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Glucose Sensing and Signalling Properties in *Saccharomyces cerevisiae* Require the Presence of at Least Two Members of the Glucose Transporter Family†

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The kinetics of glucose transport in a number of different mutants of *Saccharomyces cerevisiae* with multiple deletions in the glucose transporter gene family were determined. The deletions led to differences in maximal rate and affinity for glucose uptake by the cells, dependent on the growth conditions. At the same time, there were changes in glucose repression, as determined by expression of invertase activity. Only in the strain with genes *HXT1-4* and *SNF3* deleted but carrying *HXT6/7* were glucose uptake kinetics and invertase activity independent of the presence or concentration of glucose in the growth medium. Some degree of glucose sensitivity was recovered if the *SNF3* or *HXT2* gene was present in the multiple-deletion background. It is hypothesized that during growth on glucose, both modulation of the kinetics of glucose uptake and derepression of invertase activity require the presence of more than one active gene of the glucose transporter family.

Saccharomyces cerevisiae cells growing on glucose in batch culture exhibit an increase in affinity for glucose as the glucose in the medium is consumed, while the maximal rate of glucose transport under such conditions is constant (16). When cells growing on a high concentration of glucose (2%) are transferred to a medium containing a low concentration of glucose (0.1%), however, a similar increase in affinity for glucose is observed, but the affinity change is accompanied by an increase in the maximal rate of glucose uptake (2). This complex behavior is most likely the result of a number of factors, including modulation in expression of genes.

A variety of gene products have been implicated as playing some role in the transport of glucose into cells of *S. cerevisiae*. On the basis of kinetic analyses of mutants and sequence similarity to sugar transporters of other organisms, a homologous gene family in *S. cerevisiae* has been identified, and these genes are thought to code for hexose transporter proteins (HXT proteins). Thus far, 10 members of this gene family have been identified, and the existence of still more cannot be ruled out (14). A strain in which the genes *HXT1* to *HXT7* have been deleted does not transport (or grow on) glucose, fructose, or mannose. In this background, expression of any of the genes *HXT1*, *HXT2*, *HXT3*, *HXT4*, *HXT6*, and *HXT7* was sufficient to complement the glucose transport (and growth) defect (13). Therefore, it seems that only the products of these six genes are involved in the transport of glucose per se, at least under the conditions examined so far. The role of the other *HXT* gene products remains elusive. It has been suggested that under extreme conditions some other *HXT* genes may be expressed or that their expression is controlled by the metabolically relevant *HXT* transporters previously identified by functional complementation (13).

Recently, data on the kinetic characteristics of strains expressing each of the functional *HXT* genes individually in the *HXT* null background have started to become available (14). Some of these data seem to contradict the existing data as to the affinity for glucose of the various *HXT* gene products. For example, in a strain expressing only *HXT1*, the K_m for glucose transport was between 50 and 100 mM (14); however, deletion of *HXT1* in wild-type cells clearly caused a reduction in a high-affinity component of the glucose transport system (9). Furthermore, this reduction in high-affinity glucose transport was accompanied by a loss of high-affinity mannose transport, but no effect on fructose transport was observed (9). No such substrate specificity differences have been reported for the strains expressing individual *HXT* genes.

Similarly, a strain expressing only *HXT2* has a high affinity for glucose transport (14), but in a mutant with a deletion of *HXT2* in a wild-type background, both high- and low-affinity components were reduced compared with the wild-type levels (8).

A homologous gene that has also been implicated in glucose transport in *S. cerevisiae* is *SNF3*. On the basis of the kinetics of glucose transport in a deletion mutant, it was thought that the SNF3 gene product coded for a high-affinity glucose transporter (3), but no glucose transport has been found in a *HXT* null strain in which the *SNF3* gene is present (13).

Clearly, the kinetics of the wild-type glucose transport system are more complex than merely the sum of the kinetics of the component parts of the glucose transport system, as seen in strains that express single *HXT* genes. In such strains, interactions between gene products or between gene products and genes may have been lost. Indeed, all the *HXT* genes that have thus far been postulated to code for glucose transporters may also play a regulatory role in glucose metabolism. Both the protein coding regions and, perhaps more surprisingly, the upstream regions of at least some of the *HXT* genes, in multicopy plasmids, have been shown to suppress growth and glucose transport defects in *HTR1*, *grr1*, and *snf3* mutants (10, 12). Furthermore, similar multicopy suppression of glucose fermentative and catabolite repression defects by *HXT* sequences in *DGT1* mutants has also been reported (6).

In this paper, we report on the characteristics of glucose uptake in a number of mutants of *S. cerevisiae* in which various

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† This paper is dedicated to the memory of Wilko Kos.

TABLE 1. Growth parameters of the wild type and mutants during batch growth on various substrates[a]

Strain[b]	μ_{glu}[c]	Y_{glu}[d]	V_{glu}[e]	μ_{gal}[f]	μ_{gly}[g]
R757 (wild type)	0.36	0.046	7.9	0.30	0.23
CY287 *SNF3 HXT2 HXT6/7*	0.37	0.050	7.4	0.14	0.16
CY290 *SNF3 HXT6/7*	0.13	0.10	1.3	0.16	0.14
CY292 *HXT2 HXT6/7*	0.37	0.038	9.7	0.15	0.14
CY294 *HXT6/7*	0.29	0.051	5.6	0.16	0.16

[a] Data are the means of at least three experiments.
[b] See Materials and Methods. The notation here refers to the known functional glucose transporters which are present as well as *SNF3*. Thus, e.g., CY294 is *snf3*Δ *hxt1*Δ *hxt2*Δ *hxt3*Δ *hxt4*Δ *HXT6/7*, etc.
[c] μ_{glu}, maximum growth rate on 2% glucose (per hour).
[d] Y_{glu}, growth yield (A_{600} units per millimolar glucose used), calculated from the linear portions of plots of decrease in glucose concentration versus A_{600} of the cultures.
[e] V_{glu}, glucose consumption rate (millimolar glucose used per A_{600} unit per hour), obtained from the quotient of the previous two columns.
[f] μ_{gal}, maximum growth rate on 2% galactose (per hour).
[g] μ_{gly}, maximum growth rate on 2% glycerol (per hour).

genes related to glucose uptake (*HXT* and *SNF3*) have been deleted.

MATERIALS AND METHODS

Materials. D-[U-^{14}C]glucose was purchased from Amersham International, medium constituents were supplied by Difco, and other chemicals were purchased from Sigma Chemical Co. and were of reagent grade or better. Enzymes were purchased from Boehringer Mannheim.

Strains and growth conditions. The results presented in this paper have been obtained with a set of mutants of *S. cerevisiae* constructed and kindly supplied by R. Gaber (Evanston, Ill.). These mutants are isogenic, involve the presence of various combinations of the *SNF3* and *HXT2* genes in a *snf3* and *hxt1-4* multiple-deletion background, and have all been described previously (7). CY294 contains only the chimeric *HXT6/7* gene (*snf3*Δ *hxt1*Δ *hxt2*Δ *hxt3*Δ *hxt4*Δ *HXT6/7*), CY292 contains the chimeric *HXT6/7* and *HXT2* genes (*snf3*Δ *hxt1*Δ *HXT2 hxt3*Δ *hxt4*Δ *HXT6/7*), CY290 contains the chimeric *HXT6/7* gene and the *SNF3* gene (*SNF3 hxt1*Δ *hxt2*Δ *hxt3*Δ *hxt4*Δ *HXT6/7*), CY287 contains the chimeric *HXT6/7* and *HXT2* and *SNF3* genes (*SNF3 hxt1*Δ *HXT2 hxt3*Δ *hxt4*Δ *HXT6/7*), and R757 is wild type. The presence of one chimeric *HXT6/7* gene instead of two distinct genes, as first reported by Reifenberger and coworkers (13), in the multiple-deletion strain CY294 has been confirmed for all the mutant strains by PCR analysis (5a).

Cells were grown semiaerobically in a rotary shaker at 30°C in standard media containing either 2% glucose, 2% galactose, or 2% glycerol, 2% Bacto Peptone, and 1% yeast extract.

Growth and glucose consumption. Growth of cultures on 2% glucose originated from very small inocula (at least 10 doublings before the first measurement). Growth was monitored by measurement of the A_{600}, and the residual glucose in the medium was determined enzymatically, using hexokinase and glucose 6-phosphate dehydrogenase. Typically, a wild-type culture growing on 2% glucose has an A_{600} of approximately 4.0 at glucose exhaustion. Yields on glucose were determined from the linear portions of plots of residual glucose concentration versus A_{600}. The specific glucose consumption rate is the quotient of growth rate/yield (in millimolar glucose per A_{600} per hour).

Invertase measurement. A 1% (wet weight) cell suspension was permeabilized by treatment with 2.5% (vol/vol) isoamyl alcohol (3-methyl-1-butanol) followed by one freeze-thaw cycle in liquid nitrogen. We have found this procedure to be superior to toluene or toluene-ethanol treatment. The assay mixture consisted of 400 μl of cell suspension and 100 μl of 0.2 M sodium acetate (pH 4.7) containing 0.5 M sucrose. This mixture was incubated for 30 min at 30°C, and the reaction was terminated by adding 100 μl of the mixture to 100 μl of 0.5 M potassium phosphate (pH 7.0) and boiling the mixture for 3 min. Glucose was assayed with hexokinase and glucose 6-phosphate dehydrogenase. Blank measurements were included in assays in which the sucrose was not included but was added after termination.

Uptake kinetics. The initial rate of glucose uptake was assayed over 5 s as described previously (16, 17). The 5-s method was employed since in these mutants there is no limitation to be expected by hexose-kinase activity (15, 17). Where tested, 0.2-s uptake measurements (15, 17) yielded activities identical to those determined by 5-s measurements. Analysis of kinetic data has been described previously (16). Kinetic parameters were determined from the means of at least four experiments.

RESULTS AND DISCUSSION

In the original report (7) on the isolation of the *S. cerevisiae* mutants that were used in this study, their ability to transport glucose was assessed on the basis of the ability to grow on plates with a rich medium containing glucose. In all cases, antimycin A was included in the medium to inhibit respiration, so that oxidative growth on other substrates, such as amino acids, could not take place. A lack of growth under such conditions, however, is not necessarily indicative of a lack of glucose transport. In this study, growth (Table 1), invertase activity (Table 2), glucose transport kinetics during growth on glucose (Table 3), and glucose transport kinetics during growth on glycerol and subsequent transfer to high and low glucose concentrations (Table 4) have been compared for the four mutant strains and the wild-type strain. These properties can be best analyzed by pairwise comparison.

In mutant CY294, in which the *SNF3* and *HXT1-4* genes are

TABLE 2. Invertase activities of the wild type and mutants under various growth conditions

Strain[a]	Activity[b] in cells grown on:				
	2% glucose			2% galactose, exp	2% glycerol, exp
	exp[c]	gluc (−)[d]	stat[e]		
R757 (wild type)	68	227	233	362	237
CY287 *SNF3 HXT2 HXT6/7*	61	265	352	1,493	816
CY290 *SNF3 HXT6/7*	1,002	699	722	1,923	1,545
CY292 *HXT2 HXT6/7*	84	317	307	168	616
CY294 *HXT6/7*	552	509	590	286	194

[a] See Materials and Methods and Table 1, footnote *b*.
[b] Data are the means of at least three determinations and are expressed as nanomoles of glucose formed from sucrose per minute per milligram of protein.
[c] exp, activity determined in exponential-phase cells.
[d] gluc (−), activity determined at glucose exhaustion.
[e] stat, activity determined 12 h after glucose exhaustion.

TABLE 3. Kinetics of glucose transport of the wild type and mutants during batch growth on 2% glucose

Strain[a]	Exponential phase[b]		Glucose exhaustion[c]	
	K_m[d]	V_{max}[e]	K_m	V_{max}
R757 (wild type)	23	280	1.9	275
CY287 *SNF3 HXT2 HXT6/7*	6.9	145	1.9	145
CY290 *SNF3 HXT6/7*	4.8	38	4.2	46
CY292 *HXT2 HXT6/7*	13	560	3.1	580
CY294 *HXT6/7*	1.2	140	1.1	145

[a] See Materials and Methods and Table 1, footnote *b*.
[b] Cells harvested at early exponential phase.
[c] Cells harvested at glucose exhaustion.
[d] Expressed as millimolar.
[e] Expressed as nanomoles per minute per milligram of protein.

deleted, an almost wild-type growth rate on glucose was observed. This growth led to a higher yield and a lower glucose consumption rate than those of the wild type (Table 1). Invertase activity measurements (Table 2) indicated that this mutant is always derepressed during growth on glucose, which may explain the higher growth yield and the previously reported antimycin A sensitivity (7), since mitochondrial oxidative phosphorylation contributes to growth. The most striking effect in this strain was the absence of any modulation in the affinity of glucose uptake. During batch growth on glucose (Table 3) or transfer from glycerol to high or low glucose (Table 4), only high-affinity uptake was detected, with a relatively constant V_{max}. This is in contrast to the wild type, which exhibited essentially the same affinity modulation (Table 3) during growth on glucose as reported earlier (16) for other wild-type strains. In mutant CY294, uptake is catalyzed by the product of the chimeric *HXT6/7* gene, which is the only functional glucose transporter present in this strain, as reported by Reifenberger and coworkers (13). The product of this chimeric *HXT6/7* gene is active in this strain, because when the gene is deleted, growth and transport on glucose are completely abolished (5a).

When the *SNF3* gene is added to the multiple-deletion background, in strain CY290, poor growth on glucose was observed (Table 1), in keeping with a very low capacity for glucose uptake (Table 3). As reported by Ko and coworkers, this mutant cannot grow at all on glucose plates if antimycin A is present (7), suggesting that the growth depends on functional oxidative phosphorylation. It is possible that the very low rate of glucose uptake is compatible with the energy requirement for cell maintenance and growth only if the glucose in the medium is oxidized to a large extent, yielding ATP via oxidative phosphorylation. This is consistent with the fact that the cells are totally derepressed, as judged by the high activity of invertase under all conditions (Table 2). The glucose uptake in this mutant is also catalyzed by the chimeric *HXT6/7* gene, and the kinetic characteristics of glucose uptake during growth on glycerol for this mutant are similar to those for CY294 (Table 4). After transfer of cells to glucose-containing medium, however, the glucose transport capacity of CY290 is decreased. The glucose-dependent decrease in glucose transport in this strain does not seem to be concentration sensitive and is not likely to be a metabolic inactivation because 30 min after transfer to either high or low glucose, the cells still retained most of the glucose transport capacity. Six hours after transfer, however, the V_{max} for glucose transport had decreased to between 30 and 40 nmol·min^{-1}·mg of protein^{-1}. This suggests that the decrease in V_{max} is brought about by a combination of reduction in synthesis of new transporters and dilution of existing transporters as a result of growth of the cells. The affinity for glucose transport appears to be unchanged during the substrate transition. Thus, the addition of the *SNF3* gene to a background containing only one functional glucose transporter, namely, the *HXT6/7* gene, appears to confer some glucose sensitivity to the expression of this gene. It is possible, therefore, that the proposed involvement of *SNF3* in the regulation of *HXT2* can also be extended to other *HXT* gene products (18).

When the *HXT2* gene was added to the multiple-deletion strain, in CY292, containing two functional glucose transporters, *HXT2* and *HXT6/7*, the rate of growth on glucose was almost that of wild-type cells (Table 1). Repression of invertase activity by glucose was recovered (Table 2), and during diauxic growth on glucose, the affinity for glucose increased at a constant V_{max} (Table 3). In this comparison, the most notable effect of the presence of the *HXT2* gene was the increase in V_{max} of glucose uptake to almost double the wild-type value (Table 3). The magnitude of the affinity change during growth on glucose was somewhat less than in the wild type (K_m decreases from approximately 12 to 3 mM) and is probably limited by the presence of only two functional glucose transporter genes, *HXT2* and *HXT6/7*. The V_{max} of glucose uptake in glycerol-grown cells was much lower than that in glucose-grown cells, the difference being more pronounced than in wild-type cells (compare Tables 3 and 4). Apparently, the complete transition between growth on glycerol and growth on glucose is slower than the time scale of our measurements (several hours), because after 6 h in high-glucose medium, the V_{max} had reached only half the value for cells grown on glucose. Also, the K_m had not yet changed over this period. Strains CY292 and CY294 differ only by the presence of *HXT2*. The

TABLE 4. Kinetics of glucose transport in the wild type and mutants grown on glycerol and then shifted to high or low glucose

Strain[a]	After growth on glycerol only		After shift to low (0.2%) glucose				After shift to high (2%) glucose			
			0.5 h		6 h		0.5 h		6 h	
	K_m[b]	V_{max}[c]	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
R757 (wild type)	2.9	165	2.9/13[d]	160/90	1.6	280	2.9/13	165/90	25/2.4	220/70
CY287 *SNF3 HXT2 HXT6/7*	3.0	190	3.4	220	3.1	225	2.3	80	3.1	63
CY290 *SNF3 HXT6/7*	2.0	110	2.2	90	2.2	40	2.0	100	1.8	25
CY292 *HXT2 HXT6/7*	3.0	65	3.5	60	2.5	60	3.1/16	65/60	3.3	180
CY294 *HXT6/7*	1.4	150	1.5	135	1.2	125	1.4	150	1.3	150

[a] See Materials and Methods and Table 1, footnote *b*.
[b] K_m expressed as millimolar.
[c] V_{max} expressed as nanomoles per minute per milligram of protein.
[d] Where the data fitted more accurately to two transport components, the kinetic parameters of both components are given.

large increase in V_{max} of glucose uptake is compatible with a role for *HXT2* as a glucose transporter. The fact that the presence of *HXT2* also restores affinity modulation and repression of invertase suggests that in combination with its transport function, *HXT2* may also have a role in glucose sensing and repression. Whether this effect is specific to *HXT2* or could be substituted for by other *HXT* genes must await further study.

When both the *HXT2* and the *SNF3* genes were added to the multiple-deletion strain, yielding strain CY287, the rate of growth on glucose was also similar to that of the wild type (Table 1). Glucose repression of invertase was also similar to that in the wild type or CY292 (Table 2), and the K_m for glucose transport decreased at a constant V_{max} during growth on glucose (Table 3). In this mutant, however, the V_{max} for glucose uptake was only half that in the wild type and one-fourth of that of CY292. This suggests that the transport step has little or no control over growth in either the wild type or mutant CY292, because their growth characteristics are similar to those of mutant CY287, which has a lower V_{max} for transport. In glycerol-grown CY287 cells (Table 4), there was high-affinity glucose uptake with a higher V_{max} than in glucose-grown cells; once again, the presence of the *SNF3* gene confers some glucose sensitivity on the glucose transport system. The transition between the two situations was again very slow: interestingly, 6 h after the cells were transferred from glycerol to high glucose, the V_{max} had dropped far below that of cells grown in high glucose. Apparently, this transient situation requires several generations to adjust.

Comparison of CY292 and CY287 with wild-type cells reveals a difference upon transfer from glycerol to glucose medium. In the wild type, 6 h after transfer to high or low glucose, both V_{max} and K_m have adjusted to the values found during growth on glucose (compare Tables 3 and 4). In the mutants, 6 h after transfer to low glucose, no change in glucose uptake kinetics was detected. This may indicate either that the transition is very slow or that the sensing mechanism requires a high glucose concentration. Interestingly, in the wild type, there is a rapid (within 30 min) augmentation of V_{max} due to the addition of a low-affinity component which is not present after 6 h. Upon transfer of mutants CY292 and CY287 to high glucose, the period of 6 h appears to be too short to allow complete transition to the high-glucose steady state.

A closer look at the time course of the change in glucose uptake kinetics upon transfer from glycerol to high-glucose medium reveals further interesting phenomena. In wild-type cells, the transfer leads again to a rapid (30-min) change in V_{max} and biphasic kinetics. During the subsequent 5.5 h, the K_m readjusts to a low-affinity value, while the V_{max} remains constant. Similar behavior is seen in CY292, although for this strain it must be assumed that after even longer times the V_{max} will increase further. In CY287, a rapid decrease in V_{max} is seen during the first 30 min, without much further change in the next 5.5 h. Again, upon prolonged growth, there must be an increase in V_{max}. All this indicates that some changes occur within a period that is much shorter than the doubling time of the cells, whereas others seem to occur over several generations. An explanation for this could be that some proteins involved in transport can be rapidly mobilized to or from inactive stores. Conversely, regulatory proteins that can inhibit or activate transporter function may have to be diluted via cell growth before their influence disappears. The transport characteristics of the cells in the original inoculum, therefore, may contribute significantly to the net transport kinetics for some time after medium transfer. If there is a hysteresis in the turnover of transporters such that activation or synthesis of new transporter molecules occurs at a higher rate than the breakdown of the original transporters in the cells, or vice versa, this would result in either a culture containing mixed populations of cells with respect to their transporter components or a culture containing cells with mixed transporter properties. In each case, the net transport characteristics would be in a dynamic state of change for some time after medium transfer. This may explain the change in V_{max} that has been observed by Bisson and others (2, 4) when cells are transferred from repressing to derepressing media but which is not observed when cells are continuously grown from repressing conditions to derepressing conditions (reference 16 and this study).

In the mutants CY287 and CY292, there are two functional transporters present, *HXT2* and *HXT6/7*; these have both been characterized as high-affinity transporters (14). (In our experience, in a strain expressing only *HXT2*, the affinity is better described as intermediate, with a K_m of approximately 10 to 15 mM [12a; unpublished observations].) However, in CY287 and CY292, affinity modulation can clearly be observed, albeit over a reduced range compared with that of the wild type (Table 3). In CY287 and CY292, therefore, a differential sensitivity to glucose concentration has been recovered compared with that of strains CY290 and CY294, which contain only one functional transporter, *HXT6/7*. Perhaps the presence of two different *HXT* genes can be correlated with the ability of the cells to differentiate between high and low glucose concentrations. Such a sensing function could be a direct consequence of transporter function. Expression of *HXT2* has been described as both glucose repressible and glucose inducible (18); Reifenberger and coworkers have found that *HXT2* can be induced by 2% glucose (13), while Ozcan and Johnston have found that expression of *HXT2* is maximally induced between 0.1 and 0.5% glucose but that above 0.75% glucose, expression is reduced until a concentration of 4% glucose, at which expression reaches a basal level (11). The range of glucose concentrations over which *HXT2* is expressed, therefore, would be consistent with a glucose-sensing function. Interestingly, some conditions under which *HXT2* is expressed but does not contribute to glucose transport have been described. This implies that the *HXT2* protein may have another function or that it can be rapidly inactivated in the cell (18). In mutant CY287, which contains *SNF3*, *HXT2*, and *HXT6/7*, such a rapid inactivation (within 30 min) of glucose transport can be observed upon transfer from glycerol to high-glucose medium. If only the *HXT2* and *HXT6/7* genes are present (strain CY292), a slower activation of glucose transport is observed, whereas if only the *SNF3* and *HXT6/7* genes are present (mutant CY290), a slower inactivation of glucose transport is observed. The rapid inactivation in the presence of both *SNF3* and *HXT2* suggests some interaction between the products of these genes at the level of transporter function. This rapid inactivation is glucose concentration dependent, which implies a glucose-sensing capability. It has also been proposed that *SNF3* is a glucose sensor because it is required for glucose-induced expression of *HXT* genes (11, 18). Furthermore, *SNF3* function requires the presence of at least one *HXT* gene, and recently it has been shown that it is the cytoplasmic carboxy-terminal domain that is involved in generating the signal for both *HXT* expression (1) and transport inactivation (5). It is possible that a physical interaction between the cytoplasmic domain of *SNF3* and a glucose transporter could lead to the formation of a glucose-sensing complex, with the glucose transporter involved in initial sensing and *SNF3* involved in primary signal transduction.

A comparison of the glucose transport kinetics in the two strains lacking the *SNF3* gene, CY292 and CY294, with those of the respective strains containing the *SNF3* gene, CY287 and

CY290, clearly shows a *SNF3*-dependent, glucose-induced inactivation of glucose transport. These data are therefore also consistent with *SNF3* functioning as a glucose sensor. In galactose-grown cells, however, the presence of the *SNF3* gene, in both CY290 and CY287, correlated with invertase activities of four to five times the wild-type level (Table 2). This suggests that, although the sensing role of *SNF3* requires a *HXT* component, such a sensing role is not confined to glucose. It is pertinent to recall the high degree of homology between *HXT* proteins and galactose permease (9, 14). The invertase data for glycerol-grown cells also show some correlation between the presence of *SNF3* and high invertase activities, but this is not as clear-cut as in the galactose-grown cells (Table 2). In both cases, however, the lower invertase activities in wild-type cells suggest that other signalling components are not present in these mutants. Furthermore, the reduced growth rates of all the mutants on 2% galactose or 2% glycerol, compared with that of the wild type, suggest that *HXT1*, *HXT3*, or *HXT4* is required for maximal growth on these substrates.

Therefore, while it is tempting to speculate that some of the effects described above are due specifically to the presence or absence of *HXT2* or *SNF3*, it cannot be ruled out that another of the deleted *HXT* genes could have effects similar to those of *HXT2* found in this study or that deletion of *HXT2* or *SNF3* has allowed expression of other factors that remain to be quantified.

A minimal hypothesis would be that transport kinetics measured in strains expressing only one functional *HXT* gene give single affinity values because the more complex kinetics and glucose-dependent responses, such as affinity modulation during growth on glucose, can be observed only in strains with more than one functional *HXT* component. In mutants with at least two functional *HXT* components, metabolic effects in response to decreasing glucose concentrations can be observed, as if the cells possess a mechanism to sense changes in the external glucose concentration which is apparently not present in strains expressing only one functional *HXT* gene. Such responses may be dependent on regulatory or even physical interactions between *HXT* and other components which are not present in the strains with a single *HXT* gene. The interpretation of glucose-dependent expression patterns of *HXT* genes in strains expressing only one functional transporter, therefore, may be problematic if the glucose-sensing machinery in such strains is impaired.

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