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

Jeong-Ho Kim  
*University of Southern Mississippi, jh_kim@gwu.edu*

Mark Johnston  
*Washington University School of Medicine*

Follow this and additional works at: https://aquila.usm.edu/fac_pubs

Part of the Biochemistry, Biophysics, and Structural Biology Commons

Recommended Citation  
Kim, J., Johnston, M. (2006). Two Glucose-Sensing Pathways Converge on Rgt1 to Regulate Expression of Glucose Transporter Genes in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry, 281*(36), 26144-26149.  
Available at: https://aquila.usm.edu/fac_pubs/8533

This Article is brought to you for free and open access by The Aquila Digital Community. It has been accepted for inclusion in Faculty Publications by an authorized administrator of The Aquila Digital Community. For more information, please contact Joshua.Cromwell@usm.edu.
Two Glucose Sensing Pathways Converge on Rgt1 to Regulate Expression of Glucose Transporter Genes in *S. cerevisiae*

Jeong-Ho Kim¹, Mark Johnston²

From ¹the Center for Medical Bioscience and Bioinformatics, University of Southern Mississippi, Hattiesburg, MS 39406 and ²Department of Genetics, Campus Box 8232, Washington University School of Medicine, 660 S. Euclid Avenue, Saint Louis, MO 63110.

Running title: PKA phosphorylates Rgt1

Address correspondence to: Jeong-Ho Kim, Center for Medical Bioscience and Bioinformatics, University of Southern Mississippi, 118 College Dr. #5018, Hattiesburg, MS 39406, Tel. 601-266-4262; Fax. 601-266-5068; E-Mail: jeongho.kim@mfgn.usm.edu

The budding yeast *S. 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 alpha 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, over-expression 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 *S. 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), though how it converts from a transcriptional repressor to an activator remains unclear. The level of glucose determines the phosphorylation state of Rgt1: it is hypophosphorylated in the absence of glucose and is hyperphosphorylated 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) 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). 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 media (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 1,130 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 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 (ChIP) 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% Na-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 to 500 bp in length, were precipitated with mouse monoclonal anti-LexA antibodies (Santa Cruz). The crosslinking 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 65ºC for 6 hr 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’-ATATAATTCCCCC-TCCTGAAG-3’ (OM 3109) and 5’TGATTCAGTTTTTGCAAGC-3’ (OM3111). For Western blot analysis, 5 ml of yeast cells (O.D600 = 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% sodium dodecyl sulphate (SDS), 5% β-mercaptoethanol) and boiled for 5 min. After centrifugation, soluble proteins were resolved by SDS-PAGE, transferred to PVDF membrane (Millipore), and detected with the Anti-LexA monoclonal antibodies (Santa Cruz) 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) in NP-40 buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40). After washing with NP-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 DTT). The 119 yeast protein kinases fused to Gst (29) were overexpressed in yeast cells and affinity-purified with Glutathione Sepharose-4B beads (Amersham Bioscience). The LexA-Rgt1 and Gst-kinases were mixed in 50 μl kinase buffer containing 0.5 μCi of [γ-32P]ATP, 100 μM ATP, 10 mM MgCl2 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 amino-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 (OD600 = 1 to 1.5) at 30°C in the liquid medium containing galactose (2%), transferred to minimal medium containing galactose (2%) or glucose 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, Rockford, IL) according to the manufacturer’s instructions. Results were presented in Miller Units ((1,000 x OD_{420}) / (T x V x OD_{600}), where OD_{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, incubated with or without purified LexA-Rgt1 in buffer containing [γ-32P]-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. 1A. 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. 1B).

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 (black bar for WT in Fig. 2A) and by deletion of RGT1 (white bar for rgt1Δ in Fig. 2A), but glucose does not induce HXT1 and HXT3 gene expression in cells with greatly reduced PKA activity (tpkw = bcy1 tpk1 w1tpk2 tpk3; tpk1 allele encodes 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, over-expression 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.

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. But it has not been addressed whether Tpk3 directly phosphorylates Rgt1 (39). PKA catalyzes phosphorylation of serine or threonine in the sequence R(R/K/S)X(S/T) (phosphorylated S or T are underlined (32)). There are 9 such consensus sequences in Rgt1; four of them near the N-terminus have serines—S146, S202, S283, and S284—that are well conserved in the Rgt1 orthologs from Saccharomyces species and C. glabrata (Fig. 4A). In contrast, the serines in the other five consensus sequences (S96, S410, S480, S625, and S1130) are not conserved. Mutations altering the conserved serines S146, S202 or S283 and S284, resulted in approximately a 50% reduction in glucose-induced HXT1 expression (Fig. 4B). (Mutations altering the five non-conserved serines had no affect on HXT1 expression (data not shown)). Changing all four of the conserved serines (plus S96) to alanine (the 5S>A mutation) fully prevented glucose induction of HXT1 expression (Fig. 4B). (Mutations altering the five non-conserved serines had no affect on HXT1 expression (data not shown)). Changing all four of the conserved serines (plus S96) to alanine (the 5S>A mutation) fully prevented glucose induction of HXT1 expression (Fig. 4B), without affecting the stability of Rgt1 (Fig. 4C). 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 (lower panels of Fig. 5). 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 if PKA is responsible for this. A chromatin immunoprecipitation (ChIP) assay confirms that Rgt1 binds to the HXT1 promoter in cells grown on galactose but not glucose (Fig. 6A). However, Rgt1 binds to the HXT1 promoter in glucose-grown cells that lack PKA activity (tpkw), as does Rgt1 lacking the serines in its PKA consensus phosphorylation sites in glucose-grown wild-type cells (Fig. 6A). 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 amino 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 if 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. 6B). 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. 6B). 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 from the HXT1 promoter (Fig. 6A); 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 alpha subunit Gpa2; 2) the small GTP-binding proteins Ras1 and Ras2 stimulate adenyl cyclase in response to glucose (40). In these ways 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. 6B). 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 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.

REFERENCES

1. Lagunas, R. (1979) Mol Cell Biochem 27, 139-146
2. Lagunas, R. (1986) Yeast 2, 221-228
3. Pfeiffer, T., Schuster, S., and Bonhoeffer, S. (2001) Science 292, 504-507
4. Ozcan, S., Leong, T., and Johnston, M. (1996) Mol Cell Biol 16, 6419-6426
5. Ozcan, S., and Johnston, M. (1999) Microbiol Mol Biol Rev 63, 554-569
6. Forsberg, H., and Ljungdahl, P. O. (2001) Curr Genet 40, 91-109
7. Johnston, M., and Kim, J. H. (2005) Biochem Soc Trans 33, 247-252
8. Santangelo, G. M. (2006) Microbiol Mol Biol Rev 70, 10322-10327
9. Kim, J. H., Polish, J., and Johnston, M. (2003) Mol Cell Biol 23, 5208-5216
10. Flick, K. M., Spielvoy, N., Kalashnikova, T. I., Guadarrama, M., Zhu, Q., Chang, H. C., and Wittenberg, C. (2003) Mol Biol Cell 14, 3230-3241
11. Moriya, H., and Johnston, M. (2004) Proc Natl Acad Sci U S A 101, 1572-1577
12. Kim, J. H., Brachet, V., Moriya, H., and Johnston, M. (2006) Eukaryot Cell 5, 167-173
13. Mosley, A. L., Lakshmanan, J., Aryal, B. K., and Ozcan, S. (2003) J Biol Chem 278, 10322-10327
14. Broach, J. R. (1991) Curr Opin Genet Dev 1, 370-377
15. Thevelein, J. M., and de Winde, J. H. (1999) Mol Microbiol 33, 904-918
16. Rolland, F., De Winde, J. H., Lemaire, K., Boles, E., Thevelein, J. M., and Winderickx, J. (2000) Mol Microbiol 38, 348-358
17. Rolland, F., Winderickx, J., and Thevelein, J. M. (2002) FEMS Yeast Res 2, 183-201
18. Thevelein, J. M., Gelade, R., Holsbeeks, I., Lagatie, O., Popova, Y., Rolland, F., Stolz, F., Van de Velde, S., Van Dijck, P., Vandormael, P., Van Nuland, A., Van Roey, K., Van Zeebroeck, G., and Yan, B. (2005) Biochem Soc Trans 33, 253-256
19. Toda, T., Cameron, S., Sass, P., Zoller, M., and Wigler, M. (1987) Cell 50, 277-287
20. Cannon, J. F., and Tatchell, K. (1987) Mol Cell Biol 7, 2653-2663
21. Toda, T., Cameron, S., Sass, P., Zoller, M., Scott, J. D., McMullen, B., Hurwitz, M., Krebs, E. G., and Wigler, M. (1987) Mol Cell Biol 7, 1371-1377
22. Colombo, S., Ma, P., Cauwenberg, L., Winderickx, J., Crauwels, M., Teunissen, A., Nauwelaeirs, D., de Winde, J. H., Gorwa, M. F., Colavizza, D., and Thevelein, J. M. (1998) Embo J 17, 3326-3341
23. Kraakman, L., Lemaire, K., Ma, P., Teunissen, A. W., Donaton, M. C., Van Dijck, P., Winderickx, J., de Winde, J. H., and Thevelein, J. M. (1999) Mol Microbiol 32, 1002-1012
24. Versele, M., de Winde, J. H., and Thevelein, J. M. (1999) Embo J 18, 5577-5591
25. Edelman, A. M., Blumenthal, D. K., and Krebs, E. G. (1987) Annu Rev Biochem 56, 567-613
26. Ma, H., Kunes, S., Schatz, P. J., and Botstein, D. (1987) Gene 58, 201-216
27. Wach, A., Brachat, A., Pohlman, R., and Philippson, P. (1994) Yeast 10, 1793-1808
28. Brent, R., and Ptashne, M. (1985) Cell 43, 729-736
29. Zhu, H., Klemic, J. F., Chang, S., Bertone, P., Casamayor, A., Klemic, K. G., Smith, D., Gerstein, M., Reed, M. A., and Snyder, M. (2000) Nat Genet 26, 283-289
30. Polish, J. A., Kim, J. H., and Johnston, M. (2005) Genetics 169, 583-594
31. Cameron, S., Levin, L., Zoller, M., and Wigler, M. (1988) Cell 53, 555-566
32. Kennelly, P. J., and Krebs, E. G. (1991) J Biol Chem 266, 15555-15558
33. Kaniak, A., Xue, Z., Macool, D., Kim, J. H., and Johnston, M. (2004) *Eukaryot Cell* **3**, 221-231
34. Ozcan, S., and Johnston, M. (1996) *Mol Cell Biol* **16**, 5536-5545
35. Giaever, G., Chu, A. M., Ni, L., Connelly, C., Riles, L., Veronneau, S., Dow, S., Lucau-Danila, A., et al. (2002) *Nature* **418**, 387-391
36. Schmelze, T., Beck, T., Martin, D. E., and Hall, M. N. (2004) *Mol Cell Biol* **24**, 338-351
37. James, P., Halladay, J., and Craig, E. A. (1996) *Genetics* **144**, 1425-1436
38. Brachmann, R. K., Yu, K., Eby, Y., Pavletich, N. P., and Boeke, J. D. (1998) *Embo J* **17**, 1847-1859
39. Palomino, A., Herrero, P., and Moreno, F. (2006) *Nucleic Acids Res* **34**, 1427-1438
40. Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K., and Wigler, M. (1985) *Cell* **40**, 27-36
41. Tanaka, K., Nakafuku, M., Satoh, T., Marshall, M. S., Gibbs, J. B., Matsumoto, K., Kaziro, Y., and Toh-e, A. (1990) *Cell* **60**, 803-807
42. Tanaka, K., Lin, B. K., Wood, D. R., and Tamanoi, F. (1991) *Proc Natl Acad Sci U S A* **88**, 468-472

**ACKNOWLEDGEMENTS**

We thank Dr. Mike Wigler for strains, and our colleagues Victoria Brown-Kennerly, Jessie Sexton, Jeff Sabina, and George Santangelo for critical reading of the manuscript. We also thank Satish Pasula and David Jouandot for technical assistance. This publication was made possible by NIH grant GM32540 to M.J. and NIH Grant RR016476-04 from the MS INBRE Program of the National Center for Research Resources.

**FIGURE LEGENDS**

**Fig. 1.** Protein Kinase A (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-32P]ATP. Phosphorylation of Rgt1 was detected by autoradiography after SDS-PAGE.

**Fig. 2.** PKA is required for induction of *HXT* gene expression. Yeast cells (wild-type (FM391), rgt1Δ (FM557), tpk" (FM643), tpk"rgt1Δ (YM7308), tpk1Δ (35) tpk2Δ (35), tpk3Δ (35) and tpk2Δtpk3Δ (FM644)) expressing *pHXT1::lacZ* (pBM2636 (A)) or *pHXT3::lacZ* (pBM2817 (B)) were grown to mid-log phase (OD600 = 1 to 1.5) at 30°C in liquid minimal 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.

**Fig. 3.** Constitutive PKA activity induces *HXT1* expression. *HXT1* expression was monitored using *pHXT1::lacZ* (pKB14) in wild type (TB50a) and *bcy1Δ* (TS141) cells. Overexpression of the PKA catalytic subunits from a high copy (2 micron) 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 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.

**Fig. 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 \(S.\) \(cerevisiae\), \(S.\) \(bayanus\), \(S.\) \(castellii\), \(S.\) \(kluyveri\), and \(C.\) \(glabrata\); serine residues that are predicted to be phosphorylated are underlined. 
B, Yeast cells (\(rgt1\Delta\), FM557) containing plasmids expressing Rgt1 with mutations changing S to A 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: pBM4766 (S146A), pBM4767 (S202A), pBM4768 (S283A and S284A), and pBM4773 (5S>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.

**Fig. 5.** Serines in consensus PKA phosphorylation sites of Rgt1 are required for its phosphorylation \textit{in vitro}. Gst-Tpk1 was incubated with different forms of Rgt1. 
1\textsuperscript{st} lane: full length Rgt1 (1-1171 (pBM3580)); 
2\textsuperscript{nd} lane: full length Rgt1 with mutations altering the 9 PKA phosphorylation sites (pBM4775); 
3\textsuperscript{rd} lane: truncated Rgt1 (1-392 (pBM3832)); 
4\textsuperscript{th} lane: truncated Rgt1 (1-392) with mutations at the first 5 PKA sites (pKB12); 
5\textsuperscript{th} lane: no Rgt1. Proteins were analyzed by SDS-PAGE and autoradiography (upper). The Rgt1 proteins used in this experiment were visualized by immunoblotting (lower).

**Fig. 6.** Rgt1 phosphorylation by PKA interferes with its intermolecular interaction, resulting in its constitutive binding to DNA. 
A, A chromatin immunoprecipitation assay of Rgt1. Chromatin was prepared from the wild-type (FM391) and \(tpk\)\(^{\text{w}}\) (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% glu) 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-Rgt1-5S>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.

**Fig. 7.** Coordination of multiple glucose sensing pathways that regulate \(HXT\) expression.
### 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 gal4- gal80-GAL2-ADE2                      | (37)      |
|          | LYS2::GAL1-HIS3 met2::GAL7-lacZ                                                   |           |
| FM557    | FM391 rgt1::KanMX                                                                  | (35)      |
| FM643    | MATa his3 leu2 ura3 trp1 ade8 tpk1Δ 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 tpk1Δ tpk2::HIS3 tpk3::TRP1 bcy1::URA3                | (31)      |
| YM7308   | FM645 rgt1::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 (2μm URA3)                                                           | (4)       |
| pBM2817    | HXT3::lacZ (2μm URA3)                                                           | (4)       |
| pBM3580    | LexA-Rgt1                                                                       | (30)      |
| pBM3832    | LexA-Rgt1 (1-392)                                                               | (30)      |
| pBM4765    | pBM3580 (Rgt1 mutation; S96 to A)                                               | This study|
| pBM4766    | pBM3580 (Rgt1 mutation; S146 to A)                                              | This study|
| pBM4767    | pBM3580 (Rgt1 mutation; S202 to A)                                              | This study|
| pBM4768    | pBM3580 (Rgt1 mutations; S283 and S284 to A)                                    | This study|
| pBM4769    | pBM3580 (Rgt1 mutation; S410 to A)                                              | This study|
| pBM4770    | pBM3580 (Rgt1 mutation; S480 to A)                                              | This study|
| pBM4771    | pBM3580 (Rgt1 mutation; S625 to A)                                              | This study|
| pBM4773    | pBM3580 (Rgt1 mutations; S96, S146, S202, S283 and S284 to A)                    | This study|
| pBM4775    | pBM3580 (Rgt1 mutations; S96, S146, S202, S283, S284, S410, S480, S625, and S1,130 to A) | This study|
| pBM4616    | BD-Rgt1 (1-392)                                                                  | (30)      |
| pBM4630    | AD-Rgt1 (450-850)                                                               | (30)      |
| pKB12      | LexA-Rgt1(1-392 with mutations; S96, S146, S202, S283 and S284 to A)             | This study|
| pKB13      | BD-Rgt1 (1-392 with mutations; S96, S146, S202, S283 and S284 to A)              | This study|
| pKB14      | HXT1::lacZ (2μm HIS3)                                                            | This study|
Figure 1

A

Kinase: Pbs2  Yak1  Tpk1  Hal5  Swe2
Rgt1: +  -  +  -  +  -  +  -

LexA-Rgt1

B

Kinase: Tpk1  Tpk2  Tpk3
Rgt1: +  -  +  -  +  -

LexA-Rgt1
Figure 2

**A**

$HXT1-lacZ$

|          | HXT1-lacZ Activity (Miller Unit) |
|----------|----------------------------------|
| Wild type|                                |
| sst1     |                                |
| pka4     |                                |
| pka4-ka1 |                                |
| pka4-ka2 |                                |
| pka4-ka3 |                                |
| pka4-ka4 |                                |
| pka4-ka5 |                                |
| pka4-ka6 |                                |

**B**

$HXT3-lacZ$

|          | HXT3-lacZ Activity (Miller Unit) |
|----------|----------------------------------|
| Wild type|                                |
| sst1     |                                |
| pka4     |                                |
| pka4-ka1 |                                |
| pka4-ka2 |                                |
| pka4-ka3 |                                |
| pka4-ka4 |                                |
| pka4-ka5 |                                |
| pka4-ka6 |                                |
Figure 3

![Graph showing β-galactosidase activity (Miller Unit) for different strains: Wildtype, bgLa, Tp1, Tp2, and Tp3. The graph displays the activity levels, with Wildtype having the highest activity followed by bgLa, Tp1, Tp2, and Tp3.]
Figure 4

A

\[
\begin{align*}
S. \text{ cerevisiae} & \quad YTRST \text{IP} \ldots \text{PSRSM} \text{SVL} \ldots \text{QTRRS} \text{SS} \text{IP} \\
S. \text{ bayanus} & \quad YTRSA \text{SH} \ldots \text{PSRSM} \text{SVL} \ldots \text{QTRRS} \text{SS} \text{IP} \\
S. \text{ castellii} & \quad YTRNH \text{SK} \ldots \text{PSRSG} \text{SI} \ldots \text{QSRRS} \text{SS} \text{IP} \\
C. \text{ glabrata} & \quad \ldots \ldots \ldots \text{PSRSG} \text{SI} \ldots \text{QRRSG} \text{IE} \ldots \text{LQRRS} \text{SS} \text{IP} \\
S. \text{ kluveri} & \quad YTK \ldots Q \ldots \text{SGKNG} \text{SVS} \ldots \text{YORRS} \text{SI} \ldots \text{ERRRS} \text{NS} \text{IP}
\end{align*}
\]

B

\[\text{\(\beta\)-galactosidase activity (Miller Unit)}\]

PKA sites mutated

C

LexA-Rgt1 mutant proteins
Figure 5

\[
\begin{array}{ccc}
\text{Rgt1} & \text{Rgt1} \\
(1-1171) & (1-392) \\
\text{Rgt1:} & \text{Wt} & 5S>A & \text{Wt} & 5S>A & \text{No} & \text{Rgt1} \\
\hline
& \text{LexA-Rgt1 (1-1171 aa)} & \text{LexA-Rgt1 (1-392 aa)} & \text{LexA-Rgt1 (Western blot)}
\end{array}
\]
Figure 6

A

|       | Input | IP |
|-------|-------|----|
|       | Gal   | Glu|
| TPK   |       |    |
| tpk^w |       |    |
| Wt-Rgt1|      |    |
| Rgt1 (5S>A) | | |
| Control |      |    |

B

**BD**

UAS<sub>GAL</sub>

**AD**

lac<sub>Z</sub><sup>+</sup>

![Graph](attachment:image.png)

β-galactosidase activity (Miller Unit)

Rgt1

Wt 5S>A
Two glucose sensing pathways converge on Rgt1 to regulate expression of glucose transporter genes in S. cerevisiae

Jeong-Ho Kim and Mark Johnston

J. Biol. Chem. published online July 14, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M603636200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/early/2006/07/14/jbc.M603636200.citation.full.html#ref-list-1