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

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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). These 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^Gr1^ 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 transcription, 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 represses 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 pUG3 (pBMS884 ARSH4/CEN–HIS3–MET25 promoter–yeGFP–polylinker–CYC1 ter-

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TABLE 1. Yeast strains used in this study

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<th>Strain</th>
<th>Genotype</th>
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<td>FM391</td>
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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, genotype 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 activation.
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 _Rgt1_ 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).

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 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-

<table>
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<th>Genotype</th>
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<td>4. <em>mth1 std1</em> (YM6292)</td>
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<td>6. HTR1-23-d (YM6245)</td>
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a The plasmids used are pBM1817 (lexO::lacZ reporter) and pBM3036 (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).
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. 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.

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 rgt2Δ snf3Δ (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), FM524 (pre2-2ts), and FM524 (pre2-2ts). 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.
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<sup>Grr1</sup> 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<sup>Grr1</sup> ubiquitin-protein ligase.

Potential ubiquitin attachment sites in Std1 are required for Std1 degradation. The SCF<sup>Grr1</sup> 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,
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, 334, 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\(^{Gr}\) 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\(^{Gr}\) 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-sensing pathway. Disappearance of Mth1 is slowed in cells missing Mig1 or 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 \(HXT7\) 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.

**ACKNOWLEDGMENTS**

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**REFERENCES**


