

Integration of Transcriptional and Posttranslational Regulation in a Glucose Signal Transduction Pathway in *Saccharomyces cerevisiae*

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Expression of the *HXT* genes encoding glucose transporters in the budding yeast *Saccharomyces cerevisiae* is regulated by two interconnected glucose-signaling pathways: the Snf3/Rgt2-Rgt1 glucose induction pathway and the Snf1-Mig1 glucose repression pathway. The Snf3 and Rgt2 glucose sensors in the membrane generate a signal in the presence of glucose that inhibits the functions of Std1 and Mth1, paralogous proteins that regulate the function of the Rgt1 transcription factor, which binds to the *HXT* promoters. It is well established that glucose induces degradation of Mth1, but the fate of its parologue Std1 has been less clear. We present evidence that glucose-induced degradation of Std1 via the SCF^{Grr1} ubiquitin-protein ligase and the 26S proteasome is obscured by feedback regulation of *STD1* expression. Disappearance of Std1 in response to glucose is accelerated when glucose induction of *STD1* expression due to feedback regulation by Rgt1 is prevented. The consequence of relieving feedback regulation of *STD1* expression is that reestablishment of repression of *HXT1* expression upon removal of glucose is delayed. In contrast, degradation of Mth1 is reinforced by glucose repression of *MTH1* expression: disappearance of Mth1 is slowed when glucose repression of *MTH1* expression is prevented, and this results in a delay in induction of *HXT3* expression in response to glucose. Thus, the cellular levels of Std1 and Mth1, and, as a consequence, the kinetics of induction and repression of *HXT* gene expression, are closely regulated by interwoven transcriptional and posttranslational controls mediated by two different glucose-sensing pathways.

Glucose is an important source of carbon and energy for many organisms. This is particularly apparent in the budding yeast *Saccharomyces cerevisiae*, whose sophisticated glucose-sensing and -signaling mechanisms enable it to sense a wide range of glucose concentrations and utilize glucose efficiently (2, 7, 13). One of the first responses of yeast cells to glucose is induction of expression of the *HXT* genes, encoding glucose transporters (3, 18, 21, 28, 40). This is achieved through a signal transduction pathway that begins at the cell surface with the Snf3 and Rgt2 glucose sensors and ends in the nucleus with the Rgt1 transcription factor, which binds to *HXT* gene promoters (5, 12, 14, 27, 31).

The glucose signal generated by Rgt2 and Snf3 at the cell surface alters Rgt1 function in the nucleus by stimulating degradation of Mth1 and Std1 (4, 23), paralogous proteins that bind to Rgt1 and are necessary for it to repress transcription (20, 30, 32). Mth1 and Std1 also interact with the C-terminal tails of the Rgt2 and Snf3 glucose sensors (19, 32). This places them in proximity to the Yck1 protein kinase, which is associated with the Snf3 and Rgt2 glucose sensors and is thought to catalyze phosphorylation of Mth1 and Std1 when glucose binds to the sensors (23, 37). Phosphorylated Mth1 and Std1 are targets of the SCF^{Grr1} ubiquitin-protein ligase, which is thought to catalyze their ubiquitination, thereby targeting them for degradation by the 26S proteasome (37). In the absence of Mth1 and Std1, Rgt1 loses its ability to repress tran-

scription, leading to derepression of *HXT* gene expression (4, 20, 24, 30, 32).

While there is ample evidence that glucose induces degradation of Mth1 via the 26S proteasome, conflicting results have been reported for the effect of glucose on Std1 (4, 23, 37). *STD1* expression is induced by glucose via the Rgt2/Snf3-Rgt1 signal transduction pathway (15), and our data suggest that Std1 degradation is dampened by this glucose induction of *STD1* expression via the Rgt2/Snf3-Rgt1 pathway. By contrast, *MTH1* expression is repressed by glucose via the Snf1-Mig1 glucose repression pathway, and our results suggest that this reinforces Mth1 degradation. Thus, opposing transcriptional regulation of *MTH1* and *STD1* expression provides for rapid induction of *HXT* gene expression in response to glucose and for prompt establishment of repression of *HXT* gene expression when the available glucose has been exhausted. Thus, the course of induction and repression of the *HXT* genes is the result of close collaboration between two different glucose-sensing pathways that helps ensure efficient utilization of this key nutrient.

MATERIALS AND METHODS

Yeast strains and plasmids. The yeast strains used in this study are listed in Table 1. Cells were grown on either YP (2% Bacto peptone, 1% yeast extract) or YNB [0.67% yeast nitrogen base plus 0.5% (NH₄)₂SO₄ lacking the appropriate amino acids] medium, supplemented with the appropriate carbon sources. Genes were disrupted by homologous recombination using HisG-URA3-HisG (1) or KanMX (39) cassettes. Sequences of the primers are available on request. To construct pBM4747 (*MET25* promoter–green fluorescent protein [GFP]–*STD1*), pBM4748 (*MET25* promoter–GFP–*MTH1*), and pBM4749 (*MET25* promoter–GFP–Htr1-23), coding sequences of the genes were amplified by PCR and the resulting PCR products were cloned into the BamHI and SalI sites of pUG34 (pBM3842 *ARSH4/CEN-HIS3-MET25* promoter–yeGFP–polylinker–*CYC1* ter-

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TABLE 1. Yeast strains used in this study

Strain	Genotype
FM391	<i>MATa his3Δ1 leu2Δ ura3Δ met15Δ</i> (BY4741)
FM393	<i>MATa/MATαhis3Δ1/his3Δ1 leu2Δ/leu2Δ ura3Δ/ura3Δ met15Δ/met15Δ</i>
FM412	<i>MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ</i>
FM413	<i>MATα trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ</i>
FM436	<i>MATα ade2-1 his3-11,15 ura3-1 trp1-1</i> (CMY 18)
FM452	<i>MATa his3-11,15 leu2-3,115 ura3-1 trp1-1 ade2-1 cdc34-2</i>
FM524	<i>MATα ura3 his3-11,15 leu2-3,112 can1 pre2-2^(ts)</i>
FM535	<i>MATa cim5-1^(ts) ura3-52 his3-Δ200 leu2-Δ1</i>
YM4127	<i>MATa ura3-52 his3-200 ade2-101 lys2-801 leu2 trp1-903 tyr1-501</i>
YM4509	<i>MATa ura3-52 his3-200 ade2-101 lys2-801 leu2 trp1-903 tyr1 rgt1::hisG</i>
YM6212	<i>MATα ura3-52 his3-200 ade2-101 lys2-801 trp1-903 leu2-3,112 tyr1-501 snf3::HIS3 rgt2::HIS3</i>
YM6244	<i>MATa ura3-52 his3-11,15</i>
YM6245	<i>MATa ura3-52 his3-11,15 MTH1-23 (HTR1-23)</i>
YM6265	<i>MATa his3Δ leu2Δ met15Δ ura3Δ std1::KanMX2</i>
YM6266	<i>MATα his3Δ leu2Δ lys2Δ ura3Δ mth1::kanMX2</i>
YM6269	<i>MATα ura3-52 his3-200 ade2-101 lys2-801 leu2 trp1Δ rgt1::hisG</i>
YM6292	<i>MATα his3Δ leu2Δ met15Δ ura3Δ std1::KanMX2 mth1::kanMX2</i>
YM6328	<i>MAT his3Δ leu2Δ ura3Δ met1Δ snf3::hisG-URA3-hisG</i>
YM6452	<i>MATa ade2 ura3 his3 lys2 leu2 grr1::LEU2</i>

minator; GenBank accession number AF298784). Plasmid pBM4750 was derived from pBM4542, in which the Mig1 site was mutated (CCCCAG to CAACAG [mutated nucleotides are underlined]). To make Std1-9KR (positions K207, K287, K312, K337, K344, K347, K354, K381, and K411), Std1-7KR (positions K287, K312, K344, K347, K354, K381, and K411), and Std1-5KR (positions K312, K344, K347, K354, and K411), the lysine residues in Std1 were converted to arginine by using a gap repair technique (39).

β-Galactosidase assays. β-Galactosidase activity assays were performed using the yeast β-galactosidase assay kit (Pierce, Rockford, IL) according to the manufacturer's instructions. Results are presented in Miller units [(1,000 × OD₄₂₀)/(T × V × OD₆₀₀)], where OD₄₂₀ is the optical density at 420 nm, T is the incubation time in minutes, and V is the volume of cells in milliliters. Mean activities are averages from four to seven assays of at least eight independent transformants. Precultures were grown in selective media plus 2% galactose and 2.5% glycerol. Cells were then transferred to YP medium containing the appropriate carbon source and were incubated at 30°C for 4 h before β-galactosidase activity was measured.

Immunoprecipitation and ChIP. Yeast cell extracts were prepared by vortexing the cell pellets with glass beads in NP-40 buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40) at 4°C for 10 min. Crude protein (3 mg) was incubated with antibody-conjugated agarose beads (Santa Cruz) at 4°C for 3 h. The beads were then washed with high-salt buffer (NP-40 buffer containing 1 M NaCl) and boiled in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer at 90°C for 5 min to elute proteins. For Western blot analysis, the eluted proteins were resolved by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore). Monoclonal antibodies against LexA, GFP, and Myc (9E10) (Santa Cruz) and the ECL system (Pierce) were used for detection of proteins in the membranes as previously described (16). Binding of protein to the *HXT3* promoter in vivo was assayed by chromatin immunoprecipitation (ChIP) as described previously (16).

RESULTS

Mth1, Std1, and Rgt1 are required for repression of *HXT* gene expression. To verify the roles of Mth1, Std1, and Rgt1 in glucose signaling, we analyzed the effect of loss of these genes on expression of *HXT1*, which is induced by high levels of glucose (2%), and on expression of *HXT3*, which is induced by low levels of glucose (0.2% [a condition mimicked by 2% raffinose]), as well as by high glucose levels (28) (Table 2, genotype 1). As expected, removal of the Rgt1 repressor substantially relieved repression of the *HXT* genes (Table 2, genotype 2, Gal). Deletion of *MTH1* relieved repression of the low-glucose-induced *HXT3* gene but had little effect on expression of the high-glucose-induced *HXT1* gene (Table 2, geno-

type 3, Gal). Further deletion of *STD1* in an *mth1* mutant relieved repression of *HXT1* expression (Table 2, genotype 5). Deletion of *STD1* alone had little effect on expression of *HXT1* and *HXT3* (Table 2, genotype 4). Thus, Mth1 seems to be responsible for repression of the low-glucose-induced *HXT3* gene (and for that of *HXT4*, another low-glucose-induced gene [data not shown]), but either Std1 or Mth1 is sufficient for repression of the high-glucose-induced *HXT1* gene in the absence of glucose. These results reinforce previously reported findings (4, 20, 32) and suggest that Mth1 and Std1 work together with Rgt1 to repress expression of the high-glucose-induced *HXT1* gene but that Mth1 acts alone to regulate expression of the low-glucose-induced *HXT3* and *HXT4* genes.

Mth1 and Std1 inhibit the ability of Rgt1 to activate transcription. Rgt1 is a transcriptional activator in cells grown on high levels of glucose but not in cells grown in the absence of glucose (on galactose) (29) (Table 3, genotype 1). Deletion of both *MTH1* and *STD1* causes Rgt1 to activate transcription in cells grown on galactose (Table 3, genotype 4), suggesting that Mth1 and Std1 play a role in inhibiting transcriptional activation, in addition to their roles in promoting transcriptional

TABLE 2. Roles of Rgt1, Mth1, and Std1 in regulation of *HXT* gene expression^a

Genotype	β-Galactosidase activity (Miller units) ^b					
	<i>HXT1::lacZ</i>			<i>HXT3::lacZ</i>		
	Gal	Raf	Glu	Gal	Raf	Glu
1. Wild type	<1	29	1,220	50	629	744
2. <i>rgt1 MTH1 STD1</i>	27	57	326	225	632	559
3. <i>RGT1 mth1 STD1</i>	<1	<1	820	326	415	484
4. <i>RGT1 MTH1 std1</i>	<1	30	951	42	474	485
5. <i>RGT1 mth1 std1</i>	127	607	632	148	893	760

^a Precultures were grown to mid-log phase (OD₆₀₀, 1 to 1.5) in selective YNB medium with 2% galactose, shifted to YP medium containing the indicated sugars (all at 2%), grown for 4 h at 30°C, and then assayed for β-galactosidase activity.

^b Values are means from at least four independent experiments. Standard deviations are less than 20% in all cases. Gal, galactose; Raf, raffinose; Glu, glucose.

TABLE 3. Activation of the *lexO-lacZ* reporter^a by LexA-Rgt1

Genotype	Active molecule(s) ^b	β-Galactosidase activity (Miller units)	
		Galactose ^c	Glucose ^c
1. Wild type (FM391)	Mth1, Std1	<1	575
2. <i>mth1</i> (YM6266)	Std1	4	293
3. <i>std1</i> (YM6265)	Mth1	<1	325
4. <i>mth1 std1</i> (YM6292)		346	533
5. Wild type (YM6244)	Mth1, Std1	6	252
6. HTR1-23 ^d (YM6245)	Mth1 ^d , Std1	3	2

^a The plasmids used are pBM1817 (*lexO:lacZ* reporter) and pBM3306 (LexA-Rgt1).

^b Refers to the molecules present in the cells that act on Rgt1.

^c All sugars were present at a concentration of 2% in the growth medium.

^d Mth1 constitutive repressor caused by the *HTR1-23* mutation (6, 26, 33, 34).

repression in the absence of glucose. In *mth1* and *std1* single mutants, Rgt1 was unable to activate significant transcription in cells grown on galactose, indicating that Mth1 and Std1 are redundant inhibitors of the transcriptional activation function of Rgt1 when glucose levels are low. A form of Mth1 that is resistant to glucose-induced degradation (due to the dominant *MTH1-23* [also known as *HTR1-23*] mutation) (6, 26, 33, 34) prevents Rgt1 from activating transcription in the presence of high levels of glucose. Thus, in addition to promoting transcriptional repression by Rgt1 when glucose is absent (4, 30, 32), Mth1 and Std1 seem to inhibit transcriptional activation by Rgt1 when glucose levels are low. We believe that Mth1 and Std1 regulate Rgt1 function directly, because they interact with Rgt1 (20, 30, 38). In addition, Mth1 and Std1 are associated with *HXT* promoters (Fig. 1).

Glucose regulation of *MTH1* and *STD1* expression contributes to glucose signal transduction. It seems clear that Mth1 is degraded upon exposure of yeast cells to glucose, but there are conflicting reports regarding Std1 degradation in response to glucose (4, 23). Indeed, in our hands, degradation of Mth1 was reproducibly observed but degradation of Std1 in response to glucose was variable. We suspected that this was due to the

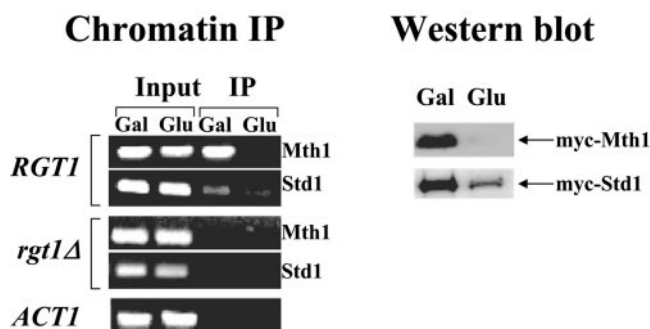


FIG. 1. Mth1 and Std1 are associated with Rgt1 at the *HXT3* promoter. Chromatin was prepared from cells expressing Mth1 (pBM4560) or Std1 (pBM4544) tagged with 9 copies of the Myc epitope grown under repressing (2% galactose) or inducing (2% glucose) conditions. Chromatin was precipitated with the anti-Myc antibody and used to detect the *HXT3* promoter by PCR amplification (ChIP). As a control, the *ACT1* gene was amplified. The proteins expressed were visualized by Western blotting. Essentially identical results were obtained when the immunoprecipitates were tested for enrichment of the *HXT1* promoter (data not shown).

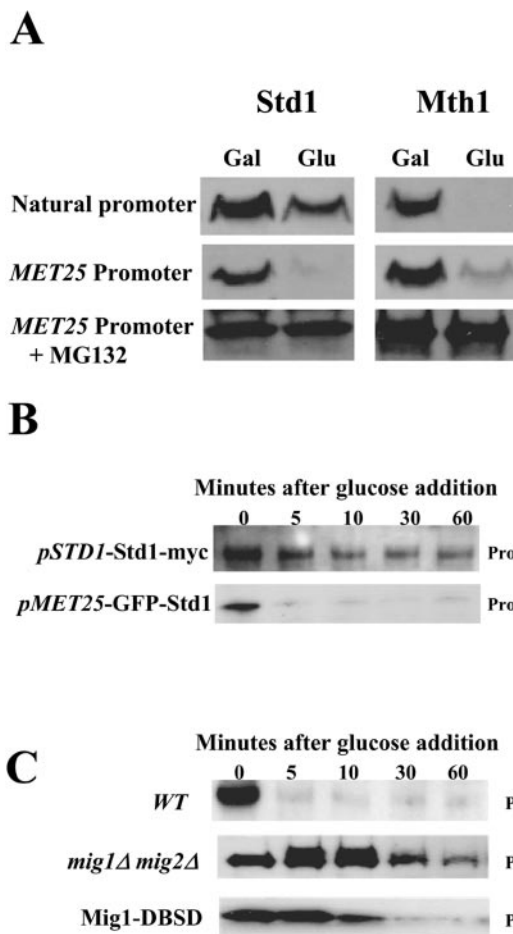


FIG. 2. Glucose induction of *STD1* expression dampens degradation of Std1. (A) Cells expressed the Std1 and Mth1 proteins from their own promoters (Std1-Myc₉ [pBM4544] or Mth1-Myc₉ [pBM4560]) or from the *MET25* promoter (GFP-Std1 [pBM4747] or GFP-Mth1 [pBM4748]). (Both versions of both proteins—with 9 copies of the Myc epitope at their C termini or with GFP at their N termini—appear to be functional, because they complement a *std1 mth1* mutant for repression of *HXT* expression in galactose-grown cells.) Cells were grown to mid-log phase in a medium selective for the plasmids containing 2% galactose. Aliquots were then transferred to a medium containing 2% galactose (Gal) or 2% glucose (Glu), incubated at 30°C for 60 min, and lysed. Cells (*ptr5Δ*) deleted for the gene encoding a drug efflux pump were also treated with MG132 (50 μg/ml) for 30 min, and the proteins were analyzed by Western blotting with anti-Myc and anti-GFP antibodies (Santa Cruz). (B and C) Time course analysis of the degradation of Std1 and Mth1. (B) Std1 was expressed from its own promoter (p*STD1-STD1*-Myc₉) and from the *MET25* promoter (p*MET25*-GFP-*STD1*). (C) Cells (wild type [FM393] or *mig1Δ mig2Δ* [YM6682]) expressing Mth1-Myc₉ (pBM4560) from its own promoter were tested for Mth1 degradation. Mig1-DBSD (pBM4750) indicates that the *MTH1* promoter carries a mutation of the Mig1 binding site (25). Cells grown on galactose medium (2%) were transferred to glucose medium (2%), and the same aliquots were harvested and analyzed at the time indicated above each lane.

different regulation of *STD1* and *MTH1* expression by glucose: *STD1* expression is induced by glucose via the Rgt2/Snf3-Rgt1 pathway, while *MTH1* expression is repressed by glucose via the Snf1-Mig1 pathway (15). Induction of *STD1* expression by glucose would be expected to counteract glucose-induced deg-

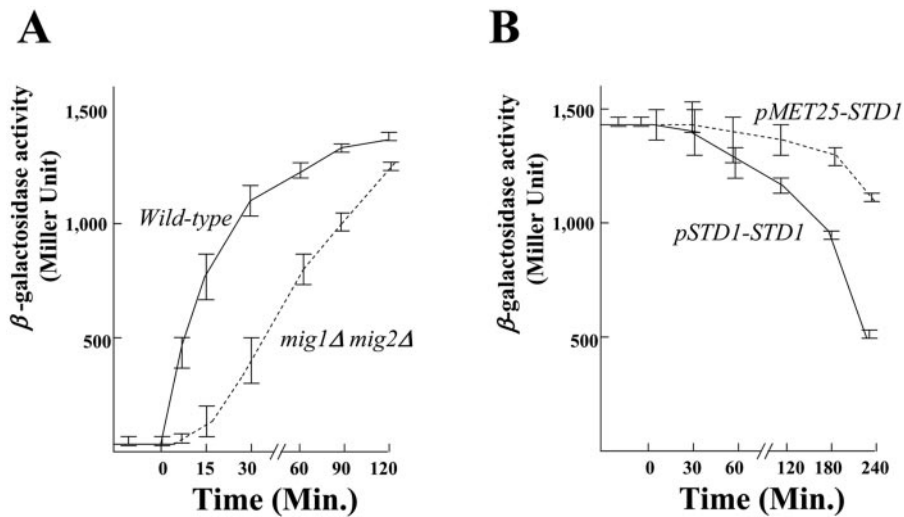


FIG. 3. Relieving transcriptional regulation of *MTH1* and *STD1* results in delayed induction and delayed repression, respectively, of *HXT* gene expression. (A) FM393 (wild type) (solid line) and YM6682 (*mig1Δ mig2Δ*) (dashed line) carrying *HXT3::lacZ* (pBM2819) were grown on 2% galactose. At time zero, 2% glucose was added to induce expression of *HXT3*. β -Galactosidase was assayed at the times indicated. (B) Cells (YM6292) carrying *HXT1::lacZ* (pBM2636) and expressing *STD1* from its own promoter (pBM4540) or from the *MET25* promoter (pBM4747) were grown in glucose. At time zero, the cells were pelleted, washed with water, and resuspended in 2% galactose to induce repression of *HXT1* expression. Aliquots of the culture were assayed for β -galactosidase activity at the times indicated. During this time the cells approximately doubled in number.

radiation of Std1 and obscure its disappearance. Conversely, repression of *MTH1* expression by glucose should reinforce the glucose-induced degradation of Mth1, thereby enhancing its disappearance upon addition of glucose to cells.

We interrupted glucose regulation of *STD1* and *MTH1* by replacing their promoters with the promoter of *MET25*, which is not regulated by glucose. Expressing *STD1* at the basal level of this promoter (by including methionine in the medium) makes degradation of Std1 in glucose-grown cells obvious (Fig.

2A, center panels) and significantly accelerates the rate of loss of Std1 after addition of glucose to cells (Fig. 2B). This suggests that induction of *STD1* expression by glucose attenuates the glucose signal to Rgt1 by slowing the disappearance of Std1. By contrast, when repression of *MTH1* expression by glucose is interrupted either by expressing *MTH1* at the basal level of the *MET25* promoter, by deleting *MIG1* and *MIG2*, or by removing the Mig1/Mig2-binding sites from the *MTH1* promoter, the extent (Fig. 2A) and rate (Fig. 2C) of degradation of *MTH1* are reduced.

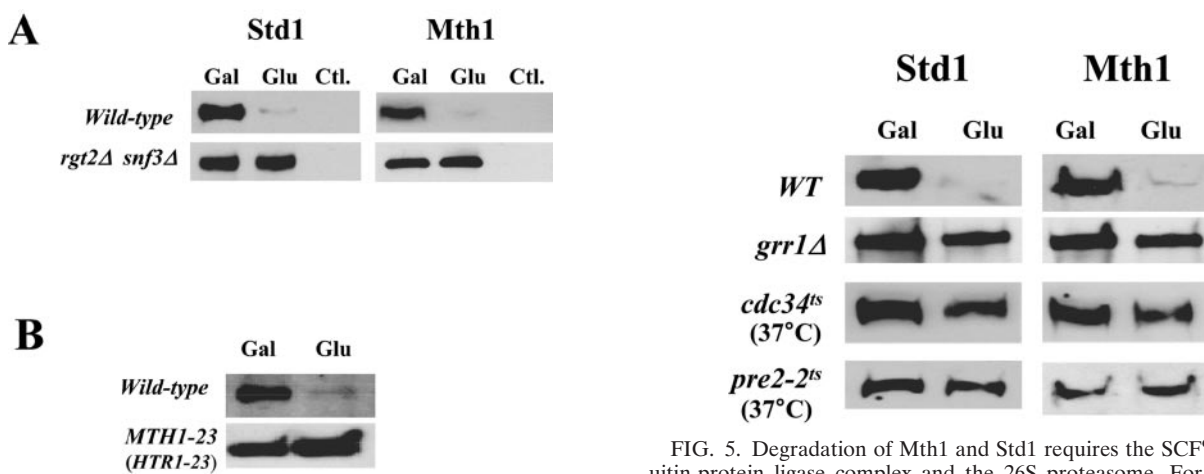


FIG. 4. Rgt2 and Snf3 promote glucose-induced degradation of Std1 and Mth1. (A) GFP-Std1 (pBM4747) or GFP-Mth1 (pBM4748) expressed in wild-type (FM391) or *rgt2 snf3* (YM6212) cells was detected by Western blotting. Control lanes (Ctl.) were loaded with extracts of cells containing the empty vector (GFP alone). (B) The dominant *HTR1-23* mutation in *MTH1* is resistant to degradation. GFP-Mth1 with the *HTR1-23* mutation (pBM4749) was expressed in FM391 (wild type) and was detected by Western blotting.

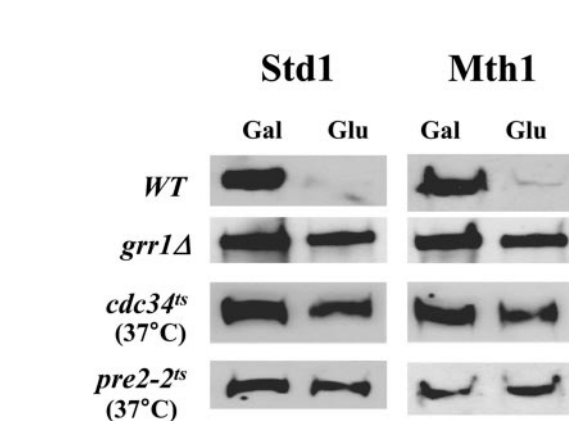


FIG. 5. Degradation of Mth1 and Std1 requires the SCF^{Grr1} ubiquitin-protein ligase complex and the 26S proteasome. For Western blotting, cell extracts were prepared from yeast cells expressing GFP-Std1 (pBM4747) or GFP-Mth1 (pBM4748) and treated as described for Fig. 2A. Strains used were YM4127 (wild type), YM6542 (*grr1Δ*), FM542 (*cdc34^{ts}*), and FM524 (*pre2-2^{ts}*). Temperature-sensitive mutant strains were grown at 30°C overnight, then shifted to a medium containing 4% glucose, and incubated for 1 h at 30°C or 37°C. The GFP-Std1 and GFP-Mth1 proteins were then detected by Western blotting.

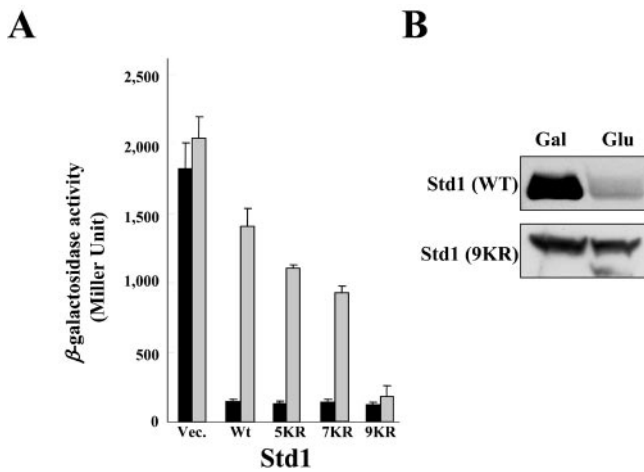


FIG. 6. Changing evolutionarily conserved lysine residues in Std1 prevents induction of *HXT1* expression and protects Std1 from degradation. (A) *HXT1::lacZ* expression was measured in yeast cells (YM6292) carrying Std1-5KR, Std1-7KR, and Std1-9KR, respectively. Black bars, cells grown in 2% galactose (*HXT*-repressing conditions); gray bars, cells grown in 4% glucose (*HXT*-inducing conditions). Vec., vector. (B) Extracts prepared from cells expressing GFP-Std1 (pBM4747) (wild type [WT]) or GFP-Std1-9KR (pBM4754) under the control of the *MET25* promoter were used for Western blotting.

The effects of transcriptional regulation of *MTH1* and *STD1* on the rate of loss of Mth1 and Std1 are expected to be translated into effects on the rates of induction and repression of *HXT* expression. We surmised that the glucose repression of *MTH1* expression and the resulting acceleration of its disappearance from the cell after addition of glucose might serve to ensure speedy induction of *HXT* expression. Indeed, in cells in

which *MTH1* expression is not repressed by glucose (due to deletion of the genes encoding the Mig1 and Mig2 glucose repressors), induction of *HXT3* expression by glucose is delayed relative to that in wild-type cells (Fig. 3A). Conversely, we speculated that glucose induction of *STD1* expression might serve to replenish Std1 after its initial glucose-induced degradation so as to enable prompt establishment of repression of *HXT* expression when glucose is exhausted in the culture. Indeed, in cells in which *STD1* expression is not induced by glucose, repression of *HXT1* expression is established more slowly than in wild-type cells after addition of galactose (Fig. 3B). Thus, transcriptional regulation of *MTH1* and *STD1* significantly affects the course of induction and repression of *HXT* gene expression.

Degradation of Std1 and Mth1 requires a glucose signal. Degradation of Std1 and Mth1 requires the glucose sensors Rgt2 and Snf3 (Fig. 4), as well as two components of the SCF^{Grr1} ubiquitin-protein ligase, Grr1 and Cdc34 (Fig. 5). Glucose addition does not cause Std1 and Mth1 to disappear in a temperature-sensitive *pre2* mutant defective in a chymotrypsin-like activity of the proteasome (8–11) or in the presence of the proteasome inhibitor MG132 (Fig. 2). These results support the view that glucose binding to the Rgt2 and Snf3 glucose sensors causes them to initiate proteasome-mediated degradation of Mth1 and Std1 by targeting them for ubiquitination by the SCF^{Grr1} ubiquitin-protein ligase.

Potential ubiquitin attachment sites in Std1 are required for Std1 degradation. The SCF^{Grr1} ubiquitin-protein ligase catalyzes the covalent attachment of ubiquitin to lysine residues of the target protein (17, 22, 35, 36). Evidence has been presented that suggests that Mth1 is ubiquitinated (37), but similar evidence that Std1 is also modified in this way is lacking. Indeed,

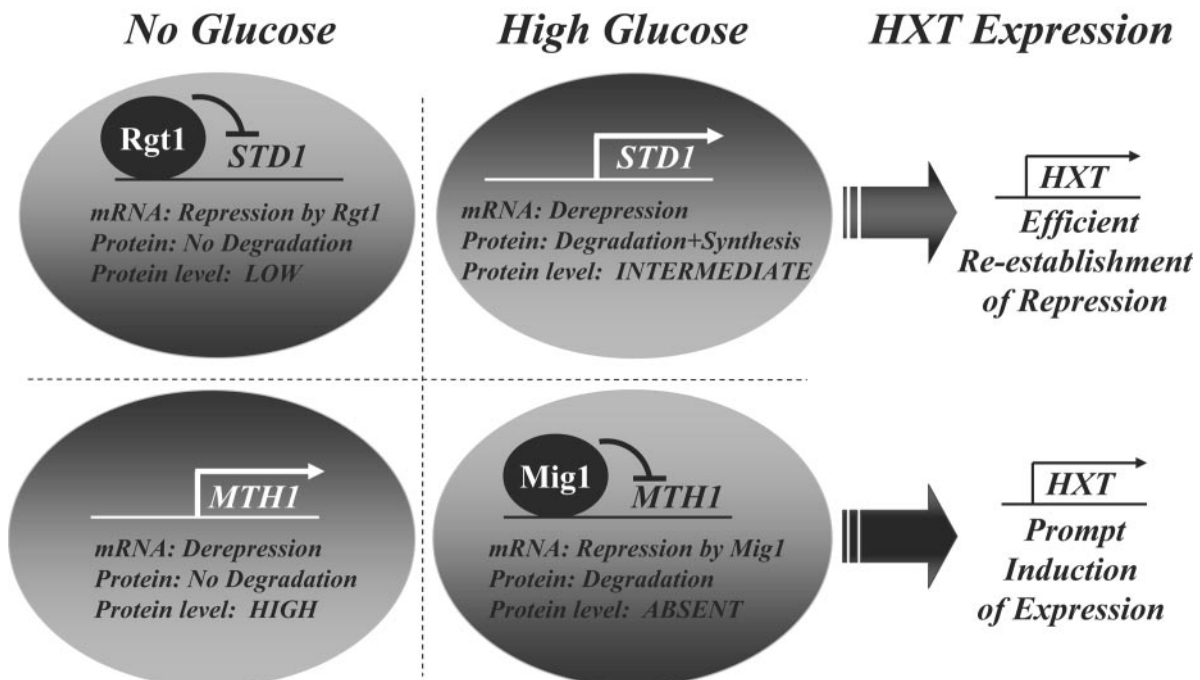


FIG. 7. Summary of transcriptional and posttranslational regulation of cellular levels of Std1 and Mth1.

our attempts to demonstrate this modification of Std1 have so far proven unsuccessful. We noticed 10 lysine residues in Std1 that are conserved in its orthologues in other yeasts (positions 207, 282, 287, 312, 337, 344, 347, 354, 381, and 411). Conversion of 9 of these lysines to arginine (9KR) prevented glucose-induced degradation of Std1 (Fig. 6B) and severely reduced derepression of *HXT1* expression (Fig. 6A). Changing fewer than 9 of these lysine residues (7KR and 5KR) had smaller effects on induction of *HXT1* expression, suggesting that ubiquitination at only a few sites of Std1 is required to target the protein for degradation. These results provide indirect evidence supporting the idea that Std1 is targeted for degradation by ubiquitination.

DISCUSSION

Degradation of Std1 and Mth1 is the central event in transmission of the glucose signal to Rgt1, which results in induction of expression of the *HXT* genes. Glucose binding to the Snf3 and Rgt2 sensors stimulates degradation of Mth1 and Std1, probably by activating casein kinase (Yck1 and Yck2), which phosphorylates Mth1 and Std1, thereby making them substrates for the SCF^{Grr1} ubiquitin-protein ligase and targeting them for degradation in the proteasome (23, 37). It has been difficult to demonstrate directly that Mth1 and Std1 become modified by ubiquitination when glucose is added to cells (37) (our unpublished results). Our observations that the SCF^{Grr1} ubiquitin-protein ligase and several lysine residues in Std1 that are conserved in evolution are required for its glucose-induced degradation (Fig. 5 and 6) provide indirect evidence that ubiquitination of Std1 (and, by inference, of Mth1) plays a role in this signal transduction pathway.

Glucose also regulates the levels of Mth1 and Std1 in cells by regulating *MTH1* and *STD1* transcription via feedback and feedforward regulatory mechanisms that operate through two different glucose signal transduction pathways (15). Glucose-induced disappearance of Std1 is attenuated by feedback regulation of *STD1* expression via the Snf3/Rgt2-Rgt1 signal transduction pathway (Fig. 2), which causes *STD1* expression to be induced by glucose, thereby replenishing Std1 soon after its degradation is initiated by addition of glucose to cells. We believe this feedback regulation evolved to provide sufficient levels of Std1 to ensure efficient reestablishment of repression of *HXT* expression as soon as cells exhaust the available glucose. Indeed, interruption of this regulation of *STD1* expression results in slower establishment of repression of *HXT1* expression upon removal of glucose from cells (Fig. 3B). In contrast, Mth1 degradation is reinforced by glucose repression of *MTH1* expression mediated by the Snf1-Mig1 glucose-signaling pathway. Disappearance of Mth1 is slowed in cells missing Mig1 and Mig2 or lacking their binding site in the *MTH1* promoter (Fig. 2). We believe the purpose of this regulation is to ensure rapid removal of Mth1 from cells when glucose becomes available so as to enable prompt induction of *HXT* gene expression. This idea is supported by our observation that interruption of this regulation results in delayed induction of *HXT3* expression in response to glucose (Fig. 3A).

Even though Std1 and Mth1 are paralogues, they appear to have different functions in the glucose induction pathway: Mth1 collaborates with Rgt1 to repress expression of *HXT1*

and *HXT3*, whereas Std1 seems to be dedicated to regulating expression of the high-glucose-induced *HXT1* gene (Table 2) (14, 32). Our results suggest that Mth1 plays a role in maintaining repression of the *HXT* genes in the absence of glucose, while Std1 may primarily be responsible for reestablishment of repression of *HXT* expression when the cells run out of glucose (Fig. 7). This intricate and highly evolved regulatory network ensures stringent regulation of glucose utilization.

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REFERENCES

- Alani, E., L. Cao, and N. Kleckner. 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. *Genetics* **116**:541–545.
- Carlson, M. 1999. Glucose repression in yeast. *Curr. Opin. Microbiol.* **2**:202–207.
- Diderich, J. A., M. Schepper, P. van Hoek, M. A. Luttkik, J. P. van Dijken, J. T. Pronk, P. Klaassen, H. F. Boelens, M. J. de Mattos, K. van Dam, and A. L. Kruckeberg. 1999. Glucose uptake kinetics and transcription of *HXT* genes in chemostat cultures of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **274**:15350–15359.
- Flick, K. M., N. Spielewoy, T. I. Kalashnikova, M. Guaderrama, Q. Zhu, H. C. Chang, and C. Wittenberg. 2003. Grr1-dependent inactivation of Mth1 mediates glucose-induced dissociation of Rgt1 from *HXT* gene promoters. *Mol. Biol. Cell* **14**:3230–3241.
- Forsberg, H., and P. O. Ljungdahl. 2001. Sensors of extracellular nutrients in *Saccharomyces cerevisiae*. *Curr. Genet.* **40**:91–109.
- Gamo, F., M. Lafuente, and C. Gancedo. 1994. The mutation DGT1-1 decreases glucose transport and alleviates carbon catabolite repression in *Saccharomyces cerevisiae*. *J. Bacteriol.* **176**:7423–7429.
- Gancedo, J. M. 1998. Yeast carbon catabolite repression. *Microbiol. Mol. Biol. Rev.* **62**:334–361.
- Ghislain, M., A. Udvardy, and C. Mann. 1993. *S. cerevisiae* 26S protease mutants arrest cell division in G₂/metaphase. *Nature* **366**:358–362.
- Heinemeyer, W., A. Gruhler, V. Mohrle, Y. Mahe, and D. H. Wolf. 1993. PRE2, highly homologous to the human major histocompatibility complex-linked RING10 gene, codes for a yeast proteasome subunit necessary for chrymotryptic activity and degradation of ubiquitinated proteins. *J. Biol. Chem.* **268**:5115–5120.
- Heinemeyer, W., J. A. Kleinschmidt, J. Saidowsky, C. Escher, and D. H. Wolf. 1991. Proteinase yscE, the yeast proteasome/multicatalytic-multifunctional proteinase: mutants unravel its function in stress induced proteolysis and uncover its necessity for cell survival. *EMBO J.* **10**:555–562.
- Hochstrasser, M., P. R. Johnson, C. S. Arendt, A. Amerik, S. Swaminathan, R. Swanson, S. J. Li, J. Laney, R. Pals-Rylandsdam, J. Nowak, and P. L. Connerly. 1999. The *Saccharomyces cerevisiae* ubiquitin-proteasome system. *Philos. Trans. R. Soc. Lond. B* **354**:1513–1522.
- Holsbeeks, L., O. Lagatie, A. Van Nuland, S. Van de Velde, and J. M. Thevelein. 2004. The eukaryotic plasma membrane as a nutrient-sensing device. *Trends Biochem. Sci.* **29**:556–564.
- Johnston, M. 1999. Feasting, fasting and fermenting. Glucose sensing in yeast and other cells. *Trends Genet.* **15**:29–33.
- Johnston, M., and J. H. Kim. 2005. Glucose as a hormone: receptor-mediated glucose sensing in the yeast *Saccharomyces cerevisiae*. *Biochem. Soc. Trans.* **33**:247–252.
- Kaniak, A., Z. Xue, D. Macool, J. H. Kim, and M. Johnston. 2004. Regulatory network connecting two glucose signal transduction pathways in *Saccharomyces cerevisiae*. *Eukaryot. Cell* **3**:221–231.
- Kim, J. H., J. Polish, and M. Johnston. 2003. Specificity and regulation of DNA binding by the yeast glucose transporter gene repressor Rgt1. *Mol. Cell. Biol.* **23**:5208–5216.
- Kishi, T., T. Seno, and F. Yamao. 1998. Grr1 functions in the ubiquitin pathway in *Saccharomyces cerevisiae* through association with Skp1. *Mol. Gen. Genet.* **257**:143–148.
- Ko, C. H., H. Liang, and R. F. Gaber. 1993. Roles of multiple glucose transporters in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13**:638–648.
- Lafuente, M. J., C. Gancedo, J. C. Jauniaux, and J. M. Gancedo. 2000. Mth1 receives the signal given by the glucose sensors Snf3 and Rgt2 in *Saccharomyces cerevisiae*. *Mol. Microbiol.* **35**:161–172.
- Lakshmanan, J., A. L. Mosley, and S. Ozcan. 2003. Repression of transcription by Rgt1 in the absence of glucose requires Std1 and Mth1. *Curr. Genet.* **44**:19–25.
- Lewis, D. A., and L. F. Bisson. 1991. The *HXT1* gene product of *Saccharomyces cerevisiae* is a new member of the family of hexose transporters. *Mol. Cell. Biol.* **11**:3804–3813.

22. Li, F. N., and M. Johnston. 1997. Grr1 of *Saccharomyces cerevisiae* is connected to the ubiquitin proteolysis machinery through Skp1: coupling glucose sensing to gene expression and the cell cycle. *EMBO J.* **16**:5629–5638.
23. Moriya, H., and M. Johnston. 2004. Glucose sensing and signaling in *Saccharomyces cerevisiae* through the Rgt2 glucose sensor and casein kinase I. *Proc. Natl. Acad. Sci. USA* **101**:1572–1577.
24. Mosley, A. L., J. Lakshmanan, B. K. Aryal, and S. Ozcan. 2003. Glucose-mediated phosphorylation converts the transcription factor Rgt1 from a repressor to an activator. *J. Biol. Chem.* **278**:10322–10327.
25. Nehlin, J. O., and H. Ronne. 1990. Yeast MIG1 repressor is related to the mammalian early growth response and Wilms' tumour finger proteins. *EMBO J.* **9**:2891–2898.
26. Ozcan, S., K. Freidel, A. Leuker, and M. Ciriacy. 1993. Glucose uptake and catabolite repression in dominant HTR1 mutants of *Saccharomyces cerevisiae*. *J. Bacteriol.* **175**:5520–5528.
27. Ozcan, S., and M. Johnston. 1999. Function and regulation of yeast hexose transporters. *Microbiol. Mol. Biol. Rev.* **63**:554–569.
28. Ozcan, S., and M. Johnston. 1995. Three different regulatory mechanisms enable yeast hexose transporter (HXT) genes to be induced by different levels of glucose. *Mol. Cell. Biol.* **15**:1564–1572.
29. Ozcan, S., T. Leong, and M. Johnston. 1996. Rgt1p of *Saccharomyces cerevisiae*, a key regulator of glucose-induced genes, is both an activator and a repressor of transcription. *Mol. Cell. Biol.* **16**:6419–6426.
30. Polish, J. A., J. H. Kim, and M. Johnston. 2005. How the Rgt1 transcription factor of *Saccharomyces cerevisiae* is regulated by glucose. *Genetics* **169**:583–594.
31. Rolland, F., J. Winderickx, and J. M. Thevelein. 2002. Glucose-sensing and -signalling mechanisms in yeast. *FEMS Yeast Res.* **2**:183–201.
32. Schmidt, M. C., R. R. McCartney, X. Zhang, T. S. Tillman, H. Solimeo, S. Wolff, C. Almonte, and S. C. Watkins. 1999. Std1 and Mth1 proteins interact with the glucose sensors to control glucose-regulated gene expression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **19**:4561–4571.
33. Schulte, F., and M. Ciriacy. 1995. *HTR1/MTH1* encodes a repressor for *HXT* genes. *Yeast* **11**:S239.
34. Schulte, F., R. Wiczorke, C. P. Hollenberg, and E. Boles. 2000. The *HTR1* gene is a dominant-negative mutant allele of *MTH1* and blocks Snf3- and Rgt2-dependent glucose signaling in yeast. *J. Bacteriol.* **182**:540–542.
35. Skowyra, D., K. L. Craig, M. Tyers, S. J. Elledge, and J. W. Harper. 1997. F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* **91**:209–219.
36. Skowyra, D., D. M. Koepp, T. Kamura, M. N. Conrad, R. C. Conaway, S. J. Elledge, and J. W. Harper. 1999. Reconstitution of G₁ cyclin ubiquitination with complexes containing SCF^{Grr1} and Rbx1. *Science* **284**:662–665.
37. Spielewoy, N., K. Flick, T. I. Kalashnikova, J. R. Walker, and C. Wittenberg. 2004. Regulation and recognition of SCF^{Grr1} targets in the glucose and amino acid signaling pathways. *Mol. Cell. Biol.* **24**:8994–9005.
38. Tomas-Cobos, L., and P. Sanz. 2002. Active Snf1 protein kinase inhibits expression of the *Saccharomyces cerevisiae* *HXT1* glucose transporter gene. *Biochem. J.* **368**:657–663.
39. Wach, A., A. Brachat, R. Pohlmann, and P. Philippsen. 1994. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* **10**:1793–1808.
40. Wendell, D. L., and L. F. Bisson. 1994. Expression of high-affinity glucose transport protein Hxt2p of *Saccharomyces cerevisiae* is both repressed and induced by glucose and appears to be regulated posttranslationally. *J. Bacteriol.* **176**:3730–3737.