

Rgt1p of *Saccharomyces cerevisiae*, a Key Regulator of Glucose-Induced Genes, Is both an Activator and a Repressor of Transcription

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The *RGTI* gene of *Saccharomyces cerevisiae* plays a central role in the glucose-induced expression of hexose transporter (*HXT*) genes. Genetic evidence suggests that it encodes a repressor of the *HXT* genes whose function is inhibited by glucose. Here, we report the isolation of *RGTI* and demonstrate that it encodes a bifunctional transcription factor. Rgt1p displays three different transcriptional modes in response to glucose: (i) in the absence of glucose, it functions as a transcriptional repressor; (ii) high concentrations of glucose cause it to function as a transcriptional activator; and (iii) in cells growing on low levels of glucose, Rgt1p has a neutral role, neither repressing nor activating transcription. Glucose alters Rgt1p function through a pathway that includes two glucose sensors, Snf3p and Rgt2p, and Grr1p. The glucose transporter Snf3p, which appears to be a low-glucose sensor, is required for inhibition of Rgt1p repressor function by low levels of glucose. Rgt2p, a glucose transporter that functions as a high-glucose sensor, is required for conversion of Rgt1p into an activator by high levels of glucose. Grr1p, a component of the glucose signaling pathway, is required both for inactivation of Rgt1p repressor function by low levels of glucose and for conversion of Rgt1p into an activator at high levels of glucose. Thus, signals generated by two different glucose sensors act through Grr1p to determine Rgt1p function.

Glucose is the preferred carbon and energy source for the yeast *Saccharomyces cerevisiae*, as well as for most mammalian cells, and has major and wide-ranging effects on gene expression. Glucose represses expression of many genes that are unnecessary for its metabolism and induces expression of many other genes required for its utilization. Among the genes whose expression is induced by glucose are several *HXT* genes encoding glucose transporters (5, 30, 31, 47). Three *HXT* genes respond differently to different levels of glucose: *HXT1* expression is induced only by high levels of glucose, whereas *HXT2* and *HXT4* are induced only by low concentrations of glucose.

We have previously shown that glucose induction of *HXT* expression is due to a repression mechanism mediated by Rgt1p (31). In cells growing in the absence of glucose, Rgt1p represses *HXT* expression; addition of glucose causes Rgt1p function to be inhibited, leading to derepression of *HXT* expression. The different responses of *HXT1* and *HXT2* (and *HXT4*) to different levels of glucose are due to two additional regulatory mechanisms that act on these genes: *HXT2* and *HXT4* are also subject to glucose repression mediated by Mig1p, a repressor that is activated by high levels of glucose (32, 47), and *HXT1* responds to an additional regulatory mechanism, whose components have not yet been identified, that requires high levels of glucose for function (31).

Glucose-stimulated inhibition of Rgt1p requires Grr1p, a leucine-rich repeat-containing protein, and Snf3p, a glucose transporter thought to be involved in sensing glucose and generating an intracellular signal (6, 9, 10, 14, 21, 31, 33). Thus, *grr1Δ* and *snf3Δ* mutants grow poorly on glucose and display severely impaired glucose transport because of reduced expres-

sion of glucose transporters (5, 6, 24, 25, 33, 46). Mutations in *RGTI* suppress the growth and glucose transport defects of *grr1Δ* and *snf3Δ* mutants by restoring *HXT* gene expression (13, 25, 31, 46). This was key evidence that led to the suggestion that Rgt1p plays a central role in regulating *HXT* gene expression in response to glucose.

RGTI appears to encode a negative regulator of the *HXT* genes, since its deletion causes constitutive (glucose-independent) *HXT* gene expression. However, high-glucose-induced expression of *HXT1* is reduced in an *rgt1Δ* mutant, suggesting an additional role for Rgt1p as a positive regulator of *HXT1* expression (31). Here, we describe the isolation of the *RGTI* gene, which encodes a Cys₆Zn₂ zinc cluster DNA-binding protein. We show that Rgt1p activates or represses transcription, depending on the concentration of glucose.

MATERIALS AND METHODS

Strains and media. The yeast strains used in this study are listed in Table 1 and are of the S288C genetic background. The bacterial strain DH5 α F' was used as a host for plasmids. Yeast cells were grown on either YEPD (2% Bacto Peptone [Difco], 1% yeast extract [Difco]) or YNB [0.67% yeast nitrogen base (Difco) plus 0.5% (NH₄)₂SO₄] medium lacking the appropriate amino acids, as described previously (31, 39).

Construction of plasmids and disruption strains. The *RGTI* clone was isolated from a yeast genomic DNA library in the shuttle vector YCp50 (36). The isolated plasmid (pBM2641) had approximately 10 kb of insert DNA. A 3.2-kb *EcoRI* fragment (containing the *PTMI* gene) of this original isolate was subcloned into the *EcoRI* site of pRS316 (pBM2856, to test for complementation of the *rgt1-1* mutation) and of pRS306 (pBM2783, to integrate into the chromosome [after cutting with *BglII*] to test for linkage to *rgt1-1*). In addition, a 5.3-kb *SpeI-SalI* fragment containing the *RGTI* gene was inserted into pRS316 (pBM2859) and into pBS(+) (pBM2858). The *RGTI* deletion plasmid (pBM2861) was constructed by replacing a 2.8-kb *BglII* fragment within the coding region with the 3.8-kb *BamHI-BglII* fragment of pNYK51, containing the *hisG-URA3-hisG* portion (1). For construction of an *RGTI* promoter fusion to *lacZ* (pBM3305), the *RGTI* upstream region was amplified by PCR using oligonucleotides OM1072 (starts at position +1 with a *BamHI* site) and OM903 (position -900, with *EcoRI*), digested with *BamHI* and *EcoRI*, and then inserted into the *lacZ* vector YEp353 (26). The *lexA-RGTI* fusion plasmid (pBM3306) was cre-

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TABLE 1. *S. cerevisiae* strains used

Strain	Genotype
YM2062	<i>MATα ura3-52 his3Δ200 ade2-101 lys2-801 met gal80Δ GAL1-lacZ</i>
YM3130	<i>MATa ura3-52 his3Δ200 ade2-101 lys2-801 met gal80Δ grr1Δ::LEU2 GAL1-lacZ lys2::GAL1-HIS3</i>
YM3866	<i>MATα ura3-52 his3Δ200 ade2-101 lys2-801 met gal80Δ grr1Δ::LEU2 rgt1-1 GAL1-lacZ lys2::GAL1-HIS3</i>
YM4498	<i>MATα ura3-52 his3Δ200 ade2-101 lys2-801 met gal80Δ rgt1Δ::hisG-URA3-hisG GAL1-lacZ</i>
YM4502	<i>MATa ura3-52 his3Δ200 ade2-101 lys2-801 gal80Δ grr1Δ::LEU2 rgt1Δ::hisG-URA3-hisG GAL1-lacZ</i>
YM4127	<i>MATa ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501</i>
YM4512	<i>MATa ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 hxx2Δ::LEU2</i>
YM4552	<i>MATa ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 reg1Δ::LEU2</i>
YM4714	<i>MATa ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 snf3Δ</i>
YM4509	<i>MATa ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 rgt1Δ::hisG</i>
YM4817	<i>MATa ura3-52 his3Δ200 ade2-101 lys2-801 leu2-3,2-112 trp1-901 tyr1-501 rgt2Δ::HIS3</i>
YM4767	<i>MATa ura3-52 his3Δ200 lys2-801 leu2-3,2-112 RGT2-1</i>
YM4576	<i>MATa ura3-52 his3Δ200 ade2-101 leu2-3,2-112 gal80 grr1Δ::hisG</i>
MCY1974 ^a	<i>MATα ura3-52 his3Δ200 ade2-101 lys2-801 trp1-901 ssn6Δ9</i>
MCY2437 ^a	<i>MATα ura3-52 his3Δ200 lys2-801 trp1-901 tup1Δ::TRP1</i>

^a Obtained from M. Carlson (42).

ated by subcloning PCR products. The coding region of the *RGT1* gene was first amplified by using primers OM669 (starts at ATG with a *Bam*HI site) and OM668 (starts at Stop with a *Sal*I site) and inserted as a *Bam*HI-*Sal*I fragment into the *lexA-GAL4* (p1027) and *lexA-SSN6* (pCK23) plasmids were described previously (8, 20). The construction of the *lexA-GAL4* (p1027) and *lexA-SSN6* (pCK23) plasmids was described previously (8, 20). The *rgt1Δ::hisG* strain (YM4509) was created by transforming the wild-type strain YM4127 to *Ura*⁺ with the 5.3-kb *Spe*I-*Sph*I fragment of pBM2861. To select for the loss of the *URA3* gene, the transformants were streaked on 5-fluoroorotic acid (5-FOA) plates.

β-Galactosidase assays. β-Galactosidase activity assays were performed with permeabilized cells as described previously (49), and activities are given in Miller units. The mean activities are the averages of four or five assays of at least three independent transformants. Precultures were grown on YNB-5% glycerol medium plus 0.5% galactose lacking the appropriate amino acids and transferred to YNB medium containing 4% glucose, 5% glycerol plus 0.5% galactose, or 5% glycerol plus 0.1% glucose and incubated for 4 to 5 h before β-galactosidase activity was assayed.

Preparation of purified Rgt1p protein from bacteria. Purified Rgt1p from bacteria was obtained as a fusion to the bacterial maltose-binding protein. For construction of the fusion plasmid (pBM3183), the complete open reading frame of the *RGT1* gene was amplified by PCR using oligonucleotides OM738 and OM740. The products of several independent PCRs were combined and digested with *Bam*HI and then subcloned into the *Bam*HI site of pMalE (New England Biolabs). Cells were grown and induced and proteins were prepared according to the manufacturer's instructions.

Gel mobility retardation assays. The 287-bp *Xba*I fragment of pBM3310 containing the *HXT1* upstream region (from positions -648 to -361 with respect to the ATG) as an *Xba*I fragment in Bluescript was labelled by filling in 5' overhangs with [α -³²P]dATP and Klenow enzyme and used as a probe for gel shift DNA-binding assays with purified Rgt1p. The binding reaction mixtures contained 10 μ l of retardation buffer (50 mM Tris-HCl [pH 7.5], 75 mM KCl, 5 mM MgCl₂, 2.5 mM dithiothreitol, 10 μ M ZnSO₄, 10% (vol/vol) glycerol, 1 μ g of salmon sperm DNA, 2 ng (30,000 cpm) of radiolabelled probe, and 50 ng of purified Rgt1p in a final volume of 20 μ l. After incubation for 10 min at 4°C, the protein-DNA complexes were separated on a native 5% polyacrylamide gel.

Gene accession number. The nucleotide sequence of the *RGT1* gene (YKL038w) was deposited in GenBank under accession no. Z28038 by Purnelle et al. (34).

RESULTS

Cloning of *RGT1*. *grr1Δ* mutants are defective in glucose repression of several genes, including *GAL1*. This is because *grr1Δ* mutants fail to express glucose transporters (5, 31, 33, 46), making them unable to sense glucose. Thus, *grr1Δ* mutants growing on glucose express *GAL1::HIS3* and *GAL1::lacZ* and are therefore His⁺ and form blue colonies on X-Gal (5-bromo-

4-chloro-3-indolyl-β-D-galactopyranoside) plates (14). Mutations in *RGT1* restore glucose repression to a *grr1Δ* mutant by restoring glucose transport (13, 31, 46). Therefore, a *grr1Δ rgt1-1* double mutant (YM3866) containing the same *GAL1* reporters behaves like the wild type and is unable to grow on glucose without histidine and forms white colonies on glucose-X-Gal plates. We isolated a CEN-based plasmid (pBM2641) from a yeast genomic library by its ability to complement the *rgt1-1* mutation and restore the His⁺ and blue colony phenotypes to a *grr1Δ rgt1-1* mutant.

Sequencing of the insert of pBM2641 from both ends revealed the location of this sequence on chromosome XI (34). The insert of pBM2641 is predicted to contain three open reading frames: one encoding a zinc finger-containing protein (YKL038w), another (a partial open reading frame) encoding UDP-glucosepyrophosphorylase (YKL035w), and the *PTM1* gene (YKL039w). Only a subclone containing the putative zinc finger gene (pBM2859) could complement the *rgt1-1* mutation (Fig. 1): a *grr1Δ rgt1-1* double mutant transformed with pBM2859 is defective in glucose repression of *GAL1* like a *grr1Δ* mutant alone (Table 2). Deletion of the zinc finger gene (YKL038w) suppresses the glucose repression defect of the *grr1Δ* mutant, like the *rgt1-1* mutation, indicating that this gene is indeed *RGT1* (Table 2, *grr1Δ rgt1Δ* mutant). *RGT1* is not required for glucose repression, since its disruption does not significantly affect *GAL1* expression on glucose (Table 2, *GRR1 rgt1Δ* mutant).

To test the linkage of the cloned DNA to the chromosomal *RGT1* locus, we integrated a plasmid containing the cloned DNA (pBM2783; see Materials and Methods) into its chromosomal locus in a *grr1Δ RGT1* mutant (YM3130). The resultant strain containing the integrated *URA3* marker was crossed to a *grr1Δ rgt1-1* mutant (YM3866). Both strains contained a *GAL1-HIS3* fusion, which provided an easily scorable phenotype for *rgt1*: a *grr1Δ rgt1* strain has normal glucose repression and is therefore His⁻ on glucose, whereas a *grr1Δ RGT1* strain is defective in glucose repression and is His⁺ on glucose. *Ura*⁺ cosegregated with His⁺ (*grr1 RGT1* phenotype) in all of 11 tetrads examined. Thus, the cloned DNA is tightly linked to the *RGT1* locus.

RGT1 encodes a putative DNA-binding protein with a zinc finger motif consisting of six cysteine residues. Thus, Rgt1p belongs to the Cys₆ zinc cluster protein family that includes Gal4p, Ppr1p, Put3p, and other transcription factors (2, 18, 34).

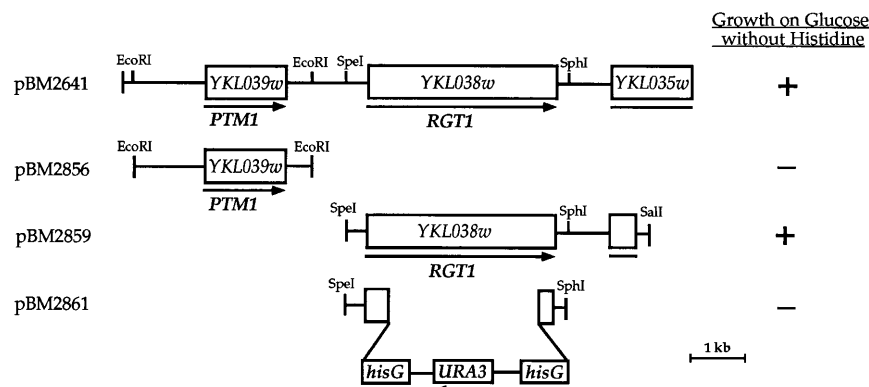


FIG. 1. Restriction map showing the genomic fragment containing the *RGT1* gene and other subclones. Symbols: →, open reading frames; + and –, presence or absence of growth, respectively. Two small questionable open reading frames (YKL036c and YKL037w) are located between *RGT1* and YKL035w.

Rgt1p binds to the *HXT1* promoter. We have previously shown that Rgt1p acts as a repressor of several *HXT* genes in the absence of glucose (31). However, it is also required for maximal expression of *HXT1* at high levels of glucose. To test if Rgt1p affects *HXT1* expression by direct binding to its promoter, we performed gel mobility retardation assays with purified Rgt1p and a 287-bp fragment containing the *HXT1* promoter (from positions –648 to –361) as a probe (Fig. 2). This region of the *HXT1* promoter is sufficient to mediate Rgt1p-dependent, high-glucose-induced expression of a reporter gene (unpublished data). Purified Rgt1p is able to bind to the *HXT1* promoter and gives rise to three shifted complexes. Indeed, this region of the *HXT1* promoter contains three putative binding sites for Rgt1p. Thus, Rgt1p has DNA-binding activity and regulates *HXT1* expression by direct binding to its promoter. We have recently demonstrated that Rgt1p also binds to the *HXT2* and *HXT4* promoters (32).

***RGT1* is expressed at low levels independent of the carbon source.** Previous results suggested that Rgt1p inhibits *HXT* gene expression in the absence of glucose and that glucose inhibits Rgt1p function through Grr1p (31). To test whether transcription of *RGT1* is regulated by glucose and by Grr1p, we measured the expression of *RGT1* using *lacZ* as the reporter. *RGT1* is expressed at low levels independent of the presence of glucose, and Grr1p has no effect on transcription of *RGT1* (Table 3). *RGT1* expression is not autoregulated. We note that because the activity of the *RGT1* promoter was assayed with a

multicopy plasmid, it is not possible to determine the exact level of expression of *RGT1* in the cell.

LexA-Rgt1p displays three different transcriptional modes in response to glucose. Since *RGT1* encodes a DNA-binding protein that is implicated as a negative regulator of *HXT* gene expression, we tested the ability of a LexA-Rgt1p fusion to repress transcription. LexA-Rgt1p causes about sevenfold repression of the *CYC1-lacZ* reporter in the absence of glucose compared with that of cells expressing only the LexA DNA-binding domain (Fig. 3A, compare lines 1 and 2). In the same assay, a LexA-Ssn6p fusion (which has been demonstrated to repress transcription [20]) mediates 13-fold repression of transcription (line 4). The repression mediated by the LexA-Rgt1p fusion is abolished at a low level of glucose (0.1%). At a high concentration of glucose (4%), LexA-Rgt1p activates transcription of the reporter gene about eightfold. For comparison,

TABLE 2. Expression of *GAL1::lacZ* in *grr1Δ* and *rgt1* mutants

Relevant genotype ^a	Plasmid	Mean β-galactosidase activity (U) ± SD ^b :		Fold <i>GAL1::lacZ</i> repression
		2% Glucose ^c	2% Galactose	
<i>GRR1 RGT1</i>	pRS316	7 ± 0.4	341 ± 43	49
<i>grr1Δ RGT1</i>	pRS316	163 ± 31	265 ± 16	1.6
<i>grr1Δ rgt1-1</i>	pRS316	23 ± 4	308 ± 31	13
<i>grr1Δ rgt1-1</i>	pBM2859	190 ± 15	224 ± 37	1.2
<i>grr1Δ rgt1Δ</i>	pRS316	31 ± 4	390 ± 60	13
<i>GRR1 rgt1Δ</i>	pRS316	20 ± 3	346 ± 62	17

^a The yeast strains assayed were YM2062 (*GRR1 RGT1*), YM3130 (*grr1Δ*), YM3866 (*grr1Δ rgt1-1*), YM4502 (*grr1Δ rgt1Δ*), and YM4498 (*rgt1Δ*).

^b Precultures were grown in YNB medium with 2% glucose lacking uracil, transferred to YNB medium containing the indicated sugars, and grown until mid-log phase before being assayed for β-galactosidase activity.

^c All strains are *gal80⁻* and thus do not require galactose to induce *GAL* gene expression.

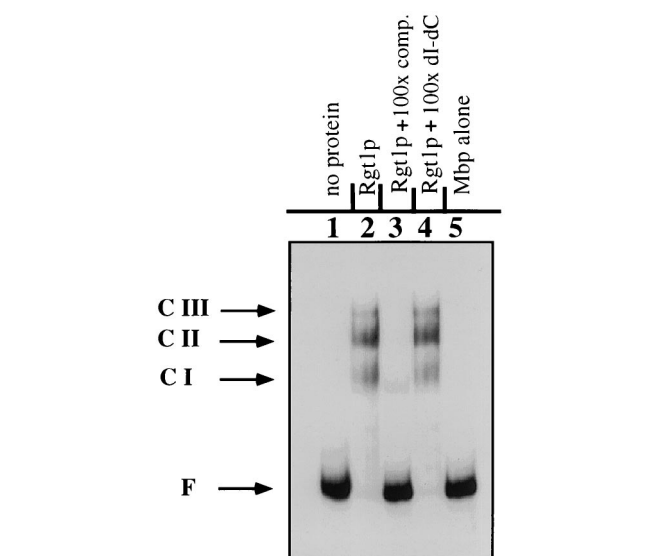


FIG. 2. Gel shift DNA-binding assays with purified Rgt1p from bacteria and the *HXT1* promoter region containing the sequences from positions –648 to –361 as a probe. The binding reaction mixtures contained no protein (lane 1), 50 ng of purified Rgt1p (lane 2), and 60 ng of purified Mbp (lane 5). Unlabelled fragment in a 100-fold molar excess (lane 3) or the same amount of poly(dI-dC) as a nonspecific competitor (lane 4) was added to the binding reaction mixtures. Unbound probe (F) and Rgt1p-DNA complexes (C I to C III) are indicated. comp., competitor.

TABLE 3. Expression of *RGT1::lacZ* in wild-type, *grr1Δ*, and *rgt1Δ* strains

Relevant genotype ^a	Mean β-galactosidase activity (U) ± SD ^b :		
	gly	gly + 0.1% glu	4% glu
WT	17 ± 2	18 ± 4	15 ± 2
<i>grr1Δ</i>	22 ± 4	13 ± 2	19 ± 4
<i>rgt1Δ</i>	12 ± 1.7	10 ± 1	11 ± 2

^a *RGT1-lacZ* was used as a reporter throughout. WT, wild type.

^b Precultures were grown on YNB medium with 5% glycerol plus 0.5% galactose lacking uracil, transferred to YNB medium containing the indicated sugars, and grown to mid-log phase (optical density at 600 nm, 1 to 2) before being assayed for β-galactosidase activity. gly, 5% glycerol plus 0.5% galactose; gly + 0.1% glu, 5% glycerol plus 0.1% glucose; 4% glu, 4% glucose.

we also determined the activation by a LexA-Gal4p fusion protein in the same assay and obtained about threefold activation of the same reporter gene on all carbon sources (Fig. 3A, line 5). Thus, LexA-Rgt1p has three different transcriptional modes: it is a repressor when glucose is absent and an activator at high levels of glucose and is neutral at low concentrations of glucose.

To test if repression and activation mediated by Rgt1p are dependent on the presence of the DNA-binding domain, we constructed LexA-Rgt1pΔ1, which lacks the zinc finger domain (with the first 75 amino-terminal residues deleted). This fusion protein gives the same level of repression and activation as observed with the full-length Rgt1p (Fig. 3A, line 3), indicating

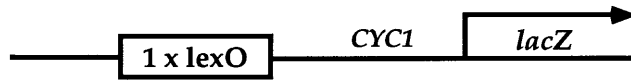
A) reporter:



Mean β-galactosidase activity (U) ± SD

construct	gly	fold repression	gly + 0.1% glu	fold activation	4% glu	fold activation
1 <i>lexA</i>	75 ± 12	1	68 ± 10	1	37 ± 8	1
2 <i>lexA-RGT1</i>	11 ± 2	6.8	81 ± 17	1.2	281 ± 47	7.6
3 <i>lexA-RGT1-Δ1</i>	9 ± 2	8.3	65 ± 12	1	434 ± 65	12
4 <i>lexA-SSN6</i>	6 ± 1	12.5	9 ± 2	0.13 (7.6)	3 ± 0.7	0.08 (12.3)
5 <i>lexA-GAL4</i>	255 ± 44	0.29 (3.4)	153 ± 18	2.2	98 ± 9	2.6

B) reporter:



Mean β-galactosidase activity (U) ± SD

relevant genotype	plasmid	gly	fold activation	gly + 0.1% glu	fold activation	4% glu	fold activation
WT	<i>lexA</i>	0.8 ± 0.22	1	1.1 ± 0.1	1	0.8 ± 0.3	1
WT	<i>lexA-RGT1</i>	1.6 ± 0.1	2	7 ± 1.5	6.4	151 ± 21	189
WT	<i>lexA-GAL4</i>	385 ± 37	481	238 ± 32	216	138 ± 7	172
<i>RGT2-1</i>	<i>lexA</i>	1.4 ± 0.3	1	0.9 ± 0.2	1	1.1 ± 0.3	1
<i>RGT2-1</i>	<i>lexA-RGT1</i>	161 ± 17	115	170 ± 13	189	215 ± 20	195
<i>rgt2Δ</i>	<i>lexA</i>	1.1 ± 0.2	1	1.0 ± 0.2	1	1.2 ± 0.3	1
<i>rgt2Δ</i>	<i>lexA-RGT1</i>	1.3 ± 0.4	1.2	1.6 ± 0.2	1.6	34 ± 7	28

FIG. 3. Rgt1p is a bifunctional transcription factor in wild-type cells. Cultures were grown as described in Materials and Methods (see also Table 3, footnote b). Numbers in parentheses are reciprocals. WT, wild type; gly, glycerol; glu, glucose.

TABLE 4. Repression by LexA-Rgt1p in the absence of glucose is dependent on Ssn6p and Tup1p^a

Relevant genotype	Plasmid genotype	Result on medium with the indicated sugar(s) ^b					
		gly		gly + 0.1% glu		4% glu	
		Activity	Fold repression	Activity	Fold activation	Activity	Fold activation
WT	<i>lexA</i>	75 ± 12	1	68 ± 10	1	37 ± 8	1
	<i>lexA-RGT1</i>	11 ± 2	6.8	81 ± 17	1.2	281 ± 47	7.6
<i>ssn6Δ</i>	<i>lexA</i>	213 ± 27	1	154 ± 21	1	103 ± 12	1
	<i>lexA-RGT1</i>	191 ± 32	1.1	182 ± 15	1.2	535 ± 51	5.2
<i>tup1Δ</i>	<i>lexA</i>	205 ± 10	1	172 ± 23	1	98 ± 17	1
	<i>lexA-RGT1</i>	198 ± 15	1	245 ± 20	1.4	636 ± 22	6.5

^a The reporter (pJK1621) is *CYCI-lacZ* containing 4 *lexA* sites upstream of the *CYCI* UAS, as in Fig. 3A.

^b See Table 3, footnote b, for definitions of sugars. Activities are mean β-galactosidase activities (in units) ± standard deviations.

that the repression and activation domain(s) is outside the DNA-binding domain.

To test if activation by LexA-Rgt1p requires the presence of a UAS element, we used a *CYCI-lacZ* reporter gene that contains one *lexA* site in place of the *CYCI* UAS (Fig. 3B). Cells expressing the LexA-Rgt1p fusion protein exhibit strong activation of transcription at high concentrations of glucose. At low levels of glucose, activation by LexA-Rgt1p is only sixfold, and it is undetectable if glucose is absent. This result indicates that Rgt1p by itself can mediate transcriptional activation in the presence of high levels of glucose. The level of activation mediated by Rgt1p is similar to that obtained with the strong LexA-Gal4p activator (Fig. 3B).

Repression by LexA-Rgt1p in the absence of glucose requires Ssn6p and Tup1p. Repression of the *HXT* genes in the absence of glucose is relieved in *ssn6Δ* and *tup1Δ* mutants (31). To determine whether repression caused by LexA-Rgt1p requires Ssn6p and Tup1p, we assayed repression in *ssn6Δ* and *tup1Δ* mutants (Table 4). The ability of the LexA-Rgt1p fusion

protein to mediate repression in the absence of glucose is completely abolished in *ssn6Δ* and *tup1Δ* mutants. Activation of transcription by LexA-Rgt1p in these mutants still requires high levels of glucose (Table 4). Thus, Rgt1p probably represses transcription by recruiting Ssn6p and Tup1p, but the activator function of Rgt1p is independent of Ssn6p and Tup1p.

Regulation of Rgt1p function requires a glucose signal. To identify components of the pathway that converts the Rgt1p repressor into an activator at high levels of glucose, we assayed LexA-Rgt1p for its activator function in various mutants defective in glucose induction of *HXT* expression (Table 5). We first tested the involvement of Rgt2p, a glucose transporter that is required for high-glucose-induced expression of *HXT1*, probably by generating a high-glucose signal (29). Activation of transcription by LexA-Rgt1p is drastically impaired at high levels of glucose in an *rgt2Δ* mutant (Table 5). Similar results were obtained with a UAS-less *CYCI* reporter (Fig. 3B). This suggests that Rgt2p is involved in generating the high-glucose

TABLE 5. Analysis of activation and repression by Rgt1p in various mutants deficient in glucose induction^a

Relevant genotype	Plasmid genotype	Result on medium with the indicated sugar(s) ^b					
		gly		gly + 0.1% glu		4% glu	
		Activity	Fold repression	Activity	Fold activation	Activity	Fold activation
WT	<i>lexA</i>	75 ± 12	1	68 ± 10	1	37 ± 8	1
	<i>lexA-RGT1</i>	11 ± 2	6.8	81 ± 17	1.2	281 ± 47	7.6
<i>rgt2Δ</i>	<i>lexA</i>	71 ± 11	1	74 ± 8	1	41 ± 11	1
	<i>lexA-RGT1</i>	14 ± 3	5.1	101 ± 19	1.4	76 ± 17	1.9
<i>RGT2-1</i>	<i>lexA</i>	83 ± 15	1	67 ± 5	1	53 ± 6	1
	<i>lexA-RGT1</i>	215 ± 34	0.3 (2.6)	238 ± 8	3.6	335 ± 54	6.3
<i>grr1Δ</i>	<i>lexA</i>	169 ± 26	1	148 ± 14	1	159 ± 22	1
	<i>lexA-RGT1</i>	19 ± 3	8.9	23 ± 3	0.16 (6.4)	20 ± 5	0.13 (8)
<i>snf3Δ</i>	<i>lexA</i>	82 ± 17	1	78 ± 7	1	39 ± 6	1
	<i>lexA-RGT1</i>	16 ± 3	5.1	18 ± 2	0.23 (4.3)	262 ± 28	6.7
<i>hvk2Δ</i>	<i>lexA</i>	68 ± 15	1	70 ± 12	1	56 ± 4	1
	<i>lexA-RGT1</i>	19 ± 4	3.6	102 ± 0.7	1.5	241 ± 34	4.3

^a The plasmids used in this assay are the *CYCI-lacZ* reporter pJK1621 (four *lexA* sites upstream of the *CYCI* UAS), pSH2-1 (*lexA*), and pBM3306 (*lexA-RGT1*). The *TRP1* versions of *lexA* and *lexA-RGT1*, pBM3307 and pBM3308, respectively, were used to assay expression in the *rgt2Δ::HIS3* mutant. Numbers in parentheses are the reciprocals.

^b See Table 3, footnote b, for definitions of sugars. Activities are mean β-galactosidase activities (in units) ± standard deviations.

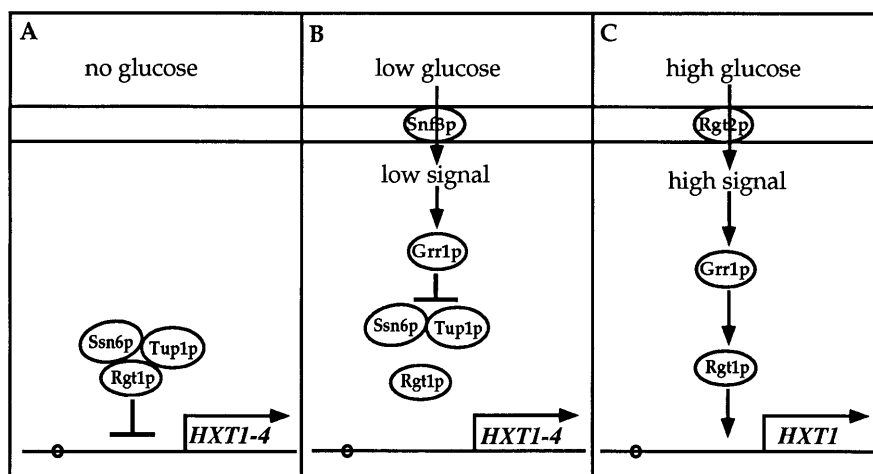


FIG. 4. Schematic model describing the three different modes of transcriptional activity of Rgt1p in response to glucose. In the absence of glucose, Rgt1p works as a transcriptional repressor (A); at low levels of glucose, Rgt1p has no transcriptional activity (B); and at high concentrations of glucose, Rgt1p activates transcription (C).

signal that converts LexA-Rgt1p into an activator. This conclusion is supported by results obtained with *RGT2-1*, a dominant mutation that causes Rgt2p to always signal glucose availability. This mutation, which leads to constitutive induction of the *HXT* genes (29), converts LexA-Rgt1p into a constitutive transcriptional activator (Table 5). The effect of the *RGT2-1* mutation on the function of LexA-Rgt1p is even more apparent when the *lexAo-CYC1-lacZ* reporter is used to assay its function: while LexA-Rgt1p activates transcription of this reporter only in the presence of high concentrations of glucose, activation is constitutive (carbon source independent) in the *RGT2-1* mutant (Fig. 3B). We conclude that Rgt2p is required to convert LexA-Rgt1p into a transcriptional activator, probably by generating a high-glucose signal.

Grr1p appears to be the next protein in the glucose induction pathway, since it is required for glucose-induced inhibition of Rgt1p (31) and acts between Rgt2p and Rgt1p in the pathway, according to the epistasis relationships of mutations in these genes (29, 31). Thus, it is not surprising that the ability of LexA-Rgt1p to activate transcription at high levels of glucose is also dependent on Grr1p function (Table 5). However, it is possible that the effects of Grr1p on Rgt1p function are indirectly due to other proteins regulated by Grr1p.

SNF3 encodes a glucose transporter that seems to be a sensor of low levels of glucose, since it is required for induction of *HXT2* and *HXT4* expression by low levels of glucose (5, 6, 10, 31). Interestingly, LexA-Rgt1p represses transcription in a *snf3*Δ mutant in cells growing on low levels of glucose, a condition that normally inhibits the repressor function of LexA-Rgt1p (Table 5). Thus, Snf3p is required for inhibition of the repressor function of Rgt1p by low levels of glucose. However, Snf3p is not required for the conversion of LexA-Rgt1p into an activator at high levels of glucose, consistent with its probable role as a sensor of low levels of glucose (5, 10, 29, 31). These results indicate that Snf3p is required to inhibit the repressor function of LexA-Rgt1p, probably by generating a low-glucose signal.

HXK2 encodes the major glucose-phosphorylating enzyme in *S. cerevisiae* and is also required for repression of several glucose-regulated genes and for induction of *HXT1* expression at high concentrations of glucose (19, 31, 35). LexA-Rgt1p displays only a slight reduction (about twofold) in transcrip-

tional activation in an *hxk2*Δ mutant growing on high levels of glucose (Table 5). Thus, *HXK2* does not appear to play a major role in converting Rgt1p into an activator.

DISCUSSION

The *RGT1* gene of the yeast *S. cerevisiae* encodes a zinc finger protein that belongs to the family of Cys₆Zn₂ zinc cluster transcription factors that includes Gal4p. We have demonstrated that it indeed binds to the *HXT1* promoter (this work), as well as to the *HXT2* and *HXT4* promoters (32), and thus likely regulates their expression directly. We have shown that Rgt1p has two effects on transcription (Fig. 4): it represses transcription when glucose is absent but activates transcription in the presence of high levels of glucose. It has no effect on transcription in the presence of low levels of glucose. Thus, Rgt1p plays two central roles in glucose induction of gene expression. It is required for repression (in the absence of glucose) and for maximal induction (at high glucose concentrations) of *HXT1* expression. Since purified Rgt1p binds to the *HXT1* promoter, it likely modulates *HXT1* expression directly. Thus, Rgt1p displays two different transcriptional activities at the same promoter in response to glucose.

LexA-Rgt1p causes about sevenfold repression in the absence of glucose, similar to the level of repression observed with a LexA-Ssn6p construct. The Ssn6p-Tup1p complex is involved in repression of transcription of several diversely regulated genes, including genes regulated by glucose repression, mating type, oxygen, and DNA damage (3, 20, 38, 42, 48). It seems that specific DNA-binding proteins recruit the Ssn6p-Tup1p repressor complex to different promoters (20, 22, 40, 44, 45). Mig1p, a Cys₂His₂ zinc finger protein, recruits Ssn6p-Tup1p to glucose-repressed genes by interacting with Ssn6p (42), and it is likely that other DNA-binding proteins that require Ssn6-Tup1 for repression, such as Rox1p (3, 11) and α2p (17, 22, 40), do the same. Since repression by LexA-Rgt1p in the absence of glucose is abolished in *ssn6*Δ and *tup1*Δ mutants, Rgt1p appears to be another DNA-binding protein that recruits Ssn6p-Tup1p, in this case to the promoters of the *HXT* genes. A LexA-Mig1p fusion protein is converted from a repressor to an activator in the absence of *SSN6* (42). While repression caused by LexA-Rgt1p in the absence of glucose is

relieved in an *ssn6Δ* mutant, Rgt1p is not converted into an activator but still requires high concentrations of glucose to activate transcription. Thus, transcriptional activation by Rgt1p is independent of Ssn6p and Tup1p.

Rgt1p could activate transcription in the presence of high levels of glucose by serving as an adapter that assists a transcriptional activator bound to the UAS, or it could stimulate transcription itself. The fact that LexA-Rgt1p causes strong activation of a *lexA*o-containing reporter gene that lacks its own UAS leads us to favor the idea that Rgt1p itself is able to function as an activator. It could activate transcription by itself, or it could recruit other (perhaps glucose-induced) proteins that provide transcriptional activation domains. The magnitude of glucose-induced activation by LexA-Rgt1p is similar to that obtained with LexA-Gal4p, suggesting that Rgt1p is a strong activator.

Regulation of Rgt1p function requires glucose signals generated by the glucose sensors Snf3p and Rgt2p. Snf3p appears to be a low-glucose sensor, since it is required for induction of expression of *HXT* genes only by low levels of glucose (29, 31). Consistent with this role, Snf3p is also required for inhibition of the repressor activity of LexA-Rgt1p by low levels of glucose (Table 5). Induction of the activator function of Rgt1p by high concentrations of glucose is independent of Snf3p but requires the high-glucose sensor Rgt2p, which is also required for maximal induction of expression of *HXT* genes by high levels of glucose (29). How the glucose signals generated by Snf3p and Rgt2p are transduced to Rgt1p remains to be discovered.

The *GRR1* gene is required for glucose-induced expression of *HXT* genes. In a *grr1Δ* mutant, LexA-Rgt1p represses transcription even in the presence of high concentrations of glucose. This suggests that Grr1p is required both for inhibition of Rgt1p repressor function in response to low levels of glucose and for conversion of Rgt1p from a repressor to an activator by high levels of glucose (Fig. 4B and C). *HXX2*, which plays a major role in glucose repression of gene expression (12, 15, 19, 28, 35, 43), also appears to be involved in glucose-induced gene expression: mutations in *HXX2* reduce induction of *HXT1* by high concentrations of glucose about five- to sixfold, similar to the levels in *rgt1Δ* mutants (31). However, the ability of LexA-Rgt1p to activate transcription at high concentrations of glucose is only slightly affected in *hxx2* mutants. Since Hxx2p is not completely required for Rgt1p activator function, it seems likely that the pathway that converts Rgt1p into an activator is different from the pathway that mediates glucose repression. In addition, this result suggests that two different pathways contribute to high-glucose induction of *HXT1* expression: while one of the pathways is Rgt1p dependent and also requires Rgt2p, the second pathway is dependent on only Hxx2p.

Since transcription of *RGT1* is not altered in response to glucose, the activity of Rgt1p is likely regulated posttranslationally. Candidates for the regulated function are DNA binding, nuclear localization, or transcriptional repression and activation. Rgt1p is probably not regulated by degradation, since it functions both in the absence of glucose (as a repressor) and in the presence of high levels of glucose (as a transcriptional activator). Since regulation of the LexA-Rgt1p chimeric protein does not require the DNA-binding domain, the DNA-binding activity of Rgt1p is probably not regulated. We expect that Rgt1p is modified in response to glucose and that this affects either the nuclear localization or the transcriptional repression and activation abilities of the protein. Because Rgt1p must be in the nucleus in cells growing both in the absence of glucose and on high glucose, nuclear localization would have to be sensitive to the levels of available glucose. Since we find it difficult to imagine how this could occur, we

currently favor the possibility that glucose regulates the transcriptional activation and repression functions of Rgt1p. Perhaps glucose (both low and high levels) inhibits the ability of Rgt1p to recruit Ssn6p and Tup1p; high levels of glucose could cause it to recruit a transcriptional activator protein.

The function of the Rgt1p is reminiscent of Ume6p, another Cys₆ zinc cluster protein that is a repressor of early meiotic genes during vegetative growth and an activator of the same set of genes under sporulation conditions (7, 37, 41). The activator function of Ume6p depends on the presence of Ime1p, an activator of meiotic genes (7). Ume6p itself appears to be a transcriptional repressor that is converted to a positive regulator by association with Ime1p, which provides the activation domain (37). Thus, both Rgt1p and Ume6p function as repressors or as activators and are regulated in response to opposing nutritional conditions.

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