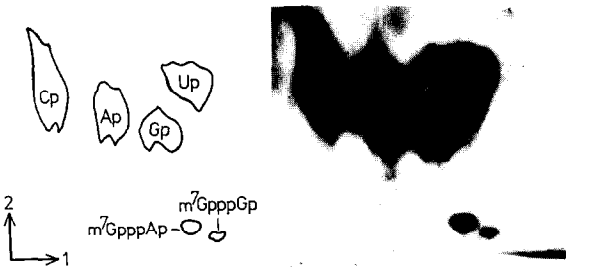

Fig.1. Two-dimensional separation of a ribonuclease T2 digest of uniformly ^{32}P-labelled yeast total polyadenylated RNA. Mononucleotides and cap structures are indicated. For experimental details see section 2.

Table 1

(a) Quantitations of 5′-caps from mRNA of 30°C pulse labellings

Pulse time (min)	7.5	15	45	120
^{32}P cpm as m^{7}GpppAp	883	1782	2416	1563
^{32}P cpm as m^{7}GpppGp	378	646	844	574
Ratio, A cap/G cap	2.30	2.75	2.85	2.70

(b) Quantitations of 5′-caps before and after a 25°C to 38°C heat shock

Pulse time relative to time of heat shock (min)	−30 to 0	0 to +20	+20 to +40	+60 to +90
^{32}P cpm as m^{7}GpppAp	1414	989	1256	1396
^{32}P cpm as m^{7}GppGp	496	328	458	511
Ratio, A cap/G cap	2.85	3.00	2.75	2.75

report of a study that indicated that yeast mRNAs with an m^{7}GpppG cap were less stable than those with an m^{7}GpppA cap. The data in table 1a are consistent with only a relatively small stability bias in favour of the m^{7}GpppA cap, and show that stability has little influence on the A cap to G cap ratio. Cap structure alone is therefore unlikely to be the major determinant of mRNA stability. This cap ratio may be fixed at the initiation of transcription by RNA polymerase II and reflect the specificity of this enzyme for purine nucleotides as the initiating, and ultimately capped, first nucleotide of the transcript. This would not prevent possible subsequent distinction of mRNAs as the basis of their caps, by cap binding proteins used in protein synthesis initiation [17] or by systems for mRNA degradation.

An experiment was also conducted to address the question of whether the massive readjustments in sequences present as cytoplasmic mRNAs during the heat shock response lead to an alteration of the A cap to G cap ratio in total cellular mRNA. A 25 to 38°C heat shock induces a heat shock response in *S. cerevisiae* which is transient, being switched off after 60–90 min at the higher temperature ([14], and references cited therein). During the response there is a dramatic induction of mRNAs for heat shock proteins and cessation of synthesis of many mRNAs existing before the shock [18]. Although many of these pre-existing mRNAs are broken down [19] a few continue to be made efficiently after the shock [14]. Yeast is also probably similar to other eukaryotes in modifying its protein synthetic apparatus to ensure preferential translation of heat shock mRNAs at high temperature [18].

30 ml cultures of *S. cerevisiae* grown at 25°C were pulse labelled with [^{32}P]orthophosphate for 20 min both prior to and during a 25 to 38°C heat shock. Total poly(A)$^+$ RNA was prepared from them, and its m^{7}GpppAp and m^{7}GpppGp cap structures resulting from ribonuclease T2 digestion separated and quantitated as for the experiment in table 1a. The results are listed in table 1b. The heat shock response induces its maximum alterations in mRNAs 20–30 min after a 25 to 38°C shift, after which the response is modulated, cells resuming pre-shift patterns of transcription and protein synthesis 60–90 min after the shift [14,18,19]. During this period of dramatic alteration of sequences expressed as mRNAs the A cap to G cap ratio in total mRNA does not undergo appreciable change (table 1b). Therefore the structure of the 5′-caps on mRNAs is not the primary determinant of any selective stabilisation or destabilisation of particular mRNA classes during heat shock.

From these and other studies [10] it is apparent that the structural basis for the existence of stability classes of yeast mRNAs must be sought within the nucleotide sequences of these mRNAs, not their 5′-caps or 3′-poly(A).

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