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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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 *Km* 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 *DG T1* 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
genes related to glucose uptake (HXT and SNF3) have been deleted.

MATERIALS AND METHODS

Materials. D-[U-14C]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.

Results and discussion. 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.

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>
<thead>
<tr>
<th>Strain</th>
<th>$\mu_{glu}$</th>
<th>$Y_{so}$</th>
<th>$V_{glu}$</th>
<th>$\mu_{gal}$</th>
<th>$Y_{so}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY287</td>
<td>0.37</td>
<td>0.50</td>
<td>9.75</td>
<td>0.14</td>
<td>1.00</td>
</tr>
<tr>
<td>CY290</td>
<td>0.13</td>
<td>0.10</td>
<td>1.35</td>
<td>0.16</td>
<td>0.60</td>
</tr>
<tr>
<td>CY292</td>
<td>0.37</td>
<td>0.038</td>
<td>9.7</td>
<td>0.15</td>
<td>1.00</td>
</tr>
<tr>
<td>CY294</td>
<td>0.29</td>
<td>0.051</td>
<td>5.6</td>
<td>0.16</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* Data are the means of at least three experiments.

† 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 snf3Δ hxt1Δ hxt2Δ hxt3Δ hxt4Δ HXT6/7, etc.

‡ $\mu_{gal}$, maximum growth rate on 2% glucose (per hour).

§ $Y_{so}$, growth yield (4$\text{so}$ units per millimolar glucose used), calculated from the linear portions of plots of decrease in glucose concentration versus $A_{so}$ of the cultures.

‖ $V_{glu}$, glucose consumption rate (millimolar glucose used per $A_{so}$ unit per hour), obtained from the quotient of the previous two columns.

§§ $\mu_{gal}$, maximum growth rate on 2% galactose (per hour).

∥∥ $\mu_{gel}$, maximum growth rate on 2% glycerol (per hour).

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

<table>
<thead>
<tr>
<th>Activity</th>
<th>2% glucose</th>
<th>2% galactose</th>
<th>2% glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>exp</td>
<td>gluc (-)</td>
<td>stat</td>
</tr>
<tr>
<td>R757 (wild type)</td>
<td>68</td>
<td>227</td>
<td>233</td>
</tr>
<tr>
<td>CY287 SNF3 HXT2 HXT6/7</td>
<td>61</td>
<td>265</td>
<td>352</td>
</tr>
<tr>
<td>CY290 SNF3 HXT6/7</td>
<td>1,002</td>
<td>699</td>
<td>722</td>
</tr>
<tr>
<td>CY292 HXT2 HXT6/7</td>
<td>84</td>
<td>317</td>
<td>307</td>
</tr>
<tr>
<td>CY294 HXT6/7</td>
<td>552</td>
<td>509</td>
<td>590</td>
</tr>
</tbody>
</table>

* See Materials and Methods and Table 1, footnote 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.

‡ exp, activity determined in exponential-phase cells.

§ gluc (-), activity determined at glucose exhaustion.

‖ 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Exponential phase</th>
<th>Glucose exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$</td>
<td>$V_{max}$</td>
</tr>
<tr>
<td>R757 (wild type)</td>
<td>23</td>
<td>280</td>
</tr>
<tr>
<td>CY287 SNF3 HXT2 HXT6/7</td>
<td>6.9</td>
<td>145</td>
</tr>
<tr>
<td>CY290 SNF3 HXT6/7</td>
<td>4.8</td>
<td>38</td>
</tr>
<tr>
<td>CY292 HXT2 HXT6/7</td>
<td>13</td>
<td>560</td>
</tr>
<tr>
<td>CY294 HXT6/7</td>
<td>1.2</td>
<td>140</td>
</tr>
</tbody>
</table>

* See Materials and Methods and Table 1, footnote b.
* Cells harvested at early exponential phase.
* Cells harvested at glucose exhaustion.
* Expressed as nanomoles per minute per milligram of protein.

When the SNF3 gene is added to the multiple-deletion strain, in strain 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.
large increase in $V_{\text{max}}$ 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 $V_{\text{max}}$ during growth on glucose (Table 3). In this mutant, however, the $V_{\text{max}}$ 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 $V_{\text{max}}$ for transport. In glycerol-grown CY287 cells (Table 4), there was high-affinity glucose uptake with a higher $V_{\text{max}}$ 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 $V_{\text{max}}$ 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 $V_{\text{max}}$ 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 $V_{\text{max}}$ 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 $V_{\text{max}}$ and biphasic kinetics. During the subsequent 5.5 h, the $K_m$ readjusts to a low-affinity value, while the $V_{\text{max}}$ remains constant. Similar behavior is seen in CY292, although for this strain it must be assumed that after even longer times the $V_{\text{max}}$ will increase further. In CY287, a rapid decrease in $V_{\text{max}}$ 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 $V_{\text{max}}$. 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 $V_{\text{max}}$ 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); Reifnerberger and coworkers have found that HXT2 can be induced by 2% glucose (13), while Ozean 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.
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 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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