New Genes Involved in Carbon Catabolite Repression and Derepression in the Yeast Saccharomyces cerevisiae

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A mutation causing resistance to carbon catabolite repression in gene *HEX2*, mutant allele *hex2-3*, causes an extreme sensitivity to maltose when in combination with the genes necessary for maltose metabolism. This provided a convenient system for the selective isolation of mutations in genes specifically required for maltose metabolism and other genes involved in general carbon catabolite repression. In addition to reversion of the *hex2-3* allele, mutations in three other genes were detected. These genes were called *CAT1*, *CAT3*, and *MUR1* and in a mutated form abolished maltose inhibition caused by mutant allele *hex2-3*. Mutant alleles *cat1* and *cat3* also restored normal repression in the presence of the *hex2-3* allele. Segregants having only mutant alleles *cat1* or *cat3* were obtained by tetrad analysis. These segregants could not grow on nonfermentable carbon sources. Mutant alleles of gene *CAT1* were allelic to a mutant allele *cat1-1* previously isolated (Zimmermann et al., Mol. Gen. Genet. 151:95–103). Such mutants prevented derepression not only of the maltose catabolizing system, the selected property, but also of glyoxylate shunt and gluconeogenic enzymes. However, respiratory activities and invertase formation were not affected under derepressing conditions. *cat3* mutants had the same phenotypic properties as *cat1* mutants. This showed that carbon metabolism in yeast cells is under a very complex and ramified control of repressing and derepressing genes, which are interdependent.

Carbon catabolite repression adapts the carbon metabolic machinery of the yeast *Saccharomyces cerevisiae* to the utilization of the most convenient carbon source. This is to say that in the presence of glucose, fructose, or mannose, enzymes of the glyoxylate shunt and gluconeogenesis are fully repressed and enzymes of the tricarboxylic acid cycle and the respiratory system are very low (17, 27, 28, 34). Other enzymatic systems like those involved in the catabolism of α-glucosides (33) and β-fructofuranosides (18) are also subject to a repression by those three hexoses. Analogous to a similar situation in *Escherichia coli*, the term catabolite repression coined by Magasanik (22) is also used in yeasts.

In *E. coli*, carbon catabolite repression is apparently under the control of a single gene coding for a protein which in combination with cAMP activates transcription at promoters of operons coding for enzymes involved in sugar catabolism (7, 9, 21, 26, 30, 32, 39). Genetic analysis in yeasts, however, has provided several mutants which alleviated carbon catabolite repression. The first report was by Montenecourt et al. (24) who selected for nonrepressible

invertase. Schamhart et al. (29) reported also on a mutant with nonrepressible synthesis of certain enzymes. The molecular basis of the defects in these mutants is so far unknown.

A very effective selection system for the isolation of mutants with nonrepressible synthesis of yeast invertase was described by Zimmermann and Scheel (38). Three mutant classes, *hex1*, *hex2* and *cat80* mutants, wild-type designations *HEX1*, *HEX2*, and *CAT80*, were identified (14, 38). *hex1* mutants were no longer repressible by glucose for invertase, maltase, malate dehydrogenase, and respiratory enzymes. Hexokinase activity was decreased to about two-thirds of wild-type activity (15). Purification of hexokinase isoenzymes PI and PII in *hex1* and wild-type mutants clearly indicated that hexokinase PII was absent in this *hex1* mutants (13). Moreover, mutants with structural gene defects for hexokinase PI or PII originally obtained by Lobo and Maitra (20) were used in allelism tests by Entian (10) who could show that *hex1* was allelic to *hxk2*, the structural gene locus for hexokinase PII. Therefore, hexokinase PII was proposed to be the recognition site of carbon

catabolite repression (13). The second class of mutants was assigned to gene *HEX2*, a centromer-linked locus close to the *TRP1* gene on chromosome IV (Zimmermann, unpublished results) *hex2* mutants showed elevated hexokinase activities when grown on fermentable sugars and were extremely sensitive to maltose (11). Increased hexokinase activity could be attributed to increased hexokinase PII synthesis in the presence of glucose in the medium (12). A third locus was called *CAT80*, the mutant derivatives of which showed normal hexokinase activity (14).

Recently, Michels and Romanowski (23) reported on nonrepressible yeast mutants with properties similar to those originally isolated by Zimmermann and Scheel (38). Although they initially felt that their mutants were different, allelism tests with hexokinase mutants also indicated allelism to *hxk2* (C. A. Michels, K. M. Hanenberger, and Y. Sylvestre, Abstr. Meeting Mol. Biol. Yeast, Cold Spring Harbor, 1981, p. 172). An entirely different type of mutation was isolated by Ciriacy (5) who found a dominant mutation in a gene *CCR80* which alleviated catabolite repression of mainly respiratory and gluconeogenic enzymes.

A different type of mutants, *cat1-1*, was described previously (37). This recessive mutation prevented derepression of enzymes of the glyoxylate shunt, gluconeogenesis, the maltose uptake system, and retarded derepression of maltase synthesis. It had only slight effects on derepression of the respiratory system and did not at all affect invertase formation. This locus *CAT1* was also identified by a dominant mutant allele *CAT1-2*^{d} which caused a rapid derepression of all enzymes after consumption of glucose in growth media. The same properties were observed in a suppressor mutation for the *cat1-1* defective mutant allele. This suppressor was recessive in contrast to dominant *CAT1-2*^{d} and called *cat2-1*, the wild-type allele being *CAT2*. Zimmermann et al. concluded that there are two sets of genes involved in catabolite repression: one set mediates repression proper, whereas an additional set of genes is involved in the derepression process. A similar set of genes was described by Ciriacy (4). They were called *CCR1*, *CCR2*, and *CCR3*. Mutants of the first gene turned out to be allelic to *cat1-1* mutants. Mutants of the other two genes were of different phenotype and did not show any effects on maltase derepression.

In this study, the extreme sensitivity of *hex2* mutants to maltose allowed to select for further genes involved in carbon catabolite repression. It turned out the alleviation of repression caused by the *hex2-3* mutation was not sufficient for a nonrepressible synthesis of maltase. There was an additional requirement for functional genes *CAT1* and *CAT3* which are also involved in the derepression process.

MATERIALS AND METHODS

The strains were SMC-1B/3 (a *his4 MAL2-8*^{c} *MAL3 SUC3 CAT1-2*^{d} *hex2*), BS.3-12A (α *leu1 MAL2-8*^{c} *MAL3 SUC3 CAT1-2*^{d} *hex2*), Z9.3A-1D (α *leu1 MAL2-8*^{c} *MAL3 SUC3 cat1-1*), cat2.3-2A/18 (a *his4 MAL2-8*^{c} *MAL3 SUC3 hex1*), and SMC-19A (α *leu1 MAL2-8*^{c} *MAL3 SUC3*). a and α refer to mating type, and *his4* and *leu1* cause nutritional requirement for histidine-respective leucin. *MAL2-8*^{c} causes largely constitutive, but still glucose-repressible, synthesis of maltase (36). *MAL3* allows for an induced synthesis of maltase, whereas *SUC3* is the structural gene for invertase (19). *CAT1-2*^{d} is a dominant mutant allele which causes a faster derepression of various enzymes after growth on glucose (37). *hex1* causes a pleiotropic defect in carbon catabolite repression (14, 38) and is the structural gene for hexokinase PII (10, 13). *hex2* causes similar defects in catabolite repression (14, 38) and causes increased hexokinase PII synthesis (12).

Media. YEP medium consisting of 1% yeast extract (Difco Laboratories) and 2% peptone (Difco) was used as the basic medium, supplemented with 4% hexoses or 3% ethanol. Minimal media contained 0.67% yeast nitrogen base (Difco) and 2% glucose. Tetrad analysis was performed as described previously (37).

Repression of enzymes was estimated in cells grown on YEP 4% glucose media for more than 16 h. Only logarithmically growing cell suspensions were harvested. For derepression, glucose-grown cells were washed twice with potassium phosphate buffer (pH 6.5) and were suspended in YEP 3% ethanol medium. Crude extracts were prepared by shaking cells with glass beads (3). We added 2 ml of potassium phosphate buffer (pH 6.5) to the suspension before centrifuging it at 5,000 × *g* for 15 min. Supernatant was used as crude extract.

Enzyme assays. Total α-glucosidases were tested with *p*-nitrophenyl-α-D-glucopyranoside as substrate (37), hexokinase as in reference 1, isocitrate lyase as in reference 8, and fructose-1,6-bisphosphatase as in reference 16. Protein was estimated with bovine serum albumine as a standard, using the microbiuret method (35). Adsorption was measured at 290 nm. Specific activities of enzymes are expressed as nanomoles of substrate converted per minute per milligram of protein. Respiratory activity was measured with a Beckman oxygen electrode as described by Sims and Barnett (31).

RESULTS

Isolation of hex2 revertants. Revertants to maltose insensitivity were selected on media consisting of YEP 2% maltose or YEP 2% maltose plus 2% glucose. Two kinds of revertants were expected: (i) reversions at the *hex2* locus and (ii) mutations in genes that are epistatic over the *hex2* allele, yielding phenotypical wild-type cells. The latter mutants were to divide into two classes (i) epistatic mutant alleles which were recessive in combination with their

TABLE 1. Specific activities of fructose-1,6-bisphosphatase, isocitrate lyase, maltase, and invertase after 15 h of derepression in wild-type *cat1*, *cat3*, and *mur1* mutants

Mutant	Sp act[a]			
	Fructose-1,6-bisphosphatase	Isocitrate lyase	Maltase	Invertase
Wild type	35	108	180	524
cat1	0.3	3.2	1.3	427
cat3	0.2	2.8	6.2	693
mur1	29	98	138	447

[a] Specific activity is expressed as nanomoles of substrate converted per minute per milligram of protein.

respective wild-type alleles and (ii) epistatic mutant alleles which were dominant. By using a replica-plating technique, all revertants obtained were automatically tested for dominance. To achieve this, all plates with revertant colonies were replica plated onto a lawn of a strain with *hex2* mutant allele of opposite mating type on plates with a glucose medium. After overnight incubation, these plates were replica plated onto plates with a minimal medium where only diploids could grow. After another overnight incubation, the minimal medium plates were replica plated onto a maltose medium where only diploids with a dominant maltose resistance could 'grow. Thus, the recessive mutant alleles were easily detected.

Recessive epistatic mutant alleles. We isolated 78 clones with recessive epistatic mutant alleles. Allelism tests showed that the clones belonged to three mutant classes. Genes were called *CAT1*, *CAT3*, and *MUR1* (maltose utilization regulatory gene), and mutant alleles were called *cat1*, *cat3* and *mur1*. One representative of each class was crossed with the *hex2-3* mutant and sporulation was followed. Growth in the presence of maltose was used as indicator for either wild-type segregants or segregants having mu-

tant allele *hex2-3* in combination with an epistatic mutant allele abolishing maltose inhibition. Most tetrads were of the tetra type consisting of three segregants that were not inhibited by maltose and one sensitive segregant.

Isolation of alleles *cat1*, *cat3*, and *mur1* depended on selection conditions. *mur1* mutants were obtained on both kinds of selection media, whereas *cat1* and *cat3* mutants were only obtained on YEP 2% glucose plus 2% maltose medium. Obviously, *cat1* and *cat3* mutants were not able to use maltose as the sole carbon source. Allelism tests showed that *cat1* mutants were allelic to the *cat1-1* mutant previously isolated (37), whereas *cat3* mutants were not allelic to this mutant allele. *cat1* and *cat3* mutants had the same phenotype. They could not derepress isocitrate lyase, fructose-1,6-bisphosphatase, and maltase and were unable to grow with glycerol as the sole carbon source, although they were not respiratory deficient. *mur1* mutants derepressed like wild-type mutants (Table 1).

Effects of alleles *cat1*, *cat3*, and *mur1* on catabolite repression. Mutants having the allelic combinations *hex2-3 cat1* or *hex2-3 cat3* were not able to grow on YEP glycerol and on YEP maltose, but were no longer inhibited by maltose. Moreover, the defect in catabolite repression was suppressed, and hexokinase activity was similar to that of the wild-type mutant (Table 2). This clearly indicated that *cat1* and *cat3* did not solely prevent maltase synthesis which provides the basis for maltose inhibition, but also abolished all defects in catabolite repression caused by mutant allele *hex2-3*. In contrast to this, the *mur1* mutant allele had no effects on the action of the *hex2-3* allele other than suppressing maltose inhibition without affecting maltase synthesis (Table 3).

When *cat1* and *cat3* mutant alleles were combined through crosses with mutant allele *hex1*, the structural gene mutant allele for hexokinase PII, which relieves catabolite repression fruc-

TABLE 2. Carbon catabolite repression, hexokinase activity, and growth behavior of wild-type, *hex2*, *cat1*, and *cat3* mutants and *hex2 cat1* and *hex2 cat3* recombinants

Mutant or recombinant	Sp act after growth on YEP 4% glucose[a]						Growth on		
	Fructose-1,6-bisphosphatase	Isocitrate lyase	Maltase	Invertase	Malate dehydrogenase	Hexokinase	Glycerol	Maltose	Glucose and maltose
Wild type	0.7	2.8	1.9	12.4	169	622	+	+	+
hex2	0.6	3.2	124	2,118	1,304	1,878	+	−	−
cat1	0.5	1.9	1.0	8.2	131	691	−	−	+
cat3	0.2	2.8	2.4	9.0	155	701	−	−	+
hex2 cat1	0.8	3.2	6.9	7.1	147	637	−	−	+
hex2 cat3	0.4	1.8	7.3	4.9	163	599	−	−	+

[a] Specific activity is expressed as nanomoles of substrate converted per minute per milligram of protein.

TABLE 3. Carbon catabolite repression, hexokinase activity, and growth behavior of wild-type, *hex2*, *cat80*, and *murl* mutants and *hex2*, *murl* and *hex2 cat80* recombinants

Mutant or recombinant	Sp act after growth on YEP 4% glucose[a]						Growth on		
	Fructose-1,6-bisphosphatase	Isocitrate lyase	Maltase	Invertase	Malate dehydrogenase	Hexokinase	Glycerol	Maltose	Glucose and maltose
Wild type	0.9	4.1	2.8	7.2	172	722	+	+	+
hex2	0.6	3.2	124	2,118	1,304	1,878	+	−	−
cat80	1.2	2.6	112	1,229	979	630	+	+	+
murl	0.3	1.2	2.2	6.3	129	582	+	+	+
hex2 murl	0.7	0.9	131	1,812	1,101	1,712	+	+	+
hex-2 cat80	1.0	2.1	147	1,629	1,106	765	+	−	−

[a] Specific activity is expressed as nanomoles of substrate converted per minute per milligram of protein.

tose-1,6-bisphosphatase (gluconeogenesis) and isocitrate lyase (glyoxylate shunt), could not be derepressed. However, carbon catabolite repression of maltase, invertase, and malate dehydrogenase was defective like in a *hexl* single mutant (Table 4). Hence, *catl* and *cat3* were not epistatic over *hexl-18*. Although maltase synthesis was not repressed in *hexl catl* and *hexl cat3* recombinants no growth with maltose as the sole carbon source was observed. Clearly, the inability of *catl* and *cat3* mutants to grow with maltose depended on the effects of these alleles on the maltose uptake system.

In the course of these experiments, the combination of the previously isolated mutant alleles *hex2-3* and *cat80-24* was investigated after appropriate crosses had been performed. The only difference between a *hex2-3* single mutant and a *hex2-3 cat80-24* double mutant was that the elevated hexokinase activity on glucose media had been eliminated in the double mutant. Hence, the *CAT80* gene product is necessary for elevated hexokinase activity in *hex2-3* mutants.

DISCUSSION

Carbon catabolite repression in yeast appears to be quite a complex system. First, there are genes which can mutate to allelic conditions

which prevent repression of enzymes whose synthesis is subject to carbon catabolite repression. Such genes are exemplified by *HEX1*, *HEX2*, and *CAT80*. Recessive alleles of those three genes alleviate repression of invertase, α-glucosidase, and malate dehydrogenase. Second, an additional set of genes can mutate to allelic conditions which do not allow derepression of several enzymes the synthesis of which is subject to carbon catabolite repression. These are genes *CAT1* and *CAT3* of this communication and the two genes of Ciriacy (4), *CCR2* and *CCR3*. An additional gene involved in the derepression process is *CAT2*, which can mutate to recessive allelic condition which accelerates derepression (37).

All mutants so far obtained showed pleiotropic regulatory defects. Analysis of interactions of these genes is complicated by the fact that different repressible enzymes are under the regulatory control of overlapping, but not identical, sets of genes. Invertase and malate dehydrogenase appear to be under the control of only genes *HEX1*, *HEX2*, and *CAT80*. Extensive searches for additional mutants affecting glucose repression of invertase have not yielded any further genes (M. K. Grossmann, Ph.D. thesis, Technischen Hochschule Darmstadt, Germany,

TABLE 4. Carbon catabolite repression, hexokinase activity, and growth behavior of wild-type, *hexl*, *catl*, and *cat3* mutants and *hexl catl* and *hexl cat3* recombinants

Mutant or recombinant	Sp act after growth on YEP 4% glucose[a]						Growth on		
	Fructose-1,6-bisphosphatase	Isocitrate lyase	Maltase	Invertase	Malate dehydrogenase	Hexokinase	Glycerol	Maltose	Glucose and maltose
Wild type	0.9	4.1	2.8	7.2	172	722	+	+	+
hexl	1.2	3.7	285	1,112	1,502	326	+	+	+
catl	0.5	1.9	1.0	8.2	131	691	−	−	+
cat3	0.2	2.8	2.4	9.0	155	701	−	−	+
hexl catl	1.1	2.3	337	1,030	1,280	293	−	−	+
hexl cat3	2.1	3.7	403	987	1,321	370	−	−	+

[a] Specific activity is expressed as nanomoles of substrate converted per minute per milligram of protein.

1981). In contrast to invertase, maltase is under a more complex regulatory surveillance. *CAT1* and *CAT3* affect the synthesis of this enzyme to some extent, whereas *CCR2* and *CCR3* have no effect on it (4).

The most stringent control is exerted on the enzymes of the glyoxylate shunt and gluconeogenesis. These two sets of enzymes are also the most sensitive to repression by all fermentable sugars, even galactose (K.-D. Entian, Ph.D. thesis, Technischen Hochschule Darmstadt, Germany, 1978). Consequently, catabolite repression can be divided into two major segments. The completely repressible sector is represented by gluconeogenic enzymes and glyoxylate cycle enzymes, which also take part in gluconeogenesis (25). These enzymes are under strict derepression control of genes *CAT1*, *CAT3*, *CCR2*, and *CCR3*. Mutation in genes *HEX1*, *HEX2*, and *CAT80* does not release them from repression. Only gene *CCR80* can mutate to a state of nonrepressible malate synthase, isocitrate lyase, and fructose-1,6-bisphosphatase (5). The other major segment affects those activities which are not repressed on a galactose medium and controlled by gene *HEX1*, the structural gene for hexokinase PII (10, 13). There are differences within this group. Respiration and some respiratory enzymes have been shown to be affected by mutation in genes *HEX1* and *HEX2*, whereas there seems to be no influence on respiration by *CAT80*. On the other hand, respiration cannot derepress properly in *CCR2* and *CCR3* (4). Further diversification can be observed within the maltose metabolizing system. Gene *HEX2* exerts an important role on maltose uptake (11) and maltase synthesis. Mutant allele *cat1-1* eliminates maltose utilization and abolishes maltose inhibition when in combination with mutant allele *hex2-3*. Apparently, it is the uptake system that is severely affected. This agrees with the observation that *hex1 cat1* recombinants did not grow with maltose as a sole carbon source, although maltase synthesis was not repressed by glucose.

Synthesis of hexokinase PII seems to be also influenced by glucose. Mutation in gene *HEX2* leads to increased hexokinase PII synthesis (12). Combination with mutant allele *cat80-24* eliminates this effect, which indicates that *CAT80* is another regulatory element involved in the regulation of hexokinase PII synthesis.

An unexpected complication for the understanding of gene interactions in carbon catabolite repression comes from recent studies (6) which led to the identification of four different genes (*TYE1*, *TYE2*, *TYE3*, and *TYE4*). Their functions are required for the expression of constitutive controlling site mutations at the structural gene *ADR2* of glucose repressible

alcohol dehydrogenase. These constitutive mutations were caused by the insertion of Ty elements (2). The *tye* mutant alleles had additional pleiotropic effects on the synthesis of several enzymes which are subject to carbon catabolite repression. However, these effects of the various *tye* mutant alleles were not as pronounced as those of the genes *CAT1* and *CAT3* or *CCR2* and *CCR3*.

In conclusion it is not possible yet to describe an entire genetic control mechanism involved in catabolite repression and derepression in yeasts. A patient cataloging of genes involved in various aspects of this central regulatory system is required with genes being identified in many different approaches.

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