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Clustering of the Genes for Allantoin Degradation in *Saccharomyces cerevisiae*

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We have shown that allantoin degradation in *Saccharomyces cerevisiae* proceeds exclusively through the intermediate formation of allantoic acid, urea, and allophanic acid. The number of reactions between allantoic acid and urea, however, remains obscure owing to our inability to isolate a mutant defective in ureidoglycolate hydrolase. Structural genes for the enzymes, allantoinase (*dal1*) and allantoicase (*dal2*) are located on chromosome IX promixal to the centromere in the order *dal1-dal2-lys1*.

Three major routes for metabolism of allantoin have been reported and are depicted in Fig. 1. In bacteria, the mode of metabolism is dictated by the availability of oxygen. The early work of Barker (2) demonstrated that aerobic and anaerobic metabolism of allantoin in *Streptococcus allantoicus* yielded quite distinct products. Subsequent investigations in the laboratories of R. Wolfe and of G. Vogels provided a knowledge of the basic reactions involved. Aerobic metabolism of allantoin in *Pseudomonas aeruginosa* yields glyoxylate and urea via the intermediate formation allantoate and ureidoglycolate (reactions 1, 2, and 3 in Fig. 1) (1, 4, 16, 22-24, 27-29, 30). In *P. acidovorans*, however, Wu et al. (34) report genetic data which support allantoate, ureidoglycine, and ureidoglycolate as the pathway intermediates (reactions 1, 6, 7, and 3 in Fig. 1). The intermediates implicated by Valentine et al. (25, 26) and Gaudy et al. (13, 14) in the anaerobic metabolism of allantoin are allantoate, ureidoglycolate, carbamoyloxamate (oxalurate), and carbamoyl phosphate (reactions 1, 2, 8, and 9 in Fig. 1).

Early investigations by DiCarlo et al. (10) demonstrated that allantoic acid was a likely intermediate in the degradative pathway of yeast. These observations were extended principally in the laboratory of Roush, who partially purified and characterized the enzymes allantoinase, allantoicase, and ureidoglycollate hydrolase (ureidoglycolatase) (8, 18). These data are consistent with yeast degrading allantoin by reactions 1, 2, and 3 in Fig. 1. To ascertain whether this was the exclusive means of allantoin degradation in *Saccharomyces cerevisiae* we have isolated mutants that cannot grow on allantoin as sole nitrogen source. Use of these mutants has permitted us to demonstrate that allantoin metabolism in this organism involves exclusively reactions 1, 2, 4, and 5. Some ambiguity, however, still surrounds reaction 3. It has also been possible to define the genetic relationships of the structural genes of these enzymes.

MATERIALS AND METHODS

Mutants unable to utilize allantoin as a nitrogen source were isolated using methods previously described (31). Nystatin enrichment was performed using the procedures of Fink (12). Ureidoglycolate and potassium allantoate were prepared using established procedures (22, 35). Purification of mutants, complementation tests, linkage determinations, and construction of prototrophic diploid strains carrying various mutant alleles were performed employing standard genetic methods (19). Enzyme assays were performed as described earlier (9, 31).

RESULTS

Growth and biochemical characteristics of isolated mutant strains. Mutants unable to utilize allantoin as sole nitrogen source were separated into four groups on the basis of their growth characteristics. The growth patterns (Table 1) are consistent with loss of the first, second, fourth, or fifth enzymes of the pathway (Fig. 1). None of the intermediates prior to the presumptively defective enzyme supported growth while all of those subsequent to the block supported good growth. The mutants were also divided into four groups on the basis of complementation (Fig. 2). Diploids heterozygous for mutant alleles belonging to different

groups could grow on allantoin, whereas diploids heterozygous for two alleles from the same group could not. Table 2 and the data reported earlier by Whitney and Cooper (31) demonstrate that these four groups of mutants lack allantoinase, allantoicase, urea carboxylase, and allophanate hydrolase activity, respectively. Conspicuously missing is a class of mutants lacking ureidoglycolate hydrolase. It may be significant that we failed to isolate any mutants of this phenotype from approximately 3,500 allantoin defective mutants; some of which were isolated specifically because of an inability to use ureidoglycolate as sole nitrogen source.

Further study of the mutant collection, by complementation, revealed two additional classes of mutants which failed to grow on allantoic acid, but complemented strains known to be deficient in allantoicase. One class is represented by mutant alleles, N-27, N-39, N-40, and N-79 (Fig. 3). Complementation occurred between these alleles, but no complementation was observed between them and the alleles of other allantoicase minus mutants. A second class is represented by alleles N-18 and N-82, between which complementation was also observed; none was observed between them and other allantoicase gene alleles. Assay of extracts derived from diploids homozygous for each of these alleles (Table 3) confirms that all lack allantoicase activity. This may represent a case of interallelic complementation. To probe the stability characteristics of hybrid allantoicase produced in diploid strains heterozygous for alleles N-39 and N-27, or N-18 and N-82,

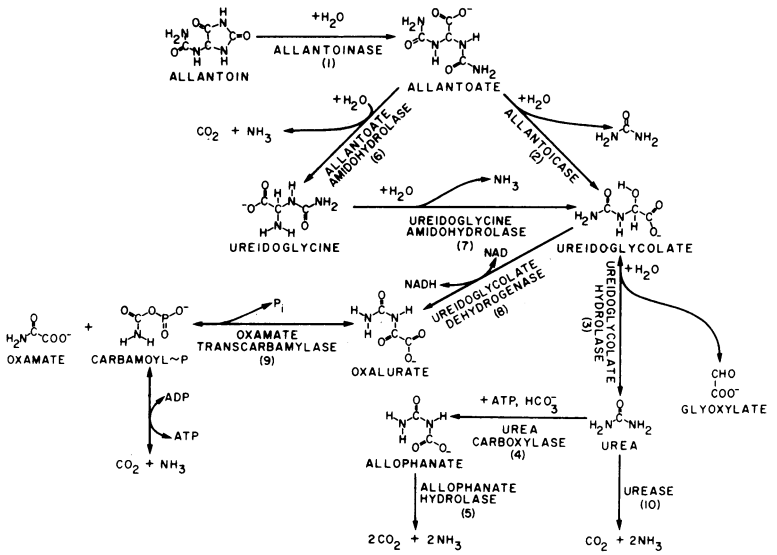

FIG. 1. *Routes of allantoin metabolism.*

TABLE 1. *Growth characteristics of various mutant strains of Saccharomyces cerevisiae*[a]

Strain	Presumptively defective enzyme	Nitrogen source				
		Allantoin	Allantoate	Ureidoglycolate	Urea	Ammonia
M-25	None	+[b]	+	+	+	+
M-85 (N-16)[c]	Allantoinase	−[d]	+	+	+	+
M-104 (N-18)	Allantoicase	−	−	+	+	+
M-62 (E-145)	Urea Carboxylase	−	−	−	−	+
M-64 (E-142)	Allophanate Hydrolase	−	−	−	−	+

[a] Diploid strains homozygous for the indicated alleles were replicated to minimal glucose medium with indicated compounds as sole nitrogen sources at a concentration of 10^{-2} M or 0.1%.

[b] +, Indicates growth.

[c] Mutant allele no.

[d] −, Indicates no growth after 48 h.

α \ a	N-16	N-17	N-21	N-28	N-35	N-18	N-19	N-26	N-44	N-46	E-143	E-145	E-152	E-174	E-176	N-12	E-142	E-148	E-153	E-157
N-16	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N-17		-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N-21			-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N-28				-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N-35					-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N-18						-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
N-19							-	-	-	-	+	+	+	+	+	+	+	+	+	+
N-26								-	-	-	+	+	+	+	+	+	+	+	+	+
N-44									-	-	+	+	+	+	+	+	+	+	+	+
N-46										-	+	+	+	+	+	+	+	+	+	+
E-143											-	-	-	-	-	+	+	+	+	+
E-145												-	-	-	-	+	+	+	+	+
E-152													-	-	-	+	+	+	+	+
E-174														-	-	+	+	+	+	+
E-176															-	+	+	+	+	+
N-12																-	-	-	-	-
E-142																	-	-	-	-
E-148																		-	-	-
E-153																			-	-
E-157																				-

FIG. 2. *Complementation of mutants unable to utilize allantoin as a sole nitrogen source. The mutants were isolated as described under Materials and Methods (N16-N46) or as previously described (E143-E157) (31) and crossed to the opposite mating type. The a and α strains were mated pairwise and the resulting prototropic diploids tested for their ability to grow on minimal medium containing 2% glucose and 0.1% allantoin as sole nitrogen source. +, Indicates growth; −, indicates no growth.*

TABLE 2. *Production of the allantoin degradative enzymes in wild-type and mutant strains of Saccharomyces cerevisiae*[a]

Diploid no.	nmol/min/mg of protein		
	Allantoinase	Allantoicase	Ureidoglycolate hydrolase
M-25	32.6	10.0	27.0
M-85 (N-16)	1.5	7.7	27.0
M-104 (N-18)	29.6	0.1	30.0

[a] Diploid strains homozygous for the indicated alleles were grown overnight on urea to a cell density of approximately 60 Klett units. Cell extracts were prepared and assays performed as indicated in Materials and Methods.

appropriate extracts were prepared. Allantoicase activity could not be found in these extracts, suggesting that the hybrid allantoicase is unstable (Table 4). This was substantiated by the observation (Table 4) that a diploid strain heterozygous for alleles N-39 and N-27 was temperature sensitive.

Mutants defective in allantoin metabolism were used to determine whether or not any of the allantoin degradative intermediates were able to bring about induction of allophanate hydrolase. Neither allantoin nor allantoic acid could serve this function if their metabolism to allophanate was inhibited (Fig. 4 and 5). This is in good agreement with our earlier observations (9, 33). It also shows that none of the allantoin degradative intermediates are able to function as physiological analogues of allophanic acid, the inducer of the allantoin degradative pathway.

Location and linkage relationships of the dal1 and dal2 loci. To facilitate a genetic discussion of the allantoin degradative system, structural gene loci for the enzymes allantoinase, allantoicase, urea carboxylase, allophanate hydrolase, and arginase will be designated

α \ a	N-16	N-17	N-21	N-28	N-35	N-37	N-45	N-48	N-49	N-52	N-80	N-81	N-19	N-26	N-30	N-44	N-46	N-53	N-27	N-79	N-39	N-40	N-18	N-82
N-16	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-17		-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-21			-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-28				-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-35					-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-37						-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-45							-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-48								-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-49									-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-52										-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-80											-	-	+	+	+	+	+	+	+	+	+	+	+	+
N-81												-	+	+	+	+	+	+	+	+	+	+	+	+
N-19													-	-	-	-	-	-	-	-	-	-	-	-
N-26														-	-	-	-	-	-	-	-	-	-	-
N-30															-	-	-	-	-	-	-	-	-	-
N-44																-	-	-	-	-	-	-	-	-
N-46																	-	-	-	-	-	-	-	-
N-53																		-	-	-	-	-	-	-
N-27																			-	-	+	+	-	-
N-79																				-	+	+	-	-
N-39																						-	-	-
N-40																							-	-
N-18																								+
N-82																								-

FIG. 3. *Complementation of mutants able to utilize urea, but unable to utilize allantoin as a sole nitrogen source. Complementation analysis was performed as shown in Fig. 2.*

TABLE 3. *Enzyme activities of mutants from various complementation groups of the allantoicase gene*[a]

Diploid no.	nmol/min/mg of protein		
	Allantoinase	Allantoicase	Ureidoglycolate hydrolase
M-25	32.3	16.5	47.0
M-104 (N-18)[b]	23.1	0.25	58.4
M-252 (N-27)	25.6	0.28	66.8
M-254 (N-39)	32.7	0.31	62.0
M-253 (N-82)	30.8	0.28	68.5

[a] Extracts from diploid strains homozygous for indicated alleles were prepared and assayed as described in Table 2.

[b] Indicates mutant allele no. in the homozygous diploid.

TABLE 4. *Growth and biochemical characteristics of wild-type and allantoicase defective strains of Saccharomyces cerevisiae*[a]

dal2 Mutant alleles carried in diploid strains used to prepare extracts	nmol/min/mg of protein			Growth on Allantoin[b]	
	Allantoinase	Allantoicase	Ureidoglycolate hydrolase	22 C	35 C
None	10.0	8.6	35.0	+	+
N-18[c]	11.1	0.0	41.8	−	−
N-82	11.4	0.0	43.2	−	−
N-18 & N-82	13.1	0.0	46.4	+	+
N-27	17.1	0.0	41.0	−	−
N-39	9.5	0.0	29.4	−	−
N-27 & N-39	9.0	0.0	44.1	+	−

[a] Heterozygous or homozygous diploids were prepared and assayed as described in Table 2.

[b] Standard minimal medium was used with allantoin as sole nitrogen source. Growth on minimal ammonia medium was normal at both temperatures.

[c] When a single allele is indicated the diploid used contained that allele in a homozygous condition.

dal1, *dal2*, *dur1*, *dur2*, and *aga2*, respectively. No linkage was observed between the *dal*, *dur*, and *aga* loci (Table 5). However, when the *his6* and *lys1* markers included in some of these crosses were analyzed with respect to the *dal1* and *dal2* loci, the observed number of nonparental ditypes was depressed (Table 6). This observation was verified by analysis of the crosses (Table 7). From these data we concluded that the *dal1*, *dal2* and *lys1* loci were closely linked. To establish the order of the loci and distances between them, three-point crosses were performed (Table 8). The average distances derived from these crosses, using the formula of Perkins (20), are the following: 4.4 centimorgans (cM) between *lys1–dal2*, 3.9 cM between *dal1–dal2*, and 7.9 cM between *lys1–dal1*. Table 9 lists the numbers and varieties of asci yielded from the above three-point crosses. In the first cross, 389 of the asci were parental ditypes. If the gene order is assumed to be *dal1–dal2–lys1*, then 67 of the remaining tetratype asci can be accounted for by a single recombinational event, and only 7 require the occurrence of two recombinational events. For the remaining three crosses, all of the observed tetratype asci can be accounted for by single recombinations. These data are consistent with a gene order of *dal1–dal2–lys1*.

To decide whether the *dal* cluster is located proximal or distal to the centromere from the *lys1* locus, crosses were prepared containing the centromere markers *leu1* and *trp1*. These mark-

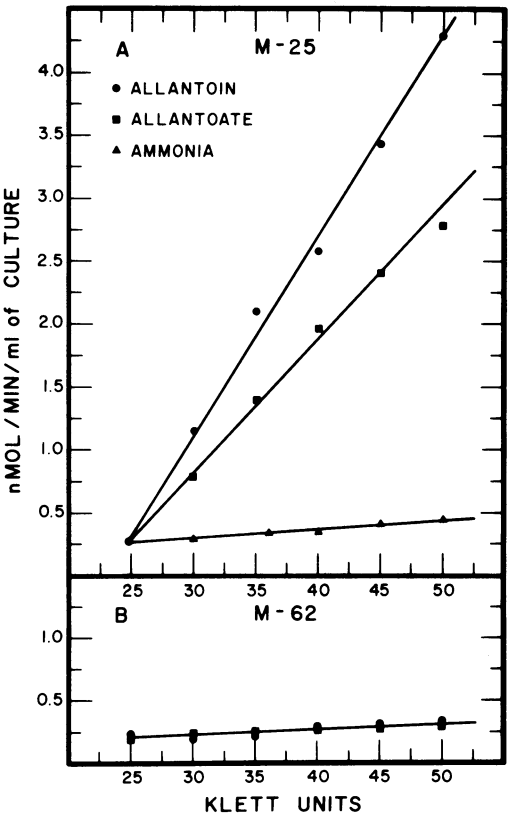

FIG. 4. *Differential rate of allophanate hydrolase synthesis in wild-type and urea carboxylase-defective strains of Saccharomyces. Cultures of M25 and M62 were grown on minimal medium containing 0.1% ammonium sulfate as sole nitrogen source. At a cell density of 25 Klett units allantoin (●) or allantoate (■) was added to a final concentration of 0.1%. Samples were removed thereafter at the cell densities indicated and assayed as described in Materials and Methods.*

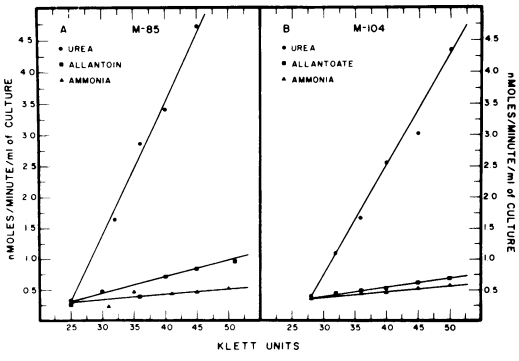

FIG. 5. *Differential rate of allophanate hydrolase synthesis in allantoinase and allantoicase deficient strains of Saccharomyces. The procedures in Fig. 4 were followed.*

TABLE 5. *Linkage of loci responsible for the allantoin, arginine and urea degradative enzymes*[a]

Gene Pair	PD[b]	NPD	T
dal1-dur1 (M160)[c]	14	17	57
dal2-dur1 (M149)	26	21	75
dal2-dur2 (M111)	23	33	101
lys1-dur1 (M135)	20	15	65
aga2-dur1 (M149)	15	26	81
aga2-dur2 (M111)	33	20	104
aga2-dal1 (M176)	6	8	31
aga2-dal2 (M111 + M149)	50	48	181

[a] Diploid strains were constructed and haploid asci obtained by standard genetic methods. Nitrogen utilization phenotypes were determined by complementation. The genotypes of the diploids used are as follows:

M111 (a, *his6, ura1, dal2* / α, *ade6, leu1, aga2, dur2*)

M135 (a, *his6, ura1* / α, *lys1, dur1*)

M149 (a, *his6, ura1, aga2, dal2* / α, *ade6, leu1, dur1*)

M160 (a, *his6, ura1, dal1* / α, *ade6, leu1, dur1*)

M176 (a, *his6, ura1, aga2* / α, *ade6, leu1, dal1*)

[b] PD, Parental ditype; NPD, nonparental ditype; T, tetratype.

[c] Number in parentheses is the diploid strain number.

TABLE 6. *Linkage of dal2 to various auxotrophic markers*[a]

Gene pair	*ura1*	*leu1*	*ade6*	*dal2*
his6	35:30[b] / 89	42:58 / 54	29:27 / 98	43:14 / 97
ura1		27:44 / 83	26:27 / 101	26:31 / 97
leu1			61:2 / 91	31:32 / 91
ade6				19:24 / 111

[a] Auxotrophic and *dal2* markers in asci derived from strain M111 were scored as described in Table 5.

[b] The numbers of parental (PD), nonparental (NPD), and tetratypes (T) are given as PD:NPD/T.

ers provided a means of identifying, with reasonable certainty, whether a given heterozygous marker segregated during the first or second division of meiosis. For genes closely linked to the centromere, the recombination frequency (distance in cM) is one half of the frequency of second division segregation (19). There is a greater amount of second division segregation in the case of the *lys1* locus than for either of the *dal* loci, suggesting that the *dal* loci are centromere proximal to *lys1* (Table 10). However, all of these genes are located sufficiently far from the centromere that the calculated distances are equivocal. Therefore, a second approach was made to this question. If the *dal* loci are assumed to be proximal to the centromere, the number of times that *lys1* segregates during first meiotic division and the *dal* loci segregate during second meiotic division should be small compared to the converse case in which the *dal* loci segregate during first meiotic division, and *lys1* segregates during second meiotic division. This is observed experimentally (Table 11) and supports the location of the *dal* cluster shown in Fig. 6.

The physiological relationships observed between allantoin and urea degradative enzymes raises questions concerning the extent of their physical association. To determine whether or

TABLE 7. *Linkage of lys1, dal1, and dal2 loci in two-point crosses*[a]

Cross	PD	NPD	T
lys1 + / + *dal1* (M108)[b]	80	0	10
lys1 + / + *sal2* (M110)	92	0	7
dal1 + / + *dal2* (M134)	94	0	9
dal1 + / + + (M137)	23	0	1
lys1 dal2 / + + (M136)	28	0	2

[a] Asci derived from the indicated strains were analyzed as described in Table 5. The genotypes of the diploid strains are as follows:

M108 (a, *his6, ura1, dal1* / α, *lys1*)

M110 (a, *his6, ura1, dal2* / α, *lys1*)

M134 (a, *his6, ura1, dal2* / α, *ade6, leu1, dal1*)

M136 (a, *lys1, dal2* / α, *ade6, leu1*)

M137 (a, *lys1, dal1* / α, *ade6, leu1*)

[b] Number in parentheses is the diploid strain number.

TABLE 8. *Linkage of lys1, dal1 and dal2 loci in three-point crosses*[a]

Cross and gene pairs	P	NP	T	Calculated distance in cM
lys1 + dal1 / *+ dal2 +* (M210)[b]				
lys1-dal2	421	0	35	3.8
lys1-dal1	396	0	60	6.6
dal2-dal1	417	0	39	4.3
lys1 dal2 + / *+ + dal1* (M215)				
lys1-dal2	193	0	17	4.1
lys1-dal1	180	0	30	7.1
dal2-dal1	197	0	13	3.1
lys1 + + / *+ dal2 dal1* (M249)				
lys1-dal2	102	0	12	5.3
lys1-dal1	94	0	20	8.8
dal2-dal1	106	0	8	3.5
lys1 dal2 dal1 / *+ + +* (M255)				
lys1-dal2	164	0	15	4.2
lys1-dal1	147	0	32	8.9
dal2-dal1	162	0	17	4.8

[a] Asci derived from the indicated strains were analyzed as described in Table 5. The genotypes of the diploid strains are as follows:

M210 (a, *his6, ura1, dal2* / α, *lys1, dal1*)

M215 (a, *his6, ura1, dal1* / α, *lys1, dal2*)

M249 (a, *leu1, met14, trp1, lys1* / α, *his6, ura1, dal1, dal2*)

M255 (a, *leu1, met14, trp1* / α, *his6, ura1, lys1, dal1, dal2*)

The *met14* marker was unscored in M249 and M255.

[b] Number in parentheses is the diploid strain number.

not allantoinase was part of the urea carboxylase: allophanate hydrolase multienzyme complex, a crude extract of wild-type cells was incubated with avidin. This was followed by addition of avidin immune serum and removal of the avidin-urea carboxylase: allophanate hydrolase complex by centrifugation using the techniques reported earlier (32). All of the allantoinase activity remains in solution, whereas allophanate hydrolase is precipitated (Fig. 7). It is clear from these results that allantoinase is not associated with the urea carboxylase: allophanate hydrolase complex.

DISCUSSION

We have demonstrated that allantoin degradation in *S. cerevisiae* proceeds through the intermediate formation of allantoate, urea, and allophanate. The reactions between ureidoglycolate and urea, however, remain obscure. Our failure to isolate a strain lacking ureidoglycolate hydrolase raises the possibilities that a ureidoglycolate hydrolase-defective cell possesses secondary characteristics which have not been provided for by our screening procedures, or that multiple reactions exist for converting ureidoglycolate to urea. In the limited number of mutants we analyzed, three complementation groups of the allantoicase gene *dal2* were found. Hybrid forms of allantoicase, produced in diploid strains heterozygous for members of these groups, were very unstable in vitro, and for one pair of alleles was also temperature-sensitive in vivo.

Our past results have shown that urea carboxylase and allophanate hydrolase are members of a multienzyme complex (32). In agreement with the observations of Fink, concerning the *his4* locus in *Saccharomyces* (11, 21), and Giles, concerning the *arom* locus in *Neurospora* (6, 15), our preliminary results suggest that the structural genes coding for the members of this complex are contiguous (Cooper and Evans, unpublished data). We have also shown here that allantoinase is not associated with that complex. Clustering of the genes for allantoicase and allantoinase on chromosome IX raises a question about the physical relationships of these two proteins. Biochemical and genetic observations, however, argue that they are not members of a multienzyme complex. Lee and Roush (18) report that allantoinase activity was absent in early fractions of a component-containing allantoicase activity eluted from diethylaminoethyl-cellulose. They did not, unfortunately, present sufficient data to ascertain whether or not the activities were totally separated. In all of the gene clusters that produce a multienzyme complex, it has been possible thus far to isolate a minor class of mutants, the members of which have lesions within the cluster and have lost the ability to synthesize two or more of the normal component activities. Case and Giles (5, 6) have reported a decrease in the sedimentation coefficients of the remaining activities isolated from such a mutant strain, and interpret this as a manifestation of the mutant strains' inability to synthesize the com-

TABLE 9. *Spore genotypes observed among asci of three-point crosses*[a]

Cross	a. *lys1* + *dal1* b. *lys1* + *dal1* c. + *dal2* + d. + *dal2* +	a. *lys1 dal2* + b. *lys1 dal2* + c. + + *dal1* d. + + *dal1*	a. *lys1* + + b. *lys1* + + c. + *dal2 dal1* d. + *dal2 dal1*	a. *lys1 dal2 dal1* b. *lys1 dal2 dal1* c. + + + d. + + +
$\frac{lys1 \; + \; dal1}{+ \; dal2 \; +}$ (M210)[b]	389	28	32	7
$\frac{lys1 \; dal2 \; +}{+ \; + \; dal1}$ (M215)	17	180	0	13
$\frac{lys1 \; + \; +}{+ \; dal2 \; dal1}$ (M249)	8	0	94	12
$\frac{lys1 \; dal2 \; dal1}{+ \; + \; +}$ (M255)	0	17	15	147

[a] Indicated crosses were analyzed as shown in Table 8.
[b] Number in parentheses is the diploid strain number.

TABLE 10. *Centromere linkage of the dal1, dal2, and lys1 loci*[a]

Cross and gene pairs	PD:NPD / T	% Second division segregation	cM distance
leu1-lys1	21:26 / 52	52.5	26.3
leu1-dal2	24:29 / 46	46.5	23.2
leu1-dal1	26:32 / 41	41.4	20.7
leu1-lys1	25:31 / 112	66.8	33.4
leu1-dal2	27:31 / 110	65.5	32.8
leu1-dal1	34:39 / 95	56.5	28.2

[a] Asci exhibiting first division segregation for *leu1* and *trp1* were analyzed for the segregation of *lys1*, *dal1*, and *dal2* as indicated. Methods are as indicated in Table 8. Lines 1 to 3, M249; lines 4 to 6, M255.

TABLE 11. *First and second division segregation of the dal1, dal2 and lys1 loci*[a]

First (F) or second (S) division segregation of *dal1*, *dal2*, and *lys1* markers			No. of tetrads observed with indicated segregation pattern	
dal1	*dal2*	*lys1*	M-249	M-255
F	F	F	44	52
F	F	S	9	6
F	S	S	7	14
S	S	S	37	92
S	S	F	2	4
S	F	F	0	0

[a] Data was obtained as described in Table 10.

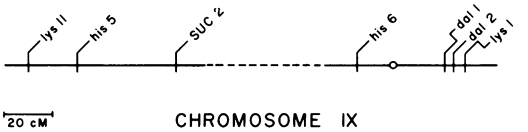

FIG. 6. *Location of markers on chromosome IX of Saccharomyces cerevisiae.*

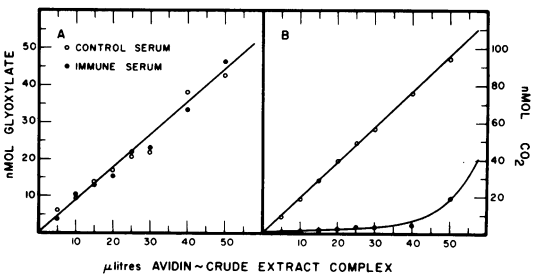

FIG. 7. *Reaction of avidin specific antibody with avidin-crude extract complex. A crude extract was concentrated by the addition of solid ammonium sulfate to a final concentration of 70% saturation. Avidin was added to the concentrated extract and the immunoprecipitate removed as described by Whitney and Cooper (32).*

plete complex due to a nonsense mutation. Pleiotrophic loss of multiple activities, as a result of a mutation mapping within one of the structural genes, can alternatively be attributed to negative complementation. In this instance, it may be argued that a mutation in one structural gene results in an altered confirmation of the cognate protein which in turn gives rise to an inappropriately assembled, and hence nonfunctional enzyme complex. These pleiotrophic mutations occur with a frequency of

about 1% in the case of the *dur1:dur2* cluster. However, none of these mutations were observed among the 3,500 allantoin-minus mutant strains. Additionally, the distance between the two *dal* loci is much greater than would be expected of contiguous loci.

Location of the *dal1* and *dal2* loci on chromosome IX, unlinked to the *dur1* and *dur2* genes, is likely another example of the fact that the structural genes for a given pathway may be widely scattered throughout the *Saccharomyces* genome. This raises an interesting question concerning the mechanistic details surrounding induction, by allophanate, of unlinked but functionally interdependent proteins.

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