# Dominant and Recessive Suppressors That Restore Glucose Transport in a Yeast *snf3* Mutant

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ABSTRACT

The *SNF3* gene of *Saccharomyces cerevisiae* encodes a high-affinity glucose transporter that is homologous to mammalian glucose transporters. To identify genes that are functionally related to *SNF3*, we selected for suppressors that remedy the growth defect of *snf3* mutants on low concentrations of glucose or fructose. We recovered 38 recessive mutations that fall into a single complementation group, designated *rgt1* (restores glucose transport). The *rgt1* mutations suppress a *snf3* null mutation and are not linked to *snf3*. A naturally occurring *rgt1* allele was identified in a laboratory strain. We also selected five dominant suppressors. At least two are tightly linked to one another and are designated *RGT2*. The *RGT2* locus was mapped 38 cM from *SNF3* on chromosome *IV*. Kinetic analysis of glucose uptake showed that the *rgt1* and *RGT2* suppressors restore glucose-repressible high-affinity glucose transport in a *snf3* mutant. These mutations identify genes that may regulate or encode additional glucose transport proteins.

THE transport of glucose into eukaryotic cells is mediated by specific carrier proteins. The genes encoding a variety of glucose transporters from mammalian cells have been sequenced, and many of the proteins are closely related, containing 12 putative membrane-spanning regions and conserved sequence motifs (MUECKLER *et al.* 1985; BIRNBAUM, HASPEL and ROSEN 1986; THORENS *et al.* 1988; FUKUMOTO *et al.* 1988; BIRNBAUM 1989; CHARRON *et al.* 1989; JAMES, STRUBE and MUECKLER 1989). Different mammalian transport systems are subject to different regulation; for example, some transporters are regulated in response to insulin (CHARRON *et al.* 1989; JAMES, STRUBE and MUECKLER 1989).

We have studied glucose transport in *Saccharomyces cerevisiae* with the view that genetic analysis should prove useful in studying a complex, highly regulated process that is essential to all eukaryotic cells. Like higher organisms, *S. cerevisiae* also appears to express multiple, differently regulated glucose transport systems. Kinetic analysis of glucose uptake in yeast has revealed at least two components, a high affinity component ($K_m$ ~1–2 mM) that is dependent on the presence of a cognate hexose kinase and a low affinity component ($K_m$ ~20–50 mM) (BISSON and FRAENKEL 1983; LANG and CIRILLO 1987). Both systems also transport fructose. The two components are differently regulated: the high-affinity system is repressed by glucose, and the low-affinity system is expressed constitutively (BISSON and FRAENKEL 1984).

A glucose transporter gene of *S. cerevisiae*, the *SNF3* gene, was first identified by isolating mutants defective in growth on sucrose or raffinose (NEIGEBORN and CARLSON 1984). These sugars are hydrolyzed extracellularly, and the resulting glucose and/or fructose, released at low concentration, must be transported into the cell. The mutants are also defective in growth on medium containing glucose at low concentration (NEIGEBORN and CARLSON 1984; NEIGEBORN *et al.* 1986). Kinetic analysis showed that the *snf3* mutants lack high-affinity glucose uptake, but exhibit normal low-affinity uptake (BISSON *et al.* 1987). The defect in high-affinity transport accounts for the growth phenotypes of *snf3* mutants.

The *SNF3* gene was cloned (NEIGEBORN *et al.* 1986) and encodes a 97-kilodalton protein, containing 12 putative membrane-spanning regions, that is homologous to mammalian glucose transporters (CELENZA, MARSHALL-CARLSON and CARLSON 1988). The SNF3 protein differs from the mammalian transporters in having additional sequences at the N and C termini. The large C-terminal extension (303 amino acids) contributes to, but is not essential for, SNF3 function (MARSHALL-CARLSON *et al.* 1990). SNF3 is also homologous to other yeast and bacterial sugar transporters (MAIDEN *et al.* 1987; SZKUTNICKA *et al.* 1989; CHENG and MICHELS 1989; NEHLIN, CARLBERG and RONNE 1989). The *SNF3* product is associated with membranes and is localized at the cell surface (CELENZA, MARSHALL-CARLSON and CARLSON 1988). Taken together, these data indicate that *SNF3* encodes a high-affinity glucose transporter.

Previous studies have identified additional genes that appear functionally related to *SNF3*. A selection for multicopy plasmids that complement the growth defect of a *snf3* mutant yielded at least five different genes (BISSON *et al.* 1987). One of these, named *HXT2*, encodes a protein that resembles other glucose transporters, and mutations in *HXT2* affect high-affinity hexose transport, although not as severely as mutations in *SNF3* (KRUCKEBERG and BISSON 1990). Low stringency blot hybridization analysis of genomic DNA suggested that the yeast genome contains a family of sequences homologous to *HXT2*, probably including additional glucose transporter genes. At least one additional transporter gene must exist, as neither *SNF3* nor *HXT2* is responsible for low-affinity transport.

In this study, we have used a different approach to identify genes that are functionally related to *SNF3*. We sought to identify genes that could mutate to suppress the transport defect caused by a *snf3* mutation. We therefore selected for suppressors that restore growth of mutants on raffinose, which requires high-affinity fructose uptake. We anticipated that this selection could yield mutations that alter other transporters so that they can bind and transport fructose with high affinity. Alternatively, the selection could yield mutations that increase expression of other transporters or allow expression of normally cryptic transporters. We describe here the isolation of two classes of suppressors that restore high-affinity uptake in *snf3* mutants: recessive *rgt1* mutations and dominant *RGT2* mutations.

## MATERIALS AND METHODS

**Strains and general genetic methods:** Strains of *S. cerevisiae* used in this study are listed in Table 1. pLS11 carries the *URA3* gene and a *SUC2-LEU2-lacZ* fusion (SAROKIN and CARLSON 1985) that is irrelevant to this study. Genetic analysis was carried out by standard methods (SHERMAN, FINK and LAWRENCE 1978). Growth phenotypes were determined by spotting cell suspensions onto plates using a 32-point inoculator and incubating the plates at 30° under anaerobic conditions in a GasPak disposable anaerobic system (BBL). Growth of single colonies was examined as described in the legend to Figure 1. Unless otherwise noted, plates contained rich medium (YEP) and 2% of the indicated carbon source.

**Glucose uptake assays:** Cells were grown in yeast nitrogen base (0.67%) containing casamino acids (0.2%), auxotrophic requirements, and the indicated carbon source. Cultures were harvested in early or mid log phase, and glucose uptake assays were performed by measuring uptake of D-[U-$^{14}$C]glucose (New England Nuclear) over the concentration range of 0.2 to 200 mM, as described previously (KRUCKEBERG and BISSON 1990). Each strain was assayed at least twice after growth under the specified conditions.

**Isolation of revertants of haploid *snf3* mutants:** Strains MCY657, MCY659, MCY714 and MCRY168 were subjected to UV mutagenesis. Single colonies were suspended in water and spread on a YEP-2% raffinose plate containing antimycin A (1 μg/ml). Cells were then exposed to 100 J/m$^2$ of UV radiation. In control experiments, 30% of the cells remained viable. The plates were incubated at 30° for 5 days. Revertants arose at frequencies of 1 to 5 × $10^{-5}$. Revertants derived from three single colonies of each strain (10 from MCY657, 8 from MCY659, 7 from MCY714, and 13 from MCRY168) were colony purified and retested.

**Complementation analysis:** Mutations were tested for dominance by crossing each revertant to a *snf3* null mutant.

**TABLE 1**

**List of *S. cerevisiae* strains**

| Strain[a] | Genotype |
|---|---|
| MCY657 | *MATα snf3-72 ura3-52 lys2-801 SUC2 (SUC7?)* |
| MCY659 | *MATa snf3-72 ura3-52 lys2-801 ade2-101 SUC2 (SUC7?)* |
| MCY714 | *MATα snf3-217 ura3-52 SUC2* |
| MCY1093 | *MATa ura3-52 lys2-801 his4-539 SUC2* |
| MCY1094 | *MATα ade2-101 ura3-52 SUC2* |
| MCY1408 | *MATa snf3-Δ4::HIS3 his3-Δ200 ura3-52 lys2-801 ade2-101 SUC2* |
| MCY1409 | *MATα snf3-Δ4::HIS3 his3-Δ200 ura3-52 lys2-801 SUC2* |
| MCY1410 | *MATα snf3-Δ4::HIS3 his3-Δ200 ade2-101 lys2-801 SUC2* |
| MCY1471 | *MATα rgt1-1 ade2-101 SUC2* |
| MCY1516 | *MATα rgt1-1 snf3-Δ4::HIS3 ura3-52 ade2-101 (his3-Δ200?) SUC2* |
| MCY1520 | *MATa rgt1-1 snf3-Δ4::HIS3 ade2-101 (his3-Δ200?) SUC2* |
| MCY1710 | *MATa RGT2-1 snf3-Δ4::HIS3 his3-Δ200 ura3-52 lys2-801 SUC2* |
| MCY1711 | *MATα RGT2-1 snf3-Δ4::HIS3 his3-Δ200 lys2-801 ade2-101 ura3-52 SUC2* |
| MCY1713 | *MATα RGT2-2 snf3-Δ4::HIS3 his3-Δ200 ura3-52 lys2-801 ade2-101 SUC2* |
| MCY1714 | *MATα* Rgt#3 *snf3-Δ4::HIS3 his3-Δ200 ura3-52 lys2-801 ade2-101 SUC2* |
| MCY1717 | *MATα* Rgt#4 *snf3-Δ4::HIS3 his3-Δ200 ura3-52 lys2-801 ade2-101 SUC2* |
| MCY1719 | *MATα* Rgt#5 *snf3-Δ4::HIS3 his3-Δ200 ura3-52 lys2-801 ade2-101 SUC2* |
| MCY1807 | *MATα ccs1 snf3-Δ4::HIS3 (his3-Δ200?) ura3-52 SUC2* |
| MCY2035 | *MATα rgt1-2 snf3-72 lys2-801 his4-539 ura3-52::pLS11 SUC2* |
| MCY2157 | *MATα RGT2-1 his3-Δ200 lys2-801 SUC2* |
| MCY2160 | *MATa cdc9 snf3-Δ4::HIS3 (his3-Δ200?) SUC2* |
| MCY2162 | *MATα leu2-3 SUF25-1 ura3-52 his4-519R SUC2* |
| MCY2166 | *MATa cdc9 snf3-Δ4::HIS3 (his3-Δ200?) lys2-801 ura3-52 SUC2* |
| MCRY168 | *MATα snf3-72 lys2-801 his4-539 ura3-52::pLS11 SUC2* |
| LBY415 | *MATα hxt2::LEU2 snf3-Δ4::HIS3 his3-Δ200 ura3-52 lys2-801 ade2-101 trp1-Δ63 leu2-Δ1 SUC2* |
| 1629[b] | *MATα leu2-3* |
| 1695[b] | *MATa leu2-3 his4-519R1 ura3-52 SUF25-1* |

[a] MCY strains are from the CARLSON laboratory, and the LBY strain is from the BISSON laboratory.

[b] Obtained from MICHAEL CULBERTSON.

To test for complementation, we constructed *snf3/snf3* diploids that were heterozygous for the suppressor mutations in pairwise combinations. Diploids were usually isolated by prototrophic selection, and when no selection was possible, single colonies were isolated and tested for mating or sporulation. Diploids were scored for anaerobic growth on raffinose after 24 and 48 h.

**Identification of *rgt1 SNF3* strains:** Segregants of genotype *rgt1 SNF3* were identified in nonparental ditype tetrads from crosses of *rgt1 snf3* strains to wild type. The presence of the *rgt1* mutation was verified by crossing the putative *rgt1 SNF3* strain to a *snf3* mutant and demonstrating segregation of the suppressor in tetrad analysis.

**Isolation of revertants carrying dominant suppressors:** Six single colonies derived from the cross of MCY1408 × MCY1409 were used to inoculate YEP-glucose liquid medium. After growth overnight, 0.3 ml of each culture ($3 \times 10^7$ cells) was spread onto YEP-raffinose medium, and plates were incubated anaerobically for 96 hr. Approximately 10–30 colonies grew on each plate. Six independent revertants, one from each plate, were colony purified twice and retested.

## RESULTS

**Isolation of revertants of *snf3* mutants:** The raffinose-nonfermenting phenotype of *snf3* mutants is caused by the defect in high-affinity glucose/fructose uptake: the mutants are unable to transport the fructose that is released at low concentration by the extracellular hydrolysis of raffinose. We selected for suppressors that restore growth of *snf3* mutants on raffinose. Four haploid *snf3* mutant strains were subjected to UV mutagenesis, and 38 revertants able to utilize raffinose were selected, as described in MATERIALS AND METHODS. The strains carried either the *snf3-72* or *snf3-217* allele. The *snf3-72* allele has been sequenced, and the mutation changes Gly-153 to Arg (MARSHALL-CARLSON *et al.* 1990).

**Dominance tests:** To test for dominance of the mutation responsible for the revertant phenotype, each revertant was crossed to a strain carrying the *snf3-Δ4::HIS3* null allele (NEIGEBORN *et al.* 1986). The resulting diploids were in each case unable to grow on raffinose anaerobically, indicating that all of the suppressor mutations are recessive.

**Complementation analysis:** Revertants derived from MCY657, MCY714 and MCRY168 were crossed to MCY1520 (*snf3-Δ4::HIS3 rgt1-1*), which was derived from MCRY168, and revertants derived from MCY659 were crossed to MCY2035 (*snf3-72 rgt1-2*) which was derived from MCRY168. All of the resulting diploids were able to ferment raffinose, indicating that the mutations fall into a single complementation group. Additional tests of other pairwise combinations also revealed no complementation. The complementation group was designated *rgt1* for *r*estores *g*lucose *t*ransport.

***rgt1* restores growth of *snf3* mutants on low glucose:** The revertants were selected for growth on raffinose, which normally requires the ability to transport the low amounts of fructose released by extracellular hydrolysis of the trisaccharide. To test whether the *rgt1* mutation also restores efficient utilization of glucose at low concentration in a *snf3* mutant, *rgt1-1 snf3-Δ4::HIS3* and control strains were streaked for single colonies on rich medium containing either 0.1% or 2% glucose. All strains produced colonies of the same size on 2% glucose. On low glucose, however, the *snf3-Δ4::HIS3* mutant formed very small colonies compared to the wild type, whereas the *rgt1-1 snf3-Δ4::HIS3* strain grew as well as the wild type (Fig. 1). Thus, the growth defect of the *snf3* mutant on low glucose was clearly remedied by *rgt1*. Diploids of genotype *snf3/snf3 rgt1/RGT1* formed small colonies on low glucose, confirming that the *rgt1* suppressor is recessive with respect to this phenotype (data not shown). In a wild-type (*SNF3*) background, *rgt1* caused no obvious phenotype.

***rgt1* is unlinked to *snf3*:** To determine whether *rgt1* is linked to *snf3*, two of the revertants (*snf3 rgt1*) were crossed to wild type. Tetrad analysis of the diploids yielded frequent raffinose nonfermenting segregants, presumably of *snf3 RGT1* genotype. Two additional crosses heterozygous for *snf3* and *rgt1* also yielded segregations of 4+:0−, 3+:1− and 2+:2− for raffinose utilization in ratios approximating 1:4:1. The ratio for the combined data from these crosses was 6:19:4. Thus, *rgt1* is not tightly linked to *snf3*.

***rgt1* suppresses a *snf3* deletion muation:** To test whether an *rgt1* allele suppresses a *snf3* null mutation, the revertant of MCRY168 carrying *rgt1-1* (*snf3-72 rgt1-1 ura3::pLS11*; pLS11 carries *URA3*) was crossed to MCY1408 (*snf3-Δ4::HIS3 ura3*). Tetrad analysis of the resulting diploid showed 2+:2− segregations for raffinose utilization in seven tetrads. Because *rgt1* is unlinked to *snf3*, these data indicate that *rgt1-1* suppresses a *snf3* null mutation. These data also confirm that *rgt1-1* behaves as a lesion in a single nuclear gene. The segregation pattern for *rgt1* and the centromere-linked marker *ura3* (5 tetratype and 2 nonparental ditype asci) did not indicate tight linkage to a centromere for *rgt1*.

Further evidence that *rgt1-1* suppresses *snf3-Δ4::HIS3* came from analysis of the cross MCY1408 (*snf3-Δ4::HIS3*) by MCY1471 (*rgt1-1 SNF3*). Segregations of 4+:0−, 3+:1− and 2+:2− for raffinose utilization were observed in the ratio 1:4:1, and the presence of the *snf3-Δ4::HIS3* or *SNF3* allele in $Raf^+$ segregants was determined by complementation. Strains MCY1516 and MCY1520 (*snf3-Δ4::HIS3 rgt1-1*) were recovered from this cross.

**A suppressor of *snf3* present in some laboratory strains is an *rgt1* allele:** The strains routinely used in this laboratory are derived from the S288C genetic background. During analysis of the cross of the S288C-derived strain MCY2160 (*snf3-Δ4::HIS3*) by

MCY2162 (*SNF3*), which was derived from strains 1629 and 1695 (obtained from M. CULBERTSON, University of Wisconsin), we observed a suppressor of *snf3* segregating. Five segregants carrying both *snf3-Δ4::HIS3* and the suppressor were identified. The suppressor was shown to be recessive by crossing each segregant to a *snf3-Δ4::HIS3* strain; the diploids were raffinose nonfermenters. To test the suppressor for complementation of *rgt1*, the five segregants were then crossed to *snf3-Δ4::HIS3 rgt1-1* strains (MCY1516 or MCY1520). All five diploids grew on raffinose, indicating that the suppressor fails to complement *rgt1*. Tetrad analysis of one of the diploids yielded no raffinose-nonfermenting segregants in seven four-spored tetrads and six triads, confirming that the suppressor is linked to *rgt1*. Thus, the suppressor is a naturally occurring *rgt1* allele.

***rgt1* is unlinked to *hxt2*:** The *HXT2* gene was identified as a multicopy suppressor of the *snf3* mutant defect in high-affinity glucose transport (BISSON *et al.* 1987) and encodes a protein homologous to glucose transporters (KRUCKEBERG and BISSON 1990). An *hxt2* null mutation reduces high-affinity glucose transport under derepressing conditions, but not as severely as a *snf3* mutation, and does not cause a strong growth defect on medium containing either high or low glucose (KRUCKEBERG and BISSON 1990). The *hxt2 snf3* double null mutants resemble *snf3* mutants in phenotype.

To determine whether the *rgt1* suppressors are alleles of *HXT2*, we carried out tetrad analysis of the diploid MCY1516 (*snf3-Δ4::HIS3 rgt1-1*) × LBY415 (*snf3-Δ4::HIS3 hxt2::LEU2 leu2*). Ten tetrads were recovered that showed 2+:2− segregations for leucine dependence, corresponding to the nonparental ditype configuration for the *leu2* and *hxt2::LEU2* markers. Tetrads of this class were easily recovered because *leu2* and *hxt2* are linked to different centromeres (D. COONS and L. BISSON, unpublished results). The *hxt2* mutation did not affect the ability of *rgt1* to suppress *snf3* because 2+:2− segregations for growth on low glucose were observed. The ten tetrads in which the segregation of *hxt2::LEU2* could be inferred included one parental ditype, one nonparental ditype and eight tetratypes with respect to *rgt1* and *hxt2*. These data indicate that *rgt1* and *hxt2* are not tightly linked.

**Selection for dominant suppressors of *snf3*:** Because all 38 suppressors of *snf3* selected in haploid strains were recessive alleles of a single locus, we next carried out a selection for suppressors in a diploid in an attempt to recover dominant mutations. We selected spontaneous raffinose-fermenting revertants of the diploid MCY1408 × MCY1409, which is homozygous for *snf3-Δ4::HIS3* (see MATERIALS AND METHODS). Six independent revertants were sporulated and subjected to tetrad analysis. For five revertants, the raffinose fermenting phenotype segregated 2+:2− in all seven tetrads tested, and Raf$^+$ segregants grew as well as the wild type on YEP-raffinose or YEP-0.1% glucose under anaerobic conditions (Figure 1). The sixth revertant showed a weak phenotype and was not characterized further.

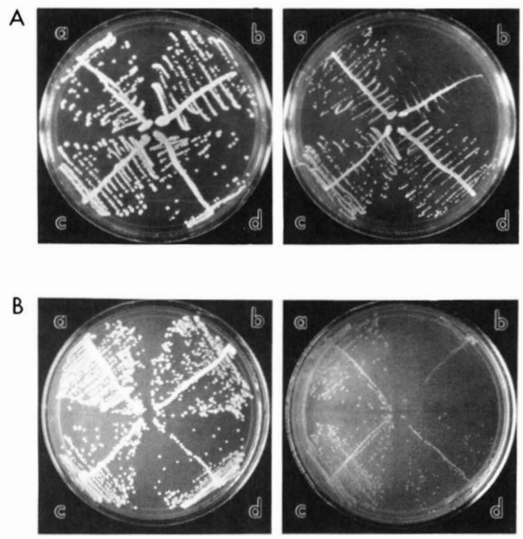


FIGURE 1.—Growth phenotypes of *snf3 rgt1* and *snf3 RGT2* strains. Strains were streaked on YEP containing 2% glucose (left panels) or 0.1% glucose (right panels). (A) Plates were incubated aerobically at 30° for 48 hr and then photographed. Relevant genotypes: (a) wild type; (b) *snf3-Δ4::HIS3*; (c) *rgt1-1*; (d) *rgt1-1 snf3-Δ4::HIS3*. The strain shown in panel (c) is MCY1471, and the others are segregants of cross MCY1471 × MCY1408. (B) Plates were incubated anaerobically at 30° for 72 hr and then photographed. Strains and relevant genotypes: (a) MCY1093 (wild type); (b) MCY1409 (*snf3-Δ4::HIS3*); (c) MCY1713 (*RGT2-2 snf3-Δ4::HIS3*); (d) MCY1711 (*RGT2-1 snf3-Δ4::HIS3*).

The dominance of these mutations was confirmed by crossing a Raf$^+$ *snf3-Δ4::HIS3* segregant from each of the five revertants to a *snf3-Δ4::HIS3* mutant. The resulting diploids each showed a Raf$^+$ phenotype. In addition, one of the diploids (MCY1410 × MCY1710) was sporulated, and tetrad analysis again showed 2+:2− segregations for growth on raffinose in seven tetrads.

**Linkage of the dominant suppressors to one another:** To determine whether the five dominant mutations are linked to one another, a segregant obtained from one of the revertants (MCY1710) was crossed to segregants from each of the other four revertants (MCY1713, MCY1714, MCY1717, MCY1719). Tetrad analysis of the resulting diploids showed 4+:0− segregations for ability to utilize raffinose (46 tetrads for MCY1710 × MCY1713 and seven tetrads for each of the other three diploids). Thus, the suppressors in MCY1710 and MCY1713 are tightly linked to one another and are probably alleles of the same locus. These mutations were designated *RGT2-1* and *RGT2-2*, respectively. The other suppressors may also be alleles of this locus.

TABLE 2

**Linkage data**

| Cross | Parents | Gene pair | No. of tetrads[a] PD | NPD | T | Map distance (cM)[b] |
|---|---|---|---|---|---|---|
| LC36 | MCY1094 × MCY1710 | *RGT2-1-snf3* | 11 | 1 | 14 | |
| LC76 | MCY1408 × MCY2157 | *RGT2-1-snf3* | 2 | 0 | 5 | |
| | | | 13 | 1 | 19 | 38 |
| LC116 | MCY1711 × MCY2166 | *RGT2-1-cdc9* | 11 | 0 | 6 | 18 |
| LC66 | MCY1710 × MCY1807 | *RGT2-1-ccs1* | 18 | 0 | 3 | |
| LC70 | MCY1408 × LC66.10A | *RGT2-1-ccs1* | 10 | 0 | 4 | |
| LC71 | MCY1409 × LC66.12A | *RGT2-1-ccs1* | 5 | 0 | 1 | |
| LC73 | MCY1408 × LC66.19A | *RGT2-1-ccs1* | 6 | 0 | 0 | |
| | | | 39 | 0 | 8 | 9 |

[a] PD, parental ditype; NPD, nonparental ditype; T, tetratype. Data were obtained from tetrads with four viable spores and showing 2:2 segregation for the markers.

[b] Genetic map distances in centimorgans were calculated from the tetrad data by the equation of PERKINS (1949): distance = 100(T + 6NPD)/2(PD + NPD + T).

**Genetic mapping of *RGT2* near *SNF3* on chromosome *IV*:** Standard meiotic linkage analysis of the cross MCY1710 × MCY1094 revealed a genetic distance of 38 cM between *RGT2-1* and *snf3* (Table 2). Previous studies established a gene order of *cdc9-snf3-SUF25* with distances of 32 cM for *cdc9-snf3* and 11 cM for *snf3-SUF25* (MARSHALL-CARLSON *et al.* 1990). To determine the location of *RGT2* relative to *cdc9*, the cross MCY1711 × MCY2166 was analyzed (Table 2). The calculated map distance is 18 cM, indicating that *RGT2* and *cdc9* lie on the same side of *snf3*. The likely gene order is *RGT2-cdc9-snf3-SUF25*; however, this could not be easily confirmed by a three-point cross due to problems inherent in scoring these markers.

Analysis of the cross MCY1710 (*snf3 RGT2*) × MCY1516 (*snf3 rgt1*) yielded parental ditype, tetratype and nonparental ditype tetrads in the ratio 3:12:3, confirming that *RGT2* and *rgt1* are not linked.

***RGT2* is not allelic to *ccs1*:** Previous work identified the recessive *ccs1* mutation, which is tightly linked to *snf3* (genetic distance 19 cM) (MARSHALL-CARLSON *et al.* 1990). This mutation causes poor growth on glucose and, in conjunction with *snf3-39* or *snf3-Δ4::HIS3* alleles, causes constitutive invertase expression. To test whether the *RGT2* mutations might be dominant alleles of the same gene, the strain MCY1710 (*RGT2-1 snf3*) was crossed to MCY1807 (*ccs1 snf3*) in cross LC66 (Table 2). Tetrad analysis yielded 3 tetratype and 18 parental ditype tetrads, as judged by the segregation of the raffinose fermentation phenotype and the small spore clone size that is characteristic of *ccs1* mutants. To confirm that the three small, $Raf^+$ recombinants (LC66.10A, LC66.12A and LC66.19A) in the tetratype asci have the genotype *RGT2 ccs1 snf3*, each was crossed to a *snf3* mutant. Two of the three diploids yielded tetratype tetrads containing both a large $Raf^+$ and a small $Raf^-$ recombinant, thereby confirming the genotypes of LC66.10A and LC66.12A (Table 2). Thus, *RGT2-1* and *ccs1* are clearly not allelic, and the calculated genetic distance is 9 cM. From the genetic distances, the likely gene order is *RGT2-ccs1-snf3*.

Assays of invertase activity in LC66.10A and LC66.12A showed that *RGT2-1* does not suppress the invertase constitutivity resulting from the combination of *snf3* and *ccs1* (data not shown). Also, assays of *RGT2 snf3* segregants showed that *RGT2-1* does not affect regulation of invertase expression (not shown).

These linkage data suggest that *ccs1* maps close to *cdc9*, and both mutations cause growth defects at 37°. We therefore tested the two mutations for complementation. The heterozygous diploid grew normally at 37°, suggesting that the two mutations complement.

***rgt1* and *RGT2* mutations restore glucose-repressible high-affinity glucose transport in a *snf3* mutant:** The $Raf^-$ phenotype of *snf3* mutants is caused by the defect in high-affinity glucose/fructose uptake, and suppression of this phenotype by the *rgt1* and *RGT2* mutations could most easily be accounted for by the restoration of hexose transport. We therefore examined the kinetics of glucose transport in *snf3-Δ4::HIS3 rgt1-1* and *snf3-Δ4::HIS3 RGT2-1* strains. Both suppressors restored high-affinity uptake in derepressed cells. *RGT2-1* restored wild-type levels of glucose transport activity, and *rgt1-1* caused wild-type or slightly elevated levels of transport (Figure 2). Similar results were obtained for *snf3* mutant strains carrying the *RGT2-2* and *rgt1-2* alleles (MCY1713 and MCY2035; data not shown).

High-affinity glucose transport in wild-type (*SNF3*) strains is regulated by glucose repression (BISSON and

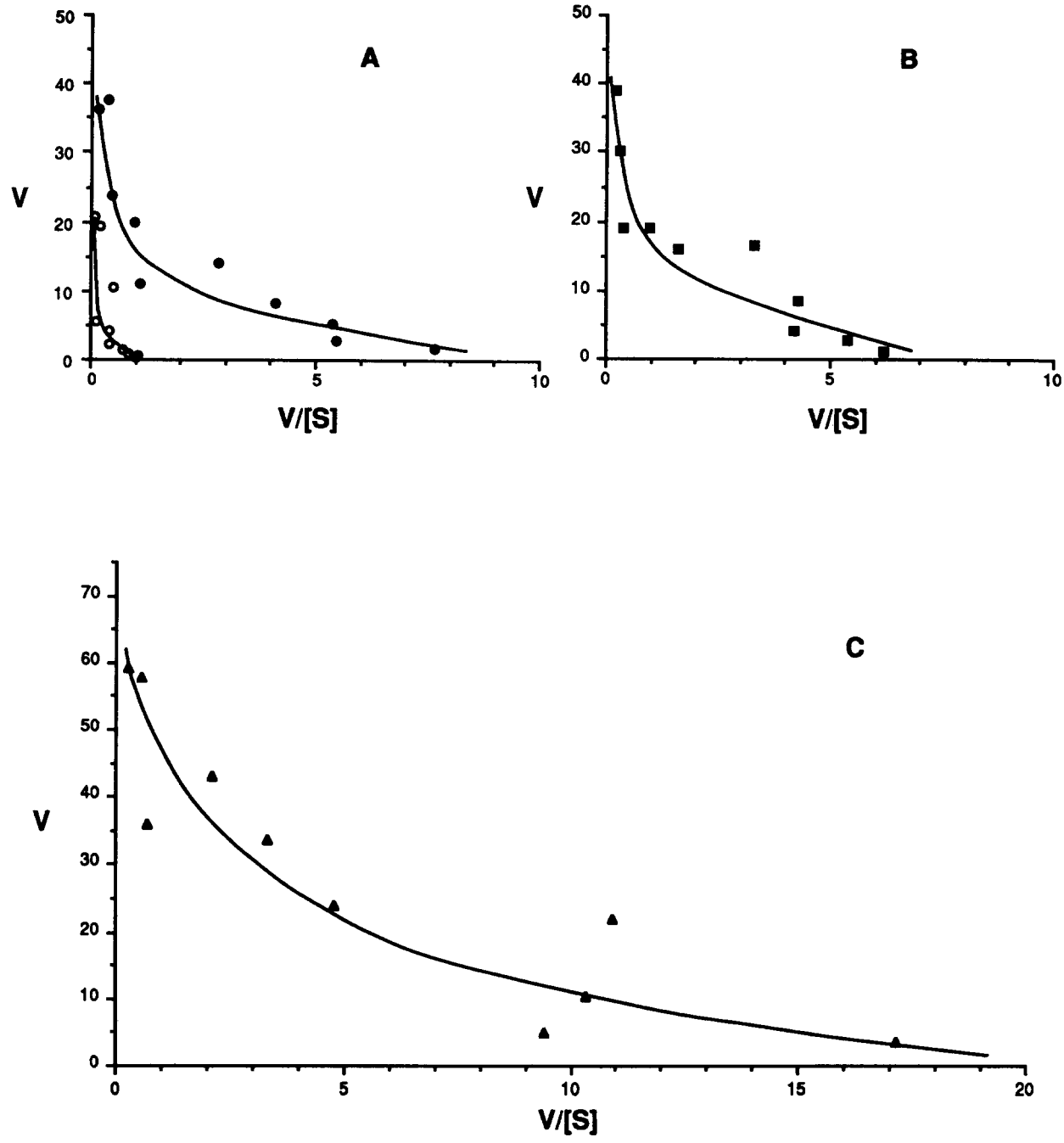


FIGURE 2.—Eadie-Hofstee plots of glucose uptake in *snf3 rgt1* and *snf3 RGT2* strains. Velocity is expressed as nanomoles of glucose per minute per milligram (dry weight); *V*/[*S*] is expressed as velocity per millimolar concentration. Cells were grown to early log phase in medium containing glycerol (2%) and lactate (2%), and assays were carried out as described in MATERIALS AND METHODS. Strains: (A) MCY1093 (wild type), filled circles, and MCY1409 (*snf3-Δ4::HIS3*), open circles; (B) MCY1710 (*snf3-Δ4::HIS3 RGT2-1*); (C) MCY1516 (*snf3-Δ4::HIS3 rgt1-1*).

FRAENKEL 1984), as is expression of the *SNF3* gene (NEIGEBORN *et al.* 1986; CELENZA, MARSHALL-CARLSON and CARLSON 1988). To determine whether the high-affinity uptake that is restored in the *rgt1-1 snf3-Δ4::HIS3* and *RGT2-1 snf3-Δ4::HIS3* strains is glucose-repressible, transport was assayed in glucose-grown cultures. The high-affinity transport expressed in these strains was glucose-repressible (Figure 3). As a control, the same cultures were also shifted to derepressing conditions, and high-affinity transport was detected (Figure 3). Assays of MCY1713 and MCY2035 also showed glucose-repressible high-affinity transport (data not shown).

## DISCUSSION

By selecting for raffinose-fermenting revertants of *snf3* mutants, we isolated two classes of suppressors

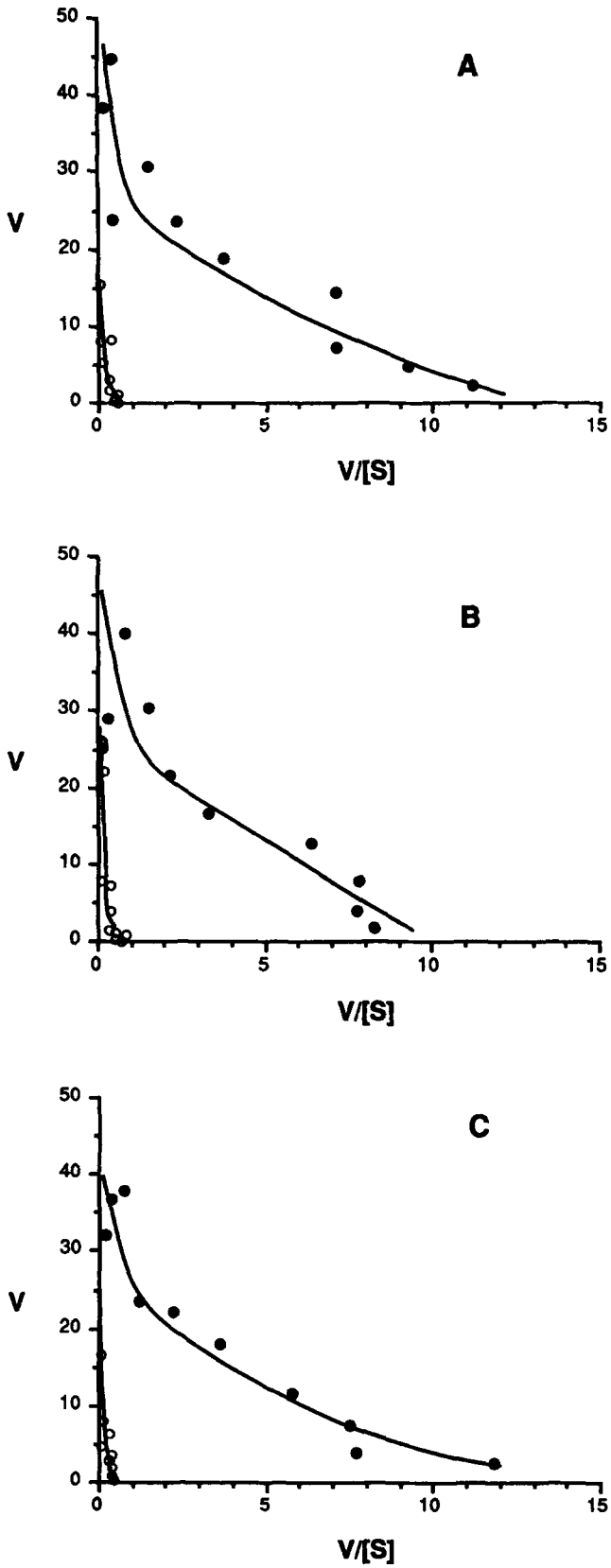


FIGURE 3.—Glucose repression of high-affinity transport in *snf3 rgt1* and *snf3 RGT2* strains. Eadie-Hofstee plots of glucose uptake are shown, as for Figure 2. Glucose-repressed cultures were grown to mid-log phase in medium containing-glucose (4%). Cells were collected by filtration, resuspended in fresh medium containing either 4% or 0.05% glucose, and incubated with aeration for 4 hr prior to assay for glucose uptake. Symbols: ○, cells resuspended in 4% glucose (repressed); ●, cells resuspended in 0.05% glucose (derepressed). Strains: (A) MCY1093 (wild type); (B) MCY1710 (*snf3-Δ4::HIS3 RGT2-1*); (C) MCY1516 (*snf3-Δ4::HIS3 rgt1-1*). These data differ from those shown in Figure 2 because growth conditions were different.

that restore high-affinity glucose transport in a *snf3* null mutant. The recovery of such revertants supports previous evidence that the yeast genome includes multiple genes that are functionally related to or homologous to the *SNF3* glucose transporter gene (BISSON *et al.* 1987; KRUCKEBERG and BISSON 1990). Presumably, the suppressor mutations allow expression of or alter the product of one of these genes.

The 38 mutations isolated in haploid strains are all recessive and define a single complementation group, *rgt1*. The recessiveness of the alleles suggests that the mutations cause loss of function. We can imagine at least two mechanisms by which an *rgt1* mutation could restore the expression of high-affinity transport. First, the wild-type *RGT1* gene product could repress expression of a gene encoding a high-affinity transporter that is functionally homologous to the SNF3 transporter. This gene might normally be expressed only at low levels or not at all. An *rgt1* mutation would then release expression of this gene to levels sufficient to confer a nearly wild-type capability for high-affinity transport. A second possibility is that the *RGT1* product acts to inhibit the function of a high-affinity transporter that is expressed but does not contribute to the high-affinity component of glucose/fructose uptake in wild-type cells.

An apparently naturally occurring *rgt1* suppressor allele was identified in a laboratory strain of *S. cerevisiae* with a genetic background different from that of S288C. Interestingly, a cross of a *snf3* mutant with the wild-type strain DFY1 (originally D585-11C from F. SHERMAN) also did not yield Mendelian segregation for failure to grow on low glucose, although the segregation pattern was not consistent with the segregation of a single suppressor of *snf3* (BISSON 1988). Perhaps *rgt1* suppressors are fairly common in *S. cerevisiae* strains, and the *RGT1* allele present in the S288C genetic background is unusual.

A selection for revertants of a homozygous *snf3* mutant diploid yielded five dominant suppressors at the *RGT2* locus. The dominance of these mutations suggests that suppression results from a change of function. The *RGT2* suppressors may elevate the expression or activate the function of a high-affinity transporter that, in wild-type cells, contributes little to the high-affinity component of uptake. It is also possible that these dominant mutations convert a low-affinity transporter into a high-affinity transporter by increasing the affinity for hexoses or by affecting interactions with hexose kinases (BISSON and FRAEN-

KEL 1983; LANG and CIRILLO 1987); however, mutations of this type might be expected to occur rarely. Molecular analysis of the *RGT2* gene will be required to determine whether it encodes a glucose transport protein. *RGT2* may prove to be the same as one of the genes cloned as a multicopy suppressor of *snf3* (BISSON *et al.* 1987), but an identity with *HXT2* is unlikely. *RGT2* is linked to *SNF3*, whereas *HXT2* is centromere-linked (D. COONS and L. BISSON, unpublished results), and no linkage was detected between *HXT2* and *SNF3* (KRUCKEBERG and BISSON 1990).

Both biochemical and genetic evidence indicate that in yeast, as in mammalian cells, glucose transport is a complex process involving the products of multiple genes. Some of these genes encode transporters, and others may regulate or otherwise affect transport. Perhaps we should expect to find functional redundancy of the genes responsible for a process so critical to survival of the cell. These studies identified two genes that affect glucose transport, and molecular analysis should help to elucidate their roles and the mechanism by which the *rgt1* and *RGT2* suppressor mutations restore transport in *snf3* mutants.

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