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Extragenic Suppressors of Yeast Glucose Derepression Mutants Leading to Constitutive Synthesis of Several Glucose-Repressible Enzymes

HANS-JOACHIM SCHÜLLER† AND KARL-DIETER ENTIAN*

Institute for Microbiology, Johann Wolfgang Goethe-Universität, Theodr-Stern-Kai 7, Haus 75A, D-6000 Frankfurt am Main, Federal Republic of Germany

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***Saccharomyces cerevisiae* regulatory genes *CAT1* and *CAT3* constitute a positive control circuit necessary for derepression of gluconeogenic and disaccharide-utilizing enzymes. Mutations within these genes are epistatic to *hxk2* and *hex2*, which cause defects in glucose repression. *cat1* and *cat3* mutants are unable to grow in the presence of nonfermentable carbon sources or maltose. Stable gene disruptions were constructed inside these genes, and the resulting growth deficiencies were used for selecting epistatic mutations. The revertants obtained were tested for glucose repression, and those showing altered regulatory properties were further investigated. Most revertants belonged to a single complementation group called *cat4*. This recessive mutation caused a defect in glucose repression of invertase, maltase, and iso-1-cytochrome *c*. Additionally, hexokinase activity was increased. Gluconeogenic enzymes are still normally repressible in *cat4* mutants. The occurrence of recombination of *cat1*::*HIS3* and *cat3*::*LEU2* with some *cat4* alleles allowed significant growth in the presence of ethanol, which could be attributed to a partial derepression of gluconeogenic enzymes. The *cat4* complementation group was tested for allelism with *hxk2*, *hex2*, *cat80*, *cid1*, *cyc8*, and *tup1* mutations, which were previously described as affecting glucose repression. Allelism tests and tetrad analysis clearly proved that the *cat4* complementation group is a new class of mutant alleles affecting carbon source-dependent gene expression.**

The yeast *Saccharomyces cerevisiae* is a suitable model organism to study the regulation of metabolism and gene expression. A very important regulatory mechanism which is referred to as glucose repression controls the expression of many enzymes involved in carbohydrate metabolism. In the presence of glucose, the synthesis of enzymes necessary for disaccharide (sucrose and maltose) or galactose utilization and for growth with nonfermentable carbon sources (ethanol, acetate, lactate, and glycerol) is repressed.

The regulatory system of glucose repression is represented by two different kinds of mutations. One group of mutations causes constitutive expression of glucose-repressible enzymes (glucose repression mutants), whereas the opposite type of mutations is characterized by its inability to derepress glucose-repressible enzymes when cells are transferred to nonfermentable carbon sources (glucose derepression mutants; for reviews, see references 5, 20, and 28).

Most glucose repression mutants were isolated by taking advantage of a selection system developed by Zimmermann and Scheel (59). Three complementation groups referred to as *hex1*, *hex2*, and *cat80* had repression defects for invertase, maltase, malate dehydrogenase, and respiratory enzymes (23). Hexokinase activity was strongly reduced in *hex1* mutants (25). Biochemical and genetic analysis showed that *hex1* corresponded to *hxk2* (18, 21), which is the structural gene of hexokinase PII (33). Several experiments proved that the function of hexokinase is indispensable in glucose repression. Although hexokinase PI is able to mediate glucose repression when overexpressed (34, 43c), isoenzyme PII is mainly responsible for glucose repression in wild-type cells because of its predominant catalytic activity. The *HEX2* gene probably encodes a nuclear protein which is supposed to act as a negatively regulating element and it turned out to be allelic to *reg1* (36, 43b). Interestingly, hexokinase PII activity was increased in *hex2* mutants (19), indicating a feedback inhibition among different components of the glucose repression system. The role of *cat80* (23) (*cat80* allelic to *grr1* [2, 43a]) and *cid1* (40) mutants also obtained by the selection system of Zimmermann and Scheel (59) is not yet known. Two other mutations causing defects in glucose repression are *cyc8=ssn6* (7, 44, 48, 53, 54) and *tup1=flk1=cyc9=umr7=amm1* (32, 44, 45, 50, 52, 53). (To facilitate understanding, allelic gene designations are shown together.)

Glucose derepression mutants show pleiotropic growth defects with different carbon sources because of their inability to derepress the enzymes required after the medium is exhausted of glucose. This regulatory system includes the *CAT1* (58) and *CAT3* (24) genes, which are allelic to *SNF1=CCR1* (6, 13, 15) and *SNF4* (39), respectively. The deduced sequence of the *CAT1=SNF1* gene product showed strong homology to protein kinase sequences and autophosphorylation was demonstrated in vitro (9). Further mutation analysis gave convincing evidence for the fact that the protein kinase activity is necessary for *CAT1=SNF1* to function in the derepression process (10). *CAT3=SNF4* shows no significant sequence similarity to genes coding for proteins of known function and probably encodes an auxiliary factor of the *CAT1=SNF1* protein kinase (10, 11, 47). Interaction of both proteins has been shown (11, 26), and cellular fractionation studies indicated a nuclear localization (47).

The stages of interaction between *trans*-acting compo-

* Corresponding author.
† Present address: Institut für Biochemie, D-852 Erlangen, Federal Republic of Germany.

TABLE 1. Strains used

Strain[a]	Genotype	Source
WAY.5-4A*	a *ura3 his3-Δ1 MAL2-8^c MAL3 SUC3*	W. Albig
WAY.6-2A*	a *ura3 leu2-3,112 his3-Δ1 HXK2::LEU2 MAL2-8^c* MAL3 SUC3	W. Albig
JS87.11-8C*	α *leu2-3,112 cat1::HIS3 MAL2-8^c MAL3 SUC3*	H.-J. Schüller
JS87.15-8A*	α *trp1 cat3::LEU2 MAL2-8^c MAL3 SUC3*	H.-J. Schüller
ENY.hex2-3A*	a *leu2-3,112 ura3-52 hex2-3 MAL2-8^c MAL3 SUC3*	K.-D. Entian
HEX2.S-6B	α *leu1 his4 hex2-3 MAL2-8^c MAL3 SUC3*	H.-J.Schüller
ENY.cat80-9B*	a *cat80-24 MAL2-8^c MAL3 SUC3*	K.-D. Entian
JS.cat80-3C*	a *his4 cat80-24 MAL2-8^c MAL3 SUC3*	H.-J. Schüller
HRX12-6D	α *trp1 ade1 cat1-12 MAL2-8^c MAL3 SUC3*	H.-J. Schüller
HRX35-2D	α *trp1 ade1 his4 cat1-35 MAL2-8^c MAL3 SUC3*	H.-J. Schüller
HRX42-10C	a *trp1 ade1 cat1-42 MAL2-8^c MAL3 SUC3*	H.-J. Schüller
HRX37-8A	a *trp1 ade1 his4 cat3-37 MAL2-8^c MAL3 SUC3*	H.-J. Schüller
RTY92	α *trp1 cyc8-20*	R. Trumbly
ENY.cyc8-2B*	α *cyc8-20 MAL2-8^c MAL3 SUC3*	K.-D. Entian
RTY110	a *ura3 leu2 his4 can1 tup1-100*	R. Trumbly
ENY.tup1-5C*	a *his3-Δ1 trp1-289 tup1-100 MAL2-8^c MAL3 SUC3*	K.-D. Entian
ENY.cid1-7C*	a *ura3-52 ade2-100$_{oc}$ cid1-226 MAL2-8^c MAL3 SUC3*	K.-D. Entian
MCY831	a *his4 ade2 cid1-226 mal0 SUC2*	M. Carlson
JS87.11-8C/39*	α *leu2-3,112 cat1::HIS3 cat4-1 MAL2-8^c MAL3 SUC3*	This work
JS87.11-8C/69*	α *leu2-3,112 cat1::HIS3 cat4-16 MAL2-8^c MAL3 SUC3*	This work
JS88.3-1A*	a *ura3 his3-Δ1 cat4-1 MAL2-8^c MAL3 SUC3*	This work
JS88.3-2A*	a *his3-Δ1 cat4-1 MAL2-8^c MAL3 SUC3*	This work
JS88.3-4D*	α *leu2-3,112 his3-1 cat4-1 MAL2-8^c MAL3 SUC3*	This work

[a] All strains marked with an asterisk are isogenic.

nents exerting different effects on carbon source-regulated genes are not known at present. Derepression genes *CAT1=SNF1* and *CAT3=SNF4* act positively on gene expression, while a negative influence is mediated by repression genes such as *HXK2*, *HEX2*, *CAT80*, *CID1*, *SSN6*, and *TUP1*. Both sets of genes regulate disaccharide- and galactose-utilizing enzymes, whereas gluconeogenic enzymes seem to be controlled only by the derepression system. The interaction of both systems was demonstrated by suppression studies and investigations of epistatic relationships. Mutant genes *cat1=snf1* and *cat3=snf4* are epistatic to *hex2=reg1* (24) and *hxk2=hex1* (40). Similarly, the *ssn6* mutant allele conferring glucose-insensitive synthesis of invertase was identified by its suppression of the non-sucrose-fermenting phenotype of a *snf1* mutant (7).

In this paper, we report on an approach to select suppressors of *cat1* and *cat3* null allele mutations. A new mutation, *cat4*, was identified, and its role in the glucose repression system was investigated.

MATERIALS AND METHODS

Yeast strains. Strains of *S. cerevisiae* used and constructed for this investigation are listed in Table 1. Auxotrophic and regulatory gene symbols have been explained previously (46). Mutations *cat80* (23), *cyc8*, *tup1* (53), and *cid1* (40) confer glucose-insensitive synthesis of invertase.

Media. Basic yeast growth medium has been described previously (46). For selecting revertants from *cat1* and *cat3* derepression mutants, synthetic complete media containing 2% raffinose, 2% maltose, and 2% galactose (SCRaf, SCMal, and SCGal, respectively) were used. For restored growth on nonfermentable carbon sources, YEP medium plus 2% glycerol and 3% ethanol (YEPGE) was used. Resistance of yeast strains to 2-deoxyglucose (DOG) was tested on SCRaf plates containing 150 ppm DOG. For derepression kinetics, cells were grown in YEPD medium, harvested by centrifugation, and subsequently resuspended in YEP medium containing 3% ethanol (YEPE).

Mutagenesis. For revertant selection of derepression mutants, the basic ethyl methanesulfonate (EMS) mutagenesis procedure was followed (27). Strains were treated with a final concentration of 1% EMS in 0.1 M potassium phosphate buffer (pH 6.5) for 1 h, giving survival rates of about 90%. After 8 h of incubation in YEPD medium, cells were washed and spread at different cell densities on selection medium SCRaf, SCMal, SCGal, and YEPGE, respectively.

Enzyme assays and protein determination. Invertase and maltase were assayed as previously described (58). β-Galactosidase was measured as previously described (30). For isocitrate lyase, the protocol given in reference 17 was followed. Specific activities are defined as nanomoles of substrate converted per minute and milligram of protein. Protein levels were estimated by the microbiuret method mentioned in reference 57.

Molecular yeast genetic techniques. Standard molecular yeast genetic techniques were performed as previously described (46).

Construction of a *cat1::HIS3* null allele. A 3.15-kb *Eco*RI fragment carrying the complete *CAT1* gene (46) was subcloned into pBR322. The resulting plasmid pJS63 was linearized at the unique *Bgl*II site and religated after insertion of the *HIS3* gene as a 1.75-kb *Bam*HI fragment, yielding plasmid pJS78 (Fig. 1). Prior to yeast transformation of strain WAY.5-4A, the *cat1::HIS3* insertion construction was released by *Eco*RI digestion.

Construction of *lacZ* fusions. A 2.2-kb *Xba*I-*Eco*RV fragment from plasmid pJS142 containing the complete *FBP1* structural gene (22) was transferred into *Xba*I-*Sma*I-cleaved *lacZ* fusion vector YEp358R (38), yielding pJS151 (*FBP1-lacZ*). A 1.9-kb *Sal*I-*Cla*I fragment from pLG669-Z (30) was inserted into YEp358R digested by the appropriate enzymes to obtain pJS177 (*CYC1-lacZ*). A *GAL10-CAT3-lacZ* fusion was constructed by ligating a 0.5-kb *Sau*3AI-*Hin*dIII frag-

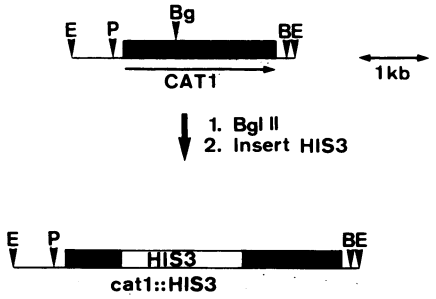

FIG. 1. Strategy of disrupting the chromosomal *CAT1* locus by insertion of a *HIS3* marker. E, *Eco*RI; P, *Pst*I; B, *Bam*HI; Bg, *Bgl*II.

ment from plasmid YEp52 (4) carrying the *GAL10* control region upstream to a promoterless *CAT3-lacZ* fusion with a *Hin*dIII linker at position −19 with respect to the start codon of *CAT3* (pJS168).

RESULTS

Construction of a *cat1::HIS3* null allele. We have previously described the isolation of yeast regulatory genes *CAT1* and *CAT3* (46). These genes are necessary for growth on maltose and on nonfermentable carbon sources. Allelism of *CAT1* with *SNF1* and *CAT3* with *SNF4* became obvious after comparing restriction maps and DNA sequences (8, 9, 11, 46, 47). Interestingly, the phenotypes of our strains carrying *cat1* and *cat3* point mutations obtained as extragenic suppressors of a *hex2* mutant allele (24) differed from those of *snf1* and *snf4* strains (6, 39). While *snf1* and *snf4* mutants were unable to utilize sucrose, raffinose, and galactose, several *cat1* and *cat3* strains grew well on these carbon sources. This phenotypic difference disappeared after several backcrosses with a new wild-type reference strain (WAY.5-4A). The same result was obtained by disruption of *CAT1* and *CAT3* genes at their chromosomal location in these wild-type strains. The correct disruption of the chromosomal *CAT1* locus was confirmed by Southern hybridization (data not shown). Backcrossing of transformant WAY.5-4A/B1 with an isogenic wild-type strain, subsequent sporulation, and final tetrad analysis gave a 2:2 segregation pattern of the progeny with respect to growth on nonfermentable carbon sources. All growth defects of a strain carrying *cat1::HIS3* null allele could be complemented by transformation with a plasmid carrying the wild-type *CAT1* gene.

Isolation of epistatic mutations suppressing *cat1* and *cat3* growth defects. For selection of extragenic suppressors, mutant strains JS87.11-8C (*cat1::HIS3*) and JS87.15-8A (*cat3::LEU2* [46]) were treated with EMS (see Materials and Methods) and subsequently spread on different selection media (SCRaf, SCMal, SCGal, and YEPGE) at a titer of 10^5 to 10^7 cells per plate. About 100 revertants were taken from each growth medium and further characterized. In a similar approach using a *snf1* strain, the *ssn6* allele showing constitutive invertase expression was identified (7). Therefore, we screened for further suppressor mutations leading to a similar phenotype. Revertants with moderate and extreme high constitutive invertase levels were obtained. Representatives showing restored growth on raffinose, maltose, and galactose as the sole carbon source were backcrossed with an isogenic wild-type strain (WAY.5-4A). All heterozygous diploid strains showed normal glucose repression, indicating recessive mutations. One of the mutants later referred to as *cat4-1* was sporulated, and tetrad analysis yielded a clear 2:2 segregation of spores with glucose-insensitive invertase expression. Backcrossing of a *cat4-1* segregant with the other revertants characterized by elevated invertase activity revealed a total of 21 allelic mutants from which 16 were obtained by suppression of the *cat1::HIS3* null allele and 5 were obtained as suppressors of the *cat3::LEU2* mutation. A summary of enzyme activities and growth behavior of different *cat1 cat4* and *cat3 cat4* mutants, respectively, is given in Table 2. The data show that all *cat4* mutant alleles were able to suppress growth defects of strains carrying *cat1* and *cat3* null alleles and grown on fermentable sugars (raffinose, maltose, and galactose), while restored growth with ethanol could be observed only with mutant alleles *cat4-2* and *cat4-16*.

Allelic relations of *cat4* to other mutant alleles conferring lack of glucose repression. The *cat4* strains were crossed with *hex1*, *hex2*, *cat80*, *cid1*, *cyc8*, and *tup1* mutants to test these mutations for possible allelism with mutations conferring a similar phenotype. In all cases, the resulting diploid strains showed complete glucose repression of invertase and maltase (Table 3), indicating *cat4* as a new mutant allele involved in glucose repression. Allelism of *cat4* and *cyc8=ssn6* (7) could also be ruled out because strains carrying those mutations have different phenotypes. The *ssn6* strains, like *tup1=flk1* mutants, showed a severe "flaky" phenotype and were unable to utilize nonfermentable carbon sources (7, 17a). This was not observed with *cat4* mutants. To ensure that *cat4* and *cyc8=ssn6* were nonallelic, tetrad analysis of a *cat4/CAT4 cyc8/CYC8* recom-

TABLE 2. Growth behavior and enzymatic characteristics of some *cat1* and *cat3* revertants belonging to the *cat4* complementation group

Strain	Genotype	Growth[a] on carbon source				Sp act (nmol/min/mg)	
		Raffinose	Maltose	Galactose	Ethanol	Invertase	Maltase
WAY.5-4A	*CAT1 CAT3 CAT4*	+	+	+	+	20	10
JS87.11-8C	*cat1::HIS3 CAT3 CAT4*	−	−	−	−	20	10
JS87.15-8A	*CAT1 cat3::LEU2 CAT4*	−	−	−	−	15	10
JS87.11-8C/39	*cat1::HIS3 CAT3 cat4-1*	+	+	+	−	5,400	230
JS87.11-8C/40	*cat1::HIS3 CAT3 cat4-14*	+	+	+	−	3,800	220
JS87.11-8C/69	*cat1::HIS3 CAT3 cat4-16*	+	+	+	+	4,400	120
JS87.15-8A/33	*CAT1 cat3::LEU2 cat4-2*	+	+	+	+	2,500	130
JS87.15-8A/35	*CAT1 cat3::LEU2 cat4-20*	+	+	+	−	4,100	75
JS87.15-8A/86	*CAT1 cat3::LEU2 cat4-21*	+	+	+	−	1,700	70

[a] +, Growth within 1 (for raffinose, maltose, and galactose) or 3 (for ethanol) days; −, no growth.

TABLE 3. Specific activity of invertase in wild type and glucose repression mutants after growth on YEP medium plus glucose

Strain	Genotype	Invertase activity (nmol/min/mg)	
		Haploid strains	Diploids obtained after mating with *cat4-1* strains
WAY.5-4A	Wild type	20	10
WAY.6-2A	*hxk2::LEU2*	2,800	70
ENY.hex-3A	*hex2-3*	2,266	40
JS.cat80-3C	*cat80-24*	2,300	10
ENY.cyc8-2B	*cyc8-20*	1,187	10
ENY.tup1	*tup1-100*	630	10
ENY.cid1-7C	*cid1-226*	1,168	60
JS88.3-1A	*cat4-1*	2,500	2,250

binant diploid was performed. This analysis resulted in a predominantly 3:1 segregation pattern of glucose-insensitive invertase synthesis and clearly proved that *cat4* and *ssn6* belong to different chromosomal loci.

Insensitivity of *cat4* mutants to DOG. Under appropriate conditions (see below), mutant alleles conferring glucose-insensitive invertase synthesis such as *hex1=hxk2* (structural gene for hexokinase PII), *hex2*, *cat80* (23), and *cid1* (40) simultaneously show insensitivity to DOG, a severe inhibitor of cell growth because of its toxic effects on cell wall synthesis (3). In fact, the selection procedure for these mutants was based exactly on this phenotypic characteristic (59). Therefore, we looked for DOG insensitivity of *cat4* strains in comparison with other mutants showing constitutive invertase synthesis. For this purpose, strains WAY.5-4A (wild type), WAY.6-2A (*hex1*), HEX2.S-6B (*hex2*), JS.cat80-3C (*cat80*), MCY831 (*cid1*), RTY92 (*cyc8*), RTY110 (*tup1*), and JS88.3-1A (*cat4*) were grown in YEPD, harvested in the early log phase, and spread on SCRaf selection medium containing DOG and as a control on SCRaf medium after complete removal of glucose. After 5 days, a similar number of colonies from all strains except wild type had grown on selection and control media, proving that all mutant strains exhibiting a repression-insensitive synthesis of invertase had comparable levels of resistance to DOG. These results show that the DOG selection system favors all glucose repression mutants investigated above.

Elevated hexokinase activity in glucose repression mutants. In a previous investigation, a significantly elevated hexokinase PII activity was observed in *hex2* mutants grown in glucose medium (19). A similar phenotype, although less pronounced, was found with the *cat80* mutant (20a; Table 4).

TABLE 4. Hexokinase activities in isogenic glucose repression mutants[a]

Heterozygous diploid strain	Genotype of segregants	No. of segregants	Sp act[b] (nmol/min/mg) of hexokinase		Q F/G
			Glucose	Fructose	
ENY.WA	Wild type	86	840	1,180	1.4
ENY.hex2	*hex2-3*	18	1,475	2,409	1.63
ENY.cat80	*cat80-24*	18	1,047	1,787	1.71
ENY.cyc8	*cyc8-20*	20	1,111	1,975	1.66
ENY.tup1	*tup1-100*	24	972	1,661	1.71
ENY.cat4	*cat4-1*	20	1,380	2,305	1.67

[a] Heterozygous diploids were sporulated, and the specific activity of hexokinase in the resulting segregants was determined after tetrad analysis.
[b] Mean values of different segregants.

The *cat4* strains also exhibited an enhanced hexokinase activity (Table 4). Hexokinase isoenzymes PI and PII differ in the ratio of phosphorylation of fructose to glucose (Q F/G; 21) which is about 3 for hexokinase PI and about 1.5 for hexokinase PII. Therefore, a Q F/G of 1.7 in *cat4*, *cat80=grr1*, *cyc8=ssn6*, and *tup1=flk1=cyc9=umr1=amm1* strains indicates that hexokinase PI expression may also be under regulatory control (Table 4). This was even more pronounced in *tup1=flk1=cyc9=umr1=amm1* strains with significantly elevated fructose phosphorylating activity, whereas glucose phosphorylating activity was nearly in the range of wild-type activity. Consequently, all glucose repression mutants (with mutations affecting disaccharide- and galactose-utilizing enzymes) with intact hexokinase structural genes show increased hexokinase activity after growth on glucose. This indicates a control on hexokinase synthesis operating in wild-type strains under repression conditions. The regulatory elements involved in this control and those regulating glucose repression are obviously identical.

Derepression of gluconeogenic pathway in *cat1::HIS cat4* recombinants. In further experiments, we examined expression of isocitrate lyase, which is a glucose-repressible enzyme representing the gluconeogenic pathway. Glucose-grown cells were harvested at early logarithmic growth phase and transferred to a medium containing ethanol as the sole carbon source. In wild-type strain WAY.5-4A, isocitrate lyase activity increased 2 h after transfer, and after 10 h, a specific activity of 85 nmol/min/mg was achieved (Fig. 2). As expected, strain JS87.11-8C carrying the *cat1::HIS3* null allele did not show any significant derepression of isocitrate lyase. However, in *cat1::HIS3 cat4* recombinants the derepression defect caused by *cat1::HIS3* was partially suppressed by the *cat4* mutation. After 10 h of derepression, strain JS87.11-8C/39 carrying mutant allele *cat4-1* had an isocitrate lyase activity of 17 nmol/min/mg and strain JS87.11-8C/69 carrying mutant allele *cat4-16* showed an isocitrate lyase activity of 32 nmol/min/mg, with both genes showing recombination with *cat1::HIS3* (Fig. 2). The *cat1::HIS3 cat4-1* recombinants derepressed isocitrate lyase levels to 17% of the wild-type level, and *cat1::HIS3 cat4-16* recombinants derepressed levels to 27% of the wild-type level. As JS87.11-8C/69 is able to utilize ethanol as the sole carbon source while JS87.11-8C/39 is not, this latter value obviously represents the threshold of isocitrate lyase activity necessary for growth under nonfermentative conditions. This partial suppression of further *cat1* defects by *cat4* mutant alleles shows that the *CAT4* gene not only influences expression of enzymes involved in disaccharide metabolism but is also of importance for the gluconeogenic pathway. The *cat4-1* mutant allele in recombination with a wild-type *CAT1* allele showed an even higher derepression rate than the wild type (Fig. 2). Our results indicate that the *CAT4* gene product has to be inactive in order to precede glucose derepression. This makes the *CAT4* protein a likely target for the *CAT1=SNF1* protein kinase.

Glucose repression in *cat4* mutants. Expression of additional genes subject to glucose repression in wild type and *cat4* mutants under repressed or derepressed conditions was determined by taking advantage of several *lacZ* fusions. A *GAL10-CAT3-lacZ* fusion was constructed for measuring galactose induction; similarly, *FBP1-lacZ* and *CYC1-lacZ* fusions represent expression of gluconeogenic (fructose-1,6-bisphosphatase) and respiratory enzymes (iso-1-cytochrome *c*). Table 5 gives a summary of specific activities of several glucose-repressible enzymes in wild-type and *cat4* mutant strains. The *cat4* mutant showed an elevation of GAL

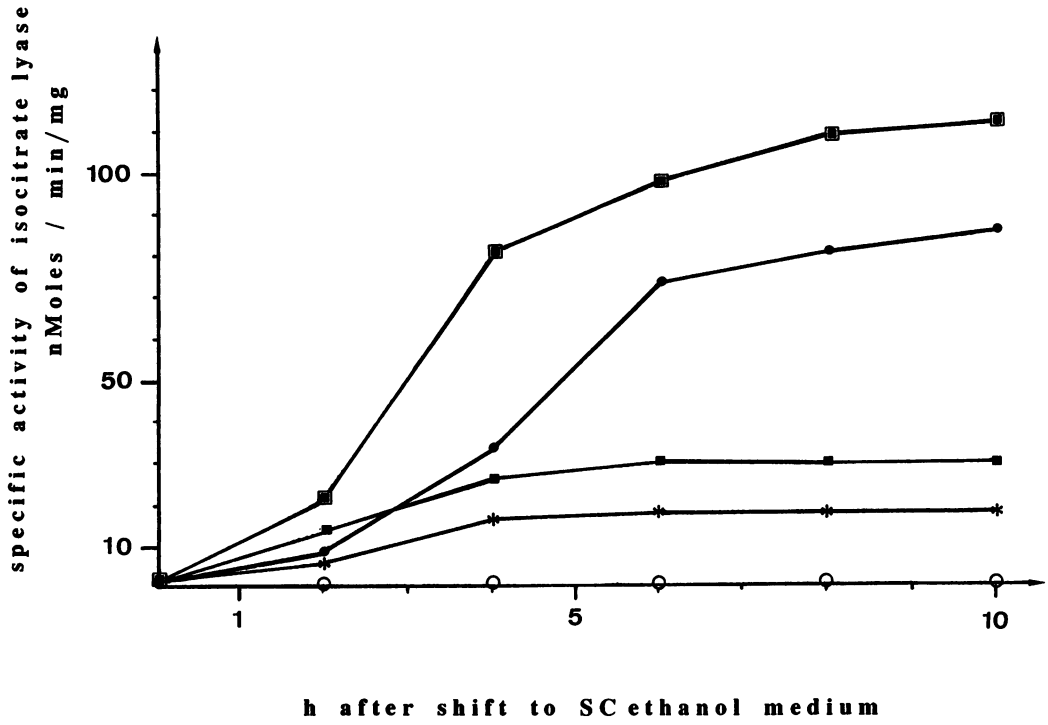

FIG. 2. Kinetics of isocitrate lyase derepression in wild type (WAY.5-4A [●]), *cat1*::*HIS3* mutant (JS87.11-8C [○]), *cat1*::*HIS3 cat4-1* recombinant (JS87.11-8C/39 [*]), *cat1*::*HIS3 cat4-16* recombinant (JS87.11-8C/69 [■]), and *cat4-1* mutant (JS88.3-1A [▣]). Glucose-grown cells were transferred to synthetic complete medium containing ethanol (see Materials and Methods).

TABLE 5. Specific activities of glucose-repressible enzymes in wild type and *cat4* mutant strains

Enzyme(s) tested	Sp act (nmol/min/mg)	
	Wild type	JS88.3-1A (*cat4*)
Invertase[a]	20	3,500
Maltase[a]	10	140
GAL enzymes[b]		
Repressed	<1	<1
Induced	1,400	1,700
Repressed/induced	7	1,200
FBPase[c]		
Repressed	7	20
Derepressed	1,400	1,200
Cytochrome *c*[d]		
Repressed	100	530
Derepressed	1,030	700

[a] Specific activities of cells grown in YEPD (glucose-repressed conditions).

[b] Specific β-galactosidase activities on strains transformed with a *GAL10-CAT3-lacZ* fusion plasmid (pJS168, see Materials and Methods). Glucose-repressed cells were grown in SCD-Ura. For *GAL10* induction, SCD-Ura-grown cells were transferred to SCGal-Ura for 6 h. To measure glucose repression under galactose-induced conditions, cells were pregrown in SCD-Ura and transferred to SCDGal-Ura medium for 6 h.

[c] Specific β-galactosidase activities of strains transformed with a *FBP1-lacZ* fusion plasmid (pJS151; see Materials and Methods). Glucose-repressed cells were grown in SCD-Ura. For derepression, SCD-Ura-grown cells were transferred for 10 h in SCE medium.

[d] Specific β-galactosidase activities of strains transformed with a *CYC1-lacZ* fusion plasmid (pJS177; see Materials and Methods). Glucose-repressed cells were grown in SCD-Ura. For derepression, SCD-Ura-grown cells were transferred for 10 h in SCE medium.

enzyme expression under repressed conditions in the presence of the inducer galactose. Glucose repression of fructose-1,6-bisphosphatase in the *cat4* mutant was similar to that in the wild type. Interestingly, repression of the respiratory enzyme iso-1-cytochrome *c* was nearly abolished in *cat4* mutants. To summarize these results, one can say that the *cat4* mutant allele conferred a pleiotropic lack of glucose repression for invertase, maltase, and respiratory functions.

DISCUSSION

Glucose repression in *S. cerevisiae* is a complex regulatory network affecting the expression of many genes encoding enzymes involved in carbohydrate and energy metabolism. Glucose and fructose (substrates which can be metabolized easily by glycolysis) suppress the synthesis of enzymes necessary only for the utilization of less favorable carbon sources. Several regulatory genes constitute a signal transduction system transmitting a change in carbon source availability from the cell surface to the transcriptional apparatus. Two different kinds of mutants with altered regulatory properties that show antagonistic effects on their target genes have been isolated. Glucose repression mutants show constitutive derepression of enzymes whose synthesis is regulated in the wild type over at least 2 orders of magnitude. On the other hand, derepression mutants exhibit a nearly constant level of repressed enzyme activities even under conditions of altered nutritional requirements leading to growth defects on several carbon sources.

Mutants lacking glucose repression have been described by many investigators. Selection for mutants which are insensitive to DOG on a raffinose medium as a result of constitutive invertase synthesis led to the identification of

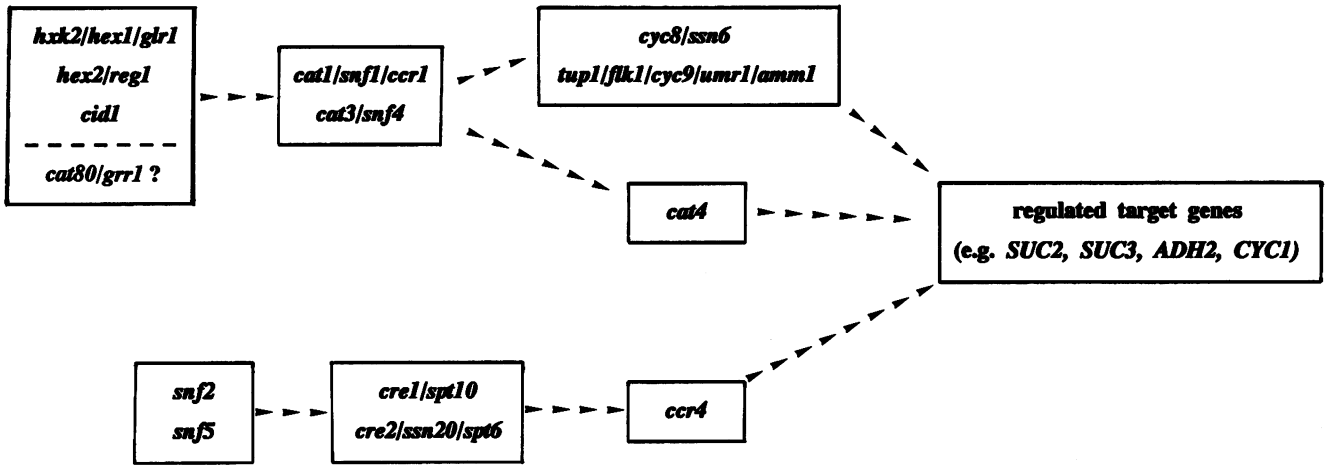

FIG. 3. Epistatic relationships of regulatory mutations involved in glucose repression. Some of them have more specific functions in glucose repression than others, which fulfill more general functions in transcription (*snf2*, *snf5*, *cre1*, *cre2*, and *ccr4*). Mutants of similar phenotypes and epistatic positions are shown in the same box. The presentation of converging pathways controlling carbon source-dependent gene expression is hypothetical and is inferred from the results of suppression studies. Interactions between components of different subgroups are not excluded but may occur at stages presently unknown. The epistatic relationships of *CAT80* to the other mutations are not known at present.

four complementation groups referred to as *hex1=hxk2=glr1* (23, 37), *hex2=reg1* (23, 36, 43b), *cat80=grr1* (2, 23, 43a, 59), and *cid1* (40). Two additional complementation groups, *cyc8=ssn6* (7, 44, 48, 53, 54) and *tup1=flk1=cyc9=umr7=amm1* (32, 45, 48, 50, 52), were isolated by quite different strategies and cover a wide range of phenotypes. Apart from constitutive expression of several glucose-regulated enzymes, both mutants show severe clumping, misregulation of mating factor production, and inability to sporulate as homozygous diploids. Additionally, *tup1* strains are characterized by uptake and incorporation of dTMP into DNA (55) and enhanced stability of minichromosomes carrying altered ARS elements (52). *CYC8=SSN6* contains the recently described tetratricopeptide repeat motif shared among some genes required for cell cycle function and RNA synthesis (49). These results support the idea that *CYC8* and *TUP1* fulfill a general function in cellular physiology, perhaps in the basic transcriptional machinery or as nonspecific DNA-binding proteins.

Similarly, mutants with pleiotropic derepression defects such as *snf2*, *snf5* (1, 39), and *ccr4* (15) might also be components of the basic apparatus of gene expression. *snf2* and *snf5* mutations are suppressed by *ssn20* (42), which is allelic to *cre2* (15) and *spt6* (56). These mutant alleles allow expression of several regulated genes such as *SUC2*, *HIS4*, and *PHO5* even in the absence of their respective upstream activation sites (14, 41). Finally, *ccr4* was proved to be epistatic to *cre1=spt10* and *cre2=ssn20=spt6* (16). A hypothetical scheme presenting epistatic relations among several mutants involved in a specific or nonspecific manner in the glucose repression network is shown in Fig. 3.

A further mutant allele conferring constitutive synthesis of several glucose-repressible enzymes is described in this paper. The *cat4* mutation, like *cyc8* and *tup1*, is epistatic to the derepression deficiencies of invertase, maltase, and GAL enzymes caused by mutant allele *cat1* or *cat3*. Interestingly, expression defects of gluconeogenic enzymes are partially suppressed by *cat4*. This is contrary to the phenotype of *cyc8* and *tup1* mutants, which cause considerable derepression defects for isocitrate lyase and malate synthase (45a), thereby explaining the growth deficiency of a strain carrying at least a *cyc8* null allele on medium containing ethanol or glycerol as the sole carbon source (7, 17a). The reason for this puzzling behavior of *cyc8* mutants (constitutive derepression of invertase, maltase, and cytochrome *c*, but lack of derepression for gluconeogenic enzymes) is not known at present. Analysis of the *cat4* mutation gave further insights into the regulatory subpathways in carbohydrate metabolism, as it suggests the existence of different control elements for enzymes involved in disaccharide utilization and in the gluconeogenic pathway necessary under nonfermentative growth conditions. Both pathways require derepression genes *CAT1=SNF1* and *CAT3=SNF4* as positive effectors; on the other hand, repression mutations, such as *hxk2*, *hex2*, *cat80*, and *cid1*, confer glucose-insensitive expression of invertase and maltase, but not for components of nonfermentative metabolism pathway. The partial suppression of derepression defects of glyoxylate cycle enzymes in *cat1 cat4* recombinants is a new phenotypic characteristic that was not found for the other pleiotropic repression mutations mentioned above. This shows that the *CAT4* gene product fulfills an important function for all subpathways of the glucose repression network. *CAT4* seems to play a specific role in the glucose repression/derepression system, as it does not show further phenotypic characteristics such as mating defects or clumping observed for *cyc8* and *tup1* strains.

As we have shown, all seven pleiotropic mutants showing glucose-insensitive expression of invertase could grow on SCRaf plates containing DOG. This is not surprising for *hxk2*, *hex2*, *cat80*, and *cid1* mutants because they were isolated this way (40, 59). Interestingly, no members of the *cyc8*, *tup1*, or *cat4* complementation group were isolated with the selection system of Zimmermann and Scheel (59). It is unlikely that these mutants might have escaped detection, as their generation times do not differ from those of *hxk2*, *hex2*, *cat80*, and *cid1* mutants. The reason for this result remains unclear, but the different genetic backgrounds of the strains used might be responsible. We used strains carrying the *SUC3* gene while previous suppression studies were done in a *SUC2* background (7). Although both *SUC* genes

show similar regulatory properties, a comparative sequence analysis of their upstream promoter elements revealed significant differences (31), possibly allowing a distinctive response to *trans*-acting components.

In examining the epistatic relationships of both subgroups with respect to *cat1* and *cat3* derepression mutants, different positions in the regulatory hierarchy became obvious (Fig. 3). *HXK2*, *HEX2*, *CAT80*, and *CID1* genes might play a role in the signal transduction requiring a functional *CAT1/CAT3* complex. On the other hand, *CYC8*, *TUP1*, and *CAT4* probably have a more direct negative effector function, as their corresponding mutant alleles can circumvent the need for a functional *CAT1* protein kinase system.

At present, the biochemical function of only a few of the regulatory components considered so far is known. Hexokinase PII (or, if adequately overexpressed, also isoenzyme PI; 34, 43c) probably plays a central role in detecting the glucose repression signal, i.e., the availability of a favorable carbon source such as glucose or fructose is recognized at the beginning of its metabolism, leading to a triggering event of unknown character. Sugar uptake systems might be also involved in this process, as indicated by the altered regulatory behavior typical for some missense mutants of the high-affinity glucose uptake system (*SNF3* gene product; 12, 43). However, *snf3* mutant analysis indicated that the repression defect might be the result of a second mutation in a gene tightly linked to *SNF3* (35). Because of the immediate transient cyclic AMP signal measured in cells transferred from nonfermentable carbon sources to a glucose medium (51), the interconnection between glucose repression and the more generally acting *CDC25/RAS/CYR* system (29) could also be of importance for signaling events. Unfortunately, components acting further downstream mediating the repressed/derepressed state at the transcriptional level are poorly understood. Such a regulatory function in the effectory part of the signal transduction pathway might be fulfilled by the *CAT4* gene product. Its importance is stressed by the suppression of all growth defects caused by a *cat1* or *cat3* mutation (at least in part) when combined with *cat4*. The epistatic position of *cat4* resembles that of *cyc8* and *tup1*. However, *cat4* mutants have a more specific phenotype that possibly indicates a specific function in glucose repression and derepression.

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