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Walsh, M.C.; Smits, H.P.; Scholte, M.E.; van Dam, K.

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Affinity of Glucose Transport in *Saccharomyces cerevisiae* Is Modulated during Growth on Glucose

MICHAEL C. WALSH, HANS P. SMITS, MARCEL SCHOLTE, AND KAREL VAN DAM*  
E. C. Slater Institute, BioCentrum Amsterdam, University of Amsterdam, 1018 TV Amsterdam, The Netherlands  
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By using a modified technique to measure glucose uptake in *Saccharomyces cerevisiae*, potential uncertainties have been identified in previous determinations. These previous determinations had led to the proposal that *S. cerevisiae* contained a constitutive low-affinity glucose transporter and a glucose-repressible high-affinity transporter. We show that, upon transition from glucose-repressed to derepressed conditions, the maximum rate of glucose transport is constant and only the affinity for glucose changes. We conclude that the transporter or group of transporters is constitutive and that regulation of glucose transport occurs via a factor that modifies the affinity of the transporters and not via the synthesis of different kinetically independent transporters. Such a mechanism could, for instance, be accommodated by the binding of kinases causing a change in affinity for glucose.

Until very recently, glucose uptake in *Saccharomyces cerevisiae* was thought to be mediated by two kinetically distinct mechanisms. A constitutive low-affinity transport system, consisting of a nonconcentrative facilitated diffusion process with a $K_m$ of approximately 20 mM (50 mM for fructose), and a kinase-dependent, glucose-repressible high-affinity transport system, also consisting of a facilitated diffusion process with a $K_m$ of approximately 1 mM (5 mM for fructose), have been described (3). The diagnostic technique used in the elucidation of these two systems was the biphasic nature of Eadie-Hofstee plots obtained from short-time-scale (s) uptake experiments using radiolabelled glucose. Analysis of such plots, however, is complex and requires computer-assisted nonlinear regression. Simple linearization of the two slopes in the biphasic plot can produce poor estimations of the kinetic parameters or, in severe cases, fail to detect all of the systems present (24, 26).

The widespread use of these experimental and analytical techniques has led to the description of high- and low-affinity glucose transporters in several *Saccharomyces* species (7) as well as other yeasts such as *Klyveromyces lactis* (23), *Klyveromyces marxianus* (9), and *Candida wickerhamii* (20).

More recent genetic studies have identified a number of genes involved in glucose transport in *S. cerevisiae*. *SNF3* (sucrose nonfermenting), a glucose-repressible component of high-affinity glucose transport (4) and *HXT1* and *HXT2* (hexose transport) are proposed components of high-affinity glucose transport (14, 16). Further studies have revealed that *HXT3*, *HXT4*, and an unidentified component are likewise involved in high-affinity glucose transport while *SNF3* may be involved only in the regulation of high-affinity transport (13). While at least six components have been identified as being involved in the high-affinity uptake, the nature of the low-affinity component has remained elusive, and this has led to questions regarding the nature of this component. Several groups have suggested that low-affinity uptake may be a consequence of passive diffusion (8, 10, 11).

By using a modified technique, we have measured glucose transport at different stages of batch growth in cells of *S. cerevisiae*, and the results strongly suggest that transition from a low-affinity to a high-affinity uptake may be a strictly regulated phenomenon not coupled to the synthesis of different transporter proteins.

**MATERIALS AND METHODS**

**Materials.** Reagents were obtained from the following sources: d-[U-14C]glucose was from Amersham International; media constituents and other chemicals were purchased from Sigma Chemical Co. and were of reagent grade or better.

**Strains and growth conditions.** This work was performed with the following strains of *S. cerevisiae*. ENY.WA-15A wild type (*MATa ura3-52 leu2-3 leu2-112 his3-Δ1*), X-2180 wild type (*MATa/a SUC2/SUC2 mal/mal gal2/gal2 CUP1/CUP1*), A364-a wild type (*MATa ade1 ade2 ura1 his7 lys2 tyr1 gal1*), and BR214-14a (*MATa cdc35-1 ade1 his7 trp1 ura1 arg*) were all obtained from the Yeast Genetic Stock Center (Berkeley, Calif.). MCY1409 (*MATa lys2-801 ura3-52 snf3-Δ4::HIS3 SUC2* GAL* his3Δ*) and MCY638 wild type (*MATa his4-539 lys2-801 ura3-52 SUC2* GAL*+) were supplied by M. Carlson (17). All strains were grown aerobically in a gyratory shaker at 23 or 30°C, usually with 1% (wt/vol) glucose in a medium containing 2% (wt/vol) yeast nitrogen base. Growth was monitored by measuring the optical density at 600 nm (OD$_{600}$).

**Glucose uptake assays.** Cultured cells were washed and filtered three times and then resuspended in 100 mM potassium phosphate buffer (pH 6.5) to a concentration of 5% (wt/vol). Uptake was measured at glucose concentrations ranging from 0.1 to 250 mM (specific radioactivity, 6 to 740 kBq·μmol$^{-1}$). Yeast cells (50 μl) and five-times-concentrated radiolabelled glucose (12.5 μl) were preincubated at the assay temperature and then mixed and incubated for 5 s (measured accurately). Uptake was then terminated by quenching in 10 ml of 100 mM potassium phosphate buffer (pH 6.5) containing 500 mM unlabelled glucose maintained at a temperature below −8°C on ice. Cells were then collected rapidly and washed on glass fiber filters with 20 ml of the quenching solution. Filters were then transferred immediately to scintillation vials containing 5 ml of scintillant, and radioactivity was measured with a Beckman liquid scintillation counter. The
control blank in each experiment consisted of labelled glucose added to the quenching solution before the yeast cells. The procedures described above were performed as quickly as possible to prevent any fermentative loss of incorporated radiolabel, and the time from mixing of cells and glucose to transfer of filter to scintillant was less than 1 min. This method represents a considerable modification of the original method of Bisson and Fraenkel (3), especially with respect to the lower temperature of the quenching solution and the inclusion of 500 mM glucose in the quenching solution. For comparison, glucose uptake was also assayed by the original method.

Data analysis. Kinetic parameters were determined by using Eadie-Hofstee plots, and all data were analyzed by computer-assisted nonlinear regression by using Enzfitter software. Standard errors were estimated by Enzfitter.

RESULTS

The modifications introduced in the assay of glucose uptake were developed because, in our hands, the original method gave inconsistent results, especially inconsistent results, in terms of nonlinearity with cell concentration and large variations in uptake values between experiments. A study of maltose uptake, in S. cerevisiae, attributed similar problems to variable nonspecific binding in the control experiments (blanks) and concluded that the low-affinity maltose uptake component was an artifact (1).

We identified four potential uncertainties in the original assay for glucose uptake (3). First, nonspecific (i.e., very-low-affinity) binding of glucose to the cells led to higher apparent uptake values. Second, incomplete quenching resulted in uptake after incubation, again causing higher values. Third, delays in processing samples led to loss of counts via CO₂ release. Finally, incorrect data analysis led to misinterpretations of data, especially where an upward curve in Eadie-Hofstee plots of glucose uptake had been observed. Errors of the first two types were reduced by including saturating concentrations of nonradioactive glucose in the quenching solution. Inclusion of saturating concentrations of nonradioactive substrate is common practice in many amino acid transport assays (2, 21). Errors of the third type were reduced by processing samples very rapidly and maintaining quench solutions on salt-ice mixtures. The high concentration of nonradioactive glucose in the quench solution prevented freezing down to a temperature of −10°C. Errors in data analysis were avoided by using computer-assisted nonlinear regression as outlined in the introduction.

Comparisons between the results obtained with the modified method and those obtained with the original method (3) showed that, at high glucose concentrations, consistently higher values were obtained when uptake was quenched in cold buffer alone. Figure 1 shows the results of kinetic analyses of glucose uptake data obtained by both methods with repressed and derepressed cells. The data for repressed cells (i.e., cells harvested at the early exponential growth phase on glucose) are presented in Fig. 1A. Fitting the data to a model of one kinetic component, i.e.,

\[ V = -K_m \cdot (v/S) + V_{\text{max}} \]

where \( V_{\text{max}} \) is the maximum rate of glucose uptake, \( v \) is the measured rate of glucose uptake, and \( S \) is the substrate concentration, gave similar \( K_m \) values whether the cells had been quenched in buffer alone (28 ± 2 mM) or in glucose-containing buffer (25 ± 2 mM). The \( V_{\text{max}} \) value of the buffer-washed cells was, however, higher (387 ± 28 nmol·min⁻¹·mg of protein⁻¹) than that of the glucose-washed cells (279 ± 11 nmol·min⁻¹·mg of protein⁻¹).

In derepressed cells (i.e., those harvested at the end of exponential growth when glucose is exhausted), the data for buffer-washed cells (Fig. 1B) exhibited the generally described
where $K_{m1}$ and $K_{m2}$ are the respective apparent affinity constants and $V_{max1}$ and $V_{max2}$ are the respective maximal rates of glucose uptake. A low-affinity component ($Km = 15 \pm 5$ mM; $Vmax = 287 \pm 46$ nmol min$^{-1}$ mg of protein$^{-1}$) and a high-affinity component ($Km = 1 \pm 1$ mM; $Vmax = 59 \pm 50$ nmol min$^{-1}$ mg of protein$^{-1}$). The total $Vmax$ value, therefore, was very similar to that for repressed cells (346.68 nmol min$^{-1}$ mg of protein$^{-1}$). The data for glucose-washed cells revealed very different kinetics (Fig. 1C); in this case, the cell data could be fitted to a model of one kinetic component (equation 1), with a $Km$ of 5 $\pm$ 1 mM and a $Vmax$ value of 259 $\pm$ 28 nmol min$^{-1}$ mg of protein$^{-1}$. A better fit, however, was obtained with a model of two components (equation 2), the high-affinity system being the major component ($Km = 4.3 \pm 0.3$ mM; $Vmax = 225 \pm 17$ nmol min$^{-1}$ mg of protein$^{-1}$) and the low-affinity component being relatively small ($Km = 24 \pm 2$ mM; $Vmax = 51 \pm 7$ nmol min$^{-1}$ mg of protein$^{-1}$). This resulted in a total $Vmax$ value of 276 $\pm$ 18 nmol min$^{-1}$ mg of protein$^{-1}$, which was very similar to the value obtained with this method for repressed cells.

The results obtained with both methods indicate that the $Vmax$ values for glucose uptake under repressed and derepressed conditions were the same, although the magnitude of the $Vmax$ was lower when the glucose wash method was used. The most obvious explanation for the difference in the $Vmax$ values obtained with the two different methods is that the incorporation measured in buffer-washed cells contains a binding component that is removed by washing in 500 mM nonradioactive glucose. This is probably a relatively low-affinity component since the glucose wash significantly affected only the uptake measurements above 50 mM glucose. Indeed, subtracting the results obtained with glucose-washed cells from those obtained with buffer-washed cells gives an apparent $Km$ for this component of 50 to 100 mM.

The difference in radiolabel incorporation between buffer-washed cells and glucose-washed cells is unlikely to represent countertransport with free intracellular glucose because this would be a relatively slow process at the low temperature of the quench buffer, as was demonstrated by Lang and Cirillo (15) for a kinaesin mutant of S. cerevisiae. Countertransport should be proportional to the $Km$ of the carrier. Since the $Km$ of the carrier is an order of magnitude higher in repressed cells than in derepressed cells, the amount of exchange should be lower in repressed cells, but the results in Fig. 1 show that the difference in counts is approximately the same at higher glucose concentrations. Countertransport would also only be effective with internal free glucose, but results obtained from very rapid (subsecond) uptake experiments have demonstrated that, in derepressed cells, less than 5% of the total radioactive incorporation is present as free glucose (22). It seems reasonable to suggest, therefore, that the biphasic plot in Fig. 1B, obtained by using the original method of Bisson and Fraenkel (3), does not describe two uptake systems but is indicative of a very-low-affinity glucose-binding component, possibly associated with the cell wall or periplasmic space.

Biphasic Eadie-Hofstee plots have also been obtained by the combination of a facilitated diffusion term and a simple diffusion term (24). Figure 2 shows an Eadie-Hofstee plot for glucose uptake in stationary-phase-grown cells by using the glucose wash method. The plot is composed of 60 separate determinations, and the data were fitted to a model consisting of one facilitated diffusion term and a simple diffusion term:}

$$v = -Km \cdot (v/S) + Vmax + D \cdot (Km + S)$$

Nonlinear regression analysis revealed a $Km$ of 1.5 $\pm$ 0.2 mM and a $Vmax$ value for 268 $\pm$ 10 nmol min$^{-1}$ mg of protein$^{-1}$ for the facilitated diffusion term and a $D$ of 0.3 $\pm$ 0.1 $\mu$mol min$^{-1}$ mg of protein$^{-1}$ for the simple diffusion term. Passive diffusion displays first-order (i.e., nonsaturable) kinetics; consequently, passive diffusion cannot account for the uptake measured under glucose-repressed conditions (Fig. 1A) since these clearly display second-order kinetics. Passive diffusion did, however, contribute to nonlinearity at higher glucose concentrations under derepressed conditions as the data in Fig. 2 clearly show.

By using the modified technique, the changes in the kinetic characteristics of glucose transport during batch growth on glucose were examined. Cells from a wild-type strain of S. cerevisiae were harvested at four different times during batch growth on 2% (wt/vol) glucose, and the uptake characteristics were investigated (Fig. 3). The most striking feature of these results was that the $Vmax$ value for glucose uptake during growth remained unchanged. Glucose uptake in cells harvested at the early logarithmic phase (OD$600$ = 0.2) exhibited low-affinity characteristics ($Km = 26 \pm 2$ mM; $Vmax = 254 \pm 18$ nmol min$^{-1}$ mg of protein$^{-1}$), and in the stationary phase (OD$600$ = 4.1), high-affinity uptake was exhibited ($Km = 1.6 \pm 0.1$ mM; $Vmax = 259 \pm 8$ nmol min$^{-1}$ mg of protein$^{-1}$). At intermediate ODs, the plots were upwardly curved, indicating the existence of at least two kinetic components (equation 2). The dashed lines in Fig. 3 represent theoretical curves generated with a model maintaining a constant $Vmax$ of 260 nmol min$^{-1}$ mg of protein$^{-1}$ for glucose uptake and varying the proportions of the low- and high-affinity components. At an OD$600$ of 0.2, all of the uptake was low affinity ($Km = 26$ mM; $Vmax = 260$ nmol min$^{-1}$ mg of protein$^{-1}$); at an OD$600$ of 2.2, low-affinity uptake was the major component ($Km = 26$ mM; $Vmax = 206$ nmol min$^{-1}$ mg of protein$^{-1}$) and there was a minor high-affinity component ($Km = 1.6$ mM; $Vmax = 56$ nmol min$^{-1}$ mg of protein$^{-1}$); at an OD$600$ of 3.3, high-affinity uptake was the major component ($Km = 1.6$ mM; $Vmax = 162$ nmol min$^{-1}$ mg of protein$^{-1}$), and there was a
minor low-affinity component \( \left( K_m = 26 \text{ mM}; \ V_{\text{max}} = 98 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg of protein}^{-1} \right) \); and at an OD_{600} of 4.1, all of the uptake was high affinity \( \left( K_m = 1.6 \text{ mM}; \ V_{\text{max}} = 260 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg of protein}^{-1} \right) \). The theoretical values generated with the model compared well with the measured uptake values (Fig. 3). Only at very high substrate concentrations was there a small deviation of the data from the model. This was due to the passive diffusion component (Fig. 2), which was not incorporated into the model.

To confirm the initial results, the experiments were repeated with another wild-type strain, A364-4, and with a mutant of this strain, ade5-1, which is a temperature-sensitive mutant with impaired glucose repression due to a lesion in adenylate cyclase (5). The change in kinetic characteristics of glucose uptake with growth in A364-4 again exhibited the appearance of higher-affinity uptake with the approach of the stationary phase, while the \( V_{\text{max}} \) remained constant. In this case, the experimental data could be fitted to a two-component uptake system by varying the proportions of the low- and high-affinity components, i.e., \( K_m \) values of 16 and 2.7 mM, respectively, and maintaining a constant total \( V_{\text{max}} \) of 300 nmol \cdot min^{-1} \cdot mg of protein^{-1} (data not shown).

In the mutant, no low-affinity uptake could be observed. In the early logarithmic phase, a \( K_m \) of 4.3 mM and a \( V_{\text{max}} \) of 242 nmol \cdot min^{-1} \cdot mg of protein^{-1} were obtained, while in the stationary phase, a \( K_m \) of 2.2 mM and a \( V_{\text{max}} \) of 220 nmol \cdot min^{-1} \cdot mg of protein^{-1} resulted. Even in this case, a decrease in \( K_m \) was observed, albeit only by a factor of two, and the \( V_{\text{max}} \) for glucose uptake still remained relatively constant (data not shown).

Mutants in the SNF3 gene are supposed to lack high-affinity glucose transport (4), but whether SNF3 is a glucose transporter or a regulator of glucose transport is still unclear. Evidence from the sequence data and point mutagenesis suggests that SNF3 is a glucose transporter (17). However, it has also been shown in mutants where the four hexose transport genes \( (HXT1 \text{ to } 4) \) have been deleted that SNF3 is not sufficient for growth on glucose (13). Figure 4 shows the variation in uptake kinetics during growth on 1% (wt/vol) glucose of a mutant in SNF3 and its corresponding parent strain. The parent strain (Fig. 4A) exhibited at the early log phase the low-affinity kinetics which has been a feature of all of the other wild-type strains examined \( \left( K_m = 23 \pm 1 \text{ mM}; \ V_{\text{max}} = 292 \pm 21 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg of protein}^{-1} \right) \), while in the mid-log phase upwardly curved kinetics, indicating the presence of at least two uptake components, were observed. Fitting the data to two kinetic components revealed a low-affinity component with a similar affinity to that at the early log phase \( \left( K_m = 25 \pm 2 \text{ mM}; \ V_{\text{max}} = 155 \pm 13 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg of protein}^{-1} \right) \) and a high-affinity component \( \left( K_m = 2.4 \pm 0.2 \text{ mM}; \ V_{\text{max}} = 158 \pm 17 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg of protein}^{-1} \right) \). Thus, the total \( V_{\text{max}} \) value was 313 \pm 22 nmol \cdot min^{-1} \cdot mg of protein^{-1}, which was similar to the value at the early log phase. In the mutant (Fig. 4B), growth was accompanied by a lowering of the \( K_m \) for glucose transport from 9.8 \pm 0.4 mM at the early exponential phase to 4.7 \pm 0.3 mM at the early stationary phase, while the \( V_{\text{max}} \) value remained relatively constant. At an intermediate OD, the data could be fitted to a 1:1 ratio of high- and low-affinity uptake, but the relatively small change in affinity reduces the significance of data gathered. Therefore, it is difficult to conclude whether one or two systems were present; the \( V_{\text{max}} \) value was, however, similar to that at the other growth phases. In our hands, therefore, a
deletion in the SNF3 gene does not result in the loss of high-affinity uptake but rather the magnitude of the change in the affinity for glucose with growth was reduced.

**DISCUSSION**

In all of the strains of *S. cerevisiae* examined, both mutants and wild types, the \( V_{\text{max}} \) values for glucose transport during batch growth remained constant. The transition from glucose-repressed to derepressed conditions is, therefore, associated with a lowering of the \( K_m \) for glucose transport but no change in the \( V_{\text{max}} \). These findings are consistent with shifting from low-affinity to high-affinity uptake, as suggested first by Serrano and Delafuente (19) and later by others (15), rather than with the development of a high-affinity transporter on top of a constitutive low-affinity transporter. The number of functional transporters in the membrane could well be constant, with the transition from low- to high-affinity uptake involving a conversion of low- to high-affinity transporters.

The uptake characteristics during growth have revealed, under fully repressed or derepressed conditions (early-exponential- and stationary-phase growth, respectively), the presence of one uptake component plus a relatively insignificant passive diffusion component. It should be stated that, even under fully repressed conditions, glucose uptake exhibits second-order kinetics and the passive diffusion component incorporated in the model cannot account for the uptake measured. This is not in agreement with the proposal of Fuhrmann and coworkers that passive diffusion accounts for low-affinity transport (8, 24), although passive diffusion could be misinterpreted as a low-affinity component under derepressed conditions (Fig. 4) (see also reference 24). The intermediate growth stages have upwardly curved kinetic plots that indicate the presence of at least two kinetic components; neither of these two components, however, are completely constitutive because the \( V_{\text{max}} \) remains constant, and therefore, the appearance of the higher-affinity component seems to be at the expense of the lower-affinity component. A similar conclusion was reached by Fuhrmann and co-workers by using a theoretical analysis (25). The observation of two kinetic components at intermediate growth stages also poses certain limits as to the rate of interconversion of these two forms, i.e., it must be a relatively slow event in comparison with the rate of glucose transport because, otherwise, only one intermediate \( K_m \) would be observed.

The cdc35-1 mutant is a temperature-sensitive mutation of adenylate cyclase and shows impaired glucose repression (5). It also shows no low-affinity glucose uptake even when grown at its permissive temperature. It does, however, show an increase in affinity with growth (the \( K_m \) for glucose uptake decreasing from 4.3 to 2.2 mM), with only one system being present in each case. Therefore, this mutant does exhibit a change, albeit a small one, in the affinity of glucose uptake with growth, while the \( V_{\text{max}} \) again remains relatively constant. This result is consistent with the conversion of low- to high-affinity uptake being a derepressive response since this mutant is known to have impaired glucose repression and the \( K_m \) change is very much smaller in this case. This result also indicates that there is probably more than one mechanism regulating glucose uptake in that in this mutant there is almost a complete lack of low-affinity uptake, but an affinity change is still observed. The maintenance of glucose transport in the low-affinity form may be a classical glucose-repressive response under the control of the cyclic AMP (cAMP) cascade; if this is impaired, then no low-affinity uptake is present. Furthermore, any subsequent affinity change will be of a smaller magnitude and, therefore, more difficult to observe. A small increase in affinity for glucose has, however, been observed in this mutant. Therefore, the mechanism of the affinity increase may be different from that involved in maintaining low-affinity uptake, for example, via a glucose sensor. In this context, it is instructive to note that, during the early to mid-exponential phase, the low-affinity uptake is predominant; only in the mid- to late exponential phase can the upwardly curved kinetics that indicate the presence of two uptake systems be observed.

The SNF gene has been identified as a component of the high-affinity uptake system (4). A deletion mutant in this gene showed a response similar to that of the cdc35-1 mutant (Fig. 4B); the change in affinity for glucose that accompanied growth was much smaller in the mutant than in the parent strain. This mutant did not seem to lack either low- or high-affinity uptake. Rather, the magnitude of the change in affinity for glucose that is associated with derepression was smaller. The results suggest that the SNF3 gene product is involved in the regulation of glucose uptake and not in the actual transport.

The terminology in the literature and poor data analysis may have contributed to the lack of progress in the area of the mechanistic elucidation of glucose transport and its regulation. Since the idea of low- and high-affinity glucose transporters is confusing, we suggest that it is the magnitude of the change in affinity for glucose that is the derepressive response and not expression of a high-affinity transporter. Glucose transport should be described as higher or lower affinity to reflect the fact that the \( V_{\text{max}} \) does not change significantly. We postulate that glucose transport is constitutive and could be composed of one or more similar transporter proteins of which the affinity for glucose can be modulated. It is the magnitude of this affinity change that is affected in some transport-sensitive mutants. Thus far, we have been unable to identify any mutants that cause a significant change in \( V_{\text{max}} \) either as a derepressive response or when compared with the parent strain.

Recently, genetic studies have implicated a number of genes as constituting the high-affinity glucose transport system; in total, SNF3, HXT7 to -4, and at least one more unidentified component are thought to make up this system (13), with the HXT family and the unidentified component being transporters while SNF3 is a negative regulator of the transporters. These conclusions were based on mutations in these genes and effects on growth on high- and low-glucose media; no transport characteristics were presented. Our data certainly suggest that glucose transport as a whole is a multicomponent system with variable affinity for glucose, and the possibility of a regulatory component, such as SNF3, binding and inactivating a proportion of transporters is a mechanism by which uptake could exhibit the constant \( V_{\text{max}} \) values we have described. The kinases must also be involved because higher-affinity uptake remains kinase dependent (3, 6, 18). Additionally, there is a requirement for a glucose sensor since it is in response to a lowering glucose concentration that the affinity increase takes place. In this case, the general glucose-sensing protein described by Hohmann et al. (15) is interesting. These authors have postulated an involvement of GSG1 in transport-associated sugar phosphorylation and demonstrated the accumulation of free glucose in gsg1 deletion mutants (12). We suggest that glucose uptake is indeed a multicomponent system where the affinity for glucose is dependent on the binding or interaction of the kinase enzymes and other regulatory components such as GSG1 or SNF3. We have found no evidence to suggest, however, that there exist distinct low- and high-affinity glucose transporters. Indeed, the results presented here suggest that the low- and high-affinity transporters are in fact different forms of the same transporter, and it is the regulation of the
conversion of one form to the other which is multicomponent. This is not to say that there is only one transporter protein existing in two forms. There may be a number of similar transporter proteins with similar kinetic properties sharing a common mechanism of affinity modulation.

In Fig. 5, we present a schematic diagram to describe how the conversion of low- to high-affinity uptake may be brought about. The binding of an effector, which could be the SNF3 gene product, the general glucose-sensing protein, hexokinase PH (or PI), one of the HXT gene products, or any combination of these, in response to the glucose concentration either directly or via a second messenger causes a change in the affinity of the transporter or transporters for glucose. This change does not necessarily have to be an increase in affinity. The binding of the effector may be what maintains the transporter in the low-affinity form, in which case the signal would cause the release of the effector. In fact, it is likely that the effector has more than one component and that the presence of both is required to cause the affinity change.

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