A CLUSTER OF THREE GENES RESPONSIBLE FOR ALLANTOIN DEGRADATION IN *SACCHAROMYCES CEREVISIAE*[1]

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ABSTRACT

Mutant strains of *Saccharomyces cerevisiae* unable to utilize allantoin as sole nitrogen source were isolated and divided into three groups on the basis of their biochemical and genetic characteristics. The three loci associated with these mutant classes were designated *dal1* (allantoinase minus), *dal2* (allantoicase minus) and *dal4* (allantoin transport minus). All three loci are located in a cluster that is proximal to the *lys1* locus on the right arm of chromosome *IX*. The gene order and intergenic distances were estimated to be: *dal1*—2.5 cM—*dal4*—1.9 cM—*dal2*—4.6 cM—*lys1*.

SACCHAROMYCES cerevisiae is able to use allantoin as sole nitrogen source by virtue of being able to degrade it, as shown in Figure 1, to ammonia, glyoxylate and carbon dioxide. Past studies have shown that production of all five enzymes associated with this catabolic pathway is inducible (COOPER and LAWTHER 1973; WHITNEY, COOPER and MAGASANIK 1973). Allophanate, the last pathway intermediate, was identified as the native inducer and an equally effective nonmetabolizable inducer (oxalurate) was also found (SUMRADA AND COOPER 1974). Allantoin degradative enzyme synthesis is also subject to nitrogen repression when strains are provided with readily used nitrogen sources (BOSSINGER, LAWTHER and COOPER 1974).

The common control characteristics of all five catabolic enzymes prompted us to ascertain the organization of the genes needed for their production. Here we report results indicating that three of the genes required for allantoin catabolism are tightly clustered on the right arm of chromosome IX. The present studies also expand on earlier observations made concerning the location of genes responsible for production of allantoinase and allantoicase (LAWTHER *et al.* 1974).

MATERIALS AND METHODS

Culture conditions: Minimal medium used in this work was that of WICKERHAM (1946). Wherever necessary, nutritional requirements were satisfied by adding the following supplements (quantities given for one l of medium): 20 mg adenine, 20 mg L-histidine hydrochlo-

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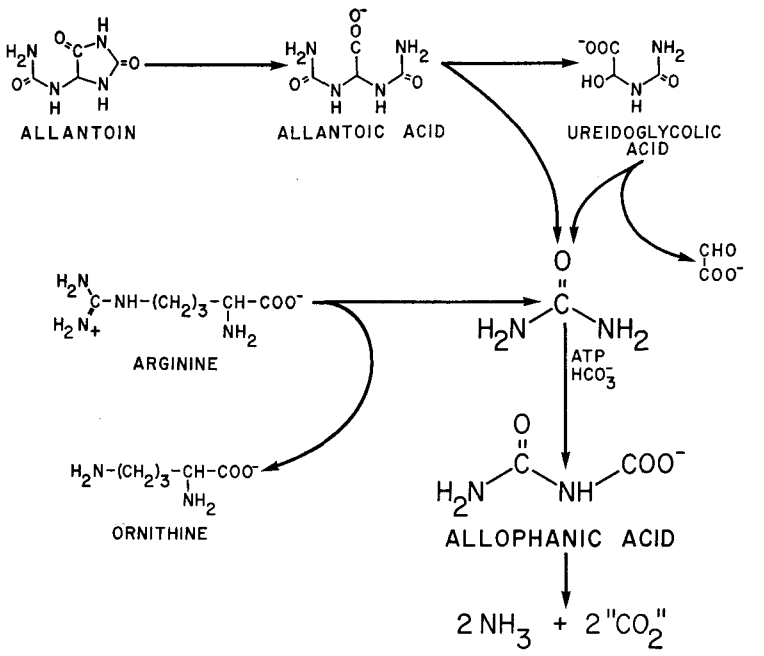

FIGURE 1.—Reactions involved in the catabolism of allantoin and arginine. Reactions associated with degradation of allantoin are catalysed sequentially by the enzymes: allantoinase, allantoicase, ureidoglycolate hydrolase, urea carboxylase and allophanate hydrolase. Arginine is converted to urea and ornithine by action of arginase.

TABLE 1

Strains used

Strain number	Genotype
M25–12b	**a**, *his6*, *ura1*
S185	*α*, *ade6*, *leu1*
MC13 *α*	*α*, *lys2*, *ino1–13*, *can*^{r}
N33, N199, N202, N203, N205, N206, N209, N211, N212, N213, N214	**a**, *his6*, *ura1*, *dal4*
M25	(*α*, *ade6*, *leu1* / **a**, *his6*, *ura1*, *lys1*)
M488	(*α*, *ade6*, *leu1*, *dal4–N33* / **a**, *his6*, *ura1*, *dal4–N33*)
M210	(*α*, *lys1*, *dal1–N16* / **a**, *his6*, *ura1*, *dal2–N18*)
M215	(**a**, *his6*, *ura1*, *dal1–N16* / *α*, *lys1*, *dal2–N18*)
M249	(*α*, *his6*, *ura1*, *dal1–N16*, *dal2–N18* / **a**, *leu1*, *met14*, *trp1*, *lys1*)
M344	(*α*, *ade6*, *leu1*, *dal1–N16*, *dal2–N18* / **a**, *lys1*, *dal4–N33*)

ride, 120 mg L-leucine, 40 mg L-lysine hydrochloride, 20 mg L-tryptophan and 20 mg uracil. Solid medium contained 1.5% agar. Nitrogen sources were 0.1% ammonium sulfate, allantoin, potassium allantoate, arginine hydrochloride, asparagine, sodium ureidoglycolate or 0.01 M urea. Growth was followed turbidimetrically using a Klett Summerson colorimeter with # 54 band pass filter (500–570 nm); 100 Klett units was approximately equivalent to 3×10^7 cells per ml of culture. The strains used are listed in Table 1.

Mutagenesis and mutant enrichment: Methods for mutagenesis were taken from SNOW (1966) and FINK (1970). Strains M25-12b and MC-13α were grown to stationary phase in YEPD medium, harvested by centrifugation, washed and then suspended in 0.05 M phosphate buffer (pH 8.0). Ethyl methanesulfonate (3% final concentration) was added to the suspension which was then incubated with gentle agitation for 90 min at 30°.

Two methods were used to enrich cultures for the mutant strains we desired. The first method involved use of nystatin as described by SNOW (1966) and FINK (1970). The second method was that of HENRY (1975) and made use of the fact that inositol auxotrophs die when deprived of inositol unless growth and division are prevented.

Purification of the mutants, complementation tests, sporulation, micromanipulation, and tetrad analysis were performed using standard genetic methods (MORTIMER and HAWTHORNE 1969; FINK 1970). Physiological and biochemical characterization was performed using prototrophic diploid strains homozygous for the mutant allele under study.

Enzyme assays: Enzymes involved in allantoin degradation were assayed in cell-free extracts to establish the biochemical phenotypes of representative mutant strains. Induced and uninduced cultures were cooled and treated with cycloheximide (10 μg/ml final concentration) on reaching cell densities of 60 to 80 Klett units. Cells were harvested by centrifugation, washed with a solution containing 0.05 M Tris buffer (pH 7.0), 5% glycerol and 3 mM mercaptoethanol and resuspended in the same buffer solution. Cells were broken with glass beads in a Braun homogenizer. Cell debris and undisrupted cells were removed by centrifugation at 11,000 $\times$ g. Extracts derived in this way were then assayed for enzyme activity.

The method of VAN DE POLL,VERWEY and KONINGSBERGER (1968) was used to assay allantoinase. The assay depends on the enzymatic conversion of allantoin to allantoate which, in the presence of dilute acid and heat, decomposes to glyoxylate which in turn condenses with phenylhydrazine to form glyoxylate phenylhydrazone. Excess phenylhydrazine was then oxidized in strong acid by potassium ferricyanide, followed by azo coupling and decarboxylation to yield the 1,5-diphenyl formazan (MATSUI, OKADA and ISHIDATE 1965).

Allantoicase was assayed by the indirect methods of LEE and ROUSH (1964), which depends on the enzymatic conversion of allantoate to ureidoglycolate and the subsequent chemical breakdown of ureidoglycolate to urea and glyoxylate. Glyoxylate thus formed reacts with phenylhydrazine to form glyoxylate phenylhydrazone, which absorbs light at 324 nm. The extinction coefficient used to convert absorbance differences to glyoxylate concentrations was 1.7×10^4 liter mole^{-1}cm^{-1} (DIXON and KORNBERG 1959).

The assay of ureidoglycolate hydrolase was essentially identical to that of allantoicase except for a change in substrate (CHO, LEE and ROUSCH 1966).

Allophanate hydrolase and urea amido-lyase were assayed using the procedures of WHITNEY and COOPER (1977).

RESULTS

Growth and biochemical characteristics of mutant strains: A large number (2,827) of mutant strains unable to utilize allantoin as sole nitrogen source were isolated and divided into three groups on the basis of their growth and complementation characteristics. Gene loci associated with the three mutant classes were designated *dal1*, *dal2*, and *dal4*. Most of the mutants isolated possessed defective enzymes involved in urea degradation (*dur1* and *dur2*). However, 1.9, 1.2 and 0.4%, respectively, of the mutants were members of the above classes.

TABLE 2

Doubling times (minutes) of wild type and dal4 *mutant strains of* Saccharomyces cerevisiae

Nitrogen source	Wild type (M25)	dal4 Mutant (M488)
Ammonia	120	159
Urea	128	215
Ureidoglycolate	157	218
Allantoate	160	161
Allantoin	150	N.G.*
Arginine	150	179
Asparagine	115	163

* N.G. indicates a lack of detectable growth after 48 hours.

All of the *dal* mutant alleles isolated were recessive to the corresponding wild-type alleles. Diploid strains heterozygous for mutant alleles belonging to different complementation groups could grow on allantoin, whereas organisms heterozygous for two alleles from the same group could not. No intragenic complementation was observed among mutants of the *dal1* and *dal4* loci, but several different examples of intragenic complementation were observed among mutants defective in the *dal2* locus (see LAWTHER *et al.* 1974 for characterization of these strains).

We previously showed that mutations in the *dal1* and *dal2* loci resulted in loss of allantoinase and allantoicase activities, respectively. Therefore, the growth and biochemical characteristics of these strains are not defined further here. The biochemical defect caused by mutation of the *dal4* locus, however, is unknown. As shown in Table 2, growth of a representative *dal4* mutant was normal when either ammonia, allantoate, arginine or asparagine was provided as sole nitrogen source. Growth using urea or ureidoglycolate was somewhat slowed (1.4- to 1.7-fold increase in the doubling time), but it is not yet clear whether this response is physiologically significant. Allantoin, on the other hand,

TABLE 3

Levels of allantoin degradative enzymes in wild type and dal4 *mutant strains of* Saccharomyces cerevisiae*

Enzyme assayed	Wild type (M25)	dal4 mutant (M488)
	nmoles/minute/mg protein	
Allantoinase	15.3	19.1
Allantoicase	9.0	7.2
Ureidoglycolate hydrolase	19.4	21.9
Urea Amidolyase	6.8	5.6
Allophanate hydrolase	62.1	52.7

* Assay procedures are described in the text. Cells were grown to a cell density of 75 Klett units in the presence of 10 mM urea. At this time extracts were made as described in the text. Note that these extracts were prepared differently from those reported earlier. Therefore, observed levels of activity are higher in some cases.

was incapable of supporting any measurable growth even after 72 hr of incubation at 30°. Similar growth behavior was also observed when a number of other *dal4* mutants were tested.

Growth characteristics of the *dal4* mutants were consistent with the idea that these strains might be deficient in allantoinase. However, as shown in Table 3, crude extracts of *dal4* mutant strains possessed wild-type levels of all enzyme activities known to be required for allantoin degradation. To assure that the mutant strains had not reverted to a wild-type phenotype during growth of the test culture, a sample of cells was removed from the cultures that we assayed and were found to possess the expected mutant phenotype. The same enzyme profile was observed for each of several *dal4* alleles tested.

We have shown previously that allantoin enters yeast cells by way of an inducible active transport system (SUMRADA and COOPER 1977; SUMRADA *et al.* 1978). A strain that was defective in allantoin uptake would likely possess the growth and biochemical characteristics observed for the *dal4* mutants. Therefore, we measured allantoin uptake in these strains. As shown in Figure 2, trans-

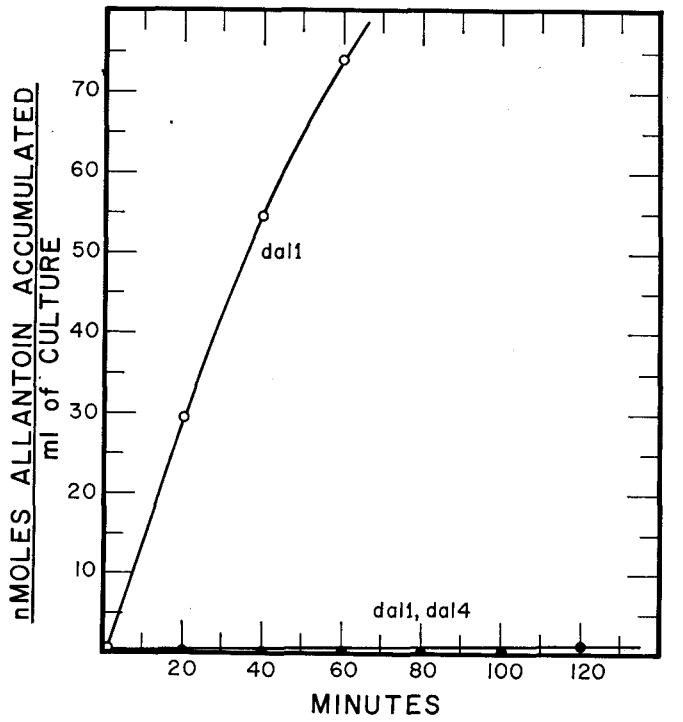

FIGURE 2.—Allantoin uptake by *dal1* and *dal1*, *dal4* mutant strains of *Saccharomyces cerevisiae*. The two strains were grown in minimal ammonia medium buffered with 1% citrate at pH 6.05. At a cell density of 45 Klett units, a sample of the cells was collected, washed with buffered glucose medium devoid of a nitrogen source and starved overnight according to the procedures described by SUMRADA and COOPER (1977). After nitrogen starvation for 18 to 24 hr, 8 ml aliquots of each culture were transferred to flasks containing ^{14}C-allantoin at a final concentration of 0.1 mM. Samples from each flask were then removed at the times indicated in the figure and processed as described by SUMRADA and COOPER (1977).

port of allantoin in *dal4* mutant strains was decreased 370-fold compared to wild type, identifying this as the likely defect in these mutants.

Linkage relationships of the dal1, dal4, dal2 *and* lys1 *loci*: LAWTHER *et al.* (1974) showed that the *dal1* and *dal2* loci were linked to one another and to the *lys1* locus on the right arm of chromosome *IX*. However, only asci in which all markers segregated in a 2+:2− fashion were analysed. Asci in which gene conversion occurred were discarded, regardless of the identity of the converted locus. Since this resulted in omitting a reasonable number of asci and did not permit analysis of the crosses for gene conversion, we have re-analysed all asci from three representative crosses of that earlier work, along with crosses that identify the location of the *dal4* locus.

As shown in Table 4, no nonparental ditypes were observed among 1,243 asci of the four crosses we analysed. Here, only asci in which all markers segregated 2+:2− were considered. Those asci in which gene conversion occurred will be discussed later and were not considered in estimates of genetic distances. In crosses M210, M215 and M249, there were always more tetratype asci for the *dal1-lys1* gene pair than for the *dal1-dal2* and *dal2-lys1* gene pairs, suggesting that the *dal2* locus was between the other two loci. The apparent genetic distance between the *dal1* and *dal2* loci was approximately 3.3 cM, whereas that between *dal2* and *lys1* was approximately 4.7 cM.

For cross M344, which involved all four loci, more tetratype asci were seen

TABLE 4

Linkage of dal1, dal4, dal2 *and* lys1 *loci on chromosome* IX

Cross	Gene pair	Number of tetrads analyzed	PD	NPD	TT	Calculated* genetic distance (cM)
M210	*dal1-dal2*	486	443	0	43	4.4
	dal1-lys1		420	0	66	6.8
	dal2-lys1		446	0	40	4.1
M215	*dal1-dal2*	228	212	0	16	3.5
	dal1-lys1		192	0	36	7.9
	dal2-lys1		208	0	20	4.4
M249	*dal1-dal2*	164	154	0	10	3.1
	dal1-lys1		138	0	26	7.9
	dal2-lys1		148	0	16	4.9
M344	*dal1-dal4*	365	347	0	18	2.5
	dal4-dal2		351	0	14	1.9
	dal1-dal2		345	0	20	2.7
	dal1-lys1		309	0	56	7.7
	dal4-lys1		320	0	45	6.2
	dal2-lys1		327	0	38	5.2

Similar results have been observed when crosses containing different *dal1*, *dal2* and *dal4* alleles were analyzed.

Crosses M210, 215 and 249 have also been discussed in a more limited way earlier (LAWTHER *et al.* 1974). It should be pointed out that the column headings in Table 9 of that work were somewhat incomplete and resulted in some values being misentered. This mistake can be seen by comparing those values with the values appearing in Table 6 of the present work.

* Genetic distances were calculated using the formula of PERKINS (1949).

for the *dal1-lys1* gene pair than for any other, again identifying these as the outside loci (Table 4). If we conclude from the data cited above that the tentative gene order was *dal1-dal2-lys1*, then *dal4* must be located either between *dal1* and *dal2* or between *dal2* and *lys1*. Forty-five tetratype asci were observed for the *dal4-lys1* gene pair, compared to only 18 for *dal4-dal1*. Since 38 tetratype were observed for the *dal2-lys1* gene pair, it is reasonable to suggest that the *dal4* locus is between *dal1* and *dal2*. Comparison of the number of tetratype asci observed for the other possible gene pairs also supports the gene order *dal1-dal4-dal2-lys-1*. Genetic distances calculated by application of PERKINS' (1949) formula were only slightly different from those observed in crosses M210, M215 and M249.

Stronger support for the above gene order comes from analysing the genotypes of observed recombinant asci. In cross M344, we observed 60 recombinant

TABLE 5

Recombinant ascospore genotypes expected from single crossovers derived for various possible gene orders

	Assumed gene order	Region where a single crossover is assumed to occur		Genotypes of recombinant ascospores*				Observed no. of asci with the indicated genotypes
1.	*dal1* I *dal4* II *dal2–lys1*	I	b.	*dal1*	*dal4*	+	*lys1*	12
			c.	+	+	*dal2*	+	
		II	b.	*dal1*	+	+	*lys1*	7
			c.	+	*dal4*	*dal2*	+	
2.	*dal1* I *dal2* II *dal4–lys1*	I	b.	*dal1*	+	*dal4*	*lys1*	12
			c.	+	*dal2*	+	+	
		II	b.	*dal1*	*dal2*	*dal4*	*lys1*	3
			c.	+	+	+	+	
3.	*dal4* I *dal1* II *dal2–lys1*	I	b.	+	+	+	*lys1*	3
			c.	*dal4*	*dal1*	*dal2*	+	
		II	b.	+	*dal1*	+	*lys1*	7
			c.	*dal4*	+	*dal2*	+	
4.	*dal2* I *dal1* II *dal4–lys1*	I	b.	*dal2*	+	*dal4*	*lys1*	1
			c.	+	*dal1*	+	+	
		II	b.	*dal2*	*dal1*	*dal4*	*lys1*	3
			c.	+	+	+	+	
5.	*dal4* I *dal2* II *dal1–lys1*	I	b.	+	+	+	*lys1*	3
			c.	*dal4*	*dal2*	*dal1*	+	
		II	b.	+	*dal2*	+	*lys1*	0
			c.	*dal4*	+	*dal1*	+	
6.	*dal2* I *dal4* II *dal1–lys1*	I	b.	*dal2*	*dal4*	+	*lys1*	1
			c.	+	+	*dal1*	+	
		II	b.	*dal2*	+	+	*lys1*	0
			c.	+	*dal4*	*dal1*	+	

* Only the recombinant ascospore genotypes appear here. The parental spore genotypes were omitted for the sake of brevity.

asci among 365 asci analyzed. Here the *lys1* locus will be assumed to be located outside the *dal* gene cluster (*dal1*, *dal4*, *dal2*). This assumption will be subsequently verified and for the moment will permit ordering of only the *dal* genes. It also eliminates 34 recombinant asci from the analysis. There are six possible orders for the genes and the expected genotypes of all recombinant ascospores derived from a single crossover event are shown in Table 5 along with the number of each ascal class we observed experimentally. Nineteen of the 26 observed recombinant asci can be accounted for by single crossovers if the gene order is assumed to be *dal1-dal4-dal2*. Only 15 asci can be accounted for in this way when the order is assumed to be *dal1-dal2-dal.4* For the remaining gene orders an even smaller percentage of observed asci can be accounted for by single crossovers.

In the above analysis we assumed that *lys1* was located outside of the *dal* gene

TABLE 6

Recombinant ascospore genotypes expected from single crossovers derived for various possible gene orders

	Assumed gene order	Region where a single crossover is assumed to occur		Genotypes of recombinant ascospores*				Observed no. of asci with the indicated genotypes
1.	*dal1–dal4* I *dal2* II *lys1*	I	b.	*dal1*	+	+	*lys1*	7
			c.	+	*dal4*	*dal2*	+	
		II	b.	*dal1*	+	*dal2*	*lys1*	34
			c.	+	*dal4*	+	+	
2.	*dal1–dal2* I *dal4* II *lys1*	I	b.	*dal1*	*dal2*	*dal4*	*lys1*	3
			c.	+	+	+	+	
		II	b.	*dal1*	*dal2*	+	*lys1*	34
			c.	+	+	*dal4*	+	
3.	*dal1–dal4* I *lys1* II *dal2*	I	b.	*dal1*	+	*lys1*	+	7
			c.	+	*dal4*	+	*dal2*	
		II	b.	*dal1*	+	+	+	1
			c.	+	*dal4*	*lys1*	*dal2*	
4.	*dal1–dal2* I *lys1* II *dal4*	I	b.	*dal1*	*dal2*	*lys1*	*dal4*	3
			c.	+	+	+	+	
		II	b.	*dal1*	*dal2*	+	*dal4*	3
			c.	+	+	*lys1*	+	
5.	*dal1–lys1* I *dal2* II*dal4*	I	b.	*dal1*	+	+	*dal4*	0
			c.	+	*lys1*	*dal2*	+	
		II	b.	*dal1*	+	*dal2*	*dal4*	3
			c.	+	*lys1*	+	+	
6.	*dal1–lys1* I *dal4* II *dal2*	I	b.	*dal1*	+	*dal4*	+	0
			c.	+	*lys1*	+	*dal2*	
		II	b.	*dal1*	+	+	+	1
			c.	+	*lys1*	*dal4*	*dal2*	

* Only the recombinant ascospore genotypes appear here. The parental spore genotypes were omitted for the sake of brevity.

cluster. We verified this assumption on the basis of observed recombinant asci as follows here we will ignore the *dal1* locus, which eliminates 12 recombinant asci from consideration. There are six possible orders for the *dal4*, *dal2* and *lys1* genes. The expected genotypes of all recombinant ascospores derived from a single crossover event are shown in Table 6 along with the number of each ascal class we observed experimentally. Forty-one of the observed recombinant asci can be accounted for by single crossovers if the gene order is assumed to be *dal4-dal2-lys1*, whereas only 37 of the asci can be accounted for in this way if the gene order is *dal2-dal4-lys1*. The remaining possible gene orders may be ignored since they account for only eight or less of the 48 recombinant asci observed. In toto, these data indicate that a maximum percentage of recombinant asci can be accounted for by single crossovers only if the gene order is *dal1-dal4-dal2-lys1*.

First and second division segregation frequency of the dal *and* lys *loci*: The *dal1*, *dal4*, *dal2* and *lys1* loci possessed second division segregation frequencies of 54, 56, 58 and 63%, respectively, suggesting that the *dal* loci are proximal to *lys1*; these estimates were corrected for errors due to second division segregation of the *leu1* marker used in this analysis. These frequencies of second division segregation are far too great to estimate genetic distances accurately, but the tentative genetic order derived from the data discussed previously generates several predictions that can be tested qualitatively by determining whether markers in the four loci segregated at first or second meiotic division. If, for example, the gene order is centromere-*dal1-dal4-dal2-lys1*, then one would predict that the number of times *dal1* and *dal2* both segregate at first meiotic division while *lys1*

TABLE 7

First and second division segregation of the dal1, dal4, dal2, *and* lys1 *loci*

Cross	First (F) and second (S) division segregation of the *dal1*, *dal4*, *dal2* and *lys1* loci				No. of tetrads observed with indicated segregation pattern
	dal1	*dal4*	*dal2*	*lys1*	
M249	F	—	F	F	71
	F	—	F	S	11
	F	—	S	S	7
	S	—	S	S	67
	S	—	S	F	2
	S	—	F	F	1
M344	F	F	F	F	122
	F	F	F	S	21
	F	F	S	S	4
	F	S	S	S	9
	S	S	S	S	189
	S	S	S	F	5
	S	S	F	F	1
	S	F	F	F	1
	S	F	S	S	3
	S	F	S	F	1

segregates at second division would be greater than the reverse situation, *i.e.*, *dal1* and *dal2* segregating at second division while *lys1* segregates at first division. As shown in Table 7, the former situation was observed 11 times in cross M249 and 21 times in cross M344 compared to the latter situation, which was observed only two and five times, respectively. Similarly the number of times *dal1* and *dal4* both segregate at first meiotic division while *dal2* and *lys1* both segregate at second division was four times greater than the reverse situation. In like manner, all of the other qualitatively predicted first and second division segregation patterns were observed experimentally (see Table 7).

Occurrence of multiple exchanges: The patterns of multiple exchanges that occurred in our crosses can be analyzed using the gene order generated above. As shown in Table 8, most of the exchanges in cross M210 were single crossovers occurring in either region II + region III or in region IV. However, there were also nine double or rank-2 crossovers among the asci of cross M210. Eight of the rank-2 crossovers involved two strands, while the ninth involved three strands (Table 8). In cross M344, most exchanges were also rank-1 crossovers in regions II, III or IV. In addition there were four rank-2 exchanges (all involving two strands) and three rank-3 exchanges (two involving two strands

TABLE 8

Analysis of recombinant asci from tetratype tetrads

dal1 *dal4* *dal2* *lys1*

—○—|— region I —|— region II —|— region III —|— region IV —|—

Cross	Tetrad rank*	Region	Number of asci	Strand relations of multiple exchanges 2-st	3-st	4-st
M210	0	——	412			
	1	II + III†	34			
		IV	31			
	2	II + III & IV‡	9	8	1	0
M215	0	——	192			
	1	II + III	16			
		IV	20			
M249	0	——	138			
	1	II + III	10			
		IV	16			
M344	0	——	305			
	1	II	12			
		III	7			
		IV	34			
	2	II & III	3	3	0	0
		III & IV	1	1	0	0
	3	II & III & IV	3	2	1	0

* Indicates the number of exchanges that have occurred within the tetrad.

† Three of the crosses (M210, M215 and M249) did not contain mutations in the *dal4* locus. Therefore, the region between the *dal1* and *dal2* loci is indicated as region II + III.

‡ A plus sign indicates the combined regions indicated as discussed in the footnote above. An ampersand indicates two different regions are being considered.

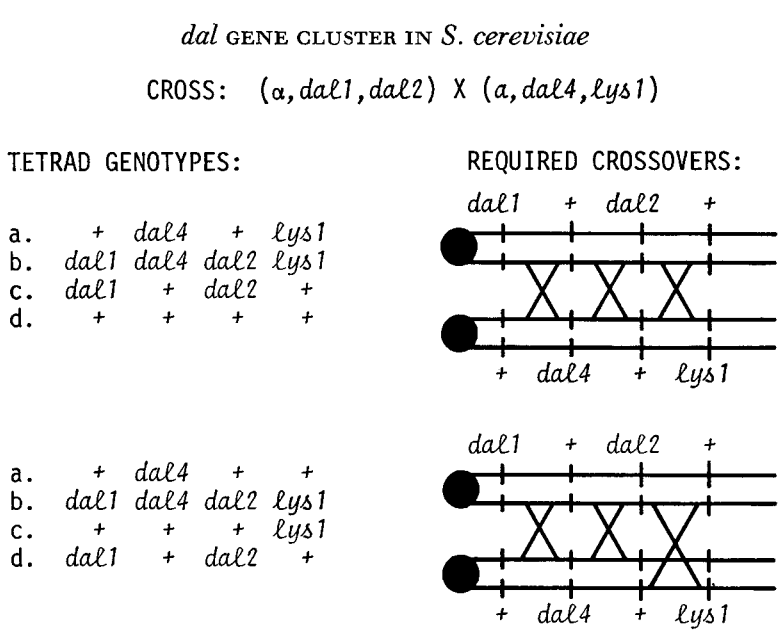

FIGURE 3.—Tetrad genotypes and crossover patterns required to account for them for the case of the rank-3 asci observed in cross M344.

and one involving three strands). The ascospore genotypes and exchange patterns for the rank-3 asci are shown in Figure 3.

Gene conversion of the dal *and* lys *loci*: As shown in Table 9, all four loci appeared to undergo gene conversion at about the same frequency (2.2, 1.6, 1.5, and 3.2% for *dal1*, *dal2*, *dal4*, and *lys1*, respectively). The behavior of markers

TABLE 9

Gene conversion of the dal1, dal4, dal2 *and* lys1 *loci*

Cross	Gene pair tested	Converted loci: *dal1* PD	*dal1* TT	*dal4* PD	*dal4* TT	*dal2* PD	*dal2* TT	*lys1* PD	*lys1* TT
		Orientation of tested loci							
M210	*dal2-lys1*	14	0	—	—	—	—	—	—
	dal1-lys1	—	—	—	—	9	4	—	—
	dal1-dal2	—	—	—	—	—	—	15	2
M215	*dal2-lys1*	6	0	—	—	—	—	—	—
	dal1-lys1	—	—	—	—	3	0	—	—
	dal1-dal2	—	—	—	—	—	—	7	0
M249	*dal2-lys1*	4	0	—	—	—	—	—	—
	dal1-lys1	—	—	—	—	1	2	—	—
	dal1-dal2	—	—	—	—	—	—	5	1
M344	*dal1-dal4*	—	—	—	—	5	1	12	0
	dal4-dal2	5	0	—	—	—	—	12	0
	dal1-dal2	—	—	4	0	—	—	12	0
	dal1-lys1	—	—	3	1	3	3	—	—
	dal4-lys1	5	0	—	—	4	2	—	—
	dal2-lys1	5	0	3	1	—	—	—	—

flanking the site of gene conversion can often be used as a circumstantial means of identifying the center locus in a set of three. FOGEL and HURST (1967) observed that markers flanking a gene conversion recombine about half of the time. Since this can be seen only for the center marker (there is no way of assaying this recombination for the flanking markers), it provides a useful test for identifying the center locus. As shown in Table 9, conversion at the *dal2* locus in crosses M210 and M249 resulted in generation of far more asci with tetratype orientations of the remaining markers than do either *dal1* or *lys1*, supporting the contention that *dal2* is the center locus of the three. For cross M344, a significant number of tetratype asci were observed on conversion at either the *dal2* or *dal4* loci, again supporting their assignment as the center loci and assignment of *dal1* and *lys1* as the flanking loci. No instances of co-coversion were observed among the asci we analyzed.

DISCUSSION

The results presented here show that three genes, whose products are needed for allantoin breakdown, are clustered on the right arm of chromosome *IX* proximal to the *lys1* locus (Figure 4). Two of these loci (*dal1* and *dal2*) are responsible for production of allantoinase and allantoicase, respectively. The *dal4* gene product appears to be required for allantoin transport since cells possessing a mutant allele of this gene were unable to accumulate allantoin from the medium.

Very few gene clusters have been found in eucaryotic cells and most of those reported in yeast have been found to code for multifunctional polypeptides. In *Saccharomyces cerevisiae* such multifunctional genes include: the *dur* region coding for urea carboxylase and allophanate hydrolase (COOPER *et al.* in preparation), the *his4* region coding for steps 3, 2 and 10 of histidine biosynthesis (FINK 1966; BIGELIS, KEESEY and FINK 1977), the *ade3* locus coding for three enzymes associated with tetrahydrofolate metabolism (JONES 1977; PAUKERT, WILLIAMS and RABINOWITZ 1977) and the *trp5* locus coding for tryptophan synthetase (MANNEY 1968). The thoroughly studied *arom* gene cluster in *Neurospora crassa* codes for a multifunctional protein that catalyzes five reactions in the synthesis of polyaromatic amino acids (GILES 1978). The *gal1,10,7* cluster, on the other hand, has been shown to code for three distinct messenger RNA molecules of different sizes (ST. JOHN and DAVIS, Abstract 9th International Conference on Yeast Genetics and Molecular Biology). The *dal* genes represent a second exam-

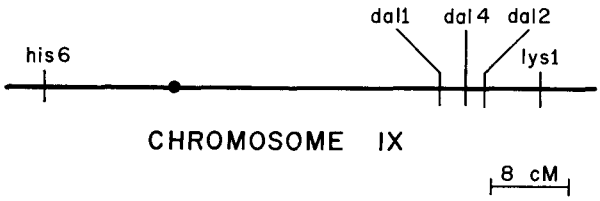

FIGURE 4.—Map of the right arm of chromosome *IX*.

ple of this type of clustering, *i.e.*, a cluster of genes that are functionally related but producing discrete polypeptides. It is intriguing to question whether the genetic material that exists between the *dal1-dal4* and *dal4-dal2* genes codes for gene products associated with the allantoin degradative pathway. Such a possibility exists because locations of the genes coding for ureidoglycolate hydrolase (the third step in allantoin degradation) and for the allantoate transport system have not yet been identified.

Two interesting genetic observations were made during these studies. First, we found a greater number of multiple crossovers than expected. In cross M210, for example, the expected number of tetrads in which double crossovers had occurred between the *dal1* and *lys1* loci may be estimated from the expression

$$\begin{matrix}\text{double}\\ \text{crossover}\\ \text{tetrads}\end{matrix} = (4)\left(\frac{\text{cM } dal1\text{-}dal2}{100}\right)\left(\frac{\text{cM } dal2\text{-}lys1}{100}\right)(\text{total asci analyzed}).$$

3.5 tetrads containing double exchanges were expected in the cross and nine were observed. For regions II and III in cross M344, 0.7 double crossovers were expected and six were observed. Similarly 1.4 double exchanges were expected in regions III and IV and four were observed. This raises the possibility of negative chromosome interference. Positive chromosome interference has been consistently observed in yeast (MORTIMER and FOGEL 1974) and several other eucaryotic micro-organisms (PERKINS 1962). In *Aspergillus nidulans*, however, negative chromosome interference was observed. We also observed an excess of two-strand double exchanges (12:1:0 = 2-strand : 3-strand : 4-strand double crossovers) among the asci we analyzed. This result suggests that some negative chromatid interference might have occurred, though the number of multiple crossover tetrads was very small. The latter findings are similar to those reported by several other investigators (FINCHAM and DAY 1971).

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