REGULATION OF GENES CONTROLLING SYNTHESIS OF THE GALACTOSE PATHWAY ENZYMES IN YEAST[1]

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THE genetic control of synthesis of the galactose pathway enzymes in *Saccharomyces cerevisiae* conforms in certain respects to the operon model proposed by JACOB and MONOD (1961) for the β-galactosidase system of *E. coli*, and shown by BUTTIN (1963a, b) to be valid for the *E. coli* galactose system as well. Three closely linked structural genes specify the galactose pathway enzymes, galactokinase, galactose-1-phosphate-uridyl transferase (transferase), and uridine diphosphogalactose-4-epimerase (epimerase) (DOUGLAS and HAWTHORNE 1964). The three loci are under the control of an unlinked regulator gene, *i*, which is recognizable by its recessive mutations that permit constitutive synthesis of the three galactose enzymes (DOUGLAS and PELROY 1963).

A key feature of the bacterial systems which appears to be absent in the yeast system in the close association of an operator gene with the structural genes (DOUGLAS and HAWTHORNE 1964). The operator locus was defined originally as a region linked to the structural genes in which two types of mutations occurred: O^c, which were expressed as *cis*-dominant for constitutive synthesis of the operon proteins, and O^o, which prevented synthesis of all of the proteins of the operon. JACOB and MONOD (1965) have redefined the operator locus in the β-galactosidase system of *E. coli* as the site of repressor recognition identified by O^c mutations. The O^o mutations in this system are now considered to be polarity mutations within the first structural gene of the operon (BECKWITH 1964).

Mutations in yeast that result in failure to synthesize the three galactose enzymes and thus resemble phenotypically mutations of the O^o type can be readily isolated. However, these are not polarity mutants nor are they mutants in which inducer uptake or metabolism is defective. They map in the GA_4 locus which segregates independently of the galactose structural genes and their phenotype is unchanged in combination with i^- (DOUGLAS and HAWTHORNE 1964). The occurrence of mutations in the yeast galactose system which display the dominant constitutive phenotype and the relationship of these mutants to the GA_4 region and to the structural genes for the galactose enzymes is the subject of the present paper.

MATERIALS AND METHODS

Isolation of dominant constitutive mutants: The method used employed respiratory-deficient ga_3 stocks, as described previously (DOUGLAS and PELROY 1963), with the exception that the

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starting material was diploid to prevent expression of i^- mutants. The recessive gene ga_3 blocks some step in the induction of the galactose pathway enzymes, since constitutive mutants, and to a lesser extent back mutants, are selected for by plating ga_3 stocks on galactose agar. An unusual feature of this system, however, is that the mutants are greatly outnumbered by phenocopies, which are gal+ clones that revert to the ga_3 phenotype when grown in the absence of galactose (WINGE and ROBERTS 1948; SPIEGELMAN, DELORENZO and CAMPBELL 1951). This problem is alleviated somewhat by using ga_3 stocks that are vegetative petites, for the respiratory-deficient condition reduces the frequency of phenocopies substantially.

To facilitate the genetic analyses it was necessary to start with a diploid that was homozygous for the mating type alleles since a respiratory-deficient diploid that is heterozygous with respect to mating type will neither mate nor sporulate. The diploid stock from which the constitutive mutants were isolated was prepared by mating two cytoplasmic respiratory-deficient ga_3, i^+ haploids of α mating type which had several complementary nutritional requirements. Matings between haploid cells of α mating type occur at a very low frequency but the diploids are readily recovered by the prototrophic selection technique.

About one cell in 10^4 of the respiratory-deficient diploid stock (#117) was capable of colony formation on galactose agar, but less than 0.1% of these were stable mutants. To increase the frequency of stable mutants, use was made of the mutagen, N-methyl-N'-nitrosoguanidine. Overnight glucose broth cultures of diploid 117 were diluted with an equal volume of fresh medium and placed on a shaker at 30°C. Three hours later a freshly prepared filter-sterilized solution of the mutagen was added to give a concentration of 20 μg/ml and the cultures were incubated for an additional hour. The treated cultures were then diluted 100-fold in fresh broth and permitted to grow to saturation by overnight incubation. Diluted samples of the overnight cultures containing approximately 10^6 cells were then spread on galactose agar, and after 4 days incubation cells from the colonies which had developed were streaked on glucose agar to obtain isolated colonies. Clones which produced confluent growth on galactose agar after this regimen were considered to be mutant.

Constitutive synthesis of the galactose pathway enzymes were determined by analyses (DOUGLAS and HAWTHORNE 1964) for transferase activity or occasionally epimerase activity, using cell extracts prepared from overnight cultures grown in glucose broth. The specific activities of the galactose enzymes in constitutive mutants were generally within the range of values observed in fully induced strains (DOUGLAS and HAWTHORNE 1964).

Conventional techniques for yeast genetics were used, and the culture media were of the same composition as described previously (DOUGLAS and HAWTHORNE 1964). The galactose phenotype of segregants, all of which were respiratory sufficient, was scored in Durham tubes containing 2% peptone, 1% yeast extract, and 2% glucose-free galactose. The inoculated tubes were sealed with a 1-inch layer of vaspar and incubated at 30°C. Under these conditions clones of the same genotype as diploid #117 failed to grow and produce gas even after several days incubation whereas constitutive clones and inducible clones containing GA_3 produced abundant growth and gas within 48 hours.

RESULTS

Seventy-two stable gal+ mutants were isolated in four separate experiments with nitrosoguanidine-treated material and one mutant was obtained in several experiments using untreated material. Twenty-three of the mutants were inducible and probably represent mutations of ga_3 to GA_3. The remaining mutants were crossed with a diploid gal+ stock of composition $aa\ i^+i^+\ GA_3GA_3$ and the resulting tetraploids were tested for constitutive synthesis. Surprisingly, the constitutive character proved dominant in only three clones, one of which was the spontaneous mutant. The constitutive strains were rechecked by crossing them to a respiratory-deficient haploid of composition $a\ i^+\ ga_3$ and then testing the

triploids for galactose fermentation. With the exception of the hybrids involving the above three mutants, the triploids were gal−. Thus, the majority of the constitutive isolates carried recessive genes, presumably i^-i^-.

The single spontaneous dominant constitutive mutant and the two mutants isolated after nitrosoguanidine treatment were subjected to additional genetic analysis. Since the results were the same in each case, only the results obtained with the spontaneous mutant will be given.

First, the tetraploid hybrid from the cross of the mutant and the respiratory-sufficient diploid described above was sporulated and asci were dissected. The diploid segregants from five tetrads were scored on galactose. In three of the asci, all the segregants were gal+; one ascus contained a single gal− segregant, and another had two gal− segregants. These results indicated that the dominant constitutive mutant had retained both ga_3 genes but had suffered a mutation at another locus, which we shall call *C*, that was able to suppress the gal− phenotype characteristic of ga_3. The analysis of the gal+ diploids from the first three asci for constitutive synthesis of transferase revealed that one ascus contained two constitutive segregants and two contained but one (Table 1). (Tetraploids with a single dominant allele can give asci with 1+:3− ratios when there is tetravalent pairing of the four homologs and multiple exchanges between the locus and the centromere (ROMAN, PHILLIPS and SANDS 1955).) From these observations we can deduce that the spontaneous mutant was heterozygous for *C*.

Diploid segregant 122-2c (Table 1), a constitutive gal+ from the above cross, was sporulated and the segregants from four asci examined for their galactose and constitutive synthesis phenotypes. In two asci all of the segregants were gal+, one ascus contained three gal+ and one gal−, and one contained two gal+ and two gal−. Constitutive synthesis by the segregants in the ascus containing three gal+ and one gal− clone was determined, and it was found that two of the gal+ were constitutive while the third was inducible (Table 2). These results indicated that diploid 122-2c was heterozygous for both ga_3 and the locus determining dominant constitutive synthesis.

Segregants 1B and 1D from diploid 122-2c were both constitutive gal+ haploids

TABLE 1

Properties of the segregants of two tetraploid asci obtained by crossing dominant constitutive mutant 117–1 with a diploid of composition aa i^+i^+ GA_3GA_3

Segregant	Mating type	Sporulation	Galactose fermentation	Constitutive synthesis
122–2a	αα	—	+	—
–2b	aa	—	+	—
–2c	aα	+	+	+
–2d	aα	+	+	+
122–3a	aa	—	—	—
–3b	aα	+	—	—
–3c	aα	+	+	+
–3d	αα	—	+	—

TABLE 2

Properties of the haploid segregants in an ascus from diploid 122–2c (Table 1)

Segregant	Mating type	Galactose fermentation	Constitutive synthesis
122–2c–1A	α	—	—
122–2c–1B	a	+	+
122–2c–1C	α	+	—
122–2c–1D	a	+	+

and could have contained either ga_3 or GA_3. To verify their genotypes crosses were made to gal+ inducible stocks and the galactose phenotypes of the segregants from ten asci of each cross were determined. In the cross with 1D all of the segregants were gal+, but in the cross with 1B approximately ¼ of the segregants were gal−. Thus segregant 1B was ga_3 and segregant 1D GA_3.

To determine whether gene *C* was linked to the structural genes for the galactose pathway enzymes, segregant 1D was crossed to an inducible stock that was mutant in the GA_1 locus which determines galactokinase synthesis. The gal+ diploid produced in this cross was constitutive as expected from the earlier results, and the segregants of five asci yielded 2:2 segregations in each ascus for galactose fermentation, indicating the segregation of a single gene (ga_1) controlling galactose fermentation. The 20 segregants of this cross were then analyzed for constitutive synthesis of transferase and in each ascus a 2:2 segregation was observed. This analysis showed that *C* recombined freely with ga_1, four of the asci being tetratype with respect to the two factors while one was a nonparental ditype.

Except for its dominance, the expression of *C* is indistinguishable from that of i^- and it was necessary, therefore, to determine if the two genes were allelic. To accomplish this, segregant 122-2c-1D, which carried *C*, was crossed with haploid 106-2a which is gal+ and possessed the recessive gene i^-. The segregants of five asci were tested for their ability to ferment galactose and for constitutive synthesis. As expected, all of the segregants were gal+. However, in only one ascus were all four of the segregants constitutive (parental ditype class), while each of the remaining four asci contained three constitutive and one inducible segregant (tetratype class). Thus, the two genes for constitutive synthesis are neither allelic nor closely linked.

It has been mentioned that dominant constitutive (O^c) and O^o mutations affecting lactose and galactose utilization in *E. coli* map within their respective operons. To determine whether the mutations with comparable phenotype were linked in our material, a cross was made between haploid segregant 122-2c-1c which carried gene *C* and haploid 279-1A which was gal− because it carried the mutation ga_4 which produces the O^o phenotype. If these two factors were closely linked, one would expect most of the asci to be of the parental ditype class, i.e., two of the segregants would be gal+ and constitutive and two would be gal−.

The diploid produced in this cross was gal+ and constitutive and 81 of the 82 asci analyzed yielded two gal+ segregants that were constitutive and two gal−

clones. The exceptional ascus (281-35) also yielded two gal+ and two gal− segregants, but one of the gal+ segregants was inducible.

The results of this cross indicated that *C* and ga_4 are closely linked and suggest that a crossover between them had occurred in ascus 281-35. The two gal− segregants are expected on this basis to be *C* ga_4, and *c* ga_4. To verify this, the two gal− segregants of this ascus were examined for the presence of *C* as follows: Both segregants were crossed to inducible gal+ stocks, but the diploids were inducible in both cases. Nevertheless, the two gal− segregants were examined further. Four independent spontaneous gal+ revertants were isolated from each segregant and tested for constitutive synthesis. The revertants from segregant 281-35D were all inducible, but the revertants from segregant 281-35B were all constitutive and produced constitutive diploids when crossed to an inducible gal+ stock. Thus, segregant 281-35B contained *C*. One of the gal+ revertants from segregant 281-35B was crossed to a gal+ haploid to determine whether the reversion was due to a back mutation at the ga_4 locus or to an unlinked suppressor. The segregants from ten asci were examined and in each ascus all four segregants were gal+. These results make it highly probable that the revertant was a back-mutant rather than a suppressed mutant.

The fact that segregant 281-35B produced an inducible diploid when crossed to an inducible stock, even though this segregant was shown by the behavior of its revertants to contain *C*, suggested that dominant constitutive synthesis required that *C* and the + allele of ga_4 be in *cis* position.

To test the validity of this conclusion, it was decided to examine the behaviour of mutants of the same composition as segregant 281-35B, *C* ga_4, but obtained in a search for ga_4 mutants in a stock carrying *C*. The results with these mutants were the same as with segregant 281-35B, for they yielded constitutive gal+ revertants which produced constitutive diploids in crosses to an inducible gal+ haploid. The mutants themselves, however, produced inducible diploids when crossed to the same inducible gal+ haploid.

DISCUSSION

The fact that expression of the constitutive phenotype mediated by mutations in the *C* locus requires the + allele of ga_4 in *cis* configuration suggests that the *C* region is not translated into a polypeptide product. In contrast to this, the region linked to *C* in which the ga_4 mutations occur is translated, for mutations in this region display interallelic complementation and are susceptible to correction by external suppressors (HAWTHORNE, unpublished).

Our observations suggest the following type of regulatory system for the structural genes controlling synthesis of the three galactose pathway enzymes in our Saccharomyces stocks. The *i* locus, which is in a separate linkage group and which can be identified by its recessive mutations that confer the constitutive phenotype, produces a repressor whose site of action is *C*, the region identified by mutations conferring the dominant constitutive phenotype. The *C* locus controls

the expression of a contiguous structural gene in which the ga_4 mutations occur, and the protein specified by the latter locus in turn exerts positive control of the expression of the complex of three linked genes that specify the structure of galactokinase, epimerase and transferase.

In comparing the yeast system with the *E. coli* lactose or galactose systems, it seems clear that the *C* locus plays essentially the same role as the bacterial operator loci in that it represents the site of repressor recognition and controls the expression of a contiguous structural gene. In the bacterial systems, the contiguous structural genes are concerned directly with the elaboration of the lactose and galactose enzymes, while in the yeast system the contiguous structural gene (GA_4) acts as an intermediary between the *C* locus and the structural genes for the galactose enzymes. Whether the product of GA_4 controls the synthesis of the galactose enzymes at the level of messenger RNA synthesis or at some subsequent step in protein synthesis is at present not known.

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SUMMARY

Regulation of synthesis of the galactose pathway enzymes in *Saccharomyces cerevisiae* involves three loci: *i*, which determines repressor synthesis; *C*, the site of repressor recognition; and GA_4, a structural gene that is closely linked to *C* and whose expression is controlled by *C*. The product of GA_4 in turn exerts positive control of the unlinked cluster of genes specifying the three galactose pathway enzymes.

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