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Yeast Glycolytic mRNAs Are Differentially Regulated

PAUL A. MOORE,† FRANCIS A. SAGLIOCCO, RACHEL M. C. WOOD, AND ALISTAIR J. P. BROWN*
Molecular & Cell Biology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, United Kingdom

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The regulation of glycolytic genes in response to carbon source in the yeast *Saccharomyces cerevisiae* has been studied. When the relative levels of each glycolytic mRNA were compared during exponential growth on glucose or lactate, the various glycolytic mRNAs were found to be induced to differing extents by glucose. No significant differences in the stabilities of the *PFK2*, *PGK1*, *PYK1*, or *PDC1* mRNAs during growth on glucose or lactate were observed. *PYK*::*lacZ* and *PGK*::*lacZ* fusions were integrated independently into the yeast genome at the *ura3* locus. The manner in which these fusions were differentially regulated in response to carbon source was similar to that of their respective wild-type loci. Therefore, the regulation of glycolytic mRNA levels is mediated at the transcriptional level. When the mRNAs are ordered with respect to the glycolytic pathway, two peaks of maximal induction are observed at phosphofructokinase and pyruvate kinase. These enzymes (i) catalyze the two essentially irreversible steps on the pathway, (ii) are the two glycolytic enzymes that are circumvented during gluconeogenesis and hence are specific to glycolysis, and (iii) are encoded by mRNAs that we have shown previously to be coregulated at the translational level in *S. cerevisiae* (P. A. Moore, A. J. Bettany, and A. J. P. Brown, NATO ASI Ser. Ser. H Cell Biol. 49:421–432, 1990). This differential regulation of glycolytic mRNA levels might therefore have a significant influence upon glycolytic flux in *S. cerevisiae*.

The glycolytic pathway plays a fundamental role in the provision of metabolic energy and intermediates during fermentative growth in the yeast *Saccharomyces cerevisiae*. Under these conditions, the glycolytic genes are among the most efficiently expressed genes in this organism, the glycolytic enzymes comprising over 30% of soluble cell protein (for reviews, see references 20 and 70). Most yeast glycolytic genes have been isolated and sequenced (2, 4, 11, 21, 23, 25, 27, 29–31, 40, 59, 62, 63, 68, 69). The high-level expression of yeast glycolytic genes is dependent upon complex interactions between a number of *cis*-acting promoter elements and *trans*-acting transcription factors which include the RAP1, ABF1, GCR1, and GAL11 proteins (3, 8, 10, 12, 13, 15, 41, 48, 55, 60, 61).

It is not clear whether the expression of all glycolytic genes is induced when yeast cultures are transferred from nonfermentative to fermentative carbon sources. Maitra and Lobo (37) observed 3- to 100-fold increases in the levels of glycolytic enzymes following the addition of glucose to yeast cultures growing on acetate. This work was performed on a hybrid yeast strain generated by a cross between *Saccharomyces fragilis* and *Saccharomyces dobzhanskii* (37). Some more recent studies appear to confirm this observation for *S. cerevisiae*. For example, analyses of the enolase (*ENO2*), phosphoglygerate kinase (*PGK1*), pyruvate kinase (*PYK1*), pyruvate decarboxylase (*PDC1*), and alcohol dehydrogenase (*ADH1*) mRNAs have suggested that their levels are regulated in response to carbon source (13, 16, 39, 47, 58). However, the levels of some other glycolytic mRNAs, for example, the phosphoglucose isomerase mRNA (*PGI1*) and an enolase mRNA (*ENO1*), appear to remain constant (1, 39).

Others have reported that the steady-state levels of most glycolytic enzymes do not differ significantly between cells grown under glycolytic conditions and cells grown under gluconeogenic conditions (3, 15). This was found to be dependent upon the activity of the *GCR1* gene, which encodes a positive activator of glycolytic gene expression (3, 15, 28). No significant glucose activation in *GCR1* wild-type cells was observed, but increased glycolytic enzyme levels in a *gcr1* mutant (15) and in strains carrying *gcr1* deletions (3) were observed during growth on glucose, albeit from significantly lower enzyme levels than those of the wild-type strains under gluconeogenic growth conditions.

These individual studies have been performed under a range of experimental conditions. In many cases, different yeast strains or gluconeogenic carbon sources (acetate, ethanol, glycerol, lactate, or pyruvate) have been used. Also, some groups have studied glycolytic gene expression under glycolytic or gluconeogenic growth conditions, whereas others have measured activity levels during the transition from gluconeogenic to glycolytic growth or vice versa (1, 12, 13, 39, 47, 66).

In this study, we compare the steady-state levels of all of the glycolytic mRNAs during exponential growth of *S. cerevisiae* on glucose or lactate. All of the measurements have been performed by using the same RNA preparations under similar conditions, and therefore the relative responses of all of the glycolytic mRNAs can be compared for the first time.

MATERIALS AND METHODS

Yeast strains and media. Strain DBY746 (*MATα his3 leu2 trp1 ura3*) was used for the analysis of glycolytic mRNA regulation in response to carbon source, and mRNA half-life measurements were performed with RY137 (*MATa ura3 his4 lys2*) and RY262 (*MATα ura3 his4 rpb1*), which were kindly donated by Richard Young (49). Yeast cultures were grown in YEPD (2% glucose, 2% bacteriological peptone, 1% yeast extract) or YEPL (3% lactate, 2% bacteriological peptone, 1% yeast extract), and their absorbance at 600 nm was monitored. Yeast transformation to generate strains carrying *lacZ* fusions was performed as described by Beggs (4).

Protein analysis. Cells from 50 ml of culture were washed

* Corresponding author.
† Present address: Department of Molecular Oncology & Virology, Roche Institute of Molecular Biology, Nutley, NJ 07100.

TABLE 1. Hybridization probes and conditions used in this study

Target RNA	Probe[a]	Hybridization conditions		Source	Reference
		Temp (°C)	% Formamide (vol/vol)		
HXK1 and *-2*	GATTGAGTGGTGTCAAAGGTACGG	37	6	This laboratory	62
PGI1	CGGTGATCTTCTTACCGGTATAACCCTTCC	37	20	This laboratory	63
PFK1	pPFK1 (3.4 kb of gene)	42	50	J. Heinisch	21
PFK2	pPFK2 (3.2 kb of gene)	42	50	J. Heinisch	21
FBA1	pHGS001 (600 bp of gene)	42	50	S. Kohlwein	59
TPI1	GTTACCACCGACAAAGAAAGTTCTAGC	37	12	This laboratory	2
TDH1, *-2*, and *-3*	GGACGAGTGATGACAACCTTCTTGGCACCAGCG	37	20	This laboratory	26
PGK1	pSPGK2 (2.9-kb *Hin*dIII fragment)	42	50	This laboratory	51a
PGM1	GACCGTGTCTAACTAAAACTAACTTTGG	42	20	This laboratory	69
ENO1 and *-2*	cDNA10 (1.2-kb cDNA clone)	42	50	This laboratory	27, 56
PYK1	pSPK2 (550 bp of coding region)	42	50	This laboratory	7
PDC1	pPDC1E (1.2 kb of gene)	42	50	S. Hohmann	57
ADH1	CCAGAATCCAGAAAGTTG	37	20	This laboratory	5
LPD1	pGP.R1 (3.6 kb of gene)	42	50	Z. Zaman	54
RAP1	pSP56RT7 (2.9 kb of gene)	42	50	A. Chambers	13
ACT1	pSPACT9 (1.5 kb of gene)	42	50	This laboratory	7
18S rRNA	pSP65R (1.0 kb of gene)	42	50	This laboratory	7

[a] Nucleotide sequences read, left to right, from 5′ ends to 3′ ends.

in 25 mM sodium phosphate, pH 7.0, containing 0.5 mM phenylmethylsulfonyl fluoride, placed at −70°C for 15 min, and resuspended in 100 μl of 25 mM sodium phosphate, pH 7.0. The cells were vortexed with glass beads for 90 s, placed at −70°C for 15 min, and vortexed again for 30 s. Extracts were centrifuged for 10 min at 10,000 × *g*, and supernatants were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (32). β-Galactosidase assays were performed as described by Legrain and Rosbach (33), and arbitrary units were calculated by the method of Miller (43).

mRNA abundance measurements. mRNA levels relative to the level of the actin mRNA were measured by Northern (RNA) analysis to correct for differences in RNA yield between samples (56). Total yeast RNA was prepared (36), denatured, and electrophoresed on agarose gels containing formaldehyde (34). The RNA was then transferred to Hybond-N membranes (64), UV fixed, and probed with either radiolabeled DNA fragments or oligonucleotides (Table 1). The adaptions made to previously published hybridization and washing conditions (7, 52) for each of the DNA or oligonucleotide probes are summarized in Table 1. Hybridizations were always performed under conditions of probe excess (56). Hybridization signals were accurately quantitated directly from the Northern filters by using the AMBIS 2D-Radioimaging System (LabLogic). Probes were then stripped from the filters in boiling 0.1% SDS, and the filters were subjected to autoradiography and then reprobed up to a maximum of four times.

Probe synthesis. Hybridization probes (Table 1) were prepared by random priming plasmid DNA or purified DNA fragments (19) or end labeling synthetic oligonucleotides (35). The oligonucleotides were synthesized by Veerabhadracharya Math and John Coggins (Glasgow University).

mRNA half-life measurements. Methods for the analysis of mRNA half-lives by using the conditional-lethal RNA polymerase II mutation (*rpb1*) (49) were adapted from methods described by Herrick and coworkers (22). The yeast strains RY137 (*RPB1*) and RY262 (*rpb1*) were each grown at 26°C in 100 ml of YEPD or YEPL in a 2-liter flask with shaking at 200 rpm to mid-exponential growth phase (A_{600}, 0.5), whereupon an equal volume of the same medium prewarmed to 48°C was added to each flask to bring the cultures immediately to the restrictive temperature (37°C). RNA was isolated (36) at various times following the temperature upshift, and equal amounts of each RNA preparation were subjected to Northern analysis as described above (15 μg per lane). RNA loadings were initially measured by the absorbance at 260 nm, but they were later measured more accurately by quantitating the 18S rRNA in each sample by dot blotting (56). Hybridization signals were accurately quantitated directly from the Northern and dot blots by using the AMBIS 2D-Radioimaging System (LabLogic).

RESULTS

Hybridization conditions for all oligonucleotide probes were optimized experimentally. A complete set of probes for all of the yeast glycolytic mRNAs was generated by obtaining some genes from other laboratories and by constructing synthetic oligonucleotides complementary to other genes (Table 1). All of the oligonucleotides were designed to hybridize with the coding regions of the target mRNAs.

Northern analyses of yeast RNA were performed to establish the optimum hybridization conditions for each oligonucleotide probe. Two parameters were varied to maximize the signal for the target mRNA while minimizing the background hybridization to other RNA species: the temperature at which the hybridization was performed and the formamide concentration in the hybridization solution (not shown). The optimum hybridization conditions for each probe used in subsequent experiments are summarized in Table 1.

For mRNA quantitation, all filter hybridizations were performed under conditions of probe excess (56). An example of this is shown in Fig. 1. Multiple dilutions of 12 RNA preparations were dotted in duplicate and probed for the 18S rRNA to control for RNA loadings on Northern blots. A comparison of the bound radioactivity with the amount of RNA dotted for each sample (Fig. 1) confirms that the response is linear and therefore that excess probe was present in the hybridization. Similar analyses for oligonucle-

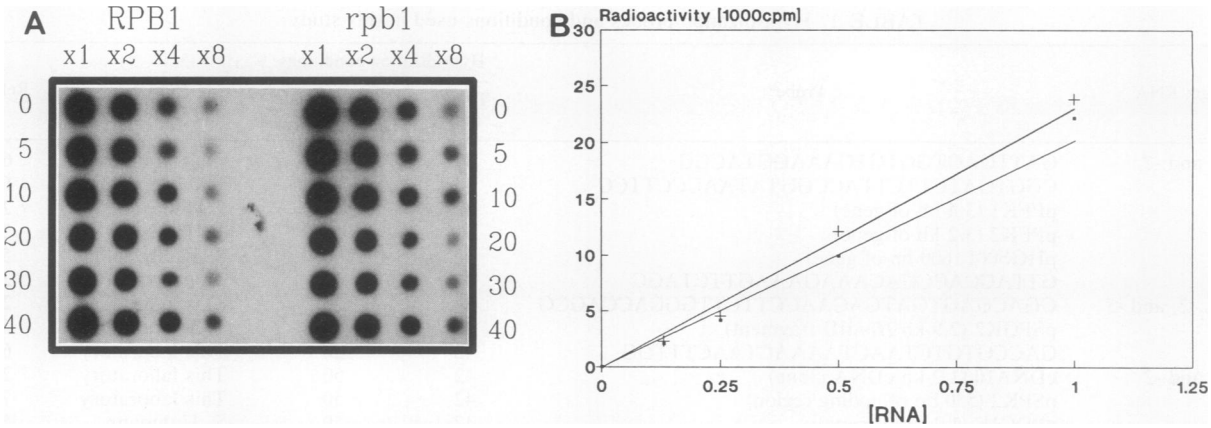

FIG. 1. Hybridizations performed under conditions of probe excess. (A) Multiple dilutions (shown at top) of 12 RNA preparations were dotted in duplicate (duplicates not shown) and hybridized with an 18S rRNA probe. The 12 preparations were obtained by preparing RNA from *RPB1* and *rpb1* yeast strains at various times (shown in minutes to left and right of the figure) following a shift from 26 to 37°C. (B) Radioactivity bound to each dot on the filter was quantitated by using an AMBIS 2D Radioimaging System, and the data for two such dilution series (indicated by dots and plus signs) are plotted against the RNA dilution.

otide probes were performed by using dot blots and Northern blots, confirming that these hybridizations were also performed under conditions of probe excess.

Glycolytic mRNAs are differentially regulated in response to carbon source. The yeast strain DBY746 was grown in 200 ml of rich medium containing glucose (YEPD) or lactate (YEPL) at 30°C with shaking at 200 rpm in a 500-ml flask. The absorbance at 600 nm was monitored for each culture, and cells were harvested for analysis during the exponential growth phase (A_{600}, 0.6). The doubling time of the lactate culture (275 min) was threefold greater than that for the glucose-grown culture (90 min), which compares favorably with results of previous studies (51).

Cells from each culture were divided into two portions: 50 ml for protein analysis and 100 ml for RNA analysis. Approximately equal amounts of total soluble protein from each culture were analyzed by SDS-PAGE (Fig. 2). Clear differences in the protein profiles, due at least in part to the derepression of many glucose-repressed gene products during growth on lactate, are evident.

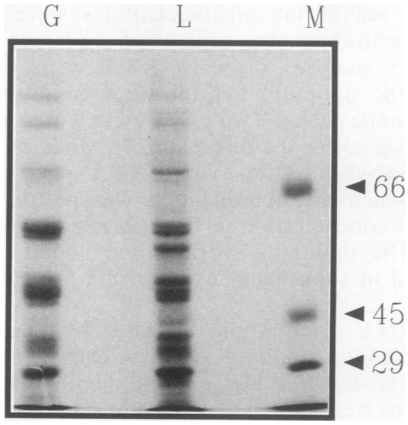

FIG. 2. Total soluble protein profiles differ between glycolyic and gluconeogenic growth. Approximately equal amounts of protein from the glucose-grown (G) and lactate-grown (L) cultures were subjected to SDS-PAGE and compared with markers (M) of known molecular mass (in kilodaltons).

RNA isolated from each culture was used to prepare several Northern blots. Each filter was probed for a specific glycolytic mRNA and autoradiographed to check for background radioactivity, and the hybridization signals were quantitated by using a 2D-Radioimaging System. The probes were then stripped from each filter, and the filter was reprobed for a second mRNA (Fig. 3). This cycle was repeated up to four times, during which each filter was probed for the actin mRNA, which acts as an internal loading control since actin mRNA levels are similar under glycolytic and gluconeogenic conditions (38). The level of each glycolytic mRNA relative to that of the actin mRNA control was calculated, and then the relative mRNA level on glucose was divided by that on lactate to give the level of glucose activation for each mRNA. Under the conditions used in this study, the glycolytic mRNAs are activated to different extents on glucose (Fig. 4). No significant induction was observed for some mRNAs (for example, *TDH* and *PGI1*), some mRNAs were moderately activated (for example, *PDC1* and *PGM1*), and other mRNAs were induced to a relatively large extent (for example, *PYK1*). Two peaks of activation along the glycolytic pathway at the *PFK2* and *PYK1* mRNAs are observed.

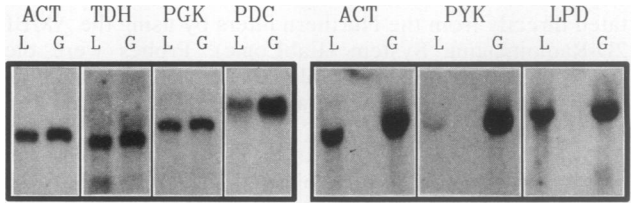

FIG. 3. Northern analysis of glycolytic mRNAs by using oligonucleotide and random-primed DNA probes. RNA samples from glucose-grown (G) and lactate-grown (L) cultures were subjected to Northern analysis, and two separate filters were sequentially probed for the actin (ACT), *TDH*, *PGK1*, and *PDC1* mRNAs or the actin, *PYK1*, and *LPD1* mRNAs. The *TDH* mRNAs were detected by using an oligonucleotide probe, whereas the other mRNAs were detected by using radiolabeled DNA fragments (Table 1). These hybridizations were used to measure the glucose activation for these mRNAs (Fig. 4).

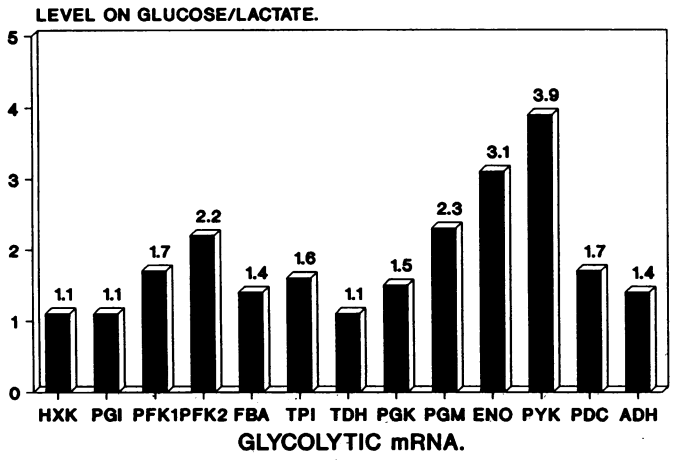

FIG. 4. Differential glucose activation of glycolytic mRNAs. The abundance of each glycolytic mRNA (relative to the actin mRNA internal control) was compared during growth on glucose or lactate. The relative abundance on glucose was divided by the equivalent value for lactate. The mRNAs are placed in the order in which they occur on the glycolytic pathway.

The different levels of activation for each glycolytic mRNA are not due to differences in the nature of the hybridization probe: similar levels of activation for the *PFK1* and *PFK2* mRNAs were observed when radiolabeled DNA fragments or oligonucleotides were used (not shown). Similarly, experimental errors (±8%) do not account for the different activation levels. Finally, the pattern of activation observed for the *PFK2*, *PGK1*, *PYK1*, and *PDC1* mRNAs in the yeast strains RY262 and RY137 (54a) is identical to that seen for DBY746 (Fig. 4).

The relative levels of the *LPD1* and *RAP1* mRNAs were measured under identical conditions. Northern analysis of the *LPD1* mRNA is shown in Fig. 3. The abundance of the *RAP1* mRNA (which encodes a transcriptional activator of a large number of loci, including many glycolytic genes) (67) remains constant, and this is consistent with previous observations (13). The level of the *LPD1* mRNA (which encodes a subunit of the pyruvate and succinate decarboxylase complexes) (53) is twofold lower during growth on glucose, as expected for an enzyme that is subject to glucose repression (17).

The differential activation of glycolytic mRNAs is not mediated at the level of mRNA degradation. Differences in transcription or mRNA degradation might account for the differential responses of the glycolytic genes to the carbon source. Therefore, the stabilities of a subset of glycolytic mRNAs were compared during growth on glucose or lactate by using the yeast strain RY262, which carries a temperature-sensitive lesion in RNA polymerase II (*rpb1*) (49). This mutation has been used previously to measure yeast mRNA half-lives (22). Strain RY137 (*RPB1*) was used to control for the effects of the temperature upshift on mRNA half-life.

RY262 and RY137 were grown at 26°C to mid-exponential phase on glucose (YEPD) or lactate (YEPL), whereupon the cultures were shifted to 37°C (see Materials and Methods). RNA was prepared from cells harvested at various time points following the temperature upshift, and equal amounts of RNA from each sample (15 μg per lane) were subjected to Northern analysis. The accuracy of the RNA loadings was checked by probing dot blots of each sample for the 18S rRNA (Fig. 1). Northern filters were then probed sequentially for glycolytic mRNAs that showed relatively high (*PFK2* and *PYK1*) or moderate (*PGK1* and *PDC1*) levels of glucose activation. Following autoradiography, the hybridization signals were quantitated by using the 2D-Radioimaging System, and these signals were corrected for minor errors in RNA loading by using the 18S rRNA control (Fig. 1). The decay of the *PYK1* and *PGK1* mRNAs following the temperature upshift in the *rpb1* strain is illustrated in Fig. 5.

In the *RPB1* control strain RY137, a threefold increase in the abundance of the *PGK1* mRNA was observed after the temperature was increased from 26 to 37°C (not shown). This was expected, because the *PGK1* promoter carries a functional heat shock element (50). As described previously (51), the heat shock induction of *PGK1* was observed only during glycolytic growth and not under gluconeogenic conditions.

mRNA half-lives were calculated by linear regression analysis by using the 10- to 40-min time points. No significant differences in the half-lives of two control mRNAs (*ACT1* and *TRP3*) were observed during glycolytic or gluconeogenic growth (Table 2). The values obtained for the half-lives of the *ACT1* and *PGK1* mRNAs are similar to

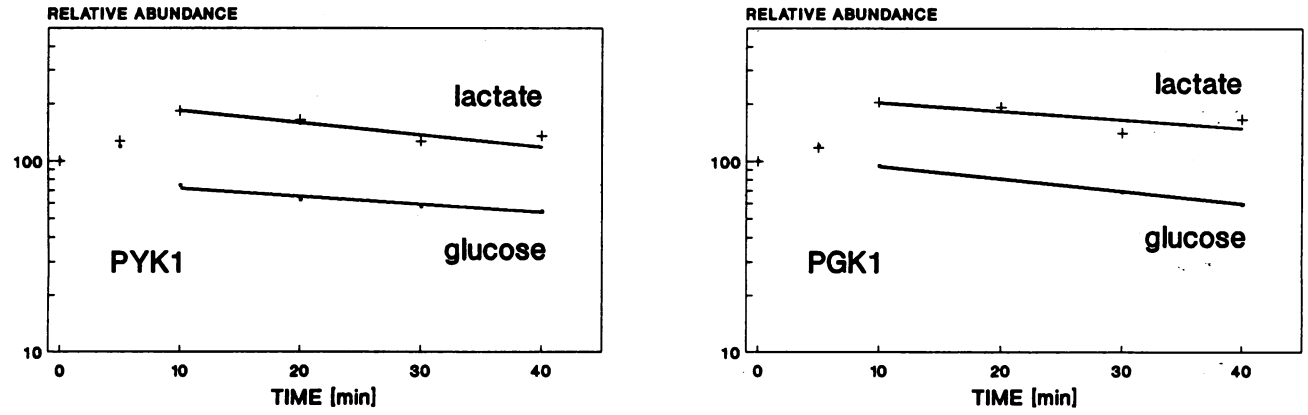

FIG. 5. The half-lives of glycolytic mRNAs do not differ significantly during growth on glucose or lactate. The decay of the *PYK1* and *PGK1* mRNAs following a shift to the restrictive temperature (time zero) during exponential growth of the *rpb1-1* strain on glucose or lactate is shown. The abundance at each time point (corrected by using the 18S rRNA control) is given relative to that at time zero (100%). Dots indicate growth on glucose, and plus signs indicate growth on lactate.

TABLE 2. The effect of carbon source on the stability of glycolytic mRNAs

mRNA	Half-life (min) with[a]:	
	Glucose	Lactate
PYK1	67 (0.968)	60 (0.884)
PGK1	45 (1.000)	74 (0.731)
PDC1	43 (0.997)	77 (0.631)
PFK2	29 (0.762)	40 (0.834)
ACT1	29 (0.872)	20 (0.991)
TRP3	12 (0.954)	12 (0.995)

[a] Approximate half-lives were calculated by linear regression analysis by using mRNA decay data from the 10- to 40-min time points. The figures in parentheses represent the correlation coefficients for the lines of best fit used to calculate each mRNA half-life.

those obtained previously by Herrick and coworkers (22) using the temperature-sensitive polymerase II mutant.

The *PFK2*, *PGK1*, and *PDC1* mRNAs are all relatively stable (Table 2), as would be expected for "housekeeping" functions (9). The *PFK2*, *PGK1*, *PYK1*, and *PDC1* mRNAs are not degraded more rapidly during growth on lactate (Table 2). Therefore, the differential glucose induction of glycolytic mRNA levels is probably mediated at the transcriptional level.

Glycolytic mRNAs are differentially regulated at the transcriptional level. To confirm that the differential glucose induction of glycolytic mRNAs is mediated at the transcriptional level, yeast strains were constructed which contain either a *PYK*::*lacZ* or a *PGK*::*lacZ* gene fusion integrated at the *ura3* locus (Fig. 6).

A *PYK*::*lacZ* gene was constructed by fusing the 5′ region of the *PYK1* gene in frame to a *lacZ* reporter. Only 36 nucleotides from the 5′ end of the *PYK1* mRNA are present in this fusion, with the *lacZ* sequences following the third codon. In addition, a *PGK*::*lacZ* gene was made by inserting the *lacZ* coding region into the *PGK1* expression cassette from pMA91 (42). An oligonucleotide linker was used to provide an in-frame initiation codon, because the 5′ *PGK1* sequences present in pMA91 terminate at −2 with respect to the ATG. The *PYK*::*lacZ* and *PGK*::*lacZ* fusions contained a *PYK1* and a *PGK1* transcriptional terminator, respectively (Fig. 6). Each *lacZ* fusion was cloned onto an integrating shuttle vector, and in independent transformations they were targeted to the *ura3* locus by using the unique *StuI* site within the wild-type *URA3* gene on each plasmid. After transformants were selected on the basis of uracil prototrophy, Southern blotting was performed on genomic DNA from individual transformants (not shown), and only those carrying a single copy of the appropriate *lacZ* fusion were taken for further analysis.

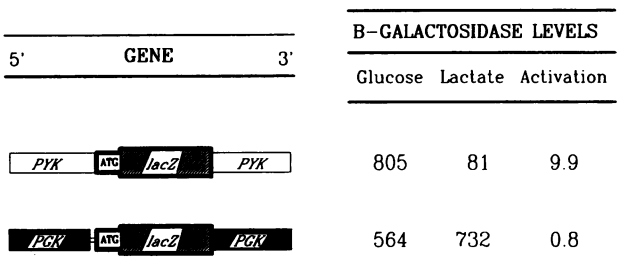

FIG. 6. *PYK*::*lacZ* and *PGK*::*lacZ* fusions are differentially regulated in response to glucose. *PYK*::*lacZ* and *PGK*::*lacZ* fusions were integrated independently at the *ura3* locus. The β-galactosidase activity in each strain was measured during growth on glucose or lactate (arbitrary units as described previously [43]). The level of glucose activation was obtained by dividing the β-galactosidase level on glucose by the equivalent value on lactate. Each assay represents the average of duplicate assays on three independent cultures (error, ±10%). For the gene fusions, open rectangles represent *PYK1* sequences, black rectangles represent *PGK1* sequences, and hatched rectangles represent the *lacZ* coding region. Areas surrounded by the thick lines represent coding sequences. The start codon in the *PYK*::*lacZ* fusion was provided by the *PYK1* sequence, the fusion occurring after the third codon. An oligonucleotide linker was used to join the *PGK1* and *lacZ* fragments in the second gene, and this linker provides the start codon.

Each *lacZ* strain was grown on either glucose (YEPD) or lactate (YEPL), cells were harvested during mid-exponential growth phase, and β-galactosidase levels in each sample were measured (Fig. 6). During growth on lactate, the *PYK*::*lacZ* gene generates lower levels of β-galactosidase than does the *PGK*::*lacZ* fusion. However, during growth on glucose, similar amounts of β-galactosidase are made by each *lacZ* fusion. No glucose activation is observed for the *PGK*::*lacZ* gene, but the *PYK*::*lacZ* gene is activated about 10-fold by glucose. Quantitative Northern analysis has revealed that a 10-fold induction of β-galactosidase in response to glucose is mediated by a 3-fold increase in *PYK*::*lacZ* mRNA (not shown). This is similar to the level of glucose activation observed for the wild-type *PYK1* mRNA (Fig. 4). Therefore, the transcription of yeast glycolytic genes is differentially regulated in response to the carbon source.

We have demonstrated previously that the *PYK1* gene is regulated at multiple levels (46). This might account for the difference between the levels of activation of *PYK*::*lacZ* mRNA (about 3-fold) and protein (about 10-fold; Fig. 6) in response to glucose.

DISCUSSION

In this study, we have measured the relative level of each glycolytic mRNA during exponential growth on glucose and lactate (Fig. 4). Since these measurements have been made with the same RNA preparations and all of the data have been related to an internal control (the actin mRNA), it is clear that the glycolytic mRNAs are differentially regulated in response to the carbon source. We have demonstrated that this differential regulation is not affected by changes in mRNA stability (Fig. 5). Furthermore, we have shown that single-copy, chromosomally located *PYK*::*lacZ* and *PGK*::*lacZ* genes respond in a manner similar to that of their respective wild-type loci (Fig. 6). Therefore, yeast glycolytic mRNAs are differentially regulated in response to glucose at the transcriptional level.

At first glance, one might expect yeast glycolytic genes to be coordinately regulated in response to the carbon source. All their gene products are required for the catabolism of glucose and all are efficiently expressed, having very strong codon biases (6) and optimal translation initiation regions (14). Furthermore, the transcription factors encoded by *ABF1*, *GCR1*, and *RAP1* are known to influence the expression of several glycolytic genes (3, 7, 13, 15, 61). However, despite a considerable amount of attention being focused on this area, the exact mechanisms by which yeast glycolytic genes are regulated remain obscure.

The positive transcriptional activator RAP1 binds to a specific sequence motif in the promoters of many glycolytic genes, the deletion of which causes a marked decrease in transcription (10, 13, 41, 59, 65). However, there is some

debate as to whether carbon source regulation is mediated through the RAP1 protein. While RAP1 binding at the *PGK1* promoter appears to be dependent upon the carbon source (13), the deletion of the RAP1 binding site from the *TPI1*, *PDC1*, or *ADH1* promoter does not seem to disrupt carbon source regulation (12, 60, 65).

The role of the RAP1 protein is further complicated by its interactions with other factors. For example, the ABF1 and RAP1 proteins bind at overlapping sites within the *ENO2* promoter in a region that imparts both positive and negative regulation (8). In addition, the GCR1 protein, which does not bind glycolytic promoters directly, seems to mediate its effect on *ADH1* transcription through the RAP1 protein (55). In contrast, GCR1 does not appear to activate the *ENO2* promoter by modulating the activity of RAP1 or ABF1 (24). Interestingly, Baker (3) has demonstrated clearly that *gcr1* gene deletions affect the expression of the glycolytic genes to different extents. Therefore, subtle differences may exist among individual glycolytic promoters with respect to their interactions with the transcription factors ABF1, GCR1, RAP1, and possibly GAL11. These differences might depend upon the juxtaposition of specific *cis*-acting sequences within the promoters (10) and the relative activities of the transcription factors under different physiological conditions.

The functions of all glycolytic enzymes are not equal. For example, the *HXK2* gene product not only catalyzes the phosphorylation of glucose but also plays a key role in glucose repression (18). During growth on glycerol, the lower part of the pathway catabolizes the carbon source, while enzymes in the upper part of the pathway fulfill an anabolic function. Also, whereas most glycolytic enzymes catalyze reversible reactions that are exploited during gluconeogenesis, phosphofructokinase and pyruvate kinase catalyze essentially irreversible reactions that are bypassed by the gluconeogenic enzymes fructose biphosphatase and phosphoenolpyruvate carboxykinase, respectively (reviewed in reference 70). Furthermore, the activities of both phosphofructokinase and pyruvate kinase are modulated by a large number of allosteric effectors (70, 71). Therefore, it is very significant that the two peaks of transcriptional activation occur at the *PFK2* and *PYK1* mRNAs (Fig. 4).

Previously, we have shown that compared with most other glycolytic genes, the *PYK1* gene is subject to additional levels of regulation (44, 45) and that the *PFK2* and *PYK1* mRNAs are coregulated at the translational level (46). It is not inconceivable that the expression of other glycolytic genes might be modulated by posttranscriptional mechanisms. Taken together, all of these observations strongly suggest that the regulation of phosphofructokinase and pyruvate kinase synthesis is significant in the control of glycolytic flux in *S. cerevisiae*.

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REFERENCES

1. **Aguilera, A., and F. K. Zimmerman.** 1986. Isolation and molecular analysis of the phosphoglucose isomerase structural gene of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **202**:83–89.
2. **Alber, T., and G. Kawasaki.** 1982. Nucleotide sequence of the triose phosphate isomerase gene of *Saccharomyces cerevisiae*. J. Mol. Appl. Genet. **1**:419–434.
3. **Baker, H. V.** 1986. Glycolytic gene expression in *Saccharomyces cerevisiae*: nucleotide sequence of *GCR1*, null mutants, and evidence for expression. Mol. Cell. Biol. **6**:3774–3784.
4. **Beggs, J. D.** 1978. Transformation of yeast by a replicating hybrid plasmid. Nature (London) **275**:104–109.
5. **Bennetzen, J. L., and B. D. Hall.** 1982. The primary structure of the *Saccharomyces cerevisiae* gene for alcohol dehydrogenase I. J. Biol. Chem. **257**:3018–3025.
6. **Bennetzen, J. L., and B. D. Hall.** 1982. Codon selection in yeast. J. Biol. Chem. **257**:3026–3031.
7. **Bettany, A. J. E., P. A. Moore, R. Cafferkey, L. D. Bell, A. R. Goodey, B. L. A. Carter, and A. J. P. Brown.** 1989. 5′-secondary structure, in contrast to a short string of non-preferred codons, inhibits the translation of the pyruvate kinase mRNA in yeast. Yeast **5**:187–198.
8. **Brindle, P. K., J. P. Holland, C. E. Willett, M. A. Innis, and M. J. Holland.** 1990. Multiple factors bind the upstream activation sites of the yeast enolase genes *ENO1* and *ENO2*: ABF1 protein, like repressor activator protein RAP1, binds *cis*-acting sequences which modulate repression or activation of transcription. Mol. Cell. Biol. **10**:4872–4885.
9. **Brown, A. J. P.** 1989. Messenger RNA stability in yeast. Yeast **5**:239–257.
10. **Buchman, A. R., N. F. Lue, and R. D. Kornberg.** 1988. Connections between transcriptional activators, silencers, and telomeres as revealed by functional analysis of a yeast DNA-binding protein. Mol. Cell. Biol. **8**:5086–5099.
11. **Burke, R. L., P. Tekamp-Olson, and R. Najarian.** 1983. The isolation, characterization, and sequence of the pyruvate kinase gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **258**:2193–2201.
12. **Butler, G., and D. J. McConnell.** 1988. Identification of an upstream activation site in the pyruvate decarboxylase structural gene (*PDC1*) of *Saccharomyces cerevisiae*. Curr. Genet. **14**:405–412.
13. **Chambers, A., J. S. H. Tsang, C. Stanway, A. J. Kingsman, and S. M. Kingsman.** 1989. Transcriptional control of the *Saccharomyces cerevisiae PGK* gene by RAP1. Mol. Cell. Biol. **9**:5516–5524.
14. **Cigan, A. M., and T. F. Donahue.** 1987. Sequence and structural features associated with translational initiator regions in yeast—a review. Gene **59**:1–18.
15. **Clifton, D., and D. G. Fraenkel.** 1981. The *gcr* (glycolysis regulation) mutation of *Saccharomyces cerevisiae*. J. Biol. Chem. **256**:13074–13078.
16. **Denis, C. L., J. Ferguson, and E. T. Young.** 1983. mRNA levels for the fermentative alcohol dehydrogenase of *Saccharomyces cerevisiae* decrease upon growth on a nonfermentable carbon source. J. Biol. Chem. **258**:1165–1171.
17. **Entian, K. D.** 1986. Glucose repression: a complex regulatory system in yeast. Microbiol. Sci. **3**:366–371.
18. **Entian, K.-D., and K.-U. Frohlich.** 1984. *Saccharomyces cerevisiae* mutants provide evidence of hexokinase PII as a bifunctional enzyme with catalytic and regulatory domains for triggering carbon catabolite repression. J. Bacteriol. **158**:29–35.
19. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
20. **Fraenkel, D. G.** 1982. Carbohydrate metabolism, p. 1–37. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Heinisch, J., R. G. Ritzel, R. C. von Borstel, A. Aguilera, R. Rodicio, and F. K. Zimmermann.** 1989. The phosphofructokinase genes of yeast evolved from two duplication events. Gene **78**:309–321.
22. **Herrick, D., R. Parker, and A. Jacobson.** 1990. Identification and comparison of stable and unstable mRNAs in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**:2269–2284.
23. **Hitzeman, R. A., F. E. Hagie, J. S. Hayflick, C. Y. Chen, P. H. Seeburg, and R. Derynck.** 1982. The primary sequence of the

Saccharomyces cerevisiae gene for 3-phosphoglycerate kinase. Nucleic Acids Res. **10**:7791–7808.
24. **Holland, J. P., P. K. Brindle, and M. J. Holland.** 1990. Sequences within an upstream activation site in the yeast enolase gene *ENO2* modulate repression of *ENO2* expression in strains carrying a null mutation in the positive regulatory gene *GCR1*. Mol. Cell. Biol. **10**:4863–4871.
25. **Holland, J. P., and M. J. Holland.** 1979. The primary structure of a glyceraldehyde-3-phosphate dehydrogenase gene from *Saccharomyces cerevisiae*. J. Biol. Chem. **254**:9839–9845.
26. **Holland, J. P., L. Labieniec, C. Swimmer, and M. J. Holland.** 1983. Homologous nucleotide sequences at the 5′-termini of messenger RNAs synthesized from the yeast enolase and glyceraldehyde-3-phosphate dehydrogenase gene families: the primary structure of a third glyceraldehyde-3-phosphate dehydrogenase gene. J. Biol. Chem. **258**:5291–5299.
27. **Holland, M. J., J. P. Holland, G. P. Thill, and K. A. Jackson.** 1981. The primary structures of two yeast enolase genes: homology between the 5′-noncoding flanking regions of yeast enolase and glyceraldehyde-3-phosphate dehydrogenase genes. J. Biol. Chem. **256**:1385–1395.
28. **Holland, M. J., T. Yokoi, J. P. Holland, K. Myambo, and M. A. Innis.** 1987. The *GCR1* gene encodes a positive transcriptional regulator of the enolase and glyceraldehyde-3-phosphate dehydrogenase gene families in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:813–820.
29. **Kawasaki, G., and D. G. Fraenkel.** 1982. Cloning of yeast glycolytic genes by complementation. Biochem. Biophys. Res. Commun. **108**:1107–1112.
30. **Kellermann, E., P. G. Seeboth, and C. P. Hollenberg.** 1986. Analysis of the primary structure and promoter function of a pyruvate decarboxylase gene (*PDC1*) from *Saccharomyces cerevisiae*. Nucleic Acids Res. **14**:8963–8977.
31. **Kopetzki, E., K.-D. Entian, and D. Mecke.** 1985. Complete nucleotide sequence of the hexokinase PI gene (*HXK1*) of *Saccharomyces cerevisiae*. Gene **39**:95–102.
32. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
33. **Legrain, P., and M. Rosbach.** 1989. Some *cis*- and *trans*-acting mutants for splicing target pre-mRNA to the cytoplasm. Cell **57**:573–583.
34. **Lehrach, R. H., D. Diamond, J. M. Wozney, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry **16**:4743–4751.
35. **Lillehaug, J. R., and K. Kleppe.** 1975. Effect of salts and polyamines on T4 polynucleotide kinase. Biochemistry **14**:1225–1230.
36. **Lindquist, S.** 1981. Regulation of protein synthesis during heat shock. Nature (London) **293**:311–314.
37. **Maitra, P. K., and Z. Lobo.** 1971. A kinetic study of glycolytic enzyme synthesis in yeast. J. Biol. Chem. **246**:475–488.
38. **Mayer, S. A., and C. L. Dieckmann.** 1989. The yeast *CBP1* gene produces two differentially regulated transcripts by alternative 3′-end formation. Mol. Cell. Biol. **9**:4161–4169.
39. **McAlister, L., and M. J. Holland.** 1982. Targeted deletion of a yeast enolase structural gene. J. Biol. Chem. **257**:7181–7188.
40. **McNally, T., I. J. Purvis, L. A. Fothergill-Gilmore, and A. J. P. Brown.** 1989. The yeast pyruvate kinase gene does not contain a string of non-preferred codons: revised nucleotide sequence. FEBS Lett. **247**:312–316.
41. **McNeil, J. B., P. Dykshoorn, J. N. Huy, and S. Small.** 1990. The DNA-binding protein RAP1 is required for efficient transcriptional activation of the yeast *PYK* glycolytic gene. Curr. Genet. **18**:405–412.
42. **Mellor, J., M. J. Dobson, N. A. Roberts, M. F. Tuite, J. S. Emtage, S. White, P. A. Lowe, T. Patel, A. J. Kingsman, and S. M. Kingsman.** 1983. Efficient synthesis of enzymatically active calf chymosin in *Saccharomyces cerevisiae*. Gene **24**:1–14.
43. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Moore, P. A., A. J. E. Bettany, and A. J. P. Brown.** 1990. The expression of a yeast glycolytic gene is subject to dosage limitation. Gene **89**:85–92.
45. **Moore, P. A., A. J. E. Bettany, and A. J. P. Brown.** 1990. Multiple copies of the pyruvate kinase gene affect yeast cell growth. J. Gen. Microbiol. **136**:2359–2366.
46. **Moore, P. A., A. J. E. Bettany, and A. J. P. Brown.** 1990. The yeast pyruvate kinase mRNA is regulated at multiple levels. NATO ASI Ser. Ser. H Cell Biol. **49**:421–432.
47. **Mountain, H. A., and P. E. Sudbery.** 1990. Regulation of the *Saccharomyces cerevisiae WHI2* gene. J. Gen. Microbiol. **136**: 727–732.
48. **Nishizawa, M., Y. Suzuki, Y. Nogi, K. Matsumoto, and T. Fukasawa.** 1990. Yeast Gal11 protein mediates the transcriptional activation signal of two different transacting factors, Gal4 and general regulatory factor I/repressor/activator site binding protein I/translation upstream factor. Proc. Natl. Acad. Sci. USA **87**:5373–5377.
49. **Nonet, M., C. Scafe, J. Sexton, and R. Young.** 1987. Eucaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. Mol. Cell. Biol. **7**:1602–1611.
50. **Piper, P. W., B. Curran, M. W. Davies, K. Hirst, A. Lockheart, J. E. Ogden, C. A. Stanway, A. J. Kingsman, and S. M. Kingsman.** 1988. A heat shock element in the phosphoglycerate kinase gene promoter of yeast. Nucleic Acids Res. **16**:1333–1348.
51. **Piper, P. W., B. Curran, M. W. Davies, K. Hirst, A. Lockheart, and K. Seward.** 1988. Catabolite control of the elevation of *PGK* mRNA levels by heat shock in *Saccharomyces cerevisiae*. Mol. Microbiol. **2**:353–361.
51a. **Purvis, I. J.** Unpublished data.
52. **Purvis, I. J., L. Loughlin, A. J. E. Bettany, and A. J. P. Brown.** 1987. Translation and stability of an *Escherichia coli* β-galactosidase mRNA expressed under the control of pyruvate kinase sequences in *Saccharomyces cerevisiae*. Nucleic Acids Res. **15**:7963–7974.
53. **Reed, L. J.** 1974. Multienzyme complexes. Accounts Chem. Res. **7**:40–46.
54. **Ross, J., G. A. Reid, and I. W. Dawes.** 1988. The nucleotide sequence of the *LPD1* gene encoding lipoamide dehydrogenase in *Saccharomyces cerevisiae*: comparison between eukaryotic and prokaryotic sequences for related enzymes and identification of potential upstream control sites. J. Gen. Microbiol. **134**:1131–1139.
54a. **Sagliocco, F. A.** Unpublished data.
55. **Santangelo, G. M., and J. Tornow.** 1990. Efficient transcription of the glycolytic gene *ADH1* and three translational component genes requires the *GCR1* product, which can act through TUF/GRF/RAP binding sites. Mol. Cell. Biol. **10**:859–862.
56. **Santiago, T. C., I. J. Purvis, A. J. E. Bettany, and A. J. P. Brown.** 1986. The relationship between mRNA stability and length in *Saccharomyces cerevisiae*. Nucleic Acids Res. **14**: 8347–8360.
57. **Schaaff, I., J. B. A. Green, D. Gozalbo, and S. Hohmann.** 1989. A deletion of the *PDC1* gene for pyruvate decarboxylase of yeast causes a different phenotype than previously isolated point mutations. Curr. Genet. **15**:75–81.
58. **Schmitt, H. D., M. Ciriacy, and F. K. Zimmermann.** 1983. The synthesis of yeast pyruvate decarboxylase is regulated by large variations in the messenger RNA level. Mol. Gen. Genet. **192**:247–252.
59. **Schwelberger, H. G., S. D. Kohlwein, and F. Paltauf.** 1989. Molecular cloning, primary structure and disruption of the structural gene of aldolase from *Saccharomyces cerevisiae*. Eur. J. Biochem. **180**:301–308.
60. **Scott, E. W., H. E. Allison, and H. V. Baker.** 1990. Characterization of *TPI* gene expression in isogenic wild-type and *gcr1*-deletion mutant strains of *Saccharomyces cerevisiae*. Nucleic Acids Res. **18**:7099–7108.
61. **Seta, F. D., S. A. Ciafre, C. Marck, B. Santoro, C. Presuti, A. Sentenac, and I. Bozzoni.** 1990. The ABF1 factor is the transcriptional activator of the L2 ribosomal protein genes of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**:2437–2441.

62. **Stachelek, C., J. Stachelek, J. Swan, D. Botstein, and W. Konigsberg.** 1986. Identification, cloning and sequence determination of the genes specifying hexokinase A and B from yeast. Nucleic Acids Res. **14**:945–963.
63. **Tekamp-Olson, P., R. Najarian, and R. L. Burke.** 1988. The isolation, characterisation and nucleotide sequence of the phosphoglucoisomerase gene of *Saccharomyces cerevisiae*. Gene **73**:153–161.
64. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
65. **Tornow, J., and G. M. Santangelo.** 1990. Efficient expression of the *Saccharomyces cerevisiae* glycolytic gene *ADH1* is dependent upon a *cis*-acting regulatory element (UAS_{RPG}) found initially in genes encoding ribosomal proteins. Gene **90**:79–85.
66. **Tuite, M. F., M. J. Dobson, N. A. Roberts, R. M. King, D. C. Burke, S. M. Kingsman, and A. J. Kingsman.** 1982. Regulated high efficiency expression of human interferon-alpha in *Saccharomyces cerevisiae*. EMBO J. **1**:603–608.
67. **Verdier, J.-M.** 1990. Regulatory DNA-binding proteins in yeast: an overview. Yeast **6**:271–297.
68. **Watson, H. C., N. P. C. Walker, P. J. Shaw, T. N. Bryant, P. L. Wendell, L. A. Fothergill, R. E. Perkins, S. C. Conroy, M. J. Dobson, M. F. Tuite, A. J. Kingsman, and S. M. Kingsman.** 1982. Sequence and structure of yeast phosphoglycerate kinase. EMBO J. **1**:1635–1640.
69. **White, M. F., and L. A. Fothergill-Gilmore.** 1988. Sequence of the gene encoding phosphoglycerate mutase from *Saccharomyces cerevisiae*. FEBS Lett. **229**:383–387.
70. **Wills, C.** 1990. Regulation of sugar and ethanol metabolism in *Saccharomyces cerevisiae*. Crit. Rev. Biochem. Mol. Biol. **25**:245–280.
71. **Yoshino, M., and K. Murakami.** 1982. AMP deaminase reaction as a control system of glycolysis in yeast: activation of phosphofructokinase and pyruvate kinase by the AMP deaminase-ammonia system. J. Biol. Chem. **257**:2822–2828.