

Function and Regulation of Yeast Hexose Transporters

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INTRODUCTION

Glucose is the preferred carbon and energy source for most cells. In addition to being a major nutrient, glucose can act as a “growth hormone” to regulate several aspects of cell growth, metabolism, and development. How a eukaryotic cell senses glucose and signals its presence, how this signal affects cellular processes, and how optimal utilization of the sugar is achieved are fundamental, unanswered questions. Defects in glucose sensing, signaling, and metabolism cause the severe and prevalent metabolic disorders in mammals known as diabetes. Thus, it is of major interest to understand these processes.

The first and limiting step of glucose metabolism is its transport across the plasma membrane. Thus, it is not surprising that in many different kinds of cells glucose ensures its own efficient metabolism by serving as an environmental stimulus that regulates the quantity, types, and activity of glucose transporters, both at the transcriptional and post-translational levels. This review focuses on regulation of expression and function of the several hexose transporter (*HXT*) genes of the baker's yeast *Saccharomyces cerevisiae*, which has proven useful for studying the mechanisms of glucose sensing, signaling, and

utilization in a eukaryotic cell. It is now apparent that multiple pathways regulate the transcription of several members of the *HXT* gene family in response to different levels of extracellular glucose. Two members of the glucose transporter gene family (*RGT2* and *SNF3*) seem to encode proteins that function not as transporters but as sensors of extracellular glucose that generate an intracellular signal for glucose-induced transcription of the *HXT* genes. These mechanisms of transcriptional regulation of the *HXT* genes by glucose are the major focus of this review. Several recent reviews focus on other aspects of yeast glucose transporters (12, 18, 28, 53, 75, 77).

HEXOSE TRANSPORTER PROTEINS

Function and Transport Kinetics

Saccharomyces cerevisiae has 20 genes that encode proteins similar to glucose (hexose) transporters (*HXT1* to *HXT17*, *GAL2*, *SNF3*, and *RGT2*) (12, 18, 28, 75). These Hxt proteins belong to the major facilitator superfamily (MFS) of transporters (89, 117). *S. cerevisiae* has the largest number of MFS transporters of any organism. MFS proteins transport their substrates by passive, energy-independent facilitated diffusion, with glucose moving down a concentration gradient (12). Because many prokaryotic and mammalian sugar transporters are MFS members, studies performed with the yeast hexose transporters will be valuable in understanding the structure, func-

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tion, and regulation of glucose transporters from a wide variety of other organisms.

Two uptake systems were described in *S. cerevisiae*: a constitutive, low-affinity system (high K_m , 15 to 20 mM) and a glucose-repressed, high-affinity system (low K_m , 1 to 2 mM) (8, 9, 11, 12, 125). It now seems clear that low-affinity and high-affinity glucose transport represent the sum of several transporters rather than being the result of individual transporters. None of these transporters are essential for growth on glucose, indicating their functional redundancy. The presence of multiple hexose transporters with different affinities for glucose in baker's yeast is not surprising, given the fact that it grows well on a broad range of glucose concentrations (from a few μM to 2 M). Indeed, the amount of glucose available dictates the expression of the appropriate glucose transporters by closely regulating *HXT* gene expression.

Because of the large number of functionally redundant *HXT* genes the organism possesses, it was difficult to isolate mutants of yeast defective in glucose uptake. The isolation of the first yeast mutants defective in glucose transport (13), and the recognition that they are defective in a gene (*SNF3*) encoding a protein similar to glucose transporters of mammalian cells (25), stimulated further efforts to identify yeast glucose transporters. Molecular insight into glucose transporter function and regulation was forthcoming once the *HXT1* to *HXT4* hexose transporter genes were isolated (72, 76, 79, 140), but most of these genes were not recognized until the sequence of the yeast genome was completed (2, 75). Of the 20 members of the *HXT* gene family, only 7 are known to encode functional glucose transporters. A strain lacking these seven *HXT* genes (*HXT1* through *HXT7*, called the *hxt* null mutant [*hxt1* Δ -*hxt7* Δ]) is unable to grow on glucose, fructose, or mannose and has no glycolytic flux (18, 82, 128). Introduction of any one of the seven *HXT* genes into the *hxt* null mutant is sufficient to allow it to grow on glucose. *HXT2*, *HXT6*, or *HXT7* are sufficient for growth on 0.1% glucose, suggesting that they encode high-affinity transporters. *HXT1*, *HXT3*, or *HXT4* enable growth only on higher glucose concentrations (more than 1%), suggesting that these genes encode low-affinity glucose transporters (128). *GAL2*, which encodes a galactose transporter similar to the Hxt proteins (100, 145), is also able to complement the glucose growth defect of the *hxt* null mutant (82), consistent with the finding that galactose-grown cultures transport glucose with the same affinity as glucose-grown cells (see references cited in reference 28). The fact that the *hxt* null mutant does not grow on glucose indicates that the remaining 10 *HXT* genes (*HXT8* through *HXT17*) encode proteins that either are unable to transport glucose or are not expressed under the conditions tested. Indeed, we observed very low level expression of genes *HXT8* to *HXT17* (see Table 3).

S. cerevisiae cells express only the glucose transporters appropriate for the amount of extracellular glucose available. This is due to the combined action of different regulatory mechanisms, including transcriptional regulation of various *HXT* genes in response to extracellular glucose (109, 110, 157), and to inactivation of Hxt proteins under certain conditions (18, 54, 74, 124, 130). Also, modulation of the affinity of certain glucose transporters for glucose and interaction between different transporters may contribute to the ability of yeast cells to adapt to different extracellular glucose concentrations. Based on measurements of the kinetics of glucose transport, it has been proposed that the rate of glucose transport (V_{max}) is constant and that only the affinity of the transporter for glucose changes when cells are shifted from a high to low levels of glucose (154). From this it was suggested that expression of the transporters is constitutive and that regulation of glucose

transport is mediated by a factor that modifies their affinity for glucose (154). However, as described below, it is clear that transcription of the genes encoding the metabolically relevant glucose transporters (*HXT1* to *HXT7*) is regulated by glucose (12, 18, 82, 109, 136). While the affinity of the different transporters for glucose may be modified posttranslationally, transcriptional regulation of the *HXT* genes is undoubtedly a major reason why the kinetics of glucose transport varies in response to the amount of glucose available.

Correlation between Transporter Regulation and Function

The way in which the *HXT* genes are transcriptionally regulated in response to glucose is consistent with their function as low- or high-affinity transporters. *HXT1* transcription is induced only by high concentrations of glucose, suggesting that it encodes a low-affinity (high K_m) transporter. Indeed, expression of *HXT1* in the *hxt* null mutant restores growth only on high concentrations of glucose (more than 1%) and provides low-affinity glucose transport ($K_m = 100$ mM) (128). *HXT3* expression is induced by both low and high levels of glucose, and it confers relatively low-affinity glucose transport ($K_m = 60$ mM) on the *hxt* null mutant, suggesting that it is a low-affinity glucose transporter. Thus, Hxt1 and Hxt3 are probably responsible for transporting glucose in cells growing on high concentrations of glucose (18, 128). *HXT2*, *HXT6*, and *HXT7* expression, on the other hand, is induced only by low concentrations of glucose, suggesting that they encode high-affinity transporters. Indeed, *HXT2*, *HXT6*, and *HXT7* enable the *hxt* null mutant to grow on low glucose concentrations (around 0.1%). *HXT6* and *HXT7* behave as high-affinity glucose transporters ($K_m = 1$ to 2 mM) when expressed in the *hxt* null mutant, while *HXT2* confers on this mutant biphasic uptake kinetics ($K_m = 1.5$ mM and $K_m = 60$ mM) (128). Perhaps the affinity of the Hxt2 transporter is modulated in response to different glucose concentrations, or perhaps Hxt2 has a regulatory role in the *hxt* null mutant and activates the expression of other transporters with low and high affinities for glucose. In any case, it is likely that Hxt2, Hxt6, and Hxt7 are responsible for transporting glucose when it is scarce. It is unclear why yeast cells need several transporters with the same affinity.

The transcriptional regulation of *HXT4*, however, is inconsistent with its predicted function. Its induction only by low levels of glucose suggests that it encodes a high-affinity transporter, but *HXT4* does not restore growth of the *hxt* null mutant on low concentrations of glucose (5 mM = 0.09%), and encodes a protein with intermediate affinity for glucose ($K_m = 9$ mM) (128). This inconsistency could be explained if transcriptional regulation of the *HXT* genes was altered in the *hxt* null mutant. It is known that the *hxt* null mutant, which is completely impaired for glucose uptake, is defective in glucose repression of *SUC2* (128). It is possible that glucose repression of *HXT4* is also abolished in the *hxt* null mutant. Alternatively, *HXT4* could have a role in regulating expression of the *HXT* genes remaining in this mutant, so the glucose transport properties of the *hxt* null mutant expressing *HXT4* could be due to other Hxt proteins.

Since most of the data on the kinetics of glucose transport mediated by the individual Hxt proteins was obtained by their expression in the *hxt* null mutant, the results do not necessarily reflect the *in vivo* function of these Hxt transporters. If the affinity of some of the Hxt proteins is modulated by their interaction with other transporters, a single Hxt protein might behave differently in this mutant than in a wild-type strain. In addition, some of the missing *HXT* genes could be necessary for regulation of other *HXT* genes. Thus, vigilance is required

TABLE 1. Regulation of *HXT1* to *HXT4* gene expression by different levels of glucose^a

Relevant genotype	Mean β -galactosidase activity (Miller units)		
	2% galactose	2% galactose + 0.1% glucose	4% glucose
<i>HXT1::lacZ</i>	0.6	1.6	254
<i>HXT2::lacZ</i>	21	145	32
<i>HXT3::lacZ</i>	18	116	210
<i>HXT4::lacZ</i>	19	163	8

^a Data are from reference 109.

in interpreting the results obtained from expression of individual hexose transporters in this strain.

TRANSCRIPTIONAL REGULATION OF *HXT* GENE EXPRESSION BY GLUCOSE

Initial studies suggested that expression of some *HXT* genes is regulated by glucose (12, 113, 115, 156, 157). Indeed, transcription of *HXT1* through *HXT4* is between 10- and 300-fold induced by glucose, depending on the gene (109) (Table 1). These four *HXT* genes exhibit three different types of regulation by glucose: (i) induction by glucose independent of sugar concentration (*HXT3*), (ii) induction only by low levels of glucose (*HXT2* and *HXT4*), and (iii) induction only by high concentrations of glucose (*HXT1*).

These three responses to glucose are due to the action of three overlapping regulatory pathways (Fig. 1). First, glucose induction of all four genes is due to a repression mechanism mediated by the Rgt1 repressor, which inhibits expression of the *HXT* genes in the absence of glucose. Both low and high concentrations of glucose induce *HXT* transcription by inhibiting Rgt1 repressor function. The Grr1 protein is required for glucose inhibition of Rgt1 function. The intracellular glucose signal responsible for Grr1-mediated inhibition of Rgt1 repressor function seems to be generated by Snf3 and Rgt2, two glucose transporter-like proteins that serve as glucose sensors for low and high concentrations of glucose, respectively. Second, *HXT2* and *HXT4* are subject to glucose repression, me-

diated by the Mig1 repressor, which acts upon many other glucose-repressed genes, and the Snf1 protein kinase, which regulates Mig1 function. Superimposition of this regulatory pathway upon the Rgt1-mediated pathway at the *HXT2* and *HXT4* promoters results in these genes being induced only by low concentrations of glucose. Third, maximal *HXT1* expression requires a high glucose-induced mechanism whose components have not yet been identified. Coupling of this regulatory pathway with the Rgt1-mediated mechanism causes *HXT1* to be expressed only in cells growing on high levels of glucose. In the next sections we describe in detail these regulatory mechanisms and how they lead to the three different responses to glucose.

Expression of genes encoding glycolytic enzymes and ribosomal proteins are also induced by glucose. However, this involves pathways that are different from the pathway responsible for glucose induction of the *HXT* genes: different regulatory proteins act upon these two types of genes, and glycolytic metabolites seem to be responsible for glucose-induced transcription of the glycolytic genes (16, 47, 98), while glucose metabolism is not required for the induction of the *HXT* genes by glucose (114).

HXT3: Induction of Transcription by Glucose Independent of Sugar Concentration

Mutations in *HXT3* were identified as suppressors of the potassium uptake defect caused by mutation of the genes encoding potassium transporters (*TRK1* and *TRK2*) (presumably due to symport of potassium ions and glucose) (72). *HXT3* also suppresses the growth defect of *snf3* mutants on raffinose when overexpressed (12, 72). Expression of *HXT3* is induced about 10-fold by both low and high concentrations of glucose (Fig. 1A) (109). This is because only the Rgt1-mediated regulatory pathway seems to act upon *HXT3*: deletion of *RGT1* causes constitutive (glucose-independent) expression of *HXT3*. Grr1 is required for glucose induction of *HXT3*, and thus plays a positive role in *HXT3* induction, by inactivating the Rgt1 repressor. The modest (about threefold) increase in *HXT3* expression caused by inactivation of various genes involved in glucose repression (such as *mig1*, *snf6*, and *tup1*) suggests that

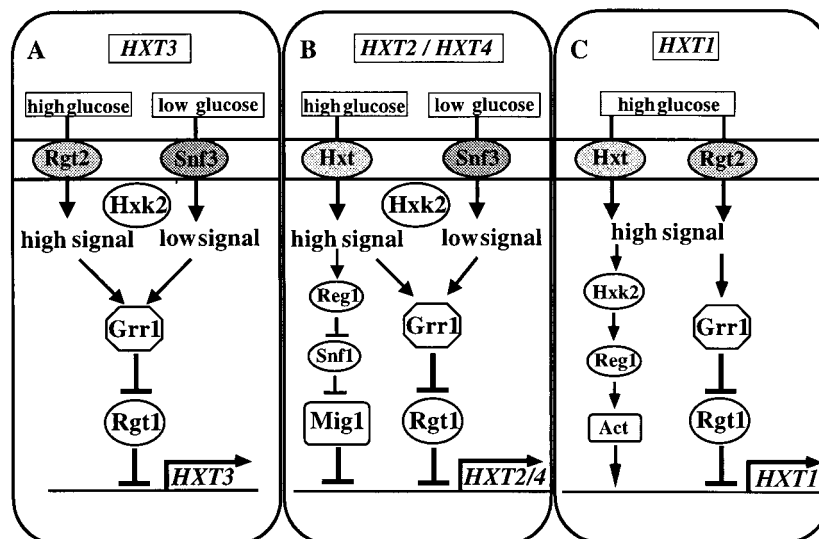


FIG. 1. Three different modes of induction of *HXT* gene transcription by different levels of glucose. An arrow implies positive regulation; a line with a bar denotes negative regulation.

glucose repression plays a minor role in regulating *HXT3* expression (109). The *HXT3* promoter contains several potential binding sites for Mig1, suggesting that this modest repression may be mediated directly by Mig1.

It has been reported that *HXT3* expression is maximal when cells enter the stationary-growth phase (72). While it is an interesting possibility that a stationary-phase-specific regulatory mechanism may act upon *HXT3*, our current knowledge about glucose induction of *HXT3* expression leads us to believe that this is due to repression of *HXT3* expression by the initial high levels of glucose (4%) in the medium into which the cells were inoculated. As the cells consume the glucose, the extracellular concentration decreases and reaches an optimal concentration where *HXT3* transcription is maximally induced.

***HXT2* and *HXT4*: Induction of Transcription by Low Levels of Glucose**

HXT2 and *HXT4* were cloned as multicopy suppressors of the high-affinity glucose uptake defect of a *snf3* Δ mutant (12, 76, 140). *HXT4* was also obtained in a screen for *S. cerevisiae* genes that can complement the low-affinity glucose uptake defect of the *Kluyveromyces lactis rag1* mutant (121). *HXT4* is the only *HXT* gene that can complement the galactose growth defect of a *gal2* mutant (121), suggesting that Hxt4 has relaxed substrate specificity.

HXT2 and *HXT4* are expressed at a low level in cells growing either in the absence of glucose or on high glucose concentrations. Their expression is induced approximately 10-fold by low levels of glucose or fructose (0.1% or 5.6 mM), and by raffinose (a trisaccharide, consisting of fructose-glucose-galactose, that is equivalent to low glucose, because most laboratory strains of *S. cerevisiae* can only inefficiently cleave the fructose-glucose bond [catalyzed by invertase] and thus obtain only low levels of fructose from it) (Fig. 1B). The induction of *HXT2* transcription by low levels of glucose correlates with a 20-fold increase in protein levels, indicating that the transcriptional regulation is indeed reflected in the amount of protein produced (156). This regulation seems physiologically relevant, because it causes these high-affinity glucose transporters to be made only when they are most useful to the cell (i.e., when glucose is scarce).

This pattern of regulation is due to the action of two independent repression mechanisms (Fig. 1B). In the absence of glucose Rgt1 binds to the *HXT2* and *HXT4* promoters and represses their expression. At high concentrations of glucose, their expression is repressed by the Mig1 repressor (99, 101, 109, 110), which is responsible for repression of many glucose-repressed genes, such as *SUC2* and *GAL* (45, 63, 133, 144). Only at low concentrations of glucose (~0.05 to ~0.4%) are both repressor proteins inactive, resulting in expression of *HXT2* and *HXT4*. Deletion of *RGT1* causes *HXT2* and *HXT4* to be expressed in the absence of glucose but has no effect on Mig1-mediated repression of these genes at high glucose concentrations. Conversely, deletion of *MIG1* causes expression of *HXT2* and *HXT4* to become inducible by high levels of glucose but has no effect on Rgt1-mediated repression in the absence of glucose. Thus, the combination of the two different repression mechanisms results in an 8- to 10-fold induction of *HXT2* and *HXT4* expression only by low levels of glucose (0.1%). The regulation of *HXT2* and *HXT4* provides a simple example of how multiple regulatory proteins that respond differently to environmental signals can combine to provide a specific and unique pattern of gene expression.

Repression by Rgt1 and Mig1 requires Ssn6 and Tup1 (109), which form a complex that functions as a general repressor of

TABLE 2. Expression of *HXT1* and *HXT2* in *rgt1*, *mig1*, *snf1*, and *ssn6* mutants^a

Relevant genotype	Mean β -galactosidase activity (Miller units)					
	<i>HXT1</i>			<i>HXT2</i>		
	Gal	Gal + Glu	Glu	Gal	Gal + Glu	Glu
Wild type	0.6	1.6	254	21	195	32
<i>rgt1</i>	14	14	42	191	334	20
<i>mig1</i>	0.3	2.3	221	23	235	82
<i>snf1</i>	ND	ND	195	ND	18	ND
<i>ssn6</i>	221	214	461	281	385	488

^a Data are from reference 109. Gal, galactose; Glu, glucose; ND, not determined.

gene expression (69, 144, 159). As shown in Table 2, expression of *HXT2* and *HXT4* is constitutive (carbon source independent) in an *ssn6* mutant, because this mutation relieves repression by both Rgt1 and Mig1. Ssn6 and Tup1 are not DNA binding proteins but are recruited to diverse promoters by several different DNA binding proteins, including Rgt1 and Mig1 (69, 142, 148, 149, 159). Ssn6 and Tup1 may repress transcription by organizing repressive regions of chromatin and/or by directly interacting with the transcriptional machinery (32, 55, 69, 126).

Glucose regulates the nuclear localization of the Mig1 repressor: in the absence of glucose it is in the cytoplasm; high concentrations of glucose cause it to move into the nucleus (36). Mig1 function is regulated by Snf1 (also known as Cat1 or Ccr1), a serine-threonine protein kinase that is required for derepression of glucose-regulated genes (22–24, 27, 48, 135). In cells growing on low levels of glucose, Snf1 is active and inhibits Mig1 repressor function by causing it to reside in the cytoplasm. High levels of glucose inhibit Snf1 activity (48), causing Mig1 to move into the nucleus and repress transcription. It is likely that Snf1 directly phosphorylates Mig1 (35, 143), though this has not been rigorously tested. Consistent with the role of Mig1 in glucose repression, induction of *HXT2* and *HXT4* by low levels of glucose is completely abolished in a *snf1* mutant, due to constant repression by Mig1p (109) (Table 2). This explains why Snf1 is required for depression of high-affinity glucose transport (8). Snf1 does not function in the glucose induction pathway, because expression of the non-glucose-repressible *HXT1* gene is not decreased in *snf1* mutants (109).

***HXT6* and *HXT7*: Repression by High Levels of Glucose**

HXT6 and *HXT7* are regulated similarly and encode nearly identical proteins (differing in only two amino acids). These two genes are arranged in tandem, immediately downstream of *HXT3*. In some strains *HXT6* and *HXT7* are fused into one gene in which the promoter is derived from *HXT7* and the coding sequence from the *HXT6* gene (82). Interestingly, a yeast strain selected for growth under glucose-limited conditions for 450 generations appears to contain more than three copies of this chimeric gene as a result of multiple tandem duplication events (20). Like *HXT2* and *HXT4*, *HXT6* expression is repressed by high concentrations of glucose (82). However, unlike *HXT2* and *HXT4*, *HXT6* expression is only modestly induced by glucose: it is high in cells growing on nonfermentable carbon sources such as glycerol and ethanol and is only about two- to threefold higher in cells growing on low concentrations of glucose or raffinose. That is, *HXT6* (and

HXT7) have a higher basal level of expression than *HXT1* to *HXT4* (18, 82, 136).

Regulation of *HXT6* differs from that of *HXT2* and *HXT4* in another significant way: *SNF3* has a negative effect on expression of *HXT6*, being required for glucose repression of its expression (82), in contrast to its positive regulatory role in *HXT2* and *HXT4* expression (109). This is curious, because *HXT6* is the only gene whose glucose repression is known to require *SNF3*. Since *SNF3* is not required for glucose repression of other genes (e.g., *ADH2*, *SUC2*, and *GAL1*), it is unlikely to encode a component of the glucose repression pathway. Snf3p also has a positive role in *HXT6* expression, because it is required for the modest induction of *HXT6* expression by raffinose or low glucose (82).

***HXT1*: Induction of Transcription by High Glucose Concentrations**

HXT1 was identified as a multicopy suppressor of the growth defect of *snf3* mutants (79). Mutations in *HXT1* also suppress the potassium transport defect of the *trk1 trk2* double mutant, which lacks potassium transporters (72). *HXT1* expression is induced about 300-fold at high concentrations (4% or 222 mM) of glucose (Table 1). Rgt1 represses the expression of *HXT1* in the absence of glucose, as it does for *HXT2*, *HXT3*, and *HXT4*. In a strain lacking Rgt1, *HXT1* expression is about 20-fold derepressed in the absence of glucose. In addition to inhibiting *HXT1* expression in the absence of glucose, Rgt1 activates transcription at high glucose concentrations (112), and this accounts for about fivefold activation of *HXT1* transcription by high levels of glucose. Since high levels of glucose still induce *HXT1* expression in *rgt1* mutants (about 5- to 10-fold, see Table 2), another regulatory mechanism (whose components have not yet been identified but which requires *HXX2* and *REG1*) mediates induction of *HXT1* expression by high levels of glucose. Thus, three different pathways, repression by Rgt1 in the absence of glucose, activation by Rgt1 at high levels of glucose, and activation by an unidentified mechanism at high levels of glucose, are responsible for high glucose induction of *HXT1* expression.

Recently, *HXT1* expression was found to be inducible by hyperosmotic stress (1 M NaCl or 1.5 M sorbitol) (49). This requires *HOG1*, a MAP kinase required for adaptation to hyperosmotic stress (19). In addition, Sln1, a histidine kinase that inhibits Hog1 kinase activity (88), negatively regulates *HXT1* (49). One possibility to explain this phenomenon is that increased glucose uptake is necessary for the synthesis of glycerol, a major osmoprotectant in yeast. Consistent with this idea, expression of two other genes encoding key enzymes of glycerol synthesis (*GLK1*, encoding glucokinase, and *GPDI*, encoding NAD⁺-dependent glycerol-3-phosphate dehydrogenase) is also induced by hyperosmotic stress (49).

Transcriptional Regulation of Other *HXT* Genes

Only limited information is available on the expression of the remaining *HXT* genes (*HXT5* and *HXT8* to *HXT17*). To test whether expression of these genes is also regulated by glucose, their upstream regulatory regions (up to the ATG) were fused to *lacZ* and their expression and regulation was monitored by different levels of glucose (Table 3) (108). Since these promoter-*lacZ* fusions are in multicopy plasmids, it is difficult to extrapolate their true expression levels, but their levels of expression can be compared to those of *HXT1*-*HXT4* promoter-*lacZ* fusions in the same reporter plasmid. Except for *HXT5* and *HXT13*, these *HXT* genes are expressed at very low levels, being expressed 30- to 300-fold less than *HXT1* and

TABLE 3. Transcriptional regulation of *HXT*-like genes by glucose

Gene	Mean β -galactosidase activity (Miller units)			Fold regulation ^a
	5% glycerol	5% glycerol + 0.1% glucose	4% glucose	
<i>HXT11</i>	0.7	0.9	0.6	Not regulated
<i>HXT12</i>	0.3	0.5	0.3	Not regulated
<i>HXT10</i>	13	11	0.8	16 \times GR
<i>HXT16</i>	2.5	3	0.22	10 \times GR
<i>HXT17</i>	3.7	5.6	0.8	4 \times GR
<i>HXT5</i>	217	746	68	3 \times GR, 3 \times LGI
<i>HXT8</i>	1.1	6.4	0.3	3 \times GR, 6 \times LGI
<i>HXT13</i>	41	163	4.2	10 \times GR, 4 \times LGI
<i>HXT14</i>	1.5	6	0.6	2 \times GR, 4 \times LGI
<i>HXT15</i>	3.9	11	1	4 \times GR, 3 \times LGI
<i>RAG1</i>	1.9	11	508	254 \times HGI
<i>KHT2</i>	113	440	469	4 \times GI

^a GI, glucose induced; LGI, low glucose induced; HGI, high glucose induced; GR, glucose repressed.

HXT2. These genes are subjected to several different modes of regulation by glucose. Expression of *HXT12* is very low and not regulated by glucose, as is expression of *HXT11*, which is 97% identical to *HXT9* (both within their coding and promoter regions). *HXT10*, *HXT16*, and *HXT17* are repressed by glucose to various degrees (4- to 16-fold). *HXT5*, *HXT8*, *HXT13*, *HXT14*, and *HXT15* are induced three- to sixfold by low levels of glucose and repressed to various degrees by high levels of glucose. Regulation of *HXT5* and *HXT13* transcription resembles that of *HXT2* and *HXT4*, except that high glucose repression of *HXT5* is not as strong (about threefold) and low glucose induction of *HXT13* is more modest (about fourfold). Thus, none of these *HXT* genes exhibit the same regulation as *HXT1* to *HXT4*.

Although these genes were named *HXT* because of their homology to the members of the *HXT* gene family, it remains to be determined whether they indeed encode glucose transporters. Some of these genes might be involved in the transport of sugars other than glucose (though there are not many candidates since *S. cerevisiae* grows on few sugars), and some could have only a regulatory function. One strategy that has been used to test whether these *HXT*-like genes encode glucose transporters is to express them in a strain that is missing all known genes encoding glucose transporters (*hxt1 Δ* through *hxt7 Δ*) and that is therefore unable to grow on glucose (128). Introduction of any one of the seven missing *HXT* genes supports growth of this *hxt* null mutant on glucose. *HXT8* appears to function as a glucose transporter since it is able to partially complement the glucose growth defect of the *hxt* null mutant (but only when overexpressed). Surprisingly, *HXT10*, which encodes a protein approximately 80% identical to Hxt2, does not complement the *hxt* null mutant, so it appears unable to transport a significant amount of glucose (18, 128).

A clue to the function of some of these *HXT*-like genes comes from the different ways some of them were obtained. *HXT11* was cloned as a multicopy suppressor of a mutation in *RAG1*, which encodes a low-affinity glucose transporter of the yeast *K. lactis* that is required for growth of this yeast at high glucose concentrations (107). This suggests that Hxt11 functions as a glucose transporter.

Hxt9 and Hxt11 may play an important role in pleiotropic drug resistance (107). Pleiotropic drug resistance in yeast is caused by overexpression of ATP-binding cassette (ABC) transporters such as Pdr5, Snq2, and Yor1 and resembles the multidrug-resistance (MDR) phenotype of mammalian cells

(5, 33). The MDR phenotype of tumor cells, which is due to increased expression of an ABC transporter, is resistance to a variety of chemotherapeutic agents. Expression of the yeast ABC transporter genes is controlled by two homologous zinc-finger-containing transcription factors, Pdr1 and Pdr3. An *in vivo* screen designed to identify target proteins of Pdr1 and Pdr3 yielded *HXT9* and *HXT11*. Expression of both genes appears to be regulated by Pdr1 and Pdr3, and Pdr3 was shown to bind directly to the *HXT11* promoter (107). Interestingly, deletion of *HXT9* and/or *HXT11* causes resistance to several drugs such as cycloheximide and sulfometuron methyl, and overexpression of *HXT11* (but not of *HXT9*) increases sensitivity to these drugs. These results suggest that Hxt11 is required to transport drugs into the cell, a function opposite to that of the ABC transporters. This is curious, since expression of *HXT11* and *PDR5* (which transports drugs out of cells) is activated by the same transcription factors. It has been speculated that these hexose transporters could negatively regulate ABC transporter function, providing a negative feedback regulation. Alternatively, Hxt11 and Hxt9 might be directly involved in the uptake of drugs (107).

The promoter region of *HXT13* was obtained in a screen for targets of the transcription factor Hap2, a transcriptional regulator of genes such as that encoding cytochrome *c* involved in respiration (28). Interestingly, *HXT13* appears to be expressed at higher levels than *HXT8* to *HXT17* and, like all Hap2-regulated genes, is also about 10-fold repressed by high concentrations of glucose. However, no difference in *HXT13* mRNA levels was observed in cells grown on ethanol compared to cells grown on 2% glucose (18).

Transcriptional Regulation of *GAL2*

The galactose permease (Gal2) is more than 60% identical to the Hxt proteins and thus belongs to the Hxt protein family. A strain lacking *GAL2* grows poorly on media containing galactose (145). *GAL2* is expressed only when galactose is available, because its transcription requires the galactose-activated transcription factor Gal4 (61, 63), and is repressed at high concentrations of glucose (mediated by Mig1) (63, 145). Gal2 is also subject to glucose-induced inactivation (also known as catabolite inactivation). This inactivation process involves the glucose-induced internalization of Gal2 by endocytosis and its subsequent degradation in the vacuole (54). Gal2 (expressed from a constitutive promoter) can also transport glucose with almost the same affinity as galactose ($K_m = 2$ mM) (82, 106, 128). This explains why glucose transport in galactose-grown cells is strongly inhibited by galactose (104) and why 6-deoxy-glucose is transported with much higher affinity by galactose-grown cells than by glucose-grown cells (73).

In contrast to glucose induction of *HXT* gene expression, which is regulated by a receptor-mediated process (where glucose serves as a ligand for the Snf3 and Rgt2 glucose receptors), stimulation of transcription by galactose requires the presence of intracellular galactose (61, 84). Galactose induction of *GAL2* is mediated by the Gal3 protein, which functions as the sensor and transducer of the galactose signal. Gal3 is similar to galactokinase (Gal1) but lacks any detectable galactokinase activity (84). Gal3 seems to bind galactose (in an ATP-dependent fashion) and inhibit Gal80 (a repressor of *GAL* genes), which is thought to enable Gal4 to activate transcription of the *GAL* genes (15, 120, 138, 162, 163).

Transcriptional Regulation of *SNF3* and *RGT2* Genes

Snf3 and Rgt2 are about 70% similar to each other but are less than 30% similar to the other members of the Hxt family

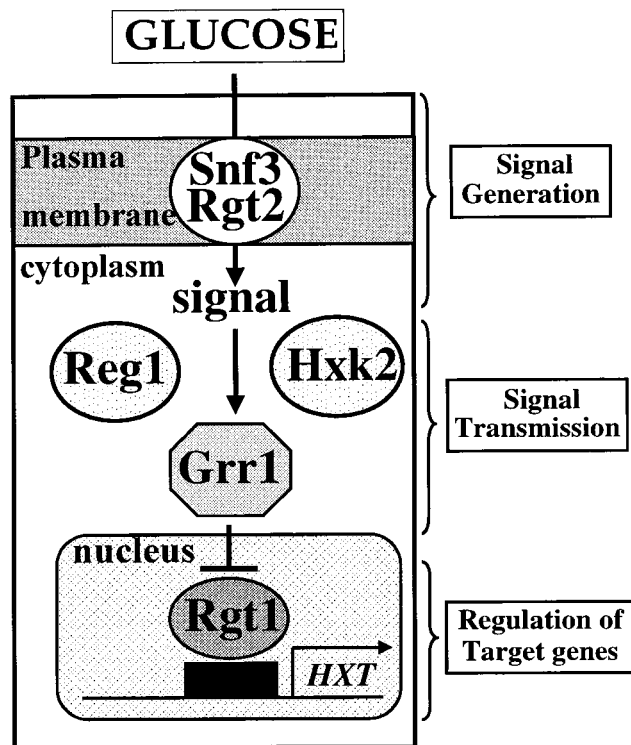


FIG. 2. The glucose induction pathway of the *HXT* genes and its components. The arrow implies positive regulation; a line with a bar denotes negative regulation.

(18, 75, 114, 160). As discussed in the next section, Snf3 and Rgt2 appear not to transport glucose but to serve as sensors of extracellular glucose that generate the intracellular signal for induction of *HXT1* to *HXT4* expression. Both genes are expressed at very low levels: about 100- to 300-fold lower than the *HXT1* to *HXT4* genes (114). Consistent with its proposed role as a high-affinity glucose sensor, *SNF3* transcription is repressed at high concentrations of glucose (91, 103, 109). Rgt2 is proposed to function as a low-affinity glucose sensor, and consistent with this role, its expression is independent of the glucose concentration (114).

COMPONENTS OF THE GLUCOSE INDUCTION SIGNALING PATHWAY

Three key components of the pathway responsible for glucose induction of *HXT* gene expression have been identified (Fig. 2): (i) the glucose sensors Snf3 and Rgt2, which are plasma membrane proteins that sense the presence of extracellular glucose and generate an intracellular signal for induction of *HXT* gene expression; (ii) the Rgt1 repressor, a C6 zinc finger DNA binding protein that binds to the promoters of the *HXT* genes and represses their transcription in the absence of glucose and activates *HXT1* transcription when high levels of glucose are present; and (iii) Grr1, which is required for glucose regulation of Rgt1 function and is a component of the SCF^{Grr1} complex of proteins that have been implicated in protein modification by ubiquitin. In addition, Hxk2 and Reg1, two other proteins known to be involved in glucose signaling, may play a role in glucose induction of the *HXT* genes.

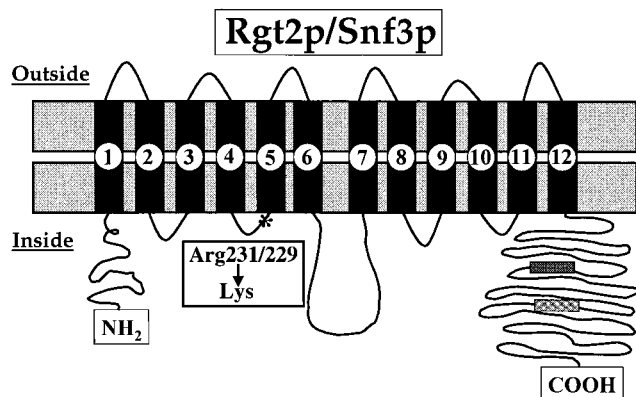


FIG. 3. The predicted transmembrane topology of the Rgt2 and Snf3 glucose transporters in the plasma membrane based on the model for Glut1 (97). The predicted transmembrane domains are numbered 1 to 12. The asterisk shows the position of the Arg-231 (in Rgt2) and Arg-229 (in Snf3) that is mutated to a lysine in the dominant mutants *RGT2-1* and *SNF3-1*, respectively. The boxes indicate the 25-amino-acid repeat in the Snf3 and Rgt2 carboxyl-terminal tail. Snf3 has two copies and Rgt2 has only one copy of this repeat.

Snf3 and Rgt2: Glucose Sensors

SNF3 and *RGT2* encode proteins with 12 predicted transmembrane-spanning domains that are about 60% identical to each other (Fig. 3). They are the most divergent members of the glucose transporter protein family, being only 26 to 30% identical to the other Hxt proteins (25, 91, 114, 160). *SNF3* was identified as a mutant that does not grow on sucrose or raffinose (102); *RGT2* mutations were obtained as dominant suppressors of the raffinose growth defect of *snf3* mutants (92).

Mutants of *snf3* were found to be defective in high-affinity glucose transport, which initially led to the conclusion that Snf3 is a high-affinity glucose transporter (13, 25). This was supported by the observation that multiple copies of *HXT1* and *HXT2* restore the raffinose growth defect of *snf3* mutants (76, 79). However, several subsequent pieces of evidence led to the view that Snf3 has a regulatory rather than a metabolic role in glucose transport. First, *SNF3* is expressed at a very low level relative to other glucose transporter genes (about 300-fold less than *HXT1*) (12, 103, 114). Second, *SNF3* has a negative effect on the growth of an *hxt1-hxt4Δ* strain on intermediate levels (0.5%) of glucose, rather than the positive effect that would be expected for a glucose transporter (72). Third, analysis of transport kinetics in a *snf3Δ* mutant suggested that the decrease in high-affinity glucose uptake is not due to loss of a single transporter (30). Finally, Snf3 was found to be required for induction of transcription of the *HXT2*, *HXT3*, and *HXT4* genes by low levels of glucose (109) (Table 4). This last observation strongly suggests that *snf3* mutants are defective in high-affinity glucose transport because they are unable to express genes encoding high-affinity glucose transporters.

Three key pieces of evidence suggest that Snf3 and Rgt2 are not glucose transporters but glucose receptors that bind glucose outside the cell and generate a signal inside the cell for induction of *HXT* gene expression (114). First, they are required for glucose induction of expression of several *HXT* genes. Snf3 is required for induction of *HXT2* and *HXT4* expression by low levels of glucose but not for induction of *HXT1* expression by high levels of glucose (Table 4). This suggests that it functions as a sensor of low levels of glucose. This conclusion is consistent with the observation that transcription of *SNF3* is maximal when glucose levels are low (*SNF3* expression is repressed about fivefold by high levels of glucose) (114).

Rgt2, on the other hand, appears to be a sensor of high levels of glucose, because it is required for maximal induction of *HXT1* expression by high concentrations of glucose but not for induction of *HXT2* and *HXT4* expression by low levels of glucose (Table 4). It is appropriate, then, that *RGT2* is expressed in cells growing on high levels of glucose (it is expressed constitutively, being neither repressed nor induced by glucose) (114). Second, Snf3 and Rgt2 are apparently unable to transport glucose. Expression of *SNF3* or *RGT2* in the *hxt* null mutant (*hxt1-hxt7Δ*) does not provide growth of this mutant on glucose, even when they are overexpressed from high-copy-number plasmids (82, 111). Thus, in contrast to the Hxt proteins, Snf3 and Rgt2 appear to have little or no ability to transport glucose.

Perhaps the most compelling observation that supports the view that Snf3 and Rgt2 are glucose sensors comes from the identification of a dominant mutation in these genes (*RGT2-1* and *SNF3-1*) that causes constitutive expression of *HXT1* to *HXT4* (i.e., in the absence of the inducer glucose) (114) (Table 4). We imagine that this mutation converts Snf3 and Rgt2 into their glucose-bound forms, thereby causing them always to generate the glucose signal that activates *HXT* expression. This and several other observations (30, 31, 72, 82, 150) led to the proposal that Snf3 and Rgt2 are membrane receptors that bind glucose outside the cell and generate a signal inside the cell for activation of gene expression (111, 114). In this view, glucose signaling by Snf3 and Rgt2 is a receptor-mediated process similar to hormone signaling in mammalian cells.

Snf3 and Rgt2 are composed of two functional domains: the 12 predicted transmembrane domains and a long C-terminal tail that is predicted to reside in the cytoplasm (91) (Fig. 3). The 12 transmembrane domains are similar to those of bona fide glucose transporters and almost certainly form the glucose-binding pocket. The considerable divergence of the transmembrane segments of Snf3 and Rgt2 from the hexose transporters probably accounts for their inability to transport glucose. The amino acid altered in the dominant, constitutive-signaling mutants lies in this region of the protein (*SNF3-1*, R229K; *RGT2-1*, R231K) (Fig. 3). This arginine, which is in the cytoplasmic loop between predicted transmembrane segments 4 and 5, is conserved in all other glucose transporters that have been identified, suggesting that it is crucial for binding or translocation of glucose. We believe that changing this residue to lysine converts the receptors into their glucose-bound and therefore glucose-signaling forms.

The C-terminal segments of Snf3 and Rgt2 that are predicted to be in the cytoplasm are unusually long (341 amino acids in Snf3 and 218 in Rgt2). They differ in this respect from

TABLE 4. Expression of *HXT1* and *HXT2* in *grt1*, *rgt1*, *snf3*, *rgt2*, *RGT2-1* and *snf3 rgt2* mutants^a

Relevant genotype	Mean β -galactosidase activity (Miller units)					
	<i>HXT1</i>			<i>HXT2</i>		
	Gal	Gal + Glu	Glu	Gal	Gal + Glu	Glu
Wild type	0.6	1.6	254	21	195	32
<i>grt1</i>	0.2	0.2	0.3	20	18	18
<i>rgt1</i>	14	14	42	191	334	20
<i>snf3</i>	0.7	1.2	227	16	23	7
<i>rgt2</i>	0.8	4.2	62	19	397	34
<i>RGT2-1</i>	30	223	393	213	415	48
<i>snf3 rgt2</i>	0.6	ND	0.9	6	14	ND

^a Data are from references 109 and 114. Gal, galactose; Glu, glucose; ND, not determined.

all other members of the hexose transporter family (in any organism), none of which have a predicted cytoplasmic C-terminal tail longer than 60 amino acids (except for two potential glucose sensors from *Neurospora crassa* and *K. lactis*, described below). The sequences of the Snf3 and Rgt2 cytoplasmic tails are only similar to one another in a stretch of 25 amino acids. Snf3 contains two of these 25-amino-acid sequences; Rgt2 contains only one (Fig. 3). These three sequences are identical at 16 of 25 positions (111).

Two observations indicate that the cytoplasmic tails of Snf3 and Rgt2 are involved in generating the signal for induction of *HXT* expression upon glucose binding. First, the tails are required for glucose induction of *HXT* expression: the Snf3 tail is required for low glucose induction of *HXT2* and *HXT4* expression (31, 111, 150), and the Rgt2 tail is required for high glucose induction of *HXT1* expression (111). Second, the cytoplasmic tail of Snf3 is sufficient for glucose-inducible signaling: attaching it to the Hxt1 or Hxt2 glucose transporters creates a chimeric protein that is able to complement the defect in glucose induction of *HXT* gene expression of *snf3* and *rgt2* mutants. That is, these modified glucose transporters sense glucose and generate an intracellular signal for induction of *HXT* expression. Another observation that implicates the C-terminal tail of Snf3 in glucose signaling is that overexpression of just the Snf3 tail suppresses the growth defect of *snf3* mutants on low levels of glucose (31) and seems to restore the low glucose induction defect of the *snf3* mutant (150). However, it is curious that this suppression does not appear to be accompanied by restoration of glucose transport (31). The 25-amino-acid sequences seem to be the functional regions of the tails, because deletions that remove them but leave the rest of the tail intact abolish signaling (12, 31, 91, 111). Furthermore, the magnitude of signaling by Snf3 correlates with the number of 25-amino-acid elements it possesses in its cytoplasmic tail: two elements (the full complement) lead to normal glucose induction of *HXT2* expression, and one element provides for partial induction (111).

The nature of the intracellular signal generated by Rgt2 and Snf3 in response to glucose is not known. Nevertheless, we can state with confidence that the primary signal is neither glucose nor one of its metabolites. The strongest justification for this statement is the observation that the dominant *SNF3-1* and *RGT2-1* mutants generate the glucose induction signal in the absence of glucose (114). In other words, these mutants do not need to transport glucose into the cell to generate the signal. Further support for this conclusion comes from analysis of a *snf3 rgt2* double mutant, which is unable to grow on glucose because of its inability to express any *HXT* genes. While expression of *HXT1* (from the *ADHI* promoter) in this mutant corrects its glucose growth defect, it does not restore glucose induction of expression of the other *HXT* genes, indicating that glucose transport and metabolism are not sufficient for generation of the glucose induction signal (111). Conversely, the *hxt1-hxt7*Δ null mutant is defective in glucose uptake but exhibits normal glucose induction of *HXT1* and *HXT2* expression (measured by using fusions of these promoters to *lacZ*) (108), further supporting the idea that glucose transport and metabolism are not necessary for generation of the glucose induction signal.

How Snf3 and Rgt2 generate the signal for induction of *HXT* gene expression in response to extracellular glucose remains to be elucidated. We imagine that the binding of glucose to the transmembrane-spanning domain induces a conformational change that is transmitted to the C-terminal signaling domain and affects its interaction with the next component(s) of the signal transduction pathway. Since the domains that are likely

responsible for signaling (the 25-amino-acid repeats) are the same in both proteins, we believe both proteins interact with the same or a similar component of the signal transduction pathway. Identifying this component is of utmost importance. A potential candidate is the protein encoded by *SKS1* (suppressor kinase of *snf3*), which was isolated as a multicopy suppressor of the *snf3* growth defect on raffinose (161). It is a serine/threonine protein kinase that is homologous to Ran1 of *Schizosaccharomyces pombe*, which regulates the switch between meiosis and vegetative growth (93). Deletion of *SKS1* has no phenotype, but perhaps this is not surprising because it has a homologue (*YDR247*) in the yeast genome. Since the *SKS1* promoter appears to contain Rgt1 binding sites (multiple copies of *SKS1* are able to suppress *snf3* mutants, presumably by titrating Rgt1 [161]), it is possible that *SKS1* itself is regulated by the glucose induction pathway.

We strongly suspect that Snf3 and Rgt2 generate the same signal, because the *SNF3-1* and *RGT2-1* dominant mutants both exhibit constitutive expression of both the low-glucose-induced genes (*HXT2* et al.) and high-glucose-induced genes (*HXT1*). We believe the different roles of Snf3 and Rgt2 in low- and high-glucose-induced gene expression is simply due to their different affinities for glucose. We imagine that Rgt2 is only involved in induction of *HXT1* expression in response to high levels of glucose because it is a low-affinity glucose receptor and that Snf3 is only involved in induction of *HXT2* expression by low levels of glucose because it is a high-affinity glucose receptor. Snf3, being a high-affinity receptor, would also be expected to bind high levels of glucose and generate a signal, but this ability is attenuated by the low levels of Snf3 that are present under this condition (due to the approximately fivefold repression of *SNF3* expression by high levels of glucose) (12, 91, 109). In fact, Snf3 does seem to contribute to high glucose signaling, because induction of *HXT1* expression is only reduced about fivefold in a *rgt2* mutant but is completely defective in the *snf3 rgt2* double mutant (Table 4). The basal level of Snf3 that is produced when glucose is abundant must be sufficient to provide for some glucose sensing and signaling in the *rgt2* mutant under these conditions. The lack of any significant reduction of *HXT1* induction by high levels of glucose in a *snf3* mutant suggests that Rgt2 is sufficient for full induction of *HXT1* expression.

Two other transporters in yeast may play a similar role in nutrient sensing and signaling. Expression of several different amino acid transporters requires Ssy1, which is similar to amino acid permeases but is apparently unable to transport amino acids into the cell (37, 64). Like the glucose sensors, Ssy1 possesses an unusually long cytoplasmic tail but in this case at the N terminus of the protein. Recently it has been shown that Ssy1 is required for transcriptional induction of the *AGP1* gene (which encodes a low-affinity, broad-specificity amino acid permease) and of five other genes, in response to multiple amino acids (57). Interestingly, induction of the *AGP1* gene by amino acids is abolished in a *grr1* mutant, indicating that Grr1 also plays an important role in this amino acid signal transduction pathway (57). From these data it seems likely that Ssy1 is an amino acid receptor that functions as a sensor of external amino acids analogous to the glucose receptors Snf3 and Rgt2. Another example is the high-affinity ammonium ion transporter Mep2, which is required for pseudohyphal differentiation at limiting concentrations of ammonium (85). It has been suggested that Mep2 is a sensor of ammonia that is involved in generating the signal for filamentous growth in response to nitrogen limitation. Mep2 does not appear to possess a long cytoplasmic tail, but a small intracellular loop of Mep2 required for mediating filamentous growth has been

identified (85). What is lacking from these two stories is conclusive evidence that these transporter-like proteins act as sensors, like that provided for the glucose sensors by their dominant, constitutive signaling mutations.

Grr1: Inhibitor of the Repressor

Mutants of *GRR1* (Glucose Repression Resistant) were originally isolated as resistant to 2-deoxyglucose (4). These mutants were found to be defective in glucose repression of several enzymes, including invertase, maltase, galactokinase, and the mitochondrial enzyme cytochrome *c* oxidase. *GRR1*, which is expressed constitutively at very low levels (43, 80), encodes a 132-kDa protein that is present in a large cytoplasmic protein complex. It appears to play several roles in yeast cells, because *grr1* mutations have pleiotropic effects, including elongated cell morphology, increased resistance to heavy metals and sulfite, increased sensitivity to osmotic stress and nitrogen starvation, loss of aromatic amino acid transport, decreased glucose uptake, and insensitivity to glucose inactivation of the maltose permease (4, 29, 43, 57, 59, 152).

Mutants of *grr1* are unable to induce *HXT* expression in response to glucose and thus are impaired in glucose uptake (109, 115, 152) (Table 4). This is due to their inability to inactivate Rgt1 in response to glucose, because mutations in *RGTI* restore expression of the *HXT* genes to *grr1* mutants (42, 92, 109). Thus, Grr1 is only indirectly involved in glucose repression: the glucose repression defect of *grr1* mutants is simply a consequence of their inability to transport significant amounts of glucose (109, 152). Other phenotypes of *grr1* mutants, such as their elongated cell morphology, are not suppressed by mutations in *RGTI* (6, 42), indicating that Grr1 acts upon other proteins in yeast.

Mutations in *grr1* convert the normally glucose-repressed gene *SUC2* into a glucose-induced gene (43, 151). Low levels of glucose induce expression of *SUC2*, and *GRR1* is required for this (116). In a *grr1* mutant, the signal for induction of *SUC2* expression appears to be shifted from low to high concentrations of glucose. Thus, *grr1* mutants are defective in growth on raffinose for two reasons: they have reduced levels of invertase due to reduced derepression of *SUC2* and reduced ability to transport the fructose that is liberated from raffinose by the action of invertase (152).

Grr1 has two protein interaction domains that are essential for its function: 12 leucine-rich repeats, preceded by an F-box motif. The F-box interacts with Skp1 (71, 80), which is part of several different but related enzyme complexes that direct protein ubiquitination (3, 50, 118, 137). These protein complexes are named SCF for their constituents: Skp1, the Cullin Cdc53 and the ubiquitin-conjugating enzyme Cdc34, and one of several F-box proteins (137). At least three different SCF complexes have been identified in yeast: SCF^{Cdc4}, SCF^{Grr1}, and SCF^{Met30} (118). They seem to differ in the F-box protein they contain, which is thought to be responsible for recruiting substrates to the complex for Cdc34-catalyzed ubiquitination. The F-box-containing protein Cdc4 recruits the cyclin-dependent kinase inhibitor Sic1 to SCF^{Cdc4} (3, 137); Grr1 recruits the G1 cyclins Cln1 and Cln2, and possibly Rgt1, to SCF^{Grr1} (6, 137). It is thought that the leucine-rich repeats of Grr1 are its substrate-recruiting domain (80).

Because it is part of an SCF complex, it seems likely that Grr1 regulates glucose-induced gene expression via a ubiquitin-directed or related process. The fact that *SKP1* and *CDC53* are required for glucose-induced *HXT1* expression (80) is consistent with this idea. It is possible that SCF^{Grr1} does not direct modification of proteins with ubiquitin but rather

with one of the two ubiquitin-related proteins (Smt3 and Rub1), whose attachment to proteins is catalyzed by conjugating enzymes specific for these proteins (60, 78). One simple view is that SCF^{Grr1} directs the modification of Rgt1 in response to glucose. However, it is also possible that SCF^{Grr1} modifies a yet unidentified regulator of Rgt1. How modification of Rgt1 (or its regulator) with ubiquitin (or with Smt3 or Rub1) alters Rgt1 function is not known. Whatever the mechanism, it is unlikely to be by degradation: Rgt1 must be present in cells grown both in the presence and absence of glucose, because it functions as a transcriptional activator in the former and a transcriptional repressor in the latter (112). Moreover, Rgt1 levels are similar in cells grown in glucose or glycerol (81).

The pleiotropic defects associated with *grr1* mutants are consistent with the idea that Grr1 recruits diverse targets to the SCF^{Grr1} complex. Among these are the G1 cyclins Cln1 and Cln2 (137), whose degradation requires Grr1 (6). The abnormal cell morphology of *grr1* mutants is probably due to overaccumulation of G1 cyclins (6). Another target of Grr1 is Gic2, an effector of the GTP-binding protein Cdc42, which is involved in actin polarization and bud emergence (58). Thus, Grr1 acts at the intersection of gene expression, cell cycle progression, and nutrient availability and could play a key role in integrating these important processes. Interestingly, the Grr1-Skp1 interaction is enhanced by high concentrations of extracellular glucose (80), which suggests a simple model for how glucose could affect these diverse cellular processes.

Rgt1: Transcriptional Repressor

RGTI was identified as a gene whose inactivation suppresses the high-affinity glucose transport defect of *snf3* mutants (42, 92, 152). Mutants of *SNF3* are unable to grow on low levels of glucose because they cannot increase expression of the high-affinity glucose transporters encoded by *HXT2* and *HXT4* under these conditions. This must be due to failure of *snf3* mutants to inactivate Rgt1 in response to glucose, because *RGTI* mutations suppress the low-glucose growth defect of *snf3* mutants. Multicopy plasmids carrying Rgt1 binding sites also suppress the low-glucose growth defect of *snf3* mutants, probably by titrating Rgt1 (113, 140). Mutations in *RGTI* also suppress the glucose repression defect of *grr1* mutants by restoring *HXT* gene expression, thereby reinstating transport of glucose and, consequently, generation of the glucose repression signal (42, 151, 152) (Table 4).

Rgt1 is a DNA binding protein with an amino-terminal C6 zinc cluster motif (Cys₆ Zn₂) like that found in the several members of the Gal4 family of transcription factors (1, 112, 123). The C6 zinc finger of Rgt1 contains the same residues that in Gal4 make base-specific contacts to DNA (Lys17 and Lys18) (90). Interestingly, Rgt1 differs from most other members of the Gal4 family in having a glycine in place of the proline residue of Gal4 (Pro26) that is critical for zinc binding (62). Another significant difference between Rgt1 and the other Gal4 family members is that Rgt1 appears to lack the dimerization domain that follows the zinc finger of nearly all of these proteins (90), suggesting that it binds to DNA as a monomer. This is unlike most of the members of this protein family, which bind as dimers to a CGG palindrome (CGGnCCG, with a different number of bases [n] separating the CGG repeats in the binding site of each protein), each subunit of the dimer recognizing one CGG sequence (1, 127). Hap1 recognizes two CGG sequences as direct repeats (119). By contrast, all four binding sites of Rgt1 that have been identified contain only one CGG sequence, supporting the idea that Rgt1 binds to DNA as

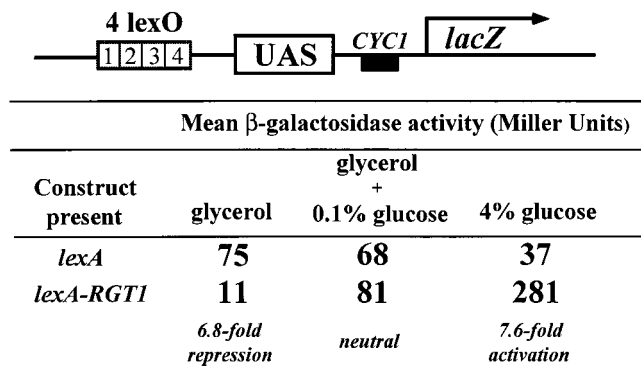


FIG. 4. Rgt1 is a bifunctional transcription factor that represses or activates transcription in response to glucose.

a monomer. ArgR11 is the only other member of the C6 zinc cluster family that appears to bind to only one CGG sequence (34, 39). The apparent simplicity of the DNA binding sites of Rgt1 and ArgR11 makes one wonder how they can achieve site specificity of DNA binding.

Rgt1 is a bifunctional transcription factor that displays three different transcriptional modes in response to glucose (Fig. 4). It functions as a transcriptional repressor in the absence of glucose, it is a transcriptional activator at high concentrations of glucose, and it is neutral (neither represses nor activates transcription) in cells growing on low levels of glucose (112). It must act directly to effect repression and activation of gene expression, because a *lexA*-Rgt1 fusion protein has these functions. In the absence of glucose Rgt1 binds to the *HXT1*-*HXT4* promoters and represses their expression (Fig. 4). Rgt1 probably represses transcription by recruiting the general repressors Ssn6 and Tup1, because repression of the *HXT* genes in the absence of glucose requires *SSN6* and *TUP1* (109) (Fig. 5). Low levels of glucose inactivate Rgt1 repressor function and thus derepress *HXT* gene expression. At high concentrations of

glucose, Rgt1 is converted into a transcriptional activator that is required for full induction of *HXT1* expression (112). As expected, the activator function of Rgt1 is independent of *SSN6* and *TUP1* (Fig. 5).

Glucose alters Rgt1 function through a pathway that requires the Snf3 and Rgt2 glucose sensors and Grr1. Grr1 is required both for conversion of Rgt1 into an activator and for inactivation of Rgt1 repressor function (112) (Fig. 5). Hxk2, which plays a major role in glucose repression of gene expression (45, 63), is also required for maximal induction of *HXT1* expression by high levels of glucose, but the ability of Rgt1 to activate transcription at high concentrations of glucose is not significantly reduced in *hxk2* mutants (112). Thus, the glucose repression pathway does not seem to be required for conversion of Rgt1 into an activator.

It is not known how glucose regulates Rgt1 function. Since transcription of *RGT1* is not regulated in response to glucose (112), the activity of Rgt1 is likely regulated posttranslationally. A *lexA*-Rgt1 chimera lacking the DNA binding domain of Rgt1 is regulated like intact Rgt1, so it is unlikely that glucose regulates its DNA binding activity. It is more likely that Rgt1 is modified in response to glucose and that this affects either its nuclear localization or its transcriptional repression and activation abilities.

Htr1/Mth1 and Msn3

MTH1 (also known as *HTR1*) may encode a component of the glucose induction mechanism that regulates *HXT* gene expression. A dominant mutation in *MTH1* (*HTR1-23*) causes defective transcription of several (probably all) *HXT* genes (113). Because of this, *HTR1-23* mutants have impaired glucose transport and consequently grow poorly on glucose and have defective glucose repression of gene expression (18, 113, 136). These phenotypes closely resemble those of *grr1* mutants (43, 115). The dominant *DGTT1-1* mutation results in phenotypes similar to that of *HTR1-23* (44), suggesting that these mutations may be allelic. Because mutations in *RGT1*, *SSN6*, and *TUP1* suppress the growth defect of *HTR1-23* on glucose

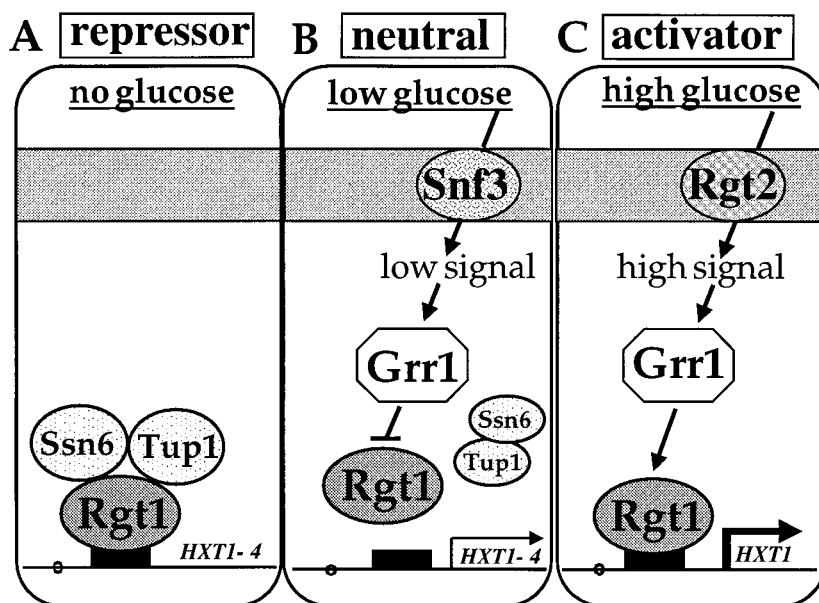


FIG. 5. Three different modes of transcriptional activity of Rgt1 in response to glucose. In the absence of glucose, Rgt1 works as a transcriptional repressor (A); at low levels of glucose, Rgt1 has no transcriptional activity (B); and at high concentrations of glucose, Rgt1 activates transcription (C).

(18, 136), Mth1/Htr1 may be involved in regulating Rgt1 activity.

MTH1 encodes a protein similar to Msn3 (56) (also known as Std1). It has been suggested that Msn3/Std1 is involved in the glucose repression pathway, because multiple copies of *MSN3/STD1* suppress the sucrose growth defect of *snf1* mutants (56, 141). However, we suspect that Mth1 and Msn3 are involved only in glucose induction of *SUC2* expression, since a strain lacking both *MTH1* and *MSN3/STD1* is defective in induction of *SUC2* by low levels of glucose (56).

The function(s) of Msn3/Std1 and Mth1 are mysterious, though several tantalizing clues have recently been uncovered. Msn3/Std1 is found in two locations in yeast cells: in (or close to) the nucleus and in the plasma membrane (17, 18, 134). The nuclear location of Msn3/Std1 may be a reflection of the fact that it physically interacts with the TATA-box binding protein (TBP) (141), which raises the possibility that it is directly involved in transcription. Interestingly, Msn3 has been found to interact with the C-terminal tails of Rgt2 and Snf3, and Mth1 interacts with the C-terminal tail of Snf3 (134), suggesting that it (and possibly also Mth1/Htr1) could be involved in transducing the glucose signal from the plasma membrane to the nucleus. However, the plasma membrane location of Msn3/Std1 is not dependent on *SNF3* and *RGT2* (17, 18, 134). Mutations in *MTH1* suppress the growth defect of *snf3* and *snf3 rgt2* mutants, probably by increasing the expression of hexose transporter genes. We imagine that the dominant *HTR1-23* mutation in *MTH1* interferes with glucose signaling by Snf3 and Rgt2. These findings are consistent with the idea that Msn3/Std1 and Mth1 might play a direct role in glucose signaling, but the precise role they play remains to be determined.

Other Components

Glucose kinases appear to play at least two roles in glucose transport. Yeast possesses three such enzymes: hexokinase 1 (Hxk1), hexokinase 2 (Hxk2), and glucokinase (Glc1) (41, 63). Hxk2 is the main glucose-phosphorylating enzyme in cells growing with abundant glucose; Hxk1 serves cells when glucose is scarce. A triple kinase mutant (*hvk1 hvk2 glk1*) lacks high-affinity glucose uptake (11), which is restored by introduction of either *HXK1* or *HXK2* or *GLK1* (9). This suggests that glucose phosphorylation is necessary for high-affinity uptake. However, high-affinity uptake of the nonphosphorylatable glucose analog 6-deoxyglucose was shown to be also kinase-dependent, suggesting that the hexokinases may have a role in high-affinity glucose uptake that is different from their catalytic function (10). Hxk2 is partially required for full induction of *HXT* expression by both low and high levels of glucose (109), suggesting that it might be involved in generating or transducing an intracellular glucose signal. It is interesting in this regard that the sole hexokinase of *K. lactis* (Rag5) is essential for glucose-induced transcription of the *RAG1* gene, which encodes a low-affinity glucose transporter (122). The different possible roles for the glucose kinases in glucose transporter function remain to be sorted out.

REG1 is a gene that appears to be involved in both glucose repression and glucose induction of gene expression. It encodes a regulatory subunit of protein phosphatase type 1 (Glc7) (146) that seems to target Glc7 to the Snf1 protein kinase for subsequent dephosphorylation (86). Mutants of *REG1* are defective in glucose repression of gene expression (45) and also in RNA processing (147). In addition, *REG1* is required for full induction of *HXT1* expression by high concentrations of glucose (109). It remains to be established

whether Reg1 is a component of the glucose induction pathway or affects *HXT1* expression indirectly.

POSTTRANSLATIONAL REGULATION

The function of several sugar transporters is regulated post-translationally. The best-understood example of this is the glucose-induced degradation of the galactose (Gal2) and maltose (Mal62) transporters (a phenomenon also called catabolite inactivation), which helps to ensure that yeast cells utilize these two sugars only if glucose is unavailable (52, 95, 124, 131, 132). Glucose-induced inactivation of Gal2 appears to be mediated by its ubiquitination, which targets it to the vacuole where it is degraded (54). Degradation of the maltose permease in response to glucose involves two signaling pathways, one dependent on glucose transport, the other one independent. The glucose-transport-independent inactivation pathway works through Rgt2p and Snf3p and requires Grr1p and Rgt1p. The glucose-transport-dependent pathway requires the function of the Hxt proteins and thus the transport and metabolism of glucose (59). Interestingly, it has recently been shown that the glucose-induced degradation of the maltose permease is ubiquitin dependent (94). In contrast to these well-studied examples, little is known about posttranslational regulation of glucose transport. Glucose inactivation of glucose transport (both low- and high-affinity components) was observed in nitrogen-starved cells (21), but the hexose transporters that are subject to glucose-induced inactivation have yet to be identified. Recent data on glucose inactivation of Hxt6 and Hxt7 indicate that these transporters are degraded in the vacuole after internalization by endocytosis. Moreover, the components of the ubiquitin machinery are required for high-glucose-induced degradation of Hxt6 and Hxt7 (74). Finally, Hxt2 transport activity might be regulated by glucose. *HXT2* expression (caused by mutation of *SSN6*) does not lead to increased glucose transport if glucose is not present in the growth medium, suggesting that low levels of glucose are required to activate Hxt2 transporter function (157).

Most of the Hxt proteins contain consensus sites for N-linked glycosylation and phosphorylation by protein kinase A and casein kinase II (12, 18, 53, 75), but it is not known if any of them are so modified. Hxt2 does not appear to be glycosylated, and likely is not phosphorylated (12, 157). Many glucose transporters, including the Hxt proteins, contain a leucine zipper motif, which is located in or near the second putative transmembrane domain. Perhaps this sequence motif, which is known to mediate protein-protein interactions, is involved in oligomerization of the hexose transporters. The mammalian glucose transporter Glut1 exists as dimers and tetramers, but oligomerization is not required for Glut1 transporter function (97).

TRANSPORTER STRUCTURE AND GENE ORGANIZATION

The mammalian glucose transporter Glut1 has 12 transmembrane domains, with its N and C termini located in the cytoplasm (97). Because of their clear similarity to Glut1, it is likely that the yeast hexose transporters have a similar topology. Mutational analysis of glucose transporters has been of limited utility for defining their functional domains, but one promising strategy for this is the construction of hybrids of different transporters. Analysis of chimeras of Hxt2 and the galactose transporter shed light on how Gal2 recognizes galactose (106). Even though glucose and galactose are nearly identical molecules (they are epimers of the C4 carbon), Hxt2

transports only glucose, while Gal2 transports galactose and (with slightly lower efficiency) glucose. Replacement of just transmembrane domains 10 to 12 (101 amino acids) of Hxt2 with those of Gal2 is sufficient to convert it into a galactose transporter (106). Further analysis revealed that the galactose recognition domain of Gal2 resides in transmembrane domain 10, which differs from that of Hxt2 in only 12 of 35 amino acids (67). Two of these (Hxt2 residues Phe431 and Tyr440) appear to be key for substrate binding, because replacing them with the corresponding Gal2 residues (Tyr446 and Trp455) enables Hxt2 to transport galactose (albeit with a lower K_m than that of Gal2) (68).

Replacement of Tyr446 in Gal2 with any of the other 19 amino acids completely abolishes galactose transport activity, suggesting that this residue is essential for the transport or recognition of galactose. By contrast, Trp455 is important but not essential for galactose recognition by Gal2 (68). More detailed mutational analysis of the two critical aromatic amino acids of Gal2 (Tyr446 and Trp455) and of Hxt2 (Phe431 and Tyr440) suggests that the presence of an aromatic amino acid in the middle of transmembrane domain 10 is essential for substrate recognition by Gal2 and Hxt2 transporters (66).

Trp455 of Gal2 corresponds to Trp388 of Glut1, which has been shown to be important for the levels, targeting, and forskolin binding of Glut1 (46, 153). A recent analysis of the two aromatic amino acids of Glut1 (Phe379 and Trp388) in transmembrane domain 10 that correspond to Tyr446 and Trp455 of Gal2 revealed a critical role for Trp388 in glucose transport (65). Thus, in contrast to Gal2, the residue at the cytoplasmic end of transmembrane domain 10 of Glut1 (Trp388, which corresponds to Trp455 of Gal2) is essential for transport activity. Another amino acid in transmembrane segment 10 of Glut1 that appears to be particularly important for Glut1 function is Pro385 (139).

The genomic organization of the *HXT* genes suggests that some of them arose by relatively recent gene duplication. Two clusters of three *HXT* genes are organized in tandem: *HXT4-HXT1-HXT5* are together on chromosome VIII, and *HXT3-HXT6-HXT7* are together on chromosome IV (129). All the others are scattered. Some are near telomeres, reminiscent of the organization of genes for sugar utilization (e.g., *SUC* and *MAL*).

REGULATION OF GLUCOSE TRANSPORTERS FROM OTHER YEASTS AND FUNGI

Genes encoding sugar transporters have also been identified in other yeasts, such as *K. lactis* (milk yeast), *Schizosaccharomyces pombe* (fission yeast), *Pichia stipitis* (a xylose-fermenting yeast) and the fungus *N. crassa*. None of these fungal species appear to have as many glucose transporters as *S. cerevisiae*.

Kluyveromyces lactis

K. lactis, like *S. cerevisiae*, has two glucose uptake systems: a constitutive one with high affinity for glucose (K_m of about 1 mM) and a glucose-inducible one with low affinity for glucose (K_m of about 20 to 50 mM) (158). Only three glucose transporter genes have been identified in *K. lactis*. The *RAG1* and *KHT2* genes encode low-affinity glucose transporters that are about 75% identical to each other and up to 70% identical to the Hxt proteins of *S. cerevisiae*. *RAG1* is required for growth on high concentrations of glucose and fructose (158). *HGT1* encodes a high-affinity glucose transporter that is only about 30% identical to the Hxt proteins (7). *HGT1* is required for growth on low concentrations of glucose, and overexpression

of *HGT1* does not allow *rag1* mutants to grow on high concentrations of glucose (7).

Transcription of the *RAG1* gene is induced by high concentrations of glucose, fructose, mannose, and raffinose (note that raffinose is equivalent to a high level of glucose for *K. lactis*, because this organism can cleave it into its constituent monosaccharides, unlike *S. cerevisiae*, for which this trisaccharide is equivalent to a low level of glucose, because it can only obtain low levels of fructose from it) (26). Curiously, galactose and lactose also induce *RAG1* transcription, even though these sugars are not substrates of the Rag1 protein. *RAG4*, *RAG5*, and *RAG8* are involved in regulating *RAG1* transcription. *RAG5* encodes the only hexokinase of *K. lactis*, which is essential for glucose-induced transcription of *RAG1* (122). *RAG8* encodes a casein kinase I very similar to the two casein kinases I of *S. cerevisiae*, Yck1 and Yck2. Indeed, overexpressed *RAG8* complements the temperature-sensitive growth defect of a *yck1 yck2* double mutant (14). *RAG4* encodes a low-affinity glucose sensor strikingly similar to Snf3 and Rgt2. It has a C-terminal extension containing the 25-amino-acid element found in the C-terminal tails of Rgt2 and Snf3 that is thought to be involved in glucose signaling. It is likely that Rag4 functions as a low-affinity glucose sensor that generates the high glucose signal required for glucose-induced transcription of the *RAG1* gene. Transcription of *HGT1* does not seem to be regulated by carbon source, but Rag5 (the hexokinase) appears to have a positive role and Rag4 (the glucose sensor) a negative role in *HGT1* expression (7).

Since the transcriptional regulation of *RAG1* by high levels of glucose resembles that of *HXT1* in *S. cerevisiae*, we tested whether high levels of glucose in *S. cerevisiae* could induce *RAG1* expression. High levels of glucose induce *RAG1* expression more than 200-fold in *S. cerevisiae* (Table 3). This is due to the Grr1-Rgt1 pathway, because deletion of *RGT1* causes a 10- to 20-fold depression when glucose is absent, and induction of *RAG1* is abolished in a *grr1* mutant (108). These results strongly suggest that the glucose induction pathway is conserved in *K. lactis* and *S. cerevisiae*. Consistent with this view, *K. lactis* appears to have a glucose sensor similar to Snf3 and Rgt2 (Rag4) that likely generates the high glucose signal for induction of *RAG1* expression. In addition, *KHT2* expression is more than fourfold induced by glucose, independent of the sugar concentration (Table 3), which resembles regulation of *HXT3* of *S. cerevisiae* (though it is not known if this is also mediated by the Grr1-Rgt1 pathway).

Schizosaccharomyces pombe

Glucose uptake mutants of *S. pombe* were isolated in a screen for mutants that are able to utilize gluconate in the presence of high concentrations of 2-deoxyglucose (51, 96). This screen was based on the fact that 2-deoxyglucose, whose uptake is mediated by a glucose transporter(s), prevents growth on gluconate-containing media, presumably because it represses expression of the gluconate transporter. The mutants obtained grew poorly on glucose and lacked any measurable glucose uptake. The gene affected in these mutants is *GHT1*, which encodes a protein with over 30% identity to the Hxt proteins. Multiple copies of *GHT1* restores the growth defect of the *hxt* null mutant of *S. cerevisiae* on 2% glucose, suggesting that it indeed encodes a glucose transporter (83).

Systematic sequencing of the *S. pombe* genome has revealed three additional genes (*GHT2*, *GHT3*, and *GHT4*), which encode proteins that are 55 to 75% identical to Ght1 and 40 to 45% identical to the Hxt proteins (18). The existence of four different *GHT* genes suggests that the original mutant of *S.*

pombe defective in glucose transport either carries a mutation in a regulatory gene required for expression of the other *GHT* genes or that *GHT2*, *GHT3*, and *GHT4* are not expressed or may not function as glucose transporters.

Pichia stipitis

The yeast *P. stipitis* is able to ferment xylose (derived from the hydrolysis of hemicelluloses of plants). It has low-affinity and high-affinity proton symport systems for this sugar which appear to be expressed constitutively (38, 70). The low-affinity xylose transport system has been shown to facilitate the uptake of glucose with a K_m of 0.3 to 1 mM (18, 70).

Three different genes, *SUT1* through *SUT3*, have been recently identified in the yeast *P. stipitis* and shown to encode glucose transporters (155). All three transporters are able to restore the growth defect of the *hxt* null mutant, indicating that Sut1 through Sut3 function as glucose transporters in *S. cerevisiae*. These transporters are also able to transport xylose and other monosaccharides when expressed in *S. cerevisiae* but with much lower affinities than for glucose (K_m for glucose is in the millimolar range). The Sut2 protein differs from Sut3 only in one amino acid (155). A strain lacking *SUT1* is not defective in growth on glucose but appears to have lost the low-affinity glucose uptake system. While transcription of the *SUT1* gene is strongly induced by glucose, *SUT2* and *SUT3* are expressed only under aerobic conditions and independent of carbon source (155).

Neurospora crassa

The glucose transporter Rco3 of the filamentous fungus *N. crassa* displays a significant homology to the Snf3 and Rgt2 sensors. It is required for glucose repression of gene expression, for conidiation, and for expression of glucose transporter activity (87). Based on these observations, it has been proposed that Rco3, like Snf3 and Rgt2, functions as a nutrient sensor. Interestingly, Rco3 also has a long C-terminal extension of 119 amino acids, but it lacks the conserved 25-amino-acid motif involved in glucose signaling that is present in the Snf3, Rgt2, and Rag4 C-terminal tails.

CONCLUSIONS AND PERSPECTIVES

Glucose induction of its own transport in *S. cerevisiae* is reminiscent of glucose-induction of glucose transport in mammalian cells. In both cases, glucose increases the number of glucose transporters in the plasma membrane. In yeast, glucose does this directly (by increasing expression of the *HXT* genes), but in mammalian cells it is accomplished indirectly through the action of the hormone insulin, which stimulates insertion of the Glut4 glucose transporter into the plasma membrane of fat and muscle cells (97). The cells that measure the concentration of glucose in the blood are mainly the insulin-producing β -cells of the pancreas. Glucose increases the amount of insulin these cells secrete in at least two ways: it rapidly stimulates secretion of insulin that is stored in vesicles and, more slowly, increases insulin gene transcription. These events require the glucose transporter Glut2 and glucokinase (105). The signal for stimulation of insulin secretion is the ATP that is generated from glucose metabolism. Because glucokinase catalyzes the rate-limiting step of glucose metabolism in β -cells, it serves as the glucose sensor (40). Thus, it is not clear whether functional analogs of Snf3 and Rgt2 exist in β -cells. It is significant that neither *Caenorhabditis elegans* nor mammals contain proteins that have the 25-amino-acid repeat found in the C-terminal cytoplasmic tails of Snf3 and Rgt2 proteins. Furthermore, none

of the candidate glucose transporters of *C. elegans* seem to have unusually long C-terminal tails. Thus, metazoans may not possess glucose sensors like those found in yeast.

A major challenge is to determine the function of the Hxt proteins that are not required for growth of yeast on glucose (Hxt8 to Hxt17). Another important goal is to determine the affinities of all the Hxt proteins for glucose and to determine whether they are modified posttranslationally. Several important questions remain regarding the issue of the transcriptional regulation of *HXT* gene expression by glucose. Paramount among these is how Snf3 and Rgt2 generate the glucose signal and how it is transmitted to the nucleus. How does Rgt1, the apparent ultimate target of the glucose-signaling pathway, display different activities in response to different levels of glucose? How does Grr1 inhibit Rgt1 function? Does Grr1 respond to the glucose signal or is some other component of the regulatory pathway sensitive to the glucose signal? It seems likely that analysis of this pathway will inform the mechanisms by which glucose regulates gene expression in other organisms.

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