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Uninducible Mutants in the *gal i* Locus of *Saccharomyces cerevisiae*

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Uninducible galactose nonfermenter mutants of *Saccharomyces cerevisiae* have been isolated and mapped in the *gal i* locus. They appear to be analogous to the i^s mutations found in the regulatory genes of the *Escherichia coli* lactose and galactose systems. The susceptibility to suppression by an ochre suppressor of an *i* allele in an $i^s i^-$ double mutant suggests that the *i* locus in *S. cerevisiae* codes for a protein.

Regulation of the galactose pathway enzymes in *Saccharomyces cerevisiae* involves the interaction of three loci: *i*, *c*, and *GAL 4*. The site of recessive constitutive mutations ($i^+ \rightarrow i^-$) is *i*, the site of dominant constitutive mutations ($c \rightarrow C$) is *c*, and the site of recessive pleiotropic mutations (*GAL 4* → *gal 4*) which prevent the synthesis of the three galactose enzymes (2, 3) is *GAL 4*. Two genes, *C* and *GAL 4*, are closely linked to each other but are not linked to *i* or to the three closely linked structural genes for the galactose pathway enzymes (2, 3). According to the operon hypothesis of Jacob and Monod (10), we have assumed that *c* represents the recognition site for a repressor specified by i^+. The expression of a *C* mutation occurs only with *C* and *GAL 4* in *cis* position, which suggests that the *c* locus controls the expression of *GAL 4* by virtue of its position rather than by its production of a cytoplasmic product. The product specified by *GAL 4* is presumably a protein, for it is known that some *gal 4* mutants respond to nonsense suppressors (D. C. Hawthorne, *unpublished data*). The complex of *c* and *GAL 4* might be looked upon as being analogous to a bacterial operon in which *GAL 4* is a structural gene whose expression is controlled by *c*, the operator. These facts suggest that the polypeptide specified by *GAL 4* exerts positive control of the expression of *GAL 1*, *GAL 7*, and *GAL 10*, the complex of three linked structural genes that specify the enzymes galactokinase (EC 2.7.1.6), galactose-1-phosphate uridyl-transferase (EC 2.7.7.10), and uridine diphospho-galactose epimerase (EC 5.1.3.2), respectively.

If the relationship between *i* and *c* in the yeast galactose system is indeed analogous to the regulator-operator gene relationship postulated in bacterial systems, one might expect to find mutants in the yeast system that are equivalent to the *lac* i^s and *gal* R^s (superrepressible) mutants described in the *Escherichia coli* lactose (12) and galactose (11) regulator genes. These uninducible mutants are thought to produce repressor molecules that are modified in such a way that they retain their capacity to react with the operator but are no longer antagonized by inducers. The bacterial superrepressible mutants are dominant to the wild type and the recessive constitutive alleles but are not expressed in the presence of operator mutations (O^c) which, by definition, have lost their potential to be recognized by repressor molecules.

We will describe the isolation and behavior of uninducible galactose-negative mutants of *S. cerevisiae* whose properties suggest that they are indeed analogous to the superrepressible mutants.

MATERIALS AND METHODS

Culture media and growth conditions. PY glucose medium contained 2.0% peptone, 1.0% yeast extract, and 2.0% glucose. Two per cent agar was added when a solid medium was used. PY galactose medium contained glucose-free galactose in place of the glucose. The galactose fermentation phenotypes were scored by observing gas formation in PY galactose broth dispensed in Durham tubes or by the use of indicator agar (6). The synthetic medium used for determining the nutritional requirements of segregants was yeast nitrogen base without amino acids (Difco), to which were added 2% agar, 1.0% glucose and the appropriate amino acid, and purine and pyrimidine supplements at 10 mg/liter. The sporulation agar contained, per liter: potassium acetate, 10.0 g; yeast extract, 2.5 g; glucose, 1.0 g; agar, 15.0

g; biotin, 2 γ; and thiamine-hydrochloride, 400 μg. The *p*H was 6.0 to 6.5.

Cultures were incubated at 30 C, except for induction of sporulation, which was carried out at room temperature.

Enzyme assays. Assays for the galactose pathway enzymes were performed with extracts prepared by alumina grinding of washed cells (2, 3). For measurement of constitutive and induced synthesis, the cells were grown overnight with shaking in fluid PY glucose medium or in PY 0.5% glucose plus 0.5% galactose medium, respectively.

Tetrad analyses. The methods used were those described previously (6).

Random ascospore preparation. Ascospores used in the random spore tests for recombination frequencies between the mutants were prepared by the sporulation of protoplasts. After the protoplasts were prepared (9), they were sedimented by centrifugation and suspended in 1.0 M sorbitol to a concentration of 10^9 per ml; 0.1-ml amounts were spread on petri plates of sporulation agar containing 1.0 M sorbitol. After 48 hr of incubation at room temperature, the sporulated and residual protoplasts were suspended in a few milliliters of water and then passed through a French pressure cell at 3,000 to 5,000 psi to disperse the clumps of spores. Dilutions of the ascospores were then plated on PY glucose-agar and, after 96 hr of incubation at 30 C, master plates were prepared by transferring cells from individual colonies to PY glucose-agar. The master plates were used to determine the nutritional requirements of the ascospore clones by replica plating. The capacity to ferment galactose was determined by inoculating PY galactose broth in Durham tubes with cells taken from the master plates.

Isolation of mutants. Mutants 8 and 16 were isolated from diploid 296 which is a vegetative petite and is homozygous for the α mating type and homozygous with respect to the wild-type alleles for galactose fermentation. Log-phase cultures were incubated with shaking at 30 C in PY glucose medium containing 20 μg of *N*-methyl-*N'*-nitro-nitrosoguanidine per ml. After 60 min of exposure to the mutagen, the cultures were diluted 100-fold with PY glucose medium and allowed to multiply to the stationary phase. Dilutions containing 50 to 100 cells were then spread on PY glucose-agar plates and, after 96 hr of incubation at 30 C, colonies were replica plated on PY galactose plates to detect galactose-nonfermenter mutants.

Two independent mutants, 8 and 16, were isolated by this technique. The diploids containing the mutations were crossed to diploid 122-2B which is an *aa* mating type, respiratory sufficient, *gal*$^+$, and homozygous for all of the wild-type galactose genes. The resulting tetraploids were sporulated and dissected, and the galactose phenotypes of the diploid segregants were determined. The viability of the segregants in this step was very poor, and many of the segregants were respiratory deficient. However, a few respiratory-sufficient nonfermenter segregants were obtained, and ascospore clones were obtained from those that would sporulate. The viability of the ascospores from these diploids was also very low, and reliable genetic ratios could not be obtained at this step. One haploid galactose-nonfermenter segregant of each mutant was chosen for further study. In subsequent crosses, spore viability was 90 to 100%.

Mutants B_2 and B_{23} were isolated as galactose-resistant mutants from galactose-sensitive (*gal 10*, epimerase deficient), respiratory-sufficient haploids (2). The *gal 10* gene was eliminated in crosses to a galactose-fermenter stock, and a single segregant of each mutant was chosen for further study.

Genetic nomenclature. The symbols used were those adopted at the Osaka Yeast Genetics Conference (Microbial Genetics Bulletin no. 31, 1969).

RESULTS

Synthesis of the galactose enzymes. The uninducible nature of the mutants is illustrated by the values for induced and constitutive levels of the galactose enzymes summarized in Table 1. Mutants 8 and 16 were found to be partially constitutive forming from 5 to 30% of the enzyme levels of an i^- strain, and these values were increased slightly or not at all upon induction. Mutants B_2 and B_{23} were also found to be uninducible, but they did not display the partially constitutive phenotype of mutants 8 and 16.

Dominance of the mutant phenotype. The dominant nature of the galactose-nonfermenter phenotype of the mutants is illustrated in Table 2 which presents the galactose fermentation phenotypes of diploids prepared by crossing the mutants to each other and to galactose-fermenter haploids of known genotypes. Intercrosses among the mutants yielded only nonfermenter diploids, and nonfermenter or slow fermenter diploids were also produced when the mutants were crossed to galactose-fermenter haploids that were i^+ or i^-. However, galactose-fermenter diploids were produced when the mutants were crossed to haploid galactose fermenters that possessed the *C* (dominant constitutive) mutation.

These results clearly indicate that the four mutations are dominant; (as in the case of B_2 and B_{23}) or semidominant (as with mutants 8 and 16) to their wild-type alleles and to i^- but are not expressed in the presence of a *C* mutation.

Allelism of the mutants. Although the four mutants yielded galactose-nonfermenter diploids when crossed to one another, this could not be taken as evidence for allelism because of the dominant nature of the mutations. Tests for recombination between the mutants were therefore made by the analyses of random ascospores produced by the six diploids derived by crossing the mutants to one another.

TABLE 1. *Constitutive and induced enzyme levels in the mutants*[a]

Mutant	Clone	Galactokinase		Transferase		Epimerase	
		-gal	+gal	-gal	+gal	-gal	+gal
8	339-1D	9	9	30	30	7	7
16	367-2A	<1.0	2.5	<1.0	5.0	<1.0	<1.0
B_2	429-6A	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
B_{23}	418-6A	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

[a] The values are expressed as the percentage of enzyme levels in an i^- strain grown under the same conditions.

TABLE 2. *Galactose fermentation by the mutants, tester stocks, and diploids prepared by crossing mutants and testers*[a]

Strains	Alone	Diploids			
		×8	×16	×B_2	×B_{23}
8	-		-	-	-
16	-			-	-
B_2	-				-
B_{23}	-				
ci^+	+	S	S	-	-
ci^-	+	S	S	-	-
Ci^+	+	+	+	+	+

[a] +, Gas in 24 hr; S, gas after 48 to 72 hr; -, no gas after 6 days.

TABLE 3. *Random ascospore analyses for galactose-fermenter recombinants*

Diploid	Mutant composition	No. of spores gal^+/no. of spores tested
375	8 × 16	0/200
432	8 × B_2	0/200
427	8 × B_{23}	0/600
433	16 × B_2	0/400
434	16 × B_{23}	0/400
436	B_2 × B_{23}	0/330

The data from these tests are summarized in Table 3. They show that the mutations are tightly linked, because no galactose-fermenter recombinants were detected in tests of 200 to 600 ascospore clones from each diploid.

Linkage studies. As anticipated, the uninducible mutants proved to be located at the *i* locus, and we will refer to them as i^s mutants. A haploid bearing mutation i_8^s was crossed with an i^- galactose-fermenter haploid (106-1a). The resulting diploid (363) was a slow galactose fermenter, and each of 10 tetrads that was examined contained 2 segregants that were galactose nonfermenters and 2 that were galactose fermenters and fully constitutive as judged by their capacity to synthesize transferase in PY glucose medium. The recovery of only parental ditype tetrads in this cross indicates close linkage for i^- and the defect in mutant i_8^s.

The use of the uninducible mutations to mark the *i* locus greatly simplifies its detection in segregants from heterozygous diploids, since one can score the i^s allele by its galactose-negative phenotype. A cross between $i_{B_2}^s$ and a *gal 4* haploid gave tetrad ratios for galactose fermentation indicative of the independent segregation of these two regulatory genes. There were nine (0+:4-) parental ditype asci, 31(1+:3) tetratype asci, and seven(2+:2-) nonparental ditype asci. There was no linkage between *i* and the cluster of three structural genes for the galactose pathway enzymes. Crosses of i_8^s and $i_{B_2}^s$ by *gal 7* (transferase) or *gal-10* (epimerase) mutants gave tetrad ratios for galactose fermentation of 5 (0+:4-), 18 (1+:3-), and 3 (4+:0-) asci. In an additional cross, very close linkage for i_8^s and *SUP 5*, an ochre-specific nonsense suppressor, was indicated by the recovery of only parental ditype asci in the 20 tetrads analyzed; however, *SUP 5* itself has not yet been placed on the genetic map of *Saccharomyces* (8).

Constitutive nature of galactose-fermenter revertants. An additional feature that characterizes uninducible mutants in the galactose and lactose systems of *E. coli* is the formation of revertants that are constitutive with respect to the synthesis of the galactose (11) or lactose (12) enzymes. This was also found to be the case for the four yeast mutants. Individual colonies of each mutant from PY glucose plates were spread over small areas on galactose agar plates, and a single revertant colony was subsequently selected from each area. These were purified by streaking on PY galactose-agar and then transferred to PY glucose agar slants for storage. Each revertant was then grown up in PY glucose broth and tested for its content of transferase. In some cases, the revertants were crossed to an i^+

strain to determine whether constitutive synthesis was dominant or recessive.

The results of these tests are summarized in Table 4. The four mutants behaved alike in that each produced galactose-fermenter revertants that contained 50 to 100% of the transferase levels of a control i^- stock. In those cases where the revertants were crossed to an i^+ haploid, constitutive synthesis was found to be recessive, except in one revertant derived from haploid 418-6A which was dominant.

Genetic analyses of one of the recessive constitutive revertants from haploid 418-6A demonstrated that it contained a second mutation closely linked to the original mutation for uninducibility and thus was of the composition $i^s i^-$. An analysis of seven tetrads from a cross of this revertant to a galactose fermenter i^+ haploid failed to reveal any galactose-nonfermenter recombinants, but an examination of 440 random ascospore clones from the same diploid yielded one such recombinant. The nonfermenter phenotype of the above recombinant was dominant, and seven asci from a cross of the recombinant to an i^+ galactose-fermenter haploid yielded only 2:2 segregations for galactose fermentation.

Genetic tests of the single dominant constitutive revertant derived from haploid 418-6A indicated that this revertant contained a second mutation closely linked to the *c*, *GAL 4* complex. A diploid prepared by crossing this revertant to a galactose-fermenter i^+ *c* haploid yielded two asci in which all four of the segregants were galactose fermenters, two that contained three fermenters and one nonfermenter and one that contained two fermenters and two nonfermenters. The nonfermenter segregants of the latter ascus were found to be uninducible and the nonfermenter phenotype was dominant, whereas the fermenter segregants displayed the dominant constitutive phenotype. One of these dominant constitutive segregants was crossed to a known *C* mutant, and the segregants from 10 asci of this diploid (461) were all galactase fermenters. One tetrad of this group was tested for synthesis of transferase after growth in PY glucose medium and all four segregants were found to be constitutive. These results indicate that the dominant constitutive revertant derived from haploid 418-6A was in all probability a double mutant of the composition i^s *C*.

TABLE 4. *Contitutive nature and dominance or recessiveness of galactose-fermenter revertants derived from i^s mutants*

i^s Mutant	Clone	No. constitutive/no. tested	Diploids with i^+ (no. constitutive/ no. tested)
8	339-1D	17/17	0/10
16	376-10C	5/5	0/5
B_2	429-6A	5/5	0/5
B_{23}	418-6A	10/10	1/5

Suppression of an i⁻ allele. By using suppressors of chain-terminating mutations, Bourgeois et al. (1) obtained evidence that the i^s phenotype in an $i^s i^-$ double mutant of *E. coli* was restored by a nonsense suppressor. This provided indirect evidence that the *i* locus in this bacterium coded for a protein.

An experiment of similar rationale was carried out with a haploid respiratory-deficient yeast containing $i^s B_2$, plus the ochre alleles *ura* 4-1, *leu* 2-1, and *iso* 1-1 and the amber alleles *trp 1-1* and *met 8-1* (4, 5). Ten galactose-fermenter revertants, presumably of the composition $i^s i^-$, were selected after ultraviolet irradiation, and these revertants were in turn plated on medium lacking either uracil, leucine, isoleucine, tryptophane, or methionine to select prototrophic revertants. The galactose-fermenter phenotypes of the prototrophs thus selected were then retested and, in one strain ($2492\text{-}5D_1, 20$) selected from a plate lacking leucine, the i^s phenotype was restored.

This galactose-negative mutant bearing the ochre-specific suppressor identified as *SUP 7* was then crossed to a haploid-inducible galactose fermenter. Surprisingly, the diploid and all four spores from each of the nine asci tested were galactose fermenters. However, since the mutant parent in this cross was a cytoplasmic petite, it was reasoned that the petite condition might be essential for the expression of the nonfermenter phenotype. When petite clones were selected after exposure of the segregants to acriflavine, the galactose-negative phenotype was seen in one-fourth of the segregants. The nine tetrads examined in this fashion showed two (4+:0−), six (3+:1−), and one (2+:2−) segregations for galactose fermentation. The nonfermenters all bore *SUP 7*, whose segregation could be followed by its action on the ochre nutritional mutants in the cross. These segregation data are consistent with the interaction of two loci assorting independently: (i) the *i* locus bearing both the original i^s defect plus an ochre nonsense mutation (i^-) alleviating this defect, and (ii) the ochre suppressor restoring the noninducible phenotype. This interpretation was verified by analyses for transferase synthesis of the petite clones from a 3+:1− tetrad: two clones were

found to be inducible, one was constitutive, and one was noninducible. Thus the genotypes of the four spore clones were as follows: spore 1, (inducible) i^+, *SUP 7*; spore 2, (inducible), i^+, *sup 7*- spore 3, (constitutive) $i^s i^-$ *sup 7;* and spore 4, (noninducible), $i^s i^-$, *SUP 7*.

DISCUSSION

The dominant mutations leading to the uninducible galactose-negative phenotype in *S. cerevisiae* are very similar to the uninducible mutations described in the lactose and galactose systems of *E. coli*. In both the bacterial and yeast systems, the mutations map within the respective regulatory loci and are dominant to the wild-type and recessive constitutive alleles of these genes. The uninducible mutations are not expressed, however, in the presence of dominant constitutive mutations in the repressor-recognition loci.

Another analogy between the bacterial and yeast systems is in the nature of the galactose-fermenter revertants that are derived from the uninducible mutants. The revertants are of two types: the recessive constitutives in which a second mutation has occurred that is closely linked to the original mutation, and the dominant constitutives in which the second mutation occurs in the repressor-recognition locus. As in the *E. coli* lactose system, we have been able to show that a nonsense suppressor introduced into a recessive constitutive revertant derived from an uninducible mutant restores the uninducible phenotype.

We interpret these observations along the lines already laid down by other investigators concerning regulation of induced enzyme synthesis in bacteria, i.e., that the *i* gal locus in *S. cerevisiae* specifies the production of a polypeptide repressor which becomes unantagonizable by galactose due to mutations within a restricted region of the gene. The uninducible phenotype produced by these mutations can be relieved by second mutations within the *i* locus which presumably block the production of an active *i* gene product, or by mutations within the *c* locus which renders this gene insensitive to the repressor.

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