Post-transcriptional modification of the poly(A) length of galactose-1-phosphate uridyl transferase mRNA in *Saccharomyces cerevisiae*

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ABSTRACT

Thermal elution poly(U)-Sepharose chromatography was utilized to fractionate yeast mRNA based on poly(A) size. Analysis of the *in vitro* translation products of the fractionated RNAs in a wheat-embryo cell-free protein synthesis system shows a heterogeneous but equal distribution of these abundant translatable mRNAs in the different poly(A) size classes. By comparing the translational activity of inducible galactose-1-phosphate uridyl transferase mRNA, which can be monitored as a function of age, to constitutive mRNAs, we demonstrate that initially galactose-1-phosphate uridyl transferase mRNA has a uniformly large poly(A) tail which becomes heterogeneous and shorter with age in the cytoplasm. These observations are consistent with the previously observed cytoplasmic poly(A) catabolism in yeast and with cytoplasmic post-transcriptional modification of the poly(A) length of galactose-1-phosphate uridyl transferase mRNA.

INTRODUCTION

Polyadenylation is a feature of most messenger RNAs from a wide variety of organisms. While the biological function of 3' polyadenylation is unclear, it has been implicated in the protection of mRNA from degradation in the cytoplasm (1-3) and in the regulation of mRNA turnover during development (4). This model accounts for observed differences in mRNA half-lives, and for observed heterogeneity and shortening in poly(A) size of mRNA (5-7). Several studies, however, argue against poly(A) stabilization of mRNA (8-10).

It has been shown, in a number of eucaryotes, that for the abundant mRNA class, heterogeneity of poly(A) tracts is present at the level of specific mRNAs as detected by their cell-free synthetic activity (6,7,11). This heterogeneity presumably occurs either at the time of primary polyadenylation of the nuclear transcripts, or after transport to the cytoplasm, as degradation and/or repolymerization of the poly(A) tails. A critical test of the possible relationship between heterogeneous poly(A) length and cellular mRNA metabolism is a characterization of the poly(A) of high regulated unique gene products. We have adopted this approach by use of the inducible

galactose pathway enzymes in *Saccharomyces cerevisiae* (12), for which quantitative *in vitro* assays for specific mRNAs have been developed (13). Half-time for the induction of galactose-1-phosphate uridylyl transferase (GalT) mRNA is approximately 15 min (J.E. Hopper, personal communication). Soon after this induction, newly synthesized transcripts should closely reflect the poly(A) length initially polymerized. Later, at steady-state, modification of the poly(A) could occur. We examined mRNA obtained 15 min after induction and compared it to that from cells grown for several generations on galactose. From our results we conclude that initially GalT mRNA has a uniformly large poly(A) tail which becomes heterogeneous with age in the cytoplasm.

MATERIALS AND METHODS

Cell growth and preparation of RNA

The haploid yeast strain 108-3c (a *trp⁻ura1*) was obtained from Dr. Howard Douglas. Cultures of cells were grown in New Brunswick fermenters in YEP medium (1% (w/v) yeast extract, 2% (w/v) Bacto-peptone) containing either 2% (w/v) galactose, (glucose-free, Sigma Chemical Co.), or 1% (w/v) ethanol and then induced for 15 min with 2% (w/v) galactose. Total cellular RNAs were obtained by cell disruption and standard H₂O-saturated phenol extraction as previously described (13,14).

RNA fractionation

The poly(A) fractionation of total yeast cellular RNA was accomplished by thermal elution chromatography on poly(U) Sepharose (Pharmacia) as described by Palatnik *et al.* (15). The poly(A) content of the fractionated RNA was determined by the method of hybridization to [³H] poly(U) (16).

Cell-free protein synthesis and immunoprecipitation

Total cellular RNAs and the chromatographically fractionated poly(A)-containing RNAs were translated in a wheat-embryo cell-free system prepared by modification of several procedures (13,14,17), with [³⁵S]-methionine (>800 Ci/mMole, Amersham) as incorporated label. Radioisotope incorporation was determined by trichloroacetic acid precipitation (13). Immunoprecipitation assays were performed by the double antibody method employing goat anti-rabbit γ -globulin and rabbit antibody against purified yeast enzymes, as previously described (13,14). Total translation products and immunoprecipitates were analyzed after treatment for 3 min at 100°C in Studier's buffer (18), by NaDodSO₄/polyacrylamide gel electrophoresis according to published methods (13,14,18). After PPO impregnation of the gels by the method of Bonner and Laskey (19) products were visualized by autoradiography

on XR-5 Royal X-omat film. Densitometry of the gel autoradiograms was accomplished with a Joyce-Loebl densitometer.

RESULTS

Thermal elution poly(U) Sepharose chromatography was used to fractionate total cellular RNA of the haploid strain 108-3c of *S. cerevisiae* based on its poly(A) content. The degree of poly(A) fractionation was assessed by hybridization to [^3H] poly(U). Data for yeast mRNA are shown in Figure 1A. The mRNA species present in these various poly(A) classes were characterized by translational analysis in a wheat-embryo cell-free system using [^{35}S]-methionine. Translations were performed under standard reaction conditions in which radioisotope incorporation was linear with RNA concentration. Five μg

Figure 1. Poly(A) content and translational efficiency of mRNA thermally eluted from poly(U) Sepharose. RNA prepared from yeast cells grown in YEP containing 2% galactose (●) or in 1% ethanol and then induced for 15 min with 2% galactose (■) was fractionated by thermal elution chromatography of the poly(A)-containing mRNA on poly(U) Sepharose at the indicated temperatures. Ft is the nonbinding, poly(A) minus fraction.₃ Shown in panel A is the percent poly(A) determined by hybridization to [^3H]-labeled poly(U). Poly(A) content was estimated assuming an average mRNA size of 1000 nucleotides. To determine the translational efficiency of the various mRNA fractions obtained by the thermal elution from the poly(U) Sepharose, mRNA was translated in a wheat-embryo translation system with [^{35}S]-methionine. Data in panel B shows radioisotope incorporation for a portion of each translation reaction assayed by trichloroacetic acid precipitation for the fractionated mRNA from cells grown on 2% (w/v) galactose. Similar results were obtained with induced mRNA.

of RNA from each fraction was used per 50 μ l reaction.

As seen in Figure 1B, the amount of radioisotope incorporation determined on a portion of each translation reaction was within a two-fold range, indicating the translational efficiency of the mRNAs (excluding the poly(A) minus, non-binding fraction) remained fairly constant. The cell-free translation products of the fractionated mRNAs were resolved by electrophoresis on NaDodSO₄/polyacrylamide slab gels. As seen in the gel autoradiogram of Figure 2, the relative amounts of a specific translation product in each of the poly(A) size classes are essentially indistinguishable, implying that the mRNAs coding for these proteins have heterogeneous poly(A) lengths. Furthermore, the data, which reflects the higher abundance yeast mRNAs, indicates their equal distribution in the various poly(A) size classes. We have made similar observations for several low abundant mRNAs coding for constitutively synthesized enzymes, by identification and quantitation of their cell-free translation products by immunoprecipitation assay (data not shown).

The heterogeneity of poly(A) lengths for the yeast mRNAs shown in Figure 2 may be explained either by initial polymerization of mRNAs with varying poly(A) lengths, or by uniform initial polymerization followed by poly(A) degradation and/or repolymerization. To distinguish between these possibilities we determined the poly(A) length of the inducible GalT mRNA both immediately after induction, and at steady-state (fully induced), when the poly(A) tail has been subject to possible modification in the cytoplasm. As an internal control the constitutively expressed glyceraldehyde-3-phosphase dehydrogenase (GPDH) mRNA (17) was analyzed in each poly(A) size class. These mRNAs were quantitated by gel-autoradiographic densitometric measurement of their cell-free synthesized products after immunoprecipitation and NaDodSO₄/

Figure 2. Distribution of poly(A) length of specific yeast mRNAs. Specific yeast mRNAs present in the various mRNA fractions obtained by the thermal elution poly(U) Sepharose chromatography of Fig. 1A were assessed by electrophoretic analysis of their cell-free translation products on a 10-15% NaDodSO₄/polyacrylamide gel. In panel A, lane (a) shows the translation products of unfractionated RNA from galactose grown cells, (b-f) are the translation products of mRNA from cells 15 min after induction with galactose eluting from the poly(U) column: (b), in the flow-through; (c), at 25°C; (d), at 35°C; (e), at 45°C; and (f), at 55°C. Lanes (g-k) are the translation products of mRNA from cells grown for several generations on galactose, eluting from the poly(U) column: (g), in the flow-through; (h), at 25°C; (i), at 35°C; (j), at 45°C; (k) at 55°C. Lane (l) shows the translation products from endogenous mRNA in the wheat-embryo system. Panel B is a composite photograph showing various exposures of the gel in panel A and is useful for comparing relative amounts of translation products in each fraction.

polyacrylamide gel electrophoresis. By this method we have previously shown that the translational efficiency of several specific yeast mRNAs is constant when the specific mRNA concentration is varied at a constant total RNA concentration in the translation reaction, such that product yield was propor-

Figure 3. Poly(A) lengths of galactose-1-phosphate uridyl transferase (GalT) mRNA at steady-state and 15 min post-induction. The specific *in vitro* translation products of GalT and GPDH mRNA present in the cell-free translations of Figures 1 and 2 were measured by immunoprecipitation with specific rabbit antibody prepared against purified galactose-1-phosphate uridyl transferase (13) and GPDH (17). Immunoprecipitates were analyzed by 10% NaDodSO₄/polyacrylamide slab gel electrophoresis and autoradiography. Panel A shows the immunoprecipitation of a cell-free translation programmed either with unfractionated RNA from (A) steady-state glucose grown cells; (B) 15 min post-induction cells, or (C) steady-state galactose grown cells, immunoprecipitated with (a) pre-immune serum; (b) anti-GalT and anti-GPDH IgG, and (c) anti-transketolase, anti-enolase, anti-alcohol dehydrogenase, and anti-GPDH IgG. The relative abundance of GalT and GPDH mRNA in the poly(A) fractionated steady-state and 15 min post-induction RNA was determined by measurement of their cell-free translation products by densitometry of gel autoradiograms as shown in panel A. In panel B the ratio of the amount of GalT and GPDH mRNA activity (cell-free product) is plotted against the poly(U) thermal elution fraction of origin for steady-state (■) and 15 min post-induction RNA (●).

tional to the specific concentration of the individual mRNA (unpublished results). RNA from pre-induced, 15 min post-induced, and steady-state galactose grown cells was translated in the wheat-embryo system and the cell-free translation products immunoprecipitated with pre-immune, anti-GalT or anti-GPDH antibody. The [35 S]-methionine labeled immunoprecipitates electrophoresed on a 10% NaDodSO₄/polyacrylamide gel are shown in the autoradiogram of Figure 3A. As seen, GPDH mRNA activity remains unchanged by growth on galactose. The amount of GPDH mRNA in the various poly(A) size classes from both induced and steady-state cultures was relative high and the same, and varied within a two-fold range. Using GPDH mRNA to normalize the data, in Figure 3B we have expressed directly the ratio of immunoprecipitable cell-free translation products of GalT to GPDH mRNA from the fully and partially (15 min) galactose induced cultures as a function of poly(A) size. As seen, a shift is observed in the poly(A) size distribution from a very narrow one soon after induction of GalT mRNA to a more disperse one at steady-state. The latter is virtually identical to that of the constitutive GPDH mRNA. These results are most easily interpreted as cytoplasmic, post-transcriptional modification of the GalT mRNA, suggesting an age dependence on poly(A) lengths.

DISCUSSION

The link between mRNA metabolism and poly(A) sequences is as yet inconclusive. Any model must take into account the observed heterogeneity in poly(A) content of the population of mRNA molecules coding for a given protein, and, as we have demonstrated, for changing lengths of poly(A). Early work has established that poly(A) catabolism occurs in the cytoplasm (20) and a possible link of catabolism to translation of mRNA has been reported (21). The metabolism of poly(A) in yeast has been investigated previously (22). Labeling kinetics experiments indicated that in a total poly(A)-containing mRNA population, long poly(A) tracts are rapidly labeled and shorter poly(A) tracts isolated from polyribosomes are labeled only after longer times. This result is analogous to the observations in human cells (21). Both nuclear and cytoplasmic forms of polyriboadenylate polymerase have been reported in yeast (23). The cytoplasmic enzyme activity has been shown to change during growth and sporulation, altering poly(A) content of the mRNA (24,25). The observations presented here argue that cytoplasmic poly(A) catabolism may well be responsible for much of the observed heterogeneity in poly(A) content for a specific mRNA. These experiments do not address directly question about poly(A) length and mRNA stability. From experiments

involving the survival of globin mRNA micro-injected into oocytes (26) it has been suggested that a minimum poly(A) length (e.g., 30 nucleotides) may be sufficient to maintain mRNA stability to nuclease attack, and it is unclear whether the poly(A) of these mRNAs physiologically decreases below this level. Furthermore, in *Dictyostelium*, Palatnik *et al.* (10) have demonstrated that vegetative mRNAs with shorter poly(A) tracts (60-65 nucleotides) are more stable. A random loss of cytoplasmic mRNA by most first-order stochastic models is not readily understood by a poly(A)-regulated sequential 3' exonuclease digestion of individual mRNAs with changing lengths. A more detailed kinetic analysis of the catabolism of the poly(A) for a specific mRNA and its relationship to both the functional and chemical stability of that mRNA will be necessary to draw any firm conclusions regarding the role of poly(A) in the regulation of a specific mRNA population. The use of inducible enzyme systems to define the initial period of synthesis of these mRNAs has obvious advantages to the study of this problem. The ability to focus on a single mRNA species of known age, and to directly measure the mRNA level (as a measure of mRNA turnover) (27) with respect to poly(A) length should allow a more detailed analysis of eucaryotic mRNA metabolism than is possible with either mRNAs for terminal differentiation products or the heterogeneous mRNA populations.

ABBREVIATIONS: NaDodSO₄, sodium dodecyl sulfate; GalT, galactose-1-phosphate uridylyl transferase; GPDH, glyceraldehyde-3-phosphate dehydrogenase.

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