Altered Regulatory Responses to Glucose Are Associated With a Glucose Transport Defect in *grr1* Mutants of *Saccharomyces cerevisiae*

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Manuscript received October 14, 1993
Accepted for publication December 27, 1993

ABSTRACT

The *GRR1* gene of *Saccharomyces cerevisiae* affects glucose repression, cell morphology, divalent cation transport and other processes. We present a kinetic analysis showing that the *grr1* mutant is also defective in high affinity glucose transport. In combination with a mutation in *SNF3*, a member of the glucose transporter gene family, *grr1* strikingly impairs growth on glucose. These findings suggest that *GRR1* and *SNF3* affect glucose transport by distinct pathways. The mutation *rgt1-1*, a suppressor of *snf3*, restores both glucose transport and glucose repression to a *grr1* mutant, but does not remedy the morphological defect. We suggest that *GRR1* affects the glucose sensing process and that the association between transport and regulation may reflect the involvement of a transporter in glucose sensing.

THE *GRR1* gene of *Saccharomyces cerevisiae* affects responses to nutrient availability and other environmental conditions. Mutations in *GRR1* were identified as relieving glucose repression of the *GAL* genes and were shown also to cause glucose-resistant expression of the *SUC2* and *MAL* genes (BAILEY and WOODWORD 1984). Subsequent studies revealed a more complex effect on *SUC2* regulation. In *grr1* mutants *SUC2* (invertase) gene expression requires glucose; that is, expression is induced, rather than repressed, by glucose (FLICK and JOHNSTON 1991; VALLIER and CARLSON 1991). As a result, *grr1* mutants are defective in growth on raffinose, a substrate of invertase. Mutations in *GRR1* cause a variety of other pleiotropic defects, including severely elongated cell morphology, slow growth on glucose, increased sensitivity to osmotic stress and nitrogen starvation, decreased divalent cation transport, and growth defects in aromatic amino acid auxotrophs (BAILEY and WOODWORD 1984; FLICK and JOHNSTON 1991; VALLIER and CARLSON 1991; CONKLIN *et al.* 1993). *GRR1* is the same (cited in SCHULLER and ENTIAN 1991) as *CAT80* (ENTIAN and ZIMMERMANN 1980) and *COT1* (CONKLIN *et al.* 1993). The *GRR1* gene was cloned and encodes a 132-kD protein containing twelve tandem leucine-rich repeats, which have been proposed to mediate protein-protein interactions (FLICK and JOHNSTON 1991; CONKLIN *et al.* 1993).

We previously proposed two models to explain the puzzling effects of a *grr1* mutation on *SUC2* regulation: the conversion from glucose repression to glucose induction (VALLIER and CARLSON 1991). First, the *grr1* mutation could alter the regulatory pathway controlling *SUC2* expression in such a way as to invert the polarity of the response. Alternatively, *grr1* could relieve glucose repression of *SUC2* and thereby expose a secondary layer of regulation, a glucose-inducible response. This idea is supported by recent evidence that glucose is required, albeit at very low levels, for maximal expression of *SUC2* in wild-type (*GRR1*) strains under nonrepressing conditions (VALLIER 1993; J. FLICK and M. JOHNSTON, personal communication). Physiologically, this glucose dependence could ensure that high level invertase expression is maintained only in the presence of suitable substrates, which upon hydrolysis yield glucose (or fructose). The *grr1* mutant differs from wild type in requiring much higher glucose levels for *SUC2* induction.

Here we explore the possibility that a primary defect in glucose transport in *grr1* mutants could relieve glucose repression and unmask a glucose-inducible regulatory response. Our rationale was that a defect in glucose transport could reduce the amount of glucose effectively available to the cell and thereby shift the apparent response curve. That is, a *grr1* mutant could fail to recognize that glucose is abundant, due to a defect in transport, and therefore respond as though glucose were limiting. This would result in glucose-insensitive expression for the *GAL* and *MAL* genes and glucose-dependent expression for the *SUC2* gene, which requires low amounts of glucose for expression.

To test this idea, we first carried out a kinetic analysis of glucose transport in *grr1* mutants. Yeast cells express both low and high affinity components of glucose transport (BISSON and FRAENKEL 1983). When glucose is abundant, the high affinity component is repressed (BISSON and FRAENKEL 1984). A family of genes that affect glucose transport has been identified and includes *SNF3* and

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TABLE 1

Strains used in this study

Strain	Genotype
MCY1093	*MATa his4-539 lys2-801 ura3-52 SUC2*
MCY1094	*MATα ura3-52 ade2-101 SUC2*
MCY1407	*MATa snf3Δ4::HIS3 his3Δ200 ura3-52 lys2-801 SUC2*
MCY1409	*MATα snf3Δ4::HIS3 his3Δ200 ura3-52 lys2-801 SUC2*
MCY1516	*MATα snf3Δ4::HIS3 rgt1-1 ura3-52 ade2-101 SUC2*
MCY1705	*MATa ssn6Δ5::URA3 ura3-52 his4-539 lys2-801 SUC2*
MCY1744	*MATa hxk2Δ::URA3 ura3-52 his4-539 lys2-801 SUC2*
MCY1803	*MATa his4-539 lys2-801 ura3-52::pLS11-URA3 SUC2*
MCY1846	*MATa snf1Δ10 ura3-52 lys2-801 SUC2*
MCY2157	*MATα RGT2-1 lys2-801 ura3-52 his3Δ200 SUC2*
MCY2218	*MATα grr1-512 ura3-52 lys2-801 ade2-101 SUC2*
MCY2270	*MATa grr1Δ::URA3 ura3-52 his3Δ200 lys2-801 met⁻ SUC2*
MCY2348	*MATα grr1Δ::URA3 snf3Δ4::HIS3 ura3-52 his3Δ200 lys2-801 ade2-101 met⁻ SUC2*
MCY2349	*MATa snf3Δ4::HIS3 grr1Δ::URA3 his3Δ200 ura3-52 lys2-801 ade2-101 SUC2*
MCY2476	*MATα grr1Δ::URA3 rgt1-1 ura3-52 his3Δ200 lys2-801 SUC2*
MCY2479	*MATα rgt1-1 ura3-52 his3Δ200 lys2-801 ade2-101 SUC2*
MCY2481	*MATα grr1Δ::URA3 snf3Δ4::HIS3 rgt1-1 ura3-52 his3Δ200 lys2-801 met⁻ SUC2*
MCY2605	*MATα grr1Δ::URA3 ura3-52 lys2-801 ade2-101 SUC2*
MCY2616	*MATa cid1-226 ura3-52 lys2-801 his3Δ200 trp1Δ1 SUC2*
MCY3332	*MATa grr1Δ::URA3 ura3-52 lys2-801 ade2-101 his4-539 SUC2*
MCY3346	*MATa grr1Δ::URA3 ura3-52 lys2-801 ade2-101 SUC2*
LV153.2A	*MATα grr1-512 his4-539 leu2-3,112 ade2-101 SUC2*
LV206.10C	*MATa snf3Δ4::HIS3 rgt1-1 ura3-52 his3Δ200 SUC2*
LV206.16B	*MATα grr1Δ::URA3 ura3-52 his3Δ200 ade2-101 SUC2*
YM3502[a]	*MATα grr1Δ::URA3 ura3-52 his3Δ200 ade2-101 lys2-801 met⁻ SUC2*

All strains have the S288C genetic background and except where indicated were constructed in this laboratory.
[a] Obtained from MARK JOHNSTON.

HXT1 through *HXT4* (CELENZA *et al.* 1988; KRUCKEBERG and BISSON 1990; LEWIS and BISSON 1991; KO *et al.* 1993). All of these genes encode proteins resembling known glucose transporters, but their roles are not yet clear. Two additional genes have also been implicated in glucose transport or its regulation: recessive *rgt1* and dominant *RGT2* mutations restore glucose transport to a *snf3* mutant (MARSHALL-CARLSON *et al.* 1991).

We show that *grr1* mutants are deficient in the high affinity component of glucose uptake. We then examine the relationship between this defect and the altered regulatory response to glucose in *grr1* mutants. We show that *rgt1-1* restores both glucose transport and glucose repression of *SUC2* to a *grr1* mutant but does not remedy the morphological defect. Finally, analysis of the interactions of *grr1* with other mutations affecting *SUC2* expression provides evidence that *GRR1* does not alter the *SUC2* regulatory pathway, except in the initial sensing of glucose.

MATERIALS AND METHODS

Yeast strains: *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. The *grr1Δ::URA3* allele (FLICK and JOHNSTON 1991) was derived from strain YM3502. Double mutant strains were obtained by tetrad analysis of crosses between YM3502 or MCY2605 (*grr1Δ*) and appropriate mutant strains. The *grr1-512* mutation (VALLIER and CARLSON 1991) was followed by its elongated cell morphology and growth defect on raffinose (Raf⁻ phenotype). The *snf1Δ10* (CELENZA and CARLSON 1989), *snf2Δ1::HIS3* (ABRAMS *et al.* 1986), *snf4Δ2* (CELENZA *et al.* 1989), and *snf5Δ2* (LAURENT *et al.* 1990) alleles were scored by their defect in glycerol utilization. The *hxk2Δ::URA3* mutation in strain MCY1744 was created by using the 2.7-kb *Eco*RI fragment from plasmid pRB528 (H. MA and D. BOTSTEIN, personal communication) to replace the chromosomal copy of *HXK2* in the wild-type strain MCY1093. *grr1Δ::URA3 hxk2Δ::URA3* double mutants were recovered from a nonparental ditype tetrad (2 Ura⁺:2 Ura⁻). The *cid1-226* mutation (NEIGEBORN and CARLSON 1987) was followed in crosses by complementation of *cid1* tester strains for growth on medium containing sucrose plus 2-deoxyglucose. The *ssn6Δ5::URA3* mutation (SCHULTZ and CARLSON 1987) was identified by clumpiness and failure to grow on glycerol. The *rgt1-1* allele was followed by suppression of the raffinose growth defect caused by *snf3*. The genotype of MCY2157 (*RGT2-1*) was confirmed by crossing to MCY2349 (*grr1Δ::URA3 snf3Δ4::HIS3*); *snf3Δ* segregants with a Raf⁺ phenotype were recovered.

General genetic methods and media: Standard genetic procedures were employed (ROSE *et al.* 1990). Utilization of glucose, sucrose, and raffinose was scored by spotting cell suspensions onto solid medium containing 1% yeast extract, 2% Bacto-peptone (YEP), 1 μg/ml antimycin A (Sigma) and 2% glucose or 2% raffinose; alternatively, cell suspensions were spotted on YEP medium containing 2% sugar and incubated in GasPak disposable anaerobic systems (BBL). Resistance to 2-deoxyglucose was tested on YEP medium containing 2% sucrose and 200 μg/ml 2-deoxyglucose.

Invertase assays: Cells were grown to mid-log phase (Δ Klett 50) in YEP-2% glucose or YEP-2% galactose, washed and resuspended in cold 10 mM sodium azide. Secreted invertase was assayed (GOLDSTEIN and LAMPEN 1975) in whole cells as previously described (VALLIER and CARLSON 1991).

Uptake of labeled glucose: Mid-log phase cultures grown in YEP-2% glucose were divided and shifted to either YEP-2% glucose or YEP-0.05% glucose, supplemented with 20 mg/liter uracil and 30 mg/liter adenine, and allowed to grow for 3 or 2 hr, respectively, before assay. The uptake of radioactively

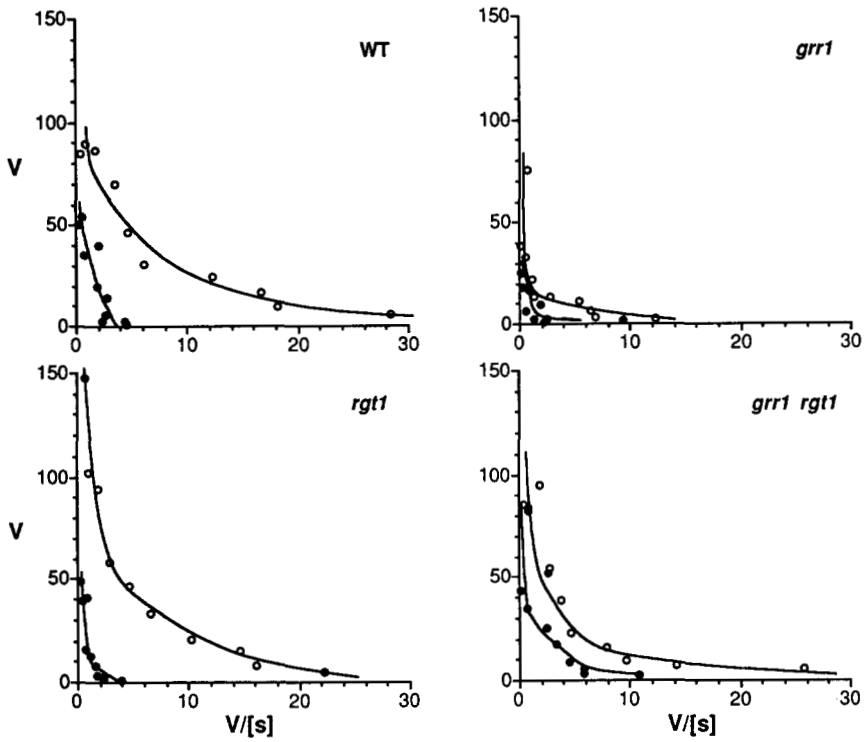

FIGURE 1.—Eadie-Hofstee plots of glucose uptake. Velocity (V) is expressed as nanomoles of glucose per minute per milligram (dry weight); $V/[S]$ is expressed as velocity per millimolar concentration. Cells were grown to mid-log phase in YEP-2% glucose and shifted to YEP-0.05% glucose (○) or YEP-2% glucose (●) medium and assayed as described in MATERIALS AND METHODS. Glucose concentrations ranged from 0.2 to 200 mM. Data are the average for two experiments. Strains assayed were MCY1093 (WT), MCY2605 (*grr1Δ::URA3*), MCY2479 (*rgt1-1*) and MCY2476 (*grr1Δ::URA3 rgt1-1*).

labeled glucose (D-[U-^{14}C]-glucose; DuPont-NEN) was measured as described previously (LEWIS and BISSON 1991).

RESULTS

***grr1* mutants are defective in high affinity glucose transport:** The kinetics of glucose uptake were examined in a *grr1Δ* mutant and wild type. Wild-type cells derepressed high-affinity uptake when shifted to medium with a low glucose concentration (0.05%) (Figure 1), as observed previously (BISSON and FRAENKEL 1983, 1984). In contrast, the *grr1Δ* mutant cells were deficient in the high affinity component of uptake, indicating that the *grr1* mutation impairs glucose transport. In this experiment, the low affinity component did not appear significantly different between the mutant and wild type. In assays of other *grr1* mutant strains, reduced low affinity transport was observed (D. COONS and L. F. BISSON, unpublished results); these differences may reflect subtle differences in strain backgrounds or variability in the assay. We also noted slower growth of a *grr1Δ* mutant on 2% glucose plates (see Figure 2).

***GRR1* and *SNF3* affect glucose transport by different pathways:** The *SNF3* gene encodes a protein that resembles other glucose transporters (CELENZA *et al.* 1988) and is required for high affinity glucose transport (BISSON *et al.* 1987). It is possible that SNF3 has a regulatory role in transport (KO *et al.* 1993). Previously, we reported that a *grr1* mutation does not impair expression of a bifunctional SNF3-β-galactosidase fusion protein or its localization to the cell membrane (VALLIER and CARLSON 1991). To examine further the functional relationship of *SNF3* and *GRR1*, we constructed *snf3Δ grr1Δ* double mutants by tetrad analysis of a cross between MCY1407 and YM3502. The double mutants were significantly less healthy on glucose than *snf3Δ* or *grr1Δ* single mutants (Figure 2). These findings suggest that *GRR1* affects glucose transport by a *SNF3*-independent mechanism.

Suppression of *grr1* growth and transport defects by the *rgt1-1* mutation: Previously, we isolated mutations in two genes, *RGT1* and *RGT2* (*r*estores *g*lucose *t*ransport), that suppressed the high-affinity glucose transport defect and associated growth defects of the *snf3Δ* mutant (MARSHALL-CARLSON *et al.* 1991). Mutations recovered in *RGT1* were recessive and those in *RGT2* were dominant. Because the *grr1* mutant is also defective in high affinity glucose transport, we asked whether these mutations could suppress the growth and glucose transport defects of a *grr1* mutant. Analysis of a cross between MCY2157 (*RGT2-1*) and MCY3346 (*grr1Δ::URA3*) showed 2+:2− segregations for growth on raffinose in seven tetrads. In addition, the cross MCY2157 (*RGT2-1*) by MCY2349 (*grr1Δ::URA3 snf3Δ4::HIS3*) yielded no Raf$^+$ *grr1Δ* segregant in seven tetrads. Because *RGT2-1* and *grr1* are unlinked (FLICK and JOHNSTON 1991; MARSHALL-CARLSON *et al.* 1991), these data indicate that *RGT2-1* does not suppress the raffinose growth defect caused by *grr1Δ*.

To examine the interaction of *rgt1* and *grr1*, we analyzed tetrads from a cross between LV206.10C (*snf3Δ4::HIS3 rgt1-1*) and LV206.16B (*grr1Δ::URA3*) (Table 2). Almost half of the *snf3*, *grr1* or *snf3 grr1* segregants from six tetrads displayed a Raf$^+$ phenotype, the result expected if *rgt1* suppresses both *snf3* and *grr1*,

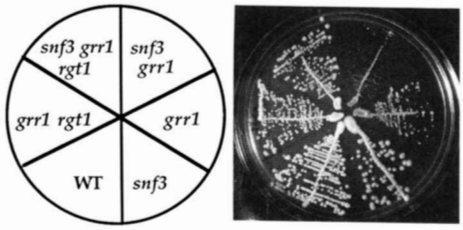

FIGURE 2.—The *rgt1* mutation restores growth on glucose to *grr1* mutants. Yeast strains were streaked onto YEP-2% glucose and incubated aerobically for 3 days at 30°. Strains shown are MCY1409, MCY2476, MCY2481, MCY2348, MCY3332 and MCY1093. WT, wild type.

TABLE 2

***rgt1-1* suppresses the raffinose growth defect of a *grr1Δ* mutant**

Relevant genotype	No. of segregants[a]	
	Raf$^+$	Raf$^-$
snf3Δ	1	2
grr1Δ	1	1
snf3Δ grr1Δ	5	5
SNF3 GRR1	9	0

[a] Tetrad analysis of LV206.10C (*snf3Δ4::HIS3 rgt1-1 his3 ura3*) crossed to LV206.16B (*grr1Δ::URA3 his3 ura3*).

alone or together; the segregation patterns in all tetrads were consistent with the segregation of a single suppressor mutation. In a similar cross, MCY1516 by MCY2270, which was heterozygous for *snf3Δ4::HIS3*, *grr1Δ::URA3* and *rgt1-1* but not homozygous for *his3*, 18 tetrads also showed segregation patterns consistent with suppression of *grr1* by *rgt1* (data not shown). These data indicate that *rgt1-1* suppresses the raffinose growth defect of *grr1* mutants. The *rgt1-1* mutation also alleviated the striking growth defect of the *snf3Δ grr1Δ* double mutants on glucose. The *snf3 grr1 rgt1* triple mutants grew nearly as well as *snf3* and wild-type strains (Figure 2). The *rgt1 grr1* double mutants also grew noticeably better than the *grr1* single mutant.

To test whether suppression of these growth defects reflects restoration of high affinity glucose transport, we carried out kinetic analysis of glucose uptake in the *grr1Δ rgt1-1* mutant (Figure 1). The *rgt1-1* mutation restored a component of high affinity uptake, although not to the wild-type level.

Thus, the *rgt1-1* mutation suppressed the glucose and raffinose growth defects and high affinity glucose transport defect in a *grr1* mutant. However, *rgt1-1* did not restore normal cell morphology to a *grr1* mutant, indicating that its effects are specific to a subset of the *grr1* mutant phenotypes. This specificity is consistent with the role of *rgt1* as a suppressor of the *snf3* glucose transport defect.

Glucose repression of *SUC2* is restored in *grr1 rgt1* double mutants: The ability of the *rgt1-1 grr1Δ* mutants to grow on raffinose suggested that *rgt1-1* suppresses the invertase regulation defect caused by *grr1Δ*. We therefore assayed invertase activity in *grr1Δ rgt1-1* double mutants after growth in YEP containing either 2% glucose or 2% galactose. The double mutants produced low level invertase activity in glucose and high level activity in galactose (Table 3); the YEP medium provides trace amounts of glucose. These mutants also showed normal derepression of invertase upon shifting to low (0.05%) glucose (data not shown). Therefore, the *rgt1* mutation restores glucose repression of *SUC2* in *grr1* mutants. These results suggest that the glucose-inducible invertase phenotype is associated with the glucose transport defect.

TABLE 3

***rgt1-1* restores glucose-repressible regulation of *SUC2* to *grr1Δ* mutants**

Relevant genotype	Invertase activity	
	Glucose	Galactose
rgt1-1	6	209
grr1Δ	124	5
grr1Δ rgt1-1	17	348

Invertase activity is expressed as micromoles of glucose released per minute per 100 mg (dry weight) of cells. Values are averages of two to six assays. Standard errors are less than 25% with one exception (35% for the *rgt1* mutant in glucose). Cultures were grown in YEP-2% glucose (Glucose) or YEP-2% galactose (Galactose).

The *grr1* mutation does not bypass the normal regulatory pathway: If the altered regulation of *SUC2* expression in a *grr1* mutant results from a defect in glucose transport, or another initial step in sensing or signaling glucose availability, then the various genes that are required for *SUC2* expression in wild type should still be important in a *grr1* mutant. On the other hand, if a *grr1* mutation alters the polarity of *SUC2* regulation by bypassing or perturbing the normal regulatory pathway, then the requirement for some of these genes might also be bypassed or altered. Expression of *SUC2* requires the *SNF1*, *SNF2*, *SNF4* and *SNF5* gene products (NEIGEBORN and CARLSON 1984). To test whether the glucose-inducible expression of *SUC2* in *grr1* mutants is still dependent on these *SNF* products, we constructed double mutants. Double mutants carrying *grr1Δ* and *snf2Δ*, *snf4Δ* or *snf5Δ* were readily recovered from appropriate crosses. None of these double mutants produced significant invertase activity when grown in glucose (data not shown).

We also crossed a *snf1Δ* strain (MCY1846) to a *grr1Δ::URA3* (YM3502) strain. Analysis of 19 tetrads yielded predominantly tetrads with two or three visible spore clones after one week at room temperature; no *snf1Δ grr1Δ* double mutant was recovered among a total of 49 healthy spore clones. After 3 weeks, several pinpoint-sized clones with inferred genotype *snf1Δ grr1Δ* were visible. These results are similar to those of FLICK and JOHNSTON (1991), who reported that *snf1Δ grr1Δ* double mutants were inviable.

TABLE 4

Invertase activity of double mutants

	Invertase activity			
	grr1Δ		*GRR1*	
Mutation	Glu	Gal	Glu	Gal
hxk2Δ	160	13	112	66
cid1-226	132	23	65	51
ssn6Δ5	75	804	493	1462
Wild type	102	19	<1	62

Invertase activity is expressed as micromoles of glucose released per minute per 100 mg (dry weight) of cells. Values represent averages of two to four assays. Standard errors were less than 25% (except 38% for the *cid1-226 grr1Δ* mutant grown in galactose). Cultures were grown in YEP-2% glucose (Glu) or YEP-2% galactose (Gal).

Because the *snf1Δ grr1Δ* double mutants were too unhealthy to assay for invertase activity, we next constructed *snf1Δ10 grr1-512* double mutants. Analysis of 11 tetrads from the cross MCY1846 × LV153.2A yielded, after germination for 12 days at room temperature, four tetrads containing three healthy spore clones and a small spore clone of the genotype *snf1Δ10 grr1-512*. Double mutant spore clones were subsequently capable of slow growth at 30°. These double mutants failed to express invertase when grown in glucose. Thus, the expression of invertase in *grr1* mutants requires each of the four *SNF* genes tested.

We also examined the interaction of *grr1* with mutations in the *HXK2* (hexokinase PII) and *CID1* genes, which relieve glucose repression of *SUC2* (MA and BOTSTEIN 1986; NEIGEBORN and CARLSON 1987). Both *grr1Δ hxk2Δ::URA3* and *grr1Δ cid1-226* double mutants still produced substantial invertase activity when grown in glucose and produced lower invertase activity than the *hxk2* or *cid1* single mutants when grown in galactose (Table 4). Thus, the double mutants resemble the *grr1* mutant with respect to its glucose-inducible phenotype and display no unexpected regulatory changes.

Mutations in *SSN6* relieve the glucose-inducible phenotype of *grr1* mutants: *SSN6* encodes a general transcriptional repressor, and mutations both relieve glucose repression and also allow invertase expression in *snf1* mutants (KELEHER *et al.* 1992; SCHULTZ and CARLSON 1987). We assessed the requirement of *SSN6* for the *grr1* glucose-inducible phenotype by constructing *grr1Δ ssn6Δ* double mutants. Both glucose- and galactose-grown double mutants expressed high level invertase activity, although activity in galactose was somewhat lower than for *ssn6* single mutants (Table 4). Thus, *ssn6* is epistatic to *grr1* with respect to *SUC2* regulation.

DISCUSSION

We present evidence that the *GRR1* gene has a major effect on glucose transport. First, kinetic analysis showed that the *grr1* mutant is defective in high affinity glucose transport. Second, in combination with a mutation in *SNF3*, a member of the glucose transporter gene family, a *grr1* mutation strikingly impairs growth on glucose: *snf3Δ grr1Δ* double mutants are much more unhealthy on glucose than either *snf3Δ* or *grr1Δ* single mutants. These results also suggest that *GRR1* and *SNF3* affect glucose transport by distinct pathways.

The basis for the high affinity glucose transport defect in *grr1* mutants is not clear. Previously we showed that a bifunctional SNF3-β-galactosidase fusion protein is expressed in a *grr1* mutant and localized to the cell membrane (VALLIER and CARLSON 1991). Preliminary evidence suggests that *GRR1* affects the expression or stability of high affinity transporters of the HXT family. A *grr1* mutant differs from the wild type in that levels of HXT2 protein do not increase in response to glucose limitation, as judged by immunoblot analysis using anti-HXT2 (D. L. WENDELL and L. F. BISSON, unpublished data).

The *RGT1* gene product could negatively regulate the expression or function of one or more high affinity glucose transporters, as proposed previously (MARSHALL-CARLSON *et al.* 1991). The genetic evidence suggests that *GRR1* is a negative regulator of the *RGT1* function. Consistent with this idea, *rgt1-1 grr1Δ* double mutants produced wild-type levels of HXT2 protein upon derepression (D. L. WENDELL and L. F. BISSON, unpublished data). Interestingly, ERICKSON and JOHNSTON (1994) directly selected for suppressors of *grr1* and recovered only *rgt1* alleles. We also note that *RGT1* may be the same as *HTR1*, which appears to encode a negative regulator of *HXT* gene expression (ÖZCAN *et al.* 1993).

Genetic evidence suggests a correlation between the defect in glucose transport and the aberrant regulatory responses to glucose observed in *grr1* mutants. The *rgt1-1* mutation not only restores glucose transport in *grr1* mutants but also restores glucose repression of *SUC2*. Although this correlation suggests a causative relation between glucose transport and regulatory defects, we cannot exclude other possibilities: for example, *grr1* could cause the two defects by separate mechanisms and *rgt1-1* independently remedies both. However, it is not the case that *rgt1-1* simply remedies all *grr1* defects, because the double mutant still exhibits elongated cell morphology.

A simple model is that in a *grr1* mutant, reduced capacity to transport glucose results in loss of glucose repression. It is not clear, however, that overall transport is reduced relative to wild type when *grr1* mutants are grown in high glucose. Although the mutant grew slower than wild type on 2% glucose (see Figure 2), the kinetic analysis was inconclusive. We note that the *grr1* and *snf3* mutations both impair high affinity glucose transport, but *snf3* null mutations do not affect invertase regulation (MARSHALL-CARLSON *et al.* 1990; NEIGEBORN *et al.*

1986). This difference is in accord with evidence that *GRR1* and *SNF3* affect glucose transport by distinct mechanisms, and it does not rule out this simple model.

Another model is that the *grr1* mutation impairs the expression or function of a protein that is responsible for sensing/signaling the availability of glucose. A defect in such a sensor or signaling protein could relieve glucose repression of many genes, such as *SUC2*, *GAL* and *MAL* genes. For *SUC2*, reduced ability to sense glucose could also unmask a glucose-inducible regulatory response. In wild type, high level *SUC2* expression under nonrepressing growth conditions is induced by trace amounts of glucose (VALLIER 1993; J. FLICK and M. JOHNSTON, personal communication). According to this model, because the glucose sensing process is impaired in *grr1* mutants, higher external concentrations of glucose would be needed to stimulate *SUC2* expression. We find this model attractive and further suggest that a member of the glucose transporter family could function as such a sensor or signaling protein.

Our evidence provides no support for the model that *grr1* perturbs the regulatory mechanism controlling *SUC2* expression, after the initial steps in glucose sensing, so as to invert the polarity of the response. All of the *SNF* genes tested (*SNF1*, *SNF2*, *SNF4* and *SNF5*) were still required for invertase expression in *grr1* mutants, and *grr1 hxk2* and *grr1 cid1* double mutants showed no unexpected phenotypes. The synthetic, nearly lethal, phenotype caused by combining mutations in *SNF1* and *GRR1* may reflect the important roles of both genes in glucose utilization. SNF1 is required for various aspects of the cell's response to glucose limitation, including derepression of high affinity glucose transport (BISSON 1988). An *ssn6* mutation caused high level constitutive invertase activity in a *grr1* background, suggesting that *ssn6* relieves all negative regulation of *SUC2*.

Additional evidence also supports the idea that a *grr1* mutant reveals a secondary regulatory response, glucose induction, for *SUC2*. Deletion of nucleotides −223 to −403 from the *SUC2* upstream region eliminates the glucose-inducible response in *grr1* mutants and allows glucose-insensitive *SUC2* expression (VALLIER 1993). This deletion does not substantially affect glucose repression of invertase in wild type (SAROKIN and CARLSON 1984). Thus, alteration of the *SUC2* locus causes *SUC2* to respond similarly to other glucose-repressed genes in a *grr1* mutant.

Besides their defects in glucose transport and glucose repression, *grr1* mutants exhibit a variety of pleiotropic phenotypes, including severely elongated cell morphology, defects in utilization of exogenous aromatic amino acids by auxotrophs, sensitivity to osmotic stress and nitrogen starvation, and defects in divalent cation transport (BAILEY and WOODWORD 1984; FLICK and JOHNSTON 1991; VALLIER and CARLSON 1991; CONKLIN *et al.* 1993). Many of these phenotypes could result from defects in different transport systems. Perhaps the GRR1 protein has a general role in expression of various transporters or in the functioning of transport processes.

We thank ARLE KRUCKEBERG for initially assaying glucose transport in the *grr1-512* mutant. We thank JEFF FLICK and MARK JOHNSTON for communicating unpublished results and for the *grr1Δ* strain, and HONG MA and DAVID BOTSTEIN for the *hxk2* disruption plasmid. We also thank a reviewer for pointing out the possible relationship of *HTR1* and *RGT1*. This work was supported by National Institutes of Health grant GM34095 to M.C. and Juvenile Diabetes Foundation Investigator grant 192223 to L.F.B.

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Communicating editor: E. W. JONES