# Analysis of the Mechanism by Which Glucose Inhibits Maltose Induction of *MAL* Gene Expression in Saccharomyces

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ABSTRACT

Expression of the *MAL* genes required for maltose fermentation in *Saccharomyces cerevisiae* is induced by maltose and repressed by glucose. Maltose-inducible regulation requires maltose permease and the *MAL*-activator protein, a DNA-binding transcription factor encoded by *MAL63* and its homologues at the other *MAL* loci. Previously, we showed that the Mig1 repressor mediates glucose repression of *MAL* gene expression. Glucose also blocks *MAL*-activator-mediated maltose induction through a Mig1p-independent mechanism that we refer to as glucose inhibition. Here we report the characterization of this process. Our results indicate that glucose inhibition is also Mig2p independent. Moreover, we show that neither overexpression of the *MAL*-activator nor elimination of inducer exclusion is sufficient to relieve glucose inhibition, suggesting that glucose acts to inhibit induction by affecting maltose sensing and/or signaling. The glucose inhibition pathway requires *HXK2*, *REG1*, and *GSF1* and appears to overlap upstream with the glucose repression pathway. The likely target of glucose inhibition is Snf1 protein kinase. Evidence is presented indicating that, in addition to its role in the inactivation of Mig1p, Snf1p is required post-transcriptionally for the synthesis of maltose permease whose function is essential for maltose induction.

IN the yeast *Saccharomyces cerevisiae*, glucose regulates the expression of a large number of genes (reviewed in Johnston 1999). A major glucose repression pathway involves the Snf1 protein kinase that acts upon Mig1 protein, a DNA-binding transcriptional repressor of the *SUC2*, *GAL*, and *MAL* genes (Nehlin and Ronne 1990; Nehlin *et al.* 1991; Hu *et al.* 1995). This repression requires glucose transport (Ozcan *et al.* 1994; Vallier *et al.* 1994; Ozcan and Johnston 1995) and several negative regulators, including Hxk2p (hexokinase PII) and Reg1p-Glc7p (protein phosphatase type-1).

Induction of *GAL* and *MAL* gene expression requires a transcriptional activator protein, Gal4p and the *MAL*-activator, respectively (for reviews, see Needleman 1991; Lohr *et al.* 1995), but other aspects of the regulation of these sugar fermentation genes appear to be quite distinct. The mechanism of galactose sensing has been elucidated by recent studies. Intracellular galactose binds to Gal3p and induces a strong interaction between Gal3p and Gal80p that in turns relieves the inhibitory effect of Gal80p on Gal4p (Suzuki-Fujimoto *et al.* 1996; Zenke *et al.* 1996; Blank *et al.* 1997; Yano and Fukasawa 1997; Platt and Reece 1998). Glucose inhibits the process by repressing *GAL3* and *GAL4* expression (Nehlin *et al.* 1991; Lamphier and Ptashne 1992; Johnston *et al.* 1994). The primary mechanism of glucose repression of *GAL* gene expression is the reduction of *GAL4* expression mediated by the Mig1p repressor (Lamphier and Ptashne 1992; Johnston *et al.* 1994). Another mechanism of glucose repression of *GAL* gene expression depends on *GAL80* and reflects a glucose inhibition of the induction process, possibly due to glucose inhibition of galactose permease (encoded by *GAL2*) that causes inducer exclusion. It is believed that the level of intracellular galactose is reduced by glucose repression of *GAL2* transcription (Nehlin *et al.* 1991) and by glucose-induced inactivation of galactose permease (Matern and Holzer 1977; Dejuan and Lagunas 1986; Horak and Wolf 1997).

Previously, we showed that Mig1p represses transcription of *MAL63*, which encodes a transcriptional activator of the *MAL* genes, as well as the *MAL* genes themselves (Hu *et al.* 1995). However, the Mig1p-binding site is missing from the promoter of the *MAL63* homologue *MAL23* due to a single base-pair deletion (Gibson *et al.* 1997). Despite this, *MAL23*-mediated *MAL* gene induction remains sensitive to glucose regulation, suggesting that, unlike the *GAL* genes, Mig1p-dependent glucose repression of *MAL*-activator expression is not sufficient to explain glucose repression of *MAL* gene expression. Since constitutive *MAL*-activators are insensitive to glucose in *mig1*Δ strains, glucose must inhibit *MAL*-activator-mediated maltose induction by a mechanism that is independent of Mig1p repression (Hu *et al.* 1995), which we refer to as glucose inhibition of maltose induction.

In addition to the *MAL*-activator, induction of the *MAL* genes by maltose requires maltose permease (Char-

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ron *et al.* 1986; Dubin 1987). Glucose inhibits transcription of the maltose-permease-encoding gene and also dramatically decreases maltose permease transport activity and protein levels by a process called glucose-induced inactivation (Medintz *et al.* 1996). Thus, like glucose regulation of *GAL* gene expression, it has been proposed that the combination of these glucose effects on maltose permease expression and activity block *MAL* gene induction by an inducer exclusion mechanism. Instead, we have found that glucose inhibition of *MAL* gene induction is not the result of inducer exclusion or reduced levels of the *MAL* transcription activator but appears to result from a novel effect that blocks maltose sensing/signaling. This requires the glucose repression regulatory pathway up to and including Snf1 protein kinase.

## MATERIALS AND METHODS

**Strains and growth media:** The *S. cerevisiae* strains used throughout this study are listed in Table 1. Strain CMY1001 contains only *MAL1* encoding maltose permease, maltase (*MAL12*), and the *MAL*-activator (*MAL13*) (Medintz *et al.* 1996). The maltose permease gene at this *MAL1* locus has been replaced with an HA-tagged maltose permease gene from *MAL6*, *MAL61/HA*. CMY1006u is the *ura3* derivative of CMY1006 isolated on 5-fluoroorotic acid-containing medium (Boeke *et al.* 1984). Yeast strains were grown at 30° in either rich media (1% yeast extract, 2% peptone) or minimal media (0.67% yeast nitrogen base with appropriate amino acids and nitrogen base supplements) plus various carbon sources as specified.

**Plasmids:** A fragment containing the *TRP1* gene was obtained from plasmid pRS304 by PCR using oligonucleotides L1 (GGAGATCTCATAAACGACATTACTAT) and L2 (GGGG TACCTGATGCGGTATTTTCTCC) that carry *Bgl*II and *Kpn*I sites, respectively. This *TRP1* fragment was inserted into pSH2-1/MAL63$_{(2\text{-}216)}$ (Hu *et al.* 1999) digested with *Bgl*II and *Kpn*I, creating plasmid pLexA/MAL63$_{(2\text{-}216)}$. An *Eco*RI-*Bam*HI fragment containing codons 216–470 of *MAL63* was inserted into pLexA/MAL63$_{(2\text{-}216)}$, resulting in plasmid pLexA/MAL63 carrying the *TRP1* gene for selection of yeast transformants. Plasmid pSH2-1/MAL63 is a *HIS3* vector with *MAL63*$_{(2\text{-}470)}$ fused to *LexA* (Hu *et al.* 1999). Plasmids pUN30/MAL63 and pUN30/MAL63/43-c are described in Gibson *et al.* (1997) and carry the inducible *MAL63* or constitutive *MAL63/43-c* genes in the CEN vector pUN30 (Elledge and Davis 1988). Construction of the reporter gene *MAL61pro-LacZ*, consisting of the *MAL61* promoter region from the 5′ AUG of the *MAL61* open reading frame (ORF), is described in Hu *et al.* (1995). Plasmid

**TABLE 1**

**List of strains**

| Strain | Genotype | Source |
|---|---|---|
| YPH500 | *MAT*α *AGT1 MAL12 mal13 MAL31 MAL32 mal33*Δ *ura3-52 his3*Δ*200 leu2-*Δ*1 trp1-*Δ*63 lys2-801 ade2-101* | |
| CMY2001 | Isogenic to YPH500, *mig1::LEU2* | This study |
| CMY2002 | Isogenic to YPH500, *hxk2::URA3* | This study |
| CMY2003 | Isogenic to YPH500, *mig1::LEU2 hxk2::URA3* | This study |
| CMY1001 | *MAT***a** *MAL61/HA MAL12 MAL13 leu2 ura3-52 lys2-801 ade2-101 trp1-*Δ*63 his2-*Δ*200* | Medintz *et al.* (1996) |
| CMY1006 | Isogenic to CMY1001, *hxk2::URA3* | Medintz *et al.* (1996) |
| CMY1015 | Isogenic to CMY1001, *hxk2::URA3 hxk1::TRP1* | Jiang *et al.* (2000a) |
| CMY1005 | Isogenic to CMY1001, *grr1::LEU2* | Jiang *et al.* (1997) |
| CMY1010 | Isogenic to CMY1001, *grr1::LEU2 rgt1::URA3* | Jiang *et al.* (1997) |
| CMY1009 | Isogenic to CMY1009, *rgt2::kan*$^R$ | Jiang *et al.* (1997) |
| KT1357 | *MAT***a** *AGT1 MAL12 mal13*Δ *MAL31 MAL32 mal33*Δ *ura3-52 leu2 his3 trp1* | Frederick and Tatchell (1996) |
| DF186 | Isogenic to KT1357, *reg1::URA3* | Frederick and Tatchell (1996) |
| FY251 | *MAT***a** *his3-*Δ*200 leu2-*Δ*1 trp1-*Δ*63 ura3-52* | Sherwood and Carlson (1997) |
| PS3851-3A | *MAT***a** *his3-*Δ*200 leu2-*Δ*1 trp1-*Δ*63 ura3-52 gsf1-1* | Sherwood and Carlson (1997) |
| PS5959-6B | *MAT*α *mig1*Δ*::URA3 his3-*Δ*200 leu2-*Δ*1 trp1-*Δ*63 ura3-52 lys2-801* | Sherwood and Carlson (1997) |
| CMY4001 | Isogenic to PS5959-6B, *mig1::LEU2* | This study |
| YM4352 | *MAT*α *ura3*Δ*::LEU2 his3-*Δ*200 ade2-101 lys2-801 trp1-901 gal80*Δ*? GAL4-HIS3::LEU2 gal4*Δ*::GAL4-URA3-TRP* | Lutfiyya and Johnston (1996) |
| YM4725 | *MAT*α *mig1*Δ*::URA3 ura3-52 his3-*Δ*200 ade2-101 lys2-801 trp1-901 gal80-*Δ*538 LEU::GAL1-LACZ* | Lutfiyya *et al.* (1998) |
| YM4664 | *MAT***a** *mig2*Δ*::HIS3 ura3-52 his3-*Δ*200 ade2-101 lys2-801 trp1-901 tyr1-501 gal80-*Δ*538 LEU::GAL1-LACZ* | Lutfiyya *et al.* (1998) |
| YM4738 | *MAT***a** *mig1*Δ*::URA3::LYS2 mig2*Δ*::HIS3 ura3-52 his3-*Δ*200 ade2-101 lys2-801 trp1-901 gal80-*Δ*538 LEU::GAL1-LACZ* | Lutfiyya *et al.* (1998) |

pBM3270 (obtained from M. Johnston) is a *CEN* plasmid carrying *URA3* and the dominant *RGT2-1* allele.

**Gene disruption and Southern analysis:** *MIG1* was disrupted in strain YPH500 to create strain CMY2001 using plasmid pJN22 as described (Nehlin and Ronne 1990). *HXK2* was disrupted in strain YPH500 (creating strain CMY2002) and isogenic CMY2001 (creating strain CMY2003) using plasmid pRB528 (obtained from David Botstein), which was digested with *Eco*RI prior to transformation. Plasmid pRB528 carries a *hxk2::URA3* deletion disruption. Strain CMY4001 was constructed from strain PS5959-6B by replacing *MIG1* with *LEU2* using the disruption fragment from plasmid pJN22 (Nehlin and Ronne 1990). All disruptions were confirmed by Southern analysis. *GSF2* was disrupted in strain CMY1001 using plasmid pΔ2-RM1 as described in Sherwood and Carlson (1997) to create strain CMY3001. This construction replaces the entire *GSF2* coding region with *TRP1*.

**Northern blot analysis:** Total RNA was prepared using the RNeasy Midi kit (QIAGEN, Chatsworth, CA) according to the manufacturer's instructions for yeast cells. The RNA was size separated in a 1.2% formaldehyde-agarose gel as described in the RNeasy kit (QIAGEN), transferred to nitrocellulose, washed, and probed according to Ausubel *et al.* (1999). Approximately 50–70 μg of total RNA was loaded per lane. PCR-amplified *MAL61* ORF and plasmid pYactI (Ng and Abelson 1980) were used as probes to the same gel. The Storm 860 PhosphorImager (Molecular Dynamics, Sunnyvale, CA) was used to visualize and quantify the hybridization signals.

**Western blot analysis:** Cells were harvested in mid-log phase. Whole-cell protein extracts were prepared as described (Ausubel *et al.* 1999). Equal amounts of protein samples were separated in SDS-polyacrylamide gel and transferred to Hybond-ECL nitrocellulose membrane (Amersham, Piscataway, NJ) as described (Ausubel *et al.* 1999). Membranes were probed with either anti-LexA antibody (obtained from Roger Brent) followed by horseradish-peroxidase-linked donkey anti-rabbit antibodies (Amersham) or anti-HA antibody (Boehringer Mannheim, Indianapolis) followed by horseradish-peroxidase-linked sheep anti-mouse antibody (Amersham). Protein bands were visualized using the enhanced chemiluminescence (ECL) Western blotting detection kit (Amersham) on ECL-Hyperfilm. The protein blots were quantified by scanning with a DU640 spectrophotometer (Beckman, Fullerton, CA).

**Enzyme assays:** Yeast cells were grown in rich media or minimal media with 3% glycerol and 2% lactate (v/v) plus 2% of the specified sugar(s) (w/v) and harvested in mid-log phase. Maltase activities were determined as described (Dubin *et al.* 1988). Maltase activity is expressed as nanomoles of *p*-nitrophenol-α-glucopyranoside (PNPG) hydrolyzed per minute per milligram of protein. The assays of β-galactosidase activity were carried out in total cell extracts, and the activity was normalized to protein concentration for calculating specific activity (Rose *et al.* 1990). Maltose transport activities were measured by the uptake of [$^{14}$C]maltose as described (Cheng and Michels 1991). Inactivation protocol for assays of glucose sensitivity of maltose permease has been described in detail in Medintz *et al.* (1996).

## RESULTS

**Loss of Mig2p does not relieve glucose inhibition:** *MIG2* encodes a protein with a $Cys_2His_2$ zinc finger similar to that of Mig1p (Lutfiyya *et al.* 1998) that represses *SUC* expression in the presence of glucose. We tested the possibility that Mig2p is responsible for the Mig1p-independent glucose effect on *MAL* gene induction. As the results in Table 2 demonstrate, glucose significantly inhibits maltase induction in the *mig1*Δ *mig2*Δ double mutant. Therefore, glucose inhibition of maltose induction is not due to residual effects of Mig2p.

**Overexpression of the *MAL*-activator does not relieve glucose inhibition:** Glucose could inhibit maltose induction by affecting the synthesis and/or stability of the Mal63 protein. Thus, we tested whether overexpression of *MAL63* abolishes glucose inhibition. A multicopy plasmid carrying a *LexA-MAL63* fusion gene abundantly expressed from the constitutive *ADH1* promoter was introduced into a *mig1*Δ strain carrying a *lexO-LacZ* reporter gene. The LexA-Mal63 fusion protein is expressed at comparable levels in both maltose-grown and glucose-grown cells, which is about twice the level seen in the glycerol/lactate-grown cells (data not shown). LexA-Mal63 protein activates transcription of both the *MAL* genes and the *lexO-LacZ* reporter gene in a maltose-dependent manner (Hu *et al.* 1999; Tables 3 and 4). As shown in Table 3, the LexA-Mal63 fusion protein fails to activate either the *LacZ* reporter gene or the maltase gene in cells grown in maltose/glucose media. Thus, unlike *GAL*, the effect of glucose on maltose induction

**TABLE 2**

**Effect of *MIG1* and *MIG2* mutations on glucose inhibition of maltase expression**

| | Maltase activity | | | |
|---|---|---|---|---|
| Relevant genotype | G/L alone | + Glucose | + Maltose | + Maltose + Glucose |
| *MIG1 MIG2* | 54.2 | 1.5 | 2082 | 1.7 |
| *mig1*Δ *MIG2* | 55.1 | 17.3 | 1998 | 17.1 |
| *MIG1 mig2*Δ | 58.2 | 3.8 | 1860 | 4.8 |
| *mig1*Δ *mig2*Δ | 55.9 | 36.2 | 1798 | 50.6 |

Isogenic strains YM4359 (*MIG1 MIG2*), YM4725 (*mig1*Δ *MIG2*), YM4664 (*MIG1 mig2*Δ), and YM4738 (*mig1*Δ *mig2*Δ) were transformed with *MAL63* carried on the CEN vector pUN30. Cells were grown in selective synthetic medium containing 2% lactate and 3% glycerol (G/L) plus 2% of the indicated sugars. Maltase activities were normalized to the protein concentration of the cell extracts. Values are averages of two to four independent transformants assayed in duplicate. Standard errors were <10%.

**TABLE 3**

**Glucose inhibition of the overexpressed LexA-Mal63 protein**

| Relevant genotype | Expressed activator protein | β-Galactosidase activity | | | Maltase activity | | |
|---|---|---|---|---|---|---|---|
| | | G/L alone | + Maltose | + Maltose + Glucose | G/L alone | + Maltose | + Maltose + Glucose |
| *mig1*Δ *HXK2* | LexA-Mal63 | 2 | 155 | 2 | 46 | 1800 | 75 |
| | LexA-Mal63$_{(\Delta 41\text{-}66)}$ | 3 | 646 | ND | 16 | 38 | ND |
| | LexA-Mal63; Mal64-R10 | 2 | 186 | 5 | 2283 | 3421 | 1042 |
| *MIG1 hxk2*Δ | LexA-Mal63 | ND | ND | ND | 73 | 1993 | 1495 |
| *mig1*Δ *hxk2*Δ | LexA-Mal63 | 1 | 398 | 116 | 65 | 1812 | 1048 |

The *LexO-LacZ* reporter plasmid used was pSH18-18. *MIG1* and *HXK2* were disrupted in strain YPH500, a *MAL*-activator null strain. The *LexA-MAL63* fusion is carried on a 2μ plasmid with *TRP1* (see materials and methods) and is able to complement the *MAL*-activator null mutation. The *MAL63*$_{(\Delta 41\text{-}66)}$ mutant allele was fused to *LexA*$_{(1\text{-}87)}$ (pSH2-1) (Hu *et al.* 1999). The constitutive *MAL64-R10* gene is carried on the *HIS3* vector pRS413 (Medintz *et al.* 1996). Cells were grown in synthetic media lacking uracil, tryptophan, and/or histidine containing 3% glycerol and 2% lactate (v/v) (G/L) plus 2% of the specified sugar (w/v). Maltase activity was assayed as described in Table 2. β-Galactosidase activity was normalized to the protein concentration of the cell extracts. Values are averages of two to four independent transformants assayed in duplicate. Standard errors were <20% for β-galactosidase activities. ND, not determined.

of the *MAL*-activator is not the result of reduced levels of the activator protein.

**Inducer exclusion is not necessary for glucose inhibition:** Maltose induction of *MAL* gene expression requires a functional maltose permease gene (Charron *et al.* 1986; Dubin 1987; X. Wang and C. A. Michels, unpublished results). Since glucose causes a rapid reduction of maltose transport activity (Medintz *et al.* 1996), glucose could block the induction signal simply by decreasing the transport of maltose (that is, by inducer exclusion). Two experiments were carried out to explore this hypothesis.

First, to test the minimal level of maltose permease activity required for induction, a *LexA-MAL63*$_{(\Delta 41\text{-}66)}$ fusion gene was transformed into a *mig1*Δ strain lacking other *MAL*-activator genes. Because it is missing its DNA-binding domain, the LexA-MAL63$_{(\Delta 41\text{-}66)}$ fusion protein is unable to activate expression of the *MAL* genes (Hu *et al.* 1999; Tables 3 and 4), causing maltose permease activity to remain at the low, uninduced basal level. Despite this, LexA-Mal63$_{(\Delta 41\text{-}66)}$ protein is able to activate transcription of the *lexO-LacZ* reporter gene in response to maltose (Table 3), indicating that this low basal level expression of maltose permease is adequate for maximal maltose induction of reporter expression.

Second, a plasmid carrying a constitutive glucose-repression-insensitive *MAL*-activator gene, *MAL64-R10*, was introduced into a *mig1*Δ strain along with the *LexA-MAL63* fusion (Dubin *et al.* 1988; Hu *et al.* 1995). *MAL64-R10* causes constitutive expression of the *MAL* genes, including maltose permease, even in the presence of glucose (Tables 3 and 4). This significantly increases maltose transport activity in these maltose/glucose-grown cells (fourfold above the basal level). Despite the elevated levels of maltose transport activity, the overexpressed inducible LexA-Mal63 activator remains sensitive to glucose inhibition of maltose induction (Table 3). Thus, neither the elimination of inducer exclusion nor the abundant expression of Mal63 protein is sufficient to relieve glucose inhibition.

***HXK2* is involved in glucose inhibition of Mal63p:** Hxk2 hexokinase reportedly generates the high glucose signal to which the glucose repression pathway responds (reviewed in Ronne 1995; Carlson 1999; Johnston 1999). To test if Hxk2p is involved in glucose inhibition, a plasmid carrying *MAL63* was introduced into wild-type and isogenic *mig1*Δ, *hxk2*Δ, and *mig1*Δ *hxk2*Δ strains, and maltase activities were determined. As was found previously (Hu *et al.* 1995), *mig1*Δ partially relieves glucose repression of maltase, but maltose induction remains mostly blocked by glucose inhibition (Table 5). In contrast, *hxk2*Δ fully relieves glucose repression of maltase expression and, more notably, maltase activity

**TABLE 4**

**Maltose permease activity in cells with the overexpressed LexA-Mal63 fusion proteins**

| Expressed activator protein | Maltose transport activity | | |
|---|---|---|---|
| | G/L alone | + Maltose | + Maltose + Glucose |
| LexA-Mal63 | 1.2 | 36.9 | <0.5 |
| LexA-Mal63$_{(\Delta 41\text{-}66)}$ | 1.0 | 1.0 | ND |
| LexA-Mal63; Mal64-R10 | 171 | 53.6 | 3.7 |

The *LexA-MAL63* fusion plasmids and pRS413/MAL64-R10, carrying the constitutive *MAL*-activator allele *MAL64-R10*, were transformed into *mig1*Δ strain CMY2001, a YPH500-derived strain lacking a *MAL*-activator gene. Cells were grown as described in Table 3. Maltose permease activities were measured as described in materials and methods and are expressed as nanomoles of $^{14}$C-maltose per milligram of dry weight per 10 minutes. ND, not determined.

**TABLE 5**

**Effect of *HXK2* disruption on glucose inhibition of MAL63 protein**

| Expressed *MAL*-activator | Relevant genotype | Maltase activity | | | |
|---|---|---|---|---|---|
| | | G/L alone | + Maltose | + Glucose | + Maltose + Glucose |
| *MAL63* | Wild type | 26 | 742 | 0.8 | 1.0 |
| | *mig1*Δ | 29 | 974 | 4.7 | 5.2 |
| | *hxk2*Δ | 22 | 433 | 26 | 72 |
| | *mig1*Δ *hxk2*Δ | 27 | 477 | 36 | 277 |

YPH500 and isogenic mutant strains were transformed with plasmid pUN30/MAL63. Cells were grown in minimal media lacking tryptophan with 3% glycerol and 2% lactate (G/L) plus 2% of various specified sugars. Maltase activities were assayed as described in Table 2.

is partially induced in the maltose/glucose-grown *hxk2*Δ cells (to about threefold higher than in glucose-grown cells). Thus, the *hxk2* mutation appears to allow some maltose induction in the presence of glucose, suggesting that Hxk2p is a component of the signaling pathway regulating both glucose inhibition and glucose repression.

Maltase expression in maltose/glucose-grown *hxk2*Δ cells is about sixfold lower than in maltose-grown cells, and we wished to explore the basis of this residual effect. Disruption of *MIG1* in the *hxk2*Δ strain allows significantly increased levels of maltase expression in the presence of glucose plus maltose, almost to the levels seen in maltose-grown cells (Table 5). Thus, it appears that the residual regulation of *MAL* gene induction seen in *hxk2* mutant strains is the result of Mig1p. We believe this is due to effects on *MAL63* expression because of the results of the following experiments.

The abundantly expressed *LexA-MAL63* activator gene was introduced into isogenic *mig1*Δ, *hxk2*Δ, and *mig1*Δ *hxk2*Δ YPH500-derived strains (Table 3). First, glucose does not inhibit maltose induction of maltase expression mediated by the abundantly expressed LexA-Mal63 activator in both the *hxk2*Δ and *mig1*Δ *hxk2*Δ strains. Significantly, no enhancement of maltase expression results from the loss of Mig1 repressor. Second, we find that disruption of *HXK2* in a strain carrying the *MAL23 MAL*-activator gene causes a complete loss of glucose sensitivity in maltose/glucose-grown cells (data not shown). *MAL23* lacks a Mig1p-binding site in its promoter (Gibson *et al.* 1997).

Taken together, these results support the view that Hxk2p is an important component of both the glucose repression and glucose inhibition signaling pathways. In the *hxk2*Δ strain, Mig1p can still confer some glucose sensitivity on *MAL63* by repressing its transcription. We suggest that in the *hxk2*Δ background another sugar kinase may at least partially substitute for the role of hexokinase PII encoded by *HXK2* in mediating glucose repression. This issue is addressed in the following section.

***HXK1* may partially mediate glucose repression in a *hxk2*Δ strain:** *S. cerevisiae* contains two hexokinases, PI (Hxk1p) and PII (Hxk2p) (Lobo and Maitra 1977). *HXK2* appears to be expressed in cells grown on high levels of glucose; expression of *HXK1* is glucose repressed (Herrero *et al.* 1995; de Winde *et al.* 1996). The functional distinction between *HXK1* and *HXK2* appears to result from their different expression patterns since multiple copies of *HXK1* in an *hxk2* mutant partially restore glucose repression (Entian *et al.* 1984; Ma and Botstein 1986; Rose *et al.* 1991). Because *HXK1* is derepressed in *hxk2*Δ cells on glucose, we reasoned that Hxk1p could play some of the roles of Hxk2p in generating/transmitting the glucose repression signal. Maltase expression on various carbon sources was not affected by disruption of *HXK1* alone (data not shown), but disruption of *HXK1* in an *hxk2* mutant further relieved glucose repression of maltase in glucose-grown cells (Table 6). These results suggest that Hxk1p can at least partially substitute for Hxk2p in mediating glucose repression.

**Glucose-induced inactivation of maltose permease is intact in the *hxk2*Δ strain:** Maltose transport activity and the amount of maltose permease protein can be followed in a strain expressing an HA-tagged maltose permease gene, allowing us to assay the sensitivity of the permease to glucose-induced inactivation. The protocol for the inactivation assay has been described in detail in Medintz *et al.* (1996). Briefly, cells are grown to early log phase in rich medium containing 2% maltose (to induce maltose permease expression), harvested, and transferred to nitrogen-starvation medium (to stop protein synthesis) with 2% glucose. Maltose transport activity and maltose permease protein levels are measured at selected time intervals within 3 hr following the transfer to glucose.

Glucose causes a decrease in both maltose transport activities and protein levels of maltose permease (Figure 1A). Disruption of *HXK2* has only a modest twofold effect on the glucose inactivation of maltose permease (Figure 1B). Moreover, the *hxk2*Δ strain pregrown in

**TABLE 6**

**Hexokinase-mediated glucose effect in a strain carrying *MAL13***

| *MAL*-activator allele | Relevant genotype | Maltase activity G/L alone | + Maltose | + Glucose | + Maltose + Glucose |
|---|---|---|---|---|---|
| *MAL13* | Wild type | 27 | 832 | 0.1 | 0.3 |
| | *hxk2*Δ | 24 | 802 | 5.8 | 160 |
| | *hxk1*Δ *hxk2*Δ | 23 | 1415 | 34 | 1153 |

Cells of CMY1001 (*HXK2*), CMY1006 (*hxk2::URA3*), and CMY1015 (*hxk1::TRP1 hxk2::URA3*) carrying *MAL13* at *MAL1* locus (Medintz *et al.* 1996; Jiang *et al.* 2000a) were grown in rich media with glycerol and lactate plus various sugars as specified. Cells were grown and maltase activities were assayed as described in Table 2.

maltose/glucose medium, which partially induces expression of maltose permease, exhibits a very rapid and complete inactivation of this permease after being transferred into glucose medium (Figure 1C). Thus, loss of Hxk2p relieves glucose inhibition of the *MAL*-activator (Table 5) but has no apparent impact on the rate of glucose-induced loss of maltose permease protein and maltose transport activity, suggesting that glucose-induced inactivation of maltose permease alone does not account for glucose inhibition.

**Effects of *reg1* and *gsf1* on glucose inhibition of Mal63p:** *REG1* encodes a regulatory subunit of the protein phosphatase type-1 (PP1) catalytic subunit encoded by *GLC7* (Tu and Carlson 1995). PP1 and Reg1p are required for glucose repression (Tu and Carlson 1994). Deletion of *REG1* fully relieves repression of maltase expression in glucose-grown cells (Table 7). More significantly, maltose induction is not glucose inhibited in the absence of Glc7p-Reg1p phosphatase activity. Thus, disruption of *REG1* appears to relieve both glucose repression of *MAL* gene expression as well as glucose inhibition of Mal63p function.

*GSF1* is proposed to encode an inhibitor of Snf1 protein kinase that responds to glucose availability and acts in the same or a parallel pathway as Reg1p (Sherwood and Carlson 1997). Loss of Gsf1p dramatically relieves glucose repression of *SUC2* and *GAL10* expression, and glucose induction of *HXT1* expression is downregulated

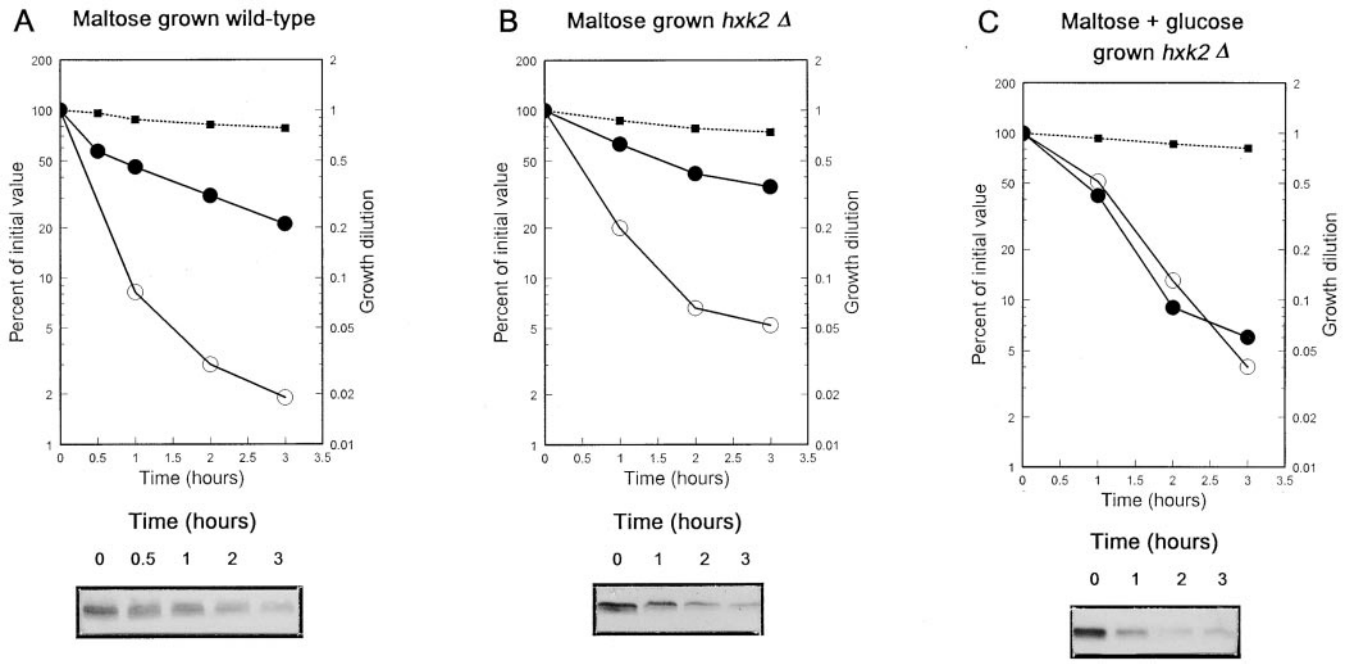


Figure 1.—Glucose-induced inactivation of maltose permease in *hxk2* mutant strains. Glucose-induced inactivation of maltose permease was followed in isogenic CMY1001 (*HXK2*) and CMY1006 (*hxk2*) as described in materials and methods. At the indicated times, maltose transport activity (open circles), relative maltose permease protein levels (solid circles), and growth dilution (dashed line, squares) were determined. A and B show strains CMY1001 and CMY1006 grown in maltose medium, respectively. C shows strain CMY1006 grown in maltose plus glucose medium.

**TABLE 7**

**Effect of a *reg1* disruption on glucose inhibition of *MAL63***

| Relevant genotype | Maltase activity | | | |
|---|---|---|---|---|
| | G/L alone | + Maltose | + Glucose | + Maltose + Glucose |
| *REG1* | 18 | 781 | 1.0 | 1.3 |
| *reg1*Δ | 17 | 814 | 23 | 331 |

Plasmid pUN30/MAL63 was transformed into strains KT1357 (*REG1*) and isogenic DF186 (*reg1*Δ). Cells were grown and maltase activities were measured as described in Table 2.

in *gsf1* mutants. It also results in partial maltose inducibility of maltase expression in maltose/glucose medium (Table 8).

***GRR1*-dependent pathway mediates inhibition of the *MAL*-activator function in response to glucose:** Since *grr1*Δ strains are defective in glucose-induced inactivation of maltose permease (Jiang *et al.* 1997) as well as glucose repression, we examined whether *grr1*Δ also relieves glucose inhibition of *MAL*-activator induction. As shown in Table 9, disruption of *GRR1* relieves glucose repression and glucose inhibition of maltase expression. Disruption of *RGT1* in the *grr1*Δ strain dramatically restores glucose repression in cells grown in glucose-containing medium (Table 9), which correlates well with the full restoration of high-affinity glucose transport in the same *grr1*Δ *rgt1*Δ strain (Jiang *et al.* 1997). However, glucose sensitivity of *MAL13* is only partially restored, and maltose-induced expression of the maltase gene is still observed in maltose/glucose-grown cells (~30-fold induction remains). Maltase expression in a *grr1*Δ *rgt1*Δ strain carrying an overexpressed *LexA-MAL63* fusion gene also is clearly induced in maltose/glucose-grown cells as compared to glucose-repressed level (Table 9). These results suggest a Grr1p-dependent glucose effect on *MAL*-activator induction that does not involve Rgt1p.

Rgt2p and Snf3p are sensors of high and low extracellular glucose, respectively (Ozcan and Johnston 1995; Ozcan *et al.* 1996a). If the *GRR1*-dependent glucose inhibition of Mal13p function requires the high-glucose sensor Rgt2p, maltase expression in a strain carrying the dominant, constitutive *RGT2-1* allele should be affected even under maltose-induced conditions. Maltose-induced expression of maltase is only slightly decreased (less than twofold) by *RGT2-1* (Table 10). Furthermore, disruption of *RGT2* only modestly relieves glucose repression of maltase expression (Table 9). Thus, it would appear that *RGT2* does not play a major role in triggering the Grr1p-dependent glucose inhibition of the *MAL*-activator.

**Snf1 protein kinase is required for maltose permease synthesis:** The results reported above indicate that the glucose sensing/signaling pathway for glucose inhibition shares several components with the glucose repression pathway that negatively regulates Snf1 protein kinase activity in response to glucose availability (reviewed in Johnston 1999). For this reason, we decided to test the possibility that the glucose inhibition signaling pathway also involves Snf1 protein kinase, suggesting that Snf1 protein kinase is required for maltose induction in addition to its role in inhibiting Mig1 repressor. *MAL* gene expression was determined in *mig1*Δ strains with or without functional *SNF1*. Loss of Snf1 kinase severely impairs *MAL* gene expression in the strain carrying the inducible *MAL63* activator gene (Table 11). The constitutive *MAL*-activator allele *MAL63/43-c* was introduced into the same *SNF1 mig1*Δ and *snf1*Δ *mig1*Δ strains, and expression of maltase, maltose transport activity, and the *MAL61pro-LacZ* reporter was compared (Table 11). Surprisingly, while maltase and the *MAL61pro-LacZ* reporter are both constitutively expressed, no maltose transport activity above background levels is detected. There is no significant difference between the level of maltose permease mRNA in the *SNF1 mig1*Δ [pMAL63/43-c] and *snf1*Δ *mig1*Δ [pMAL63/43-c] strains (Figure 2). These results suggest that the lack of maltose transport activity in the *snf1*Δ *mig1*Δ [pMAL63/43-c] strain is the result of a post-transcriptional block in maltose permease synthesis. Consistent with this, the introduction of additional plasmid-borne copies of *MAL61* slightly increases the level of maltose transport activity expressed in this strain. Thus, these results suggest that

**TABLE 8**

**Effect of *GSF1* on glucose inhibition of maltose induction**

| Expressed *MAL*-activator | Relevant genotype | Maltase activity | | | |
|---|---|---|---|---|---|
| | | G/L alone | + Maltose | + Glucose | + Maltose + Glucose |
| *MAL63* | *GSF1* | 13 | 790 | 0.5 | 0.6 |
| | *gsf1-1* | 13 | 741 | 20 | 124 |

FY251 and the congenic *gsf1-1* mutant strain PS3851-3A (Sherwood and Carlson 1997) were transformed with plasmid pUN30/MAL63. Cells were grown in minimal media lacking the appropriate nutrients for plasmid selection with 3% glycerol and 2% lactate plus 2% of various specified sugars. Maltase activities were assayed as described in Table 2.

**TABLE 9**

***GRR1*-dependent glucose inhibition of *MAL*-activator**

| Relevant genotype | Maltase activity | | |
|---|---|---|---|
| | + Glucose | + Maltose | + Maltose + Glucose |
| Wild type | 0.1 | 0.3 | 832 |
| *grr1*Δ | 13 | 1197 | 1691 |
| *grr1*Δ *rgt1*Δ | 1.4 | 49 | 619 |
| *grr1*Δ *rgt1*Δ [pLexA-MAL63] | 3.0 | 142 | ND |
| *rgt2*Δ | 0.2 | 2.6 | 447 |

*GRR1* and *RGT2* were disrupted in wild-type strain CMY1001 carrying a *MAL13* gene (Jiang *et al.* 1997). *RGT1* was disrupted in CMY1005 (*grr1*Δ). Plasmid pSH2-1/MAL63, a *HIS3* vector carrying a *LexA-MAL63* fusion gene under the control of the *ADH1* promoter, was transformed into strain CMY1010 (*grr1*Δ *rgt1*Δ). Cells were grown in rich media or synthetic media minus histidine with glycerol/lactate plus 2% of specified sugar for maltase assay. ND, not determined.

the inability of maltose to induce *MAL* gene expression in *snf1*Δ *mig1*Δ strains is due to the block in synthesis of maltose permease. In uninduced cells, the low basal level of maltose permease must play an essential role in induction.

## DISCUSSION

Although *MAL63* transcription is repressed by Mig1p, maltose induction of *MAL* gene expression is still inhibited by glucose in *mig1*Δ strains (Hu *et al.* 1995). We refer to this Mig1p-independent regulatory mechanism as glucose inhibition of maltose induction. It does not result from residual effects of Mig2p (Table 2).

Overexpression of *LexA-MAL63* fails to overcome glucose inhibition. It is unlikely that the ability of the overexpressed LexA-Mal63 fusion protein to bind to DNA is affected directly by glucose, because LexA-MAL63 fusion protein binds to the LexA operators regardless of carbon sources (Z. Hu, unpublished data). Also, abundant Mal63p is able to compete with Mig1p for binding to their adjacent binding sites in the *MAL61-MAL62* promoter, suggesting that overexpressed Mal63p is able to bind to $UAS_{MAL}$ in the presence of glucose (Wang *et al.* 1997). Thus, we favor the view that glucose inhibition does not affect nuclear entry or DNA-binding of the *MAL*-activator, but instead acts on some other function required for induction such as maltose sensing/signaling.

**TABLE 10**

**Effect of an *RGT2-1* allele on maltase expression**

| *MAL*-activator allele | Relevant genotype | Maltase activity | |
|---|---|---|---|
| | | G/L alone | + Maltose |
| *MAL13* | *RGT2* | 27 | 832 |
| | *RGT2* (p*RGT2-1*) | 24 | 562 |
| *MAL63* | *RGT2* | 26 | 742 |
| | *RGT2* (p*RGT2-1*) | 30 | 410 |

Plasmid pBM3270 carrying an *RGT2-1* allele (obtained from M. Johnston) was transformed into strain CMY1001 containing *MAL13* or strain YPH500 carrying a *MAL63* plasmid. Cells were grown in rich media or synthetic media minus uracil or uracil and tryptophan plus glycerol/lactate with or without 2% maltose. Maltase activities were assayed as described in Table 2.

**The relationship between maltose permease and glucose inhibition of maltose induction:** *MAL*-activator-mediated maltose induction requires the presence of a functional maltose permease gene (Charron *et al.* 1986; Dubin 1987; X. Wang and C. A. Michels, unpublished results). It has been suggested that this role is to transport the inducer, maltose, into the cell, thereby making it available for *MAL*-activator stimulation. It is equally possible that maltose permease plays the role of a "maltose sensor" and signals the presence of extracellular maltose via an intracellular signaling pathway, as Snf3p and Rgt2p do for glucose (Ozcan *et al.* 1996a). If this were the case, changes in maltose transport activity would not necessarily correlate with changes in glucose inhibition sensitivity. This is what we see.

The expression level of maltose permease required for induction is remarkably low. Several *LexA-MAL63* mutant fusions activate the transcription of the *lexO-LacZ* reporter even in strains capable of expressing only the extremely low basal level of maltose transport activity. Moreover, raising maltose transport activity significantly above this basal level does not relieve glucose inhibition of the LexA-$Mal63_{(\Delta 41\text{-}66)}$ activator. This is evident from our finding that overexpression of maltose permease does not relieve glucose inhibition (Tables 3 and 4). Additionally, glucose-induced inactivation of maltose permease, which inhibits maltose transport activity and increases the degradation rate of maltose permease protein, does not contribute significantly to the glucose inhibition of maltose induction. Strains carrying

**TABLE 11**

**Effect of *SNF1* mutation on the expression of the *MAL* genes and the maltose fermentation enzymes**

| Relevant genotype | *MAL*-activator allele | Maltase activity[a] | β-Galactosidase activity[a] | Maltose transport activity |
|---|---|---|---|---|
| *SNF1 mig1*Δ | *MAL63* | 299 | 182 | 9.4 |
| *snf1*Δ *mig1*Δ | *MAL63* | 19 | 47 | <0.5 |
| *SNF1 mig1*Δ | *MAL63/43-c* | 798 | 338 | 22.5 |
| *snf1*Δ *mig1*Δ | *MAL63/43-c* | 658 | 710 | 0.8 |
| *snf1*Δ *mig1*Δ [pUN70/MAL61] | *MAL63/43-c* | 1469 | ND | 4.3 |

Strains PS5959-6B (*SNF1 mig1*Δ) and CMY4001 (*snf1*Δ *mig1*Δ) were transformed with plasmid pUN30/MAL63 carrying the inducible *MAL*-activator gene *MAL63* or plasmid pUN30/MAL63/43-c carrying the constitutive *MAL*-activator gene *MAL63/43-c*. Cells were grown in selective synthetic medium containing 0.1% glucose to mid-log phase and then were transferred to the same selective synthetic medium containing 2% maltose for 6 hr before the assays were done. Plasmid pUN70/MAL61 carries the maltose permease gene *MAL61* on the CEN vector pUN70 (Elledge and Davis 1988).

[a] A plasmid carrying the *MAL61pro-LacZ* reporter gene was introduced to the indicated strains for this assay.

a disruption of *HXK2* are significantly relieved of glucose inhibition of maltose induction, yet these strains maintain almost normal rates of glucose-induced inactivation of maltose permease (Figure 1).

Thus, even without inducer exclusion and downregulation of *MAL*-activator protein levels, glucose inhibits maltose induction. Moreover, the results reported here support the possibility that maltose permease has a signaling function in *MAL* gene induction. However, we cannot exclude the possibility that the combination of both glucose repression of maltose permease transcription and glucose-induced inactivation of maltose permease protein can act synergistically to functionally eliminate maltose permease completely in cells grown on glucose plus maltose.

Our findings indicate that it is unlikely that the N-terminal half of the *MAL*-activator containing the DNA-binding and transcription activation domains is a direct target of glucose inhibition. Two constitutive activators lacking the C-terminal maltose-regulatory domain but possessing the DNA-binding and transcriptional activation domains are insensitive to glucose inhibition (Hu *et al.* 1995; Z. Hu, unpublished results). Furthermore, the full-length constitutive mutations of *MAL23* and *MAL43*, as well as those of *MAL63* (when their expression is not repressed by Mig1p), all become resistant to glucose inhibition (Charron and Michels 1987; Wang and Needleman 1996; Gibson *et al.* 1997). Taken together, these findings suggest a correlation between maltose inducibility and glucose inhibition sensitivity.

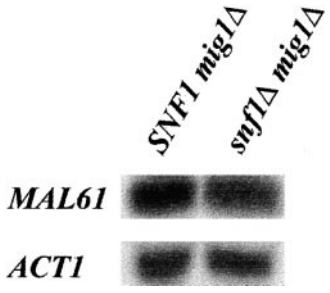


Figure 2.—Maltose permease mRNA expression in *snf1 mig1* mutant strains. Northern blot analysis was carried out on strains PS5959-6B (*SNF1 mig1*Δ) and CMY4001 (*snf1*Δ *mig1*Δ) transformed with plasmid pUN30-MAL63/43-c carrying the constitutive *MAL*-activator gene *MAL63/43-c* according to the procedures in materials and methods. Cells were grown as described in Table 11. PCR-amplified *MAL61* ORF was used as the probe for maltose permease mRNA and normalized to the level of *ACT1* mRNA using plasmid pYact1 as probe (Ng and Abelson 1980).

**The glucose inhibition pathway shares components with the glucose repression pathway and is distinct from the Rgt2p-dependent glucose induction pathway:** The results reported here demonstrate that several upstream negative regulators in the glucose repression pathway are also components of the glucose inhibition signaling pathway, including Hxk2p, Reg1p, and Gsf1p (reviewed in Johnston 1999). *HXK2* is proposed to act at an early, possibly signal-generating step in the glucose repression pathway. The nature of the glucose signal controlling glucose repression is unknown, but, based on the homology between components of the Snf1 kinase complex and the mammalian AMP-activated protein kinase, the AMP/ATP ratio is a possible candidate (Woods *et al.* 1994; Wilson *et al.* 1996). *HXK2* is the predominant hexokinase in glucose-grown Saccharomyces cells and acts as a gatekeeper regulating the rate of glucose fermentation and thus, indirectly, the AMP/ATP ratio. Our results indicate that the signal generated by hexokinase regulates glucose inhibition of maltose induction as well as glucose repression. Deletion of *HXK2* allows for some, but not full, maltose induction of maltase expression in the presence of glucose (Table 5). This residual glucose inhibition is relieved by deletion of *HXK1* (Table 6), deletion of *MIG1* (Table 5), or overexpression of the *MAL*-activator (Table 3). Thus, a glucose signal is still

generated in the absence of Hxk2p by Hxk1 hexokinase, and this reduced signal is able to repress expression of certain *MAL*-activator genes via Mig1p. In summary, hexokinase, particularly Hxk2p, is an important early negative regulator of glucose inhibition.

*REG1* encodes a glucose-repression-responsive regulatory subunit of protein phosphatase type-1 (Tu and Carlson 1995; reviewed in Johnston 1999). Interaction between Reg1p and the catalytic subunit of protein phosphatase type-1, Glc7p, is stimulated in high glucose conditions (Tu and Carlson 1995). Reg1p is believed to target Glc7p phosphatase activity to Snf1p, altering Snf1p- and Snf4p-binding and thereby blocking Snf1 kinase activation (Ludin *et al.* 1998). We show that loss of Reg1p fully relieves both glucose repression of *MAL* gene expression and glucose inhibition of maltose induction. Thus, Reg1p is a component of both signaling pathways.

Sherwood and Carlson (1997) suggest that Gsf1p may act in conjunction with Reg1p to stimulate or target Glc7 phosphatase activity toward the Snf1 kinase. Indeed, physical interaction of Snf1p and Reg1p has been reported (Ludin *et al.* 1998). Alternately, Gsf1p could be a substrate of a Reg1-Glc7 phosphatase. Our results are consistent with these reports. We find that *gsf1-1* mutant strains exhibit a relief of glucose inhibition similar to that of *reg1*Δ strains (compare Tables 7 and 8) but the extent is more modest, which could reflect the possibility that *gsf1-1* is a partial loss-of-function allele. Nevertheless, deregulation of Snf1 kinase activity appears to correlate with the release of glucose inhibition of maltose induction in the *gsf1-1* strain.

Glucose-induced expression of *HXT1* is mediated by the high-glucose sensor Rgt2p and is dependent upon Grr1p (Ozcan *et al.* 1996a,b). Our data indicate that Grr1p is involved in the glucose inhibition of maltose induction, but not Rgt2p or the glucose induction pathway. Disruption of *RGT1* in *grr1*Δ strain fully restores glucose transport rate and glucose repression of maltase expression, but only partially restores glucose inhibition, as evidenced by the partial induction of maltase expression in maltose/glucose medium (Table 9). Additionally, loss of Rgt2p has no significant effect on glucose inhibition sensitivity (Table 9), nor does introduction of the dominant constitutive signaling allele *RGT2-1* cause glucose inhibition (Table 10). We conclude from these results that the role of Grr1p in mediating glucose inhibition of *MAL*-activator induction is probably indirect, and is in addition to and distinct from its role in the inactivation of the Rgt1 repressor. Results reported elsewhere indicate that loss of Grr1p leads to reduced levels of Reg1 protein (Jiang *et al.* 2000b; F. Li and M. Johnston, unpublished results). This decreased Reg1p is likely to be the basis of the *GRR1*-mediated effect on glucose inhibition of maltose induction. Thus, we do not suggest that Grr1p is directly involved in glucose inhibition.

**Snf1 protein kinase is required post-transcriptionally for the synthesis of maltose permease:** Snf1p is known to directly regulate transcription factors such as Mig1p and Sip4p (Celenza and Carlson 1986; Vallier and Carlson 1994; Devit *et al.* 1997; Vincent and Carlson 1998). Two targets of AMP-activated protein kinase, the mammalian homologue of Snf1 protein kinase, are HMG-CoA reductase and acetyl-CoA carboxylase, key enzymes in sterol biosynthesis (reviewed in Hardie and Carling 1997). Similarly in Saccharomyces, Snf1 kinase may activate acetyl-CoA carboxylase in glucose-derepressed conditions (Woods *et al.* 1994). Our results suggest the possibility that Snf1p might have broader function in yeast.

*SNF1* is required for maltose fermentation. If the only role of Snf1 kinase in *MAL* gene expression were to inhibit Mig1 repressor in low-glucose growth conditions, *snf1 mig1* double-mutant strains should be able to induce *MAL* structural gene expression and ferment maltose. They cannot. Expression of the maltose fermentation enzymes is still blocked in the *snf1 mig1* strain carrying the inducible *MAL63 MAL*-activator (Table 11 and Figure 2). In contrast, the *snf1*Δ *mig1*Δ *MAL*-activator constitutive and *SNF1 mig1*Δ *MAL*-activator constitutive strains transcribe the *MAL* structural genes at comparable rates. Taken together, these results indicate that Snf1p is required for maltose sensing/signaling. We found that despite the constitutive expression of the maltose permease gene, maltose transport activity is not expressed. This clearly suggests that Snf1 kinase plays a post-transcriptional role in maltose permease synthesis, and, since maltose permease is essential for maltose sensing/signaling, *snf1* mutant strains are not maltose inducible. One can conceive of several points in the maltose permease synthesis where Snf1p might act, including translation of maltose permease mRNA, transit of nascent maltose permease through the secretory pathway, stability of the nascent protein during this process, or its maturation to an active integral membrane protein. Moreover, Snf1p need not act directly on maltose permease synthesis but could regulate the expression or activity of other functions required for this process. The nature of this role of Snf1 kinase is currently under investigation.

**Mechanism of glucose inhibition of maltose induction:** We have uncovered a novel mechanism by which glucose inhibits maltose induction of the *MAL*-activator. Snf1 kinase is a key factor in glucose inhibition, as it is in glucose repression, but in this case Snf1p is necessary for the synthesis of maltose permease as opposed to the inactivation of Mig1 repressor. Thus, Snf1 kinase is the branchpoint of the glucose repression and glucose inhibition pathways. Upstream of Snf1 kinase the glucose sensing/signaling pathways controlling glucose repression and glucose inhibition appear to be the same since both utilize *HXK2*, *REG1*, and *GSF1* as negative regulators. One of the major unresolved questions in Sacchar-

omyces glucose repression is whether Snf1p, Reg1p, or both respond directly to the glucose signal. Our results suggest that Reg1p or factors controlling the Reg1p-Glc7p interaction respond directly to high-glucose growth conditions which, in turn, regulates Snf1 kinase activity. If instead, or in addition, the Snf1 kinase complex were the direct target of the glucose signal, loss of Reg1p would not be so effective in eliminating glucose inhibition and glucose repression of *MAL* gene induction.

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