Mutations Causing Constitutive Invertase Synthesis in Yeast: Genetic Interactions with *snf* Mutations

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Manuscript received August 15, 1986
Revised copy accepted November 10, 1986

ABSTRACT

We have selected 210 mutants able to grow on sucrose in the presence of 2-deoxyglucose. We identified recessive mutations in three major complementation groups that cause constitutive (glucose-insensitive) secreted invertase synthesis. Two groups comprise alleles of the previously identified *HXK2* and *REG1* genes, and the third group was designated *cid1* (*c*onstitutive *i*nvertase *d*erepression). The effect of *cid1* on *SUC2* expression is mediated by the *SUC2* upstream regulatory region, as judged by the constitutive expression of a *SUC2-LEU2-lacZ* fusion in which the *LEU2* promoter is under control of *SUC2* upstream sequences. A *cid1* mutation also causes glucose-insensitive expression of maltase. The previously isolated constitutive mutation *ssn6* is epistatic to *cid1*, *reg1* and *hxk2* for very high level constitutive invertase expression. Mutations in *SNF* genes that prevent derepression of invertase are epistatic to *cid1*, *reg1* and *hxk2*; we have previously shown that *ssn6* has different epistasis relationships with *snf* mutations. The constitutive mutation *tup1* was found to resemble *ssn6* in its genetic interactions with *snf* mutations. These findings suggest that *CID1*, *REG1* and *HXK2* are functionally distinct from *SSN6* and *TUP1*.

GLUCOSE repression, or carbon catabolite repression, is a global regulatory system affecting the expression of many genes in *S. cerevisiae*. The *SUC2* gene has proved a useful "reporter" gene for studies of this regulatory system because expression of *SUC2* is regulated only by glucose repression and is modulated over a greater than 200-fold range. The *SUC2* gene encodes two forms of invertase: a secreted invertase that is responsible for the extracellular hydrolysis of sucrose and raffinose and an intracellular invertase with no apparent physiological role. The gene encodes these two enzymes via two mRNAs: secreted invertase is encoded by a glucose-repressible 1.9-kb mRNA, and intracellular invertase is encoded by a constitutive 1.8-kb mRNA with a different 5′ end (CARLSON and BOTSTEIN 1982; CARLSON *et al.* 1983; PERLMAN, HALVORSON and CANNON 1982).

We have previously isolated recessive mutations in six genes, *SNF1* through *SNF6* (*s*ucrose-*n*onfermenting), that caused defects in derepression of secreted invertase (CARLSON, OSMOND and BOTSTEIN 1981; NEIGEBORN and CARLSON 1984). Analysis of the genetic interactions of *snf* mutations with extragenic suppressors suggested that *SNF1* and *SNF4* are functionally related and that *SNF2*, *SNF5* and *SNF6* are related (NEIGEBORN and CARLSON 1984; NEIGEBORN, RUBIN and CARLSON 1986). The *SNF1* gene encodes a protein kinase (CELENZA and CARLSON 1986). The *SNF3* gene is required for high-affinity glucose transport (L. BISSON, L.N., M.C. and D. FRAENKEL, unpublished results). Disruption of *SNF3* impairs growth on sucrose and raffinose, but has no effect on *SUC2* expression (NEIGEBORN *et al.* 1986); in contrast, the *snf3* missense mutations that were originally isolated cause aberrant regulation of *SUC2* expression (NEIGEBORN and CARLSON 1984).

We have also previously isolated mutations causing constitutive (glucose-insensitive) secreted invertase synthesis by selecting for suppressors of the sucrose-nonfermenting phenotype of *snf1*. The *ssn6* (suppressor of *snf*) mutations cause high-level constitutive invertase expression in both *snf1* and wild-type (*SNF1*) genetic backgrounds (CARLSON *et al.* 1984). The *ssn6* mutations are pleiotropic, causing clumpiness, mating defects, and failure to sporulate in homozygous diploids (CARLSON *et al.* 1984); moreover, *ssn6* was found to be allelic to *cyc8*, a mutation causing overproduction of iso-2-cytochrome *c* (ROTHSTEIN and SHERMAN 1980). Recently, TRUMBLY (1986) has isolated additional *cyc8/ssn6* alleles by screening for mutants constitutive for invertase.

Other workers have also identified constitutive mutations. ZIMMERMANN and SCHEEL (1977) isolated mutations in three complementation groups that cause constitutivity for invertase, maltase and malate dehydrogenase: *hex1*, *hex2* and *cat80* (ENTIAN and ZIMMERMANN 1980). *HEX1* is the same gene (ENTIAN 1980) as *HXK2*, the structural gene for the hexokinase PII isozyme (LOBO and MAITRA 1977), which has been postulated to play both catalytic and regulatory roles

in carbon source utilization (ENTIAN and FROHLICH 1984; ENTIAN *et al.* 1985). Genetic mapping suggests that *hex2* (ENTIAN and FROHLICH 1984) is probably allelic to *reg1* (MATSUMOTO, YOSHIMATSU and OSHIMA 1983), which causes glucose-insensitive expression of galactokinase and invertase. Mutations at a locus called variously *tup1*, *umr7*, *flk1* and *cyc9* cause constitutivity for invertase, maltase, α-methyl glucosidase and succinate dehydrogenase, clumpiness, mating and sporulation defects, elevated levels of iso-2-cytochrome *c*, utilization of exogenous deoxythymidine monophosphate, and other abnormalities (WICKNER 1974; LEMONTT, FUGIT and MACKAY 1980; SCHAMHART, TEN BERGE and VAN DE POLL 1975; STARK, FUGIT and MOWSHOWITZ 1980; ROTHSTEIN and SHERMAN 1980; TRUMBLY 1986).

One would expect that constitutivity for invertase synthesis could result from defects in a variety of functions, such as direct negative regulators of *SUC2* expression and sensory or signaling functions that enable the cell to evaluate the availability of glucose in the environment. To define the role in glucose repression played by genes with constitutive alleles, it was important to examine the interactions of these constitutive mutations with the *snf* mutations and with one another. Such studies are ideally carried out using isogenic strains to eliminate any effects of variation in genetic background. We therefore undertook the isolation of new constitutive mutations in the S288C genetic background, in which all of our previous mutations have been isolated. A second motivation for a new mutant search was the likelihood that previous studies had not identified all classes of mutations that can cause constitutivity for invertase.

To select constitutive mutants, we employed a genetic selection similar to that of ZIMMERMANN and SCHEEL (1977). We selected for mutants able to grow on medium containing sucrose plus the glucose analog 2-deoxyglucose, which causes repression of glucose-repressible genes but is not utilized as a carbon source (WITT, KRONAU and HOLZER 1966). In the presence of 2-deoxyglucose, wild-type cells do not synthesize secreted invertase and are consequently unable to utilize sucrose. We used sucrose as the carbon source, instead of raffinose as had ZIMMERMANN and SCHEEL, to reduce the stringency of the selection; growth on raffinose requires higher levels of invertase than does growth on sucrose.

We report here the isolation of mutants constitutive for invertase synthesis. In addition to *reg1* and *hxk2* alleles, we identified mutations at a locus designated *cid1* (*c*onstitutive *i*nvertase *d*erepression). We examined the epistasis relationships between these constitutive mutations and *snf1* through *snf6* and *sn6*. We also tested the interactions of a previously isolated *tup1* mutation with *snf1* and *snf2*. Our findings suggest that these constitutive mutations fall into two classes, one comprising *cid1*, *reg1* and *hxk2* and the other comprising *ssn6* and *tup1*.

TABLE 1

List of yeast strains

Strain	Genotype	Source
MCY87	*MATα ade2-101 his4-619 SUC3 MAL3*	This laboratory
MCY317	*MATα his4-539 ade2-101 SUC2*	This laboratory
MCY638	*MATa his4-539 lys2-801 ura3-52 SUC2*	This laboratory
MCY730	*MATa ade2-101 ura3-52 Δtrp1 lys2-801 Δhis3 SUC2*	This laboratory
A364A-T18	*MATa ade1 ade2 lys2 gal1 tyr1 his7 ura1 tup1-18*	R. WICKNER
F445	*MATα ade1 his2 trp1 met14 suc° hxk1-1 hxk2-2*	G. FINK
YG5-4C	*MATa leu1 reg1-1 GAL81-12 gal7-2*	K. MATSUMOTO
ENY100-7C	*MATa trp1 ura3-52 cat80 MAL3 SUC3*	K.-D. ENTIAN
ENY100-8A	*MATα leu1 ura3-52 cat80 MAL3 SUC3*	K.-D. ENTIAN

MATERIALS AND METHODS

Strains: The genotypes and sources of *S. cerevisiae* strains used in this study are listed in Table 1. All strains were isogenic or congenic to S288C, except those containing *hxk2-2*, *reg1-1*, *MAL3*, *cat80* and *tup1-18*. The *hxk2-2* mutation was previously introduced into our genetic background from F445 by three serial backcrosses (NEIGEBORN and CARLSON 1984). Strains carrying *reg1-1* were derived from YG5-4C for use in complementation analysis. *tup1-18* was introduced into our background from A364A-T18 by crossing, and a *tup1 SUC2* segregant from such a cross was used for the construction of *snf1 tup1* and *snf2 tup1* double mutants. Since strains derived from S288C are maltose nonfermenters, *cid1 MAL3* strains were constructed by crossing a *cid1* mutant to MCY87 (*MAL3*).

General genetic methods: Standard genetic procedures of crossing, sporulation and tetrad analysis were followed (SHERMAN, FINK and LAWRENCE 1978). Carbon source utilization was scored under anaerobic conditions, as described (CARLSON, OSMOND and BOTSTEIN 1981); cell suspensions were spotted onto rich medium (YEP) containing the appropriate carbon source.

Isolation of mutants: Cells were mutagenized with ethyl methanesulfonate as described by CARLSON, OSMOND and BOTSTEIN (1981). Surviving cells were spread onto plates of rich medium containing sucrose and 200 μg/ml 2-deoxy-D-glucose (Sigma) and incubated anaerobically at 30° for 4 days.

Complementation analysis: To test pairs of mutations for complementation, heterozygous diploids were constructed and isolated by prototrophic selection. Diploids were tested for constitutive production of secreted invertase activity by assaying invertase activity under glucose-repressing conditions.

Construction of double mutants: Heterozygous diploids were sporulated and subjected to tetrad analysis. Complete tetrads were tested for genetic markers and utilization of raffinose and sucrose plus or minus 2-deoxyglucose. The

genotypes of double mutants were determined by complementation analysis: complementation of *snf1* through *snf6* was judged by testing diploids for ability to utilize raffinose, and complementation of *ssn6*, *regl*, *hxk2*, and *cid1* was determined by assaying glucose-repressed diploids for invertase.

Assay for invertase: Preparation of glucose-repressed and -derepressed cells was as described by CELENZA and CARLSON (1984); repressed cells were grown to exponential phase in YEP medium containing 2% glucose, and derepressed cells were prepared by shifting repressed cells to YEP medium containing 0.05% glucose for 2.5 hr. Secreted invertase activity was assayed (GOLDSTEIN and LAMPEN 1975) in whole cells as described (CELENZA and CARLSON 1984).

Assays for β-galactosidase and maltase: β-Galactosidase was assayed in permeabilized cells (GUARENTE 1983). For maltase assays, cells were broken by vortexing with glass beads and assayed as described by KHAN and EATON (1967) in the presence of 40 μg/ml phenylmethylsulfonyl fluoride and 1 mM EDTA. Protein concentrations were determined using the Bio-Rad Protein Assay.

RESULTS

Isolation of mutants: Mutants able to form colonies on rich medium containing sucrose and 2-deoxyglucose were isolated from strains MCY638 and MCY317, as described in MATERIALS AND METHODS. 2-Deoxyglucose-resistant colonies were obtained at a frequency of 5×10^{-4}, and 256 colonies were purified for characterization. To identify mutants in which resistance resulted from constitutive (glucose-insensitive) invertase synthesis, the mutants were grown in glucose-repressing conditions and assayed for secreted invertase activity: 210 isolates produced significant constitutive invertase activity (>10% of the wild-type derepressed level) and were analyzed further.

To test for dominance, each of these 210 mutants was crossed to a wild-type strain. The resulting diploids showed normal glucose repression of secreted invertase activity and failed to grow on medium containing sucrose plus 2-deoxyglucose, indicating that all mutations are recessive.

Complementation analysis: The 210 new mutations were tested for ability to complement previously isolated mutations causing constitutive production of secreted invertase: *reg1-1*, *hxk2-2*, *ssn6-1*, and *tup1-18*. Forty mutations failed to complement *hxk2*, and 140 mutations failed to complement *reg1*, as judged by assaying secreted invertase in glucose-repressed diploids. No *ssn6* or *tup1* alleles were identified.

The 30 remaining mutants were tested for ability to complement one another. One new complementation group containing 21 alleles was identified and designated *cid1* for *c*onstitutive *i*nvertase *d*erepression. The remaining nine mutants represent at least two additional complementation groups; however, because none produces invertase constitutively at a level higher than 20% of that of the derepressed wild type, they were not characterized further.

TABLE 2

Secreted invertase activity in mutants

Relevant genotype	Secreted invertase activity[a] R	D
Wild type	<1	100
cid1-226	60	100
reg1-69	70	95
hxk2-66	70	100
ssn6-1	300	380
cid1-226 reg1-69	82	ND
cid1-226 hxk2-66	69	ND
reg1-69 hxk2-66	84	ND
ssn6-1 cid1-226	290	ND
ssn6-1 reg1-69	300	ND
ssn6-1 hxk2-66	300	ND

[a] Micromoles glucose released/minute/100 mg (dry weight) of cells. Values are the average of assays of two or more strains. Standard errors were <15%. R, repressed; D, derepressed; ND, not determined.

We attempted to test the allelism of *cid1* to the previously identified *cat80* mutations (ENTIAN and ZIMMERMANN 1980); however, we were unable to detect constitutive secreted invertase activity in *cat80* mutants grown to mid-log phase in rich medium containing 2% glucose. Diploids heterozygous for *cat80* and *cid1* synthesized no detectable invertase under glucose-repressing conditions.

Table 2 shows the invertase activity detected in the *cid1*, *reg1* and *hxk2* mutants with the highest constitutive levels. All produce wild-type derepressed levels of invertase. The intracellular invertase is present at normal levels in glucose-repressed *cid1*, *reg1* and *hxk2* mutants, as judged by an *in situ* gel assay (CARLSON, OSMOND and BOTSTEIN 1981) (data not shown).

***cid1* segregates as a single nuclear mutation:** A representative mutant (*cid1-226*) was backcrossed to wild type, and the diploid was subjected to tetrad analysis. In eight tetrads, resistance to 2-deoxyglucose and invertase constitutivity segregated 2:2, indicating that *cid1-226* segregates as a single nuclear mutation.

Linkage analysis of *cid1*: Analyses of crosses including *cid1* and the centromere-linked markers *ura3* and *trp1* showed that a majority of the tetrads were tetratype, indicating that *cid1* is not tightly linked to a centromere. No tight linkage was detected between *cid1* and *his4*, *lys2*, *ade2*, *MAT*, *snf1* through *snf6*, *reg1*, *hxk2*, *ssn6*, or *MAL3*.

Effects of *cid1* on expression of other glucose-repressible genes: A *cid1* mutant was examined for pleiotropic defects in the regulation of other glucose-repressible genes. To test the effects of *cid1* on maltase expression, *cid1-226 MAL3* and *CID1 MAL3* strains were constructed. Maltase activity was assayed in cultures grown under glucose-repressing and nonrepressing conditions in the presence of the inducer maltose (Table 3). Glucose repression was defective: the *cid1*

TABLE 3

Glucose-insensitive maltase synthesis in mutants

Relevant genotype	Maltase activity[a]	
	Repressed	Induced
cid1-226 MAL3	1900	3200
CID1 MAL3	82	3100

[a] Nanomoles of *p*-nitrophenyl-α-D-glucopyranoside cleaved/minute/mg protein. Cultures were grown to mid-log phase in rich medium (YEP) containing 5% glucose and 2% maltose (repressed) or 2% maltose (induced). Values are the average of assays of two segregants of each genotype, and standard errors were <10%.

TABLE 4

Constitutive expression of a *SUC2-LEU2-lacZ* fusion in a *cid1* mutant

Relevant genotype	β-Galactosidase activity[a]	
	R	D
CID1 (pLS7)	1.4	380[b]
cid1-226 (pLS7)	86	260

[a] Units of activity normalized for OD_{600} of the culture were calculated as described by MILLER (1972). Repressed cultures were grown to mid-log phase in synthetic minimal medium lacking uracil and containing 2% glucose; for derepression, cultures were shifted to similar medium containing 0.05% glucose for 3 hr prior to assaying. Values are the average of assays of two transformants; standard errors were <10%. R, repressed; D, derepressed.

[b] Values reported by SAROKIN and CARLSON (1985).

mutants produced almost 25-fold more maltase under glucose-repressing conditions than did the wild type (*CID1*) strains.

To determine whether *cid1* affects glucose repression of *GAL10* expression, a *cid1-226* mutant and the wild type were transformed with pRY123, an episomal plasmid carrying a *GAL10-lacZ* gene fusion (WEST, YOCUM and PTASHNE 1984). Transformants were assayed for β-galactosidase activity after growth under conditions of glucose repression (2% glucose, 2% galactose, 3% glycerol) or induction (2% galactose, 3% glycerol). The *cid1-226* mutation did not affect regulation of *GAL10-lacZ* fusion expression (data not shown).

The *cid1-226* mutants were also examined for pleiotropic growth defects. The mutants were able to grow at 37° and to utilize a variety of carbon sources: glucose, sucrose, raffinose, galactose, ethanol or glycerol. Diploids homozygous for *cid1-226*, or *cid1-28*, were unable to sporulate.

***cid1* affects regulation by the *SUC2* upstream region:** Previous studies have identified a *SUC2* upstream regulatory region that is required for derepression of secreted invertase. (SAROKIN and CARLSON 1984) and confers glucose-repressible expression to the heterologous *LEU2* promoter (SAROKIN and CARLSON 1985). To test whether a *cid1* mutation affects regulation by the *SUC2* upstream region, a *cid1-226* mutant was transformed with pLS7, a multicopy plasmid carrying a *LEU2-lacZ* fusion under control of the *SUC2* upstream regulatory region (SAROKIN and CARLSON, 1985). Expression of β-galactosidase was resistant to glucose repression in *cid1* mutants (Table 4). This finding suggests that *CID1* function is required for regulation of transcription by the *SUC2* upstream region.

***ssn6* is epistatic to *cid1*, *reg1* and *hxk2*:** To examine the interactions between different mutations conferring invertase constitutivity, we constructed double mutants between *cid1-226*, *reg1-69*, *hxk1-66* and *ssn6-1* in all pairwise combinations. In each cross, resistance to 2-deoxyglucose segregated 4+:0−, 3+:1− and 2+:2−, in ratios indicating that none of the mutations are tightly linked. Double mutants were identified by complementation, and all were resistant to 2-deoxyglucose and produced secreted invertase activity constitutively (Table 2). Segregants carrying combinations of *cid1*, *reg1* and *hxk2* showed no unexpected phenotypes. Double mutants carrying the *ssn6* allele showed the high level constitutivity and clumpiness characteristic of *ssn6* mutants, indicating that *ssn6* is epistatic to *cid1*, *reg1* and *hxk2*.

***snf* mutations are epistatic to *cid1* and *reg1*:** The *snf1* through *snf6* mutations cause defects in derepression of secreted invertase and utilization of sucrose and/or raffinose (NEIGEBORN and CARLSON 1984). To determine the epistasis relationships, *cid1-226* and *reg1-69* mutants were crossed with strains carrying one of the mutations *snf1* through *snf6*. Tetrad analysis of each cross showed 2:2 segregations for ability to utilize raffinose, indicating that the *snf* mutations are epistatic to *cid1* and *reg1* for this phenotype. Ability to grow on sucrose in the presence of 2-deoxyglucose segregated 0+:4−, 1+:3− and 2+:2−, in ratios indicating that *cid1* and *reg1* are not tightly linked to any of the *snf* loci. Two or more *cid1 snf* and *reg1 snf* double mutants from each cross were identified by complementation analysis. With the exception of double mutants carrying a *snf3* allele (see below), all *snf cid1* and *snf reg1* strains resemble their *snf* parent with respect to their raffinose-nonfermenting phenotype and secreted invertase activity (Table 5). We have previously shown that *snf1* and *snf2* are epistatic to *hxk2* (NEIGEBORN and CARLSON 1984).

The epistasis relationships with *snf3* are a special case. *snf3* null mutations do not cause aberrant regulation of invertase expression (NEIGEBORN *et al.* 1986). *SNF3* is required for high affinity glucose transport (L. BISSON, L.N., M.C. and D. FRAENKEL, unpublished results), and the raffinose-nonfermenting phenotype of *snf3* mutants results from the defect in hexose uptake. Double mutants carrying the null allele *snf3-Δ4::HIS3* exhibit the constitutivity for invertase characteristic of the *cid1*, *reg1* or *hxk2* parent; however, because these strains remain defective in hexose up-

TABLE 5

The *snf* mutations are epistatic to *reg1*, *cid1* and *hxk2*

Relevant genotype	Secreted invertase activity[a]							
	reg1-69		*cid1-226*		*hxk2-66*		Wild type	
	R	D	R	D	R	D	R	D
SNF	70	95	60	110	70	100	<1	200
snf1-Δ3	<1	<1	<1	<1	<1	2[b]	<1	<1
snf2-50	<1	3	<1	5	<1	3[b]	<1	4
snf3-Δ4::HIS3	70	200	90	160	70	180	1	170
snf4-319	<1	1	<1	1	ND	ND	<1	1
snf5-18	<1	3	<1	5	ND	ND	<1	4
snf6-719	1	15	1	20	ND	ND	<1	20

[a] Micromoles glucose released/minute/100 mg (dry weight) of cells. Values are the average of assays of two strains of the indicated genotype, and standard errors were <15%. R, repressed; D, derepressed; ND, not determined.

[b] Data taken from NEIGEBORN and CARLSON (1984); alleles were *hxk2-2* and *snf1-28*.

TABLE 6

Interactions of *tup1* with *snf1* and *snf2* mutations

Relevant genotype[b]	Secreted invertase activity[a]		Clumpy
	R	D	
Wild type	<1	200	No
snf1	<1	<1	No
snf2	<1	5	No
tup1	70	150	Yes
ssn6	300	380[c]	Yes
snf1 tup1	50	110	Yes
snf1 ssn6	200	270[c]	Yes
snf2 tup1	10	60	No
snf2 ssn6	10	80[c]	No

[a] Micromoles of glucose released/minute/100 mg (dry weight) of cells. Values are the average of assays of two spore clones. Standard errors were <15%. R, repressed; D, derepressed.

[b] Alleles were *snf1-28*, *snf2-50*, *tup1-18* and *ssn6-1*.

[c] Data from NEIGEBORN and CARLSON (1984).

take, they are unable to ferment raffinose or grow on medium containing sucrose plus 2-deoxyglucose.

***tup1* resembles *ssn6* in its interactions with *snf1* and *snf2*:** The *ssn6* mutations were previously shown to have different genetic interactions with the *snf* mutations than do *cid1*, *reg1* and *hxk2*. The *ssn6* mutations are epistatic to *snf1* and *snf4* with respect to invertase expression, and *ssn6 snf2* and *ssn6 snf5* double mutants display a phenotype intermediate between that of the parents: low-level constitutivity and moderate-level derepression (CARLSON *et al.* 1984; NEIGEBORN and CARLSON 1984). Another gene in which constitutive mutations have been isolated is *TUP1*. Phenotypically, *tup1* mutants resemble *ssn6* mutants in their invertase constitutivity, mating and sporulation defects and flocculence (WICKNER 1974; SCHAMHART, TEN BERGE and VAN DE POLL 1975; STARK, FUGIT and MOWSHOWITZ 1980). To determine whether *tup1* resembles *ssn6* in its genetic interactions with *snf* mutations, we examined the ability of *tup1* mutations to suppress the defects in invertase expression caused by *snf1* and *snf2*.

Since we did not recover any *tup1* alleles in this study, the *tup1-18* mutation (WICKNER 1974) was introduced into our genetic background (see MATERIALS AND METHODS). Tetrad analysis of diploids heterozygous for *tup1* and *snf1* or *snf2* showed 3+:1− segregations for raffinose utilization, indicating that *tup1* suppresses the raffinose-nonfermenting phenotype caused by *snf1* and *snf2*. Double mutants were assayed for secreted invertase under conditions of glucose repression or derepression (Table 6). Although strains carrying *tup1* are not isogenic to other strains in this study, the effects of *tup1* on invertase expression in *snf1* or *snf2* mutants are clearly similar to those of *ssn6*. Moreover, *tup1 snf1* and *ssn6 snf1* double mutants are as clumpy as the parent *tup1* and *ssn6* mutants, and *tup1 snf2* and *ssn6 snf2* strains are significantly less clumpy. These findings indicate that *tup1* resembles *ssn6* in its interactions with *snf1* and *snf2*.

DISCUSSION

We have selected mutants that are able to utilize sucrose in the presence of 2-deoxyglucose. We isolated 210 recessive mutations causing constitutive expression of secreted invertase, which fall into three major complementation groups. Two of these groups comprise alleles of the previously identified *REG1* (*HEX2*) and *HXK2* (*HEX1*) loci (ENTIAN and ZIMMERMANN 1980; MATSUMOTO, YOSHIMATSU and OSHIMA 1983). The third group identifies a new locus designated *CID1* (*c*onstitutive *i*nvertase *d*erepression). We were unable formally to exclude the possibility that *cid1* is allelic to *cat80* as *cat80* mutants were not constitutive in our hands. Studies carried out by K.-D. ENTIAN (personal communication) indicated that *cid1* and *cat80* complement. We did not recover any new *ssn6* or *tup1* alleles, although previously isolated mutations cause high-level constitutivity sufficient for growth on the selective medium.

The *cid1*, *reg1* and *hxk2* mutants produce moderate to high invertase activity under glucose-repressing conditions and produce wild-type levels under derepressing conditions. None shows the very high levels of constitutive and derepressed activity exhibited by *ssn6* mutants. Previous studies have shown that *reg1* and *hxk2* cause glucose-insensitive expression of other glucose-repressible genes (ENTIAN and ZIMMERMANN 1980; MATSUMOTO, YOSHIMATSU and OSHIMA 1983). In this work, we showed that a *cid1* mutation causes glucose-insensitive expression of maltase, but the allele that was tested does not affect the expression of a *GAL10-lacZ* fusion.

We report here analyses of the epistasis relationships between *cid1* and *reg1* and the *snf* mutations.

Double mutants carrying *cid1* or *reg1* and any one of the mutations *snf1*, *snf2*, *snf4*, *snf5* or *snf6* resemble the *snf* parent with respect to invertase expression. We have previously reported that *snf1* and *snf2* are epistatic to *hxk2* (NEIGEBORN and CARLSON 1984). These epistasis relationships suggest that *CID1*, *REG1* and *HXK2* function at earlier steps in the regulatory circuitry than do the *SNF* genes (except for *SNF3*). The hexokinase PII isozyme encoded by *HXK2* has an early catalytic function in glycolysis; however, the nature of its putative regulatory role in glucose repression (ENTIAN and FROHLICH 1983; ENTIAN *et al.* 1985) is not yet known. We suggest that *CID1*, *REG1* and *HXK2* may have sensory or signaling functions involved in monitoring the availability of glucose and perhaps regulate the activity of the protein kinase encoded by *SNF1* (CELENZA and CARLSON 1986).

Our findings also suggest that the *CID1*, *REG1* and *HXK2* genes are functionally distinct from *SSN6*. The *cid1*, *reg1* and *hxk2* mutants do not share many of the pleiotropic properties of *ssn6* mutants, and the epistasis relationships of *cid1*, *reg1* and *hxk2* with *snf* mutations are different from those of *ssn6*. The finding that *ssn6* is epistatic to *cid1*, *reg1* and *hxk2* for very high level constitutive invertase expression suggests that *SSN6* affects gene expression more directly than do *CID1*, *REG1* and *HXK2*. The phenotypic similarities between *ssn6* and *tup1* mutants prompted us to examine the genetic interactions between *tup1* and *snf1* and *snf2*. The epistasis relationships resemble those of *ssn6*, suggesting that *TUP1* may be functionally related to *SSN6*. We have previously suggested that the *SSN6* gene product is a candidate for a substrate of the *SNF1* protein kinase and for a direct negative regulator of *SUC2*, although other functions are also possible (CARLSON *et al.* 1984; CELENZA and CARLSON 1986).

We thank J. CELENZA for optimizing the conditions used for selecting mutants. We thank K.-D. ENTIAN for providing strains and communicating results. This work was supported by Public Health Service grant GM34095 from the National Institutes of Health and by an Irma T. Hirschl Research Career Award to M.C.

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Communicating editor: E. JONES