Glucose Repression in *Saccharomyces cerevisiae* Is Related to the Glucose Concentration Rather Than the Glucose Flux*

(Received for publication, March 26, 1998, and in revised form, June 11, 1998)

Michelle M. C. Meijer‡§, Johannes Boonstra‡, Arie J. Verkleij‡, and C. Theo Verrips‡¶

From the ‡Utrecht University, Department of Molecular Cell Biology/Institute for Biomembranes, Padualaan 8, 3584 CH Utrecht and §Unilever Research Laboratory, Olivier van Noortlaan 120, 3133 AT, Vlaardingen, The Netherlands

Glucose plays an important regulatory role in the yeast *Saccharomyces cerevisiae*, which is mostly reflected at the transcriptional level by glucose repression. The signal that initiates glucose repression is unknown, but data indicate that it is located at or above the level of glucose 6-phosphate, suggesting the involvement of either the intracellular or extracellular glucose concentration or the glucose flux in triggering glucose repression. We have investigated the role of the glucose flux and the extracellular glucose concentration in glucose repression by growing the cells in continuous culture under nitrogen limitation. By a step-wise increase in the glucose feed concentration, the glucose flux and extracellular glucose concentrations were modulated independently of each other by increasing the dilution rate or by the use of fructose as a substrate. Using these approaches we demonstrate that glucose repression is related to the extracellular (or intracellular) glucose concentration rather than the glucose flux. At external glucose concentrations lower than 14 mM, glucose repression of *SUC2* gene transcription was not triggered, whereas glucose repression of this gene was activated when the glucose concentration exceeded 18 mM. A comparable effect was observed for the glucose-repressible carbon source fructose.

In addition to its function as a nutrient, glucose plays an important regulatory role in the metabolism of the yeast *Saccharomyces cerevisiae*. The addition of glucose to cells growing on nonfermentable carbon sources causes induction of a variety of signal transduction pathways and the activation and inactivation, respectively, of several proteins (1–5). The regulatory role of glucose is most prominent at the level of transcription. The ability of glucose to repress gene expression by inhibition of transcription is called carbon catabolite repression or glucose repression. Genes that are under the control of glucose repression (6–8) encode enzymes that are involved in gluconeogenesis, the Krebs cycle, respiration, mitochondrial development, and the utilization of carbon sources other than glucose, fructose, or mannose.

Although several proteins and protein-protein interactions in the nucleus and the cytoplasm have been shown to be involved in glucose repression, the signal that initiates the signal transduction pathway leading to glucose repression has not yet been identified. Based on the observation that galactose (9–11) and maltose (12, 13) are unable to induce glucose repression, it has been suggested that the trigger for glucose repression is located at or above the level of glucose 6-phosphate in the glycolytic pathway. This suggestion is supported by the observations that a mutant defective in hexokinase isoenzyme 2 is unable to exhibit glucose repression (14–16), whereas the glucose analogue 2-deoxyglucose, which can be phosphorylated after uptake but not further metabolized, is able to cause glucose repression of invertase. In agreement with these data Schaff et al. (17) showed that various intermediates of glycolysis do not contribute to the repression phenomenon. These observations suggest that the following parameters may play a role in the generation of the initial signal for glucose repression: 1) an increase in glucose concentration, either extracellular or intracellular, or 2) an increase in glucose flux over the plasma membrane.

The aim of this study was to determine the role of the extracellular glucose concentration and glucose flux, respectively, in glucose repression. Yeast cells were grown under continuous culture conditions in a chemostat, which generated a physiologically stable and well defined environment. Using nitrogen limitation, both glucose flux and extracellular glucose concentration could be modulated by cultivating the cells at different but constant glucose concentrations at different but fixed growth rates. Using this approach we demonstrate the possibility of discriminating between the effects of a change in the glucose flux and the glucose concentration, since these parameters were modulated independently of each other. The expression of the glucose-repressible invertase (*SUC2*) gene was investigated under these conditions. It was shown that glucose repression of *SUC2* was activated at external glucose concentrations between 14 and 18 mM at all growth rates used. No relationship was found between glucose repression and the glucose flux. In conclusion, the glucose concentration is most likely to be involved in the activation of glucose repression, whereas the glucose flux is not important in this respect.

**EXPERIMENTAL PROCEDURES**

Reagents—Chemicals were purchased from Merck and Sigma and were reagent grade or better. Enzymes were purchased from Boehringer Mannheim.

Strain and Growth Conditions—Commercial bakers’ yeast *S. cerevisiae* strain SU32 was grown in a 2 l BiofloII chemostat (New Brunswick Scientific; Nijmegen, The Netherlands) that was connected to a computer control unit running on Advanced Fermentation Software (New Brunswick Scientific). SU32 was inoculated in the medium as described previously (18), and after batch growth overnight, a continuous feed was connected. The medium for continuous cultivation was as described previously (18) in which the NH₄Cl concentration was 1.5 g/liter, and the glucose or fructose feed concentration was changed for each steady state. At a dilution rate of 0.15 h⁻¹, steady state analyses were performed at glucose feed concentrations of 196, 226, 238, 280, 290, 310, and 345 mM, respectively. At a dilution rate of 0.2 h⁻¹, steady state analyses were performed at glucose feed concentrations of 98, 134,
Yeast cells were cultivated under nitrogen limitation in a continuous culture at a constant dilution rate under step-wise increasing dilution rates. Yeast cells were grown in chemostats at a fixed glucose concentration of 121, 147, 175, 201, 264, and 309 mM, respectively. The pH of the cultures was kept constant automatically at pH 5.0 by the addition of 5 mM KOH. The temperature was kept at 30 °C. The air flow and stirrer speed were 2 liter/min and 800 rpm, respectively, resulting in an oxygen tension of 50% or higher. Carbon dioxide production (rCO₂ in mmol/(g dry weight)/h) and oxygen consumption (qO₂ in mmol/(g dry weight)/h) were measured on line by connection of the headspace of the chemostat to a Ura3G CO₂ analyzer and a Magneto4G O₂ analyzer (Hartmann & Braun; Delft, The Netherlands). Samples for the determination of external sugar concentrations and dry mass per liter of culture liquid as well as samples for the isolation of RNA were taken from the chemostat under steady state conditions and prepared or determined as described previously (18).

**Determination of the “in Vivo” Sugar Uptake Rate in the Chemostat—**Glucose and fructose concentrations in the feed medium and in the chemostat were determined enzymatically. The "in vivo" glucose and fructose flux (in mmol/g/h) in the chemostat are defined as described previously (18) according to Equation 1, in which S_feed and S_extracellular represent the sugar concentration in the feed medium and the extracellular sugar concentration, respectively (both in mM), D represents the dilution rate (in h⁻¹), and DW represents the dry weight (in g/liter).

\[
\Phi_{\text{in vivo}} = (S_{\text{feed}} - S_{\text{extracellular}}) \cdot D / \text{DW}
\]

Error estimations in “in vivo” sugar flux rates are less than 10%.

**Northern Blot Analyses—**To analyze mRNA levels, total RNA was isolated from yeast cells under steady state conditions. Yeast cells (~2 ml, OD₆₀₀ = 20) were lysed by shaking the cells with 1 g of glass beads in phenol and 1% SDS in RNA extraction buffer (1 mM EDTA, 100 mM LiCl, 100 mM Tris-HCl, pH 7.5, 10 mM iodiumacetate) at maximum speed on a vortex mixer for 30 s cycles with 30 s intervals on ice. Lysed cells were separated from the glass beads and cell debris by centrifugation. A chloroform/phenol extraction was performed, and total RNA was precipitated by 40% potassium acetate in ethanol. RNA was suspended in DEPC-treated water. Total RNA samples (5 μg) were separated on a denaturing formaldehyde/formamide/agarose gel and blotted on Hybond paper (Amersham; Den Bosch, The Netherlands) by capillary blotting. The RNA was cross-linked to the blot membrane by exposure to UV light. Blots were prehybridized for at least 2 h and hybridized according to Sierks et al. (19). For the Northern blot analysis, the following oligonucleotides were used: 5'-TGGGTTCAGTGGAGAGAAGTGGCCGCAAGC-3' for SUC2 detection and SUC2 gene and 5'-TGCTTGGTACCGACGATGATGGGAAAGCTG-3' for ACT1 detection. Oligonucleotides were labeled by incubating 15 pmol with 1 unit of T4 polynucleotide kinase and 50 μCi of [³²P]ATP.

**RESULTS**

**Flux and Extracellular Glucose Concentration as a Function of the Glucose Feed Concentration at a Constant Growth Rate—**To determine the role of the glucose flux and the extracellular glucose concentration in glucose repression, it is important to modulate these parameters independently of each other. Studying the growth of wild-type S. cerevisiae in batch culture results in continuous changes in the glucose flux as well as the glucose concentration. To provide the required constant growth conditions for S. cerevisiae, the cells can be grown in continuous culture under nitrogen limitation at a constant dilution rate. The medium that is fed to the culture contains all nutrients in excess except for one (in this case the nitrogen source). The latter, the limiting nutrient, determines biomass in the culture. Under steady state conditions, the concentration of all nutrients, the glucose consumption rate, and the growth rate are constant in time, resulting in a constant physiological state of the yeast cells (20–23). Culturing S. cerevisiae in a nitrogen-limited continuous culture in the presence of various concentrations of glucose in the feed medium allows the possibility of modulating the glucose concentration in the medium and, hence, the glucose flux without changing the growth rate.

S. cerevisiae was cultivated in a nitrogen-limited continuous culture at a fixed growth rate of 0.15 h⁻¹. The sugar concentration in the feed medium was increased step-wise from 196 to 345 mM as described by Meijer et al. (18). Under these conditions, no changes in cell number or biomass were observed (data not shown). The minimum glucose concentration at which the culture could grow under nitrogen limitation was determined by growing the cells at a constant dilution rate with a feed containing 1.5 g/liter NH₄Cl and increasing glucose concentrations. At glucose concentrations of 40 mM and higher no further increase in biomass was observed, indicating that under these conditions the cells in the culture were growing under nitrogen limitation. Increases in the nitrogen concentration under these conditions directly affected biomass (data not shown) (24).

Under steady state conditions, a constant biomass was obtained of ~5.4 g of dry weight/liter, independent of the glucose concentration in the feed medium. These observations demonstrate the nitrogen-limited nature of the culture. Biomass was constant at all glucose feed concentrations used, which indicated that glucose metabolism changed as the glucose concentration was increased, which was reflected in an increase in the production of CO₂, ethanol, and glycerol (data not shown). At each steady state, the extracellular glucose concentration in the chemostat and the glucose flux (Eq. 1) were determined. When the glucose concentration in the feed medium was increased from 196 to 345 mM, the extracellular glucose concentration in the chemostat increased biphasically from 0.9 to 29.3 mM as is shown in Fig. 1A. The extracellular glucose concen-
Glucose Repression Is Related to the Glucose Concentration

Tration was initially low but increased sharply at concentrations in the feed higher than 280 mM, corresponding to an extracellular glucose concentration of 5 mM. However, in contrast to the biphasic increase in the external glucose concentration, the glucose flux increased linearly under these conditions from 5.6 to 8.9 mmol/g/h (Fig. 1A). These observations demonstrate that continuous cultivation of S. cerevisiae under nitrogen limitation provides conditions in which the glucose flux and the external glucose concentration can be modulated independently in an accurate way by increasing the glucose feed concentration.

Relationship of the Glucose Flux and Glucose Concentration to the Growth Rate—The increase in glucose flux and extracellular glucose concentration is not only determined by the glucose concentration in the feed medium but also by the growth rate, i.e., the dilution rate in a continuous culture. Therefore, similar experiments were performed at different growth rates corresponding with a dilution rate of 0.2 h⁻¹ and 0.3 h⁻¹, respectively. At a dilution rate of 0.2 h⁻¹, the glucose concentration in the feed medium was increased step-wise from 98 to 360 mM. Under steady state conditions, at all glucose feed concentrations, a constant biomass of 5.5 g of dry weight/liter was again obtained, indicating the nitrogen-limiting condition. The step-wise increase in the glucose feed concentration resulted in a linear increase in the glucose flux from 3.7 to 13.0 mmol/g/h, comparable with that found for the dilution rate of 0.15 h⁻¹, although with a steeper slope. At the same time, the extracellular glucose concentration increased from 0.2 to 40.0 mM (Fig. 1B) but, as was also shown in Fig. 1A, this increase was clearly biphasic. Interestingly, the sharp increase in extracellular glucose concentration was seen at a concentration between 3.3 and 9.4 mM, as was also found at a dilution rate of 0.15 h⁻¹.

At a dilution rate of 0.3 h⁻¹, the glucose concentration in the feed medium was increased from 136 to 303 mM. As a consequence of this, the extracellular glucose concentration increased biphasically from 2.0 to 73.8 mM, whereas the glucose flux increased linearly from 9.6 to 16.1 mmol/g/h (Fig. 1C). At this dilution rate, under steady state conditions biomass was ~4.5 g of dry weight/liter, whereas ethanol and glycerol were produced at all glucose feed concentrations used. The changes in flux and concentration at the dilution rates of 0.3 h⁻¹ were quite similar to those presented in Fig. 1, A and B, at dilution rates of 0.15 and 0.2 h⁻¹, but they differed in kinetics. At a dilution rate of 0.3 h⁻¹, the sharp increase in extracellular glucose concentration occurred between 7.5 and 14.0 mM. The increase in flux at a dilution rate of 0.3 h⁻¹ was again steeper than at a dilution rate of 0.15 or 0.2 h⁻¹.

The dependence of the glucose flux rate on the extracellular glucose concentration at different dilution rates is more clearly shown in Fig. 2. At a particular external glucose concentration in the chemostat, the respective glucose flux was higher as the dilution rate increased. From these data, the conclusion is drawn that glucose flux and glucose concentration can be investigated independently of each other. Therefore, this set of experiments at different dilution rates allows discrimination between effects that are a result of an increase in the glucose flux rate or effects that are a result of a change in extracellular glucose concentration.

Expression Levels of SUC2—In S. cerevisiae, invertase is encoded by the glucose-repressible gene SUC2. SUC2 expression is solely regulated by glucose. At very low concentrations SUC2 is induced by glucose (25), and at high glucose concentrations, expression is repressed by glucose (6, 26, 27). Under the conditions used in the continuous culture experiments, the extracellular glucose concentration needed for induction of SUC2 was always attained. Consequently, SUC2 is an excellent reporter gene to study glucose repression in its most basic form. To study the influence of the increase in glucose flux or concentration on glucose repression, SUC2-mRNA expression levels were measured in the steady states of the three experiments at different dilution rates in the experimental set up as described above. Fig. 3 represents Northern blot analyses, which show SUC2 expression levels as a function of the glucose concentration in the feed medium at the particular dilution rates. The level of ACT1 mRNA was used for normalization of the Northern. ACT1 levels were compared with the intensity of the ribosomal RNA bands (data not shown), and no differences were observed between the samples. Therefore we concluded that, under the conditions used, ACT1 mRNA represented a good internal control for the Northern blot analyses. At a dilution rate of 0.15 h⁻¹, SUC2 was expressed at glucose feed concentrations from 196 to 280 mM and repressed at glucose feed concentrations above 310 mM. As the dilution rate was increased to 0.2 h⁻¹, repression was not triggered at glucose feed concentrations from 98 to 287 mM but was observed at glucose feed concentrations above 311 mM. At a dilution rate of 0.3 h⁻¹, SUC2 was expressed when the glucose feed concentrations were below 232 mM. The relationship between these expression levels and the extracellular glucose concentration or on the glucose flux is presented in Fig. 4, in which the SUC2 expression levels are plotted against the extracellular glucose concentration (panel A) and against the glucose flux (panel B). From Fig. 4A it is concluded that SUC2 was expressed (derepressed) under all conditions in a range of glucose concentrations lower than 14 mM, but at glucose concentrations higher than 18 mM SUC2 was always repressed. This implies that glucose repression was only triggered at glucose concentration of 18 mM or higher. A striking observation was that, independent of the dilution rate, repression occurred at the point where the extracellular glucose concentration commenced its sharp increase in the biphasic pattern shown in Fig. 1.

On the other hand no relationship was found between SUC2 expression and the glucose flux (Fig. 4B). For any given value for glucose flux, examples could be found of SUC2 expression or
repression, which implies that the mechanism for glucose repression could be active or inactive at these flux values. These data demonstrate that the flux itself is not important for glucose repression.

**Fructose as a Substrate**—To obtain further indications that the extracellular hexose concentration is important for glucose repression rather than the hexose flux, the cells were grown in a nitrogen-limited continuous culture as described above but using fructose instead of glucose as a carbon source. Fructose is able to induce glucose repression and is taken up by the same transporters as glucose. The affinity of the hexose uptake system for fructose is lower than for glucose (28, 29). This means that when, under steady state conditions at a constant dilution rate, cells are grown at the same extracellular glucose or fructose concentration, this will result in a different flux for glucose than for fructose. This provides an additional approach to discriminate between flux and concentration in relation to their respective role in glucose repression. The fructose concentration in the feed medium was increased from 121 to 309 mM at a constant dilution rate of $D = 0.3 \text{ h}^{-1}$ as described for the former experiments. As soon as a steady state was established, fructose flux, extracellular fructose concentration, and SUC2 expression levels were determined. The increase in fructose feed concentration was accompanied by a linear increase in fructose flux from 7.6 to 15.8 mmol/g/h and a biphasic increase in fructose concentration from 3 to 70 mM (Fig. 5). Fig. 6 shows the accompanying Northern blot analysis, which demonstrates that SUC2 was only expressed at fructose feed concentrations of 121 to 201 mM. According to these data SUC2 was still expressed when the extracellular fructose concentration was 13 mM but repressed at a higher extracellular fructose concentration of 31 mM. The expression pattern corresponded with a derepressive fructose flux of 11.3 mmol/g/h and a repressive fructose flux of 14.1 mmol/g/h. These results are in accordance with the conclusion that extracellular concentrations under 14 mM are derepressive, whereas concentrations above 18 mM are repressive and that the repression can occur at a variety of flux values. Taken together, these data demonstrate that the extracellular (or intracellular) hexose concentration in some way plays a role in glucose repression.

**DISCUSSION**

Glucose regulates carbon metabolism in *S. cerevisiae* by transcription inhibition of a number of genes involved in the utilization of carbon sources other than glucose. The initial signal that induces glucose repression has not yet been identified, but several observations imply that the trigger is most likely to be localized at the level of glucose uptake. This indicates that the glucose flux or the intracellular or extracellular glucose concentration represent possible candidates in the generation of the trigger for glucose repression. Therefore we investigated the role of the glucose flux and the extracellular glucose concentration on glucose repression. In batch cultures it is difficult to accurately determine glucose flux and extracellular glucose concentrations because of the changing environmental conditions, due to the continuous change in parameters such as growth rate, acidification, nutrient concentrations, etc. To eliminate the varying external conditions, yeast cells were grown in a physiologically well defined environment in a nitrogen-limited continuous culture. The nitrogen-limited environment provides the possibility to modulate the extracellular glucose concentration as well as the glucose flux by a step-wise increment of the glucose concentration in the feed medium. Under these conditions the glucose flux increases in a linear manner, whereas the extracellular glucose concentration increases biphasically. By altering the growth rate but maintaining a constant growth rate within one series of experiments, the independent modulation of glucose flux and extracellular glucose concentration can be realized. At the highest dilution rate of $D = 0.3 \text{ h}^{-1}$ the biomass formed in a steady state appeared to be lower than the dry weight at dilution rates of $D = 0.15$ and $0.2 \text{ h}^{-1}$ and was accompanied by a continuous production of ethanol independent of the glucose concentration in the feed medium. The strain used, SU32, is a Crabtree-positive strain (30, 31), meaning that this strain is capable of aerobic fermentation under fully adapted, steady state conditions at high growth rates. Above the critical growth rate it ferments and produces ethanol independently of the glucose feed concentration. The critical dilution rate for SU32 was shown to be $0.275 \text{ h}^{-1}$ (19). Apparently, at a high dilution rate of $0.3 \text{ h}^{-1}$, glucose is converted into biomass and ethanol, independently of the glucose feed concentration, which explains the lower biomass at this dilution compared with the lower dilution rates. However, Sierkstra et al. (19) have shown that the occurrence of the Crabtree effect is not related to glucose repression, whereas SUC2 mRNA is expressed independently of the dilution rate.

The approach described above allows a proper discrimination between effects due to changes in the glucose flux on one hand or changes in the extracellular glucose concentration on the other. Under the steady state conditions, the expression pattern of SUC2 was investigated to determine whether glucose repression was triggered or not. Two different approaches, an increase of the dilution rate and the use of the glucose-repressible carbon source fructose, which has a lower affinity for the hexose uptake system, have shown that the hexose flux is not associated with glucose repression of SUC2. However, a distinct relationship was observed between glucose repression and the extracellular glucose concentration. At all growth rates used, it was shown that glucose-induced repression of SUC2 occurred at an external glucose concentration of 18 mM and higher. At glucose concentrations of 14 mM and lower, SUC2 was derepressed, which means that the mechanism of glucose repression must be activated between 14 and 18 mM extracellular glucose. For a second glucose-repressible carbon source such as fructose, repression was triggered between 13 and 31 mM.

These observations clearly demonstrate that either the extracellular or the intracellular glucose concentration constitute the activation signal of glucose repression. If extracellular glu-
Glucose Repression Is Related to the Glucose Concentration

**Fig. 4.** SUC2 expression is dependent on the extracellular glucose concentration. SUC2 expression levels are plotted against the extracellular glucose concentration (A) or at the glucose flux at different dilution rates: $D = 0.15 \text{ h}^{-1}$ (●), $D = 0.2 \text{ h}^{-1}$ (□), $D = 0.3 \text{ h}^{-1}$ (▲) (B). Expression levels from Fig. 3 were determined by measuring radio signal on the film in a densitometer. Expression levels were corrected for an internal standard, ACT1 mRNA.

**Fig. 5.** The fructose flux and the extracellular fructose concentration as a function of the fructose feed concentration at a dilution rate of $D = 0.3 \text{ h}^{-1}$. Yeast cells were cultivated under nitrogen limitation in a continuous culture under step-wise increasing fructose concentrations in the feed medium as described under “Experimental Procedures.” The growth rate was kept constant by a fixed dilution rate of $D = 0.3 \text{ h}^{-1}$. Under steady state conditions the fructose flux (in mmol/g Vh$^{-1}$) (●) and the extracellular fructose concentration in the chemostat (in mM) (▲) were determined at each fructose feed concentration.

**Fig. 6.** Northern blot analysis of SUC2 mRNA. Cells were continuously grown on step-wise increased fructose concentrations at a dilution rate of $D = 0.3 \text{ h}^{-1}$. At steady state conditions total RNA was isolated, separated on a denaturing agarose gel, and blotted by capillary blotting. RNA was cross-linked to the membrane by exposure to UV. The blot was hybridized with $^{32}P$-labeled oligonucleotides homologous to SUC2 and ACT1 sequences. The level of ACT1 mRNA was used to indicate the relative amount of RNA applied in each lane.

cose concentration is involved, the concentration has to be sensed by a membrane-localized protein and subsequently transmitted to the cytoplasmic side of the plasma membrane. If the intracellular glucose concentration is involved, the underlying molecular mechanism is much more complex. The intracellular glucose concentration is determined by the activity of the glucose transport systems and by the rate of glucose consumption. Expression of the essential glucose- (and fructose-) transporters, i.e., HXT1–7, is also dependent on the extracellular glucose concentration (32–35). Expression of the high affinity HXT2 and HXT4 has been reported in a range around 14–18 mM glucose (32). However, the $K_m$ of the high affinity transport systems (HXT2, HXT4, HXT6, HXT7) ranges around 1–2 mM for glucose and 5–7 mM for fructose (28, 35, 36), and therefore, a regulating role of the high affinity transport systems at 14–18 mM seems unlikely. If transport is in some way involved in glucose repression, only transporters with a higher $K_m$ could be involved. Candidates for this are the low affinity hexose transporters, i.e., the glucose-inducible HXT1, with a $K_m$ of 100 mM, and HXT3, with a $K_m$ of 50 mM, which is expressed at a more or less constitutive level once it has been induced by the presence of glucose. It should be realized in this context that all $K_m$ values for the respective hexose transporters have been determined in mutants in which all essential HXT genes were deleted except one. If, under wild-type conditions, the affinity of a single Hxt protein is influenced by the regulation of other Hxt proteins, it is questionable whether the transport properties in mutants are identical to a wild-type setting. Furthermore, affinity constants for Hxt proteins have been calculated from “in vitro” hexose uptake experiments. Recently, it has been shown that “in vitro” hexose transport kinetics can differ significantly from “in vivo” transport kinetics (18). This difference will obviously also affect the determination of a $K_m$.

These considerations open the possibility for a role of the high affinity transport system in addition to the low affinity transport system in regulation of glucose repression. To investigate the in vivo role of the respective hexose transporters in glucose repression more precisely, we are currently studying the expression levels of the HXT genes in the experiments described above. Furthermore, it would be interesting to study glucose repression in high affinity or low affinity glucose transport mutants in which either all high or low affinity hexose transporters are deleted. At the moment we are constructing such mutants.

Another possibility is that the transport system is not involved in triggering glucose repression. An alternative mechanism would be that the intracellular glucose concentration is sensed by a protein like Snf3p, which is thought to be a regulator of the (high affinity) glucose uptake system (32, 36–39) or Rgt2p, a possible regulator of the low affinity glucose uptake system (40). Both Snf3p and Rgt2p have, unlike the homologous Hxt proteins, an unusual C-terminal domain, which is localized in the cytoplasmic side of the plasma membrane. The C terminus contains several possible phosphorylation consensus sites for different protein kinases, which imply a possible link to further downstream signaling. The determination of intracellular glucose concentration has been shown to be possible in batch cultures$^1$ (41, 42). We are currently investigating the possibilities of the measurement of the intracellular glucose

---

$^1$ M. C. Walsh and K. van Dam, personal communication.
concentrations in a continuous culture in the experimental set-up as described in this paper.

REFERENCES
1. Broach, J. R. (1991) Trends Genet. 7, 28–32
2. Thevelein, J. M. (1991) Mol. Microbiol. 5, 1301–1307
3. Thevelein, J. M. (1992) Antonie van Leeuwenhoek 62, 109–130
4. Thevelein, J. M. (1994) Yeast 10, 1753–1790
5. Hawkins, P. T., Stephens, L. R., and Piggot, J. R. (1993) J. Biol. Chem. 268, 3374–3383
6. Trumbly, R. J. (1992) Mol. Microbiol. 6, 15–21
7. Thevelein, J. M. (1991) Mol. Microbiol. 5, 1301–1307
8. Ronne, H. (1995) Trends Genet. 11, 12–17
9. Johnston, M. (1987) Microbiol. Rev. 51, 458–476
10. Johnston, M., and Carlson, M. (1992) in The Molecular and Cellular Biology of the Yeast Saccharomyces (Jones, E. W., Pringle, J. R., and Broach, J. R., eds) pp. 193-281, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
11. Sierkstra, L. N., Nouwen, N. P., Verbakel, J. M. A., and Verrips, C. Th. (1993) Yeast 9, 787–795
12. Needleman, R. (1991) Mol. Microbiol. 5, 2079–2084
13. Hu, Z., Nehlin, J. O., Ronne, H., and Michels, C. A. (1995) Curr. Genet. 28, 258–266
14. Entian, K. D., Kopetzki, E., Frohlich, K. U., and Mecke, D. (1984) Mol. Gen. Genet. 198, 50–54
15. Ma, H., Bloom, L. M., Walsh, C. T., and Botstein, D. (1989) Mol. Cell. Biol. 9, 5643–5649
16. Rose, M., Albig, W., and Entian, K. (1991) Eur. J. Biochem. 199, 511–518
17. Schaff, I., Heinisch, J., and Zimmermann, F. K. (1989) Yeast 5, 285–290
18. Meijer, M. C., Bonnstra, J., Verklej, A. J., and Verrips, C. Th. (1996) Biochim. Biophys. Acta 1326, 209–216
19. Sierkstra, L. N., Verbakel, J. M. A., and Verrips, C. Th. (1992) J. Gen. Microbiol. 138, 2559–2566
20. Harder, W., and Dijkhuizen, L. (1976) in Continuous Culture 6: Applications and New Fields (Dean, A. C. R., Ellwood, D. C., Evans, C. G., Evans, T., and Melling, J., eds) Ellis Horwood, Chichester, England
21. Fiechter, A., Fuhrmann, G. F., and Kapelli, O. (1981) Adv. Microb. Physiol. 22, 123–183
22. Weusthuis, R. A., Pront, J. T., Van den Broek, P. J. A., and Van Dijken, J. P. (1994) Microbiol. Rev. 58, 616–630
23. Ter Schure, E. G., Stappaerts, J. H. W., Raes, L. J. M., Bonnstra, J., Verklej, A. J., and Verrips, C. Th. (1995) Microbiology (Reading) 141, 1101–1108
24. Oezcan, S., Vallier, L., Flick, J., Carlson, M., and Johnston, M. (1997) Yeast 13, 127–137
25. Neigeborn, L., and Carlson, M. (1984) Genetics 108, 845–858
26. Carlson, M. (1987) J. Bacteriol. 169, 4873–4877
27. Bisson, L. F., and Fraenkel, D. G. (1983) Proc. Natl. Acad. Sci. U. S. A. 30, 1730–1734
28. Bisson, L. F., Coons, D., Krackeberg, A. L., and Lewis, D. A. (1993) Crit. Rev. Biochem. Mol. Biol. 28, 259–308
29. Van Dijken, J. P., and Scheffers, W. A. (1986) FEMS Microbiol. Rev. 32, 199–224
30. Postma, E., Verduyn, C., Scheffers, W. A., and Van Dijken, J. P. (1989) Appl. Environ. Microbiol. 55, 468–477
31. Ozcan, S., and Johnston, M. (1995) Mol. Cell. Biol. 15, 1564–1572
32. Ozcan, S., and Johnston, M. (1996) Mol. Cell. Biol. 16, 5536–5545
33. Reifenberger, E., Freidel, K., and Ciriacy, M. (1995) Mol. Microbiol. 16, 157–167
34. Reifenberger, E., Boles, E., and Ciriacy, M. (1997) Eur. J. Biochem. 245, 324–333
35. Wendell, D. L., and Bisson, L. F. (1993) J. Bacteriol. 175, 7688–7696
36. Ko, C. H., Liang, H., and Gaber, R. F. (1993) Mol. Cell. Biol. 13, 638–648
37. Coons, D. M., Boulton, R. B., and Bisson, L. F. (1995) J. Bacteriol. 177, 3251–3258
38. Walsh, M. C., Smits, H. P., Scholte, M., and Van Dam, K. (1994) J. Bacteriol. 176, 953–958
39. Ozcan, S., Doever, J., Rosenwald, A. G., Wolff, S., and Johnston, M. (1996) Genetics 143, 12428–12432
40. de Koning, W., and Van Dam, K. (1992) Anal. Biochem. 204, 118–123
41. Smits, H. P., Smits, G. J., Postma, P. W., Walsh, M. C., and Van Dam, K. (1996) Yeast 12, 439–447