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Growth and Glucose Repression Are Controlled by Glucose Transport in *Saccharomyces cerevisiae* Cells Containing Only One Glucose Transporter

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A set of *Saccharomyces cerevisiae* strains with variable expression of only the high-affinity Hxt7 glucose transporter was constructed by partial deletion of the *HXT7* promoter in vitro and integration of the gene at various copy numbers into the genome of an *hxt1-7 gal2* deletion strain. The glucose transport capacity increased in strains with higher levels of *HXT7* expression. The consequences for various physiological properties of varying the glucose transport capacity were examined. The control coefficient of glucose transport with respect to growth rate was 0.54. At high extracellular glucose concentrations, both invertase activity and the rate of oxidative glucose metabolism increased manyfold with decreasing glucose transport capacity, which is indicative of release from glucose repression. These results suggest that the intracellular glucose concentration produces the signal for glucose repression.

Metabolism of glucose in *Saccharomyces cerevisiae* proceeds via sugar transport across the plasma membrane and oxidation to pyruvate via the common glycolytic pathway. The flux through these steps determines the rates of fermentation and respiration. The contribution of the individual enzymatic steps of glycolysis to flux through the pathway has been examined in *S. cerevisiae*, with the surprising conclusion that the enzymes can be overexpressed up to 10-fold without significant effects on growth or ethanol production (26, 27). These observations lend support to the proposal (11) that glucose transport limits the rate of glycolysis. Control of flux by the transport step may be more pronounced in cells growing at low glucose concentrations and expressing high-affinity glucose transporters (28).

Evaluation of the degree to which glycolysis in *S. cerevisiae* is limited by glucose transport is complicated by the large number of transporters expressed by that yeast (16). Metabolic control analysis offers both a theoretical basis and a set of experimental approaches for that evaluation. Metabolic control analysis describes the control of flux through a metabolic pathway in terms of the control coefficient of each step. In principle, every step in a pathway shares the control of that pathway; the sum of the control coefficients in a pathway is 1 (7, 15). In order to estimate the control coefficient of an individual step under defined conditions, its activity should be varied by small amounts and the magnitude of the effect of each variation on flux should be measured.

Glucose plays a regulatory role in yeast in addition to its importance as a nutrient. When glucose is available, it is used preferentially to other carbon sources. This is achieved, in part, by transcriptional repression of genes that are required for respiratory metabolism and utilization of other carbon sources (12). The molecular mechanisms of this signal transduction pathway have been described in considerable detail (2, 14). However, the nature of the signal that the cell perceives from glucose in its environment is still unknown.

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The promoter of the *HXT7* gene in plasmid p21 (encoding the high-affinity glucose transporter Hxt7; reference 25) was progressively deleted by exonuclease III deletion (13). Selected deletion mutants were integrated at the *URA3* locus of *S. cerevisiae* KY73 (*MAT*α *hxt1*Δ*::HIS3::*Δ*hxt4 hxt5::LEU2 hxt2*Δ*::HIS3 hxt3*Δ*::LEU2::*Δ*hxt6 hxt7*Δ*::HIS3 gal2*Δ*::DR ura3-52 MAL2 SUC2 MEL*; reference 17). The endpoints of the deletions were determined by DNA sequence analysis, and the locations and copy numbers of the integrated *HXT7* genes were determined by Southern blotting with an *HXT7*-specific oligonucleotide probe (4) and a DNA probe from the *URA3* gene. Isolates with various *HXT7* promoter lengths and copy numbers were selected for further study based on their growth characteristics on solid glucose medium (unpublished data).

Cells of four selected *HXT7* integrant strains and isogenic strains MC996A (wild type; *MAT*α *ura3-52 his3-11,15 leu2-3,112 MAL2 SUC2 GAL MEL*; reference 25) and RE607B (*HXT7* only; *MAT*α *hxt1*Δ*::HIS3::*Δ*hxt4 hxt5::LEU2 hxt2*Δ*::HIS3 hxt3*Δ*::LEU2::*Δ*hxt6 ura3-52 MAL2 SUC2 GAL MEL*; reference 25) were grown in liquid medium containing 1% yeast extract, 2% peptone, and 1% (approximately 55 mM) glucose. Growth was monitored by measurement of the optical density at 600 nm (OD_{600}) at various time points. The residual glucose in the medium at each time point was determined enzymatically (1). Cells were harvested at a residual glucose concentration of approximately 40 mM for the following analyses. Transport of glucose was measured with the 5-s [^{14}C]glucose uptake assay described by Walsh et al. (30). For strains containing only *HXT7*, the glucose concentrations in the assay were 1 and 10 mM; for wild-type strain MC996A, transport was assayed at 10 glucose concentrations ranging from 0.25 to 250 mM. Invertase activity was measured as described by Walsh et al. (29). The rate of oxygen consumption by the cultures was measured in an Oxygraph equipped with a Clark oxygen electrode. Hxt7 protein abundance was estimated by Western blotting of 10-μg samples of whole-cell extracts with anti-Hxt7 antibody (kind gift of E. Boles) as previously described (17) and densitometric scanning of the resulting chemiluminograms. Total cell protein was estimated by the method of Lowry et al. (18) using bovine

TABLE 1. Effect of glucose transport on growth rate and glucose repression of wild-type and Hxt7-only *S. cerevisiae* strains[a]

Strain	*HXT7* promoter length (bp)	*HXT7* copy no.	Growth rate (h^{-1})	Hxt7 expression level (arbitrary units)	Glucose transport capacity[b] (nmol · min^{-1} · mg of $protein^{-1}$)	Invertase activity (nmol · min^{-1} · mg of $protein^{-1}$)	Oxygen consumption rate (nmol · min^{-1} · mg of $protein^{-1}$)	Glucose flux rate[c] (nmol · min^{-1} · mg of $protein^{-1}$)
MC996A	1,148	1	0.39 ± 0.02 (100)	ND[d]	364 ± 26 (100)	183 ± 80 (100)	41 ± 1 (100)	494 ± 56 (100)
LYY4	729	12	0.32 ± 0.03 (82)	100 ± 1	245 ± 22 (67)	229 ± 111 (125)	53 ± 2 (129)	340 ± 1 (69)
LYY0	1,148	2	0.31 ± 0.03 (79)	93 ± 3	217 ± 14 (60)	429 ± 105 (234)	59 ± 4 (144)	309 ± 35 (63)
RE607B	1,148	1	0.30 ± 0.03 (77)	69 ± 3	201 ± 22 (55)	430 ± 108 (235)	61 ± 8 (149)	328 ± 4 (66)
LYY8	507	1	0.27 ± 0.01 (69)	50 ± 1	195 ± 9 (54)	973 ± 2 (532)	75 ± 6 (184)	234 ± 21 (47)
LYY16	179	8	0.11 ± 0.02 (28)	ND	27 ± 1 (7)	1406 ± 163 (768)	144 ± 13 (351)	47 ± 2 (10)

[a] Values are means ± standard deviations. Values represent at least two independent experiments as described in the text. The glucose concentrations of these cultures varied from 30 to 47 mM at the time of harvest. Values in parentheses are percentages of the wild-type value.

[b] The transport capacity of the strains is the maximal velocity determined by fitting the zero-*trans* influx rates to the Michaelis-Menten equation, with a K_m of 16 mM for the wild-type strain and a K_m of 2.2 mM for the *HXT7*-only strains.

[c] Estimation of the glucose flux rate was based on the total consumption of extracellular glucose (millimolar concentration of glucose per unit of OD_{600}) and the rate of growth at the time of harvest. The mean protein concentration was 0.3 (range, 0.25 to 0.35) mg · ml^{-1} · $OD_{600}U^{-1}$.

[d] ND, not detectable.

serum albumin as the standard. The results of these analyses are shown in Table 1.

Glucose transport exerts a high level of control over growth. Wild-type strain MC996A grew faster than all other strains. At this stage of growth, Hxt7 protein was not detected in the wild-type strain; *HXT7* expression is low in wild-type *S. cerevisiae* at glucose concentrations of >20 mM (data not shown; see also reference 4). For the *HXT7*-only strains, the growth rate correlated well with the level of Hxt7 protein expressed.

The glucose transport capacity of these strains was also correlated with the growth rate. When the logarithm of the growth rate is plotted against the logarithm of the V_{max} for glucose transport (Fig. 1), the data fall on a straight line with a slope of 0.54 ± 0.04 (R^2 = 0.96). According to metabolic control analysis theory, the slope of the line produced by plotting a flux versus a catalytic activity in double-logarithmic space is equal to the control coefficient of that activity over that flux (8).

These results are consistent with previous reports that glucose transport exerts a high level of control over growth and glycolytic flux by *Saccharomyces*. When maltose was used to inhibit glucose transport in wild-type *S. bayanus*, it was found that the control coefficient ranged from 0.5 to 1, depending on the extracellular glucose concentration (5). In another study, the coefficients of glucose transport control over glycolytic flux in nongrowing *S. cerevisiae* cell suspensions were 0.64 at pH 5.5 and 0.83 at pH 4.5; under the same conditions, the control coefficients for phosphofructokinase were 0.10 and 0.12, respectively (9). The control coefficient for phosphofructokinase (often considered to be "the rate-limiting step of glycolysis" [11]) has been calculated by other investigators to be 0.3 (3, 8).

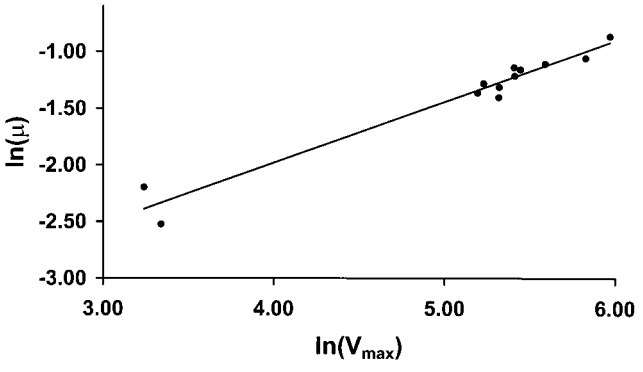

FIG. 1. Control of growth rate by glucose transport. The rate of growth of the wild-type MC996A strain and strains expressing only *HXT7* to various levels is plotted as a function of the maximal velocity of glucose transport (logarithmic scales). Cells were harvested during exponential phase, 8 h after inoculation (OD_{600}, 0.4 to 1.1; residual glucose, 30 to 47 mM).

Glucose transport affects glucose repression. The status of glucose repression in these cultures was determined by measuring their invertase activity and oxygen consumption rate. The strains with reduced glucose transport capacity expressed higher levels of invertase activity (Table 1). Similarly, the specific oxygen consumption rate was inversely correlated with transport capacity. The invertase assay used here measures the total cellular activity of this enzyme. Using standard culture conditions for repression and derepression of secreted invertase (23), we found that the repressed level of total invertase in the wild-type MC996A strain was 361 nmol · min^{-1} · mg of $protein^{-1}$, and the derepressed level is 3,897 nmol · min^{-1} · mg of $protein^{-1}$. By comparison with Table 1, these values demonstrate that invertase was fully repressed at the highest glucose uptake capacities and was significantly derepressed at the lowest uptake capacity.

Lower levels of glucose transport activity in yeast have previously been found to diminish glucose repression. In *Kluyveromyces lactis* strains containing two low-affinity glucose transporter genes, endogenous β-galactosidase activity was fully repressed during growth on glucose. Null mutations of either gene resulted in partial derepression of β-galactosidase, and in a double null mutant strain, the activity was completely derepressed (31). In *S. cerevisiae*, the dominant mutations *HTR1-23* and *DGT1-1* resulted in decreased levels of *HXT* gene expression and glucose transport activity. Both mutations alleviated glucose repression of enzymes such as invertase, maltase, malate dehydrogenase, glutamate dehydrogenase, and cytochrome *c* oxidase (10, 22). However, it was not resolved whether the reduced repression levels were consequences of the mutations or of the reduced glucose transport activities. In an *S. cerevisiae* strain with null mutations in *HXT1* to *HXT7*, glucose repression of maltase was completely relieved. In related strains with single *HXT* genes, the extent of glucose repression was strongly correlated with the glucose consumption rate of the strain. In particular, increasing the copy number of *HXT1* stepwise from 1 to 3 in this *hxt* null strain increased the glucose consumption rate and decreased the maltase activity (24).

In contrast to these results that suggest that the flux of

glucose into the cell determines the degree of glucose repression, Meijer et al. (20) found that repression of the *SUC2* gene was dependent on the external glucose concentration and was fully derepressed at glucose concentrations of <14 mM. In contrast, the level of *SUC2* expression was independent of the glucose flux.

Mutations of *HXK2*, encoding hexokinase II, also lead to relief of glucose repression (6, 19, 21). It has been pointed out that intracellular glucose is the metabolite that links glucose transport and hexokinase and that the intracellular glucose concentration is a likely signal for the glucose repression pathway (28).

We observed that at lower rates of transport, a higher fraction of glucose was oxidized via the respiratory pathway (Table 1). Therefore, the effect on the growth rate of the decrease in glucose uptake was partly compensated for by a difference in glucose metabolism, with relatively more glucose being metabolized by oxidative phosphorylation (which generates more ATP per mole of glucose) at low rates of glucose uptake. These results demonstrate that glucose transport plays important roles in determining the relative activities of the fermentative and respiratory pathways of glucose metabolism, both by delivering glucose across the plasma membrane to the glycolytic pathway and by influencing the glucose repression status of various metabolic activities.

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