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Genetic Order of the Galactose Structural Genes in *Saccharomyces cerevisiae*

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The galactose structural genes of *Saccharomyces cerevisiae* were ordered by determining the genotypes of mitotic and meiotic recombinants from crosses heterozygous for the three genes. The most probable order is centromere-*gal7-gal10-gal1*. Nonreciprocal recombination was more frequent than reciprocal exchange, and both mitotic and meiotic co-conversions involving mutant sites in all three genes were observed.

There are three structural genes in the galactose fermentation pathway of *Saccharomyces cerevisiae*. These genes specify the enzymes galactokinase, galactose-1-phosphate uridylyl transferase, and galactose-4-epimerase; the locus designations are *gal1* (kinase), *gal7* (transferase), and *gal10* (epimerase).

In *S. cerevisiae*, *gal1*, *gal7*, and *gal10* have not been found to recombine meiotically and therefore are thought to comprise a tightly linked cluster (1). Here we report on the ordering of the three galactose structural genes by the genotypic analysis of mitotic and recombinants from crosses heterozygous for *gal1*, *gal7*, and *gal10*.

MATERIALS AND METHODS

Mitotic recombination experiments. The galactose-negative strains used in this investigation were obtained by treating a galactose-positive strain (S416B, Berkeley Culture collection) with ethyl methane sulfonate (Eastman Chemical Co.) by the method of Lindegren et al. (5). Mutant strains with complementary mating types were derived from crosses to a galactose-positive stock culture.

The genotype of the galactose-positive diploid used in the mitotic recombination studies is as follows:

$$\text{Diploid no. 580}\ \frac{gal7\ +\ gal1\ +}{+\ gal10\ +\ lys2}\quad\frac{met1}{+}\ \frac{a}{\alpha}\ \frac{ura1}{+}\ \frac{ade6}{+}\ \frac{ade2}{+}\ \frac{ade3}{+}$$

The presence of galactose inhibits the growth of bacterial mutants with defects equivalent to *gal7* and *gal10* yeast strains, and these mutants have been found to accumulate large quantities of the metabolic intermediates gal-1-phosphate and uridine diphosphogalactose (UDP gal; reference 8). In yeast *gal7* and *gal10* mutants show a similar sensitivity to galactose and this phenomenon can be utilized to select double mutants by plating either a *gal7* or a *gal10* strain on an agar medium containing galactose (0.3%) and ethanol (0.5%, reference 1). On this medium any additional mutations which relieve the galactose sensitivity, presumably by preventing the accumulation of gal-1-phosphate and UDP gal, can be selected since they will allow growth at the expense of ethanol. The *gal7-gal1* parental strain was obtained by employing this technique. This strain grows well on a galactose-ethanol medium and does not complement with either *gal7* or *gal1* tester strains.

The diploid culture was grown overnight on YEPD-agar (peptone 2%, yeast extract 1%, dextrose 2%, agar 2%), diluted in distilled water and plated on YEPD-agar to give approximately 300 cells/plate. The plates were then irradiated with X rays (10 kr) and incubated for 48 hr at 30 C. The resultant colonies were replica plated to YEP Gal plates (peptone, 2%; yeast extract, 1%; agar, 2%; purified galactose, Sigma Chemical Co., 0.5%). Both the galactose-positive and the galactose-negative sides of half-sectored colonies were transferred to YEPD master plates and were scored for the presence or absence of a lysine requirement by replica plating to synthetic omission media. Half-sectored colonies were used exclusively so that only cells with recombinant strands might be recovered. Because quarter sectors, for instance, sometimes result from one round of replication prior to an exchange, the use of such colonies might mean that the larger sector side would contain cells carrying nonrecombinant strands and their presence would interfere with the genetic analysis. To determine which galactose markers were present in the recombinant colonies, five asci each from the sporulated-sector sides were dissected and the segregants were crossed with *gal1*, *gal7*, and *gal10* tester stocks. The resulting diploids were then tested for growth on YEP Gal media. Since the presence of a defective gene results in no growth when mated with a strain containing an allelic mutation or growth when crossed with a strain defective in a complementary gene, the geno-

types of the recombinant diploids could be inferred.

The procedures and media for sporulation, tetrad dissection, allelic complementation, and scoring genetic markers have been described (4, 6).

Meiotic recombination experiments. The genotype of the galactose-positive diploid from which unselected tetrads were isolated and analyzed is as follows:

$$\text{Diploid no. 593}\quad \frac{gal7\ \ gal10\ \ +\ \ lys2}{+\ \ +\ \ gal1\ \ +} \quad \frac{\alpha}{a}\ \ \frac{+}{trp1}\ \ \frac{+}{met14}\ \ \frac{+}{ura3}$$

The *gal7-gal10* double mutant was recovered from the mitotic recombination experiments. It was necessary to construct a different diploid for the meiotic experiments to introduce the centromere markers *trp1*, *met14*, and *ura3*. Approximately 95% of the spores isolated from diploid no. 593 were viable.

RESULTS

Table 1 presents the results of a genetic analysis of half-sectored diploid clones derived from diploid no. 580 which was heterozygous for *gal1*, *gal7*, *gal10* and a linked, distal marker, *lys2*. All of the clones sectored on galactose were the result of nonreciprocal recombinations (gene conversions) involving at least two and more often all three of the galactose markers. Simultaneous gene conversions involving different alleles within a gene or markers in linked genes have been termed co-conversions.

Table 2 presents data on meiotic recombination among unselected tetrads dissected from diploid no. 593 which was again heterozygous for *gal1*, *gal7*, *gal10* and the independently segregating centromere markers *trp1*, *met14*, and *ura3*. We found only five recombinant asci among 997 tetrads. Only one ascus, no. 386, showed a reciprocal exchange between the *gal* markers. In addition to the single reciprocal case, there were four tetrads showing gene conversions, including one triple-site co-conversion and two double-site co-conversions.

DISCUSSION

Two assumptions are useful in making inferences from the data presented in Tables 1 and 2 as to the order of the galactose structural genes in *S. cerevisiae*. The first assumption is that the mitotic and meiotic co-conversions affecting the gal markers are the result of a single event that overlaps the co-converted sites and not the result of multiple, independent exchanges. This would seem to be a reasonable assumption in view of the total absence of the single convertant classes in the mitotic results and the preponderance of co-conversion in the meiotic results. The observation that, with one exception (Table 1, clone 1), the co-converted strands faithfully reproduce the allelic arrays of one or the other of the parental strands offers additional support to the assumption that the co-convertants are not the result of multiple, independent exchanges.

This assumption has been made with regard to meiotic co-conversions in *S. cerevisiae* on the basis of data showing that the closer two alleles are to each other the more frequently they co-convert and that these co-conversions occur at the expense of the single convertant classes. To account for these observations, it has been proposed that the mechanism of meiotic gene conversion involves the replacement of genetic information, some hundreds of nucleotides in length, with that of an homologous, nonsister chromatid (2, 3). Mitotic gene conversion has not been as extensively studied as its meiotic counterpart; however, mitotic co-conversions involving widely separated allelic sites have been observed (9).

On the basis of many data showing that outside marker recombination is associated with 50% of meiotic gene conversions, it has been suggested that these two phenomena are not independent but are manifestations of a single, underlying genetic mechanism (2). Similarly, mitotic gene conversion and outside marker recombination have been found to occur coincidentally at a rate far higher than the product of their individual frequencies would predict (9). In this study we also found a high incidence of outside marker recombination correlated with mitotic gene conversion. The frequency of sectoring for *lys2* alone was about 1 in 500 colonies, whereas about 1 in 900 colonies was sectored for galactose utilization. Among the colonies showing gene conversions affecting the galactose genes, however, approximately one in five (7 of 38 colonies) was also recombinant for the distal marker, *lys2*.

These observations are similar to those which have been made in the case of meiotic gene conversion and suggest that mitotic gene conversion and outside marker recombination are also related to a single genetic event.

On the basis of the data presented in Tables 1 and 2, a probable order for the galactose structural genes can be inferred. Table 1 shows that three sectored colonies (clones 5, 6, and 7) all co-converted for *gal10* and *gal1* and showed reciprocal exchanges for the distal marker, *lys2*, whereas *gal7* remained heterozygous in both sector halves. Under the assumption that co-conversions are not independent, it is clear that *gal7* is not the central gene. Furthermore, under the assumption that mitotic gene conversion and outside marker recombination are not inde-

TABLE 1. *Mitotic recombination: genotypes of sectored diploid clones*

Genotypes	Clone no.	*gal* Sectors	Sector genotypes[a]				*gal* Cistrons converted
			gal7	*gal10*	*gal1*	*lys2*	
Genotypes of parental diploid			− / +	+ / −	− / +	+ / −	
Genotypes of clones sectored on both galactose and lysine	1	Pos	− / +	+ / −	+ / +	+ / +	7, 10, 1
		Neg	+ / +	− / −	− / +	− / −	
	2	Pos	− / +	+ / −	− / +	− / −	7, 10, 1
		Neg	− / −	+ / +	− / −	+ / +	
	3	Pos	− / +	+ / −	− / +	+ / −	7, 10, 1
		Neg	+ / +	− / −	+ / +	− / −	
	4	Pos	− / +	+ / −	− / +	+ / +	7, 10, 1
		Neg	+ / +	− / −	+ / +	− / −	
	5	Pos	− / +	+ / −	− / +	+ / +	10, 1
		Neg	− / +	+ / +	− / −	− / −	
	6, 7	Pos	− / +	+ / −	− / +	+ / +	10, 1
		Neg	− / +	− / −	+ / +	− / −	
Genotypes of clones sectored on galactose	8	Pos	− / +	+ / −	− / +	+ / −	7, 10, 1
		Neg	− / −	+ / +	− / −	+ / −	
	9	Pos	− / +	+ / −	− / +	+ / −	7, 10, 1
		Neg	− / −	+ / +	− / −	+ / +	
	10, 11, 12	Pos	− / +	+ / −	− / +	+ / −	7, 10, 1
		Neg	+ / +	− / −	+ / +	+ / −	
	13	Pos	− / +	+ / −	− / +	+ / −	10, 1
		Neg	− / +	− / −	+ / +	+ / −	

[a] Galactose genotypes scored by complementation tests with *gal7*, *gal10*, and *gal1* tester strains; *lys2* scored by replica plating to a synthetic omission medium.

TABLE 2. *Meiotic recombination: genotypes of recombinant tetrads among 997 unselected asci*

	Ascus no.	Spore	1st Division spore array[a]	Spore genotypes[b]				gal cistrons	
				gal7	*gal10*	*gal1*	*lys2*	2nd Division segregation	Converted[c]
Genotype of parental diploid	–	–	–	– +	– +	+ –	– +	–	–
Genotype of reciprocal recombinant	386	A B C D	● ○ ● ○	+ – + –	+ – – +	– + + –	– + + –	10, 1	–
Genotype of nonreciprocal recombinants	152	A B C D	● ○ ○ ●	– + + +	– + + +	+ – – –	– – + +	–	7, 10, 1
	258	A B C D	● ○ ● ○	+ + + –	+ – + –	– + – +	+ + – –	–	7
	519	A B C D	● ○ ○ ●	+ – + +	+ – + +	– + + –	+ – – +	–	7, 10
	756	A B C D	● ○ ○ ●	– + + –	– – + –	+ + – +	– + + –		10, 1

[a] First division spore array deduced from the segregation of known centromere markers: *tryl*, *met14*, *ura3* (7). *Combination of closed and open symbols indicates first division spore array.*

[b] The *tryl*, *met14*, *ura3*, *lys2*, and mating type showed 2:2 segregations in the five recombinant asci; all the genotype tests were repeated at least once.

[c] Gene conversion frequencies: *gal7*, 0.3%; *gal10*, 0.3%; *gal1*, 0.2%; *tryl*, 0.4%; *met14*, 0.5%; mating type, 0.5%; *ura3*, 1.5%; *lys2*, 1.9%.

pendent genetic events, the proximal location for *gal7* is favored since this marker did not show recombination in three separate instances in which *gal10* and *gal1* co-conversions occurred in conjunction with reciprocal exchanges affecting *lys2*.

The data discussed thus far favor two gene orders: centromere-*gal7*-*gal10*-*gal1* or centromere-*gal7*-*gal1*-*gal10*. There is some additional evidence, however, that supports the former, since in one sectored colony (Table 1, no. 1) *gal7* and *gal10* co-converted in a parental array, whereas *gal1* converted independently. As a whole, the mitotic data are most consistent with the order: centromere-*gal7*-*gal10*-*gal1*.

The meiotic data presented in Table 2 are consistent with two gene orders. Only one ascus, no. 386, showed a reciprocal exchange between the *gal* markers. In this tetrad, *gal10* and *gal1* exhibited second-division segregation and *gal7* exhibited first-division segregation. This ascus was unusual in another respect, since it also represented a four-strand double exchange between the distal marker, *lys2*, and *gal1*. Given this result, the same number of exchanges are consistent with *gal7* being on either the proximal or the distal end of the cluster.

In addition to the single reciprocal case, there were four tetrads showing gene conversions, including a co-conversion involving *gal7* and *gal10* and another co-conversion involving *gal10* and *gal1*. As a whole, the meiotic data are consistent with either centromere-*gal7*-*gal10*-*gal1* or centromere-*gal1*-*gal10*-*gal7*.

Taken together, the mitotic and meiotic data are consistent with each other and indicate that

the most probable gene order is centromere-*gal7-gal10-gal1*. This agrees with another report which has indicated that *gal10* is proximal to *gal1* (6).

In addition to establishing a probable order for the galactose structural genes, the data presented in Tables 1 and 2 indicate that both mitotic and meiotic co-conversions are able to span a genetic length extending into at least three adjacent genes. Meiotic co-conversions involving sites in two adjacent genes have been reported previously (2, 7).

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