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Two Glucose-sensing Pathways Converge on Rgt1 to Regulate Expression of Glucose Transporter Genes in *Saccharomyces cerevisiae**

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The yeast *Saccharomyces cerevisiae* deploys two different types of glucose sensors on its cell surface that operate in distinct glucose signaling pathways: the glucose transporter-like Snf3 and Rgt2 proteins and the Gpr1 receptor that is coupled to Gpa2, a G-protein α subunit. The ultimate target of the Snf3/Rgt2 pathway is Rgt1, a transcription factor that regulates expression of *HXT* genes encoding glucose transporters. We have found that the cAMP-dependent protein kinase A (PKA), which is activated by the Gpr1/Gpa2 glucose-sensing pathway and by a glucose-sensing pathway that works through Ras1 and Ras2, catalyzes phosphorylation of Rgt1 and regulates its function. Rgt1 is phosphorylated *in vitro* by all three isoforms of PKA, and this requires several serine residues located in PKA consensus sequences within Rgt1. PKA and the consensus serine residues of Rgt1 are required for glucose-induced removal of Rgt1 from the *HXT* promoters and for induction of *HXT* expression. Conversely, overexpression of the *TPK* genes led to constitutive expression of the *HXT* genes. The PKA consensus phosphorylation sites of Rgt1 are required for an intramolecular interaction that is thought to regulate its DNA binding activity. Thus, two different glucose signal transduction pathways converge on Rgt1 to regulate expression of glucose transporters.

The budding yeast *Saccharomyces cerevisiae* prefers to ferment glucose even when oxygen is available (1–3). This specialized mode of metabolism yields only two ATPs per molecule of glucose fermented, requiring yeast cells to pump large amounts of glucose through glycolysis. They do this by enhancing the rate-limiting step of glucose metabolism, its transport into cells, by increasing expression of the *HXT* genes encoding glucose transporters (*HXT*). Glucose induction of *HXT* expression is achieved through the Snf3/Rgt2-Rgt1 signal transduction pathway, in which the glucose signal generated by the Snf3 and Rgt2 glucose sensors ultimately alters function of the Rgt1 transcription factor (4–8).

Rgt1 functions differently in cells exposed to different levels of glucose. In the absence of glucose, Rgt1 represses *HXT* expression in conjunction with Mth1 and Std1 (4) by binding to *HXT* gene promoters and recruiting the Ssn6 and Tup1 corepressors (4, 9). Induction of *HXT* gene expression is achieved by relieving Rgt1-mediated repression through glucose-induced degradation of Mth1 and Std1 (10–12). Rgt1 also serves as a transcriptional activator that is required for full induction of *HXT1* expression when glucose levels are high (4), although how it converts from a transcriptional repressor to an activator remains unclear. The level of glucose determines the phosphorylation state of Rgt1; it is *hypo*phosphorylated in the absence of glucose and is *hyper*phosphorylated when glucose levels are high (9, 10, 13). It seems that glucose induces phosphorylation of Rgt1, which prevents it from binding to the *HXT* promoters and thus inhibits its repressor function (9, 10, 13).

The cAMP-dependent protein kinase A (PKA)[2] is involved in many different cellular processes including cell growth, stress resistance, and metabolism (8, 14–18). PKA is inactive during non-fermentative growth, existing as a tetrameric holoenzyme composed of two catalytic subunits encoded by one of three redundant *TPK* genes (*TPK1*, *TPK2*, and *TPK3*) and two regulatory subunits encoded by *BCY1* (19–21). The addition of glucose to cells induces a rapid elevation of the cAMP level due to activation of adenylate cyclase (Cyr1) via the Gpr1/Gpa2 and the Ras1/Ras2 pathways (22–24, 40–42). Binding of cAMP to the Bcy1 inhibitory subunit of PKA liberates the catalytic subunits, resulting in their activation (25). We report that glucose-activated PKA catalyzes phosphorylation of Rgt1, which results in altered Rgt1 function and relief of repression of the *HXT* genes.

EXPERIMENTAL PROCEDURES

Yeast Strains—The yeast strains used in this study are listed in Table 1. Yeast cells were grown on YP (2% bacto-peptone, 1% yeast extract) or synthetic yeast nitrogen base medium (0.17% yeast nitrogen base with 0.5% ammonium sulfate) supplemented with the appropriate amino acids.

Plasmids—Serine codons 96, 146, 202, 283, 284, 410, 480, 625, and 1130 of *RGT1* were converted to alanine codons by "gap repair" of a plasmid (26, 27) (Table 2). Briefly, two oligonucleotides carrying complementary nucleotide changes that result in a single nucleotide substitution that change a Ser

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[2] The abbreviation used is: PKA, cAMP-dependent protein kinase A.

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codon to Ala were used as primers along with the oligonucleotides flanking *RGT1* (OM4631 for 5′ and OM4632 for 3′) to amplify in separate reactions the 5′ and 3′ portions of *RGT1*, using pBM3306 or pBM3307 (4) as template. The *rgt1Δ* cells (FM557) were cotransformed with the PCR products (which overlap by the length of the PCR primers) and a *URA3*-containing plasmid that carries *lexA* (pSH2-1) (28), linearized by digestion with BamHI, selecting for Ura^+ cells. The PCR products are incorporated into the plasmid by homologous recombination regenerating Rgt1 (but with the mutation changing Ser to Ala).

Chromatin Immunoprecipitation and Western Blot Analysis—Yeast cells grown to mid-log phase were treated with formaldehyde (1% final concentration) for 20 min at room temperature, and the cross-linking reaction was quenched by adding glycine (125 mM final concentration). Yeast cell extracts were prepared by vortexing cell pellets with glass beads in lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate). After centrifugation the, cell lysates were sonicated five times with 10-s pulses using a microtip. Proteins with their attached genomic DNA fragments, which averaged 200–500 bp in length, were precipitated with mouse monoclonal anti-LexA antibodies (Santa Cruz Biotechnology). The cross-linking of the precipitated DNA to the protein was reversed by incubating them in elution buffer (50 mM Tris-HCl, pH 8.0, 1% SDS, 10 mM EDTA) at 65°C for 6 h, and 1/100 of the input DNA was used as template in a 25-cycle PCR. The sequences of the PCR primer pair used to detect the *HXT1* promoter were 5′-ATATAATTCCCCCCTCCTGAAG-3′ (OM 3109) and 5′TGATTCTACGTTTTTGCAAGC-3′ (OM3111). For Western blot analysis, 5 ml of yeast cells (A_{600} = 1.2) grown on different carbon sources (*i.e.* either 2% galactose or 4% glucose) were collected by centrifugation. The cells were resuspended in 100 μl of SDS-buffer (50 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol) and boiled for 5 min. After centrifugation, soluble proteins were resolved by SDS-PAGE, transferred to polyvinylidene difluoride membrane (Millipore), and detected with the Anti-LexA monoclonal antibodies (Santa Cruz Biotechnology) and ECL system (Pierce).

In Vitro Protein Kinase Assay—Rgt1 fused to LexA (pBM3307 (4)) was harvested from yeast cell extracts with anti-LexA conjugated to agarose beads (Santa Cruz Biotechnology) in Nonidet P-40 buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40). After washing with Nonidet P-40 buffer containing 1 M NaCl, the LexA-Rgt1 beads were equilibrated with kinase buffer (50 mM Tris-Cl, pH 6.8, 150 mM NaCl, 0.5% Triton X-100, 1 mM dithiothreitol). The 119 yeast protein kinases fused to GST (29) were overexpressed in yeast cells and affinity-purified with glutathione-Sepharose-4B beads (Amersham Biosciences). The LexA-Rgt1 and glutathione *S*-transferase kinases were mixed in 50 μl of kinase buffer containing 0.5 μCi of [γ^{32}P]ATP, 100 μM ATP, 10 mM $MgCl_2$ and incubated at 28 °C for 30 min. After washing the beads with the kinase buffer containing 0.5 M NaCl, the proteins were eluted by boiling the beads in SDS-sample buffer for 5 min. The eluted proteins were resolved by SDS-PAGE and detected by autoradiography. Each set of *in vitro* kinase assays was independently repeated twice.

Two-hybrid Assay—To construct Gal4 DNA-binding domain hybrids (Gal4 DBD-Rgt1), the N-terminal region of RGT1 (encoding amino acids 1–392) was amplified by the PCR using pBM3580 (30) as a template, and the PCR products were incorporated into the GAL4-DBD plasmid (pBM3593 (30)) by gap repair (26, 27). These plasmids were combined with the GAL4 activation domain hybrid (GAL4-AD-Rgt1, encoding amino acids 450–850 (30)) and used to transform yeast cells (FM413) to Leu^+ Trp^+. Yeast cells carrying both plasmids were grown to mid-log phase (A_{600} = 1–1.5) at 30 °C in the liquid medium containing galactose (2%), transferred to minimal medium containing galactose (2%) or glu-

TABLE 1

Yeast strains used in this study

Strain	Relevant genotype	Reference
FM391	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 (BY4741)*	38
FM413	*MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δgal80ΔGAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ*	37
FM557	FM391 *rgt1Δ::KanMX*	35
FM643	*MATa his3 leu2 ura3 trp1 ade8 tpk1^{w1} tpk2::HIS3 tpk3::TRP1 bcy1::LEU2*	31
FM644	*MATa his3 leu2 ura3 trp1 ade8 TPK1 tpk2::HIS3 tpk3::TRP1 BCY1*	31
FM645	*MATa his3 leu2 ura3 trp1 ade8 tpk1^{w2} tpk2::HIS3 tpk3::TRP1 bcy1::URA3*	31
YM7308	FM645 *rgt1Δ::KanMX*	This study
TB50a	*MATa leu2-3,112 ura3-52 trp1 his3 rme1 HMLa*	36
TS141	TB50a *bcy1::HIS3MX6*	36

TABLE 2

Plasmids used in this study

Plasmid	Characteristic	Reference
pBM2636	*HXT1::lacZ* (2 μm *URA3*)	4
pBM2817	*HXT3::lacZ* (2 μm *URA3*)	4
pBM3580	LexA-Rgt1	30
pBM3832	LexA-Rgt1-(1–392)	30
pBM4765	pBM3580 (Rgt1 mutation; Ser-96 to Ala)	This study
pBM4766	pBM3580 (Rgt1 mutation; Ser-146 to Ala)	This study
pBM4767	pBM3580 (Rgt1 mutation; Ser-202 to Ala)	This study
pBM4768	pBM3580 (Rgt1 mutations; Ser-283 and Ser-284 to Ala)	This study
pBM4769	pBM3580 (Rgt1 mutation; Ser-410 to Ala)	This study
pBM4770	pBM3580 (Rgt1 mutation; Ser-480 to Ala)	This study
pBM4771	pBM3580 (Rgt1 mutation; Ser-625 to Ala)	This study
pBM4773	pBM3580 (Rgt1 mutations; Ser-96, Ser-146, Ser-202, Ser-283, and Ser-284 to Ala)	This study
pBM4775	pBM3580 (Rgt1 mutations; Ser-96, Ser-146, Ser-202, Ser-283, Ser-284, Ser-410, Ser-480, Ser-625, and Ser-1130 to Ala)	This study
pBM4616	BD-Rgt1-(1–392)	30
pBM4630	AD-Rgt1-(450–850)	30
pKB12	LexA-Rgt1(1–392 with mutations; Ser-96, Ser-146, Ser-202, Ser-283, and Ser-284 to Ala)	This study
pKB13	BD-Rgt1-(1–392 with mutations; Ser-96, Ser-146, Ser-202, Ser-283, and Ser-284 to Ala)	This study
pKB14	*HXT1::lacZ* (2 μm *HIS3*)	This study

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cose and grown for 45 min, and then assayed for β-galactosidase activity.

β-Galactosidase activity assays were performed using the yeast β-galactosidase assay kit (Pierce) according to the manufacturer's instructions. Results were presented in Miller units ((1,000 × A_{420})/(T × V × A_{600}), where A_{420} is the optical density at 420 nm, T is the incubation time in minutes, and V is the volume of cells in milliliters). The reported lacZ activities are averages of results from triplicate of usually three different transformants.

RESULTS

PKA Catalyzes Phosphorylation of Rgt1—To identify potential Rgt1 protein kinases, 119 known and predicted protein kinases were tested for their ability to catalyze phosphorylation of Rgt1 *in vitro*. The protein kinases were expressed as glutathione *S*-transferase fusion proteins (29), affinity-purified from yeast cell extracts, and incubated with or without purified LexA-Rgt1 in buffer containing γ^{32}P-labeled ATP, and the radiolabeled proteins were detected by autoradiography after separating them by SDS-PAGE. Assays of a representative set of protein kinases are shown in Fig. 1*A*. The Tpk1 isoform of protein kinase A seemed to exhibit the strongest activity on Rgt1. The two other PKA isoforms, Tpk2 and Tpk3, also catalyzed phosphorylation of Rgt1 (Fig. 1*B*).

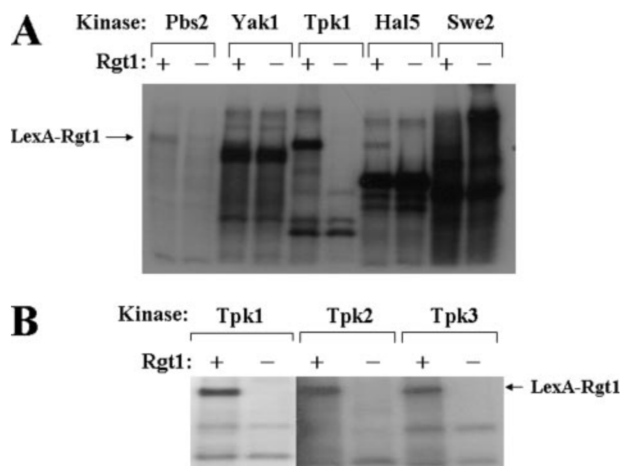

FIGURE 1. **PKA phosphorylates Rgt1 *in vitro*.** *A*, to identify potential Rgt1 kinases, 119 known and predicted protein kinases of *S. cerevisiae* were tested for their ability to phosphorylate LexA-Rgt1 *in vitro*. Many protein kinases exhibited the low level of activity on Rgt1 apparent for Pbs2 and Hal5; the PKAs exhibited much stronger activity on Rgt1. *B*, three PKA catalytic subunits (Tpk1, Tpk2, and Tpk3) phosphorylate LexA-Rgt1. Kinases were incubated with (+) or without (−) Rgt1 in buffer containing [γ^{32}P]ATP. Phosphorylation of Rgt1 was detected by autoradiography after SDS-PAGE.

The TPK Genes Are Required for Induction of HXT Expression—We explored the role of the *TPK* genes in *HXT* gene expression. Expression of the *HXT* genes is induced by glucose (Fig. 2*A*, *black bar* for wild type) and by deletion of *RGT1* (Fig. 2*A*, *white bar* for *rgt1*Δ), but glucose does not induce *HXT1* and *HXT3* gene expression in cells with greatly reduced PKA activity (tpk^w = *bcy1* $tpk1^{w1}$*tpk2 tpk3*; tpk^{w1} allele encodes a functionally attenuated PKA catalytic subunit (31)) (Fig. 2). Deletion of *RGT1* suppresses this defect, consistent with the idea that PKA functions through Rgt1. Deletion of any single *TPK* gene reduced induction of *HXT1* expression by only 30–50%; deletion of both *TPK2* and *TPK3* reduced *HXT1* expression by about 75%. The *TPK* genes contribute significantly to regulation of the high glucose-induced *HXT1* and *HXT3* genes but seem to be less involved in regulation of the low glucose-induced *HXT2* gene (data not shown). This might reflect the fact that PKA activity is maximal when glucose levels are high.

HXT1 expression was constitutive when PKA was rendered active by eliminating its Bcy1 regulatory subunit (Fig. 3). Similarly, overexpression of any one of the *TPK* genes induces *HXT1* expression (Fig. 3), presumably because high levels of the catalytic subunit of PKA overwhelms the Bcy1 regulatory subunit. These results suggest that PKA exerts its function through Rgt1 to effect expression of the *HXT* genes.

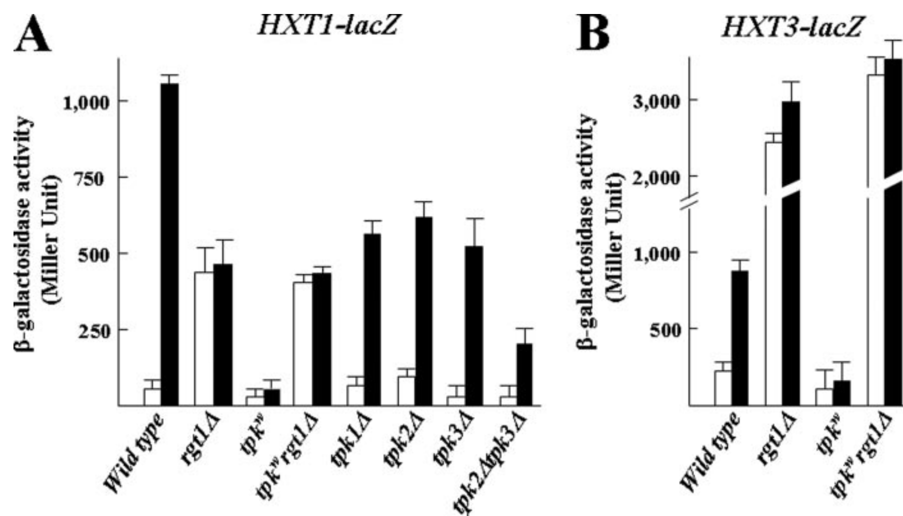

FIGURE 2. **PKA is required for induction of *HXT* gene expression.** Yeast cells (wild-type (FM391), *rgt1*Δ (FM557), tpk^w (FM643), tpk^w*rgt1*Δ (YM7308), *tpk1*Δ (35) *tpk2*Δ (35), *tpk3*Δ (35), and *tpk2*Δ*tpk3*Δ (FM644)) expressing *pHXT1:lacZ* (pBM2636 (*A*)) or *pHXT3::lacZ* (pBM2817 (*B*)) were grown to mid-log phase (A_{600} = 1–1.5) at 30 °C in liquid medium containing 2% galactose. Aliquots were transferred to minimal medium containing 2% galactose (*white bars*) or 4% glucose (*black bars*), grown for 90 min, and then assayed for β-galactosidase activity.

Serines in PKA Consensus Sequences of Rgt1 Are Required for Derepression of HXT1 Expression and Phosphorylation of Rgt1 by PKA—It has been previously suggested that Tpk3 has a role in modulation of Rgt1 activity. However, whether Tpk3 directly phosphorylates Rgt1 (39) has not been addressed. PKA catalyzes phosphorylation of serine or threonine in the sequence R(R/K/S)*X*(S/T) (phosphorylated Ser or Thr are underlined (32)). There are nine such consensus sequences in Rgt1; four of them near the N terminus have serines, Ser-146, Ser-202, Ser-283, and Ser-284, that are well conserved in the Rgt1 orthologs from *Saccharomyces* species and *Candida glabrata* (Fig. 4*A*). In contrast, the serines in the other five consensus sequences (Ser-96, Ser-410, Ser-480, Ser-625, and Ser-1130) are not conserved. Mutations

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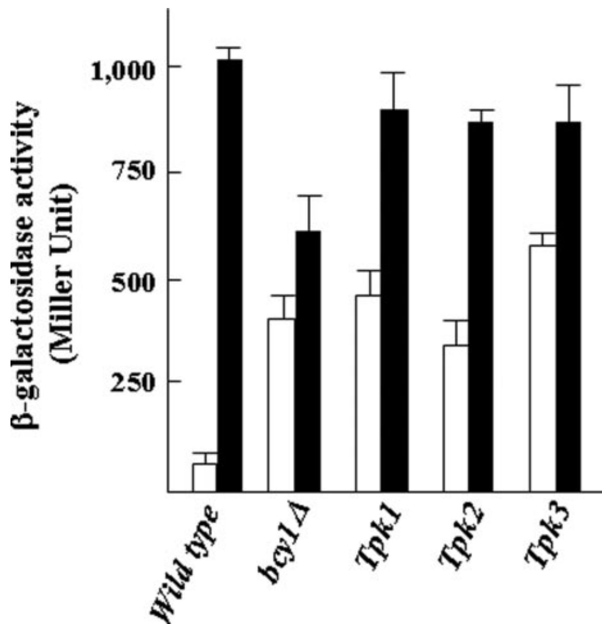

FIGURE 3. **Constitutive PKA activity induces *HXT1* expression.** *HXT1* expression was monitored using *pHXT1::lacZ* (pKB14) in wild type (TB50a) and *bcy1*Δ (TS141) cells. Overexpression of the PKA catalytic subunits from a high copy (2 μm) plasmid (*TPK1*, *TPK2*, *TPK3*) (29) causes constitutive induction of *HXT1* expression. Yeast cells were grown in minimal medium containing 2% galactose (*white bars*) or 4% glucose (*black bars*) as described in the legend for Fig. 2 and assayed for β-galactosidase activity. For overexpression of PKA, cells were grown on glucose medium (4%) and switched to medium containing galactose (2%) to induce expression of the *TPK* genes.

altering the conserved serines Ser-146, Ser-202, or Ser-283 and Ser-284 resulted in approximately a 50% reduction in glucose-induced *HXT1* expression (Fig. 4*B*). (Mutations altering the 5 non-conserved serines had no effect on *HXT1* expression (data not shown).) Changing all 4 of the conserved serines (plus Ser-96) to alanine (the S5A mutation) fully prevented glucose induction of *HXT1* expression (Fig. 4*B*) without affecting the stability of Rgt1 (Fig. 4*C*). Thus, the four conserved consensus sequences in Rgt1 for phosphorylation by PKA are crucial for regulation of Rgt1 function by glucose.

PKA catalyzes phosphorylation of wild-type Rgt1 *in vitro* but not of Rgt1 with alanines in place of the 5 serines in the conserved PKA consensus sequences (Fig. 5). This is true for full-length Rgt1 and for an N-terminal fragment of Rgt1-(1–392) that contains the evolutionarily conserved PKA consensus phosphorylation sites. The phosphorylation of Rgt1 by PKA *in vitro* is also apparent from the increased mobility in SDS-PAGE it causes (Fig. 5, *lower panels*). We conclude that PKA phosphorylates Rgt1 at one or more of its consensus phosphorylation sites.

Phosphorylation of Rgt1 Regulates Its Function—It is well known that glucose promotes phosphorylation of Rgt1 and its dissociation from the *HXT* gene promoters (9–10, 13), so we sought to determine whether PKA is responsible for this. A chromatin immunoprecipitation assay confirms that Rgt1 binds to the *HXT1* promoter in cells grown on galactose but not glucose (Fig. 6*A*). However, Rgt1 binds to the *HXT1* promoter in glucose-grown cells that lack PKA activity (tpk^w), as does Rgt1 lacking the serines in its PKA consensus phosphorylation sites in glucose-grown wild-type cells (Fig. 6*A*). These results suggest that phosphorylation of Rgt1 by PKA in response to glucose inhibits its DNA binding activity.

Rgt1 function is regulated by an intramolecular interaction between the N terminus and middle region of Rgt1 that has been suggested to inhibit function of the DNA-binding domain of Rgt1 (30). We used a two-hybrid assay to test whether the PKA consensus phosphorylation sites of Rgt1 are necessary for this intramolecular interaction. We used as "bait" the N-terminal region of Rgt1-(1–392) fused to the Gal4 DNA-binding domain and as "prey" the central region of Rgt1-(450–850) fused to the Gal4 transcriptional activation domain (Fig. 6*B*). Interaction between these two parts of Rgt1 is induced by glucose, but the interaction was not observed if the 5 serines of the consensus PKA phosphorylation sites of Rgt1 are changed to alanine (Fig. 6*B*).

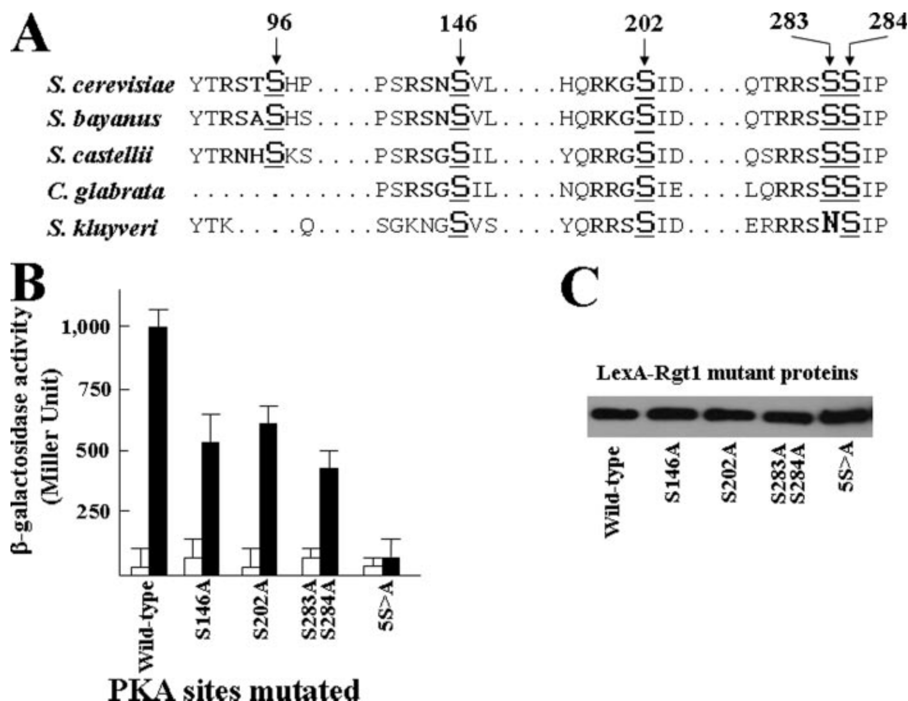

FIGURE 4. **Mutations at the PKA consensus sites in Rgt1 eliminate induction of *HXT1* expression.** *A*, the consensus PKA sites of Rgt1 are evolutionarily conserved in yeast species. ClustalW alignment of Rgt1 orthologs from *S. cerevisiae*, *Saccharomyces bayanus*, *Saccharomyces castellii*, *Saccharomyces kluyveri*, and *C. glabrata*; serine residues that are predicted to be phosphorylated are *underlined*. *B*, yeast cells (*rgt1*Δ, FM557) containing plasmids expressing Rgt1with mutations changing Ser to Ala in the PKA consensus phosphorylation sites and *pHXT1::lacZ* (pBM2636) were assayed for *HXT1* expression by assaying β-galactosidase activity after growth on 2% galactose (*white bars*) or 4% glucose (*black bars*). Plasmids used are: pBM4766 (S146A), pBM4767 (S202A), pBM4768 (S283A and S284A), and pBM4773 (S5A (indicated as *S > A*); S96A, S146A, S202A, S283A, and S284A). *C*, expression of the mutant LexA-Rgt1 proteins used in *panel B* was tested by immunoblotting proteins separated by SDS-PAGE with the LexA antibody.

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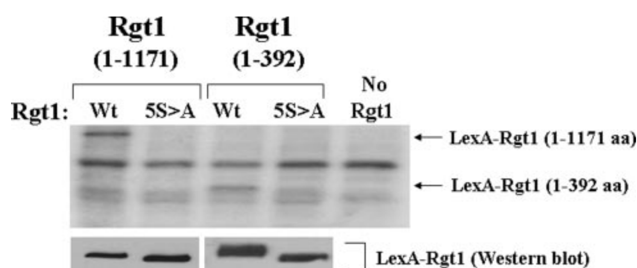

FIGURE 5. **Serines in consensus PKA phosphorylation sites of Rgt1 are required for its phosphorylation *in vitro*.** GST-Tpk1 was incubated with different forms of Rgt1. *First lane*, full-length Rgt1-(1–1171 (pBM3580)); *second lane*, full-length Rgt1 with mutations altering the nine PKA phosphorylation sites (pBM4775); *third lane*, truncated Rgt1-(1–392 (pBM3832)); *fourth lane*, truncated Rgt1-(1–392) with mutations at the first five PKA sites (pKB12); *fifth lane*, no Rgt1. Proteins were analyzed by SDS-PAGE and autoradiography (*upper*). The Rgt1 proteins used in this experiment were visualized by immunoblotting (*lower*). *Wt*, wild type; *aa*, amino acids.

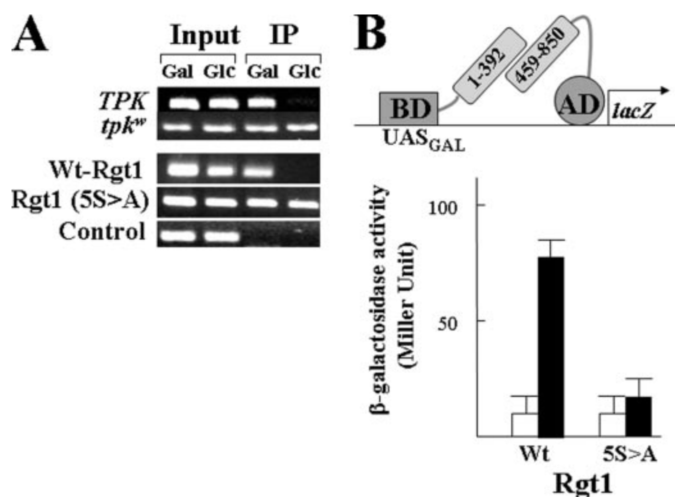

FIGURE 6. **Rgt1 phosphorylation by PKA interferes with its intermolecular interaction, resulting in its constitutive binding to DNA.** *A*, a chromatin immunoprecipitation (*IP*) assay of Rgt1. Chromatin was prepared from the wild-type (*Wt*) (FM391) and *tpk*^{w} (FM643) cells and cells expressing Rgt1 with the 5 serines in consensus PKA phosphorylation sequences changed to alanine (pBM4773). Cells were grown on repressing (2% Gal) or inducing (4% Glc) conditions, and chromatin was precipitated with antibody to Rgt1 (9). *B*, yeast two-hybrid assay. FM413 (37) was cotransformed with the BD-Rgt1 (amino acids 1–392 (pBM4614)) or the BD-Rgt1-S5A (indicated as *S* > *A*)) (pKB13) plasmids and the AD-Rgt1 plasmid (amino acids 450–850 (pBM4630)) and grown in minimal medium containing 2% galactose (*white bars*). Cells were transferred to medium with 4% glucose (*black bars*) for 20 min and assayed for β-galactosidase activity.

These results suggest that PKA phosphorylates Rgt1 when glucose is available and that this is required for the Rgt1 intramolecular interaction that inhibits its DNA binding activity, thereby dissociating Rgt1 from the *HXT* promoters.

DISCUSSION

We have presented three pieces of evidence that support the view that PKA contributes to glucose induction of *HXT* gene expression by catalyzing phosphorylation of Rgt1. 1) Glucose fails to induce *HXT1* and *HXT3* expression in yeast cells deficient in PKA activity (Fig. 2), and *HXT1* expression is constitutive in cells with constitutive PKA activity (Fig. 3); 2) evolutionarily conserved serine residues in PKA consensus phosphorylation sequences of Rgt1 are essential for glucose to induce *HXT1* expression (Fig. 4) and to cause release of Rgt1

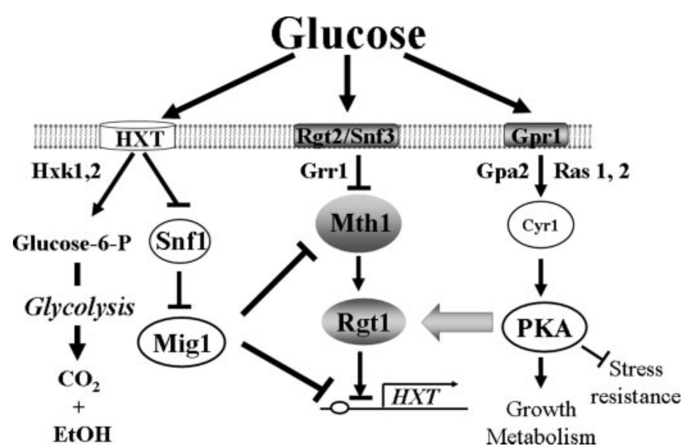

FIGURE 7. **Coordination of multiple glucose-sensing pathways that regulate *HXT* expression.**

from the *HXT1* promoter (Fig. 6*A*); and 3) PKA catalyzes phosphorylation of Rgt1 *in vitro* but not of Rgt1 devoid of PKA consensus phosphorylation sites (Fig. 5).

PKA activity is regulated in two ways. 1) A glucose-sensing pathway that starts at the plasma membrane with Gpr1, a G-protein-coupled receptor (17), stimulates adenylate cyclase via the G-protein α subunit Gpa2, and 2) the small GTP-binding proteins Ras1 and Ras2 stimulate adenyl cyclase in response to glucose (40). In these ways, the addition of glucose to yeast cells leads to an increase in the intracellular level of cAMP, which binds to the Bcy1 inhibitory subunit of PKA and dissociates it from the enzyme, thereby activating the protein kinase activity of PKA. These two routes of PKA activation seem to be redundant because deletion of *GPR1* or *RAS1* and *RAS2* does not affect glucose induction of *HXT* gene expression (data not shown).

Glucose induces *HXT* gene expression by ultimately effecting the release of the Rgt1 repressor from the *HXT* promoters (9–10, 13). We previously provided evidence that this is due to an intramolecular interaction between the N-terminal region of Rgt1 that contains its zinc cluster DNA-binding domain and the middle region of the protein (30). That intramolecular interaction requires phosphorylation of Rgt1, and our results suggest that PKA is the protein kinase responsible for this event (Fig. 6*B*). Mth1 and Std1 inhibit this intramolecular interaction (30). Thus, two different glucose-induced events must occur for this intramolecular interaction to take place and release Rgt1 from the *HXT* promoters; Mth1 and Std1 must be degraded via the Snf3-Rgt2 glucose-sensing pathway, and Rgt1 must become phosphorylated via the Gpr1-PKA glucose-sensing pathway.

Std1 does not completely disappear when glucose is added to cells because glucose induces expression of *STD1* (via the Snf3/Rgt2-Rgt1 glucose signaling pathway (12, 33)). Enough Std1 could remain in glucose-grown cells to attenuate the intramolecular interaction of Rgt1, and this would dampen induction of *HXT* expression. This may necessitate a device to lock Rgt1 in a conformation that enables full induction of *HXT* expression, and we propose that PKA could provide such a device. We surmise that yeast cells take advantage of this strategy to induce different *HXT* genes in response to different levels of glucose. When glucose levels are low, Mth1 would be degraded, but Rgt1

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would not be fully phosphorylated because PKA is not fully active in this condition. This might result in induction only of *HXT* genes encoding high affinity glucose transporters (*e.g. HXT2*). When glucose levels are high, Mth1 would be degraded, and Rgt1 would be fully phosphorylated because PKA is fully active. This would drive to completion the intramolecular interaction of Rgt1 and result in full induction of the high glucose-induced *HXT* genes (*i.e. HXT1* and *HXT3*).

This is the third glucose-sensing pathway known to affect expression of the *HXT* genes encoding glucose transporters (Fig. 7). The Snf3/Rgt2-Rgt1 pathway is responsible for glucose induction of *HXT* expression. The glucose repression pathway that operates through the Snf1 protein kinase and the Mig1 transcriptional repressor contributes to regulation of *HXT* expression by repressing expression of *MTH1* (33), which reinforces glucose-induced degradation of Mth1 and results in rapid glucose induction of *HXT* expression (12). Mig1 also represses expression of the *HXT2* and *HXT4* genes when glucose levels are high (34), ensuring that the high affinity glucose transporters encoded by these genes are only expressed when glucose levels are low. The results described here indicate that the Gpr1-Ras1/Ras2-PKA glucose-sensing pathway also contributes to regulation of *HXT* expression by regulating Rgt1 function. By integrating the signals generated in three different glucose-sensing pathways, yeast cells are able to respond rapidly and decisively to fluctuating levels of glucose.

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