Asymmetric Signal Transduction through Paralogs That Comprise a Genetic Switch for Sugar Sensing in Saccharomyces cerevisiae

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Efficient uptake of glucose is especially critical to Saccharomyces cerevisiae because its preference to ferment this carbon source demands high flux through glycolysis. Glucose induces expression of HXT genes encoding hexose transporters through a signal generated by the Snf3 and Rgt2 glucose sensors that leads to depletion of the transcriptional regulators Mth1 and Std1. These paralogous proteins bind to Rgt1 and enable it to repress expression of HXT genes. Here we show that Mth1 and Std1 can substitute for one another and provide nearly normal regulation of their targets. However, their roles in the glucose signal transduction cascade have diverged significantly. Mth1 is the prominent effector of Rgt1 function because it is the more abundant of the two paralogs under conditions in which both are active (in the absence of glucose). Moreover, the cellular level of Mth1 is quite sensitive to the amount of available glucose. The abundance of Std1 protein, on the other hand, remains essentially constant over a similar range of glucose concentrations. The signal generated by low levels of glucose is amplified by rapid depletion of Mth1; the velocity of this depletion is dependent on both its rate of degradation and swift repression of MTH1 transcription by the Snf1-Mig1 glucose repression pathway. Quantitation of the contributions of Mth1 and Std1 to regulation of HXT expression reveals the unique roles played by each paralog in integrating nutrient availability with metabolic capacity: Mth1 is the primary regulator; Std1 serves to buffer the response to glucose.

Nutrient sensors provide cells with vital information about their environment, enabling rapid and efficient assimilation of specific nutrients (1). Efficient utilization of glucose is especially important to the yeast Saccharomyces cerevisiae because of its propensity to ferment this primary carbon source. The central components of the signaling pathway that controls expression of HXT (hexose transporter) genes encoding the hexose transporters are Mth1 and Std1. These two proteins are paralogs that arose from the whole genome duplication event that occurred about 100 million years ago, before the divergence of the sensu stricto clade of Saccharomyces species (2, 3). Information about the presence of glucose in the environment is transduced from the cell surface to the nucleus through Mth1 and Std1. Because they are functionally redundant under most experimental conditions, one of these paralogs should suffice, and indeed, yeasts that diverged before the whole genome duplication make do with a single Mth1/Std1 ortholog (4). The advantage conferred by having both Mth1 and Std1 (or indeed, any paralogs) cannot be fully appreciated without an understanding of how each contributes to the behavior of the signaling machinery. To that end, we enumerated the properties of Mth1 and Std1 that inform a larger question: Why retain paralogs?

Yeast cells sense extracellular glucose through the Snf3 and Rgt2 glucose sensors (reviewed in Refs. 5, 6), which are thought to be glucose receptors with high (Snf3) and low (Rgt2) affinity for glucose (7). The signal they generate upon binding of glucose is transduced to the Mth1 and Std1 proteins through the Yck1 protein kinase, which phosphorylates them, thereby targeting them for ubiquitylation by the SCFGrr1 ubiquitin-protein ligase, dooming them to destruction (8–10). Because Mth1 and Std1 are required for the function of Rgt1, a transcriptional repressor of HXT genes (11–13), their destruction results in derepression of HXT gene transcription, leading to a rapid influx of glucose into cells (14).

Rgt1 repressor function is controlled by the abundance of Mth1 and Std1. These two proteins have maintained 58% amino acid identity since their divergence 100 million years ago, but their own transcriptional regulation differs significantly; STD1 expression is induced by glucose because it is controlled by Rgt1, and MTH1 expression is repressed by glucose through the Snf1-Mig1 glucose repression pathway (14). Repression of MTH1 transcription by glucose augments the glucose-stimulated degradation of Mth1, leading to a rapid and significant decline in the abundance of Mth1 protein (15). However, the levels of Std1 protein change little after cells are exposed to glucose, because glucose induction of its transcription offsets degradation of the protein (15). The different roles played by these central components of this glucose signal transduction pathway in integrating the glucose signal are not well understood. Different consequences of loss of Mth1 or Std1 or their over expression have been observed (16–18). We discov-
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TABLE 1

Yeast strains used

The references are given for strains that were not created in this study.

| Strain | Genotype | Reference |
|--------|-----------|-----------|
| FM391  | MATa his3Δ leu2Δ trp1Δ met15Δ (BY4741) | 49 |
| FM392  | MATa his3Δ leu2Δ trp1Δ his3Δ lys2Δ (BY4742) | 49 |
| FM393  | MATa/MATa his3Δ leu2Δ/trp1Δ met15Δ/MET LYS/lys2Δ (BY4743) | 49 |
| FM686  | FM392, std1Δ:kanMX | 50 |
| FM688  | FM395, mth1Δ:kanMX/mth1Δ:kanMX | 50 |
| FM689  | FM395, std1Δ:kanMX/std1Δ:kanMX | 50 |
| YM6266 | FM392, mth1Δ:kanMX | 50 |
| YM6294 | FM391, mth1Δ:kanMX std1Δ:kanMX | V. Brachet, unpublished strain |
| YM6292 | FM392, mth1Δ:kanMX std1Δ:kanMX | 15 |
| YM7393 | FM393, mth1Δ:kanMX/mth1Δ:kanMX std1Δ:kanMX | 15 |
| YM7374 | YM6294 + pUG34 + pBM3212 |
| YM7350 | YM6294 + pBM4881 + pBM3212 |
| YM7353 | YM6294 + pBM4884 + pBM3212 |
| YM7354 | YM6294 + pBM4885 + pBM3212 |
| YM7355 | YM6294 + pBM4886 + pBM3212 |
| YM7356 | YM6294 + pBM4887 + pBM3212 |
| YM7646 | FM686 + pBM4544 |
| YM7648 | FM666 + pBM4560 |
| YM7677–78 | YM6292 + pBM5167 + pBM4476 + pBM3118 |
| YM7679–80 | YM6292 + pBM4886 + pBM4940 + pBM3118 |
| YM7681–82 | YM6292 + pBM5021 + pBM4884 + pBM3118 |
| YM7683–84 | YM6292 + pBM4882 + pBM5121 + pBM3118 |
| YM7685–86 | YM6292 + pBM4886 + pBM5121 + pBM3118 |
| YM7687–88 | YM6292 + pBM4886 + pBM5121 + pBM3118 |
| YM7689–90 | YM6292 + pUG34 + pRS316 + pBM3118 |

TABLE 2

Plasmids used in this study

The references are given for strains that were not created in this study.

| Plasmid | Description | Reference |
|---------|-------------|-----------|
| pBM4476 | pRS316 + STD1 locus (1017 bp upstream, STD1 open reading frame, 499 bp downstream) | A. Kaniak, unpublished plasmid |
| pBM4881–82 | HIS3 marker replaced URA3 in pBM4498 |
| pBM4884–85 | MTH1 promoter replaced by STD1 (1017 bp) promoter in pBM4498 |
| pBM4940 | HIS3 marker replaced URA3 in pBM4499 |
| pBM4940 | pBM4476, S-tag fused to C terminus of Std1 |
| pBM5021 | pBM4881, S-tag fused to C terminus of Mth1 |
| pBM5121 | pBM4476, Std1 replaced by Mth1-S-tag |
| pBM5167 | pBM4886, S-tag fused to C terminus of Std1 |
| pBM5237 | pBM5155, Mth1-S-tag inserted after TetO |
| pBM5238 | pBM5155, Std1-S-tag inserted after TetO |
| pBM3212 | HXT1 promoter lacZ fusion |
| pBM4544 | P24tdx-ST1-9xMyc |
| pBM4560 | P24tdx-MTH1-9xMyc |
| pRS316 | Centromeric yeast shuttle vector |
| pBM4498 | pRS316 + MTH1 locus (1491 bp upstream, MTH1 open reading frame, 858 bp downstream) | A. Kaniak, unpublished plasmid |
| pBM4499 | MTH1 promoter (1491 bp) replaced STD1 promoter in pBM4476 |
| pBM3118 | HXT2 promoter lacZ fusion |

It was noted that these differences do not arise from functional independence of Mth1 and Std1 but rather result from differences in their transcriptional regulation.

We demonstrate that Mth1 and Std1 are largely dedicated to regulating Rgt1 function and that they are functionally interchangeable in the glucose-sensing pathway. Because of the significant glucose-induced changes in Mth1 protein levels, we believe that Mth1 serves as a primary regulator of Rgt1 function and thus of HXT expression. In addition, Mth1 protein levels provide information about the availability of glucose to the transcriptional program regulating glucose uptake and utilization. Std1, on the other hand, with its low and static abundance, may provide a buffer that modulates the speed and extent of repression and induction of HXT gene expression.

EXPERIMENTAL PROCEDURES

Yeast Growth, Strains, and Plasmids—S. cerevisiae strains used in this study are listed in Table 1, and plasmids are listed in Table 2. All of the yeast cultivation was done at 30 °C, in flasks, shaken at 325 rpm unless otherwise specified. Synthetic complete medium (SC) lacking the appropriate amino acid (2 g/liter synthetic dropout mix (US Biological), 1.7 g/liter yeast nitrogen base, 5 g/liter ammonium sulfate) and supplemented with the indicated carbon source was used in all cultivations in which prototrophic selection was necessary. Growth of YM7660 and YM7661 was carried out in YPD plus 100 μg/ml cloNAT (Warner) to ensure plasmid maintenance. All of the yeast transformations were performed according to standard methods (19).

All of the plasmids were constructed via gap repair (13) by PCR amplification of sequence to be inserted, flanked by 20–25 bp of homology to the recipient linearized plasmid. Plasmids in positively selected clones were recovered from yeast and transformed into Escherichia coli GC10 Thunderbolt (GeneChoice, Inc.) electrocompetent cells for amplification and DNA sequencing. The DNA sequence coding for the S-tag (5'-AAA GAA ACC GCT GCT GCT AAA TTC GAA CGC CAG CAC...
ATG GAC TCT-3’) was introduced into plasmids pBM5021 or pBM4940 by PCR amplification of Mth1 or Std1 with the reverse primer containing the first 35 bp of S-tag coding region in-frame with the rest of the open reading frame, and gap repair was performed on this product and one containing the region downstream of the STOP codon with the last 34 bp of S-tag coding region in the sense primer into a plasmid containing the entire MTH1 or STD1 locus (pBM4881 or pBM4476, respectively). Plasmids containing promoter exchanges were constructed by PCR-amplifying the promoter region of MTH1 or STD1 and gap repairing this product into plasmids already containing the S-tagged or native version of the protein. Plasmid pBM5155 (a gift from C. T. Hittinger, unpublished plasmid) contains the reverse-tetracycline transactivator and seven copies of the tetO sequence (TetO7), allowing tetracycline-inducible gene expression in yeast. It was used as the recipient for S-tagged Mth1 or Std1 PCR-amplified from pBM5021 or pBM4940, where the S-tag had already been added. Plasmids pBM4882 (P<sub>MTH1</sub>-MTH1) and pBM4886 (P<sub>STD1</sub>-STD1) were derived from pBM4498 and pBM4499 (A. Kaniak, unpublished plasmid) after change of the selective marker from LEU2 to HIS3 by gap repair with a Pvull fragment from pUG34 (20).

The homozygous diploid ∆mth1/∆std1 knock-out strain (YM7393) was constructed by mating haploid ∆mth1/∆std1 strains (YM6292 (15) and YM6294 (V. Brachet, unpublished strain)). Mating types were verified by screening against mating type tester strains.

**Transcription Profiling**—Duplicate cultures of diploid wild type (FM393) and homozygous deletion mutants of mth1 (FM688), std1 (FM689), or mth1/stand1 (YM7393) were grown to A<sub>600</sub> = 1–1.2 in SC medium + 2% galactose and further incubated for 2 h before the cells were harvested by centrifugation at 3000 × g for 5 min at room temperature. The cell pellets were snap-frozen in liquid nitrogen and stored at −80 °C. Total RNA was prepared as described (21) by extraction with hot phenol and chloroform, followed by isopropanol precipitation and reconstitution in TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA). Fluorescent labeling, hybridization, and quantification were performed by the microarray core in the Genome Center at Washington University in St. Louis as previously described (22). Each RNA sample from the mutant strains was hybridized with a single sample from the wild type strain grown in galactose, resulting in four measurements for each gene in each mutant, compared with wild type (2 on-chip × 2 biological replicates). Genes whose expression change was significant (<p < 0.01), altered 2-fold or greater and present in the ∆mth1/∆std1 mutant and in one or both of the single deletion mutants are listed in supplemental Table S1 and were subject to DNA sequence motif analysis (supplemental Table S3). Raw microarray data have been deposited in NCBI Gene Expression Omnibus (accession number GSE17437).

**Motif Analysis**—Promoter sequences consisting of 1 kb of DNA upstream of the ATG start codon and including the first five codons of each open reading frame in *S. cerevisiae* were scanned using PATSER (version 3) (23) in combination with position-specific weight matrices for Mig1 (accession number M00061)-binding motifs acquired from TRANSFAC public data base (24) and a matrix for Rgt1 derived from *HXT* promoter binding data (25). Using this Rgt1 matrix, 87% (5856 of 6713 promoters) of the promoters in the genome were scored as a hit. This matrix is derived from *in vitro* binding data at one class of Rgt1 targets (*HXTs* 1–3) that results in a quite degenerate consensus sequence (5’-CGGANNNA-3’), expected to occur roughly once in a random 1-kb stretch. In addition, nearly half of the consensus sites in the region upstream of *HXT1* open reading frame are dispensable for Rgt1 repression (26). To have less biased matrix, AlignACE version 3.0 (27) was used to extract overrepresented motifs in the upstream regions of the top Rgt1 target genes taken from Ref. 14 (supplemental Table S2). A single motif was found that resembled the Rgt1 consensus and was then used to generate the list of upstream regions containing Rgt1-binding motifs. The significance of enrichment of gene sets for those containing each binding motif was calculated using the binomial distribution, where the number of successes was defined as the number of promoters that contain at least one match to the matrix, scoring above the significance threshold determined by PATSER. The results of these analyses are shown in supplemental Table S3.

**Protein Quantitation Using S-tag**—The S-tag is a 15-amino acid peptide fragment of pancreatic ribonuclease that associates with the remaining fragment, called the S-protein, to reconstitute RNase activity (28). When fused to a protein of interest, the fusion protein associates with S-protein in a 1:1 ratio, and the degree of this association can be measured by monitoring RNase activity (28), a process enhanced by the use of a fluorophore/quencher, dually labeled RNA substrate (29).

Previously, this has been utilized in bacterial systems to quantify protein yield (30); this is the first case of its use in yeast cells.

Kinetic measurements of changes in S-tagged protein abundance were carried out as follows. The cultures were grown to A<sub>600</sub> = 1–1.2 at 30 °C, and the stated carbon source was added. At the times indicated before and after addition, duplicate 1.5-ml aliquots of culture were removed, and 100 μl of each was pipetted into a clear, flat-bottom 96-well plate resting on a dry ice slab, and the A<sub>600</sub> was later determined en masse for all time points. The cells were pelleted from the remaining 1.4 ml of culture, and the media were removed. Each tube was immediately plunged into a dry ice-ethanol bath and stored at −80 °C until assayed. Frozen pellets were resuspended by the addition of 10 μl of Y-PEL (Pierce) and thoroughly mixed. The extracts were incubated at room temperature for an additional 2 h to ensure complete permeabilization and clarified by centrifugation at 19,000 × g in a microcentrifuge. The FRETWorks S-tag assay kit (Novagen) was used to assess the quantity of S-tagged protein in each sample. Briefly, 5 μl of lysate prepared as described was mixed with 95 μl of reaction mix in a flat-bottom, black, 96-well plate. The reaction was monitored by incubating the 96-well plate at 30 °C in a Bio-Tek Synergy HT microplate reader (excitation, 485 ± 20 nm; emission, 528 ± 20 nm; sensitivity, 80), measuring AFU<sup>3</sup> every 80 s for 1 h. The initial velocity of the reaction (AFU min<sup>−1</sup>) was converted to femtmoles of S-tag in the reaction using a standard curve of known

<sup>3</sup>The abbreviation used is: arbitrary fluorescence unit(s).
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S-tag concentrations (an S-tag standard is provided in the kit). A standard curve was run on every plate of samples assayed. The S units in each sample were then calculated using the following formula,

\[ \text{S units} = \frac{\text{fmol of S-tag}}{A_{600} \times V_{\text{culture}} \times F_{\text{extract}}} \]  

(Eq. 1)

where \( A_{600} \) is measured from 100-\( \mu \)l aliquots frozen during the time course, \( V_{\text{culture}} \) is the volume of culture taken for each time point (i.e. 1.5 ml), and \( F_{\text{extract}} \) is the fraction of extract assayed, in this case, 0.5. The resulting quantity is similar in formulation to the Miller unit used to describe the activity of \( \beta \)-galactosidase (31).

Western Blotting—Total protein extract was prepared (32) from cultures of YM7648 and YM7646 expressing Mth1–9xMyc, Std1–9xMyc, or YM7345 expressing a tandem affinity purification-tagged Mth1 protein as a negative control. Cell pellets from 25 ml of culture were resuspended in lysis buffer (250 mm sucrose, 60 mm KCl, 14 mm NaCl, 5 mm MgCl\(_2\), 1 mm CaCl\(_2\), 0.8% Triton X-100) and vortexed for 20 min at 4 °C with ~150 \( \mu \)l of 0.5-mm glass beads (BioSpec). Lysate was separated from beads and centrifuged at 19,000 \( \times g \) for 10 min at 4 °C. The pellet was resuspended in 25 ml of SDS loading buffer, and proteins were resolved by SDS-PAGE. The proteins were transferred to polyvinylidene difluoride membrane (Millipore) using a semi-dry blot apparatus (Bio-Rad) for 1 h at 16 V. The membrane was blocked for 20 min in TBS (10 mm Tris pH 7.5, 40 \( \mu \)M MgCl\(_2\), 70 \( \mu \)M CaCl\(_2\), 150 mm NaCl) plus 5% milk and incubated with primary antibody (mouse anti-c-Myc; Santa Cruz Biotechnology) at 1:3000 dilution in TBS + 1% milk, overnight at 4 °C. Extensive washing with TBST (TBS plus 0.5% Tween20 (v/v)) was followed by a 30-min incubation with a 1:10,000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG in TBS + 0.5% milk. The bands were visualized by exposure to film after treatment of the membrane with Supersignal West Pico chemiluminescence kit (Pierce).

\( \beta \)-Galactosidase Assay—Enzyme activity was measured in aliquots of cell culture lysed in Y-PER (Pierce). The enzymatic activity in each lysate was determined by monitoring the increase in fluorescence caused by the liberation of 4-methylumbellifereone through cleavage of the glycosidic bond by the \( \beta \)-galactosidase enzyme. This assay is based on the Betafluor \( \beta \)-galactosidase assay kit (Novagen). Briefly, the \( A_{600} \) of 100 \( \mu \)l of cell culture was measured on a Bio-Tek Synergy HT and immediately following the reading, 50 \( \mu \)l of culture was added to 25 \( \mu \)l of Y-PER reagent to lyse the cells present. To this lysate, 75 \( \mu \)l of reaction mix was added. The reaction mix contains Z buffer, pH 7.0 (60 mm Na\(_2\)HPO\(_4\), 40 mm NaH\(_2\)PO\(_4\), 10 mm KCl, 1 mm MgSO\(_4\), 1 mm dithiothreitol) (33), and the fluorogenic substrate 4-methylumbelliferyl \( \beta \)-d-galactopyranoside (Sigma) dissolved in dimethyl sulfoxide to 20 mg/ml and present at a final concentration of 1 mg/ml. The progress of the reaction was monitored at 30 °C in a Bio-Tek Synergy HT plate reader (excitation, 360 ± 40 nm; emission, 460 ± 40 nm; sensitivity, 50), with readings taken every 2 min after 3 s of shaking. The \( \beta \)-galactosidase units reported were calculated according to the following formula.

\[ \beta\text{-gal units} = \frac{\text{Initial velocity (AFU min}^{-1})}{A_{600} \times V_{\text{culture}}(\mu l)} \]  

(Eq. 2)

Protein Turnover Determination—Protein synthesis was inhibited by the addition of 35 \( \mu \)g/ml of cycloheximide in dimethyl sulfoxide to cultures grown to \( A_{600} = 1\)–1.2 in the indicated media, and the time course of protein decay was carried out and quantified by the S-tag assay, as described above. The addition of dimethyl sulfoxide alone was used in uninhibited cultures. For turnover rates measured after glucose addition (see Fig. 4), a single 100-ml culture was grown to \( A_{600} = 1 \) and split into four flasks, and cycloheximide or dimethyl sulfoxide was added 5 min prior to the addition of glucose. The samples were taken at times indicated and assayed as described. The data points were log transformed, and the degradation rate constant was determined from the slope of the best fit line (see Fig. 4A and supplemental Fig. S1).

Steady State Protein Abundance across Glucose Spectrum—A single 50-ml culture of the indicated strain was grown in SC-HUL + 2% glucose, and the cells were pelleted, washed once with 5 ml of SC-HUL + 5% glycerol, resuspended in fresh SC-HUL + 3% glycerol, and further grown for 6–12 h in a roller at 30 °C. This glycerol-acclimated culture was dispensed in 160-\( \mu \)l aliquots in duplicate into a 96-well PCR plate. To each well, 40 \( \mu \)l of 5 \( \times \) concentrated glycerol was added, to give final concentrations ranging from 0.001 to 10%. An equivalent volume of water was added to the 0% glycerol wells. A presterilized, gas-permeable, plate-sealing film was applied, and the cells were thoroughly mixed by vortexing and incubated for 90 min at 30 °C. After incubation, 25 \( \mu \)l of the culture was removed for \( A_{600} \) determination; the cells were pelleted at 3000 \( \times g \) for 5 min, the medium was removed, and S-tag protein abundance was assayed.

RESULTS

Analysis of Gene Expression Reveals an Overlap of Targets Regulated by Mth1 and Std1—To determine whether Mth1 and Std1 regulate expression of distinct sets of genes, we profiled gene expression of strains missing MTH1, STD1, or both. Transcriptional profiles for each of the mutants relative to wild type cells grown in galactose (in which Rgt1-Mth1/Std1 is a functional repressor) were measured. Expression of 115 genes was altered >2-fold in the \( \Delta \text{mth1/\text{std1}} \) double mutant and in one or both of the single deletion mutants (supplemental Table S1).

Transcription of 55 of these genes is increased by deletion of \( \text{mth1, std1} \), or both genes; 17 of these are known targets of Rgt1 (14). We believe most of the other 38 genes are also Rgt1 targets because their upstream sequences are enriched in Rgt1-binding sites (23 of 38 sites; \( p \) value < 0.0005; see “Experimental Procedures”), but it is possible that Mth1 or Std1, acting through a repressor other than Rgt1, regulates expression of these genes. The majority of the genes affected in the double deletion mutant can be accounted for by the effect of simply deleting \( \text{mth1} \) (30/55 or 55% of affected genes); expression of only 5 of these 55 genes is altered by deletion of \( \text{std1} \) alone (expression of all five is also affected by deletion of \( \text{mth1} \)). Detailed breakdown of the overlap between these sets of genes can be found in supplemental Table S3. These results make it clear that both pro-
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Mth1 Is More Abundant than Std1 When Glucose Is Absent—In the absence of glucose, Mth1 appears to be the major regulator of Rgt1 function (see above). To determine the relative Mth1 and Std1 levels and their changes after a glucose pulse, we monitored their protein levels (using the S-tag assay) and HXT1 expression (using a lacZ reporter) after adding glucose to cells. The Mth1 level rapidly decreases when cells are exposed to glucose, and this is quickly followed by induction of HXT1 expression (Fig. 1A). The level of Std1 did not change appreciably, remaining roughly 5-fold lower than that of Mth1 at time 0 (Fig. 1A, filled circles). In another experiment, the cells were grown in glucose, a condition in which both Mth1 and Std1 are present in cells at low levels, and then transferred to medium with 5% glycerol as the carbon source. The level of Mth1 increased rapidly during the first 30 min after glucose withdrawal and approached a steady state (Fig. 1B, filled triangles), with kinetics mirroring its depletion after glucose addition (Fig. 1A). The Std1 level was largely unperturbed, remaining 6–8-fold lower than Mth1 (Fig. 1B, filled circles), similar to the 5-fold difference measured in cells growing on galactose (Fig. 1A). The difference in relative abundance of Mth1 and Std1 was confirmed qualitatively by Western blots of extracts expressing Myc-tagged versions of these two proteins (Fig. 1C). An excess of Mth1 over Std1 in cells growing on galactose is consistent with our observation that Mth1 is the chief regulator of Rgt1 function. This suggests that the difference in the roles of Mth1 and Std1 in glucose sensing is likely a consequence of their abundance rather than the result of the two proteins having different functions.

Mth1 and Std1 Proteins Are Interchangeable in the Glucose-sensing System—If Mth1 and Std1 protein functions overlap completely, the proteins should be able to be exchanged or replaced with no effect on function of the signaling pathway. To test this idea, each protein was expressed from the promoter of the other; P_{MTH1}-STD1 and P_{STD1}-MTH1 constructs were created. The Δmth1/Δstd1 strain served as the host for these hybrid genes, along with a P_{HXT1}-lacZ reporter. Previous work demonstrated that either MTH1 or STD1 alone is sufficient for repression of the HXT1 reporter used here (15). As expected, both P_{MTH1}-STD1 and P_{STD1}-MTH1 are capable of repressing HXT1 expression in galactose compared with a strain lacking both co-repressors (Fig. 2A, white bars). Induction of HXT1 expression is similar in P_{STD1}-MTH1 and wild type (P_{MTH1}-MTH1) strains (Fig. 2A, black bars); however, the degree of HXT1 induction is reduced in the strain expressing STD1 from the stronger, MTH1 promoter (P_{MTH1}-STD1) (Fig. 2A, P_{MTH1}-STD1 versus P_{MTH1}-MTH1). This suggests that Std1 is a stronger co-repressor than Mth1 and/or that Std1 is depleted to a lesser degree upon exposure to glucose. Evidence supporting the latter possibility is presented in Table 3, which shows the differences in the turnover of the Mth1 and Std1 proteins. In this experiment, the half-life of Mth1 or Std1 was measured when either protein was expressed from a tetracycline-inducible promoter (insensitive to glucose) in cells grown on galactose or glucose. Turnover of the Mth1 protein in this setting is faster than Std1 turnover in both galactose (Mth1 half-life, 100 min versus 150 min for Std1) and in glucose (Mth1, 50 min versus 130 min for Std1).

Because both Mth1 and Std1 promote repression through Rgt1, the simplest model predicts that the sum of Mth1 + Std1...
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![Graph](image)

FIGURE 2. HXT expression in strains with wild type and shuffled MTH1 or STD1 genes. A, the glucose induction of a P_{HXT3}-lacZ reporter was measured in the Δmth1/Δstd1 strain (Empty Vector) or in Δmth1/Δstd1 cells expressing Mth1 protein from its native promoter (P_{MTH1}-MTH1), Mth1 protein from the STD1 promoter (P_{STD1}-MTH1), or Std1 protein from the STD1 promoter (P_{STD1}-STD1). The white bars indicated activity in galactose; the black bars denote activity in glucose after 2 h. Assays of two independent transformants are shown for P_{STD1}-MTH1 and P_{STD1}-STD1. Error bars showing the standard error of the mean for duplicate measurements are absent from empty vector and native measurements at 2 h. B, the glucose induction of a P_{HXT3}-lacZ reporter was measured after 2.5 h in the Δmth1/Δstd1 mutant expressing MTH1 and STD1 alone or in combination from the indicated promoters. The error bars denote the standard error (n = 8).

TABLE 3
Turnover rates of Mth1 and Std1 expressed from P_{TetO7} promoter in Δmth1/Δstd1 mutant

| Galactose | Glucose |
|----------|---------|
|          |         |
| Mth1     | Std1    |
| k (min⁻¹) | 0.007   | 0.005   |
| Std1      | 0.014   | 0.005   |
|          | Standard error | 0.001  | 0.001  |
|          | Ratio Mth1 to Std1 | 1.6    | 2.5    |

Protein levels in aliquots were measured using S-tag after cycloheximide arrest of protein synthesis.

The two glucose sensors, of high and low affinity, are thought to contribute to the degradation of Mth1 in response to glucose (7). In a cell expressing both sensors, half of the Mth1 is depleted at ~0.05% glucose (2.75 mM), whereas in a strain lacking the high affinity glucose sensor (Snf3), a higher concentration of glucose, ~0.5%, is necessary to induce degradation of Mth1 to the same extent. Sensitivity at such a low glucose level is similar to that reported for the high affinity glucose sensor of *Candida albicans* (21). Interestingly, glucose levels higher than...
2% do not reduce Mth1 abundance in the snf3 mutant to the same extent as in the wild type (Fig. 3B, compare circles to diamonds). This illustrates how both the high and low affinity sensors, acting in concert, provide a sensitive readout of extracellular glucose over a wide range of concentrations.

Both a Decrease in Its Production and an Increase in Its Degradation Contribute to Rapid Depletion of Mth1 after Glucose Addition—The disappearance of Mth1 after the addition of glucose is a consequence of both its glucose sensor-mediated degradation and a cessation of its production. While growing in galactose, the steady state level of Mth1 is constant, reflecting the balance between degradation and production of new protein (Fig. 4B, dashed line). To isolate the contributions that each mechanism makes to Mth1 depletion after glucose addition, the turnover rate of Mth1 in cells growing in galactose and in cells after glucose addition was determined (Fig. 4A). The turnover rate in cells grown on galactose is near to that measured in glucose (0.013 versus 0.018 in glucose) and the fold change (glucose rate/galactose rate) measured here (1.3-fold) is comparable with that measured for Mth1 (above 2-fold). Given this nominal change in degradation rate, a larger alteration in the rate of Mth1 production (3–4-fold) must also occur to account for the 5-fold or greater drop in Mth1 abundance seen after glucose addition, a feature absent from the regulatory program governing Std1. Transcriptional repression of MTH1 expression by the Snf1-Mig1 pathway is the likely candidate for this
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slow down in production, although acceleration of mRNA degradation, like that observed for gluconeogenic genes FBP1 and PCK1 (41, 42), may also contribute.

To illustrate the effects of the change in production necessary to cause the observed drop, the following function was fit to data from a parallel time course of the Mth1 level after glucose addition (Fig. 4B).

\[ L(t) = \frac{p_0}{k_0} + \left( \frac{p_0}{k_0} - \frac{p_1}{k_1} \right) \cdot e^{-k_1 \cdot t} \]  

(Eq. 3)

This function describes the exponential decay in protein level \( L \) from one steady state, defined by the ratio of production rate \( (p_0) \) to degradation rate constant \( (k_0) \) at \( t = 0 \), to another steady state, defined by the new production rate \( (p_1) \) and degradation rate constants \( (k_1) \) (43). The degradation rate constants measured for Mth1 in galactose and glucose, determined in Fig. 4A, were used as constraints, \( k_0 \) and \( k_1 \), respectively, whereas the production rate parameters were allowed to vary, achieving an optimal fit. The resulting blue line (Fig. 4B), which represents the net effects of glucose-induced Mth1 degradation and repression of MTH1 expression, follows the data for Mth1 depletion after glucose addition and extrapolates this to longer times, resulting in an overall reduction of Mth1 levels by ~6-fold. The effect on Mth1 levels of only the measured change in degradation (Fig. 4B, green line) or only the inferred decrease in its production (Fig. 4B, red line) was then simulated in isolation. From these simple simulations, it is clear that the effect of glucose on both degradation (through the sensor and SCFGrr1) and production (through repression of MTH1 expression) of Mth1 protein contribute substantially to adjusting its level to reflect the amount of available glucose, intimately linking detection of glucose at the membrane to its progress through metabolism within the cell.

DISCUSSION

Nutrient detection through transporter-like sensors is an ancient, unique mechanism by which yeast cells monitor their environment (21, 44, 45). The glucose sensor-dependent regulation of Mth1 level enables yeast cells to respond quickly to glucose over a wide range of concentrations. The behavior of Mth1 resembles the dose-dependent regulatory response of the level of Stp1 in the related amino acid sensing system that employs Ssy1, a transporter-like amino acid sensor (46).

A large number of the genes whose expression is altered by deletion of MTH1 and/or STD1 are known or likely targets of Rgt1, suggesting that Mth1 and Std1 are dedicated to this signal transduction pathway. Mth1 is clearly the prime player because it accounts for the majority of the effects on gene expression observed in the Δmth1/Δstd1 mutant. This may be explained by a greater abundance of Mth1 protein relative to Std1 in the absence of glucose.

The functional overlap is not complete, however; a strain containing only the Std1 protein expressed from both the MTH1 and STD1 promoters is not as sensitive to glucose as its wild type equivalent, being unable to achieve the same magnitude of induction of HXT expression (Fig. 2B). The relative insensitivity of Std1 protein to depletion after glucose addition, even when it is expressed from the glucose-repressed MTH1 promoter, may reflect inherent differences in the ability of Mth1 or Std1 to interact productively with the signaling apparatus (18). Thus, although the individual contributions of Mth1 and Std1 to the logic of the glucose-sensing circuit are primarily a consequence of dissimilar transcriptional regulation, differences in the proteins also contribute to the characteristics of the glucose response. This is not surprising, because these two genes diverged from their common ancestor at least 100 million years ago.

Although the role played by Mth1 seems clear, the contributions made by Std1 to the signaling pathway remain enigmatic. It is possible that the primary function of Std1 lies outside of the glucose-sensing pathway or that it is active under conditions other than simple addition or depletion of glucose. Such a role may require it to work with proteins other than Rgt1, because Rgt1 seems to be primarily (perhaps exclusively) dedicated to regulating the transcription of HXT genes and their regulators (14). Under the conditions tested here, Std1 does not appear necessary for the major characteristics of the glucose-signaling network. Because of its low, constant level of expression, we imagine it serves as a buffer to ensure that a minimum level of active Rgt1 is always present, even at high glucose concentrations. It is likely that any role found for Std1 in the glucose-sensing network will be subtle in nature, perhaps endowing the signaling machinery with properties more discernable at the population level.

The whole genome duplication that occurred in the lineage that led to S. cerevisiae provided this and related yeasts with two co-repressors for their glucose-sensing network that came to be expressed at different levels and regulated in different ways. Yeasts such as Kluyveromyces lactis and C. albicans diverged from S. cerevisiae before the whole genome duplication and are thus more constrained because they possess only a single MTH1/STD1 ortholog. In the yeasts in which that ortholog has been studied, its expression is under Rgt1 control and is therefore induced by glucose (47, 48), like STD1 in S. cerevisiae. This suggests that glucose induction of STD1 expression is the ancestral state of the system. This is supported by recent work to reconstruct the gene order of the pre-whole genome duplication ancestor, which suggests that duplication of the ancestral locus resulted in a maintenance of synteny on both sides of present day STD1, whereas a chromosomal translocation occurred in the region upstream of MTH1 (4). The specialization of the Mth1 and Std1 signal transducers was complemented by specialization of the duplicated glucose sensors, resulting in paralogs with high (Snf3) and low affinity (Rgt2) for glucose. The release of functional constraint ushered in by the arrival of paralogs allowed S. cerevisiae to exploit alternative wiring schemes of the glucose-sensing network that were not accessible to species lacking this duplication. It appears that S. cerevisiae has tuned the expression and regulation of Mth1 and Std1 to optimize sensitivity to a wide range of glucose concentrations (0.01–2%). The combined effects of glucose-induced Mth1 degradation and glucose-stimulated shutdown of Mth1 production serve to amplify a weak signal generated by the low amounts of glucose detected through Snf3. S. cerevisiae undergoes an “all-or-none” switch from oxidative to fermenta-
tive growth when an amount of glucose sufficient for that life-style is detected. The amplification of a signal at low levels of glucose (via Snf3), sensitivity to high levels of glucose (via Rgt1), and coupling both production and degradation of a central regulator (Mth1) to glucose availability are elegant examples of how gene duplication enabled new regulatory wirings, providing a competitive advantage in this niche.

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