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Genetic Co-Regulation of Galactose and Melibiose Utilization in *Saccharomyces*

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The *gal*3 mutation of *Saccharomyces*, which is associated with an impairment in the utilization of galactose, has been shown to be pleiotropic, causing similar impairments in the utilization of melibiose and maltose. Melibiose utilization and α-galactosidase production are directly controlled by the galactose regulatory elements *i*, *c*, and *GAL*4. The fermentation of maltose and the induction of α-glucosidase are regulated independently of the *i*,*c*,*GAL*4 system. The production of α-galactosidase and galactose-1-phosphate uridyl transferase is coordinate in galactokinaseless strains. Galactose serves as a nonmetabolized, gratuitous inducer of α-galactosidase in strains lacking the genes for one or more of the Leloir pathway enzymes.

In *Saccharomyces*, the products of at least five genes participate in the uptake of exogenous galactose and its conversion to glucose-6-phosphate. The requisite conversions are catalyzed, in order, by a specific galactose transport protein (7, 9), by the Leloir pathway enzymes galactokinase (EC 2.7.1.6), galactose-1-phosphate uridyl transferase ("transferase," EC 2.7.7.10), and uridine diphosphogalactose-4-epimerase ("epimerase," EC 5.1.3.2), and by the major isozyme of phosphoglucomutase (EC 2.7.5.1; 5, 8). The first four gene products are induced by exogenous galactose (7, 10); the phosphoglucomutase is produced constitutively (10).

Galactose-nonfermenting mutants have been isolated which produce reduced levels of one or more of the required activities. Mutations affecting the individual production of transferase, epimerase, and galactokinase, respectively, map in three closely linked, probably contiguous, complementation groups, *gal*7-*gal*10-*gal*1 (3, 10). The galactose gene cluster, which is thought to contain the structural genes for the Leloir enzymes, maps near the centromere of chromosome II (3, 22). Mutations occurring in the presumptive structural gene for phosphoglucomutase-2, *gal*5, and a component of the galactose transport system, *gal*2, map separately from the Leloir enzyme gene cluster and from each other (10).

Mutations occurring within the regulatory genes of the galactose system are recognized by their pleiotropic effects on the production of the Leloir enzymes (10) and the galactose transport system (V. P. Cirillo, personal communication). The mutations map in four genes, *i*, *c*, *GAL*4, and *GAL*3. The *i* gene is identified by three allelic configurations: i^+, wild type, inducible; i^-, recessive to i^+ and i^s, constitutive; and i^s, dominant to i^+ and i^-, uninducible (10, 12, 13). The *i* gene is thought to specify the production of a repressor protein that interacts with an operator, *c* (11, 12). A second class of constitutive mutations, *C*, is dominant to i^s. The genetic properties of the *C* allele resemble those of a constitutive operator mutation since the dominant constitutive phenotype is expressed only when the *C* mutation occurs in a position *cis* to a functional *GAL*4 gene (11). Recessive mutations of the *gal*4 class are pleiotropic and confer a phenotype of uninducibility for the galactose pathway activities (10). Mutants bearing the *gal*3 allele display a complex phenotypic pattern originally described as long-term adaptation to galactose (24, 30). None of the four regulatory genes is linked to any of the galactose structural genes, and only *c* and *GAL*4, which may be adjacent, are linked to each other (10, 11).

The initial descriptions of the long-term adaptation phenomenon by Winge and Roberts suggested that the effects of the *gal*3 mutation were restricted to the galactose fermentation system (30, 31). The recent studies of Tsuyumu and Adams appeared to further suggest a nonpleiotropic phenotype for the *gal*3 mutation (28). In contrast to these earlier reports, we

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present evidence in this paper that the *gal*3 mutation is pleiotropic and blocks the efficient utilization of galactose, melibiose, and maltose. Our results indicate further that the production of the melibiose-hydrolyzing enzyme α-galactosidase (EC 3.2.1.22) is under the control of the galactose regulatory genes *i*, *c*, and *GAL*4.

MATERIALS AND METHODS

Yeast strains. The strains used in this study are derived from two yeast species, *S. cerevisiae*, a melibiose nonfermenter, and a closely related melibiose-fermenting species, *S. carlsbergensis*. All melibiose-fermenting strains described in this report displayed segregation patterns consistent with their carrying a single melibiose allele. The nomenclature of the several melibiose alleles is not currently well defined (23). Because the melibiose fermentation gene of *S. carlsbergensis* was the first to be examined physiologically (4) and genetically (20), we refer to this allele as *MEL*1.

The *gal*$^{\nabla}$ strain 2894-3C was given to us by D. C. Hawthorne. The *MAL*1 allele in our strains was derived from strain X123, obtained from the collection of H. L. Roman.

Growth conditions. Solid media were based on YEP (1% yeast extract, 2% peptone) or YNB (Difco yeast nitrogen base) solidified with 2% agar and supplemented with the desired carbon sources (generally to 2%, wt/vol). Liquid media were based on either YEP or SBY. The latter medium consists of (grams per liter): yeast nitrogen base, 6.7; yeast extract, 2; peptone, 4; succinic acid, 10; and potassium hydroxide, 3.7; the pH adjusted to 4.5. SBY was used because it is well buffered near the pH optimum (4.0) of α-galactosidase and supports rapid growth of our cultures in both fermentable and nonfermentable substrates. SBY was supplemented with 25 μg of each growth factor required by the strain cultivated per ml. Except in the case of glucose, carbon sources were sterilized separately from the medium base.

Fluid cultures were grown aerobically at 30 C in reciprocating shakers and harvested while they were in the exponential phase of growth. To insure good agitation, culture flasks were filled to no more than 20% capacity with the fluid medium.

Enzyme assays. Transferase activity was measured by the method of Douglas (8). Cell extracts were prepared by grinding washed cells with alumina (10, 11). One unit of transferase is that amount of enzyme sufficient to cause an optical density increase at 340 nm of 0.001/min.

α-Galactosidase activity was determined by measuring the amount of *p*-nitrophenol liberated upon the hydrolysis of *p*-nitrophenyl-α-D-galactopyranoside (PNPGal). Assays were performed at 30 C in 5.0 mM PNPGal buffered at pH 4.0 with 39 mM KH_2PO_4 and 31 mM citric acid. Advantage was taken of the fact that α-galactosidase is an extracellular enzyme (14), and most assays were conducted with whole cells. Accurate and reproducible values were obtained with as few as 10^5 induced cells. Reactions were started by the introduction of whole cells (or cell extracts) to the assay reagents. At periodic intervals, 0.1-ml samples were transferred to 0.9 ml of 0.1 M Na_2CO_3. Further hydrolysis of PNPGal was effectively stopped by this step. The optical densities of the carbonate-developed samples were measured at 400 nm. One unit of α-galactosidase is that amount capable of hydrolyzing 1.0 nmol of PNPGal per min under the described assay conditions.

α-Glucosidase (EC 3.2.1.20) activity was measured in permeabilized cells by the procedures of Adams (1). The assay system contained 2 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) and 0.1 mM dithiothreitol in 50 mM KPO_4^{2-} buffer, pH 6.8. The samples were diluted into 0.1 M Na_2CO_3 and analyzed spectrophotometrically as for α-galactosidase. One unit of α-glucosidase catalyzes the hydrolysis of 1.0 nmol of PNPG in 1 min.

Constitutive production of transferase was scored in the tetrad analyses by growing spore cultures for 2 days in YEP broth containing 0.25% glucose and 2% potassium acetate. Washed cells were permeabilized by suspension of each culture for 30 min at 30 C in 5.0 ml of 40% dimethyl sulfoxide (1). After two washings in water, the cells were suspended in 2.0 ml of complete transferase assay reagent (8) and incubated for 30 min at 30 C. The suspensions were then centrifuged, and the optical densities of the supernatants were determined at 340 nm. The optical density ratios between constitutive and uninduced spore cultures obtained from a single ascus were greater than 10.

Constitutive production of α-galactosidase in the spore cultures was examined by suspending a small amount of cells from YEP-2% glucose replica plates into 0.20 ml of α-galactosidase assay reagent. Color was developed by adding a drop of 1.0 M Na_2CO_3 to each tube after 15 min of incubation at room temperature. α-Galactosidase-positive tubes were bright yellow, and α-galactosidase-negative tubes were virtually colorless.

Proteins were estimated by the method of Lowry et al. (21).

Genetic methods. Tetrad analyses were performed as described by Mortimer and Hawthorne (22). Fermentation characters were scored in Durham fermentation tubes containing YEP and 2% of the desired sugar.

The presence of the *gal*3 gene was scored in several ways. Respiratory-sufficient *gal*3 strains generally produced gas in galactose fermentation tubes 4 to 5 days after inoculation. However, a few *GAL*3 strains displayed a lag prior to the onset of fermentation so that this scoring method was not entirely reliable. The *gal*3 phenotypes were rechecked by replica plating onto YEP agar containing 2% of either galactose, melibiose, or maltose. Provided that only a small amount of cells was transferred at the replica plating step, *GAL*3 strains could be distinguished from *gal*3 mutants by the greater confluence of their colonies after 1 to 2 days of incubation. The difference in the degree of growth confluence could be enhanced by the addition of 10 μg of ethidium bromide per ml to the test plates,

which preferentially inhibits the growth of *gal3* strains on the test sugars.

Respiratory-deficient ("petite," ρ^-) mutants were generated by plating respiratory-sufficient ("grande," ρ^+) cells onto YEP-2% glucose agar containing 10 μg of ethidium bromide per ml. Petite clones were identified by their inability to grow at the expense of nonfermentable substrates.

RESULTS

Pleiotropic nature of the gal3 mutation. The existence of pleiotropic effects of the *gal3* mutation was initially suggested by the observations of Douglas and Hawthorne (unpublished data) in which the fermentations of melibiose and maltose, in addition to that of galactose, were delayed in *gal3* strains. To examine these effects further, we studied the fermentation and growth properties of a large number of *gal3* mutant strains. Included among these were derivatives of four independently selected *gal3* mutants, as well as *S. chevalieri* Y1345, a direct descendant of the culture used by Spiegelman and his colleagues in their studies of the naturally occurring g_a gene (24), which is an allele of *gal3* (20). All of the *gal3* strains tested responded similarly with regard to their utilization of galactose and, when the *MEL1* and *MAL* alleles also were present, in their utilization of melibiose and galactose as well. The properties of the diploid strains Z661 (Table 1) are representative of those of *gal3* and *GAL3* strains, respectively. Use of diploid strains greatly reduced the appearance of fermenter clones consisting of *gal3* i^- double mutants.

Respiratory-sufficient and respiratory-deficient cultures of Z661 and Z669 were harvested after exponential growth in YEP-2% glucose broth. Washed cells were plated onto YEP-based media containing 2% glucose, galactose, melibiose, or maltose. Colonies were counted after 5 days of incubation at 30 C.

Both grande and petite *GAL3* strains were able to form equivalent numbers of colonies on each medium. Furthermore, both strains were capable of rapid fermentation of the test sugars, generally producing gas within 12 h on maltose, 16 h on melibiose, and 48 h on galactose.

Respiratory-sufficient *gal3* strains also formed colonies efficiently on all four sugars. Approximately 10% fewer colonies developed on plates containing the test sugars than formed on glucose agar. This value correlates with the fraction of petite clones arising on glucose agar. All clones growing on the alternative sugars were grande. The fewest number of grande *gal3* clones developed on galactose agar. A reduced efficiency of plating was frequently obtained with cultures of both *gal3* and *GAL3* strains; plating efficiencies as low as 0.40 were occasionally observed. Similar effects have been reported by other workers (25), but the phenotype has not been associated with any known genotype. The rates of colony development and the onsets of fermentation were retarded in every case for respiratory-competent *gal3* strains.

The unusual heterogeneity of colony morphology of *gal3* cultures plated on galactose, first reported by Spiegelman and co-workers (24), was also seen with our strains. All clones apparently contained galactose-fermenting cells since similar patterns occurred on YNB containing 2% galactose. The *gal3* grande clones forming on glucose, melibiose, and maltose agar were disk shaped and generally of uniform size.

Respiratory-deficient *gal3* strains were severely restricted in their ability to produce colonies at the expense of galactose, melibiose, or maltose. In each case, only a small fraction of the cells plated gave rise to macroscopically visible clones within 5 days. By the described plating procedures, the number of colonies arising on melibiose agar is 10-fold less than

TABLE 1. *Comparison of GAL3 and gal3 strains in their abilities to utilize galactose, melibiose, and maltose*[a]

Strain	Plating efficiency[b]			Fermentation rates[c]		
	Galactose	Melibiose	Maltose	Galactose	Melibiose	Maltose
Z669ρ^+	1.0	1.0	1.0	48	16	12
Z669ρ^-	1.0	1.0	1.0	48	16	12
Z661ρ^+	0.85	0.92	0.90	120	96	48
Z661ρ^-	2×10^{-4}	2×10^{-5}	3×10^{-3}	—	96	48

[a] Strain genotypes: Z669, *GAL3/GAL3 MEL1/MEL1 MAL1/MAL1*; Z661, *gal3/gal3 MEL1/MEL1 MAL1/MAL1*.

[b] Plating efficiencies are expressed as the number of colonies forming on the indicated carbon source as a fraction of the number forming on glucose agar.

[c] Fermentation rates are expressed as average number of hours prior to onset of vigorous fermentation in Durham fermentation tubes. The dash indicates the absence of fermentation after 5 days of incubation at 30 C.

that on galactose agar and 150-fold less than that found on maltose agar. Although the relative order of the initial plating efficiency maltose > galactose > melibiose was obtained with other *gal3* ρ^- strains as well, the numbers of fermenter clones increased with time; the rate of increase occurred in the order maltose > melibiose > galactose.

The onset of fermentation was delayed for each test sugar in petite *gal3* strains.

The inhibitory effects of the *gal3* mutation on maltose utilization are probably not restricted to the *MAL1* allele. Many strains in our stocks carry the maltose alleles *MAL2*, *MAL3*, and *MAL4*. Several other *gal3* strains carrying *MAL* genes of undetermined allelism displayed the same phenotypes as were observed with Z661.

No inhibitory effects on sucrose utilization were observed in *gal3* strains. The *GAL3* product appears not to be involved in the induction of the yeast respiratory system since the durations of the glucose-ethanol diauxies were approximately equal in both *gal3* and *GAL3* grandes.

Regulation of galactose and melibiose utilization by the genes i, c, and GAL4. The fermentation phenotypes and the state of induction of the associated enzyme systems were examined in *MEL1* strains, each bearing a mutant allele of one of the galactose regulatory genes. To minimize complications arising from strain differences or from possible fortuitous combinations of independent genes, each mutant was crossed to a wild-type strain, and the meiotic products were analyzed for recombinants (Table 2).

In every cross, only parental types were observed in each of the 10 tetrads examined. The first cross demonstrated that the phenotypes of the delayed fermentation of galactose, melibiose, and maltose consistently remained associated through meiosis. The parental *gal3* mutant carried the allele *trp1*, which is closely linked to *gal3* (17). In every ascus, the long-term adaptation phenotype was associated with the *trp1* spores. The delayed fermentation of all three sugars is a recessive trait.

Tetrads derived from an i^+/i^- heterozygote showed cosegregation of the abilities to produce transferase and α-galactosidase constitutively (Table 2, cross 2). The levels of transferase provide an indication of the state of induction of all of the galactose pathway enzymes (10, 11). As expected, the constitutive phenotype was recessive in the hybrid. The conclusion that the *i* gene is involved in the induction of both the Leloir pathway enzymes and α-galactosidase is supported further by the fact that i^- mutants can be readily isolated from *gal3* ρ^- cultures plated on melibiose.

TABLE 2. *Co-regulation of the galactose and melibiose fermentation systems by the regulatory genes GAL3, i, c, and GAL4*

Cross	Parents	Pertinent genotypes[a]	Fermentation capabilities[b]						Segregant classes[c] (PD: NPD:T)
			Parental strains			Diploid hybrids			
			Galactose	Melibiose	Maltose	Galactose	Melibiose	Maltose	
1	650-5D	*gal3 MAL1*	$+^s$	$+^s$	$+^s$	$+^f$	$+^f$	$+^f$	10:0:0
	650-6A	*GAL3 MAL1*	$+^f$	$+^f$	$+^f$				
2	653-5C	i^-	$+^c$	$+^c$	NT	$+^i$	$+^i$	NT	10:0:0
	108-3C	i^+	$+^i$	$+^i$	NT				
3	218-2C	*C*	$+^c$	$+^c$	NT	$+^c$	$+^c$	NT	10:0:0
	108-3C	*c*	$+^i$	$+^i$	NT				
4	278-1B	*gal4*	−	−	NT	+	+	NT	10:0:0
	108-3C	*GAL4*	+	+	NT				

[a] Except as indicated, all strains were wild type for the galactose structural and regulatory genes and carried one *MEL1* allele per haploid genome.

[b] A plus indicates occurrence of fermentation and a minus indicates absence of evidence of fermentation within 7 days of incubation at 30 C. Analysis of cross was also performed on agar plates as described in the text. Superscripts denote properties of fermentation system: s, delayed initiation of fermentation (slow phenotype); f, fast initiation of fermentation; c, constitutive synthesis of transferase and α-galactosidase; i, inducible production of transferase and α-galactosidase. NT indicates that the properties were not tested.

[c] Segregant classes expressed as a ratio of parental ditype (PD), nonparental ditype (NPD), and tetratype (T) asci. In cross 1, only parental types were observed for each pair of fermentation characters scored.

In cross 3, similar 2:2 cosegregation patterns for constitutivity were observed for spores derived from a *C/c* heterozygote. Constitutivity was dominant to inducibility in the hybrid for both transferase and α-galactosidase.

All *gal*4 segregants obtained from cross 4 were unable to ferment either galactose or melibiose. To rule out the possibility that the parental *gal*4 strain 278-1B carried a defect in a gene closely linked to *gal*4, which might be specifically required for melibiose fermentation, a series of revertants was isolated on melibiose and galactose agar. Five melibiose-fermenting and five galactose-fermenting revertants were selected from strain 278-1B and from the nonfermenter segregants Z8-1A and Z8-1C. In every case, revertants selected for the ability to ferment one sugar were also able to ferment the other rapidly.

Non-participation of the galactose structural gene products in the induction of the melibiose fermentation system. The preceding data, although demonstrating the requirement for the functional allele of *GAL*4 in the fermentation of melibiose, do not prove that the *GAL*4 product is involved directly in the induction of α-galactosidase. Inasmuch as the induction of the Leloir pathway enzymes and the galactose transport system is controlled by *GAL*4, the possibility remained that *gal*4 mutants were uninducible for α-galactosidase as a direct consequence of a specific deficiency of one or more products of the galactose structural genes.

To examine the role of the Leloir enzymes in the induction of the melibiose fermentation system, use was made of a mutant strain that was unable to produce the three Leloir enzymes. This strain carried a mutation, which we designate *gal*$^{\nabla}$, having the genetic properties of a deletion of the *GAL*7-*GAL*10-*GAL*1 structural gene cluster (D. C. Hawthorne, personal communication). The meiotic products obtained from the hybrid *gal*$^{\nabla}$ *mel*/*GAL*$^{+}$ *MEL*1 showed 2:2 segregation of the capacities to ferment galactose and melibiose. However, the tetrad ratios obtained were 0PD:1NPD:9T (Table 3, cross 5). The existence of 11 recombinant spore cultures capable of melibiose fermentation in *gal*$^{\nabla}$ backgrounds demonstrates the dispensability of the Leloir enzymes in the induction of the melibiose fermentation system.

Similar conclusions were reached regarding the dispensability of the galactose transport system and phosphoglucomutase-2 activities for melibiose fermentation. In the crosses *gal*2 *mel* × *GAL*$^{+}$ *MEL*1 and *gal*5 *mel* × *GAL*$^{+}$ *MEL*1 (Table 3, crosses 6 and 7), the capacities to ferment galactose and melibiose segregated independently, such that 9 melibiose-fermenting recombinant cultures of the class *gal*2 *MEL*1 and 10 of the class *gal*5 *MEL*1 were obtained.

These data indicate that *GAL*4 controls the melibiose fermentation system directly and that the galactose structural genes play no obligatory role in the induction of the *MEL*1 system.

Effects of the i^s allele on melibiose fermentation and α-galactosidase production. Mutations of the i^s class behave as though they specify the production of dominant "superrepressors" and block the induction of the Leloir pathway enzymes in all genetic backgrounds except *C* (12). The effects of the i^s allele on

TABLE 3. *Independence of the ability to ferment melibiose from the functional state of the galactose structural genes*

Cross	Parental strains	Pertinent genotypes[a]	Fermentation capabilities[b]				Segregant classes[c] (PD:NPD:T)
			Parental strains		Diploid hybrids		
			Galactose	Melibiose	Galactose	Melibiose	
5	2894-3C	*gal*$^{\nabla}$ *mel*	–	–	+	+	0:1:9
	108-3C	*GAL*$^{+}$ *MEL*1	+	+			
6	346-3D	*gal*2 *mel*	–	–	+	+	2:1:7
	108-3C	*GAL*2 *MEL*1	+	+			
7	146-2D	*gal*5 *mel*	–	–	+	+	0:2:8
	108-3C	*GAL*5 *MEL*1	+	+			

[a] Except as indicated, all strains were wild type for the galactose structural and regulatory genes.

[b] A plus indicates occurrence of fermentation and a minus indicates the absence of evidence of fermentation within 7 days of incubation at 30 C.

[c] Segregant classes expressed as a ratio of parental ditype (PD), nonparental ditype (NPD), and tetratype (T) asci.

melibiose fermentation and α-galactosidase production were tested in different genetic combinations (Table 4). The response of the melibiose fermentation system completely paralleled that of the galactose system. The phenotype of uninducibility of α-galactosidase and nonfermentation of melibiose, expressed in haploid i^s strains, is dominant in i^s/i^+ and i^s/i^- heterozygotes. The presence of a *C* allele in an i^s strain allows melibiose fermentation and constitutive production of α-galactosidase. Furthermore, the i^- and *C* haploid strains were hyperinducible for α-galactosidase in the presence of galactose. Although the *C* allele allowed production of high levels of α-galactosidase within the haploid i^+ background, enzyme levels were reduced four- to fivefold in combination with the i^s gene in Z636.

Relationship between the GAL genes and maltose utilization. Although the *gal*3 mutation has pleiotropic effects on the fermentations of galactose, melibiose, and maltose, only the utilization of the first two sugars is regulated by the genes *i*, *c*, and *GAL*4. Strains bearing the *MAL*1 allele retained their abilities to ferment maltose and produce α-glucosidase inducibly in *gal*4 or i^s mutants (Table 5) and did not constitutively produce α-glucosidase in combination with i^- or *C* genetic backgrounds. Identical results were obtained with strains carrying other *MAL* fermentation genes as well. Furthermore, maltose-fermenting clones obtained from respiratory-deficient *gal*3 strains were generally unable to ferment galactose or melibiose. On the other hand, *gal*3 petite clones isolated from galactose or melibiose agar were able to utilize all three sugars efficiently. These latter observations suggest that the minimal metabolic conditions necessary to overcome the *gal*3 block of maltose utilization are not sufficient to allow the utilization of galactose and melibiose and that the execution of at least one additional step is required to initiate the fermentation of the latter sugars.

Coordinate induction of α-galactosidase and transferase. A possible consequence of the common genetic regulation of the galactose and melibiose utilization systems might be the coordinate induction of the representative enzymes transferase and α-galactosidase. To test this hypothesis, the steady-state specific activities of the two enzymes were compared in cells grown in the presence of different concentrations of galactose. A galactokinaseless diploid, homozygous for *gal*1 and *MEL*1, was grown for approximately 10 generations in SBY-2% ethanol in each of 10 cultures containing different galactose concentrations. Galactose served as a nonmetabolized inducer for all cultures throughout the growth period, as none showed evidence of reversion of the *gal*1 mutation.

The results (Fig. 1) indicate that, over a 10,000-fold concentration range, the steady-state specific activities of transferase and α-galactosidase exhibited equivalent degrees of induction for each concentration of galactose in the culture. The steady-state specific activities of both enzymes rose sharply over the concentration range 100 to 312 μM. Galactose concentrations less than 100 μM did not raise the enzyme levels above basal values. The concentration of galactose stimulating half-maximal

TABLE 4. *Effect of i^s allele on melibiose fermentation and α-galactosidase production*

Strain	Pertinent genotype[a]	Fermentation[b]		α-Galactosidase sp act[c]		Transferase sp act[d]	
		Melibiose	Galactose	− Galactose	+ Galactose	− Galactose	+ Galactose
108-3C	i^+c	+	+	<1	137	<5	2,000
102-4C	i^-c	+	+	169	216	2,192	2,400
218-2A	i^+C	+	+	146	177	1,020	1,200
418-6A	i^sc	−	−	<1	<1	<5	<5
Z634	i^sc/i^+c	−[e]	−[e]	<1	<1	<5	<5
Z635	i^sc/i^-c	−[e]	−[e]	<1	<1	<5	<5
Z636	i^sc/i^+C	+	+	27	41	535	717

[a] Except as indicated, all strains carried a wild-type complement of galactose structural and regulatory genes and one *MEL*1 allele per haploid genome.

[b] Fermentation was scored in fermentation tubes and on agar containing ethidium bromide. Symbols are as described in Table 2.

[c] α-Galactosidase specific activities are expressed as units per 1.0 ml of cells at a density of 100 Klett units (540 nm).

[d] Transferase specific activities are expressed as units per milligram of protein in cell extracts.

[e] Very slow rate of fermentation observed.

induction was estimated to be 270 μM, somewhat higher than the 100 μM concentration of D-fucose necessary for half-maximal induction of galactokinase in *Escherichia coli* (6).

It was concluded that, at least under gratuitous conditions, α-galactosidase and transferase are induced coordinately by galactose. Furthermore, in the steady-state, the amount of α-galactosidase activity detected in intact cells is proportional to the amount found in cell extracts.

DISCUSSION

Our observations on the pleiotropic effects of the *gal*3 mutation suggest that the *GAL*3 product plays a central role in the initiation of induction of at least two otherwise independently regulated fermentation systems. One *GAL*3-dependent system, providing for the fermentation of galactose and melibiose and the induction of the corresponding catabolic enzymes, is under the control of the regulatory genes *i*, *c*, and *GAL*4. On the other hand, the second system definitively shown to be dependent on *GAL*3, the *MAL*1-specified fermentation of maltose and production of α-glucosidase, is not regulated by the latter three genes. Our preliminary results, however, suggest that the inhibitory effects of the *gal*3 mutation on maltose fermentation is not restricted to that mediated by the *MAL*1 allele.

The demonstration of a requirement for the *GAL*3 function for efficient maltose fermentation increases the number of genes, in addition to the classical *MAL* alleles, known to affect maltose fermentation in *Saccharomyces*. At least seven genes, designated *dsf*, are required for the fermentation of maltose and other disaccharides (32). None of the *dsf* mutations, however, appears to be identical to *gal*3 since all *dsf* mutants have been reported to be capable of galactose fermentation (32).

The nature of the *Saccharomyces MAL* alleles themselves is not very clear. Early observations suggested that the *MAL* alleles represented structural rather than regulatory genes (15). Recent reports, however, have described the existence of constitutive regulatory mutations within the *MAL* genes (18, 19, 26, 27). Also, maltose-nonfermenting strains obtained as segregational nonfermenters (26) or as mu-

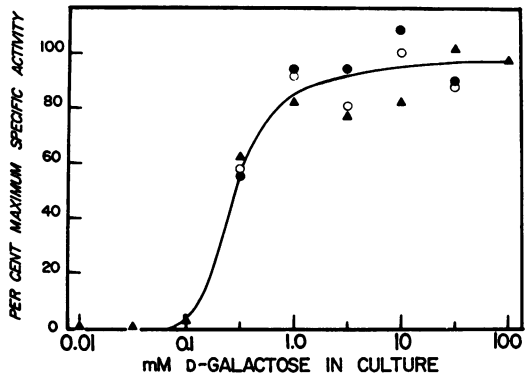

FIG. 1. *Coordinate induction of α-galactosidase and transferase by galactose. Symbols represent normalized enzyme specific activities and the values in parentheses are the respective specific activities, which have been normalized to 100:* (○) *α-galactosidase in whole cells (94 U/1.0 ml of cells at 100 Klett units at 540 nm);* (●) *α-galactosidase in cell extracts (240 U/mg of protein);* (▲) *transferase in cell extracts (1,700 U/mg of protein).*

TABLE 5. *Independence of α-glucosidase production from the functional state of the regulatory genes i, c, and GAL4*

Strain	Pertinent genotype[a]	Sp act					
		Ethanol[b]		Ethanol galactose[b]		Ethanol maltose[b]	
		α-Gal[c]	α-Glu[d]	α-Gal	α-Glu	α-Gal	α-Glu
650-1B	*GAL*$^+$	<1	1.5	123	3.6	<1	140
651-3C	*gal*4	<1	<1	<1	2.1	<1	95
652-3A	*C*	92	3.2	170	7.6	81	130
653-5C	*i*$^-$	81	2.0	112	1.9	120	19
654-3B	*i*^{s}	<1	<1	5.8	<1	<1	37

[a] Except as indicated, all strains carried a wild-type complement of galactose structural and regulatory genes, one *MEL*1, and one *MAL*1 allele per haploid genome.

[b] Cells were grown in SBY medium containing 2% (wt/vol) of the indicated carbon sources.

[c] α-Galactosidase (α-Gal) specific activities are expressed as units per 1.0 ml of cells at a density of 100 Klett units (540 nm).

[d] α-Glucosidase (α-Glu) specific activities are expressed as units per 1.0 ml of permeabilized cells at a density of 100 Klett units (540 nm).

tants selected from fermenter strains (19, 26) continue to produce the uninduced levels of α-glucosidase. The current evidence suggests that the *MAL* alleles may be regulatory genes specifying the production of positive regulators that act upon a series of maltase structural genes whose genetic positions have not yet been determined.

The findings in the *MAL* system are particularly relevant to melibiose fermentation in yeast. At least five other *MEL* genes, in addition to *MEL*1, are known to occur in yeasts closely related to *S. cerevisiae* (23). As was the case for the *MAL* alleles, the polymeric *MEL* genes were, on the basis of gene dosage experiments, originally thought to consist of α-galactosidase structural genes (16). This conclusion, however, may require modification in view of the observations in the maltose system. An interesting question, as yet unexplored, is whether the galactose regulatory elements control the other *MEL* genes as well.

The results presented in this report do not suggest a mechanism for the function of the *GAL*3 gene product. It is clear, though, that any mechanistic model must account for the observed pleiotropy of the *gal*3 mutation. Recently, Tsuyumu and Adams presented a model for the functional role of the *GAL*3 product (29). The model was partly based upon their findings that two of the three chromatographically distinct uridine 5'-diphosphate (UDP)-glucose pyrophosphorylase activities present in wild-type cells were absent in *gal*3 mutants (29). According to their hypothesis, UDP-glucose, in addition to galactose, serves as an obligate co-inducer of the galactose pathway enzymes. The *gal*3 phenotype, then, is proposed to result from a deficiency of UDP-glucose. Although the Tsuyumu-Adams model was formulated on the assumption that the *gal*3 defect was not pleiotropic (28), our results do not preclude a role for UDP-glucose in the induction of both the galactose-melibiose and maltose fermentation systems. UDP-glucose is a precursor in the biosynthesis of trehalose, which is thought to serve as an obligate intermediate in the catabolism of maltose in *Saccharomyces* (2). As suggested by Adams (personal communication), UDP-glucose may act as both a catabolic intermediate and endogenous co-inducer for both inducible systems.

The demonstration of the co-regulation of the galactose and melibiose fermentation systems provides an additional dimension in the analysis of the common regulatory system. Since the induction of α-galactosidase in grandes is independent of the state of the galactose structural genes, determination of the state of induction in galactose structural gene mutants may generally be achieved with melibiose fermentation tubes or plates. This same property allows the use of galactose as a nonmetabolized inducer of α-galactosidase when applied to *gal*1 or *gal* ∇ strains, such that the effects of inducer catabolism can be eliminated from the physiological analyses. Furthermore, the PNPGal assay for α-galactosidase presents the advantages of sensitivity, enzyme, substrate, and product stability, as well as convenience of assay.

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LITERATURE CITED

1. Adams, B. G. 1972. Method for decryptification of α-glucosidase in yeast with dimethyl sulfoxide. Anal. Biochem. **45**:137–146.
2. Avigad, G. 1960. Accumulation of trehalose and sucrose in relation to the metabolism of α-glucosides in yeast of defined genotype. Biochim. Biophys. Acta **40**:124–134.
3. Bassel, J., and R. K. Mortimer. 1971. Genetic order of the galactose structural genes in *Saccharomyces cerevisiae*. J. Bacteriol. **108**:179–183.
4. Bau, A. 1895. Über ein neues Enzyme der Hefe. Chem.-Ztg. **19**:1873.
5. Bevan, P., and H. C. Douglas. 1969. Genetic control of phosphoglucomutase variants in *Saccharomyces cerevisiae*. J. Bacteriol. **98**:532–535.
6. Buttin, G. 1963. Mécanismes régulateurs dans la biosynthèse des enzymes du métabolisme du galactose chez *Escherichia coli* K12. I. La biosynthese induite de la galactokinase et l'induction simultanée de la séquence enzymatique. J. Mol. Biol. **7**:164–182.
7. Cirillo, V. P. 1968. Galactose transport in *Saccharomyces cerevisiae*. I. Nonmetabolized sugars as substrates and inducers of the galactose transport system. J. Bacteriol. **95**:1727–1731.
8. Douglas, H. C. 1961. A mutation in *Saccharomyces* that affects phosphoglucomutase activity and galactose utilization. Biochim. Biophys. Acta **52**:209–211.
9. Douglas, H. C., and F. Condie. 1954. The genetic control of galactose utilization in *Saccharomyces*. J. Bacteriol. **68**:662–670.
10. Douglas, H. C., and D. C. Hawthorne. 1964. Enzymatic expression and genetic linkage of genes controlling galactose utilization in *Saccharomyces*. Genetics **49**:837–844.
11. Douglas, H. C., and D. C. Hawthorne. 1966. Regulation of genes controlling synthesis of the galactose pathway enzymes in yeast. Genetics **54**:911–916.
12. Douglas, H. C., and D. C. Hawthorne. 1972. Uninducible mutants in the *gal i* locus of *Saccharomyces cerevisiae*. J. Bacteriol. **109**:1139–1143.
13. Douglas, H. C., and G. Pelroy. 1963. A gene controlling inducibility of the galactose pathway enzymes in *Saccharomyces*. Biochim. Biophys. Acta **68**:155–156.
14. Friis, J., and P. Ottolenghi. 1959. Localization of melibiase in a strain of yeast. C. R. Trav. Lab. Carlsberg **31**:272–281.
15. Halvorson, H., S. Winderman, and J. Gorman. 1963.

Comparison of α-glucosidases of *Saccharomyces* produced in response to five nonallelic maltose genes. Biochim. Biophys. Acta **67**:42–53.
16. Haupt, W., and H. Alps. 1963. Über die Vergarung der Melibiose durch *Saccharomyces*-Stamme. Arch. Mikrobiol. **45**:179–187.
17. Hawthorne, D. C. 1956. Genetics of galactose fermentation. C. R. Trav. Lab. Carlsberg. Ser. Physiol. **26**: 149–160.
18. Khan, N. A., and N. R. Eaton. 1971. Genetic control of maltase formation in yeast. I. Strains producing high and low basal levels of enzyme. Mol. Gen. Genet. **112**:317–322.
19. Khan, N. A., F. K. Zimmermann, and N. R. Eaton. 1973. Genetic control of maltase formation in yeast. II. Evidence for a gene regulating the level of maltase production. Mol. Gen. Genet. **124**:365–367.
20. Lindegren, C. C., S. Spiegelman, and G. Lindegren. 1944. Mendelian inheritance of adaptive enzymes in yeast. Proc. Natl. Acad. Sci. U.S.A. **30**:346–352.
21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
22. Mortimer, R. K., and D. C. Hawthorne. 1969. Yeast genetics, p. 385–460. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, vol. 1. Academic Press Inc., New York.
23. Roberts, C., A. T. Ganesan, and W. Haupt. 1959. Genetics of melibiose fermentation in *Saccharomyces italicus* var. *melibiosi*. Heredity **13**:499–517.
24. Spiegelman, S., R. Sussman, and E. Pinska. 1950. On the cytoplasmic nature of long-term adaptation in yeast. Proc. Natl. Acad. Sci. U.S.A. **36**:591–606.
25. ten Berge, A. M. A. 1972. Genes for the fermentation of maltose and α-methylglucoside in *Saccharomyces carlsbergensis*. Mol. Gen. Genet. **115**:80–88.
26. ten Berge, A. M. A., G. Zoutewelle, and K. W. van de Poll. 1973. Regulation of maltose fermentation in *Saccharomyces carlsbergensis*. I. The function of the gene *MAL6*, as recognized by *mal6*$^-$ mutants. Mol. Gen. Genet. **123**:233–246.
27. ten Berge, A. M. A., G. Zoutewelle, K. W. van de Poll, and H. P. J. Bloemers. 1973. Regulation of maltose fermentation in *Saccharomyces carlsbergensis*. II. Properties of a constitutive *MAL6* mutant. Mol. Gen. Genet. **125**:139–146.
28. Tsuyumu, S., and B. G. Adams. 1973. Population analysis of the deinduction kinetics of galactose long-term adaptation mutants in yeast. Proc. Natl. Acad. Sci. U.S.A. **70**:919–923.
29. Tsuyumu, S., and B. G. Adams. 1974. Dilution kinetics of yeast populations: *in vivo* aggregation of galactose utilizing enzymes and positive regulator molecules. Genetics **77**:491–505.
30. Winge, O., and C. Roberts. 1948. Inheritance of enzymatic characters in yeasts, and the phenomenon of long-term adaptation. C. R. Trav. Lab. Carlsberg. Ser. Physiol. **24**:263–315.
31. Winge, O., and C. Roberts. 1957. A genetic analysis of melibiose and raffinose fermentation. C. R. Trav. Lab. Carlsberg. Ser. Physiol. **25**:419–459.
32. Zimmermann, F. K., N. A. Khan, and N. R. Eaton. 1973. Identification of new genes involved in disaccharide fermentation in yeast. Mol. Gen. Genet. **123**:29–41.