Molecular and Cellular Biology, Mar. 1995, p. 1564–1572 Vol. 15, No. 3
0270-7306/95/$04.00+0
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Three Different Regulatory Mechanisms Enable Yeast Hexose Transporter (*HXT*) Genes To Be Induced by Different Levels of Glucose

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Received 17 August 1994/Returned for modification 11 October 1994/Accepted 8 December 1994

The *HXT* genes (*HXT1* to *HXT4*) of the yeast *Saccharomyces cerevisiae* encode hexose transporters. We found that transcription of these genes is induced 10- to 300-fold by glucose. Analysis of glucose induction of *HXT* gene expression revealed three types of regulation: (i) induction by glucose independent of sugar concentration (*HXT3*); (ii) induction by low levels of glucose and repression at high glucose concentrations (*HXT2* and *HXT4*); and (iii) induction only at high glucose concentrations (*HXT1*). The lack of expression of all four *HXT* genes in the absence of glucose is due to a repression mechanism that requires Rgt1p and Ssn6p. *GRR1* seems to encode a positive regulator of *HXT* expression, since *grr1* mutants are defective in glucose induction of all four *HXT* genes. Mutations in *RGT1* suppress the defect in *HXT* expression caused by *grr1* mutations, leading us to propose that glucose induces *HXT* expression by activating Grr1p, which inhibits the function of the Rgt1p repressor. *HXT1* expression is also induced by high glucose levels through another regulatory mechanism: *rgt1* mutants still require high levels of glucose for maximal induction of *HXT1* expression. The lack of induction of *HXT2* and *HXT4* expression on high levels of glucose is due to glucose repression: these genes become induced at high glucose concentrations in glucose repression mutants (*hxk2*, *reg1*, *ssn6*, *tup1*, or *mig1*). Components of the glucose repression pathway (Hxk2p and Reg1p) are also required for generation of the high-level glucose induction signal for expression of the *HXT1* gene. Thus, the glucose repression and glucose induction mechanisms share some of the same components and may share the same primary signal generated from glucose.

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In the yeast *Saccharomyces cerevisiae*, glucose is a key metabolite that has significant effects on the expression of many genes: expression of a large number of genes is repressed by glucose; expression of others is induced (for review, see reference 21). Glucose repression and glucose induction of gene expression act mainly at the level of transcription.

Many genes required for the metabolism of alternate carbon sources (galactose, maltose, sucrose, glycerol, and ethanol) are repressed during growth on glucose. Several genes are required for glucose repression (*HXK2*, *REG1*/*HEX2*, *GAL82*, *GAL83*, *GRR1*, *TUP1*, *SSN6*/*CYC8*, and *MIG1*); two genes are necessary for derepression of glucose repressible genes (*SNF1* and *SNF4*) (for reviews, see references 13, 18, 21, and 46). A central component of the glucose repression mechanism is Mig1p, which binds to sites in the promoters of several glucose-sensitive genes and is thought to recruit Ssn6p and Tup1p to form a repressor complex (23, 52). The *SNF1* gene encodes a protein kinase that, together with Snf4p, appears to inhibit Mig1p function (8, 22, 31). *HXK2* encodes hexokinase PII, one of the three glucose-phosphorylating enzymes that has been implicated as an intracellular sensor for glucose (37). Reg1p may be involved in the transmission of the glucose repression signal to Snf1p, and Gal83p (and possibly Gal82p) probably acts in association with Snf1p (7a, 14).

It is not clear where Grr1p acts in the glucose repression pathway. Mutations in *GRR1* not only cause loss of glucose repression, but also result in several other pleiotropic defects, including elongated cell morphology, reduced growth rate on glucose, impaired expression of hexose transporter genes, increased sensitivity to osmotic stress and nitrogen starvation, resistance to divalent cations, and possible reduction in transport of aromatic amino acids (10, 16, 35, 47, 48). The glucose repression defect and the growth defect of *grr1* mutants are suppressed by mutations in *RGT1* (14), which also suppress the high-affinity glucose transport defect of *snf3*Δ mutants (28).

The expression of many genes involved in glucose utilization is induced during growth on glucose. The glycolytic genes *ENO2*, *PGK1*, *PYK1*, *PDC1*, and *ADH1* are inducible by glucose about 2- to 30-fold at the transcriptional level (29), and glucose-induced expression of some of the *HXT* genes, encoding hexose transporters, has been reported (4, 34, 35, 45, 50). In contrast to glucose repression, the components of the glucose induction pathway have not been extensively characterized. High-level expression of the glycolytic genes requires several *trans*-acting factors, including the DNA-binding proteins Gcr1p, Rap1p, Reb1p, and Abf1p, but how, or if, these proteins respond to glucose is unknown.

To begin to understand how glucose induces gene expression in yeast cells and to identify the components of this signal transduction pathway, we studied the glucose-induced expression of four different hexose transporter genes (*HXT1* to *HXT4*). Previous results suggested that expression of some of these genes is induced by glucose (4, 34, 35, 50). We discovered that expression of these genes exhibits three distinct types of glucose induction in response to different levels of glucose. A central component of all three mechanisms of glucose induction is Rgt1p, which we propose is a repressor of *HXT* gene expression that is inhibited by Grr1p.

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TABLE 1. List of yeast strains used in this study

Strain	Relevant genotype
YM2061	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801*
YM4127	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501*
YM2955	*MATα ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 grr1Δ::LEU2*
YM4428	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 snf3Δ::HIS3*
YM4509	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 rgt1Δ*
YM4510	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 grr1Δ::LEU2 rgt1Δ*
YM4511	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 snf3Δ::HIS3 rgt1Δ*
YM4512	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 hxk2Δ::LEU2*
YM4513	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 tup1Δ::TRP1*
YM4552	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 reg1Δ::LEU2*
YM4553	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 mig1Δ::LEU2*
YM4554	*MAT***a** *ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 ssn6Δ::ura3::LEU2*
MCY1551[a]	*MAT***a** *ura3-52 ade2-101 lys2-801 leu2::HIS3 snf1Δ3*

[a] Obtained from M. Carlson; see reference 8.

MATERIALS AND METHODS

Strains and growth media. The yeast strains used in this study are listed in Table 1. Yeast cells were grown on standard media: YEP (2% Bacto Peptone [Difco], 1% yeast extract [Difco]) or YNB (0.67% yeast nitrogen base [Difco]) supplemented with the appropriate amino acids and containing either 4% glucose, 2% galactose, 2% raffinose, or 5% glycerol as the carbon source (43). Plasmids were transformed into yeast cells by the freeze method (11). *Escherichia coli* DH5αF′ was used as a host for all plasmids.

Construction of plasmids and of disruption mutants. The *HXT* and *SNF3* promoters, up through the ATG, without any coding region, were fused to *lacZ* by using the vector YEp357R (30). For the *HXT1::lacZ* fusion (pBM2636), the 1.3-kb *Hin*dIII-*Eco*RI fragment of the regulatory region was used (26). Plasmid pBM2819, containing the *HXT3::lacZ* construct, was created by using the 1.8-kb *Bam*HI-*Eco*RI fragment of the *HXT3* upstream region (24). *HXT2* and *HXT4* promoter fusions to *lacZ* (pBM2717 and pBM2800) were constructed by cloning PCR products (primers: OM562-OM572 for *HXT2*, +1 to −618, and OM583-OM609 for *HXT4*, +1 to −946) with either an *Eco*RI site (starting at ATG) or a *Bam*HI site (25, 45). The *SNF3* promoter *lacZ* fusion (pBM2982) was constructed by subcloning the 0.86-kb *Sal*I-*Bam*HI fragment of the upstream region into the vector YEp357R. Plasmid pBM2773, which contains a *SUC2::lacZ* fusion, was constructed in two steps. First, the *SUC2* promoter region from −135 to −784 was inserted in front of the *HIS3* gene (starts at −79) by using the vector pBM1530 (15), yielding pBM2755. The 1.04-kb *Bam*HI-*Hin*dIII fragment of pBM2755, which contains the *SUC2* promoter and a part of the *HIS3* gene (codon 102, *Hin*dIII site), was then fused in frame to *lacZ* by using the vector YEp356 (30). pBM2639, which contains the entire *HXT1* gene in Bluescript (4-kb *Hin*dIII fragment [26]), was used in sequencing reactions.

All disruptions except for *grr1Δ::LEU2* (pBM1829 [16]) and *snf1Δ3* (MCY1551 [8]) were constructed by using the wild-type strain YM4127 (see Table 1). The plasmids used to disrupt genes involved in glucose repression are as follows: pBM1966 containing *reg1Δ::LEU2* (J. Flick), pMR226 (*hxk2Δ::LEU2* [37]), pBM2861 (*rgt1Δ::hisG-URA3-hisG*), pJS22 (*ssn6-Δ6::URA3* [41]), pBM2677 (*snf3Δ::HIS3*), pFW36 (*tup1Δ::TRP1* [51]), and pJN22 (*mig1Δ::LEU2* [32]). The *URA3* gene in the *ssn6Δ::URA3* strain was disrupted in a second step with the *ura3::LEU2* gene by using the plasmid pJH672 (obtained from Jim Haber). All of these gene disruptions produce null alleles.

β-Galactosidase assays. β-Galactosidase activity was assayed in permeabilized cells grown to mid-log phase as described previously (15). Activities are given in Miller units and are the averages of three to six assays of two to three independent transformants. Cells were pregrown on YNB-2% galactose lacking uracil and transferred to YNB-medium containing 4% glucose, 2% galactose, or 2% raffinose without uracil. For determination of glucose dependence of induction (Fig. 1), cells were pregrown on YNB-2% galactose without uracil until early log phase and transferred to YNB-2% galactose with different concentrations of glucose (0 to 8%). After incubation for 4 h at 30°C, the cultures were assayed for β-galactosidase activity. The data presented are the average values of two independent cultures. Derepression of *HXT* genes in *snf1* mutants was determined by incubating glucose-grown cells in either 4% glucose or 2% raffinose for 3 to 4 h.

RNA analysis. Total yeast RNA was isolated as described previously (12). Transcripts were detected on Northern (RNA) blots by using ^{32}P-labeled strand-specific RNA probes. The riboprobes were synthesized with T7 RNA polymerase by an in vitro runoff transcription reaction as described by Selleck and Majors (42). The template for the *HXT1* probe was a 0.83-kb *Eco*RI-*Hpa*I fragment cloned into the *Eco*RI-*Sma*I site of pT7-4 (pBM2651) and cut with *Ssp*I prior to RNA synthesis. The *HXT2* riboprobe was synthesized by using the plasmid pBM2668 cut with *Hpa*I (contains the 1.2-kb *Eco*RI-*Hin*dIII fragment of the *HXT2* coding region in pT7-4). Plasmid pBM2962 contains the 0.78-kb *Eco*RI-*Hpa*I fragment of the *HXT3* coding region in pT7-4 and was used as a template to synthesize the *HXT3* riboprobe. *LEU2* was used as an internal control for loading; the template for the *LEU2* probe (pBM1117) was described previously (15). Primer extension analysis was carried out with 5′-end-labeled oligonucleotides +28/+51 for *HXT1* (OM808) and +13/+40 for LEU2 (OM28 [1]) with 50 μg of total RNA per reaction, as described previously (38). The primer extension products were separated on a 6% polyacrylamide–7.0 M urea gel.

RESULTS

Expression of the *HXT* genes is inducible by glucose. To determine whether expression of the hexose transporter genes is inducible by glucose at the transcriptional level, we fused the regulatory regions of all four genes (up to the ATG) to the reporter gene *lacZ* on a plasmid and monitored expression in cells grown on different carbon sources. The results, shown in Table 2, reveal three patterns of regulation of these four genes. First, *HXT1* expression is induced about 300-fold by high levels (4%) of glucose. Second, *HXT2* and *HXT4* expression is induced 8- to 10-fold on low levels (0.1%) of glucose and on raffinose. Third, expression of *HXT3* is inducible by glucose about 10-fold, independent of the sugar concentration. Expression of *HXT2*, *HXT3*, and *HXT4* is somewhat higher on raffinose than on 0.1% glucose. We believe that this is because cleavage of the trisaccharide raffinose (galactose-glucose-fructose) by invertase likely provides continuously low levels of sugar (fructose) for induction, whereas low levels of glucose are rapidly consumed. For subsequent assays, we used raffinose

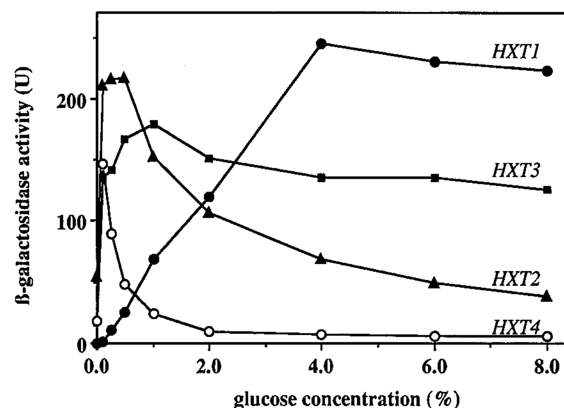

FIG. 1. Induction of *HXT* gene expression as a function of extracellular glucose concentration. Cells were pregrown to early log phase on YNB–2% galactose without uracil and transferred to YNB–2% galactose medium with various concentrations of glucose (0.1 to 8%). After 3 h of incubation, cultures were assayed for β-galactosidase activity. Symbols: ●, *HXT1*; ▲, *HXT2*; ■, *HXT3*; ○, *HXT4*.

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TABLE 2. Regulation of *HXT* gene expression in wild-type cells[a]

Relevant genotype	Mean β-galactosidase activity (Miller units) ± SD				
	5% glycerol	2% galactose	2% raffinose	2% galactose + 0.1% glucose[b]	4% glucose
HXT1::*lacZ*	0.7 ± 0.07	0.6 ± 0.1	1.4 ± 0.28	1.6 ± 0.35	254 ± 27
HXT2::*lacZ*	44 ± 5	21 ± 2.4	195 ± 20	145 ± 14	32 ± 3
HXT4::*lacZ*	51 ± 2.8	19 ± 3.2	230 ± 12	163 ± 9	8 ± 2.4
HXT3::*lacZ*	29 ± 1.7	18 ± 3.1	160 ± 17	116 ± 10	210 ± 26

[a] Precultures were grown on YNB medium lacking uracil with 2% galactose and transferred to minimal medium without uracil containing the indicated sugars and grown until mid-log phase before assay.

[b] Similar levels of expression were obtained when glycerol was substituted for galactose.

as a source of low levels of hexose and galactose as noninducing media.

Induction as a function of glucose concentration. As shown in Table 2, expression of the *HXT* genes responds to different levels of glucose. To determine more precisely the dependence of induction of each *HXT* gene on external glucose concentration, we monitored the expression of all four genes in cells growing on different glucose concentrations (Fig. 1). *HXT1* expression increases linearly with increasing concentrations of glucose and achieves full induction at 4% glucose. Expression of *HXT2* is fully induced at initial glucose concentrations ranging from 0.1 to 0.5%. Glucose concentrations above 0.75% reduce *HXT2* expression until 4% glucose, at which *HXT2* expression reaches the basal level. Thus, expression of *HXT2* is repressed at high glucose concentrations. Similar results were also obtained for *HXT4*, which seems to be more sensitive to repression by high levels of glucose. *HXT3* expression is induced at glucose concentrations between 0.1 and 1% and decreases only slightly at higher glucose levels. Thus, these four *HXT* genes exhibit three different types of regulation by glucose: (i) induction at all glucose concentrations (*HXT3*), (ii) induction only at high levels of glucose (*HXT1*), and (iii) induction at low levels and repression at high levels of glucose (*HXT2* and *HXT4*).

RNA analysis of *HXT* gene expression. To confirm the regulation of the *HXT* genes by glucose observed with *HXT*::*lacZ* constructs, we detected the transcripts of the chromosomal copy of each *HXT* gene in cells growing on different carbon sources. Consistent with the β-galactosidase assays, *HXT2* mRNA is abundant only in cells grown on raffinose and is repressed by high glucose concentrations (Fig. 2). As expected, transcription of the *HXT3* gene is induced in cells grown on both low and high levels of glucose. *HXT1* expression is maximal in cells grown on high glucose, but the levels in cells grown on low glucose are unexpectedly high (see below). In any case, none of the *HXT* transcripts are detectable in cells grown without glucose (on galactose), confirming that expression of these genes is induced by glucose. Compared with the *LEU2* transcript, the levels of the *HXT1*, *HXT2*, and *HXT3* transcripts are moderately high, suggesting that these *HXT* genes are relatively highly expressed in the cell.

The similar levels of *HXT1* transcript in cells grown on high glucose and on raffinose (Fig. 2) are inconsistent with the observed regulation of the *HXT1*::*lacZ* construct. Since there are at least 11 *HXT*-related genes whose sequences are highly similar (about 60 to 85% identical [4, 45]), the strong signal

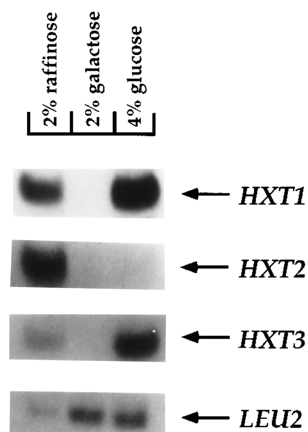

FIG. 2. Northern blot analysis of *HXT* gene expression. RNA was prepared from the wild-type strain YM2061 grown on YNB medium containing either 4% glucose, 2% galactose, or 2% raffinose. Total RNA (20 μg per lane) was separated on a 1% agarose gel, blotted to a membrane, and probed for *HXT1*, *HXT2*, *HXT3*, and *LEU2* transcripts.

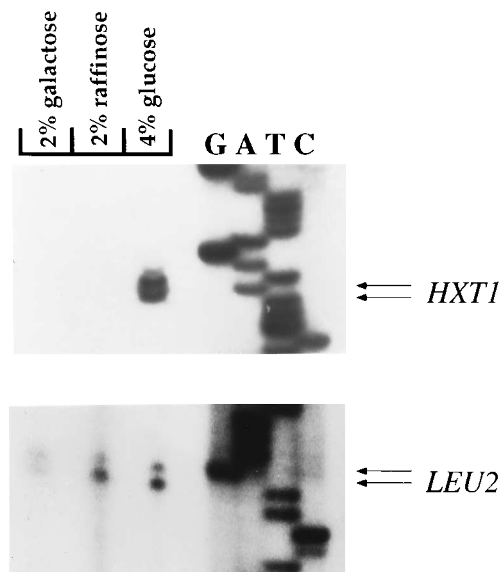

FIG. 3. Primer extension analysis of *HXT1* transcript on different carbon sources. The same oligonucleotide primer (OM808) used in primer extension was also used in sequencing reactions with an *HXT1* DNA template (pBM2639). The level of *LEU2* transcript was also analyzed by primer extension to demonstrate that approximately equal amounts of total RNA were used in each reaction. The major RNA start site for the *LEU2* gene under repressing conditions (in the presence of leucine) is at position −16 from the initiation codon AUG (1).

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TABLE 3. *HXT* gene expression in *grr1*, *snf3*, and *rgt1* mutants

Relevant genotype	Mean β-galactosidase activity[a] (U) ± SD											
	HXT1			*HXT2*			*HXT3*			*HXT4*		
	Gal	Raf	Glu	Gal	Raf	Glu	Gal	Raf	Glu	Gal	Raf	Glu
Wild type	0.6 ± 0.1	1.4 ± 0.28	254 ± 27	21 ± 2.4	195 ± 20	32 ± 3	18 ± 3.1	160 ± 17	210 ± 26	19 ± 3.2	230 ± 12	8 ± 2.4
grr1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	20 ± 2.0	18 ± 0.5	18 ± 1.0	7 ± 2	15 ± 5	59 ± 9	1.2 ± 0.7	1.5 ± 0.5	8 ± 0.5
grr1 rgt1	12 ± 0.5	14 ± 1.5	194 ± 22	249 ± 25	302 ± 28	23 ± 1.0	200 ± 24	271 ± 37	161 ± 14	264 ± 34	295 ± 42	28 ± 1.4
rgt1	14 ± 1.8	14 ± 1.4	42 ± 5	191 ± 17	334 ± 48	20 ± 2.5	287 ± 86	514 ± 46	152 ± 36	203 ± 15	330 ± 23	15 ± 1.5
snf3	0.4 ± 0.08	0.3 ± 0.07	195 ± 15	24 ± 1.8	38 ± 5	31 ± 1.5	7 ± 1.7	47 ± 7	172 ± 37	11 ± 1.0	44 ± 0.7	6 ± 1.3
snf3 rgt1	14 ± 2.0	18 ± 2.5	50 ± 8	207 ± 23	325 ± 18	26 ± 4.5	258 ± 21	362 ± 13	162 ± 12	238 ± 14	362 ± 67	12 ± 1.2

[a] Gal, 2% galactose; Raf, 2% raffinose; Glu, 4% glucose.

obtained on raffinose with the *HXT1* probe is likely due to cross-hybridization with mRNA of genes induced at low glucose concentrations (*HXT2*, *HXT3*, *HXT4*, and probably others). To avoid this problem, we carried out a primer extension analysis of *HXT1* transcripts with an oligonucleotide primer with minimal homology to other known *HXT* genes (Fig. 3). The results reveal that *HXT1* is indeed highly expressed only in the presence of high glucose (4%), consistent with the β-galactosidase data. Two start sites are apparent for *HXT1* transcription, at −42 and −43. Analysis of *HXT2*, *HXT3*, and *HXT4* regulation by primer extension verified the regulation by glucose observed by using *HXT*::*lacZ* constructs and Northern blots (data not shown).

***GRR1* and *RGT1* are involved in glucose induction.** *grr1* mutants have a number of pleiotropic defects, including loss of glucose repression of the *SUC2*, *GAL*, and *MAL* genes (16, 35, 47) and reduced glucose transport (35, 48). The impaired glucose transport of *grr1* mutants is probably due to reduced expression of several *HXT* genes in this mutant (34, 35, 48). To determine the importance of Grr1p for glucose-induced gene expression, we assayed expression of all four *HXT* genes in a *grr1* mutant. As shown in Table 3, glucose-induced expression of all *HXT* genes is severely impaired in *grr1* mutants. Thus, Grr1p is required for induction of *HXT* gene expression by both high and low levels of glucose. Surprisingly, *HXT3* expression is still inducible to some extent by high concentrations of glucose in the absence of Grr1p function, while induction by low levels of glucose is completely abolished (Table 3).

Mutations in *RGT1* suppress the glucose repression defect in *grr1* mutants (14) and also the glucose transport defect of *snf3* mutants (28). The fact that mutations in *RGT1* restore high-affinity glucose transport in *grr1* mutants (48) suggested that Rgt1p functions primarily in regulation of glucose transport. Indeed, the glucose induction defect of the *HXT2*, *HXT3*, and *HXT4* genes in *grr1* mutants is completely suppressed by the *rgt1* mutation (Table 3). In addition, the expression of *HXT2*, *HXT3*, and *HXT4* is constitutive (nearly fully induced on galactose) in *rgt1* mutants (Table 3). These data show that Rgt1p plays a negative role in expression of the *HXT2*, *HXT3*, and *HXT4* genes and reveal that glucose induction of these *HXT* genes is due to a repression mechanism that requires Rgt1p.

The defect in *HXT1* expression on high levels of glucose caused by the *grr1* mutation is also suppressed by *rgt1* (Table 3). However, while *HXT1* expression in *rgt1* mutants growing on galactose is 20-fold higher than in wild-type cells, it is not fully induced (Table 3). *HXT1* expression in a *grr1 rgt1* double mutant is induced a further 15-fold by 4% glucose. This result implies that another regulatory pathway that is independent of Rgt1p contributes to the glucose induction of *HXT1* expression. The *RGT1*-dependent pathway is responsible for about 20-fold induction of *HXT1* expression (compare the wild type and *grr1 rgt1* in Table 3 for galactose), while the Rgt1p-independent pathway seems to be responsible for about 15-fold induction (compare Gal and Glu for *grr1 rgt1* in Table 3). It is noteworthy that both regulatory pathways working together are predicted to result in about 300-fold induction of *HXT1* expression (15 times 20), which is what we observe in wild-type cells (compare Gal and Glu for the wild type in Table 3). *HXT1* expression in *rgt1* cells growing on high glucose is unexpectedly low, suggesting that Rgt1p also has a positive role in *HXT1* expression. We do not understand why the requirement for Rgt1p for maximal levels of *HXT1* expression is relieved by a *grr1* mutation (compare *grr1 rgt1* and *rgt1* in Table 3 for Glu).

***SNF3* is required for induction by low levels of glucose.** *SNF3* encodes a glucose transporter similar to yeast and mammalian glucose transporters (7). Like *GRR1*, deletion of the *SNF3* gene results in an inability to grow on raffinose or low levels of glucose (when respiration is blocked) due to the loss of high-affinity glucose transport (7, 47). The similar phenotypes of *snf3* and *grr1* mutants (defective in high-affinity transport and impaired growth on low levels of glucose) raised the possibility that Snf3p could be required for glucose induction of *HXT* expression. Snf3p is indeed required for full induction of *HXT* expression on low levels of glucose (Table 3): induction of *HXT2*, *HXT3*, and *HXT4* is strongly diminished in *snf3* mutants. By contrast, expression of the *HXT1* and *HXT3* genes induced by high levels of glucose is not significantly reduced. These data clearly demonstrate the requirement of Snf3p for induction of *HXT* gene expression by low levels of glucose.

***SNF3* is weakly expressed.** *SNF3* expression is about 500-fold weaker than for the *HXT* genes (Table 4). Importantly, *SNF3* expression is not induced by glucose but instead is repressed by high glucose (Table 4). Consistent with this observation, glucose repression of the *SNF3* gene is relieved in *grr1* and *mig1* mutants (Table 4). In contrast to the *HXT* genes, expression of *SNF3* does not require Grr1p as a positive regulator. Thus, we conclude that expression of the *SNF3* gene is

TABLE 4. Expression of the *SNF3* gene on different carbon sources

Relevant genotype	Mean β-galactosidase activity (U) ± SD		
	2% galactose	2% raffinose	4% glucose
Wild type	0.23 ± 0.02	0.37 ± 0.05	0.03 ± 0.01
grr1	4.1 ± 0.7	1.9 ± 0.3	4.9 ± 0.8
mig1	0.5 ± 0.09	1.5 ± 0.1	1.3 ± 0.2
rgt1	0.32 ± 0.3	0.41 ± 0.5	0.015 ± 0.004
grr1 rgt1	0.86 ± 0.1	0.7 ± 0.2	0.64 ± 0.08

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TABLE 5. Expression of the *HXT* genes in various glucose repression mutants[a]

Relevant genotype	Mean β-galactosidase activity[b] (U) ± SD											
	HXT1			*HXT2*			*HXT3*			*HXT4*		
	Gal	Raf	Glu	Gal	Raf	Glu	Gal	Raf	Glu	Gal	Raf	Glu
Wild type	0.6 ± 0.1	1.4 ± 0.28	254 ± 27	21 ± 2.4	195 ± 20	32 ± 3	18 ± 3.1	160 ± 17	210 ± 26	19 ± 3.2	230 ± 12	8 ± 2.4
hxk2	0.13 ± 0.04	0.6 ± 0.07	40 ± 4	22 ± 3	43 ± 7	109 ± 6	18 ± 0.3	67 ± 12	288 ± 43	15 ± 1.5	89 ± 30	88 ± 6
reg1	1.0 ± 0.1	1.4 ± 0.1	42 ± 7	44 ± 9	202 ± 22	179 ± 12	42 ± 6	196 ± 26	265 ± 19	37 ± 6	263 ± 31	272 ± 16
mig1	0.3 ± 0.1	2.3 ± 0.3	221 ± 37	23 ± 3	235 ± 15	82 ± 14	14 ± 2	285 ± 86	628 ± 43	47 ± 5	301 ± 36	436 ± 52
ssn6	221 ± 40	214 ± 31	461 ± 50	281 ± 23	385 ± 26	488 ± 67	288 ± 25	411 ± 38	938 ± 162	339 ± 76	553 ± 43	785 ± 56
tup1	2.1 ± 0.4	3.6 ± 0.6	133 ± 13	72 ± 17	226 ± 16	253 ± 24	94 ± 11	215 ± 14	546 ± 55	70 ± 9	270 ± 13	433 ± 59

[a] All the glucose repression genes were disrupted in the same genetic background (YM4127 [Table 1]).
[b] See Table 3, footnote *a*.

not under the control of the glucose induction pathway that regulates expression of the *HXT* genes.

The low level of *SNF3* expression relative to *HXT* expression is inconsistent with a role for Snf3p as a major glucose transporter, but instead suggests a regulatory role for this protein. This is consistent with our observation that it is required for induction of *HXT* gene expression by low levels of glucose.

***HXT* gene expression is affected in various glucose repression mutants.** The fact that *GRR1* is required for glucose induction of the *HXT* genes and for glucose repression raises the possibility that the glucose induction and the glucose repression mechanisms could share components. To test this idea, we assayed expression of all four *HXT* genes in various glucose repression mutants. It has been suggested that Hxk2p, one of the three hexose kinases, functions as an intracellular sensor for glucose. Thus, we were interested to know if Hxk2p is required for glucose induction of *HXT* gene expression. As shown in Table 5, derepression of all four *HXT* genes is reduced about three- to sixfold in *hxk2* mutants. Thus, Hxk2p is required for full induction of *HXT* genes by both high and low levels of glucose. Expression of *HXT2* and *HXT4* in *hxk2* mutants is about fivefold higher on high levels of glucose, confirming that both genes are indeed glucose repressed. In contrast to *hxk2* mutants, mutations in the *REG1* gene seem only to affect induction by high levels of glucose (Table 5). While expression of *HXT2*, *HXT3*, and *HXT4* on raffinose is not changed, *HXT1* expression on high glucose is reduced about fivefold in the *reg1* mutant, suggesting that Reg1p and Hxk2p proteins are both required for the high-glucose-induction pathway. As expected, glucose repression of *HXT2* and *HXT4* is relieved by the *reg1* mutation. Surprisingly, induction of the *HXT3* gene at high glucose concentrations is independent of *REG1* and *HXK2* function, suggesting that *HXT3* expression and *HXT1* expression may be regulated differently.

Mig1p is a repressor of glucose-repressed genes (*SUC2*, *GAL1*, and *GAL4* [19, 32]). Thus, it is not surprising that the *mig1* mutation relieves glucose repression of *HXT2* and *HXT4* expression (Table 5). Consistent with our other data (Fig. 2 and Table 2), *HXT4* expression seems to be more stringently glucose repressed than that of the *HXT2* gene. While glucose induction of *HXT1* expression is not significantly affected by the *mig1* mutation, expression of *HXT3* is increased about threefold on high glucose in the *mig1* mutant, suggesting that *HXT3* may be somewhat sensitive to glucose repression.

Ssn6p and Tup1p form a complex that functions as a general repressor that is required for glucose repression (23, 52). Expression of all four *HXT* genes is constitutive in *ssn6* mutants (Table 5), suggesting that Ssn6p, in addition to Rgt1p, is required for repression in the absence of glucose. As expected, glucose repression of *HXT2* and *HXT4* expression is relieved in *ssn6* strains (Table 5). *HXT3* expression on high glucose is about fourfold higher in the *ssn6* background, again suggesting that *HXT3* expression may be weakly glucose repressed. Another interesting result is that *HXT1* expression is fully induced (on galactose) in an *ssn6* strain (221 U) and much higher than in an *rgt1* mutant (14 U). This indicates that Ssn6p not only is required for Rgt1p-dependent repression in the absence of glucose but is also involved in the Rgt1p-independent induction mechanism that operates on *HXT1*. Furthermore, it suggests that the Rgt1p-independent induction is due to a repression mechanism. Surprisingly, the effect of the *tup1* mutation on *HXT* expression is different from that of *ssn6* (Table 5). While glucose repression of *HXT2* and *HXT4* is relieved in the *tup1* mutant, repression in the absence of glucose is only partially relieved. Perhaps Ssn6p has a different partner when acting (presumably with Rgt1p) as a repressor of glucose-induced genes.

***HXT* expression in *snf1* mutants.** The *SNF1* (*CAT1*/*CCR1*) gene encodes a serine/threonine protein kinase that is essential for release from glucose repression (8, 9, 39). Consistent with the role of Snf1p in glucose repression, induction of the glucose-repressible hexose transporter genes *HXT2* and *HXT4* by low levels of glucose is completely abolished in *snf1* mutants (Table 6). Thus, expression of *HXT2* and *HXT4*, like that of *SUC2*, requires a functional Snf1p. This is likely due to constitutive repression by Mig1p, which is normally inhibited by Snf1p in the absence of glucose (22, 31). Induction of the non-glucose-repressible *HXT1* and *HXT3* genes is not significantly decreased in *snf1* mutants, suggesting that Snf1p does not function in the glucose induction pathway.

TABLE 6. Derepression of the *HXT* genes in *snf1* mutants[a]

lacZ reporter	Mean β-galactosidase activity (U) ± SD			
	Wild type		*snf1*	
	2% raffinose	4% glucose	2% raffinose	4% glucose
HXT1		253 ± 32		195 ± 8
HXT2	181 ± 9		18 ± 2	
HXT3	235 ± 12	274 ± 27	150 ± 23	233 ± 17
HXT4	199 ± 16		6 ± 1	
SUC2	15 ± 2		<0.1	

[a] Cells were pregrown on YNB–4% glucose lacking uracil to mid-log phase, harvested, washed, and transferred to either YNB–4% glucose (*HXT1* and *HXT3*) or YNB–2% raffinose (*HXT2*, *HXT3*, and *HXT4*) without uracil. After 4 h of incubation, derepression of the *HXT* genes was scored by determination of β-galactosidase activity. The *snf1Δ3* strain (MCY1551; see Table 1 and reference 8) was kindly provided by M. Carlson. The construction of the *SUC2::lacZ* fusion used in this study is described in Materials and Methods.

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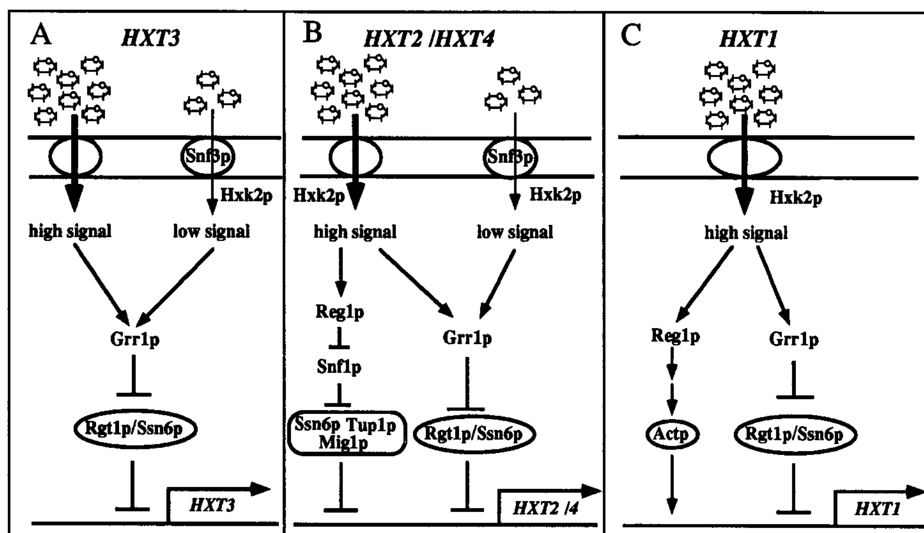

FIG. 4. Schematic representation of the three different mechanisms of induction by different levels of glucose. A line with an arrowhead implies positive regulation; a line with a bar denotes negative regulation. (A) *HXT3*; (B) *HXT2* and *HXT4*; (C) *HXT1*.

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DISCUSSION

Expression of the several yeast hexose transporter (*HXT*) genes is inducible by glucose. We identified three different patterns of glucose-induced expression of *HXT1*, *HXT2*, *HXT3*, and *HXT4*. The simplest type of regulation is exhibited by *HXT3*: its expression is inducible by glucose independent of the sugar concentration. *HXT1* expression is maximally induced only at high levels of glucose; *HXT2* and *HXT4* expression is induced by low levels of glucose and repressed at high concentrations of glucose.

Three different regulatory mechanisms acting separately or together on these genes can account for these three patterns of regulation. The induction of *HXT2*, *HXT3*, and *HXT4* by low levels of glucose appears to be due to a repression mechanism in which Rgt1p plays a major role as a repressor. Genetic evidence suggests that Grr1p is an inhibitor of Rgt1p (14). Our results lead us to suggest that in the presence of glucose, Grr1p is activated and inhibits the Rgt1p repressor (Fig. 4). In the absence of glucose (on galactose), the positive regulator Grr1p is inactive and therefore unable to inactivate Rgt1p, which represses expression of all four *HXT* genes. Consistent with this model, expression of all four *HXT* genes is severely impaired in *grr1* mutants and is constitutive in *rgt1* mutants (Table 3). Since Rgt1p contains a zinc finger DNA-binding domain (25a), we imagine that it mediates repression in the absence of glucose directly by binding to the upstream regulatory regions of the *HXT* genes, or possibly indirectly by repressing the expression of their activators. We further propose that Grr1p function is activated by low as well as high levels of glucose. Thus, the induction of *HXT3* expression independent of the glucose concentration can be accounted for only by the Rgt1p-mediated regulatory mechanism (Fig. 4A). Recently, it was demonstrated that upstream regulatory regions of all four *HXT* genes, when present on multicopy vectors, can suppress the growth defect of *snf3*Δ mutants on low glucose, probably by titrating a negative regulator of *HXT* genes (34, 45). These promoter elements (called DDSE, for DNA sequence-dependent suppressing element) possibly contain binding sites for the Rgt1p repressor.

In contrast to *HXT3*, expression of *HXT2* and *HXT4* is not inducible at high glucose concentrations because of glucose repression. Thus, both genes are inducible by high glucose levels in mutants defective in glucose repression (*hxk2*, *reg1*, *tup1*, *ssn6*, or *mig1*) (Table 5) and uninducible in *snf1* mutants, which are defective in glucose derepression (Table 6). Since *mig1* mutations relieve the glucose repression of *HXT2* and *HXT4* expression, we propose that the Mig1p repressor, which is known to be required for glucose repression (32), acts directly upon these promoters (Fig. 4B). Alternatively, glucose repression of *HXT2* and *HXT4* expression could be indirect, possibly due to glucose repression of a gene required for their expression (such as the glucose sensor). Snf1p is apparently required to inactivate Mig1p (8, 22, 31, 40), which would account for the inability of *snf1* mutants to express *HXT2* and *HXT4*. Thus, in our model, *HXT2* and *HXT4* expression is prevented in the absence of glucose by Rgt1p and at high glucose levels by Mig1p. Only at low levels of glucose are both Rgt1p and Mig1p inactive, leading to maximum gene expression. It is noteworthy that *HXT4* is more strongly glucose repressed than *HXT2* (Table 5 and Fig. 2). Perhaps *HXT4* contains more Mig1p binding sites in its promoter.

The regulatory mechanism responsible for induction of *HXT1* expression at high glucose levels is more complex (Fig. 4C). In addition to the Grr1p/Rgt1p-dependent pathway, *HXT1* expression appears to be subject to another regulatory mechanism that responds to high levels of glucose. We reach this conclusion because of our observation that, in the absence of Rgt1p, *HXT1* expression is still inducible by high glucose concentrations (Table 3). We imagine that high levels of glucose are required for maximal function of the activator of *HXT1* (or some other protein required for *HXT1* expression). Thus, in our model, *HXT1* expression is prevented in the absence of glucose by Rgt1p and at low glucose concentrations by an inactive transcriptional activator. Only at high glucose concentrations are both Rgt1p inactive and the activator functioning. Another possibility is that this second regulatory mechanism is due to yet another repression mechanism that involves Ssn6p and is inactivated by high levels of glucose. In addition, Rgt1p also seems to be required for full induction of *HXT1* expression at high glucose concentrations (Table 3, compare the wild type and *rgt1* for *HXT1* on Glu) and hence may play an

additional role as an activator of *HXT1* expression under these conditions.

The Ssn6 and Tup1 proteins have been proposed to form a complex that acts as a general repressor by being recruited to diverse promoters by several different DNA-binding proteins. Ssn6p and Tup1p are required for glucose repression mediated by Mig1p as well as for repression of cell-type-specific genes by the α2 repressor (23) and of oxygen-regulated genes by the Rox1p repressor (2). Our results suggest that Rgt1p is yet another DNA-binding protein that mediates repression by Ssn6p and Tup1p, since *ssn6* and *tup1* mutations relieve repression of the *HXT* genes (Table 5). However, the requirement for Tup1p for Rgt1p-mediated repression appears to be less stringent than for repression mediated by the other DNA-binding proteins: *tup1* mutations cause only partial relief of repression of *HXT2*, *HXT3*, and *HXT4* on galactose (Table 5), while *ssn6* mutations lead to fully derepressed levels under the same conditions (Table 5). Surprisingly, *tup1* mutations have very little effect on repression of *HXT1* expression (Table 5). Perhaps Ssn6p has a different partner when acting through Rgt1p. However, mutations in *TUP1* are known to affect plasmid stability and result in a decreased copy number, and this may account for these unexpected results. Note that both *SSN6* and *TUP1* are required for glucose repression of *HXT2* and *HXT4*, consistent with our model that they work through Mig1p to effect glucose repression of these genes.

Induction of *HXT2*, *HXT3*, and *HXT4* expression by low glucose concentrations requires the glucose transporter protein Snf3p (Table 3), which we suggest functions as a glucose sensor (Fig. 4A and B). The expression of the *SNF3* gene is regulated in a manner different from that of the *HXT* genes: it is repressible by high glucose concentrations and is not controlled by the Rgt1p-dependent pathway (Table 4). The requirement for Snf3p for expression of glucose transporters at low glucose concentrations probably explains why *snf3* mutants exhibit greatly reduced high-affinity glucose transport and grow poorly on low levels of glucose (in the presence of antimycin A [7]). The fact that mutants with deletions of all four *HXT* genes are unable to grow on low levels of glucose (in the absence of respiration) even if the *SNF3* gene is present suggests that Snf3p is not a major glucose transporter, consistent with the idea that it fulfills a regulatory function (24). Also consistent with this view of Snf3p function is the low level of *SNF3* expression (about 500 times weaker than that of the *HXT* genes; Table 4) (4). It is possible, however, that this regulatory function of Snf3p is dependent on its metabolic role as a glucose transporter. We note that *HXT2*, *HXT3*, and *HXT4* expression is still modestly induced by low levels of glucose in *snf3* mutants, suggesting that some other transporter partially fulfills the role of the glucose sensor. There must be another glucose transporter(s) that functions as a sensor of high glucose levels, since induction of *HXT1* and *HXT3* expression by high levels of glucose is not significantly affected in *snf3* mutants. The finding that Snf3p is not involved in sensing high levels of glucose is not surprising, given the fact that expression of the *SNF3* gene itself is about 5- to 10-fold repressible by glucose (Table 4) (33).

The induction of expression of *HXT2*, *HXT3*, and *HXT4* by low levels of glucose is significantly reduced in *hxk2* mutants (Table 5). This raises the possibility that Hxk2p could play a role in sensing low levels of glucose, in addition to its role in the glucose repression pathway (37). The reduced expression of low-glucose-induced genes in *hxk2* mutants is perhaps not surprising, since a mutant unable to phosphorylate glucose (*hxk1 hxk2 glk1*) lacks high-affinity glucose transport (5, 6). While previous kinetic analysis of glucose transport in *snf3* and in glucose kinase mutants (*hxk1*, *hxk2*, or *glk1*) supports the idea that the hexose kinases interact directly with hexose transporter proteins and modulate their affinity for glucose (4, 6, 24), the decreased expression of *HXT2*, *HXT3*, and *HXT4* in *hxk2* mutants suggests that the kinase dependence of high-affinity glucose transport may also be due to transcriptional regulation of genes that encode high-affinity glucose transporters. It is interesting in this regard that the sole hexokinase of *Kluyveromyces lactis* (encoded by *RAG5*) is essential for glucose-induced transcription of the *RAG1* gene, which encodes a low-affinity glucose transporter (36).

HXK2 and *REG1* are also required for full induction of *HXT1* expression by high levels of glucose, suggesting that Hxk2p and Reg1p are required for the Rgt1p-independent glucose induction mechanism that we propose operates on *HXT1*. Therefore, both the glucose repression and glucose induction pathways seem to have some of the same primary components and may respond to the same primary signal derived from glucose. By contrast, other components of the glucose repression mechanism that act more directly on glucose-repressed genes (Snf1p and Mig1p) do not affect the high-glucose-induction pathway. While both *HXK2* and *REG1* are clearly required for normal induction of *HXT1*, we do not understand why these same genes are not required for induction of *HXT3* expression by high levels of glucose (Table 5). Perhaps another regulatory mechanism contributing to the induction of *HXT3* expression remains to be revealed.

Grr1p, in addition to being required for glucose-induced expression of all four *HXT* genes, is also essential for glucose repression (16). This suggests that there is a tight connection between the glucose repression and glucose induction pathways. The loss of glucose repression in *grr1* mutants is likely due to impaired glucose induction of the *HXT* genes, which reduces transport of glucose (35, 48), thereby preventing generation of the glucose repression signal. Consistent with this idea is the observation that mutations in *RGT1* restore both glucose-induced expression of the *HXT* genes (48) and glucose repression in *grr1* mutants (14).

The different regulation of the various *HXT* genes may reflect their roles during growth on different glucose concentrations. We predict that *HXT2* and *HXT4*, being inducible at low concentrations of glucose, encode high-affinity glucose transporters. Consistent with this view are the results of kinetic analysis of glucose transport, which revealed a glucose-repressible high-affinity uptake system for glucose (3). We imagine that *HXT1*, which is maximally expressed at high glucose concentrations, encodes a low-affinity glucose transporter.

In addition to their function of providing the cell with glucose for metabolism, some of the Hxt proteins could also have a regulatory function as initial sensors of glucose and therefore be essential for both the glucose repression and glucose induction pathways. We speculate that Snf3p, which may be among the only glucose transporters expressed in the absence of glucose, is the initial sensor of glucose. In this view, glucose added to a culture of cells growing without glucose would be initially transported by Snf3p, which would lead to induction of *HXT2*, *HXT3*, and *HXT4* expression. The increased glucose influx provided by these (and possibly other) transporters would ultimately lead to induction of *HXT1* expression. If Hxt1p is indeed a low-affinity, high-capacity transporter, it could provide the cell with high levels of glucose for rapid growth and would lead to glucose repression of the presumably high-affinity, low-capacity glucose transporters encoded by *HXT2*, *HXT4*, and *SNF3* (and possibly others).

The glucose induction pathway in *S. cerevisiae*, which regulates the expression of hexose transporter (*HXT*) genes, is in

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some aspects similar to the pathway in mammalian cells, which results in glucose-induced expression of insulin. The hormone insulin stimulates transcription and secretion of the glucose transporter Glut4p to the cell surface (44). The glucose-induced expression of insulin occurs in β-pancreatic cells and requires the glucose transporter Glut2p, which has been implicated as a sensor for glucose (20). In addition, this mammalian glucose induction pathway also requires a glucokinase as a component of the glucose-sensing apparatus (17). Glut2p and glucokinase function in mammalian cells thus shares similarities with the proposed function of the Snf3p and Hxk2p in yeast cells. Perhaps the mechanisms of glucose sensing and signal transduction in yeast and mammalian cells are similar.

ACKNOWLEDGMENTS

We thank Tony Leong for providing information about the sequence of *RGT1* and M. Rose and K.-D. Entian for the *HXK2* disruption plasmid. We thank M. Ciriacy for sharing information prior to publication and for helpful discussions. We thank the members of the Johnston laboratory for helpful discussions.

This work was supported by NIH grant GM32540. S.Ö. is supported by a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft (DFG).

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