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The Residual Enzymatic Phosphorylation Activity of Hexokinase II Mutants Is Correlated with Glucose Repression in *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae **mutants containing different point mutations in the *HXK2* gene were used to study the relationship between phosphorylation by hexokinase II and glucose repression in yeast cells. Mutants showing different levels of hexokinase activity were examined for the degree of glucose repression as indicated by the levels of invertase activity. The levels of hexokinase activity and invertase activity showed a strong inverse correlation, with a few exceptions attributable to very unstable hexokinase II proteins. The in vivo hexokinase II activity was determined by measuring growth rates, using fructose as a carbon source. This in vivo hexokinase II activity was similarly inversely correlated with invertase activity. Several *hxk2* alleles were transferred to multicopy plasmids to study the effects of increasing the amounts of mutant proteins. The cells that contained the multicopy plasmids exhibited less invertase and more hexokinase activity, further strengthening the correlation. These results strongly support the hypothesis that the phosphorylation activity of hexokinase II is correlated with glucose repression.**

Hexokinase II is one of the two hexokinase isoenzymes of the budding yeast *Saccharomyces cerevisiae*. It functions in vivo both in glycolysis (16, 17) and in catabolite repression (6, 20). Among the mutations that cause failures in glucose repression (25), some have reduced levels of hexokinase activity; these mutations were determined to be alleles of the structural gene of hexokinase II, *HXK2* (6). Biochemical analysis of one of the mutants (10) indicated that hexokinase II was indeed defective. The synthesis of invertase from the *SUC3* gene (reviewed in references 3 and 4) was derepressed in these mutants even when cells were grown in medium with a high glucose concentration. These results suggest that hexokinase II mediates glucose repression in yeast cells. Mutations in another gene, *HEX2* (which may be the same as *REG1* [22]), also cause failures in glucose repression, but *hex2* strains have levels of hexokinase II activity higher than the wild-type level (7).

It is difficult to interpret these results because mutations that either reduce (*hxk2*) or increase (*hex2*) hexokinase II catalytic activity can cause failures of glucose repression. To determine whether hexokinase II protein is required for glucose repression or derepression, we constructed null mutations in the *HXK1* (encoding hexokinase I) and *HXK2* genes and studied their effects on glucose repression (20). The result that *hxk2* null mutants do not show glucose repression indicates that hexokinase II is indeed required for glucose repression. Although hexokinase I is normally not required, it can partially suppress the *hxk2* defect in glucose repression if overproduced.

Entian and Frohlich (8) isolated mutations in the *HXK2* gene that cause defects in glucose repression yet still retain hexose phosphorylation activity. On the basis of these results and the conformational change observed in yeast hexokinases upon binding glucose, it was proposed that hexokinase II has two domains, one for catalytic phosphorylation of hexoses and the other for glucose repression (8, 9). The nature of the regulatory domain was not specified; however, three possible mechanisms were proposed (9): (i) hexokinase II bound to glucose has a new conformation and can now phosphorylate a protein (peptide) or a metabolite, which in turn carries the signal for glucose repression; (ii) the conformational change upon binding of glucose by hexokinase II releases some regulatory protein that was associated with hexokinase II; and (iii) hexokinase II can be cleaved at certain positions without affecting catalytic activity, and the conformational change releases from hexokinase II a peptide that is regulatory. All of these hypotheses depend on a conformational change of hexokinase II upon glucose binding. These researchers also proposed another site(s) on the hexokinase II that is required for glucose repression but not for phosphorylation. These hypotheses, however, do not directly address the role of phosphorylation at the normal catalytic site in glucose repression.

We have been interested in determining whether the normal ATP-linked hexose phosphorylation catalytic activity of hexokinase II is required for glucose repression. During preliminary analysis of the phenotypes of *hxk2* mutants, we noticed that mutants that failed to grow on fructose (such as hexokinase I and II double-null mutants) produced high levels of invertase activity, whereas those that grew well on fructose produced relatively lower levels of invertase (19). Since growth on fructose requires a catalytically functional hexokinase, this behavior led us to hypothesize that the catalytic activity of hexokinase II is required for glucose repression. Here we present the results of testing our hypothesis with a series of hexokinase II mutants. The mutants were chosen from a larger number of ones that were generated by random mutagenesis and isolated on the basis of phenotype (19), and they were selected

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TABLE 1. Construction of plasmids by recombination in yeast cells

New plasmid[a]	*hxk2* allele	Linearized plasmid[b]
pRB1155	*517*	pRB517
pRB1156	*035*	pRB946
pRB1157	*090*	pRB983
pRB1158	*098*	pRB991
pRB1159	*119*	pRB1012
pRB1160	*192*	pRB1049
pRB1161	*195*	pRB1052
pRB1162	*196*	pRB1053
pRB1163	*201*	pRB1058
pRB1164	*217*	pRB1074
pRB1165	*218*	pRB1075
pRB1166	*224*	pRB1081

[a] Each is a new YEp420 (22) derivative constructed by homologous recombination in yeast cells. Each linearized centromere plasmid was used with a fragment generated from YEp420 by restriction digestion with *Eco*RV, *Pst*I, and *Sma*I to transform a *ura3 hxk1 hxk2* strain, with Ura$^+$ selection.

[b] Centromere plasmid containing the corresponding *hxk2* allele, linearized by digestion with *Bgl*II and *Xho*I. The *hxk2* alleles are described elsewhere (19).

for this study because they are capable of growing on fructose to various extents.

MATERIALS AND METHODS

Strains and plasmids. The yeast strains used are isogenic to S288C (*MAT***a** *SUC2 gal2*). Unless otherwise indicated, they all have chromosomal null alleles of *HXK1* and *HXK2*, *hxk1*::*LEU2* and *hxk2-202* (20). Individual strains contain different plasmids carrying either the *HXK2* allele or different *hxk2* alleles. The *hxk2* mutants were isolated among the transformants of a *hxk1 hxk2* double-null strain with randomly mutagenized yeast centromere plasmids (YCp50 derivatives) carrying the *HXK2* gene. The plasmids were mutagenized by one of the following methods: (i) passage through an *Escherichia coli mutD* host or (ii) in vitro mutagenesis of misincorporation by omission, which involves repairing small single-stranded gaps in the absence of one of the four nucleoside triphosphates, forcing misincorporation. The mutants were isolated on the basis of their phenotypes in one or more screens. Details of the mutagenesis and mutant isolation are described in the accompanying paper (19). Daphne Preuss very kindly provided strain DBY2617 (*MAT***a** *ura3-52 lys2-801 his4-539 suc2-438*); the secretion-defective *suc2-438* (14) allele was crossed into *hxk1*::*LEU2* HXK2 and *hxk1*::*LEU2 hxk2-202* backgrounds.

Growth media, transformation, and genetic methods. The growth media and genetic manipulations used have been described previously (24). Yeast cells were transformed by using a modification (T. Stearns, H. Ma, and D. Botstein, Methods Enzymol., in press) of the alkali cation method (13, 15). Cell density was measured either by Klett colorimeter (Klett Manufacturing Co. Inc., New York, N.Y.) reading or by determination of optical density at 600 nm (OD_{600}).

Construction of plasmids by in vivo methods. A number of plasmids were constructed in vivo by using yeast homologous recombination as described previously (21). A set of YCp50 derivatives (19) bearing wild-type and mutant *HXK2* alleles was converted to YEp420 (21) derivatives by replacing the *CEN4 ARS1* sequences with a fragment containing the origin of the 2μm circle (Table 1). Plasmid pRB702 was constructed in a similar fashion from linearized pRB701 (a pSI4 [2] derivative; 19) and a 3.7-kilobase-pair *Eco*RI fragment of the *HXK2* gene (21).

Enzyme assays and immunofluorescence. To determine the levels of hexokinase II and invertase, cells were grown in medium containing 5% glucose, fructose, or mannose and harvested in the mid-exponential phase (OD_{600} of 1.0 to 2.0). Cells were harvested, chilled on ice, and washed with 50 mM NaN_3 to stop metabolism. A portion of cells from each strain was used to determine invertase activity, and another portion was used to prepare crude protein extracts for determining hexokinase activity. Invertase activity was measured on whole cells as described by Goldstein and Lampen (12). Hexokinase activity was assayed in crude extracts and purified enzymes essentially as previously described (1), with slight modifications of volumes and concentrations of reagents. Each reaction mix contained the following: 500 μl of 50 mM triethanolamine (pH 7.6), 500 μl of 100-mg/ml sugar in 50 mM triethanolamine (pH 7.6), 40 μl of 10-mg/ml ATP (sodium salt), 40 μl of 10-mg/ml NADP (sodium salt), 5 μl of 1 M $MgCl_2$; and 3 μl of 1-mg/ml glucose-6-phosphate dehydrogenase. For fructose as a substrate, 3 μl of 2-mg/ml phosphoglucose isomerase was also included. Protein content was determined by the method of Lowry et al. (18). Immunofluorescence of yeast cells was determined as described by C. A. Kaiser and D. Botstein (manuscript in preparation).

RESULTS

Hexokinase and invertase activities. To determine the relationship between catalytic phosphate transfer to hexose and catabolite repression, invertase (encoded by the *SUC2* gene) activity and hexokinase activity were determined for strains representing the range of fructose growth between the levels of wild type and null mutants (Table 2; Fig. 1). With a few exceptions, hexokinase II and invertase activities showed a strong inverse correlation. An inverse correlation is expected if increasing the ATP-linked hexose phosphorylation improves the efficiency of glucose repression, since invertase levels decrease under repressing conditions. The correlation broke down (boxed region in Fig. 1) for mutants that had low levels of hexokinase activity in vitro (Table 3) which are incompatible with the rates of growth on fructose. The cells with alleles *hxk2-090*, *-119*, *-192*, and *-195* could grow on fructose at rates not much slower than the wild-type rate. Anomalously, their extracts had hexokinase activities even lower than those of mutants that grew more slowly on fructose (*hxk2-088*, and *-098*).

The anomalous alleles were transferred to 2μm plasmids in order to obtain enough hexokinase II protein to examine the deviation from the inverse correlation more thoroughly. In addition, the protease inhibitor phenylmethylsulfonyl fluoride was included in the buffer used during cell lysis. As a result, much more activity was detected (Table 3) for three of the four mutant alleles. The hexokinase activity in these extracts was still unstable, however, as indicated by the fact that after storage at −20°C for several days, the same extracts showed lower levels of hexokinase activity (data not shown). It is also likely that the hexokinase activity declined during preparation of the extracts and throughout the time that the extracts were stored on ice before and after assaying.

To investigate the instability further, the extracts of wild type and one (*hxk2-195*) of the mutants were incubated at room temperature for several hours, after which time hexokinase activity was determined. The hexokinase activity in the wild-type extract was almost constant, whereas that of the mutant YEp420 derivative with *hxk2-195* exhibited a

TABLE 2. Hexokinase and invertase activities of yeast strains with *HXK2* or *hxk2* alleles

Plasmid[a]	Plasmid-borne *hxk2* allele	Hexokinase activity (U)[b]	1/doubling time (h^{-1})[c]	Invertase activity (U)[d]	Amino acid alteration(s)[e]
pRB312	Wild type	3.0	0.57	2.9	
pRB1079	*222*	2.6	ND[f]	1.4	ND
pRB997	*104*	0.69	0.53	2.5	ND
pRB1008	*115*	0.06[g]	0.37	6.6	Gly-55→Asp
pRB983	*090*	0.05[g]	0.52	8.5	Leu-216→Ser
pRB1049	*192*	0.06[g]	0.50	9.3	Glu-193→Pro
pRB1052	*195*	0.88	0.46	11	Gln-163→Arg
pRB988	*095*	0.29[g]	ND	12	Glu-78→Cys
pRB982	*089*	0.04[g]	0.54	12	Ser-305→Pro
pRB1000	*107*	0.06[g]	ND	12	Ser-305→Pro
pRB1012	*119*	0.06[g]	0.48	13	Ser-305→Pro
pRB978	*085*	1.05	0.49	14	ND
pRB1080	*223*	0.12[g]	0.42	16	ND
pRB950	*040*	0.67	ND	42	Phe-178→Ile
pRB947	*036*	0.42	0.53	51	ND
pRB1053	*196*	0.35	0.28	53	Val-94→Ala, Leu-99→Trp
pRB1075	*218*	0.32	0.37	55	Asp-286→His
pRB1074	*217*	0.31	0.27	64	Ala-239→Val
pRB981	*088*	0.42	0.44	65	Gln-376→Glu, Arg-391→Gly
pRB1076	*219*	0.35	0.42	81	ND
pRB1058	*201*	0.32	0.35	100	Tyr-220→His, Met-228→Lys
pRB1059	*402*	0.23	0.29	100	ND
pRB1081	*224*	0.09	0.22	110	Ala-239→Val
pRB991	*098*	0.16	0.24	210	Thr-90→Ile
YCp50	None	0.05	0.01	280	

[a] Cells are DBY2212 (*hxk1::LEU2 hxk2-202*) harboring one of these centomere plasmids.
[b] Crude extract from cells grown in medium containing glucose was measured for hexokinase activity, using fructose as the substrate. One unit = 1 μmol of fructose phosphorylated min^{-1} (mg of total protein)$^{-1}$.
[c] Cell growth in medium with 5% fructose was monitored by measuring turbidity with a Klett meter.
[d] Cells were grown on 5% glucose, and invertase assays were done on whole cells. One unit = 1 μg of glucose produced min^{-1} (ml of cells at 1 OD_{600} unit)$^{-1}$.
[e] Changes already determined (20).
[f] ND, Not determined.
[g] Value is so low because of the extreme sensitivity of the altered hexokinase II to protease degradation; see text and Tables 3 and 4.

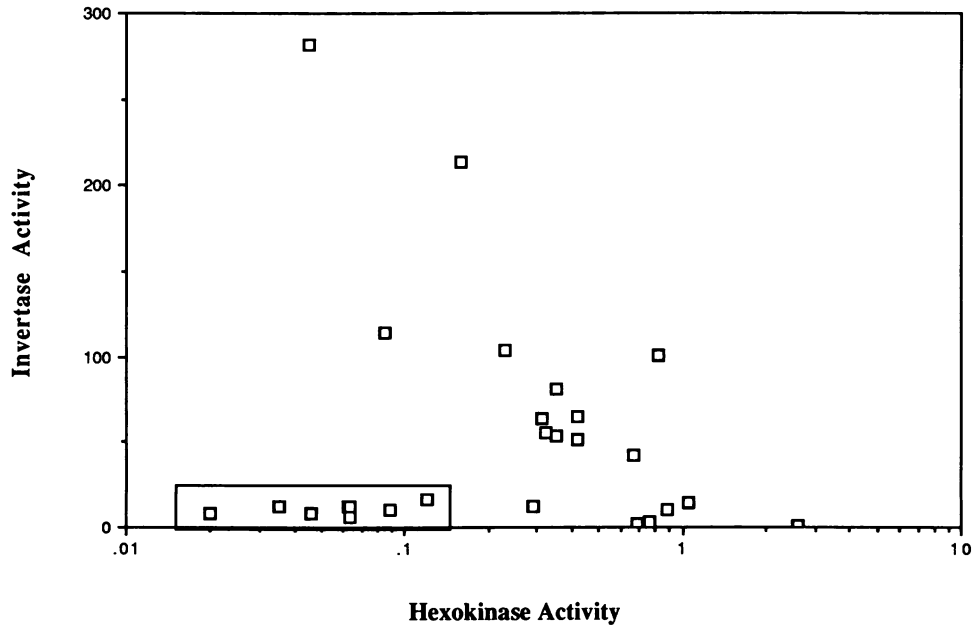

FIG. 1. Correlation of hexokinase and invertase activities for *hxk2* mutants. Hexokinase activity was determined by using fructose as a substrate; 1 = 1 μmol of fructose phosphorylated U min^{-1} (mg of total protein)$^{-1}$. Invertase activity was determined as described in Materials and Methods; 1 = 1 μg of glucose produced U min^{-1} (1 ml of cells at OD_{600} of 1)$^{-1}$. The boxed points were from mutants producing extremely unstable proteins; see text and Tables 3 and 4.

TABLE 3. Inconsistency of in vivo and in vitro hexokinase activities for some hexokinase II mutants

hxk2 allele[a]	Doubling time (min) on fructose[b]	Hexokinase activity (U)[c]	
		CEN[d]	2μm[e]
Wild type	105	2.25	27
090	115	0.05	6
119	126	0.06	1.2
192	121	0.13	0.25
195	132	0.16	17
088	138	0.42	
098	251	0.16	
202	No growth	0.01	

[a] Allele on plasmids; the chromosomes have *hxk1* and *hxk2* null alleles.
[b] Cells carrying centromere plasmids were grown in SD medium with Casamino Acids (Difco Laboratories) and fructose instead of glucose.
[c] Crude extracts from cells grown in medium containing glucose were measured for hexokinase activity, using fructose as the substrate. One unit = 1 μmol of fructose phosphorylated min^{-1} (mg of total protein)$^{-1}$.
[d] Extracts were from cells with centromere plasmids, and the cell lysis buffer did not include phenylmethylsulfonyl fluoride.
[e] Extracts were from cells with YEp420 derivatives, and phenylmethylsulfonyl fluoride was included in the cell lysis buffer.

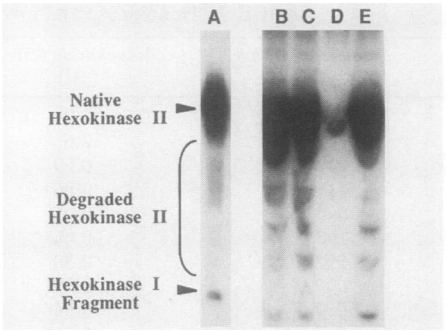

FIG. 2. Autoradiogram of Western blot on extracts from wild type and mutants with unstable hexokinase II. Lanes: A, wild type; B, *hxk2-090*; C, *hxk2-119*; D, *hxk2-192*; E, *hxk2-195*. The extracts were used soon after they were obtained; hexokinase activities are shown in Table 3.

continuing decline of hexokinase activity during the incubation at room temperature (Table 4). When equal volumes of the two extracts were mixed and then incubated at room temperature for 30 h, the hexokinase activity did not decline more than for the wild-type extract alone (data not shown). This result rules out the possibility that the mutant extract contained an inhibitor of hexokinase function or had higher protease levels. Western blot (immunoblot) analysis of the mutant extracts indicated that hexokinase was degraded (Fig. 2). It is not clear, however, how much of the proteolysis occurred before cell lysis. We conclude that hexokinase II activity and invertase activity show a strong inverse correlation. Deviations from the correlation arise largely because the mutant proteins are unstable in vitro.

Growth rates on fructose and invertase levels. Because glucose repression is an in vivo phenomenon, we wanted to find an in vivo measure of hexokinase II catalytic activity for the mutants. In these cells, because of the absence of chromosomal hexokinases, the only hexokinase is encoded by the plasmid. Since fructose utilization requires hexokinase function, the growth rate on fructose should reflect the in vivo catalytic function of the altered hexokinase II proteins. Strains with plasmids bearing different alleles of *HXK2*, including wild-type and null alleles, were grown in fructose-containing medium. Cell density was monitored by measuring Klett meter units, and the doubling times of these strains were determined. The growth rates, expressed as the inverse of doubling time, were plotted against invertase activity from glucose-grown cells of each mutant (Table 2; Fig. 3). These results showed an inverse correlation between growth rate on fructose and level of glucose repression, supporting the hypothesis that the catalytic activity of hexokinase II is related to its function in glucose repression.

TABLE 4. Effect of copy number on hexokinase II and invertase activities

hxk2 allele[a]	Activity (U)				Amino acid alteration
	Hexokinase[b]		Invertase[c]		
	CEN[d]	2μm[e]	CEN	2μm	
Wild type	2.6	27	2.3	8.6	
217	0.38	2.54	55	20	Ala-239→Thr
224	0.12	1.46	67	19	Ala-239→Val
196	0.33	1.68	67	24	Val-94→Ala
218	0.54	2.89	71	28	Asp-286→Leu
201	0.27	1.47	135	34	Tyr-220→His
098	0.06	0.48	146	50	Thr-90→Ile
517	0.05	0.03	144	108	Ser-306→Phe
202	0.01	0.02	162	120	

[a] Alleles on plasmids (see Table 1); the chromosomal alleles were *hxk1*::*LEU2* and *hxk2-202*.
[b] Cells were grown on 5% glucose, and activity was determined with fructose as the substrate. One unit = 1 μmol of fructose phosphorylated min^{-1} (mg of total protein)$^{-1}$.
[c] Cells were grown on 5% glucose. One unit = 1 μg of glucose produced min^{-1} (ml of cells at 1 OD_{600} unit)$^{-1}$.
[d] Measured in cells containing a low-copy-number centromere plasmid.
[e] Measured in cells containing a multicopy 2μm plasmid.

Multicopy plasmids containing *hxk2* mutant alleles. Although the inverse correlations between hexokinase and invertase activities (Fig. 1) or between growth on fructose and invertase activity (Fig. 3) over 2 to 3 orders of magnitude suggest the involvement of hexokinase II phosphorylation activity in glucose repression, it could still be argued that the mutations cause, in addition to defects in the phosphorylation of fructose, defects in another activity of hexokinase II that is independent of the amount of the hexokinase II protein and controls catabolite repression. To test further whether the degree of glucose repression is linked to the level of catalytic phosphorylation activity, we examined the effect of copy number on the mutants. We transferred several mutant alleles exhibiting different levels of growth on fructose from their original low-copy-number centromere plasmids to multicopy 2μm plasmids by homologous recombination in yeast cells (Materials and Methods; 21). Strains containing these plasmids were then analyzed in conjunction with the strains containing the original low-copy-number plasmids. Simply increasing the copy number of a mutant allele could result in more glucose repression (Table 4; Fig. 4). This result further strengthens the argument that catalytic activity and glucose repression are closely associated: the higher the catalytic activity, the more the repression, as reflected by lower invertase levels.

For the wild-type protein or one of a few very active mutant forms of hexokinase II, the cells with multicopy plasmids had more invertase than did the cells with centromere plasmids (Table 5). This result seemed to be contra-

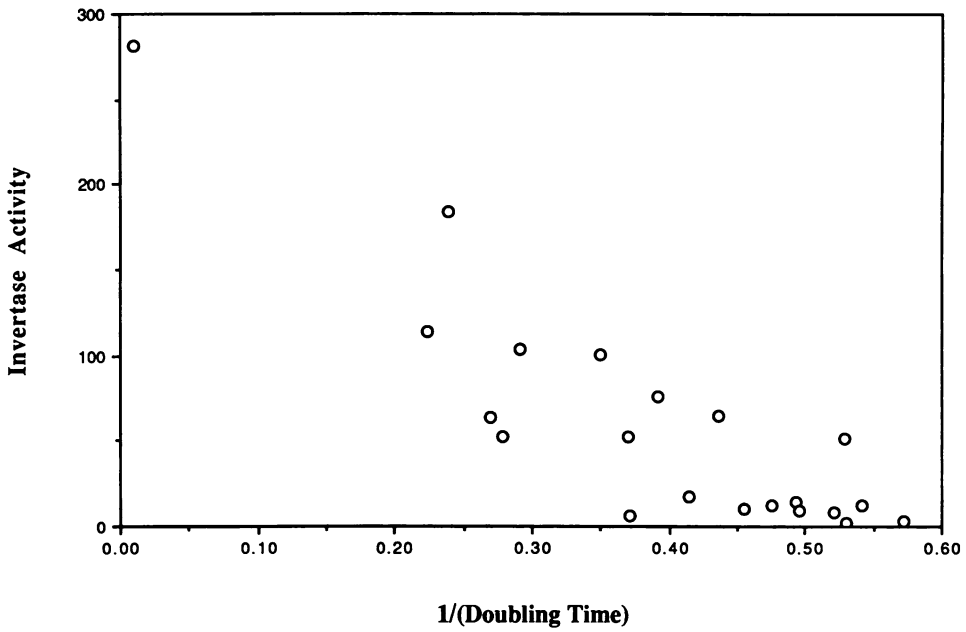

FIG. 3. Correlation of growth rate on fructose and invertase activity for *hxk2* mutants. Growth rate is expressed in hours. The invertase activity was determined as described in Materials and Methods; 1 U = 1 μg of glucose produced min^{-1} (ml of cells at OD_{600} of 1)$^{-1}$.

dictory to the correlation, but the deviation proved to be due to plasmid instability. The slight increase in invertase activity for the strains with 2μm plasmids was due to the presence of a small fraction of cells that had lost the plasmids and produced large amounts of invertase, as shown by the following experiment. A *hxk1 hxk2 ura3* strain with *suc2-438*, an invertase allele lacking a functional signal sequence (14), was constructed and transformed with plasmids containing different alleles of *HXK2*. In addition, an isogenic *hxk1 HXK2 ura3 suc2-438* strain was also transformed with YEp420. These transformants were grown in medium containing 5% glucose, and cells were examined by immunofluorescence with anti-invertase serum. A small percentage of cells from strains with 2μm plasmids carrying a wild-type allele or one of the active mutant alleles of *HXK2* had bright invertase staining, whereas strains with *HXK2* on a centromere plasmid or the chromosome had a 1% or lower detection limit of staining, respectively (Table 5). As controls, most cells without hexokinase II or those with inactive enzymes were stained (Table 5), and all cells were stained with anti-actin serum (data not shown). The values of invertase levels estimated from the percentage of stained

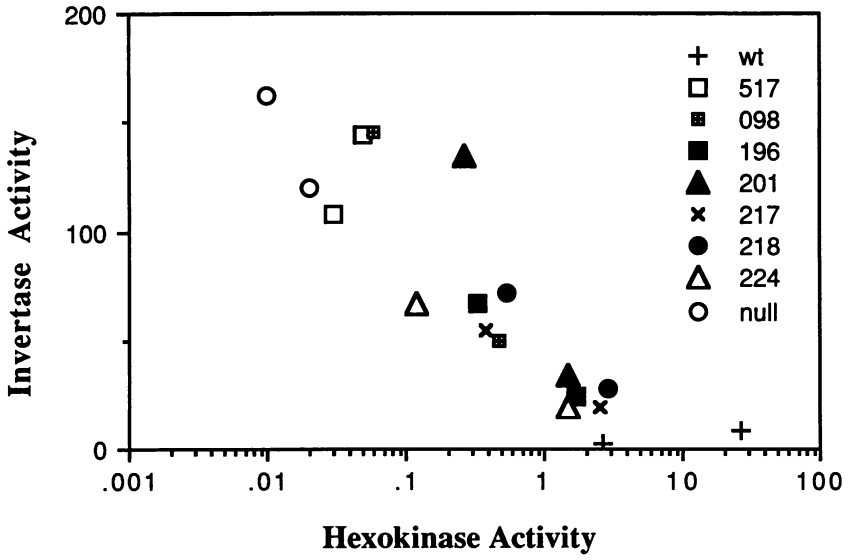

FIG. 4. Correlation of hexokinase and invertase activities for strains with *HXK2* alleles on either centromere plasmids or 2μm plasmids. Hexokinase activity was determined by using fructose as a substrate; 1 U = 1 μmol of fructose phosphorylated min^{-1} (mg of total protein)$^{-1}$. Invertase activity was determined as described in Materials and Methods; 1 U = 1 μg of glucose produced min^{-1} (ml of cells at OD_{600} of 1)$^{-1}$. Numbers in key to symbols are allele numbers. wt, Wild type.

TABLE 5. Invertase activity and immunofluorescence with anti-invertase serum for cells with wild-type or active mutant hexokinase II

hxk2 allele[a]	Invertase activity (U)[b]			% of cells stained with anti-invertase[c]		
	CEN[d]	2μm[e]	Calculated[f]	CEN	2μm	Chrom[g]
Wild type	2.3	8.6	12	1	10	0
090	5.7	24	22		19	
119	7.9	19	20		17	
195	8.6	20	14		12	
517	144	108	115		96	
202	162	120	114		95	

[a] Cells containing the alleles on plasmids were grown on medium with 5% glucose.

[b] Determined on mid-exponential-phase cells. One unit = 1 μg of glucose produced min^{-1} (ml of cells at 1 OD_{600} unit)$^{-1}$.

[c] Percentage stained of total cells stained with 4',6'-diamidino-2-phenylindole.

[d] Cells contained YCp50 derivatives.

[e] Cells contained YEp420 derivatives.

[f] Calculated as follows: 120 (value of null) × value of percentage of cells stained with anti-invertase serum.

[g] *HXK2* was on the chromosome.

cells, assuming that the stained cells produced as much as did hexokinase null mutants, agree well with the observed results.

DISCUSSION

Although hexokinase II is known to be required for glucose-mediated catabolite repression in yeast cells, the mechanism of its involvement is not understood. Because hexokinase catalyzes the first reaction in glycolysis, it is reasonable to think that its role in glucose repression is in some way to signal the presence of high levels of glucose. Entian and colleagues proposed (9) three related schemes by which hexokinase II might generate such a signal. The differences between the models concern the nature of the signal messenger and the precise mechanism of its generation. Prominent in the proposals was the concept of a regulatory domain of hexokinase II distinct from the catalytic domain. These hypotheses did not directly address the possible role of catalytic phosphorylation by hexokinase in glucose repression. Our results in fact show a strong correlation between the phosphorylation activity of hexokinase II when fructose is used as a substrate and the level of glucose repression in the cells. The inverse correlation was obtained from the study of several mutations in the yeast *HXK2* gene that lead to a range of residual values of V_{max} catalytic activity for hexose phosphorylation. Use of these mutant *hxk2* genes in an *hxk1 hxk2* double-null background permits precise control of hexokinase activity levels in the cell. The *SUC2*-encoded invertase activity is measured as a prototypic catabolite repression enzyme. The strong inverse correlation between catalytic activity and invertase activity suggests that the catalytic activity of hexokinase II is closely associated with glucose repression, possibly with the signaling process. For example, hexokinase II might be involved in the generation of a phosphorylated metabolite. Hexokinase is known not to undergo autophosphorylation in normal catalysis but will inactivate itself by autophosphorylation in the presence of certain pentoses, such as xylose (5, 11, 23).

All mutants that completely failed to grow on fructose also lacked glucose repression. Although we have isolated many mutants of this type, only one (*hxk2-098*) is shown in Fig. 1 and 3 and only two (*hxk2-098* and *hxk2-517*) are shown in Fig. 4. The failure to isolate any mutants that lack catalytic activity yet retain glucose repression supports, but cannot prove, the hypothesis that catalytic activity is correlated with glucose repression. Entian and colleagues argued for the presence of two domains in hexokinase II on the basis of the isolation of mutants that have defects in glucose repression yet retain high levels of catalytic activity (8, 9). Although the precise natures of their mutants are not known, they could be similar to some of our mutants (*hxk2-036*, *hxk2-088*, and *hxk2-219*), which have intermediate levels of activity in both glucose repression and catalysis. It is worth noting that other factors, such as strain background, type of plasmid, and genes coding for invertase, that differ between our study and that of Entian and colleagues may contribute to some of the differences in the results. Although our results strongly support the involvement of catalytic site in glucose repression, they do not eliminate the possibility of a second site that is required for repression but not for catalysis. Nevertheless, the simplest proposal is that there is no separate regulatory domain in yeast hexokinase II and that the catalytic activity is closely coupled to glucose repression. Subsequent studies may then focus on identification of a phosphorylation activity or a phosphorylated metabolite that acts as a signal molecule for repression.

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