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Recessive Mutations Conferring Resistance to Carbon Catabolite Repression of Galactokinase Synthesis in *Saccharomyces cerevisiae*

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A total of 37 recessive mutations showing enhanced resistance to the glucose repression of galactokinase synthesis have been isolated by a selection procedure with a *GAL81 gal7* double mutant. These mutations were grouped into three different complementation classes. One class, *reg1*, contains mutants arising from mutations at a site close to, but complementing, the *gal3* locus. The *reg1* mutant also showed resistance to the glucose repression of invertase synthesis but not to that of α-D-glucosidase. The two other classes were identified as arising from recessive mutations at the *GAL82* locus and the *GAL83* locus, respectively, at which various dominant mutations were isolated previously. When in a constitutive background due to the *GAL81* or *gal80* mutation, the *GAL82* and *GAL83* mutations did not show a mutually additive effect on the resistance to glucose repression of galactokinase synthesis, while the *reg1* and *GAL82* (or *GAL83*) mutations did. Based upon the specific behavior of cells with various genotypes for the above genes in response to the concentration of galactose and glucose in the medium, we propose a model involving three independent circuits for glucose signals in the regulation of the structural genes for the galactose pathway enzymes.

Carbon catabolite repression is a central regulatory mechanism for the synthesis of many of the enzymes involved in carbon catabolism. Although several attempts have been made to elucidate the mechanism of glucose repression in yeasts by use of mutants with altered regulatory properties (4–7, 9, 17, 18), a comprehensive theory explaining all of the phenomena of carbon catabolite repression has not yet emerged. To investigate the mechanism of carbon catabolite repression in *Saccharomyces cerevisiae*, we looked at the regulatory system for the synthesis of galactose pathway enzymes, as we have long been engaged in the elucidation of this system in connection with the inducer function of galactose. The current hypothesis is that expression of the genes encoding the galactose pathway enzymes is regulated by two regulatory factors, a positive factor which is encoded by the *GAL4* gene and a negative factor specified by the *GAL80* gene (13). The positive factor is indispensable for the expression of the structural genes (10), and the negative factor interacts with the inducer and modulates the function of the positive factor. The galactose pathway enzymes are also under the control of carbon catabolite repression (1, 15, 24).

In a previous study (15), we isolated two classes of dominant mutations, *GAL82* and *GAL83*, from strains bearing *GAL81 gal7* double mutations. The *GAL81* mutation, which is dominant over its wild-type counterpart *gal81*$^+$ and confers the constitutive production of galactose pathway enzymes, was shown to occur in the *GAL4* locus and to give rise to a positive factor insensitive to the function of the negative factor (13). The *gal7* mutation occurs on the structural gene of α-D-galactose-1-phosphate uridyltransferase (EC 2.7.7.12) (8) and results in deficiency of enzyme activity. The *GAL82* and *GAL83* mutations are probably specific for galactose metabolism; they showed greatly enhanced resistance to glucose repression of galactokinase synthesis only when one or both of these mutations were combined with the *GAL81* or *gal80* constitutive mutation or with both (15). It is, however, possible to isolate by the same selection procedure mutants similar to *GAL82* and *GAL83*, but which are recessive to the wild-type counterpart.

This communication is a follow-up to the previous one (15) and deals with isolation and characterization of 37 recessive mutations which were classified into three different complementation groups. One class, *reg1*, consists of mutations which occur at a site close to, but comple-

menting, the *gal3* locus on chromosome IV. Two other classes of mutants were identified as recessive mutations at either the *GAL82* or *GAL83* locus. The *reg1*, *GAL81* (or *gal80*), and *GAL82* (or *GAL83*) mutations have an additive effect with each other on the resistance to glucose repression of galactokinase synthesis. The *reg1* mutation also releases the glucose repression of invertase synthesis. Based upon the specific behavior of cells with various genotypes for the above genes in response to the concentrations of galactose and glucose in the medium, we propose a regulatory model involving three independent circuits which convey the glucose signals to the *GAL* structural genes.

MATERIALS AND METHODS

Yeast strains. Genotypes and sources of the strains used are listed in Table 1. A *gal3* mutant allele was introduced into the test strains from strain 107-1C, which was provided by H. C. Douglas of Washington University, Seattle.

Media. General usage and composition of nutrient, minimal, sporulation, YPGlu (20 g of polypeptone, 10 g of yeast extract, and 50 g of glucose dissolved in and made up to 1 liter with distilled water), YPGly (20 g of glycerol per liter replaced the glucose in YPGlu), YPEth (5 g of ethanol per liter replaced the glucose in YPGlu), YPEthGal (2 g of galactose added per liter of YPEth), YPGluGal (10 g of galactose added per liter of YPGlu), YPGluGly (20 g of glycerol added per liter of YPGlu), YPGlyGal (20 g of galactose added per liter of YPGly), YPGluGlyGal (20 g of galactose added per liter of YPGluGly), and EBGal (20 g of polypeptone, 10 g of yeast extract, 20 g of galactose, and 20 mg of ethidium bromide per liter) media were described previously (14, 15, 20). To investigate the response of various mutants to different concentrations of effectors, modified YPGluGlyGal medium, in which the glucose concentration was reduced from 5 to 1% and that of galactose was increased from 2 to 5% (abbreviated as YPGlu[1%]GlyGal[5%]), modified YPGlyGal (YPGlyGal[5%]), in which galactose concentration was increased from 2 to 5%, and modified YPGluGly (YPGlu[1%]Gly), in which glucose concentration was decreased from 5 to 1%, were prepared. YPGlyMal medium was YPGly medium plus 50 g of maltose per liter. YPGluGlyMal medium was YPGlyMal medium plus 10 g of glucose per liter. In these two maltose media, the maltose concentration was increased to 5% and that of glucose in YPGluGlyMal medium was decreased to 1% from the previous formula (15).

Techniques. All of the techniques for mutant isolation, assay of enzyme activities (galactokinase [EC 2.7.1.6], β-D-fructofuranoside fructohydrolase [EC 3.2.1.26], and α-D-glucosidase [EC 3.2.1.20]), permeabilization of cells for galactokinase and α-D-glucosidase assays, and genetic analysis were as described in the previous publications (15, 20).

RESULTS

Isolation and classification of mutants. Respiratory-competent cells of a *GAL81 gal7* double mutant are able to grow on YPEth medium but not on YPEthGal medium because of the sensitivity to galactose conferred by the transferase mutation, *gal7*. However, the same mutant can grow on YPGluGal medium. This can be explained by the catabolite repression of galactokinase synthesis by glucose in YPGluGal medium. Catabolite repression-resistant mutations for the galactokinase synthesis can be isolated by picking up colonies which failed to grow on YPGluGal medium as described previously (15). According to this protocol, strain YG2-4D (α *GAL81-12 gal7-2*) was subjected to ethyl methane sulfonate mutagenesis (11) followed by nystatin selection (22), as described previously (15), and the treated cells were spread on YPGlu plates. Colonies that developed on the plates after incubation at 30°C for 4 days were screened for their inability to grow on YPGluGal plates by replica plating, and 37 mutants were isolated.

Diploids were constructed by crossing each isolate to strain YG1-1A (**a** *GAL81-12 gal7-2*). Because all of the resulting diploids could grow on YPGluGal medium, the mutations which confer the inability to grow on the YPGluGal medium, probably by the resistance to carbon catabolite repression of the galactokinase synthesis, are recessive to the wild-type counterpart. Though we found seven dominant mutations among 52 primary isolates in the previous study (15), none were detected in the present study. The reason for this is obscure, although we did use different strains in the two studies. When the diploids obtained by the above crosses were sporulated and dissected, all tetrads showed 2+:2− segregation for growth on YPGluGal medium for 6 to 12 asci tested in each cross (data not shown). Thus, each mutant carries a single mutation on a chromosome. Complementation tests among these mutants revealed three distinct classes of mutations: 8 of the 37 primary isolates fell into class I, 16 into class II, and the remaining 13 into class III. Tetrad analyses of the diploids constructed to have a genotype heterozygous for two mutations of different complementation classes and homozygous for the *GAL81-12* and *gal7-2* mutant alleles confirmed the assignment of the above complementation tests. The compiled data for the heterogeneous combinations showed the 0+:4− (corresponding to the parental ditype tetrade [PD]), 1+:3− (tetratype tetrad [T]), and 2+:2− (non-parental ditype tetrad [NPD]) segregations for the growth on YPGluGal medium in the ratio of 11:10:6 for the combination of mutation classes I and II, 2:7:1 for classes I and III, and 5:9:6 for classes II and III.

Allelism tests were then made with the dominant glucose-resistant mutations, *GAL82* and *GAL83*, isolated in the previous study (15). Some of the segregants having the new mutant

TABLE 1. List of yeast strains[a]

Strain	Genotype	Source
P-28-24C	a wild type	Our stock culture
G211-2A	a *gal7-2*	Matsumoto et al. (13)
G291-4C	a *gal3*	Segregant from 107-1C (α *gal3 ade2 ade6 his trp1 met*; H. C. Douglas) × P-28-24C cross
G373-4B	α *GAL81-12*	Matsumoto et al. (15)
G427-1C	a wild type	Segregant from P-28-24C × P612B (α *gal4-2* [13]) cross
G432-2A	a *gal80-2 gal7-2*	Constructed by repeated crosses and tetrad dissections among G183-1C (α *gal80-2* [15]), G251-5B (a *GAL80^s-1 gal7-2* [13]), and G211-6A (α *gal7-2* [13])
CR3-1B	a *GAL81-12 GAL82-R1*	Matsumoto et al. (15)
CR6-3D	α *GAL81-12 gal7-2 GAL83-R4*	Segregant from G373-1BR-4 (a *GAL81-12 gal7-2 GAL83-R4* [15]) × G373-6B (α *GAL81-12 gal7-2* [15]) cross
CR8-1D	α *GAL82-R1*	Matsumoto et al. (15)
CR21-1D	α *GAL81-12 gal7-2 GAL82-R1*	Segregant from CR3-1B × G373-3C (α *GAL81-12 gal7-2*; a haploid clone from the same family as G373-4B) cross
YG1-1A	a *GAL81-12 gal7-2*	Segregant from G373-1B (a *GAL81-12 gal7-2* [15]) × AX2-7A (α wild type; our stock culture) cross
YG2-4D	α *GAL81-12 gal7-2*	Segregant from G373-3C (α *GAL81-12 gal7-2*; a haploid clone from the same family as G373-4B) × G211-2A cross
YG2-4DR-35	α *GAL81-12 gal7-2 reg1-1*	Mutant from YG2-4D
YG5-4C	a *GAL81-12 gal7-2 reg1-1*	Segregant from YG2-4DR-35 × YG1-1A cross
YG5-6C	α *GAL81-12 gal7-2 reg1-1*	Segregant from YG2-4DR-35 × YG1-1A cross
YG8-2A	a *GAL81-12 reg1-1*	Selected from an ascus showing 2+:1− segregation on EBGal (13) and 4+:0− on YPGluGal medium from YG5-4C × G373-4B cross
YG11-7C	α *gal7-2 reg1-1*	Segregant from YG5-6C × G211-2A cross
YG16-8B	α *GAL81-12 gal7-2 trp1*	Constructed by repeated crosses and tetrad dissections among S2072D (α *arg4 leu1 trp1 thr4*; obtained from C. C. Lindegren), P-28-24C, G373-6B (α *GAL81-12 gal7-2* [15]), and YG2-4D
YG17-2C	α *reg1-1*	Constructed by repeated crosses and tetrad dissections among YG11-7C, P-28-24C, and AM1-9C (α wild type [16])
YG20-2D	a *reg1-1*	Segregant from YG8-2A × YG17-2C cross
YG22-1A	α *GAL81-12 GAL83-R4 reg1-1*	Constructed by repeated crosses and tetrad dissections among YG5-4C, CR6-3D, and YG8-2A
YG23-7D	α *GAL81-12 GAL82-R1 reg1-1*	Constructed by repeated crosses and tetrad dissections among YG5-4C, CR21-1D, and YG8-2A
YG24-6B	α *GAL83-R4 reg1-1*	Segregant from YG20-2D × YG22-1A cross

[a] The genetic symbols are those described by Broach (3), whereas the symbols for mating types (a and α) follow conventional usage. Genotypes for markers other than the mating type and *gal* are omitted from the table, except for strains 107-1C, S2072D, and YG16-8B.

alleles selected from the above crosses were crossed with the *GAL81-12 GAL82-R1 gal7-2* (CR21-1D) and *GAL81-12 GAL83-R4 gal7-2* (CR6-3D) strains, and diploids were subjected to tetrad analysis. Two of the three classes, classes II and III, are allelic or close to *GAL83* and *GAL82*, respectively (Table 2). A total of 2 of the 56 asci tested in the combination of class II and *GAL83* mutations showed 1+:3− segregations and might have arisen through interallelic recombination between two independent mutations at the *GAL83* locus. Thus, we designated the mutations of classes II and III as *gal83* and *gal82*, respectively. Since we have isolated both the dominant and recessive mutations in these loci, we use symbols *gal82*, *GAL82*, and *GAL82*$^+$ for the recessive mutant, dominant mutant, and wild-type alleles, respectively, for the *GAL82* gene and similar symbols for those of the *GAL83* gene. It is, however, uncertain whether the dominant and recessive mutations in each locus occur in the same cistron. Class I mutations segregated independently of the *GAL82* and *GAL83* loci and obviously occurred at a different site from the *gal7* and *GAL81* mutations. It is shown later that the class I

TABLE 2. Allelism tests of the new mutations with the *GAL82* and *GAL83* mutations

Cross[a]	No. of asci showing these segregations for growth on YPGluGal		
	0+:4−	1+:3−	2+:2−
Class I × *GAL82*	2	10	4
Class I × *GAL83*	8	4	4
Class II × *GAL82*	3	12	2
Class II × *GAL83*	54	2	0
Class III × *GAL82*	38	0	0
Class III × *GAL83*	1	5	2

[a] All of the crosses have a homozygous combination of the *GAL81-12* and *gal7-2* mutant alleles. A detailed description of the combination in each cross is omitted.

mutations release the glucose repression of invertase synthesis and occur at a different site from the *gal80* mutation. Hence we designated the locus of the class I mutations as *regI* (resistance to glucose repression).

Gal phenotype of the *regI* mutants. To study the galactokinase synthesis in the *GAL81-12 regI-1* cells, the *GAL81-12 regI-1 GAL7*$^+$ (YG8-2A) and *GAL81-12 REGI*$^+$ *GAL7*$^+$ (G373-4B) strains were grown at 30°C in YPGly and YPGluGly media. We eliminated the *gal7* mutation from the test strains used in this experiment as this mutation has undefined effects on the expression of the *GAL* structural genes. The time course of galactokinase appearance was followed by using suspensions of permeabilized cells as the enzyme source (Fig. 1). The *GAL81-12 REGI*$^+$ strain produced about 4% of the galactokinase activity in YPGluGly medium compared with that of cells grown in YPGly medium. On the other hand, the *GAL81-12 regI-1* strain showed about 40% of the enzyme activity in YPGluGly compared with that of cells cultivated in YPGly medium. No essential differences in cellular growth rates were observed between these strains, but higher growth rates were observed in YPGluGly medium than in YPGly medium for both strains.

Next we studied the *regI* mutation in the wild-type background for the *GAL81* locus. Strain YG5-6C (α *GAL81-12 regI-1 gal7-2*) was crossed with strain G211-2A (**a** *gal81*$^+$ *REGI*$^+$ *gal7-2*), the diploid was sporulated, and four-spored asci were dissected. Three ascus types, namely, 4+:0− (corresponding to the NPD tetrad), 3+:1− (T), and 2+:2− (PD) segregations for the growth of segregants on YPGluGal medium, were observed in a ratio of 4:12:2. This observation indicates that the catabolite repression-resistant phenotype might occur by a combination of the *regI* and *GAL81* mutations. To test this possibility for the other constitutive mutation, *gal80*, strain YG11-7C (α *regI-1 gal7-2*) was crossed with the *gal80-2 gal7-2* strain (G432-2A). Both the haploid strains and the resultant diploid could grow on YPGluGal medium. Tetrad analysis of the diploid gave the three ascus types, 4+:0− (PD), 3+:1− (T), and 2+:2− (NPD) segregations for the growth of segregants on YPGluGal medium, in a ratio of 4:5:0. This segregation pattern indicates that a cell with the *gal80 regI gal7* genotype is unable to grow on YPGluGal and is sensitive to galactose because of the *gal7* mutation even in the presence of glucose. Hence the *gal80 regI* genotype gives rise to the resistant phenotype for catabolite repression of the galactokinase synthesis, and the *regI GAL80*$^+$ *gal81*$^+$ genotype gives rise to the catabolite repression-sensitive phenotype for the galactokinase synthesis in YPGluGal medium. These tetrad data are also consistent with the conclusion that *regI* is unlinked to *gal80* (see below).

Effect of *regI* mutation on the invertase synthesis. To determine whether the *regI* mutation affects catabolic enzymes other than galactokinase, the activity levels of α-D-glucosidase and invertase, whose syntheses are normally repressed by glucose, were investigated. Although

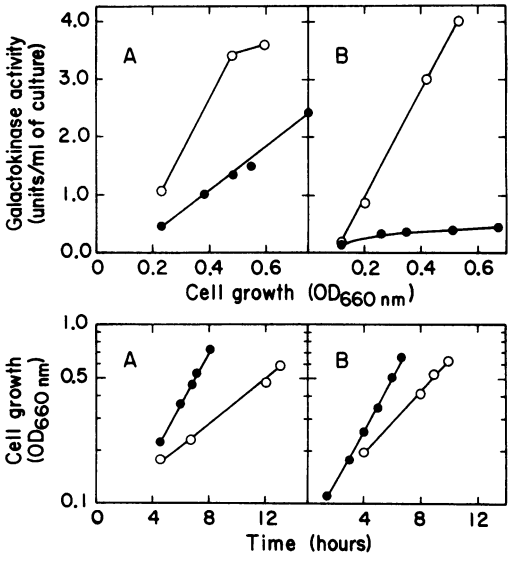

FIG. 1. Time courses of galactokinase synthesis in strains with the *GAL81-12 regI-1* and *GAL81-12 REGI*$^+$ genotypes. Cells of strains (A) YG8-2A (*GAL81-12 regI-1*) and (B) G373-4B (*GAL81-12 REGI*$^+$) were shaken at 30°C for 48 h in YPGly medium. A 0.5-ml portion of each culture was inoculated into 100 ml of YPGly (○) or YPGluGly (●) medium and shaken at 30°C. Galactokinase activity (upper panels) as a function of cell growth (optical density of the culture at 660 nm [$OD_{660\,nm}$], lower panels) was determined by using a permeabilized cell suspension as the enzyme source.

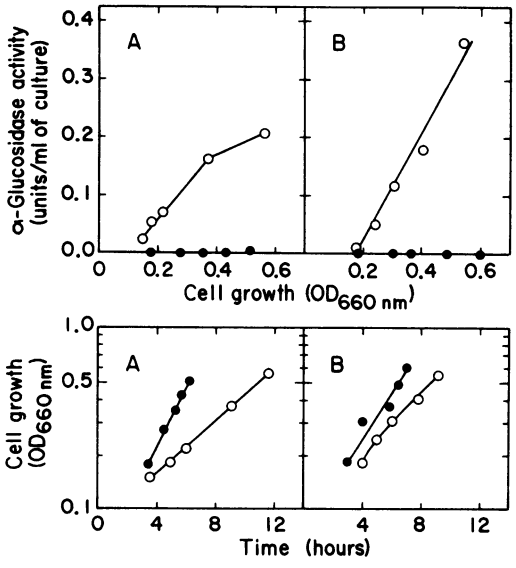

FIG. 2. Time courses of α-D-glucosidase synthesis in strains with the *GAL81-12 regl-1* and *GAL81-12 REG1*$^+$ genotypes. Cells of strains (A) YG8-2A (*GAL81-12 regl-1*) and (B) G373-4B (*GAL81-12 REG1*$^+$) were shaken at 30°C for 48 h in YPGly medium. A 0.5-ml portion of each culture was inoculated into 100 ml of YPGlyMal (○) or YPGluGlyMal (●) medium and shaken at 30°C. α-D-Glucosidase activity (upper panels) as a function of cell growth (optical density of the culture at 660 nm [$OD_{660\ nm}$], lower panels) was determined by using a permeabilized cell suspension as the enzyme source.

we do not know exactly their genotypes for maltose and sucrose utilization, strains YG8-2A (*GAL81-12 regl-1*) and G373-4B (*GAL81-12 REG1*$^+$) are able to utilize these substrates. They were cultivated at 30°C in YPGlyMal and YPGluGlyMal media to test α-D-glucosidase synthesis and in YPGly and YPGluGly media to test invertase synthesis. The time courses of the enzyme activities were followed. α-D-Glucosidase formation in both strains was fully repressed by the presence of glucose in the medium (Fig. 2). Invertase formation of the *GAL81-12 REG1*$^+$ strain was also repressed in YPGluGly medium, while the *GAL81-12 regl-1* strain showed a considerable level of invertase activity in the presence of glucose (Fig. 3). To confirm that the *regl* mutation is responsible for the synthesis of invertase in YPGluGly medium, five tetrads were randomly selected from the cross between YG2-4DR-35 (the original *regl-1* mutant; α *GAL81-12 regl-1 gal7-2*) and YG1-1A (**a** *GAL81-12 REG1*$^+$ *gal7-2*), and the activity level of invertase was determined for the segregant cells grown on YPGly and YPGluGly media. Tetrad clones in each ascus showed a two-to-two segregation of higher (11 to 75% of the activity of those grown on YPGly; mean = 25.5%) and lower (2 to 9%; mean = 5.4%) invertase activity on YPGluGly. The segregants showing the higher invertase activity all failed to grow on YPGluGal medium. These results indicate that the *regl* mutation is effective for release of the synthesis of both galactokinase and invertase from carbon catabolite repression.

Since the above experiments were done with the *regl* mutants marked with the *GAL81-12* mutation, we examined the invertase activities of the *regl* single mutant, YG20-2D, cultivated in YPGly and YPGluGly media and compared them with those of the wild-type strain, G427-1C, in the same way as in the above experiments. The specific activity of invertase in cells of the *regl* single mutant was found to be 3.42 U/ml per optical density unit at 660 nm in the culture on YPGly and 1.61 U on YPGluGly, while the wild-type strain produced 5.19 U on YPGly and 0.25 U on YPGluGly. The activity ratio (100 × units in YPGluGly/units in YPGly) of the *regl* mutant was 47%, and that of the wild-type strain was 5%. Thus, it is possible to

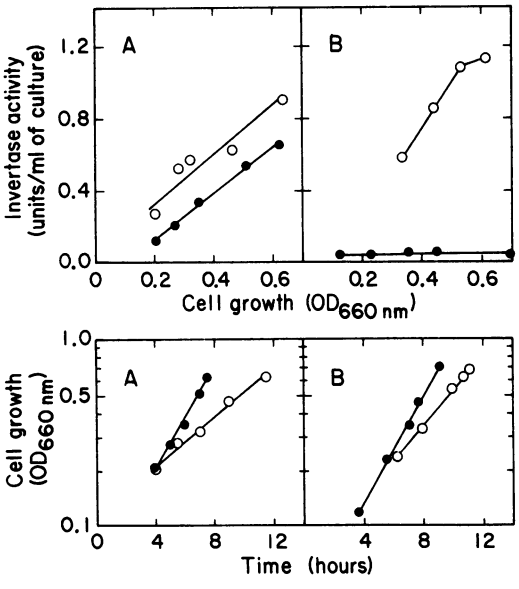

FIG. 3. Time courses of invertase synthesis in strains with the *GAL81-12 regl-1* and *GAL81-12 REG1*$^+$ genotypes. Cells of strains (A) YG8-2A (*GAL81-12 regl-1*) and (B) G373-4B (*GAL81-12 REG1*$^+$) were shaken at 30°C for 48 h in YPGly medium. A 0.5-ml portion of each culture was inoculated into 100 ml of YPGly (○) or YPGluGly (●) medium and shaken at 30°C. Invertase activity (upper panels) as a function of cell growth (optical density of the culture at 660 nm [$OD_{660\ nm}$], lower panels) was determined by using an intact-cell suspension as the enzyme source.

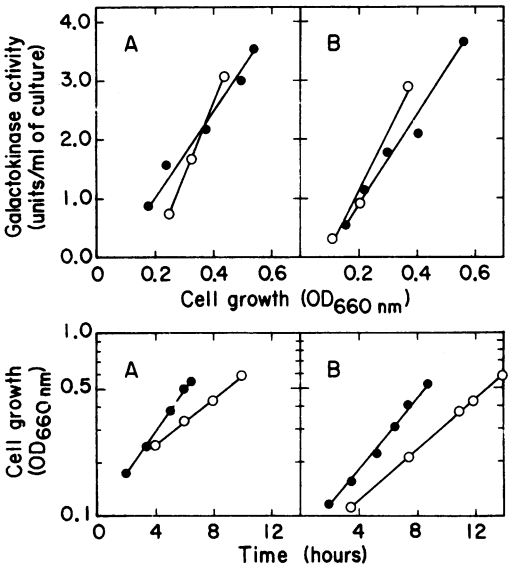

FIG. 4. Time courses of galactokinase synthesis in strains with the *GAL81-12 regl-1 GAL82-R1* and *GAL81-12 regl-1 GAL83-R4* genotypes. Cells of strains (A) YG23-7D (*GAL81-12 regl-1 GAL82-R1*) and (B) YG22-1A (*GAL81-12 regl-1 GAL83-R4*) were shaken at 30°C for 48 h in YPGly medium. A 0.5-ml portion of each culture was inoculated into 100 ml of YPGly (○) or YPGluGly (●) medium and shaken at 30°C. Galactokinase activity (upper panels) as a function of cell growth (optical density of the culture at 660 nm [$OD_{660\ nm}$], lower panels) was determined by using a permeabilized cell suspension as the enzyme source.

conclude that the *regl* single mutation releases the invertase synthesis from the catabolite repression and that the *GAL81-12* mutation is not concerned with invertase activity.

Additive effect of *regl* with *GAL82* and *GAL83*. The *GAL81 GAL82* and *GAL81 GAL83* double mutants produced about 50% as much galactokinase activity in YPGluGly as in YPGly medium (15), and *GAL82* and *GAL83* lack an additive effect on the resistance to glucose repression of galactokinase synthesis. The *GAL81 regl* double mutant showed approximately 40% as much galactokinase activity in YPGluGly as in YPGly medium (Fig. 1). The *GAL81 regl GAL82* and *GAL81 regl GAL83* cells showed about 79 to 90% as much galactokinase activity in YPGluGly as in YPGlu medium (Fig. 4). These triple mutants are therefore almost fully resistant to the glucose repression of galactokinase synthesis, although they showed severely reduced growth rates on the medium containing galactose (data not shown). The *regl* mutation thus has an additive effect with the *GAL82* and *GAL83* mutations on the resistance to glucose repression in combination with the *GAL81* (or *gal80*) mutation.

To examine in detail the combined effects of the *GAL81* (or *gal80*), *GAL82* (or *GAL83*), and *regl* mutations, strains with various genotypes for these genes were cultivated in YPGluGly, YPGluGlyGal, YPGlyGal, and YPGly. We also used the modified media, YPGlu[1%]Gly, YPGlu[1%]GlyGal[5%], and YPGlyGal[5%], to examine the response of the mutants to different concentrations of the effectors. During the cultivation, galactokinase activities were determined at appropriate intervals, and the highest values attained by each culture are listed in Table 3. These data showed specific features of the correspondence between the genotypes and the phenotypes. (i) The *regl* mutation has an additive effect with the *GAL82* and *GAL83* mutations, while the *GAL82* and *GAL83* mutations have no additive effect with each other (15). (ii) The *GAL81* (or *gal80*) *regl* mutants have phenotypes equivalent to those of the *GAL81* (or *gal80*) *GAL82* (or *GAL83*) double mutants. (iii) No single mutation of the *regl*, *GAL82*, and *GAL83* loci is enough to confer full resistance to the glucose effect, even in combination with the *GAL81* or *gal80* mutation. (iv) The triple mutant with the *regl GAL82* (or *GAL83*) *GAL81* (or *gal80*) genotype always showed a fully induced level irrespective of the composition of the culture medium, while the triple mutants showed severely reduced growth rates in the medium containing galactose. (v) Irrespective of the genotype of the *regl*, *GAL82*, *GAL83*, *GAL81*, and *gal80* genes, the phenotype was influenced by the effector concentration, the effect being particularly noted in the galactokinase activity levels of cells cultivated in YPGlu[1%]GlyGal[5%] and YPGluGlyGal media. It is noteworthy that the *regl* mutant showed a significantly reduced growth rate in media containing high amounts of galactose. For example, the generation time of YG17-2C was 6.6 h in YPGlyGal[1%] and 3.2 h in YPGlu[1%]GlyGal[5%] compared with that of the wild-type strain, G427-1C, of 3.6 h in YPGlyGal[5%] and 2.2 h in YPGlu[1%]GlyGal[5%]. This slow growth reduced the accuracy of the galactokinase assay, which requires a dense inoculation of cells.

Close linkage but nonallelic relationship of *regl* and *gal3*. A diploid prepared by crossing YG5-4C (**a** *GAL81-12 gal7-1 regl-1*) and YG5-4C (α *GAL81-12 gal7-1 GAL83-R4*) showed 0+:4− (corresponding to the PD tetrad), 1+3− (T), and 2+:2− (NPD) segregations on YPGluGal medium in the ratio of 8:4:4. This result suggests that both the *regl* mutation and the *GAL83* mutation are located close to a centromere on two different chromosomes, and indeed, we already know that the *GAL83* locus is on the right arm of

TABLE 3. Effect of the *reg1*, *GAL82* (or *GAL83*), and *GAL81* (or *gal80*) mutations on galactokinase synthesis in various media

Representative strain	Genotype[a]			Expt I[b]			Expt II[c]		
	reg1	*GAL82* (*GAL83*)	*GAL81* (*gal80*)	Glu (5%)	Gal (2%)	Sp act[d]	Glu (1%)	Gal (5%)	Sp act[d]
P-28-24C	WT	WT	WT	+	−	− (<0.01)	+	−	− (<0.01)
				+	+	− (<0.01)	+	+	± (0.3)
				−	+	+++ (10.1)	−	+	+++ (7.0)
				−	−	− (<0.01)	−	−	− (<0.01)
G373-4B	WT	WT	MU	+	−	± (0.4)	+	−	+ (0.8)
				+	+	+ (0.6)	+	+	+ (1.3)
				−	+	+++ (9.6)	−	+	+++ (9.3)
				−	−	+++ (9.6)	−	−	+++ (9.6)
CR8-1D	WT	MU	WT	+	−	− (0.01)	+	−	− (<0.01)
				+	+	− (0.01)	+	+	+++ (9.2)
				−	+	+++ (8.9)	−	+	+++ (9.0)
				−	−	− (<0.01)	−	−	− (<0.01)
CR3-1B	WT	MU	MU	+	−	++ (3.8)	+	−	++ (4.1)
				+	+	++ (2.8)	+	+	+++ (7.8)
				−	+	+++ (8.1)	−	+	+++ (8.4)
				−	−	+++ (8.0)	−	−	+++ (8.0)
YG17-2C	MU	WT	WT	+	−	− (<0.01)	+	−	− (<0.01)
				+	+	− (<0.01)	+	+	+++ (7.0)
				−	+	+++ (8.8)	−	+	+++ (9.5)
				−	−	− (<0.01)	−	−	− (<0.01)
YG8-2A	MU	WT	MU	+	−	++ (4.0)	+	−	++ (4.1)
				+	+	++ (4.6)	+	+	+++ (8.1)
				−	+	+++ (7.7)	−	+	+++ (8.5)
				−	−	+++ (7.0)	−	−	+++ (7.0)
YG24-6B	MU	MU	WT	+	−	− (<0.01)	+	−	− (<0.01)
				+	+	− (1.1)	+	+	+++ (8.2)
				−	+	+++ (8.8)	−	+	+++ (9.0)
				−	−	− (<0.01)	−	−	− (<0.01)
YG22-1A	MU	MU	MU	+	−	+++ (8.0)	+	−	+++ (7.8)
				+	+	+++ (8.1)	+	+	+++ (8.0)
				−	+	+++ (7.8)	−	+	+++ (8.4)
				−	−	+++ (9.0)	−	−	+++ (9.0)

[a] WT and MU indicate wild-type and mutant alleles in the respective locus.

[b] In experiment I, test strains were cultivated on YPGlu, YPGluGlyGal, YPGlyGal, and YPGly media. Cultural conditions are expressed by the presence (+) or absence (−) of effectors (5% glucose and 2% galactose) in the medium.

[c] In experiment II, test strains were cultivated as in experiment I, and cultural conditions are similarly expressed by the presence or absence of effectors, in this case 1% glucose and 5% galactose.

[d] Specific activity of galactokinase for each genotype is symbolized as follows: +++, induced level of the wild-type cells; ++, medium level; +, low level; ±, trace; −, no activity. The specific galactokinase activity (units per milliliter per optical density unit at 660 nm of the culture) observed with the representative strain is listed as an example.

chromosome V at a site approximately 8 centimorgans from the centromere (15). Conclusive data on the close linkage of the *reg1* locus to a centromere were given by tetrad analysis of the diploid constructed by crossing YG16-8B (α *GAL81-12 gal7-2 REG1*$^+$ *trp1*) and YG5-4C (a *GAL81-12 gal7-2 reg1-1 TRP1*$^+$). The tetrad data showed a PD/NPD/T ratio of 102:0:3 for the *reg1* and *trp1* genes. Since the *trp1* locus is tightly linked to the centromere on the right arm of chromosome IV (19), the *reg1* locus must be located on one arm of chromosome IV approximately 1.5 centimorgans from the centromere. This also means that *reg1* is closely linked to the

gal3 locus, because *gal3* was mapped on the same chromosome at a site approximately 0.4 centimorgans distal from *trp1* (19).

It is known that the *gal3* mutation gives rise to a phenotype showing long-term adaptation for synthesis of the galactose pathway enzymes (23). To examine the interaction between the *reg1* mutation and the *gal3* mutation, a *reg1* mutant, YG17-2C, was crossed to the *gal3* strain, G291-4C. The resultant diploid was compared with diploids of the *reg1/reg1* (YG17-2C × YG20-2D) and *reg1/REG1*$^+$ (YG17-2C × G427-1C) genotypes in galactokinase synthesis. Since the *reg1* mutation alone afforded resistance to the glucose repression of galactokinase synthesis in YPGlu[1%]GlyGal[5%] medium (Table 3), these strains were cultivated in the same low-glucose and high-galactose medium. Fully repressed levels of the enzyme activity were observed in the *reg1/gal3* and *reg1-1/REG1*$^+$ diploids, while the *reg1-1/reg1-1* homozygous diploid showed a significant level of the enzyme activity (Fig. 5). In addition, the *reg1* mutant showed enhanced resistance to the glucose repression of invertase synthesis (Fig. 3), whereas we confirmed with our strain that the *gal3* mutation has no effect on the invertase activity. The invertase activity of the *gal3* strain, G291-4C, was repressed by glucose: the specific activity of invertase in the cells at logarithmic growth phase in YPGluGly at 30°C was 0.04 U, as compared with 1.54 U/ml per optical density unit at 660 nm in the culture of the same cells in YPGly medium under the same conditions. These results suggest that the *reg1* mutation might not be allelic with *gal3* but might belong to a different gene. This problem needs further study, however, since the possibility remains that the different phenotypes represent multiallelomorphs of a single gene.

DISCUSSION

In the previous communication (15), we postulated a double circuit system for the glucose repression of galactokinase synthesis. One circuit consists of the *GAL80*$^+$ and *gal81*$^+$-*GAL4* proteins and is the circuit which transmits the galactose signals. The other circuit involves the *GAL82*$^+$ and *GAL83*$^+$ gene products. The isolation of an additional class of mutants, the *reg1* mutants, in the present study necessitates the revision of this hypothesis by the addition of a circuit mediated by the *REG1*$^+$ product, which is also concerned with the glucose repression. This circuit is independent of the *GAL82*$^+$–*GAL83*$^+$ and *GAL80*$^+$–*gal81*$^+$-*GAL4* circuits, as the *reg1* mutation has additive effects with the

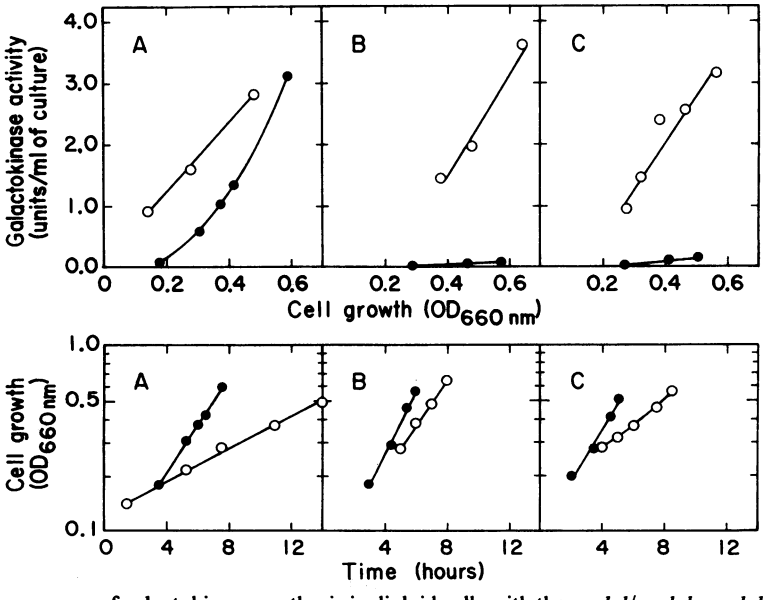

FIG. 5. Time courses of galactokinase synthesis in diploid cells with the *reg1-1/reg1-1*, *reg1-1/gal3*, and *reg1-1/REG1*$^+$ genotypes in YPGlu[1%]GlyGal[5%] medium. Cells of diploids constructed by (A) YG17-2C × YG20-2D (*reg1-1/reg1-1*), (B) YG17-2C × G291-4C (*reg1-1/gal3*), and (C) YG17-2C × G427-1C (*reg1-1/REG1*$^+$) crosses were shaken at 30°C for 48 h in YPGly medium. A 0.5-ml portion of each culture was inoculated into YPGlyGal[5%] (○) or YPGlu[1%]GlyGal[5%] (●) medium and shaken at 30°C. Galactokinase activity (upper panels) as a function of cell growth (optical density of the culture at 660 nm [$OD_{660\ nm}$], lower panels) was determined by using a permeabilized cell suspension as the enzyme source.

mutations of these circuits. It may have a more generalized function in the glucose repression than the other circuits, as it is effective for certain catabolic enzymes other than the galactose pathway enzymes, for example, invertase.

Cells with the wild-type alleles of the *GAL81* and *gal80* genes showed no galactokinase activity or a severely reduced level in YPGluGlyGal medium, even in combination with the *regl* or *GAL82* (or *GAL83*) mutation, or both of them, but a significant elevation of the enzyme activity when they were cultivated in YPGlu[1%]GlyGal[5%] (Table 3). This elevation can be explained by the involvement of the *GAL80*$^+$-*gal81*$^+$-*GAL4* circuit in the transmittance of the glucose signals, probably by the following mechanisms; namely, the intracellular levels of galactose are strongly affected by glucose through the inhibition of the galactose uptake system (12). In YPGluGlyGal medium, the intracellular level of galactose is insufficient to overcome the competition with glucose for the negative factor or is lower than the necessary threshold level to inactivate the function of the negative factor; in YPGlu[1%]GlyGal[5%], galactokinase should be produced to some extent due to the penetration of galactose into the cells at a sufficient level to overcome the glucose competition or to inactivate the negative factor to activate the positive factor. Since the *GAL81* (or *gal80*) mutation abolishes the requirement for the inducer for expression of the *GAL* structural genes in cells with the *regl GAL82* (or *GAL83*) *GAL81* (or *gal80*) genotype and the other two glucose circuits are interrupted by the *regl* and *GAL82* (or *GAL83*) mutations, these cells should show the fully induced level of galactokinase activity irrespective of the presence or absence of glucose and galactose. These arguments are summarized in the model illustrated in Fig. 6. Whether the competition or threshold level mechanism or both are involved in the glucose repression remains to be elucidated. All of these arguments, however, are based on the assumption

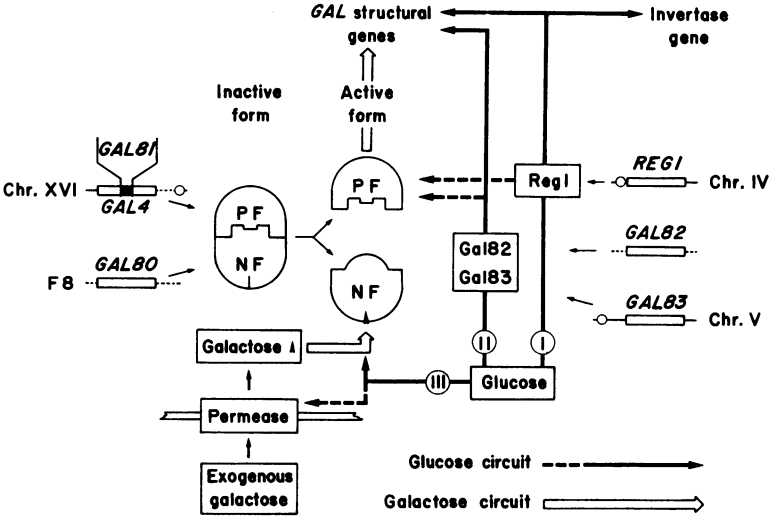

FIG. 6. Model for the function of regulatory genes in regulation of the galactose pathway enzymes. The structural genes coding for the galactose pathway enzymes are coordinately controlled in their expression by a positive factor (PF) and a negative factor (NF), which are coded and constitutively produced by the *gal81*$^+$-*GAL4* and *GAL80* genes, respectively (13). These factors interact in the cytoplasm or nucleoplasm and convey signals of the presence or absence of inducer (galactose) to the structural gene. The presence of inducer inhibits the function of the negative factor or dissociates it from the positive factor, allowing the positive factor to activate the expression of the structural genes of the galactose pathway enzymes at the transcriptional level (10). The expression of the structural genes is also controlled by the carbon catabolite repression. The observations described in this communication suggest that the glucose signals of the presence or absence of enough glucose to cause carbon catabolite repression are transmitted by three independent circuits, I, II, and III. Circuit I, which is mediated by the *REG1* product, is effective for repression of various catabolic enzymes, including galactokinase and invertase, in the presence of glucose. Circuit II is mediated by the *GAL82* and *GAL83* products and is specific for the galactose pathway enzymes (15). Circuit III exerts its function by the competitive interaction of the effectors. glucose and galactose, on the negative factor in the cytoplasm or by decreasing the inducer concentration lower than the necessary threshold level to express the *GAL* structural genes, through the allosteric inhibition of galactose permease on the cell membrane (12). Whether circuits I and II convey the glucose signals to the positive factor or directly to the structural genes is still obscure.

that the *reg1*, *GAL82*, and *GAL83* genes are involved in the regulation of transcription of the structural genes for the galactose pathway enzymes, as has been found for *GAL4* regulation (10).

Since cyclic AMP plays an essential role in the mechanism of catabolite repression in bacteria (see review; 2), much work has been devoted to investigating the similar regulatory system in yeasts. Though a correlation between the concentration of glucose in the medium and the intracellular level of cyclic AMP in yeasts has been reported (21, 25), the correlation does not conform to the details of the time course and specificity of catabolite repression. In a recent study in our laboratories (16) with a mutant capable of utilizing cyclic AMP as an adenine source, we obtained evidence that cyclic AMP may not be concerned in the mechanism of glucose repression in *S. cerevisiae*. This evidence further suggests that the circuits for the glucose repression illustrated in Fig. 6 do not involve cyclic AMP as a cofactor.

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