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Intracellular Maltose Is Sufficient To Induce *MAL* Gene Expression in *Saccharomyces cerevisiae*

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The presence of maltose induces *MAL* gene expression in *Saccharomyces* cells, but little is known about how maltose is sensed. Strains with all maltose permease genes deleted are unable to induce *MAL* gene expression. In this study, we examined the role of maltose permease in maltose sensing by substituting a heterologous transporter for the native maltose permease. *PmSUC2* encodes a sucrose transporter from the dicot plant *Plantago major* that exhibits no significant sequence homology to maltose permease. When expressed in *Saccharomyces cerevisiae*, PmSUC2 is capable of transporting maltose, albeit at a reduced rate. We showed that introduction of *PmSUC2* restores maltose-inducible *MAL* gene expression to a maltose permease-null mutant and that this induction requires the *MAL* activator. These data indicate that intracellular maltose is sufficient to induce *MAL* gene expression independently of the mechanism of maltose transport. By using strains expressing defective *mal61* mutant alleles, we demonstrated a correlation between the rate of maltose transport and the level of the induction, which is particularly evident in medium containing very limiting concentrations of maltose. Moreover, our results indicate that a rather low concentration of intracellular maltose is needed to trigger *MAL* gene expression. We also showed that constitutive overexpression of either *MAL61* maltose permease or *PmSUC2* suppresses the noninducible phenotype of a defective *mal13 MAL*-activator allele, suggesting that this suppression is solely a function of maltose transport activity and is not specific to the sequence of the permease. Our studies indicate that maltose permease does not function as the maltose sensor in *S. cerevisiae*.

Saccharomyces cerevisiae responds to the presence of a wide variety of environmental nutrients via sensing and signaling pathways capable of identifying the nutrients, determining their approximate concentrations, and integrating the information from these several signals to regulate gene expression and cell growth, proliferation, and morphology. Few nutrient or metabolite sensors have been identified despite extensive efforts in a variety of regulated systems. For the most part, those nutrient sensors identified to date fall into two categories: integral membrane receptor-like proteins and cytoplasmic nutrient/metabolite-binding proteins. Reports suggesting a dual role for certain nutrient transporters as both transporters and sensors have been presented, but conclusive evidence for such, in the form of constitutive alleles of the transporter genes, is lacking (20).

We are interested in identifying the *Saccharomyces* maltose sensor. Previous studies demonstrated that maltose permease plays an essential role in maltose induction of *MAL* gene expression (5). Moreover, the ability of an α-glucoside sugar to serve as an inducer of *MAL* gene expression appears to be dependent on the substrate specificity of the transporter (15). These results suggested the possibility that the *Saccharomyces* maltose permease can also play the role of the maltose sensor.

Five nearly identical *MAL* loci have been identified in *S. cerevisiae*, each located at a telomere-associated site: *MAL1* (chromosome *VII*), *MAL2* (chromosome *III*), *MAL3* (chromosome *II*), *MAL4* (chromosome *XI*), and *MAL6* (chromosome *VIII*) (7). Different maltose-fermenting strains carry at least one of these fully functional alleles, but often two or more loci are present in a strain (27). A typical *MAL* locus is a cluster of three genes, all of which are required for maltose fermentation. Gene 1 encodes maltose permease, a member of the 12-transmembrane domain family of sugar transporters; gene 2 encodes maltase, an α-glucoside hydrolase; and gene 3 encodes the *MAL*-activator, a DNA-binding transcription activator of the *MAL* genes (7). Genetic nomenclature uses both the locus number and the gene number. For example, *MAL61* encodes maltose permease at the *MAL6* locus. Natural variants of *MAL1* and *MAL3* containing nonfunctional alleles of gene 1 or gene 3 have been identified in strains from the wild and in common laboratory strains (6, 26). Induction of the *MAL* structural genes requires an inducer, usually maltose, but certain other α-glucosides will also act as inducers in strains encoding an appropriate transport protein, such as maltose permease, and the *MAL*-activator (5, 15). Does maltose permease function simply for the accumulation of intracellular maltose, whose presence is then monitored by some other mechanisms, or is maltose permease itself a maltose sensor capable of responding to extracellular maltose by initiating an intracellular signal?

Integral membrane proteins, particularly transporter-like homologues, are known to be utilized as sensors (34). Well-studied examples are Snf3p and Rgt2p, integral membrane receptors that sense, respectively, low and high extracellular concentrations of glucose (18). The Snf3 and Rgt2 proteins are

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structural and sequence homologues of the Hxt glucose transporters but are distinguished from the Hxt proteins by the presence of a long C-terminal cytoplasmic domain. Dominant gain-of-function mutations of *SNF3* and *RGT2* cause constitutive expression of the *HXT* genes. Moreover, the *HXT* genes cannot restore the signaling defect of *SNF3*- or *RGT2*-null mutations, and neither Snf3p nor Rgt2p is able to function as a glucose transport protein. Thus, although transporter like, Snf3p and Rgt2p appear to serve solely as glucose sensors (18).

Additional members of the sugar transporter superfamily with unique structural features similar to those of Snf3p and Rgt2p have been identified as putative sugar sensors in other eukaryotes. The Rco3 protein, a regulator of conidiation in *Neurospora crassa* (22), and the Mst1 protein from *Amanita muscaria* (28) appear to act as glucose sensors. *Arabidopsis SUT2* has been proposed to encode a sucrose sensor in sieve element cells (1).

Utilization of transporter-like proteins as nutrient sensors is not unique to sugars. The *Saccharomyces* Ssy1 protein is a member of the large superfamily of amino acid permeases but is distinguishable from these permeases by its elongated N-terminal cytoplasmic domain (17). This cytoplasmic extension is reminiscent of the C-terminal domain of Snf3p and Rgt2p, but no shared sequence homology has been identified. Results suggest that the Ssy1 protein functions as a sensor of extracellular amino acids, including leucine, isoleucine, and tryptophan. Similar to Snf3p and Rgt2p, Ssy1p requires the F-box protein Grr1p as a downstream effector in the amino acid signaling pathway.

Recent reports suggest that members of the G protein-coupled receptor class of proteins also function as glucose receptors in fungi. *Saccharomyces GPR1* encodes a member of the seven-transmembrane domain family of G protein-coupled receptors that includes Ste2p and Ste3p, the mating type pheromone receptors (9, 10, 19, 21, 38). In conjunction with its G protein alpha subunit Gpa2p, Gpr1p regulates pseudohyphal differentiation in response to glucose. The *git3* gene of *Schizosaccharomyces pombe* encodes another member of the G protein-coupled receptor family responsible for monitoring of extracellular glucose (36). Interestingly, both the *Saccharomyces GPR1*-dependent pathway and the *S. pombe git3*-dependent pathway regulate the activity of cyclic-AMP-dependent protein kinase, in particular, the *Saccharomyces* Tpk2p isoform (21, 36, 39).

S. cerevisiae also utilizes intracellular nutrient sensors. The Snf1 protein kinase signaling pathway responds to high rates of glucose metabolism, possibly by monitoring changes in the ATP/AMP ratio produced by rapid glycolysis (4). Snf1 protein kinase is the catalytic subunit of a large protein complex that exhibits homology to mammalian AMP-activated protein kinases. It has been proposed that this kinase complex is the metabolite sensor, but this remains to be demonstrated in *S. cerevisiae.* The *Saccharomyces* Gal3 protein, a homologue of galactokinase (Gal1p), is the galactose sensor. Binding of galactose to Gal3p promotes Gal3p-Gal80p interaction, thereby releasing the Gal4p transcription activation domain from Gal80p repression (33). Recent reports indicate that the Gal3p-Gal80p interaction occurs in the cytoplasm exclusively and effectively shifts the subcellular localization of Gal80p from the nucleus to the cytoplasm in the presence of galactose (30).

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Reference
CMY1001	*MAT***a** *MAL61/HA MAL12 MAL13 GAL leu2 ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-200*	23
CMY1050	*mal61Δ::HIS3* (isogenic to CMY1001)	24
CMY1061	*mal61Δ::HIS3 mal13Δ::G418* (isogenic to CMY1001)	This study
YPH500	*MATα leu2-Δ1 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 ura3-52 AGT1 MAL12 mal13 MAL31 MAL32 mal33*	32
CMY1071	*mal13Δ::G418* (isogenic to YPH500)	This study
CMY1072	*mal33Δ::HygB* (isogenic to YPH500)	This study
CMY1073	*mal13Δ::G418 mal33Δ::HygB* (isogenic to YPH500)	This study

In the context of these studies on nutrient sensing in *Saccharomyces* and other fungi, we proposed to explore the role of the maltose transport protein, maltose permease, in the regulation of *MAL* gene expression. Our approach to resolving questions regarding the role of maltose permease in maltose sensing is based on the assumption that, if maltose permease were also to serve as a maltose sensor, this function would require specialized sequence features of maltose permease protein that could not be replaced by a heterologous protein capable of maltose transport. For this study, we chose to use the high-affinity sucrose transporter from *Plantago major* encoded by *PmSUC2* (13). The PmSUC2 protein is a member of the 12-transmembrane domain superfamily of sugar transporters, and although it exhibits little sequence homology with the *Saccharomyces* maltose permease, it is capable of transporting maltose, albeit at lower affinity. We report here that expression of PmSUC2 in *Saccharomyces* restores maltose-inducible *MAL* gene expression to maltose permease-null mutants. Moreover, by other parameters explored in this study, we show that the PmSUC2 transporter is able to fully replace maltose permease as a regulator of *MAL* gene expression.

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MATERIALS AND METHODS

Yeast strains. The strains used in this study are listed in Table 1. CMY1001 has been described by Medintz et al. (23). It contains a single *MAL1* locus at which the *MAL11* maltose permease gene is replaced by hemagglutinin (HA)-tagged *MAL61*. No other *MAL* genes are present in this strain. Strain CMY1050 is a *MAL61/HA*-null derivative of CMY1001 and was constructed by the PCR-based gene disruption described by Medintz et al. (24). Strain CMY1061 is a *MAL13* deletion disruption of CMY1050 and was constructed as follows. The appropriate upstream (5′-CCATGTAATCGCTGCATTCAGCGCAATTTGAACTGCACTCAGCTGAAGCTTCGTACGC) and downstream (5′-CGGTGCAAACAATAGTATGTCATGATTCGAAATATGTCGGCATAGGCCACTAGTGGATCTG) primers were used to amplify the G418 resistance marker gene with pFA2-kanMX2 as the template (35). Bases homologous to the template are underlined. The resulting PCR product, which has homology to the *MAL13* sequence at both the 5′ and 3′ ends of the open reading frame, was then used for one-step replacement of *MAL13*. Candidate disruptants were confirmed by PCR analysis.

A similar PCR-based process was used to construct CMY1071 (*mal13Δ::G418*), CMY1072 (*mal33Δ::HygB*), and CMY1073 (*mal13Δ::G418 mal33Δ::HygB*) in strain YPH500. Strain YPH500 is isogenic to S288C, and

TABLE 2. Effect of *PmSUC2* on expression of *MAL* genes[a]

Relevant *MAL* genotype (strain)	Plasmid	Maltose transport activity[b]		Maltase activity[c]	
		G/L	G/L + M	0 h	6 h
MAL61 MAL12 MAL13 (CMY1001)	Vector	0.17	5.61	48	576
*mal61*Δ *MAL12 MAL13* (CMY1050)	Vector	0.09	0.11	21	20
	pPTE18 (*PmSUC2*)	0.68	ND[d]	27	706
	pADH1-MAL61/HA	5.98	ND	26	643
*mal61*Δ *MAL12 mal13*Δ (CMY1061)	Vector	0.11	ND	27	21
	pPTE18 (*PmSUC2*)	0.56	ND	12	25
	pADH1-MAL61/HA	6.10	ND	12	18

[a] Strains CMY1050 (*MAL61*Δ::*HIS3*) and CMY1061 (*MAL611*Δ::*HIS3 MAL13*Δ::*G418*) were transformed with plasmid pUN70, pPTE18 (*PmSUC2*), or pADH1-MAL61. Maltose transport activity was determined in early mid-log-phase cells grown in selective medium containing 3% glycerol and 2% lactate (G/L) or G/L plus 2% maltose (G/L + M). For maltase activity, cells were grown to early mid-log phase in selective synthetic medium containing G/L and then transferred to G/L + M. Maltase activity was determined at the time of transfer to maltose (0 h) and 6 h after the transfer. Maltase activity and transport activity were assayed as described in Materials and Methods. Experimental variation for maltose transport activity was <20%, and that for maltase activity was <30%.

[b] Maltose transport activity is expressed as nanomoles of maltose transported per milligram of cells per minute.

[c] Maltase activity is expressed as nanomoles of PNPG hydrolyzed per milligram of total protein per minute.

[d] ND, not determined.

primers for the deletion of *mal13* and *mal33* were determined on the basis of the sequence of S288C, which is available at the *Saccharomyces* Genome Database website (http://genome-www.stanford.edu/Saccharomyces/). The primers 5′-ACTTTAACTAAGCAAACATGCGCCAAGCAGGCATGCGACTGCTGTCGATCAGCTGAAGCTTCGTACGC and 5′-ATCAAGGGTCTATGTCTTCATTATCCTTGGGATAACCATCCAATTGTAAAGCATAGGCCACTAGTGGAT were used to amplify *G418*^{R} for *mal13* disruption. Primers 5′-ACTTTAGTCAAGTATGCATGCGACTATTGTCGTGTCCGTCGAGTAAAGTGCAGCTGAAGCTTCGTACGC and 5′-AGGAATTATGTCGTCTTCATCTTTGGAATCATCATTTAGGCGCAGTGGTCGCATAGGCCACTAGTGGAT were used to amplify *Hyg*^{R} for *mal33* disruption.

Plasmid construction and mutagenesis. *MAL61/HA* was constructed from *MAL61* by inserting a 12-codon sequence containing a single of copy of the HA epitope tag at the 5′ end of the open reading frame (23). *MAL61/HA* was subcloned into pUN30, yielding pMAL61/HA. The native *MAL61* promoter in pMAL61/HA was removed and replaced with the *ADH1* promoter at the −12-bp position of *MAL61*, yielding pADH1-MAL61/HA, which has been described by Medintz et al. (24). Plasmids pMAL61/HA and pADH1-MAL61/HA were used as templates for in vitro mutagenesis with a Bio-Rad Mutagene Kit (Bio-Rad, Richmond, Calif.) to construct pMAL61/HA(Δ61-90) (25), pMAL61/HA(Δ571-580), pADH1-MAL61/HA(Δ61-90), pADH1-MAL61/HA(Δ571-580), and a series of C-terminal nonsense mutations (pMAL61/HA-581NS, pMAL61/HA-575NS, pMAL61/HA-570NS, and pMAL61/HA-560NS).

Plasmid pPTE18 was obtained from Norbert Sauer, University of Regensburg, Regensburg, Germany. *PmSUC2* cDNA encoding a *P. major* sucrose transporter was ligated into the *Eco*RI site of vector NEV-E, yielding pPTE18 (13). Expression of *PmSUC2* is controlled by the *S. cerevisiae PMA1* promoter.

Maltose transport assay. Maltose transport was measured as the rate of uptake of 1 mM [^{14}C]maltose as described by Cheng and Michels (8) and Medintz et al. (23). Assays were done in duplicate on two or three transformants. V_{max} was determined by Lineweaver-Burk analysis as described by Medintz et al. (23). Maltose transport activity and V_{max} are expressed as nanomoles of maltose transported per milligram of cells per minute.

Maltase assay. Maltase activity was determined in total cell extracts as described by Dubin et al. (11). Activity is expressed as nanomoles of *p*-nitrophenyl β-D-glucoside (PNPG) hydrolyzed per milligram of total protein per minute. The values reported are averages of duplicate assays obtained with extracts from at least two separate cultures.

Western blot analysis. Western blot analysis was carried out as described previously (23). Mal61/HA protein was detected by using anti-HA-specific antibody, the Vistra-ECF kit (Amersham), and the Storm 860 image analyzer (Molecular Dynamics). Relative protein levels were determined by using the Storm 860 image capture software. Western blot analysis was done in duplicate on extracts prepared from duplicate experiments carried out with at least two independent transformants.

RESULTS

PmSUC2 suppresses the noninducible phenotype of a maltose permease deletion. CMY1001 is a maltose-fermenting strain containing *MAL1* as the sole *MAL* locus (23). The *MAL1* locus of CMY1001 encodes maltose permease (*MAL61/HA*), maltase (*MAL12*), and the *MAL* activator (*MAL13*). Charron et al. (5) showed that loss of either maltose permease or the *MAL* activator blocks maltose induction of *MAL* gene expression but loss of maltase has no obvious effect on induction. *MAL61/HA* was deleted from strain CMY1001 to create CMY1050 (*mal61*Δ*::HIS3*), and maltose induction of maltase expression was determined (Table 2). Under uninduced conditions (3% glycerol, 2% lactate), CMY1001 expresses a low but significant level of maltose transport activity (0.17 nmol/min/mg [dry weight] of cells). Deletion of the maltose permease gene reduces this rate to a background rate of 0.09 nmol/min/mg that probably represents the nonspecific low-affinity binding activity described by Benito and Lagunas (2) and not true transport. Table 2 also confirms that maltose induction is dependent on maltose permease. A 6-h induction period is sufficient for full induction of maltase expression in CMY1001, while permease deletion strain CMY1050 exhibits no induction during the same period.

PmSUC2 encodes a sucrose transporter from *P. major* that is a member of the 12-transmembrane domain family of sugar transporters (13). Blast analysis comparing the amino acid sequence of PmSUC2 to that of Mal61p indicates that these transporters do not share significant sequence homology (only 10% identity largely in transmembrane domains). When expressed in *Saccharomyces*, PmSUC2 was found to be capable of transporting sucrose and this sucrose transport was inhibited by maltose, indicating that PmSUC2 could also be capable of transporting maltose (13). Plasmid pPTE18, which carries the *PmSUC2* gene under the control of the constitutive *Saccharomyces PMA1* promoter, was introduced into maltose permease deletion strain CMY1050, and the ability to support maltose induction was tested. As shown in Table 2, growth in glycerol-lactate allows expression of *PmSUC2* and produces a low but

TABLE 3. Maltose induction of *MAL* gene expression in strains carrying *MAL61/HA*(Δ*61–90*)[a]

MAL61-HA allele	Maltose fermentation[b]	V_{max}[c]	Maltose transport activity[c]	Maltase activity[d]
MAL61/HA	+	7.14	5.96	1,652
mal61/HA(Δ*61–90*)	−	0.21	0.07	320
ADH1-mal61/HA(Δ*61–90*)	−	1.42	0.21	463
Vector control	−	0.08	0.06	35

[a] Strain CMY1050 was transformed with a plasmid carrying the indicated *MAL61/HA* allele in vector pUN30 (12). Cells were grown in selective induced medium with 3% glycerol, 2% lactate, and 2% maltose to mid-log phase. Maltase activity and maltose transport activity were determined as described in Materials and Methods. Fermentation was determined as growth in rich medium (YP) + Maltose medium plus the production of gas within 7 days. V_{max} was calculated by Lineweaver-Burk plotting of maltose transport activity over a range of substrate concentrations as described by Medintz et al. (23).

[b] +, maltose fermented; −, no maltose fermented.

[c] Expressed as nanomoles of maltose transported per milligram of cells per minute.

[d] Expressed as nanomoles of PNPG hydrolyzed per milligram of total protein per minute.

TABLE 4. Maltose induction of *MAL* gene expression in strains carrying mutations in the C-terminal cytoplasmic domain of Mal61/HA maltose permease[a]

MAL61/HA allele	Maltose fermentation[b]	Maltase activity[c]	Maltose transport activity[d]	Relative level of Mal61/HA protein
MAL61/HA	+	2,231	5.45	100
MAL61/HA-581NS	+	2,117	2.68	79
MAL61/HA-575NS	+	1,582	0.63	41
MAL61/HA-570NS	−	69	0.06	<1
MAL61/HA-560NS	−	77	0.06	<1
MAL61/HA (Δ*571–580*)	−	702	0.31	32
Vector control	−	42	0.06	<1

[a] Strain CMY1050 was transformed with a plasmid carrying the indicated *MAL61/HA* allele in vector pUN30. Cells were grown in selective induced medium with 3% glycerol, 2% lactate, and 2% maltose to mid-log phase. Fermentation was determined as described in Table 3, footnote *a*. Maltase activity and maltose transport activity were determined as described in Materials and Methods. The relative levels of Mal61/HA permease protein were determined by comparison of Western blots using total cell extracts from cells grown under maltose-induced conditions.

[b] +, maltose fermented; −, maltose not fermented.

[c] Expressed as nanomoles of PNPG hydrolyzed per milligram of total protein per minute.

[d] Expressed as nanomoles of maltose transported per milligram of cells per minute.

significant level of maltose transport activity. The maltose transport rate in cells expressing *PMA1promoter-PmSUC2* is approximately 10-fold lower than that in cells carrying *ADH1-MAL61*, but this reduced transport rate is nevertheless sufficient to cause wild-type levels of maltase induction. CMY1050 carrying constitutively expressed *ADH1promoter-MAL61* is presented as a control.

To test whether PmSUC2-mediated induction is dependent on the *MAL*-activator, *MAL13* was deleted from CMY1050, creating CMY1061 (*mal61*Δ *mal13*Δ) and maltose induction of maltase was assayed in transformants constitutively expressing either Mal61p or PmSUC2. The results in Table 2 demonstrate that loss of the *MAL13* activator gene blocks maltose-induced maltase expression by both the PmSUC2 transporter and Mal61/HA permease. Thus, *PmSUC2*-dependent maltose induction requires the *MAL*-activator. Taken together, theses results indicate that intracellular maltose is sufficient to induce *MAL* gene expression independently of the mechanism of maltose transport and that induction by intracellular maltose is dependent on the *MAL*-activator.

The concentration of intracellular maltose correlates with the level of the induction. Medintz et al. (24) constructed a series of deletions of the N-terminal cytoplasmic domain of Mal61/HA permease as part of a study done to localize the target site of glucose-induced endocytosis and proteolysis. One interesting mutation, a deletion of residues 61 to 90, exhibited little or no transport activity but nonetheless was able to induce *MAL* gene expression. Further analysis of this mutant is shown in Table 3. The maltose transport activity of strains expressing *mal61/HA*(Δ*61-90*) from the native promoter, when measured at a substrate concentration of 1 mM maltose (standard assay condition), does not differ significantly from that of the vector control. Measurement of V_{max} by Lineweaver-Burk analysis found an approximately threefold increase in V_{max} compared to that of the vector control, and this suggests that the low level of maltose induction exhibited by this strain could be a function of this very low rate of maltose transport (Table 3). The K_m of the Mal61/HA(Δ61-90) permease is unaffected (approximately 1.1 mM for both alleles). Expression of *mal61/HA*(Δ*61-90*) from the constitutive *ADH1* promoter allowed a modest increase in maltose transport activity and a slight increase in maltase induction levels. These activities were still too low to allow rapid maltose fermentation. As a result, the strain grows slowly on maltose as the sole carbon source and growth is not associated with carbon dioxide bubble formation.

An additional series of *MAL61/HA* mutants containing deletions of the C-terminal cytoplasmic domain were constructed by creation of a translation stop site at codon 560, 570, 575, or 581 and by removal of codons 571 to 580 as described in Materials and Methods. Plasmid-borne copies of these mutant alleles were transformed into strain CMY1050, and the transformants were tested for the ability to induce maltase activity. The results are shown in Table 4. Also shown are the levels of maltose transport activity and permease protein expressed by the transformants. A clear correlation can be observed between the levels of maltose transport activity and maltase induction. Additionally, residues 571 to 580 appear to play an important role in maltose transport activity but not an essential role in induction. Truncation of Mal61p to residue 580 has only a modest effect on transport activity, while truncation to residue 574 or 559 or deletion of residues 571 to 580 severely reduces or eliminates transport (Table 4). Deletion of residues 571 to 580 reduces transport activity almost 20-fold, but maltase induction is reduced to about one-third of the wild-type level (Table 4). The results reported in Tables 3 and 4 suggest that extremely low levels of intracellular maltose are able to trigger *MAL* gene induction and that, at these limiting intracellular maltose concentrations, the level of induction correlates with the presumed level of intracellular maltose.

Low rates of maltose transport activity cause a delay in induction by very low extracellular concentrations of maltose. The finding that, at very low levels of maltose transport activity, induction correlates with transport rates suggests that the threshold level of intracellular maltose needed to induce the *MAL* genes is very low. If a strain expressing *mal61/HA*(Δ*61-*

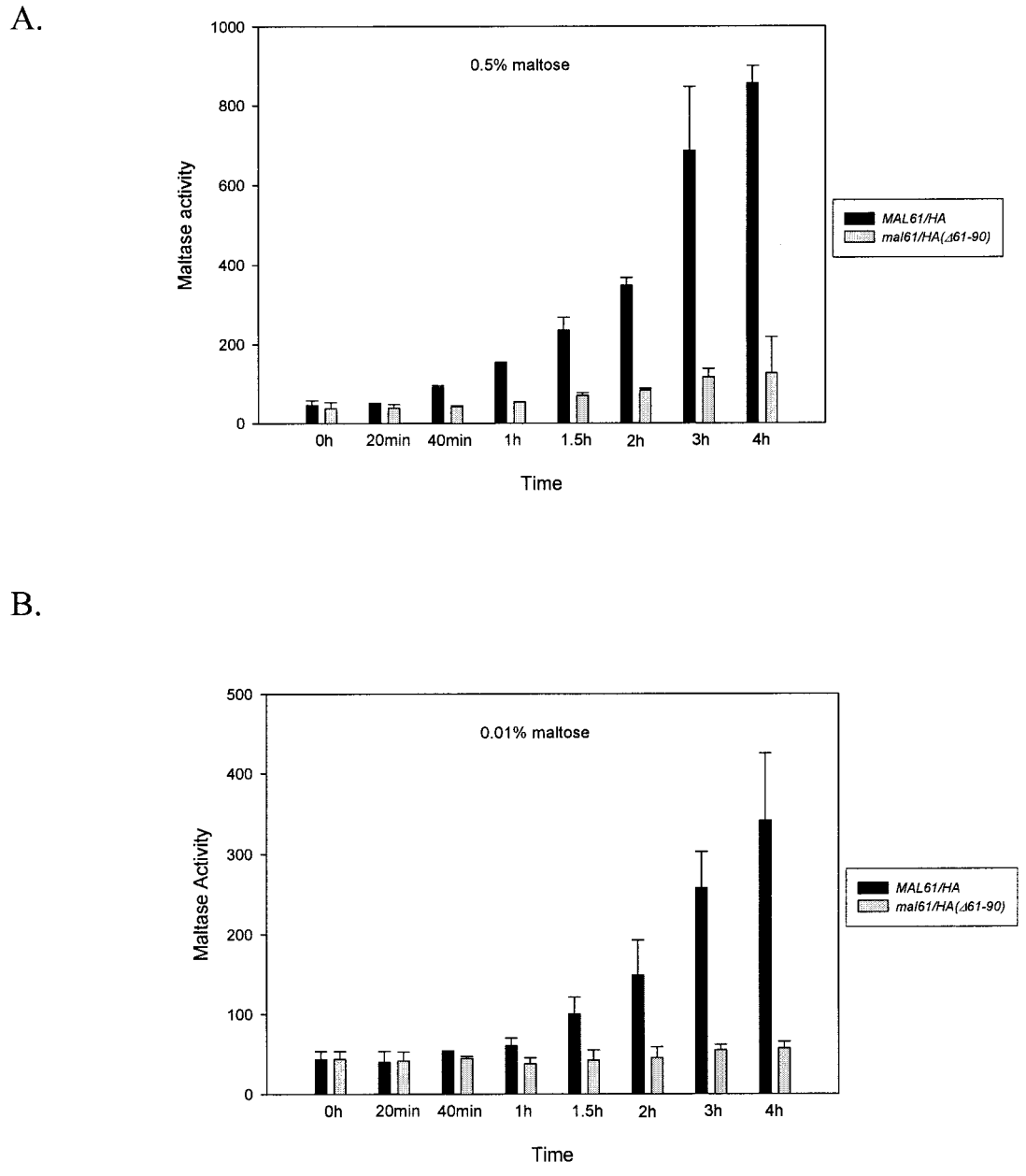

FIG. 1. Time course of maltase induction in strains expressing either *MAL61*/*HA* or *mal61*/*HA*(Δ*61-90*) maltose permease. CMY1050 strains expressing either *mal61*/*HA*(Δ*61-90*) or *MAL61*/*HA* were grown to early log phase under uninduced conditions (3% glycerol and 2% lactate) and transferred to inducing medium containing 3% glycerol–2% lactate plus either 0.5% (13.9 mM) (A) or 0.01% (0.3 mM) (B) maltose. Cells were collected at the indicated time points, and maltase activity was assayed as described in Materials and Methods. The data represent the averages and standard deviations of results obtained in at least two independent assays for cultures grown under both conditions.

90) from its native promoter were grown in medium containing a very low concentration of maltose, we would expect a delay in induction because greater time would be needed to accumulate sufficient intracellular maltose to reach this threshold level. We wished to test this prediction. Strains expressing either *mal61*/*HA*(Δ*61-90*) or *MAL61*/*HA* were grown to early log phase under uninduced conditions and transferred to medium containing either 0.5 or 0.01% maltose. The time courses of maltase induction at these two concentrations are compared in Fig. 1. When transferred to medium containing 0.5% maltose, the strain expressing wild-type maltose permease initiates induction at approximately 30 min while induction is delayed to about 1 to 1.5 h in the strain expressing mutant permease. Moreover, the rate of increase in maltase activity is far more rapid in cells expressing wild-type permease. In contrast, when the time course of induction is followed in medium containing 0.01% maltose, induction in the wild-type strain still initiates at about 30 min, although the rate of increase in maltase activity is slowed, but no significant induction is observed in the strain expressing mutant maltose permease, even after 4 h. These

TABLE 5. Constitutive *MAL61* expression suppresses the defective *mal13 MAL* activator but not *mal33* of strain YPH500[a]

MAL activator alleles	Plasmid	Maltase activity[b]	
		G/L	G/L + maltose
mal13 mal33 [*MAL63*]	Vector	40	1,177
mal13 mal33 [*MAL63*]	pADH1-MAL61/HA	37	1,522
mal13 mal33	Vector	50	129
mal13 mal33	pADH1-MAL61/HA	16	1,180
*mal13*Δ *mal33*	Vector	30	38
*mal13*Δ *mal33*	pADH1-MAL61/HA	56	97
*mal13 mal33*Δ	Vector	30	53
*mal13 mal33*Δ	pADH1-MAL61/HA	42	1,240
*mal13*Δ *mal33*Δ	Vector	38	32
*mal13*Δ *mal33*Δ	pADH1-MAL61/HA	12	134

[a] Strain YPH500 and isogenic disruptions of defective *mal13* (CMY1071), *mal33* (CMY1072), and both *mal13* and *mal33* (CMY1073) were transformed with plasmid pADH1-MAL61/HA or the vector control pUN30. As a control, YPH500 was also transformed with the functional *MAL*-activator *MAL63* carried in vector pUN70 (lines 1 and 2). Cells were grown in selective uninduced (3% glycerol and 2% lactate) and induced (3% glycerol, 2% lactate, and 2% maltose) media to mid-log phase. Maltase activity was assayed as described in Materials and Methods.

[b] Expressed as nanomoles of PNPG hydrolyzed per milligram of total protein per minute.

TABLE 6. Constitutive *PmSUC2* suppresses the defective *MAL* activator genes of strain YPH500[a]

Plasmid	Maltose transport activity[b]	Maltase activity[c]	
		G/L	G/L + maltose
Vector	0.08	50	129
pADH1-MAL61/HA	1.98	16	1,180
pADH1-mal61/HA(Δ61–90)	0.34	16	290
pPTE18 (*PmSUC2*)	0.50	38	525

[a] Strain YPH500 was transformed with the indicated plasmids that constitutively express *MAL61, mal61/HA*(*Δ61–90*), or *PmSUC2*. Transformants were grown in selective uninduced (3% glycerol and 2% lactate) and induced (3% glycerol, 2% lactate, and 2% maltose) media to mid-log phase. Maltase and maltose transport activities were assayed as described in Materials and Methods. Maltose transport activity is reported for cells grown under induced conditions.

[b] Expressed as nanomoles of maltose transported per milligram of cells per minute.

[c] Expressed as nanomoles of PNPG hydrolyzed per milligram of total protein per minute.

results are consistent with the proposal that a threshold level of intracellular maltose is needed in order to trigger *MAL* gene induction and that this threshold level is rather low.

Overexpression of Mal61 maltose permease rescues the defect in *MAL* induction caused by the *mal13*, but not the *mal33*, mutant activator. Strain YPH500, which is essentially isogenic to S288C, contains two *MAL* loci mapping to the right telomeres of chromosomes *VII* (*MAL1*) and *II* (*MAL3*) but is unable to ferment maltose and is defective for maltose induction (Table 5, line 3). This induction defect is complemented by a plasmid-borne copy of *MAL63* encoding the *MAL*-activator from *MAL6* (Table 5, line 1), indicating that the noninducible phenotype of YPH500 results from a lack of a functional *MAL* activator. The amino acid sequences of the defective mal13p and mal33p *MAL* activators encoded by YPH500 are 70.7 and 71.2% identical to Mal63p, respectively.

As part of a separate study of the *MAL* activator, we introduced a plasmid carrying the *ADH1promoter-MAL61* gene into YPH500. Much to our surprise, we found that constitutive high-level expression of Mal61 permease is able to restore maltose inducibility of maltase expression to wild-type levels in strain YPH500 despite its defective *MAL*-activators (Table 5, lines 1, 2, and 4).

To determine whether rescue by *ADH1promoter-MAL61/HA* is dependent on *mal13*, *mal33*, or both, single and double deletions of both genes were constructed in strain YPH500, yielding CMY1071, CMY1072, and CMY1073, and maltose induction of maltase was assayed. Loss of *mal13* blocked the suppressing effect of constitutive Mal61 permease (Table 5, lines 6 and 10). The strain lacking only *mal33* was still rescued by overexpressed Mal61 permease, as well as strain YPH500 (Table 5, line 8). These results indicate that constitutive, high-level Mal61 permease expression rescues the induction defect caused by *mal13* but not that caused by *mal33*.

Rescue of the defective *mal13 MAL*-activator is not specifically dependent on Mal61 maltose permease. The results shown in Table 5 may be interpreted in two ways. First, if the maltose permease protein itself were to play a direct role in induction, simple restoration of maltose transport activity by an unrelated transport protein such as PmSUC2 permease should not be able to rescue the defective *mal13 MAL*-activator. An example of such is the direct binding of the *Escherichia coli* MalT transcription activator to the maltose transport complex ($MalEFGK_2$) (29). Alternatively, if rapid delivery of an initially very high concentration of intracellular maltose were capable of triggering *MAL* gene induction by the defective *mal13 MAL*-activator, different permeases with varied transport activities should vary in the ability to rescue *mal13*. Thus, even the *PmSUC2*-encoded transporter should be capable of rescuing *mal13* despite its lack of sequence homology to Mal61 permease. To test this, plasmids constitutively expressing different versions of maltose permease genes [*MAL61/HA*, *mal61/HA*(*Δ61-90*), and *PmSUC2*] were introduced into YPH500 and maltase induction was assayed. The results in Table 6 indicate that PmSUC2 permease is able to rescue the defective *mal13 MAL*-activator. Moreover, the significantly reduced level of maltose transport by PmSUC2 has a relatively modest impact on *mal13* suppression, suggesting that the suppression is solely a function of the maltose transport activity of PmSUC2 permease and is unrelated to any specific sequence feature of this protein.

DISCUSSION

Intracellular maltose is sufficient to induce *MAL* gene expression. We provide several lines of evidence demonstrating that intracellular maltose is sufficient to induce *MAL* gene expression in *Saccharomyces*. PmSUC2, a sucrose transporter from *P. major*, was used as a surrogate for the *Saccharomyces* maltose permease to transport maltose into a strain lacking the native maltose permease. Blast analysis shows that PmSUC2 has no significant sequence homology to the Mal61 and Agt1 maltose permeases. Sequence identity among these transport-

ers is about 10% and is largely confined to the transmembrane domains. Given the lack of significant sequence homology, we feel confident in concluding that PmSUC2 is only functioning as a maltose transporter in our *Saccharomyces* strains. The finding that PmSUC2 is able to replace the *Saccharomyces* maltose permease therefore provides strong evidence that accumulation of intracellular maltose concentrations is sufficient to stimulate *MAL* gene induction. Our results do not exclude the possibility that the true inducer is a metabolite of maltose and not maltose itself, but we believe this to be unlikely.

The level of intracellular maltose required to initiate induction and to maintain the induced state appears to be quite low. Strains expressing defective maltose permeases exhibit a significant reduction in their maximal induced levels of maltase activity (Tables 3 and 4), and induction correlates well with the reduced rate of maltose transport capacity of these strains. Moreover, very low levels of maltose transport cause a delay in maltase induction when extracellular maltose concentrations are limiting. Both findings suggest that accumulation of intracellular maltose to a threshold level is needed to initiate the induction and that the threshold level is rather low.

Maltose permease does not appear to function as a maltose sensor. Demonstrating that intracellular maltose is sufficient to stimulate *MAL* gene induction does not exclude the possibility that the *Saccharomyces* maltose permease also has a sensor-like role in induction. Our finding that constitutive high-level expression of Mal61 maltose permease suppresses the defective *mal13*-encoded *MAL*-activator suggested a possible role in induction for maltose permease similar to that of the *E. coli* maltose transport complex in the bacterial maltose utilization system. In the *E. coli* maltose-maltodextrin system, uptake is mediated by a periplasmic binding protein-dependent ABC transporter (the $MalEFGK_2$ complex). MalT, a transcriptional activator, is sequestered at the plasma membrane in the inactive monomeric form by binding to the idling transporter complex via MalK. Transport of maltose by the transporter complex releases MalT from MalK-MalT interaction, enabling it to form active oligomeric MalT in the presence of the effectors ATP and maltotriose (3).

One possible mechanism by which constitutive Mal61 permease expression could suppress the defective *mal13* gene is specific binding of the activator. Under uninduced growth conditions, the permease might sequester the *MAL*-activator at the plasma membrane and thereby prevent the *MAL*-activator from entering the nucleus and activating *MAL* gene transcription. Conformational changes in maltose permease induced by maltose transport might destabilize the putative interaction between maltose permease and the *MAL*-activator, resulting in *MAL*-activator release, nuclear entry, and *MAL* gene activation. Binding of the defective *mal13* activator to the constitutive elevated levels of maltose permease might protect it from degradation or other forms of inactivation, thereby suppressing this mutant form of the activator.

Our finding that constitutive expression of *PmSUC2* also suppresses the defective *mal13 MAL*-activator argues against the specific-binding model and supports a second model. In this model, the large bolus of maltose transported into the cell via the abundant maltose permease suppresses the defect of mal13p, suggesting that the defect of the mutant activator protein is in its ability to respond to the maltose signal.

On the basis of these results, we propose that maltose permease serves solely as a maltose transporter. It accumulates intracellular concentrations of maltose to levels sufficient to both induce *MAL* gene expression and provide an energy source for growth. In cells grown under uninduced conditions, the basal low level of maltose permease expression is sufficient to transport enough maltose into the cell to allow the accumulation of intracellular maltose to levels that are adequate to promote the activation of the *MAL* activator and induce further structural gene expression. Wykoff and O'Shea (37) similarly concluded that the phosphate transporter Pho84p, which is essential during phosphate starvation, does not serve as the phosphate sensor and suggested that the intracellular phosphate concentration regulates *PHO* gene expression.

So, what is the maltose sensor? One likely possibility is that the *MAL* activator itself binds to maltose directly. In work to be reported elsewhere, we have shown that maltase induction is dependent on the Hsp90 molecular chaperone complex (B. Zhang, M. Bali, K. Morano, and C. A. Michels, unpublished data). Several other transcriptional activators are known to associate with Hsp90 (16). Members of the steroid receptor family of activators are released from the chaperone complex following direct binding of the cognate steroid (31). Similarly, Hap1 activator release from the Hsp90 chaperone complex is dependent on heme binding (14, 40). An alternate possibility is that a Gal3-like protein serves as the maltose sensor. We do not favor this hypothesis because, despite extensive genetic analysis, specific maltose-nonfermenting mutants with mutations in genes unlinked to the *MAL* loci have not been isolated. Currently, we are using genetic approaches to identify the maltose-binding sensor.

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