Isolation and Identification of Yeast Messenger Ribonucleic Acids Coding for Enolase, Glyceraldehyde-3-phosphate Dehydrogenase, and Phosphoglycerate Kinase†

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ABSTRACT: Yeast poly(adenylic acid)-containing messenger ribonucleic acid isolated from two strains of *Saccharomyces cerevisiae* was fractionated by preparative polyacrylamide gel electrophoresis in the presence of formamide. Three messenger ribonucleic acids, present at high intracellular concentration, were electrophoretically eluted from the polyacrylamide gels and translated in a wheat germ cell-free extract. The in vitro synthesized polypeptides were identified by tryptic peptide analysis. Messenger ribonucleic acids coding for enolase and glyceraldehyde-3-phosphate dehydrogenase were isolated from commercially grown baker's yeast (strain F1), and messenger ribonucleic acid coding for phosphoglycerate kinase was isolated from *Saccharomyces cerevisiae* (ATCC 24657). Significant differences in the spectrum of abundant messenger ribonucleic acids isolated from commercially grown baker's yeast (strain F1) and strain 24657 were observed. When both strains were grown under identical conditions, however, the spectrum of messenger ribonucleic acid isolated from the cells is indistinguishable.

The facultative anaerobe *Saccharomyces cerevisiae* maintains high intracellular levels of the glycolytic enzymes when grown on a fermentable carbon source such as glucose (Hess et al., 1968). Further studies have demonstrated that the intracellular concentrations of glycolytic enzymes in yeast are coordinately regulated (Hommes, 1966; Maitra and Lobo, 1974). Commercial baker's yeast contains an abundant population of messenger RNAs comprising approximately 25% of the cellular mRNA (Holland et al., 1977) which codes for approximately ten proteins of average molecular weight 25 000. The principal polypeptides synthesized in vitro from yeast poly(A)[1]-containing mRNA are glycolytic enzyme polypeptides (Holland et al., 1977). These data suggest that mRNAs coding for the glycolytic enzymes are present at sufficiently high intracellular concentration to permit a practical purification from yeast. Although these mRNAs comprise a substantial proportion of the cellular mRNA pool, each of the major glycolytic enzymes represents no more than 1–5% of the total. In order to obtain suitable hybridization probes for cloning bacterial cells carrying hybrid plasmids containing the structural genes which code for specific glycolytic enzymes, the fractionation and identification of the abundant messenger RNAs of yeast were carried out.

In this report, we describe the isolation and identification of yeast mRNAs coding for enolase, glyceraldehyde-3-phosphate dehydrogenase, and phosphoglycerate kinase. These data provide direct evidence that there is a small number of mRNAs in yeast which are present at high intracellular concentration and that the most abundant mRNA species in yeast code for glycolytic enzymes. Furthermore, the intracellular concentration of specific glycolytic messenger RNAs in *S. cereviseae* is highly dependent on cellular growth conditions, suggesting that expression of glycolytic genes in yeast is regulated at the transcriptional level.

Experimental Procedure

Materials. Heparin (grade I), cyclohexamide, iodoacetic acid, Hepes, Tris, creatine phosphate, creatine phosphokinase, iodoacetamide, and enolase (type III, from yeast) were purchased from Sigma Chemical Co. Dithiothreitol and ethidium bromide were obtained from Calbiochem. Trypsin–TPCK and bovine pancreatic ribonuclease were from Worthington. Casamino acids, yeast extract, and peptone were purchased from Difco. Poly(U) was obtained from P-L Biochemicals. Omnifluor and L-[^{35}S]methionine (500 Ci/mmol) were purchased from New England Nuclear. L-[^{3}H]Leucine (46 Ci/mmol) was from ICN. Fluorescamine (Fluoram) was supplied through Roche Diagnostics. Phosphoglycerate kinase was obtained from Boehringer Mannheim, and glyceraldehyde-3-phosphate dehydrogenase was prepared by the method of Holland and Westhead (1973). Wheat germ was obtained from the "bar Rav" Mill, Tel Aviv, Israel. Acetonitrile (UV) was from Burdick and Jackson Laboratories, Inc.

Cells. Early log-phase baker's yeast cells (*Saccharomyces cerevisiae,* strain F1) were kindly donated by the Red Star Corp. (Division of United Foods), Oakland, Calif. *Saccharomyces cerevisiae* ATCC 24657 was grown in cultures containing 2% peptone, 1% yeast extract, and 2% glucose with vigorous aeration at 30 °C. Cells were harvested in early log-phase growth (A_{660} = 0.9–1.0). Yeast cells were washed twice in distilled water and suspended in breaking buffer. The cell suspension contained 85 g of wet weight cells, 10 mL of glycerol, and 5 mL of buffer containing 1 M Tris (pH 7.5), 0.1 M $MgCl_2$, and 0.2 M iodoacetate. The suspension was frozen in small pellets by dripping into liquid nitrogen and stored in liquid nitrogen.

Isolation of Yeast Poly(A)-Containing mRNA. Liquid nitrogen frozen cells were disrupted in an Eaton press, and total cellular RNA was prepared as previously described (Holland

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[1] Abbreviations used: poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); cDNA, complementary deoxyribonucleic acid; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; TPCK, (1-tosylamido-2-phenyl)ethyl chloromethyl ketone; EDTA, (ethylenedinitrilo)tetraacetic acid; Temed, *N,N,N′,N′*-tetramethylenediamine. Enzyme commission numbers: enolase, 4.2.1.11; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 1.2.1.12; phosphoglycerate kinase (PGK), 2.7.2.3.

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et al., 1977). Poly(A)-containing mRNA was isolated from total cellular RNA by affinity chromatography on poly(U)-cellulose. Total cellular RNA was suspended at a concentration of 2–3 mg/mL in buffer containing 10 mM Tris (pH 7.5), 1 mM EDTA, and 0.1 M NaCl. The RNA solution was loaded onto a poly(U)-cellulose column (1 × 10 cm) at a flow rate of 0.5 mL/min. After loading, the column was washed with 3 column volumes of buffer containing 10 mM Tris (pH 7.5), 1 mM EDTA, and 0.1 M NaCl, followed by 3 column volumes of buffer containing 10 mM Tris (pH 7.6), 1 mM EDTA, and 30% formamide. Poly(A)-containing mRNA was then eluted with buffer containing 10 mM Tris (pH 7.6), 1 mM EDTA, and 90% formamide. All chromatography steps were carried out at 4 °C.

RNA Polyacrylamide Gel Electrophoresis. RNA samples were electrophoresed in the presence of 99% formamide according to the procedure of Pinder et al. (1974) with the following modifications. Poly(A)-containing mRNA (300 μg) was suspended in 90% deionized formamide which was buffered with 0.02 M sodium phosphate (pH 7.6) and applied directly to a 13.5 × 10 × 0.3 cm, 4% acrylamide slab gel. Gels containing 3.4% acrylamide, 0.6% bisacrylamide, 0.02 M sodium phosphate (pH 7.6), 1.2% ammonium persulfate, 0.2% Temed, and 99% deionized formamide were electrophoresed at 40 V for 20 h in 0.02 M sodium phosphate (pH 7.6) running buffer. In order to maintain constant pH, a circulating reservoir system, as described by Pinder et al. (1974), was employed.

After electrophoresis, the gel was placed on a screen support and submerged in a solution of 1 mM EDTA (pH 7.6) for 30 min with stirring. This procedure removed the formamide in the gel. The gel was then placed in a solution containing ethidium bromide (0.5 μg/mL) for 30 min and then transferred to a glass plate. RNA species were visualized with a long ultraviolet wavelength lamp (Mineralight lamp Model XX-1SC, Ultra-Violet Products, Inc.) and sliced from the gel. The gel slices, suspended in 3 mL of elution buffer containing 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA (pH 7.8), and 1.5% sodium dodecyl sulfate, were placed in a constricted 20-cm disc gel tube which contained a 3% acrylamide plug and were electrophoresed into a dialysis bag for 20 h at 7.5 mA/gel. The RNA was then adjusted to 0.1 M Tris (pH 9.0) and 0.1 M KCl and extracted with phenol–chloroform–isoamyl alcohol (50:49:1). The sample was chilled for 10 min on ice and then centrifuged at 3000*g* for 10 min at 4 °C. The aqueous phase was removed and adjusted to 0.3 M sodium chloride and precipitated with 2 volumes of ethanol at −20 °C overnight.

In Vitro Translation of Purified mRNAs. Wheat germ S-30 fractions were prepared as described by Roberts and Paterson (1973). In vitro translations were carried out at 22 °C for 120 min at 25-μL reaction mixtures containing 10 μL of the S-30 fraction (OD_{280} units = 0.85), 12 mM Hepes buffer (pH 7.6), 2 mM dithiothreitol, 1 mM ATP, 0.2 mM GTP, 8 mM creatine phosphate, 40 μg/mL creatine phosphokinase, 0.1 M potassium acetate, 2.0 mM magnesium acetate, 10 μCi of [^{35}S]methionine (sp act. 500 Ci/mmol), and 30 μM each of the unlabeled amino acids. Magnesium and potassium optima were determined for each preparation of wheat germ S-30. Reaction mixtures contained 50–70 μg of total cellular RNA, 1–15 μg of poly(A)-containing mRNA, or 0.1–0.2 μg of purified glycolytic mRNA (based on a comparison of incorporation of [^{35}S]methionine in response to added poly(A)-containing mRNA). Following incubation, aminoacyl-tRNAs were hydrolyzed by adjusting the reaction mixture to 0.1 N NaOH and incubating for an additional 15 min at 37 °C. The reaction was finally adjusted to 0.3% in casamino acids and precipitated with 10% trichloroacetic acid. The precipitate was collected on GF/C filter disks and counted in toluene Omnifluor.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Analysis. Polyacrylamide gel analysis of proteins was carried out using the discontinuous buffer system described by Laemmli (1970). Slab gels, 13.5 × 10 × 0.15 cm, containing a stacking gel and a 10% running gel, were electrophoresed for 15 h at 30 V. Up to 10 μL of the translation reaction mixture was added to a lane on the gel without affecting resolution. The purified glycolytic enzymes phosphoglycerate kinase, enolase, and glyceraldehyde-3-phosphate dehydrogenase were used as markers. Slab gels were stained with 0.25% Coomassie blue–25% 2-propanol–10% acetic acid overnight and destained by washing two times for 4 h with 10% acetic acid–10% 2-propanol. Following washing with 10% acetic acid containing 1% glycerol for two hr the gel was dried under vacuum in a Hoeffer Gel Dryer. Autoradiograms were analyzed by cutting lanes from the exposed film and scanning the film strips at 660 nm in a McPherson scanning device.

Tryptic Peptide Analysis. Method I. Tryptic peptides of in vitro synthesized proteins were prepared according to the procedure of Kochman and Rutter (1968) with the following modifications: 5 μL of the in vitro translation mixture was adjusted to 10 mM EDTA (pH 7.4) and incubated with 0.5 μg of pancreatic ribonuclease for 30 min at 37 °C. After ribonuclease treatment, the sample was dissolved in 1 mL of 8 M urea in 0.1 M ammonium bicarbonate (pH 8.2) and 10 mM dithiothreitol, and 50 μg of the appropriate purified glycolytic enzyme was added. With a 4-h incubation at 45 °C, the sample was adjusted to 0.1 M iodoacetamide and incubated for 15 min at 25 °C, followed by the addition of dithiothreitol to a final concentration of 0.2 M. The sample was dialyzed extensively against 0.2 M ammonium bicarbonate (pH 8.6). Following dialysis, 1 μg of trypsin–TPCK was added, and the sample was allowed to incubate for 4 h at 25 °C. A second aliquot of trypsin–TPCK (1 μg) was then added, and the sample was allowed to incubate for an additional 4 h at 25 °C. The tryptic peptides were lyophilized and suspended in 5–10 μL of deionized water and spotted on a 20 × 20 cm 250-μm silica gel G thin-layer chromatographic plate (Analtech). Chromatography, according to a modified procedure of Baum et al. (1975) was carried out in the first dimension in an ascending system of chloroform–methanol–ammonium hydroxide (2:2:1) until the solvent form had traveled a distance of 18 cm. After air-drying, chromatography in the second dimension was carried out in butanol–acetic acid–water (4:1:1). The resulting peptide map was air-dried overnight, sprayed with 20 mL of fluorescamine in acetone (0.15 mg/mL), and examined under long ultraviolet light. The peptide map was then analyzed by autoradiography after 2–6 weeks exposure time.

Method II. Analysis of tryptic peptides by reverse-phase high-pressure liquid chromatography was carried out with polypeptides synthesized in a wheat germ cell-free extract containing 20 μCi of [^{3}H]leucine (sp act. 46 Ci/mmol) as described above. Digestion with trypsin was as described in method I, except that 1 mg of the appropriate purified glycolytic enzyme was added and 20 μg of trypsin–TPCK was added to each incubation. Lyophilized tryptic peptides were suspended in deionized water, centrifuged at 25 000*g* for 10 min to remove traces of insoluble material, and injected directly onto a 25 × 0.4 cm LiChrosorb RP-18 reverse-phase chromatography column (Altex). A Micromeritics Model 7000 LC apparatus was used for these analyses. Tryptic peptides derived from the added glycolytic enzyme were monitored spectrophotometrically at 215 nm. The analyses were carried out at

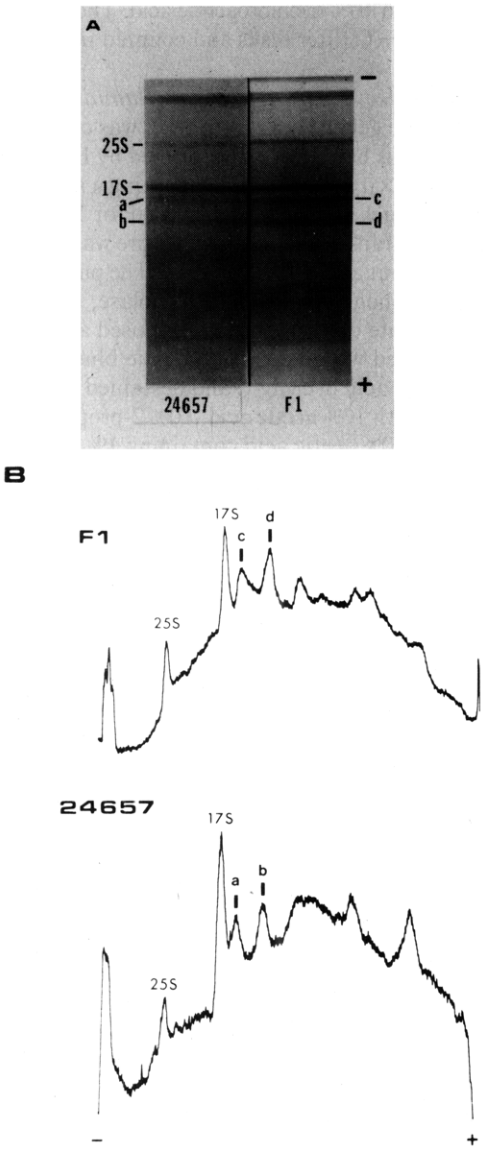


FIGURE 1: (A) Formamide–polyacrylamide slab gels of poly(A)-containing mRNA isolated from *Saccharomyces cerevisiae* strains F1 and ATCC 24657. Gels were stained with ethidium bromide, and RNA was visualized with long-wavelength ultraviolet light as described under Experimental Procedures. The mobilities of the 25S and 17S rRNAs are indicated by arrows. (B) Densitometer tracings of photographic negatives of ethidium bromide stained formamide–polyacrylamide slab gels of poly(A)-containing mRNA isolated from *S. cerevisiae* strains F1 and ATCC 24657 shown above.

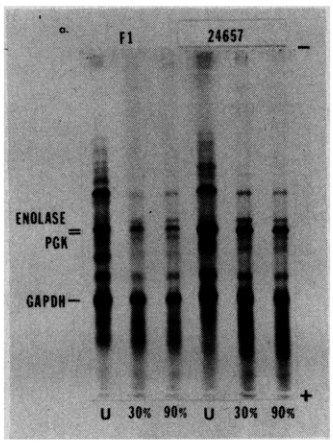
a flow rate of 0.5 mL/min with a linear gradient from 0 to 50% acetonitrile containing 10 mM potassium phosphate buffer (pH 5.8). Fractions were collected at 0.3-min intervals, and the [^{3}H]leucine-containing peptides were quantitated by scintillation counting utilizing a Triton X-100–toluene–omnifluor scintillation fluid (1 part Triton X-100, 2 parts toluene, 15 g of omnifluor/gal).

Results

Fractionation of Yeast Poly(A)-Containing mRNA by Polyacrylamide Gel Electrophoresis in the Presence of Formamide. Total yeast poly(A)-containing mRNA was isolated by poly(U)-cellulose affinity chromatography. Figure 1A shows preparative polyacrylamide slab gels of total poly(A)-containing mRNA isolated from commercially grown baker's yeast (strain F1) and *S. cerevisiae* ATCC 24657 grown as described under Experimental Procedure. Messenger RNA was visualized by staining in a solution of 0.5 μg/mL ethidium bromide after removal of formamide as described under Experimental Procedure. Ethidium bromide bound to RNA in the gel can be visualized with long-wavelength ultraviolet light, making it possible to accurately slice RNA bands from the gel. While there are a number of RNA species, including contaminating 25S and 17S rRNA, which are identical in migration in the two slab gels, there are also major components of the populations which are either not present or are present at significantly different concentrations in the two preparations. The mRNA fractionation shown in the first figure demonstrates that there are major mRNAs in yeast. The relative concentration of these major mRNAs is best illustrated by the densitometer tracings of the slab gels shown in Figure 1B. It is clear from these data that the abundant mRNAs are present on a large background of other cellular mRNAs. Slicing these mRNAs from the gel would, therefore, give a significant enrichment but would not yield homogeneous species. With this reservation in mind, and the knowledge that mRNAs coding for glycolytic enzymes are present in high concentration in yeast, we sought to further analyze the major mRNA species (labeled a–d in Figure 1) in order to determine the identity of the polypeptides coded for by each of the four mRNAs.

In Vitro Translation of Isolated mRNAs in a Wheat Germ Cell-Free Extract. In order to identify the major mRNAs present in commercially grown baker's yeast and *S. cerevisiae* ATCC 24657, the polypeptides synthesized in vitro under the direction of the respective unfractionated total poly(A)-containing mRNA were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Figure 2 shows the pattern of polypeptides synthesized from total unfractionated cellular RNA, poly(A)-containing mRNA eluted from poly(U)-cellulose with a step elution with 30% formamide, and finally a subsequent step elution with 90% formamide. On inspection of the polypeptides synthesized by the three fractions of RNA it is clear that the bulk of the polypeptides synthesized from these fractions are qualitatively and quantitatively similar. The major exception is a polypeptide which migrates more slowly than the enolase marker and is present in all of the translation products synthesized from poly(U)-cellulose fractionated RNA. This species is not detectable in the polypeptides synthesized from unfractionated RNA. The major polypeptides synthesized from mRNA isolated from commercial baker's yeast coelectrophorese with purified enolase and glyceraldehyde-3-phosphate dehydrogenase (Figure 2b). In contrast, the major polypeptide synthesized from *S. cerevisiae* ATCC 24657 migrates slightly faster than enolase and coelectrophoreses with purified phosphoglycerate kinase (Figure 2b). The subunit molecular weights of yeast enolase, phosphoglycerate kinase, and glyceraldehyde-3-phosphate dehydrogenase are 44 000 (Wold, 1971), 45 000 (Scopes, 1973), and 36 000 (Jones and Harris, 1972), respectively. The molecular weight of phosphoglycerate kinase, determined from amino acid composition (Scopes, 1973), is greater than that reported for enolase; however, we observe more rapid mobility of phosphoglycerate kinase relative to enolase in $NaDodSO_4$–polyacrylamide gels. The identity of both enolase and phosphoglycerate kinase marker proteins has been verified by enzymatic activity measurement and amino acid composition. Close inspection of the total polypeptide pattern obtained from the two mRNA populations reveals a number of differences, suggesting that the spectrum of major mRNAs is different in the two populations, in agreement with the mRNA analysis (Figure 1).

In order to determine if the major mRNA species resolved



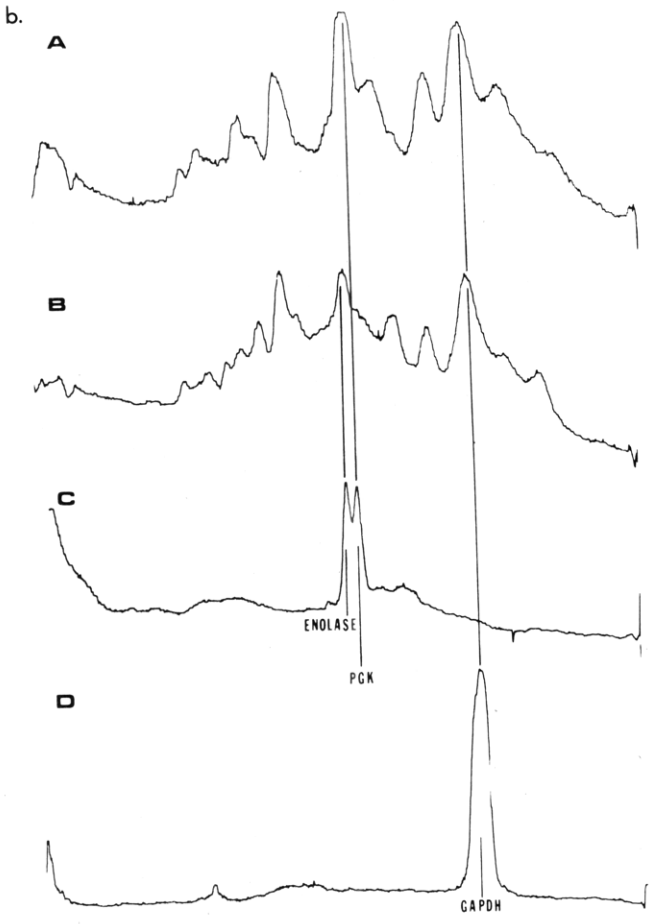


FIGURE 2: (a) Autoradiogram of a sodium dodecyl sulfate–polyacrylamide slab gel of [^{35}S]methionine-labeled polypeptides synthesized in vitro in a wheat germ cell-free extract in response to (U) unfractionated total cellular RNA; (30%) poly(A)-containing mRNA eluted from a poly(U)-cellulose column with buffer containing 30% formamide, 10 mM Tris (pH 7.5), and 1 mM EDTA; (90%) poly(A)-containing mRNA eluted from a poly(U)-cellulose column with buffer containing 90% formamide, 10 mM Tris (pH 7.5), and 1 mM EDTA after the 30% formamide elution described above. RNA fractions were isolated from *S. cerevisiae* strains F1 and ATCC 24657. The mobilities of purified yeast enolase, phosphoglycerate kinase (PGK), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) markers are indicated by arrows. (b) Densitometer tracings of autoradiograms of a sodium dodecyl sulfate–polyacrylamide gel of [^{35}S]methionine-labeled polypeptides synthesized in vitro in a wheat germ cell-free extract in response to unfractionated poly(A)-containing mRNA isolated from *S. cerevisiae* strain ATCC 24657 (A) and commercially grown baker's yeast (strain F1) (B). Panels C and D are densitometer tracings of Coomassie blue stained enolase plus phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase marker proteins. The marker proteins were electrophoresed in parallel lanes with the [^{35}S]-methionine-labeled polypeptides.

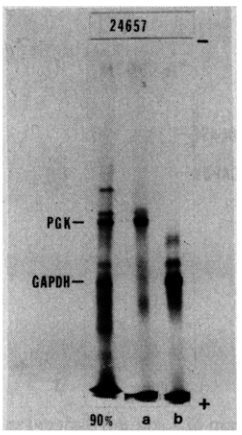


FIGURE 3: Autoradiogram of a sodium dodecyl sulfate–polyacrylamide slab gel of [^{35}S]methionine-labeled polypeptides synthesized in vitro in a wheat germ cell-free extract in response to (90%) unfractionated poly(A)-containing mRNA and (a and b) mRNA electrophoretically eluted from the a and b bands shown in the formamide–polyacrylamide slab gel in Figure 1. RNA fractions were isolated from *S. cerevisiae* strain ATCC 24657. The mobilities of purified yeast phosphoglycerate kinase (PGK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) markers are indicated by arrows.

by polyacrylamide gel electrophoresis in the presence of formamide, in fact, code for the principal polypeptides synthesized in response to unfractionated poly(A)-containing mRNA, mRNA species a–d were sliced from the formamide slab gels and eluted electrophoretically. After translation of the fractionated mRNAs in a wheat germ cell-free extract, the polypeptides were compared to those synthesized from unfractionated poly(A)-containing mRNA on sodium dodecyl sulfate–polyacrylamide gels. Figure 3 shows a polyacrylamide gel of polypeptides synthesized in vitro from unfractionated poly(A)-containing mRNA isolated from *S. cerevisiae* ATCC 24657 and from two major species (a and b) isolated from a preparative polyacrylamide gel. It is clear that the two major mRNA species do, in fact, code for the two major polypeptides synthesized from unfractionated poly(A)-containing mRNA. In addition, it appears that the two polypeptides coelectrophorese with phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase. A similar analysis of the polypeptides synthesized in vitro from unfractionated poly(A)-containing mRNA isolated from commercially grown baker's yeast and the two major mRNAs (c and d) isolated from a preparative polyacrylamide gel in formamide is shown in Figure 4. Again the polypeptides synthesized from the two major mRNAs coelectrophorese with the two major polypeptides synthesized from unfractionated poly(A)-containing mRNA. In contrast, however, these polypeptides coelectrophorese with purified enolase and glyceraldehyde-3-phosphate dehydrogenase.

It is clear from the in vitro translation data obtained from the major mRNAs isolated from preparative polyacrylamide gels that the fractionation yields a substantial enrichment of each mRNA. It should also be noted that each of these fractionated mRNAs contains contaminating mRNAs which code for unidentified polypeptides but which coelectrophorese with polypeptides synthesized from unfractionated poly(A)-containing mRNA. Since the analysis of polypeptides synthesized in vitro is biased toward the major species present in the mRNA population, it is not possible from these data to make an accurate estimate of the actual purity of the fractionated mRNA. A preliminary estimate of the purity of the mRNAs has been made on the basis of hybridization complexity anal-

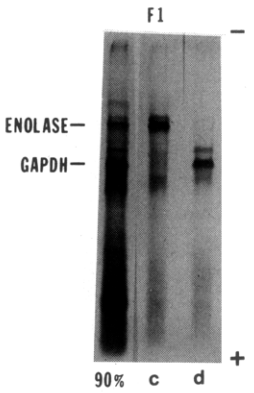


FIGURE 4: Autoradiogram of a sodium dodecyl sulfate–polyacrylamide slab gel of [^{35}S]methionine-labeled polypeptides synthesized in vitro in a wheat germ cell-free extract in response to (90%) unfractionated poly(A)-containing mRNA and (c and d) mRNA electrophoretically eluted from the c and d bands shown in the formamide–polyacrylamide slab gel in Figure 1. RNA fractions were isolated from *S. cerevisiae* strain F1. The mobilities of purified yeast enolase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) markers are indicated by arrows.

ysis. Complementary DNA synthesized from each fractionated mRNA with reverse transcriptase was hybridized with each respective fractionated mRNA, and the kinetics of hybridization were analyzed. By measuring the percentage of cDNA which rapidly annealed with each mRNA, we estimated that these mRNAs are 15–40% pure.

Identification of Fractionated mRNAs by Tryptic Peptide Analysis of Polypeptides Synthesized in Vitro. The major mRNAs isolated from the two cells have been tentatively identified on the basis of coelectrophoresis with purified marker proteins. In order to rigorously identify these mRNAs, the polypeptides synthesized in vitro from each of the fractionated mRNAs were subjected to tryptic peptide analysis. *Total* wheat germ cell-free extracts containing newly synthesized polypeptides were denatured, alkylated, and digested with trypsin without prior fractionation of newly synthesized polypeptides. This was done in order to eliminate the possibility that prior fractionation would alter the relative concentration of the polypeptide of interest. The major disadvantage of this approach is that the large amount of unlabeled protein present in the wheat germ extract severely limits the amount of material which can be analyzed on a thin-layer chromatography plate. The standard tryptic peptides are influenced in migration by the wheat germ extract, making comparison of an in vitro plate to a standard plate very difficult. Thus, it is necessary to include the purified unlabeled glycolytic marker protein with the wheat germ extract. The tryptic peptide maps of polypeptides synthesized in vitro from the major mRNAs isolated from *S. cerevisiae* ATCC 24657 and F1 are shown in Figure 5. In the lower panel is a peptide analysis of wheat germ polypeptides synthesized in the absence of added mRNA. There are two major unlabeled fluorescamine-staining species present in the mock wheat germ S-30 as well as a number of minor species. Of these wheat germ species two are labeled to a low level with [^{35}S]methionine. The intensity and migration of these wheat germ peptides are such that a large "window" exists for the analysis of peptides synthesized in response to added mRNA. The intensity of the two major unlabeled wheat germ peptides varies in the three maps as a function of the amount of S-30 applied to the plate. Unlabeled marker peptides were visualized with fluorescamine (shown on the left in Figure 5), and [^{35}S]methionine-labeled peptides were visualized by autoradiography (shown on the right in Figure 5). [^{35}S]Methionine-labeled peptides which are coincident with

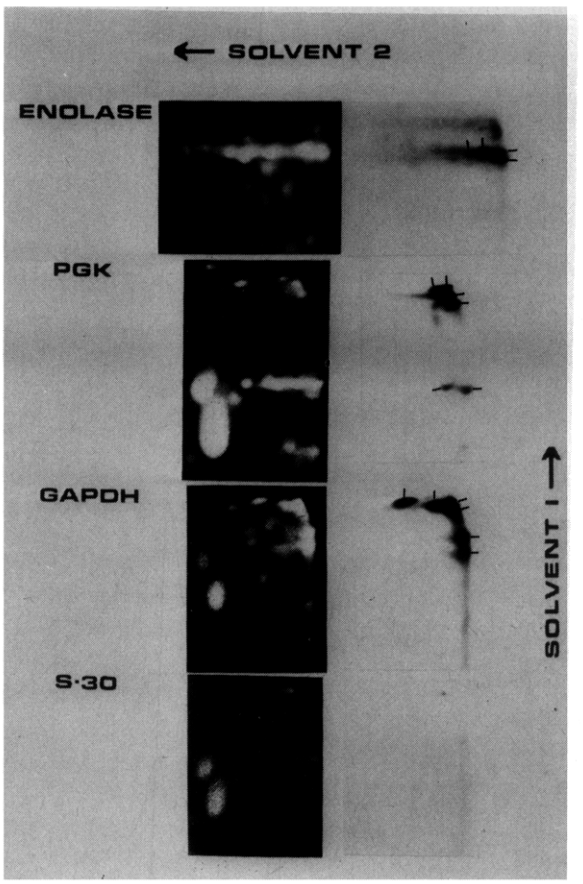


FIGURE 5: Tryptic peptide fingerprints of purified yeast enolase, phosphoglycerate kinase (PGK), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) marker polypeptides mixed with [^{35}S]methionine-labeled polypeptides synthesized in a wheat germ cell-free extract in response to the a, c, and d mRNAs, respectively, isolated from preparative formamide–polyacrylamide slab gels. Following synthesis of [^{35}S]methionine-labeled polypeptides in response to each isolated mRNA, the appropriate purified marker protein was added to the wheat germ S-30 reaction and the mixture was digested with trypsin as described under Experimental Procedure. Tryptic peptides were subjected to two-dimensional chromatography (solvent 1, chloroform–methanol–ammonium hydroxide (2:2:1); solvent 2, butanol–acetic acid–water (4:1:1) on a silica gel thin-layer plate. Unlabeled peptides were visualized with fluorescamine (left panels) and the [^{35}S]methionine-labeled peptides were visualized by autoradiography (right panels). The lower panels (S-30) show the unlabeled and [^{35}S]methionine-labeled tryptic peptides derived from the wheat germ extract in the absence of added mRNA or marker protein. [^{35}S]-Methionine-labeled peptides which overlap with unlabeled marker peptides are indicated by arrows.

fluorescamine-stained marker peptides are indicated by arrows.

Enolase contains six methionine residues per subunit (Chinn et al., 1978). Two of these methionine residues are in a single tryptic peptide. Four major [^{35}S]methionine-labeled tryptic peptides were identified by autoradiography (Figure 5). Three of these peptides clearly overlap with fluorescamine-stained enolase peptides and the fourth migrates in a region which contains a smear of fluorescamine-stained peptides. In addition to the four major [^{35}S]methionine-labeled peptides, there are four labeled peptides in the upper right corner of the autoradiogram which are coincident with fluorescamine-staining peptides. These species probably represent peptides which arise from incomplete tryptic digestion of the protein as well as the fifth methionine-containing peptide.

Published amino acid compositions of phosphoglycerate kinase indicate that there are three methionines per subunit (Scopes, 1973). We have observed a total of six major ^{35}S-

labeled tryptic peptides after digestion of the polypeptides synthesized in vitro under the direction of the putative phosphoglycerate kinase mRNA. All of these labeled peptides are coincident with fluorescamine-stained tryptic peptides obtained after cleavage of purified phosphoglycerate kinase, suggesting that the mRNA does, in fact, code for phosphoglycerate kinase. We have examined the amino acid composition of purified phosphoglycerate kinase under a variety of hydrolysis conditions, and all of the compositions indicate three methionine residues per subunit. We conclude, therefore, that under our experimental conditions trypsin digestion of phosphoglycerate kinase is incomplete.

The amino acid sequence of yeast glyceraldehyde-3-phosphate dehydrogenase has been determined, and the enzyme contains six methionine residues per subunit (Jones and Harris, 1972). A total of six [^{35}S]methionine-labeled peptides were obtained from the polypeptides synthesized in vitro under the direction of the major mRNA isolated from *S. cerevisiae* F1. All of these labeled peptides are coincident with fluorescamine-staining marker peptides, demonstrating that this mRNA codes for glyceraldehyde-3-phosphate dehydrogenase.

Tryptic peptides derived from polypeptides synthesized in vitro under the direction of each fractionated mRNA in the presence of [^{3}H]leucine were analyzed by reverse-phase high-pressure liquid chromatography. Tryptic peptides derived from the appropriate purified glycolytic enzyme were monitored spectrophotometrically at 215 nm (absorption at 215 nm is proportional to the number of peptide bonds per peptide), and the [^{3}H]leucine-labeled peptides were monitored by scintillation counting fractions eluted from the column. The results of the analysis for enolase, phosphoglycerate kinase, and glyceraldehyde-3-phosphate dehydrogenase are shown in Figure 6. The lower panel (S-30) shows the results obtained from an analysis of the wheat germ cell-free extract in the absence of added mRNA. No major [^{3}H]leucine-labeled or unlabeled peptides (A 215 nm) were observed even though five- to tenfold more S-30 extract was analyzed in this experiment than in those with added mRNA. Based on the amino acid sequence of yeast glyceraldehyde-3-phosphate dehydrogenase (Jones and Harris, 1972), there are 32 predicted tryptic peptides containing at least two amino acid residues and 16 of these peptides contain at least one leucine residue. Approximately 40 peptides were observed on the high-pressure liquid chromatogram (A_{215nm}), and 18 of these peptides cochromatograph with [^{3}H]leucine-labeled peptides when the polypeptide synthesized from the putative glyceraldehyde-3-phosphate dehydrogenase mRNA was analyzed. We have routinely observed more than the expected number of tryptic peptides for each of the glycolytic enzymes tested. While we do not know the origin of the additional peptides, it is possible, given the extremely high resolving power of the reverse-phase column, that they represent chemically altered peptides (e.g., carbamylated peptides) or, alternatively, submolar quantities of incompletely digested peptides. When each of the polypeptides synthesized in vitro was examined (Figure 6), a large number of [^{3}H]leucine-labeled peptides are resolved which cochromatograph with peptides derived from the glycolytic marker protein. The number of [^{3}H]leucine-labeled peptides which cochromatograph with the enolase, phosphoglycerate kinase, and glyceraldehyde-3-phosphate dehydrogenase marker peptides is 34, 24, and 18, respectively.

From the analyses shown in Figures 5 and 6 the identification of each mRNA can be made as follows: phosphoglycerate kinase, a mRNA; enolase, c mRNA; and glyceraldehyde-3-phosphate dehydrogenase, d mRNA. These data confirm the observation that the a and c mRNAs obtained from *S. cerevisiae* ATCC 24657 and F1, respectively, do code for different glycolytic proteins. The other mRNA species (b mRNA) isolated from *S. cerevisiae* ATCC 24657 codes for a polypeptide which coelectrophoreses with glyceraldehyde-3-phosphate dehydrogenase. A tryptic peptide analysis of this in vitro synthesized polypeptide was carried out (data not shown) to verify that the b mRNA does, in fact, code for glyceraldehyde-3-phosphate dehydrogenase.

Effect of Cellular Growth Conditions on the Distribution of Abundant Messenger RNAs in Saccharomyces cerevisiae. In order to determine if the differences in the distribution of abundant mRNA species between commercially grown baker's yeast (strain F1) and strain 24657 are related to the methods of propagating the cells, the baker's yeast (strain F1) was grown in YPD medium as described under Experimental Procedure for strain 24657. Poly(A)-containing mRNA was isolated from these cells and translated in the wheat germ cell-free extract. The [^{35}S]methionine-labeled polypeptides synthesized under the direction of poly(A)-containing mRNA isolated from commercially grown strain F1 were compared to those synthesized from poly(A)-containing mRNA isolated from strain F1 and strain 24657 grown under identical conditions utilizing $NaDodSO_4$–polyacrylamide gel electrophoresis (Figure 7). The distribution of polypeptides synthesized under the direction of mRNA isolated from both strains, propagated under the same conditions, is indistinguishable (Figure 7, panels A and B). These results demonstrate that the distribution of abundant mRNAs in *S. cerevisiae* is highly dependent on the conditions used to propagate the cells. These results are consistent with published reports that the intracellular concentrations of glycolytic enzymes in *S. cerevisiae* are highly regulated and vary, for example, as a function of the glucose concentration in the growth medium (Hommes, 1966; Maitra and Lobo, 1974). These results further demonstrate that the glycolytic pathway in yeast is regulated at the level of transcription.

Discussion

A significant purification of the major yeast messenger RNAs can be achieved by preparative polyacrylamide gel electrophoresis in the presence of 99% formamide. Under these conditions, mRNA aggregation can be eliminated, yielding mRNA species which migrate in a unique position on the gel. Similar analysis in neutral sucrose gradients revealed the presence of substantial amounts of each of these mRNAs sedimenting with the large rRNAs as well as at their expected positions in the gradient (unpublished observations). The high resolution of these gels, as well as the ability to visualize mRNA bands with ethidium bromide, provides a generally useful means of purifying abundant cellular mRNAs. As evidenced by the densitometer scans of the polyacrylamide gels (Figure 1b), the mRNAs are not homogeneous. Nevertheless, with the level of purification achieved, it should now be possible to carry out further enrichment of complementary DNAs synthesized from the respective mRNAs by partial annealing of the cDNA to purified mRNA and by subsequent fractionation of the hybrids by hydroxylapatite chromatography (Taylor et al., 1974). Complementary DNAs purified in this manner will serve as suitable probes for the isolation of bacterial clones containing specific glycolytic gene–bacterial plasmid hybrid DNAs.

The identity of the isolated mRNAs was first suggested by coelectrophoresis of polypeptides synthesized in vitro under the direction of fractionated mRNAs with purified glycolytic proteins. These tentative identifications were confirmed by the tryptic peptide analyses shown in Figures 5 and 6. We conclude

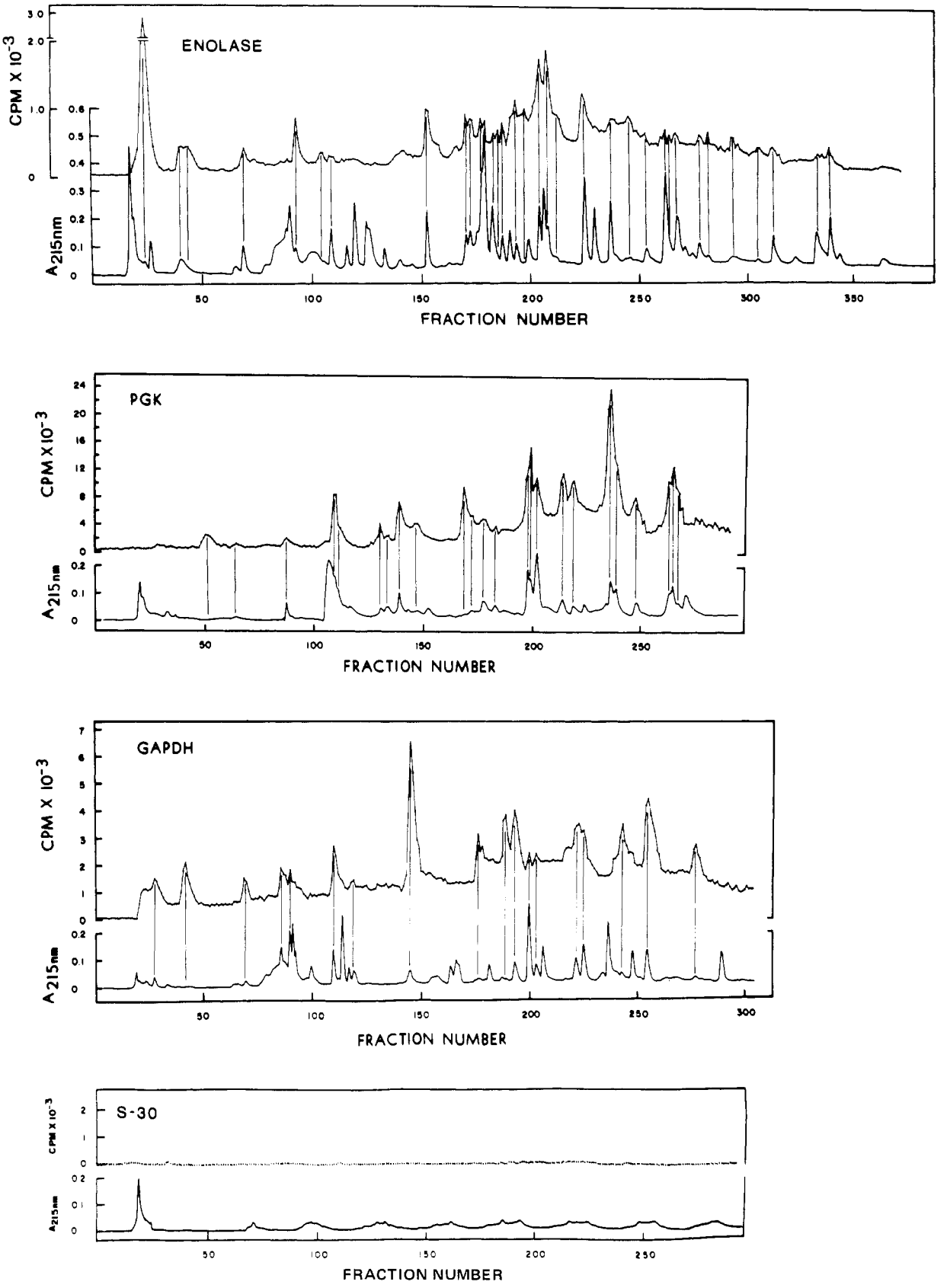


FIGURE 6: Reverse-phase high-pressure liquid chromatograms of tryptic peptides derived from purified yeast enolase, phosphoglycerate kinase (PGK) and glyceraldehyde-3-phosphate dehydrogenase marker polypeptides mixed with [^{3}H]leucine-labeled polypeptides synthesized in a wheat germ cell-free extract in response to the a, c, and d mRNAs, respectively. The appropriate marker polypeptide was added to the wheat germ S-30 reaction, and the mixture was digested with trypsin as described under Experimental Procedure. Reverse-phase high-pressure liquid chromatography was carried out as described under Experimental Procedure. Unlabeled marker peptides were monitored spectrophotometrically at 215 nm. [^{3}H]Leucine-labeled peptides were monitored by scintillation counting. The lower panel (S-30) shows the analysis of peptides derived from the wheat germ extract in the absence of added mRNA or marker polypeptide (five to ten times more S-30 extract was analyzed in this experiment than in any of the experiments shown in the upper three panels).

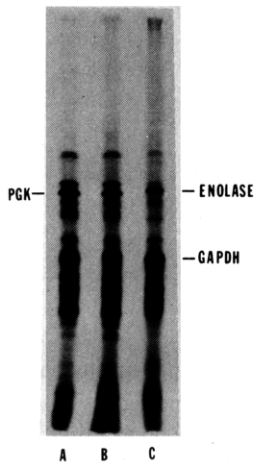


FIGURE 7: Autoradiogram of a sodium dodecyl sulfate–polyacrylamide gel of [^{35}S]methionine-labeled polypeptides synthesized in vitro in a wheat germ cell-free extract in response to poly(A)-containing mRNA isolated from: (A) *S. cerevisiae* strain F1 grown in YPD media as described under Experimental Procedure. (B) *S. cerevisiae* strain ATCC 24657 grown in YPD media as described under Experimental Procedure. (C) Commercially grown baker's yeast (strain F1). Poly(A)-containing mRNA was isolated by poly(U) cellulose chromatography as described under Experimental Procedure. The mobilities of purified yeast enolase, phosphoglycerate kinase (PGK), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) markers are indicated by arrows.

on the basis of these results that the major mRNA species isolated from commercially grown *S. cerevisiae* strain F1 code for glyceraldehyde-3-phosphate dehydrogenase and enolase. The identification of enolase mRNA reported in this manuscript is in agreement with a previously published identification of the enolase polypeptide synthesized in vitro under the direction of unfractionated total poly(A)-containing mRNA isolated from commercially grown *S. cerevisiae* strain F1 (Holland et al., 1977). This identification was based on specific immune precipitation of in vitro synthesized enolase with an antibody prepared against purified yeast enolase. In marked contrast, the major mRNA species isolated from *S. cerevisiae* ATCC 24657 codes for phosphoglycerate kinase.

The relative concentrations of enolase and phosphoglycerate kinase mRNAs in the two strains of yeast are dramatically different. This observation can be attributed to differences in the conditions for propagating the cells rather than genetic differences between the strains (Figure 7). The commercial cells were grown in the presence of a limiting carbon source (usually molasses) and oxygen unlike *S. cerevisiae* strain ATCC 24657 which was grown in the presence of 2% glucose with aeration. It is useful to point out that a number of glycolytic enzymes, including enolase and glyceraldehyde-3-phosphate dehydrogenase, have been isolated in large quantity from commercial baker's yeast. Glyceraldehyde-3-phosphate dehydrogenase was shown by Krebs (1953) to be the most abundant protein in commercial cells and makes up 5% of the dry weight of the cell. Westhead and McLain (1964) showed that enolase comprises 2–5% of the soluble protein in commercial baker's yeast. Analysis of the intracellular concentration of glycolytic enzymes in yeast cells propagated in the presence of high glucose by Hess and his colleagues (1968) shows that phosphoglycerate kinase is the most abundant enzyme in these cells and is 6.5 times more abundant than enolase. It is apparent that additional studies on the regulation of the intracellular levels of glycolytic enzymes will be necessary before a clear understanding of these differences in enzyme concentration can be achieved. It is of interest, however, that the relative intracellular concentrations of the mRNAs investigated here do correlate well with reported intracellular levels of the respective glycolytic enzymes in *Saccharomyces cerevisiae,* suggesting that the coordinate expression of glycolytic enzymes in yeast is regulated at the transcriptional level.

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