Saccharomyces cerevisiae Null Mutants in Glucose Phosphorylation: Metabolism and Invertase Expression

R. Brian Walsh,[1] Drago Clifton, Jaroslav Horak[2] and Dan G. Fraenkel

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

Manuscript received December 7, 1990
Accepted for publication April 1, 1991

ABSTRACT

A congenic series of *Saccharomyces cerevisiae* strains has been constructed which carry, in all combinations, null mutations in the three genes for glucose phosphorylation: *HXK1*, *HXK2* and *GLK1*, coding hexokinase 1 (also called PI or A), hexokinase 2 (PII or B), and glucokinase, respectively: *i.e.*, eight strains, all of which grow on glucose except for the triple mutant. All or several of the strains were characterized in their steady state batch growth with 0.2% or 2% glucose, in aerobic as well as respiration-inhibited conditions, with respect to growth rate, yield, and ethanol formation. Glucose flux values were generally similar for different strains and conditions, provided they contained either hexokinase 1 or hexokinase 2. And their aerobic growth, as known for wild type, was largely fermentative with *ca.* 1.5 mol ethanol made per mol glucose used. The strain lacking both hexokinases and containing glucokinase was an exception in having reduced flux, a result fitting with its maximal rate of glucose phosphorylation *in vitro*. Aerobic growth of even the latter strain was largely fermentative (*ca.* 1 mol ethanol per mol glucose). Invertase expression was determined for a variety of media. All strains with *HXK2* showed repression in growth on glucose and the others did not. Derepression in the wild-type strain occurred at *ca.* 1 mM glucose. The metabolic data do not support–or disprove–a model with *HXK2* having only a secondary role in catabolite repression related to more rapid metabolism.

SACCHAROMYCES cerevisiae has three enzymes for glucose phosphorylation: hexokinase 1 (also known as PI or A), hexokinase 2 (PII or B), and glucokinase. Their obvious function is in the first step of glucose metabolism, and it is known primarily through the work of MAITRA and LOBO with deficiency mutants that any one of these enzymes is sufficient for growth on glucose (*e.g.*, LOBO and MAITRA 1977a,b; MAITRA and LOBO 1983). Even with regard to growth, however, the individual functions of the three enzymes in wild-type strains are not so clear. At least in some strains, hexokinase 2 predominates on glucose and hexokinase 1 in its absence (GANCEDO, CLIFTON and FRAENKEL 1977; MURATSUBAKI and KATSUME 1979; R. B. WALSH, to be reported).

Aside from catalyzing the first step of glucose metabolism, the three kinases have a role in high affinity glucose uptake (BISSON and FRAENKEL 1983; LANG and CIRILLO 1987; MCCLELLAN and BISSON 1988). Also, hexokinase 2 seems to have a special role in carbon catabolite repression, deficiency mutants having typically derepressed levels of a sensitive enzyme, such as invertase, in the presence of glucose concentrations which would normally repress its synthesis (*e.g.*, ENTIAN and MECKE 1982; MICHELS, HAHENBERGER and SYLVESTRE 1983; MA and BOTSTEIN 1986; for reviews, see ENTIAN 1986; GANCEDO and GANCEDO 1986; CARLSON 1987; JOHNSTON 1987).

Thus, neither the common nor individual roles of the three kinases are yet understood. In the present paper we report construction of a set of congenic strains carrying null mutations *hxk1::LEU2*, *hxk2::LEU2* and *glk1::LEU2*–eight strains, seven of which grow on glucose–and their characterization as to growth, cell yield, glucose flux, ethanol formation and expression of invertase. Many of these functions have, over the years, been assessed in a variety of wild type strains, and certain ones recently even in kinase null mutants [*e.g.*, MA and BOTSTEIN (1986) on catabolite repression; see also MCCLELLAN and BISSON (1988), on glucose transport]. But a congenic complete set has not been evaluated in this way. Perhaps the most extensive previous study of the role of these enzymes in glucose metabolism is that of LOBO and MAITRA (1977b), and in part the present paper is an extension of that work to the new strains emphasizing metabolism in growth. The other emphasis is on invertase expression, and in this respect the present work complements, with the various combinations of null mutants, the detailed recent studies of hexokinase 2 point mutants (MA *et al.* 1989a,b).

[1] Present address: Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California 95616.

[2] Present address: Department of Membrane Transport, Institute of Physiology, 142 20 Prague 4, Czechoslovakia.

MATERIALS AND METHODS

Strains: The strains (see Table 1) are DFY1 and seven kinase mutants congenic with it. Each strain also carries *lys1-1* and *SUC* and, except DFY1, *leu2-1* at normal chromosomal position; strains DFY1, -566, -567, and -581 are mating type **a** and the others α. Their construction employed subcloning of clones pBW111-113 (WALSH, KAWASAKI and FRAENKEL 1983) and insertion of a *ca.* 4-kbp *Nco*I fragment (SEDIVY and FRAENKEL 1985) containing *LEU2*. The genes have been sequenced (FRÖHLICH, ENTIAN and MECKE 1985; KOPETZKI, ENTIAN and MECKE 1985; STACHELEK *et al.* 1986; ALBIG and ENTIAN 1988), and the mutations are *hxk1::LEU2*, a *ca.* 0.9-kbp substitution extending from codon 15 in the 5′ direction; *hxk2::LEU2*, an insertion at codon 15; and *glk1::LEU2*, a *ca.* 1-kbp substitution from codon 105 in the 5′ direction. Each mutation abolished complementation of the original triple mutant strain DFY437. Strains congenic with DFY1 but carrying *leu2-1* and *hxk1-1*, *hxk2-1*, or both (from DFY87, WALSH, KAWASAKI and FRAENKEL 1983) were obtained by five or more backcrosses, and the null mutations substituted into the chromosome by transplacement with Leu$^+$ selection; galactose was permissive carbon source. The chromosomal substitutions were confirmed by gel transfer hybridization (Southern analyses) and genetic mapping (linkage of *hxk1::LEU2* with *met10* and *hxk2::LEU2* with *ade5*). Further crosses and assays allowed identification of the three single gene null mutants. The double mutant, *hxk1::leu2 hxk2::LEU2*, was then obtained, crossed with the *glk1::LEU2* strain and the seven mutant strains identified among segregants.

Growth and enzyme assays: Medium R61 (FRAENKEL 1985) was supplemented with the indicated carbon sources (1% galactose, etc.). For "anaerobic" metabolism the respiration inhibitor antimycin A was included at 1 μg/ml. All cultures originated from small inocula (*e.g.*, A_{580} values of 0.002) and, for enzyme assays (unless indicated otherwise) were harvested long before glucose exhaustion: *e.g.*, at A_{580} values of *ca.* 2 for cells from media originally with 2% glucose, and of 0.5 for cultures from 0.2% glucose (glucose exhaustion typically occurs at A_{580} of *ca.* 2 in the latter medium, except for strain DFY568). Cyclohexamide, 20 μg/ml, was added just before harvest and also included in washing and resuspension buffer (YEB, 50 mM KH_2PO_4, 2 mM Na_2EDTA, 2 mM 2-mercaptoethanol, pH 7.4); as was 2 mM phenylmethylsulfonyl fluoride in the final resuspension, which was usually to *ca.* 1/100 of initial culture volume.

Hexokinase or glucokinase, and glucose 6-P dehydrogenase assay used extracts made with a French press and 30-min centrifugation at 15,000 × *g*; similar values were obtained without centrifugation. The assay employed 5 mM triethanolamine with 10 mM $MgCl_2$, pH 7.4, 0.3 mM NADP, and was supplemented with 1 mM ATP, 5 mM glucose or fructose, and 5 μl of a 1:1 mixture of phosphoglucose isomerase (Boehringer-Mannheim 127–396, 2 mg/ml) and glucose 6-P dehydrogenase (Boehringer-Mannheim 127–035, 1 mg/ml) or, for glucose 6-P dehydrogenase, 1 mM glucose 6-P. Activities at 30° were calculated from increase in absorbance at 340 nm. For Table 1, specific activities were calculated according to protein assay (BRADFORD 1976) with bovine plasma albumin as standard or, values in parentheses, according to A_{580} values in cell suspension before treatment with the French press.

For invertase, cells prepared as above were treated with toluene (1 drop per ml cell suspension), 30 sec on a vortex mixer and 30-min vigorous shaking in a 37° bath. (Similar activities were obtained with French press extraction, about twice the values with glass bead extracts.) The assay mixture contained 75 μl 0.2 M sodium acetate, pH 4.7, 25 μl 0.5 M sucrose (or water), and cells plus YEB to 140 μl. After 60 min at 30°, 100 μl 0.5 M potassium phosphate, pH 7.0, was added and the reaction stopped by 3 min at 100°. Glucose in the supernatant was assayed with hexokinase and glucose 6-P dehydrogenase. Activities were normalized to A_{580} in the cell suspension before toluene treatment and, in some cases, protein was measured (LOWRY *et al.* 1951) after precipitation of cells (before toluene treatment) with 0.5 M trichloroacetic acid and resuspension in 1 M NaOH. Protein values were between 80 and 120 μg per A_{580}.

Yield: Cultures grown from low A_{580} were sampled over a range of *ca.* 0.5 to 2 A_{580}. For cultures with initial glucose concentration 0.2%, glucose was measured by enzymatic assay (as above), and 1/yield values obtained from the (linear) slopes of plots of glucose *vs.* A_{580}. V_{glu} is $1/Y \times \mu$ in (the first order growth rate constant, hr^{-1}), *i.e.*, μmol per hr per A_{580}. Yields of ethanol were determined from the same samples, with ethanol assay according to Sigma technical bulletin 331-UV.

For growth in media initially containing 2% glucose, the method (adapted from ROGNSTAD, CLARK and KATZ 1973; KATZ and ROGNSTAD 1976) was to measure transfer of radioactivity from 2[^{3}H]glucose (initially 10^5 cpm/ml, TRK.361, Amersham) to medium. Columns containing *ca.* 2 ml AG1-X8 (Bio-Rad Laboratories) were washed with water, 5 volumes 0.5 M $(NH_4)_2B_4O_7$ and then water again to a pH of *ca.* 6. The usual culture samples, 0.5 ml were diluted with 1.5 ml R61, filtered (GF/A, 2.4 cm filters, Whatman) and 0.8 ml applied to the column followed by five 1-ml portions of water and 10 1-ml portions of 0.5 M ammonium tetraborate. Fractions (1 ml) were collected and radioactivity determined in 10 ml Aquasol (New England Nuclear). Fractions 2–5 were summed, fraction 1 was subtracted as a blank from each, and constitute the unbound fraction (*i.e.*, ^3H_2O); the remaining fractions (from the borate elution) were summed with, likewise, the first one subtracted from each as a blank and constitute the bound fraction (*i.e.*, remaining 2-[^{3}H]glucose). Data were plotted as percent of initial glucose used (*i.e.*, unbound/(unbound + bound)), *vs.* A_{580} and recorded as $1/Y$ and V_{glu} expressed in the same units as above.

One percent or less of counts or a sample of tritiated glucose alone in growth medium eluted from such a column, and this value did not increase with 18-hr incubation on a shaker before assay, or decrease if the tritiated glucose sample had first been lyophilized. [^{14}C]Glucose contained *ca.* 0.5% of counts not binding to the column, while tritiated water was 100% unbound.

RESULTS AND DISCUSSION

Kinase levels: Table 1 shows enzyme activities for cells obtained from exponential growth on glucose. The three strains carrying just a single kinase gene had fructose/glucose phosphorylation ratios in extracts in line with knowledge of the individual pure enzymes: values of *ca.* 3 for hexokinase 1, 1 for hexokinase 2, and less than 0.1 for glucokinase. For strains with more than one intact kinase gene interpretation is less easy. However, as mentioned, experiments where the enzymes have been individually assessed show hexokinase 2 to predominate in growth on glucose, which fits with the present data, both for the wild type and for any strain with *HXK2*. Also, as

TABLE 1

Strains and *in vivo* enzyme activities

Strain[a]	Enzyme activity[b] Hxk-Glu	Hxk-Fru	G6PDH
1. DFY1 (+++)	1.15 (5.81)	1.70	0.13
2. DFY566 (−++)	1.30 (7.00)	1.63	0.13
3. DFY567 (+−+)	0.47 (2.07)	1.43	0.19
4. DFY581 (++−)	1.50 (7.92)	1.70	0.13
5. DFY568 (−−+)	0.17 (0.68)	0.01	0.19
6. DFY582 (−+−)	1.23 (7.57)	1.25	0.12
7. DFY583 (+−−)	0.40 (2.29)	1.29	0.15
8. DFY570 (−−−)[c]			
9. DFY646 (++ ++ ++)	1.21 (5.63)	1.15	0.18
10. DFY647 (++ +− ++)	0.56 (3.17)	0.60	0.12

The strains were harvested (see MATERIALS AND METHODS) from growth in medium with 2% glucose and extracts assayed for glucose and fructose phosphorylation activities (Hxk-Glu and Hxk-Fru, respectively), as well as glucose-6-P dehydrogenase (G6PDH).

[a] The notation in parentheses is +, wild type allele, and −, null allele, for *HXK1*, *HXK2* and *GLK1*, respectively. Strains 9 and 10 are diploids, wild type, and heterozygous for *HXK2*, respectively.

[b] Enzyme activities are expressed as units (μmol/min) per mg protein; in the Hxk-Glu column values are also given, in parentheses, as μmol/hr/A_{580}; see text.

[c] This strain does not grow on glucose, hence no entries in the table. Assays from growth in a permissive medium revealed no glucose or fructose phosphorylation activities.

TABLE 2

Growth on plates

Strain	Colony size (mm) with carbon source: —	Glu	Fru	Man	Mal	Suc	Gal	—	Pyr
DFY1 (+++)	1.1	3.8	3.3	3.2	2.6	3.0	2.4	1.6	2.0
DFY566 (−++)	1.0	3.8	3.3	3.4	2.6	3.6	2.2	1.3	1.8
DFY567 (+−+)	0.8	3.3	3.2	3.0	1.7	3.2	2.3	1.2	2.0
DFY581 (++−)	1.1	2.4	3.0	3.0	2.7	3.2	2.2	1.4	1.9
DFY568 (−−+)	0.9	2.7	0.8	1.5	0.2	2.2	2.2	1.2	1.8
DFY582 (−+−)	0.9	3.7	3.1	3.0	2.0	3.2	2.3	1.2	1.7
DFY583 (+−−)	1.0	3.0	2.9	2.5	0.7	2.9	2.5	1.3	2.0
DFY570 (−−−)	0.6	0.7	0.7	0.6	0.0	0.2	2.0	0.9	1.6

Cultures were diluted and spread on R61 plates with the indicated carbon sources, and average colony size estimated after 3 days (for the sugars) or 4 days (for pyruvate) of incubation at 30 C. Abbreviations are Glu, glucose; Fru, fructose; Man, mannose; Mal, maltose; Suc, sucrose; Gal, galactose, and Pyr, pyruvate. A "—" means no carbon source added to the R61 medium, the left hand column being the entry for 3 days of incubation and the penultimate column for 4 days of incubation. Strain designations are as in Table 1.

seen in the three strains with single intact kinase genes, putatively maximal hexokinase 1 activity was lower than hexokinase 2, and glucokinase activity was even lower (relative values of *ca.* 0.3 and 0.1, respectively).

Growth on various sources: The two enzymes hexokinase 1 and hexokinase 2 have been long studied (COLOWICK 1973) and known to phosphorylate glucose, fructose and mannose. Yeast glucokinase is less well known (MAITRA 1975). Contrary to its name, it does phosphorylate, but with lower V_{max} and higher K_m, other sugars and derivatives, including mannose, but fructose is barely employed. Earlier work with undefined deficiency mutants showed that growth of yeast on fructose required either hexokinase 1 or hexokinase 2; that a strain lacking both still grew on glucose, as did strains with only hexokinase 1 or hexokinase 2; and that a strain lacking all three did not grow on glucose (LOBO and MAITRA 1977a; GANCEDO, CLIFTON and FRAENKEL 1977).

The present strains with null mutations grow in accord with findings from previous mutants. Thus, as judged on plates (Table 2), all the mutant strains grew normally on galactose and all except the triple mutant grew well on glucose. Neither the triple mutant DFY570 nor the double mutant DFY568 (*hxk1 hxk2*) grew on fructose, and the double mutant was intermediate in its growth on mannose. And, obviously, the triple null mutant strain is clearly viable (as also reported by ALBIG and ENTIAN 1988). [DFY570 did not notably differ in its growth from the triple mutant previously employed, DFY437 (WALSH, KAWASAKI and FRAENKEL 1983). Tests with antiserum showed cross reacting material to hexokinase 2 but not to hexokinase 1 in the latter strain, but not when the allele was *hxk2::LEU2* instead of *hxk2-1* (Vojtek 1988).]

There are other observations on these growth patterns:

1. An enriched medium was employed, so there was some growth in the absence of added carbon source; this was reduced by use of minimal medium or by the respiration inhibitor antimycin A. Thus, on a minimal plate with glucose and 3 days of incubation, colonies of the triple mutant strain were of *ca.* 0.1-mm diameter *vs.* 0.8-mm colonies for the double mutant strain DFY568. However, prolonged incubation of the triple mutant on a minimal glucose plate showed slight growth (0.7-mm colonies after 6 days), and there were similar indications on enriched medium that the double and triple mutant strains DFY568 and DFY570 are not completely inert to fructose, or fructose and glucose, respectively. The possibility of marginal metabolism of these sugars, by whatever pathway, is not excluded.

2. The strains unable to phosphorylate glucose, or fructose, were indifferent to these sugars in their residual growth on the enriched medium. In data not presented, the same result of no apparent inhibition of the triple mutant by glucose was seen on plates containing both pyruvate and glucose. Glucose was somewhat inhibitory on a plate containing galactose, direct interference with galactose uptake being a likely explanation.

3. In spite of lack of glucose toxicity in the triple mutant, maltose and sucrose were inhibitory. Maltose even inhibited growth of the double mutant strain DFY568, a surprising finding in view of its growth on

TABLE 3

Parameters of growth on glucose

Strain[a]	Medium[b]	μ[c]	$1/Y_{Glu}$[d]	V_{Glu}[e]	Ethanol/glucose[f]
DFY1 (+++)	0.2% G	0.38	6.0	2.5	1.5
DFY566 (−++)	0.2% G	0.46	6.8	3.1	—[g]
DFY567 (+−+)	0.2% G	0.41	5.6	2.3	—
DFY581 (++−)	0.2% G	0.46	6.9	3.2	—
DFY568 (−−+)	0.2% G	0.31	2.6	0.8	0.9
DFY582 (−+−)	0.2% G	0.36	6.2	2.2	1.4
DFY583 (+−−)	0.2% G	0.39	4.8	1.9	1.5
DFY646 (++ ++ ++)	0.2% G	0.50	5.1	2.6	—
DFY647 (++ +− ++)	0.2% G	0.47	5.6	2.7	—
DFY1 (+++)	0.2% G + A	0.24	6.9	1.7	2.0
DFY568 (−−+)	0.2% G + A	0.18	5.8	1.0	1.7
DFY582 (−+−)	0.2% G + A	0.28	7.1	2.0	1.9
DFY583 (+−−)	0.2% G + A	0.26	7.7	2.0	1.6
DFY1 (+++)	2.0% G	0.35	6.5	2.3	—
DFY566 (−++)	2.0% G	0.35	6.5	2.3	—
DFY567 (+−+)	2.0% G	0.34	4.8	1.6	—
DFY581 (++−)	2.0% G	0.36	6.5	2.4	—
DFY568 (−−+)	2.0% G	0.24	3.3	0.8	—
DFY582 (−+−)	2.0% G	0.37	6.4	2.4	—
DFY583 (+−−)	2.0% G	0.35	5.1	1.8	—

[a] Designated as in Table 1.
[b] Medium R61 was supplemented with 0.2% or 2% glucose (G); A, antimycin also present.
[c] Hr^{-1}.
[d] Micromoles glucose used per A_{580} increment.
[e] Micromoles glucose used per A_{580} per hr (product of previous 2 columns).
[f] Ratio of slopes, (ethanol made *vs.* A_{580})/(glucose used *vs.* A_{580}).
[g] "—" indicates not measured.

glucose. Experiments on this matter will be reported elsewhere.

Rate and yield of glucose metabolism in growth: Although growth on glucose, as judged by colony sizes (Table 2) did not differ much between the seven strains with one or more kinase, actual growth rates might significantly differ and metabolic flux of glucose might differ even more. After all, *S. cerevisiae* has a relatively inefficient glucose metabolism which is largely fermentative even aerobically (FRAENKEL 1982), so large differences in glucose flux in mutants might be compensated by respiration.

We therefore determined (see MATERIALS AND METHODS) growth rate, yield, and glucose flux values (Table 3). For some of the strains ethanol was also assayed, and the determinations also done for growth in the presence of antimycin A. The following points may be made.

1. All seven strains grew well on glucose in liquid medium, as expected from growth on plates. The only strain whose growth consistently was slower than the others, doubling time of *ca.* 125 min instead of *ca.* 95 min, was the strain containing just glucokinase, DFY568, which has the lowest value for glucose phosphorylation *in vitro* (Table 1). That level is clearly adequate for relatively normal growth.

2. Each strain grew at similar rate (and yield) on 0.2% (11 mM) and 2% (110 mM) glucose. Growth rates only decreased in the range of 2 mM glucose (data not shown), which is near the K_m value of high affinity glucose transport (BISSON and FRAENKEL 1983; LANG and CIRILLO 1987).

3. Just as the growth rates of the seven strains did not differ much, neither did the yields (glucose used per A_{580} increment), a significant exception, again, being the strain with only glucokinase, DFY568. whose yield was twice or more that of the other strains. Thus, V_{glu}, the rate of glucose metabolism, was lowest in this strain, values in the other strains being similar to each other.

4. Comparison of glucose flux (Table 3) and *in vitro* maximum glucose phosphorylation values (Table 1) for the cultures growing on 2% glucose shows that strains with *HXK2* have an *in vitro* capacity to phosphorylate glucose in excess of their rate of glucose metabolism, while in strains with *HXK1*, but not *HXK2*, the two values were similar. The strain with only *GLK1* had similar and low kinase and flux values.

5. DFY1, like other wild-type strains, performed an ethanolic fermentation in its aerobic growth, *ca.* 1.5 mol ethanol being made per mol glucose used in its period of steady state growth before glucose exhaustion from 0.2% glucose concentration. Nonetheless, antimycin A did somewhat increase its ethanol yield

TABLE 4

Invertase activity

Strain[a]	[Units/A_{580} or units/mg protein][b] in growth medium:[c]				
	2% G	0.2% G	0.2% G + A	After 0.2% G	1% Gal
DFY1 (+++)	0.02	0.05	0.14	0.63	0.87
	(0.16)	(0.34)	(0.92)	(6.7)	(9.8)
DFY566 (−++)	0.02	0.07	—[d]	0.89	1.01
DFY567 (+−+)	1.00	0.67	—	1.07	1.09
	(6.9)	(7.3)		(16.5)	
DFY581 (++−)	0.01	0.14	—	1.12	0.78
				(12.7)	
DFY568 (−−+)	1.38	1.17	2.03	1.12	0.74
	(18.1)	(14.1)			(10.1)
DFY582 (−+−)	0.02	0.05	0.16	0.58	0.47
DFY583 (+−−)	1.07	0.94	0.65	0.96	0.96
	(9.4)	(9.7)			(11.5)
DFY570 (−−−)	—	—	—	—	0.61
					(6.9)
DFY646 (++ ++ ++)	0.02	—	—	—	0.47
DFY647 (++ +− ++)	0.02	—	—	—	0.62

[a] As in Table 1.

[b] Invertase activity is expressed as units (μmol glucose formed from sucrose per min) per A_{580}, as well as, for many samples, units per mg protein (values in parentheses); see MATERIALS AND METHODS.

[c] The media contained glucose (G, or, last column, galactose) at the initial concentrations indicated; in column 3, A means that antimycin was also present. Cells were harvested (see MATERIALS AND METHODS) from a low absorbance, so that most of the sugar was still unused, except for column 4 samples, which were taken after glucose exhaustion ("After 0.2%").

[d] "—" means not done. Note that strain DFY570 does not grow on glucose.

and decrease cell yield and growth rate (Table 3), as if this strain has significant respiratory metabolism in these conditions. The same pattern was observed for mutant strains containing or *HXK2* or *HXK1*. Mere absence of hexokinase 2, therefore, has a marginal effect on fermentation itself.

For the strain with just glucokinase, DFY568, aerobic ethanol yield was 0.9 mol per mol glucose–somewhat less than the other strains–and antimycin A markedly slowed growth, decreased cell yield and doubled ethanol yield. Thus, the contribution of respiratory metabolism is larger in this strain than in the others.

Invertase: Table 4 reports total cell associated invertase in the same conditions used for glucose flux, as well as some other ones. In spite of differences in methodology, these data are in many ways confirmatory of the known picture of hexokinase 2 involvement in catabolite repression. Thus (i), in the four strains with *HXK2* invertase was repressed in growth on 2% glucose (values of *ca.* 0.02 unit/A_{580}) while *hxk2* mutants were not repressed (*ca.* 1 unit/A_{580}). (ii) About the same result as with 2% glucose was obtained for medium with 0.2% glucose (column 2), but with somewhat higher repressed levels. (iii) For growth in the presence of antimycin A (column 3), repressed values were higher than in normal aerobic growth, with, again, typical derepressed values in the *hxk2* strains. (iv) Invertase was derepressed in *HXK2* strains after glucose exhaustion from 0.2% glucose cultures (column 4), with similar levels to cultures in exponential growth on galactose (column 5). Derepressed levels of invertase were similar throughout the set of strains. (v) A pair of congenic diploids of the same series, *HXK2/HXK2* and *hxk2/HXK2*, showed normal repressed level of invertase on 2% glucose and normal derepressed level on galactose. (vi) For the wild-type strain, as cultures depleted glucose from 0.2% concentration, half-maximal invertase level was observed at *ca.* 1 mM glucose (data not shown).

Comments: invertase expression and glucose metabolism: Comparing invertase with glucose flux (Tables 3 and 4), cultures with high rates of glucose metabolism were repressed and ones with low rates were derepressed. One interpretation might be that repression is a secondary consequence of high catabolic rate: perhaps some ordinary metabolite whose amount differs in the two situations governs gene expression. The partial restoration of catabolite repression in a *hxk2* mutant strain by additional copies of *HXK1* (MA and BOTSTEIN 1986) might fit this model. However, since strains with similar V_{glu} values, *ca.* 2.2, may be repressed (*HXK2*) or derepressed (*hxk2*), differences in flux beyond the accuracy of our data would have to be significant. The same comment applies to metabolite levels, found as relatively normal (ENTIAN and FRÖHLICH 1984). But it should be remarked that if a function of catabolite repression were, say, maintenance of ATP, then levels of a signaling metabolite (*e.g.*, ATP itself) might indeed be similar in the two steady states.

A different model, not contradicted by the present results, has been emphasized (ENTIAN 1986): a special function for hexokinase 2 separate from its metabolic role. An important line of evidence has been mutants with adequate catalysis but defective in repression (ENTIAN and FRÖHLICH 1984). However, proof that the altered enzyme is normal in catalysis (ENTIAN *et al.* 1985) is incomplete. Indeed, an extensive study of hexokinase 2 mutants showed a correlation between glucose phosphorylation activity and catabolic repression (MA *et al.* 1989a,b). Perhaps, as suggested by S. KUCHIN (personal communication), there is a catalytic activity of hexokinase 2 which is different from normal glucose phosphorylation. Hexokinase 2 has been reported as a protein kinase (HERRERO, FERNANDEZ and MORENO 1989), and both hexokinases as being phosphoproteins *in vivo* (VOJTEK and FRAENKEL 1990).

Finally, it need be emphasized that our data are only for invertase, and other systems subject to catabolite repression and/or *hxk2* derepression might differ in sensitivity or mechanism. One example is high affinity glucose uptake (BISSON and FRAENKEL 1984; BISSON 1988), which, considering K_m values and that metabolism may be limited at uptake (BECKER and BETZ 1972) is likely in some of the present experiments to be derepressed when invertase is repressed.

This work was supported by grant GB21098 from the National Institute of General Medical Sciences, National Institutes of Health.

LITERATURE CITED

ALBIG, W., and K.-D. ENTIAN, 1988 Structure of yeast glucokinase, a strongly diverged aldo-hexose-phosphorylating enzyme. Gene **73:** 141–152.

BECKER, J.-U., and A. BETZ, 1972 Membrane transport as controlling pacemaker of glycolysis in *Saccharomyces carlsbergensis.* Biochim. Biophys. Acta **272:** 584–597.

BISSON, L. F., 1988 High affinity glucose transport in *Saccharomyces cerevisiae* is under general glucose repression control. J. Bacteriol. **170:** 4838–4845.

BISSON, L. F., and D. G. FRAENKEL, 1983 Involvement of kinases in glucose and fructose uptake by *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **80:** 1730–1734.

BISSON, L. F., and D. G. FRAENKEL, 1984 Expression of kinase dependent uptake in *Saccharomyces cerevisiae.* J. Bacteriol. **159:** 1013–1017.

BRADFORD, M., 1976 A quick and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248–254.

CARLSON, M., 1987 Regulation of sugar utilization in *Saccharomyces* species. J. Bacteriol. **169:** 4873–4877.

COLOWICK, S. P., 1973 The hexokinases, pp.1–48 in *The Enzymes,* Ed. 3, edited by P. D. BOYER. Academic Press, New York.

ENTIAN, K.-D., 1986 Glucose repression: a complex regulatory system in yeast. Microbiol. Sci. **3:** 366–371.

ENTIAN, K.-D., and K.-U. FRÖHLICH, 1984 *Saccharomyces cerevisiae* mutants provide evidence of hexokinase PII as a bifunctional enzyme with catalytic and regulatory domains for triggering repression. J. Bacteriol. **158:** 29–35.

ENTIAN, K.-D., and D. MECKE, 1982 Genetic evidence for a role of hexokinase isoenzyme PII in carbon catabolite repression in *Saccharomyces cerevisiae.* J. Biol. Chem. **257:** 870–874.

ENTIAN, K.-D., F. HILBERG, H. OPITZ and D. MECKE, 1985 Cloning of hexokinase structural genes from *Saccharomyces cerevisiae* mutants with regulatory mutations responsible for glucose repression. Mol. Cell. Biol. **5:** 3035–3040.

FRAENKEL, D. G., 1982 Carbohydrate metabolism, in *The Molecular Biology of the Yeast Saccharomyces. Metabolism and Gene Expression,* edited by J. N. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

FRAENKEL, D. G., 1985 On ras gene function in yeast. Proc. Natl. Acad. Sci. USA **82:** 4740–4744.

FROHLICH, K.-U., K.-D. ENTIAN and D. MECKE, 1985 The primary structure of the yeast hexokinase PII gene which is responsible for glucose repression. Gene **36:** 105–111.

GANCEDO, J. M., D. CLIFTON and D. G. FRAENKEL, 1977 Yeast hexokinase mutants. J. Biol. Chem. **252:** 4443–4444.

GANCEDO, J. M., and C. GANCEDO, 1986 Catabolite repression mutants of yeast. FEMS Microbiol. Rev. **32:** 179–187.

HERRERO, P., R. FERNANDEZ and F. MORENO, 1989 The hexokinase isoenzyme PII of *Saccharomyces cerevisiae* is a protein kinase. J. Gen. Microbiol. **135:** 1209–1216.

JOHNSTON, M., 1987 A model fungal regulatory mechanism: the *GAL* genes of *Saccharomyces cerevisiae.* Microbiol. Rev. **51:** 458–476.

KATZ, J., and R. ROGNSTAD,1976 Futile cycles in the metabolism of glucose. Curr. Top. Cell. Regul. **10:** 237–289.

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

LANG, J. M., and , V. P. CIRILLO, 1987 Glucose transport in a kinaseless *Saccharomyces cerevisiae* mutant. J. Bacteriol. **169:** 2932–2937.

LOBO, Z., and P. K. MAITRA, 1977a Genetics of yeast hexokinase. Genetics **86:** 727–744.

LOBO, Z., and P. K. MAITRA, 1977b Physiological role of glucose phosphorylating enzymes in *Saccharomyces cerevisiae.* Arch. Biochem. Biophys. **182:** 639–645.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, 1951 Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275.

MA, H., and D. BOTSTEIN, 1986 Effects of null mutations in the hexokinase genes of *Saccharomyces cerevisiae* on catabolite repression. Mol. Cell. Biol. **6:** 4046–4052.

MA, H., L. M. BLOOM, Z. ZHU, C. T. WALSH and D. BOTSTEIN, 1989a Isolation and characterization of mutations in the *HXK2* gene of *Saccharomyces cerevisiae.* Mol. Cell. Biol. **9:** 5630–5642.

MA, H., L. M. BLOOM, C. T. WALSH and D. BOTSTEIN, 1989b The residual enzymatic phosphorylation activity of hexokinase II mutants is correlated with glucose repression in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **9:** 5643–5649.

MAITRA, P. K., 1975 Glucokinase from yeast. Methods Enzymol. **42:** 25–30.

MAITRA, P. K., and Z. LOBO, 1983 Genetics of yeast glucokinase. Genetics **105:** 501–515.

MCCLELLAN, C. J., and L. F. BISSON, 1988 Glucose uptake in *Saccharomyces cerevisiae* grown under anaerobic conditions: effect of null mutation in the hexokinase and glucokinase structural genes. J. Bacteriol. **170:** 5396–5400.

MICHELS, C. A., K. M. HAHNENBERGER and Y. SYLVESTRE, 1983 Pleiotropic mutations regulating resistance to glucose repression in *Saccharomyces carlsbergensis* are allelic to the structural gene for hexokinase B. J. Bacteriol. **153:** 574–578.

MURATSUBAKI, H., and T. KATSUME, 1979 Distribution of hexokinase isoenzymes depending on a carbon source in *Saccharomyces cerevisiae.* Biochem. Biophys. Res. Commun. **86:** 1030–1036.

ROGNSTAD, R., D. G. CLARK and J. KATZ, 1973 Relationship between isotopic reversibility and futile cycles in isolated rat

liver parenchymal cells. Biochem. Biophys. Res. Commun. **54:** 1149–1156.

SEDIVY, J. M., and D. G. FRAENKEL, 1985 Fructose bisphosphatase of *Saccharomyces cerevisiae*. Cloning, disruption and regulation of the *FBP1* structural gene. J. Mol. Biol. **186:** 307–319.

STACHELEK, C., J. STACHELEK, J. SWAN, D. BOTSTEIN and W. KONIGSBERG, 1986 Identification, cloning, and sequence determination of the genes specifying hexokinases A and B from yeast. Nucleic Acid Res. **14:** 945–963.

VOJTEK, A. B., 1988 Ph.D. thesis, Harvard University.

VOJTEK, A. B., and D. G. FRAENKEL, 1990 Phosphorylation of yeast hexokinase. Eur. J. Biochem. **190:** 371–375.

WALSH, R. B., G. KAWASAKI and D. G. FRAENKEL, 1983 Cloning of genes that complement yeast hexokinas and glucokinase mutants. J. Bacteriol. **154:** 1002–1004.

Communicating editor: M. CARLSON