BIPARTITE STRUCTURE OF THE *ade3* LOCUS OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Forty *ade3* mutants were examined with respect to their growth requirements, levels of the tetrahydrofolate interconversion enzymes, and/or map positions. Four deletions were detected. Mutations that result in a requirement for adenine and histidine map in one region of the locus; those which result in a requirement for adenine only map in a quite separate region of the locus, a region not disclosed in previous studies. No correlation was observed between growth properties of the strains and enzyme levels.

POINT mutations in the *ade3* locus of *Saccharomyces cerevisiae* may result in a requirement for adenine and histidine (ROMAN 1956), for adenine only (LOMAX, GROSS and WOODS 1971; LAM and JONES 1973), or for adenine or histidine (LOMAX, GROSS and WOODS 1971). For mutations of the *ade3* locus isolated as white or pink derivatives of red *ade2*-bearing strains, at least 70% of the mutations recovered are nonsense mutations (JONES 1972b). The fine structure profile of mutations at this locus was derived using strains (1) which required both adenine and histidine, and (2) which had low reversion rates (JONES 1972a). Later work revealed that the restriction of the mapping to mutations that met these two criteria had resulted in the mapping of mutations which were not representative of all mutations at the locus (JONES 1972b).

The *ade3* mutants have been shown to be deficient for formyltetrahydrofolate synthetase (EC6.3.4.3) and methenyltetrahydrofolate cyclohydrolase (EC3.5.4.9), and to lack one of two isoenzymes of methylenetetrahydrofolate dehydrogenase (EC1.5.1.5) (JONES and MAGASANIK 1967a; LAZOWSKA and LUZZATTI 1970a,b; LOMAX, GROSS and WOODS 1971; LAM and JONES 1973). LOMAX, GROSS and WOODS (1971) found some heterogeneity in the levels of formyltetrahydrofolate synthetase among the *ade3* mutations, depending on the particular nutritional phenotype of the mutant strain.

This paper reports results of genetic, physiological, and enzymatic studies on a random sample of *ade3* mutations isolated as white or pink derivatives of red *ade2*-bearing strains. These studies define two regions of the locus. Mutations which require both adenine and histidine map in one region of the locus; those with less stringent requirements map in a second region of the locus.

MATERIALS AND METHODS

Yeast strains: New *ade3* mutations were isolated by the method of ROMAN (1956) in C62-30B of genotype α *ade2-1 trp5-2 lys2 ura1-1 SUC1*. Tester alleles used in mapping have been previously described (JONES 1972a,b).

Media: YEPD, sporulation medium, and omission medium (for mapping) are as previously described (JONES 1972a). Minimal medium for determination of growth rate constants was according to the recipe for yeast nitrogen base without amino acids as described in the DIFCO manual (1953). This medium also contained (in mg/l) lysine (50), tryptophan (40) and uracil (10). Adenine and histidine when indicated were supplied at 20 mg/l.

Growth experiments: Cultures were grown in 300 ml nephelometric flasks at 30° in shaking water baths. Inocula for growth experiments were grown in 10 ml minimal medium containing adenine, histidine, lysine, tryptophan and uracil to a cell density of approximately 2×10^7 cells/ml (125–150 Klett units), at which time the flasks were refrigerated. The following day, 9 ml of the cell suspension were removed and the cells pelleted by centrifugation. The pellet was resuspended in 1 ml of minimal medium containing lysine, tryptophan and uracil. 0.05 to 0.1 ml of this cell suspension was then inoculated into 10 ml of the same medium. Appropriate additions of adenine and histidine were then made. Growth was monitored nephelometrically in a Klett-Summerson photoelectric colorimeter equipped with a 520 nm filter.

Genetic methods: Isolation of diploids was as previously described (JONES 1972a). Recombination frequencies, expressed as recombinants/10^3 asci, are as given in JONES (1972a) with the following exceptions. Platings for determination of viable counts were made on YEPD rather than SC. Colonies on adenine omission medium were counted after three days unless the heteroallelic diploid was a bradytroph (showed discernible growth on adenine omission medium). For such diploids, colonies were counted after 36–40 hours. The frequency of adenine-independent colonies after sporulation was multiplied by two since all diploids were heterozygous for *ade2-1* and *ade2-1* is unlinked to *ade3*.

Spot tests for deletion mapping were accomplished by replicating YEPD master plates containing the relevant diploids to Petri dishes containing sporulation medium. After five days incubation at room temperature the sporulated diploids were replica plated to medium lacking adenine. Diploids showing papillae were scored as +, those lacking them as 0.

Preparation of cell free extracts: Cells were grown and harvested and extracts prepared as described in LAM and JONES (1973).

Enzyme assays: Formyltetrahydrofolate synthetase, methenyltetrahydrofolate cyclohydrolase and methylenetetrahydrofolate dehydrogenase were assayed as previously described (LAM and JONES 1973).

RESULTS

Allelic mapping: In order to map the new, randomly chosen, sample of *ade3* mutations, the *ade2 ade3*-bearing strain was crossed to a series of strains bearing previously mapped tester alleles including *ade3-59*, *-39*, *-67*, *-40*, *-45*, *-34*, *-38*, *-26*, *-41* and *-58* and deletions *ade3-10* and *ade3-63*. Four of these new mutations gave patterns of recombination indicative of deletion mutations. The profiles of recombination for heteroallelic diploids involving the ten tester alleles and the four presumed deletions (*102*, *108*, *118* and *130*), as well as for heteroallelic diploids involving the previously described deletions *ade3-10* and *ade3-63*, are given in Table 1.

The tester alleles listed across the top of the table are given in order within the map. The order of alleles *41* and *58* has been reversed in comparison to the previous map (JONES 1972a), for *41* fails to recombine with *118*, whereas *58* shows recombination with *118*.

TABLE 1

*Detection of recombinants in crosses of tester alleles to presumed deletion mutations**

Deletion mutation	Tester allele *59*	*39*	*67*	*40*	*45*	*34*	*38*	*26*	*41*	*58*
102	+	+	+	+	+	+	+	0	0	0
108	+		0	0	+	+	+	+	nt	+
118	+	0	0	0	0	0	0	0	0	+
130	0	0	0	0	0	0	0	0	0	0
10	+	0	0	0	0	0	0	+	+	+
63	+	+	+	+	0	0	0	+	nt	nt

* + signifies recombinants were detected, 0 signifies no or very few recombinants were detected, n.t. signifies not tested. Tested alleles are arranged in the linear order previously described (JONES 1972a.)

No entry is given for the heteroallelic diploid *39* × *108*. This diploid was constructed twice. In both cases the diploid showed no requirement for adenine. There are at least two possible explanations for this observation. The mitotic recombination frequency may be high. If it is high and recombination occurs early during mitotic growth of the diploid culture, adenine independent cells will be generated. Such ADE+ cells have an enormous selective advantage over ADE− cells in YEPD and will take over the culture (unpublished observations). The diploid will give the impression of being prototrophic even though a fraction of the population still requires adenine. Alternatively the two alleles may complement. That this latter explanation is not impossible, even though one mutation may be a deletion, will be discussed later with respect to diploids involving *ade3–109* and *ade3–10*.

The four strains bearing the presumed new deletions were crossed to a wild-type strain in order to recover the new alleles free of *ade2* and in the opposite mating type. Strains bearing the six presumed deletions were crossed in all possible combinations. The results of these intercrosses are given in Table 2. Of the 15 heteroallelic diploids, only two yield recombinants. From these results one can infer that *102* and *108* do not overlap, nor do *102* and *63*.

The results of Tables 1 and 2 have been used in constructing Figure 1. Dele-

TABLE 2

*Detection of recombinants in crosses of deletion mutations with one another**

	102	*108*	*118*	*130*	*10*	*63*
102	0	+	0	0	0	+
108		0	0	0	0	0
118			0	0	0	0
130				0	0	0
10					0	0
63						0

* + signifies recombinants were detected, 0 that none were detected.

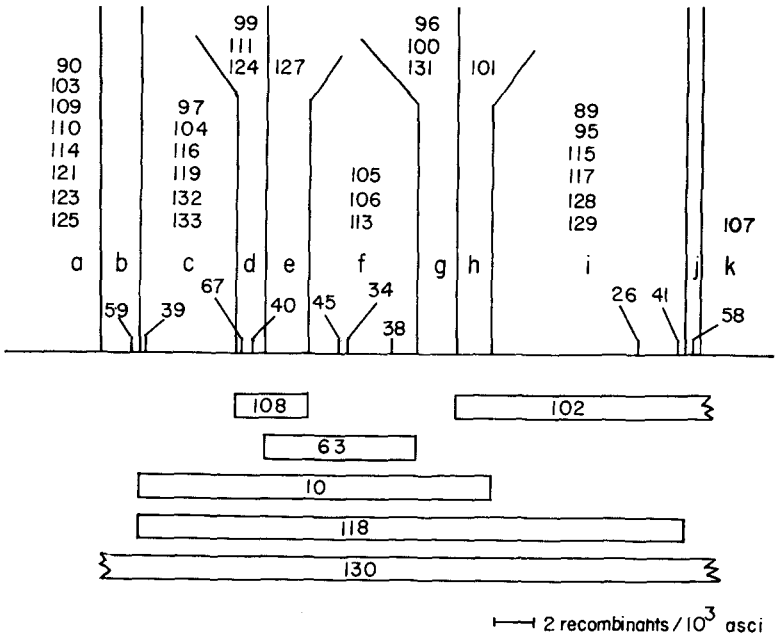

FIGURE 1.—Deletion map of the *ade3* locus. The solid line represents the chromosome in the *ade3* region. Each number on the line represents a previously mapped mutation (JONES 1972a). Distances between alleles are as previously determined (JONES 1972a). Open bars represent deletions. Lower case letters signify regions defined by crosses among the deletions and the previously mapped alleles. Numbers above the lower case letters signify alleles mapping within the regions designated by lower case letters. This region constitutes roughly half of the locus. See Figure 2 and text for details.

tions are indicated as open bars enclosing the allele number. Previously mapped tester alleles are arranged just above the line. The map is roughly to scale except that small distances have been expanded.

The locus in Figure 1 has been divided into eleven regions labelled a through k. The division is based largely on the results presented in Tables 1 and 2. Two regions, a and k, contain alleles which show recombination with all six deletions. That both ends of deletion *130* lie within the *ade3* locus, that the right end of *102* lies within the locus, and that regions a and k exist will be shown in the next section. No tester alleles lie within regions g and h. These two distinct regions must exist, however, for *102* fails to recombine with *10*, but shows recombination with *63*. Similar justifications can be made for the other divisions of the locus.

Thirty-four new *ade3* alleles were crossed to each of the six presumed deletions. The pattern of recombination expected for alleles lying within the stipulated regions are given in Table 3, where + signifies that recombinants were obtained, 0 that none (or very few) were obtained. Alleles lying in regions a and k will show recombination with all six deletions when placed in heteroallelic combination. The assignment of an allele to one region or the other can be made if quantitative data are obtained, however. Similarly, alleles lying within regions

TABLE 3

Profile of recombination for alleles falling within indicated regions of the ade3 *locus*

Region	Deletions *108*	*118*	*130*	*10*	*63*	*102*	Alleles showing pattern Old	New
a	+	+	+	+	+	+	none	*90,103,109*,110, 114,121,123,125*
b	+	+	0	+	+	+	*59*	*none*
c	+	0	0	0	+	+	*39*	*97,104,116,119,132,133*
d	0	0	0	0	+	+	*40,67*	*99,111,124*
e	0	0	0	0	0	+	none	*127*
f	+	0	0	0	0	+	*45,34,38*	*105,106,113*
g	+	0	0	0	+	+	none	*96,100,131*
h	+	0	0	0	+	0	none	*101*
i	+	0	0	+	+	0	*26,41*	*89,95,115,117,128,129*
j	+	+	0	+	+	0	*58*	none
k	+	+	+	+	+	+	none	*107*

* See text for qualification.

c and g will show similar patterns of recombination in diploids involving these six deletions. Assignment can be made using quantitative data. Thirty-two alleles have been assigned to one of the eleven regions shown in Figure 1. These 32 alleles are listed in column 8. For comparison, the tester alleles are indicated in column 7. Quantitative data allowing placement in regions a *versus* k or c *versus* g are given in Table 4. The alleles are also shown emplaced in regions in Figure 1.

An attempt has been made to position the new alleles within the regions defined above. Recombination frequencies for heteroallelic diploids involving the ten tester alleles and the thirty-two new alleles are given in Table 4. The tester alleles are in map order, and the new alleles are grouped by region. (The placements are very rough.) Consider alleles in region a; these alleles showed recombination with all deletions, and they show uniformly high recombination frequencies in crosses to alleles at the right end of the locus, namely, *26*, *41*, and *58*. When alleles in region a are crossed to alleles at the left end of the locus, the frequencies of recombination are lower than when crossed to alleles at the right end of the locus. As an example, consider allele *109*. This allele shows low frequencies of recombination in crosses to *59* and *39*. As one moves to the right, the recombination frequencies steadily increase. Hence *109* was placed in region a, two units to the left of *59*. Allele *123* has also been placed in this region. It shows high frequencies of recombination in nearly all crosses. The frequencies obtained in crosses to alleles at the right end of the locus appear higher than those obtained in crosses to alleles at the left end. Allele *107* also recombines with all deletions; it has been placed in region k because it shows low frequencies of recombination in crosses involving alleles in the right end and high frequencies of recombination in crosses involving alleles in the left end of the locus.

Alleles placed in region c show low frequencies in crosses to alleles *59*, *39*, *67* and *40* but higher frequencies in crosses to alleles near the g region (*45*, *34*, *38*).

Alleles in the i region can be placed rather easily, for four of them fail to recombine with a tester allele. Allele *129* probably lies between *26* and *41*, for the distance between *26* and *41* is 2.06 units.

Some alleles are clearly difficult to place. Allele *101* is placed in region h on the basis of crosses involving deletions; recombination frequencies would place it much closer to *38*. By a similar argument, allele *100* is placed in region g on

TABLE 4

Meiotic recombination frequencies for crosses of new ade3 *alleles to previously mapped tester alleles**

	New *ade3* allele	Tester *ade3* allele									
		59	*39*	*67*	*40*	*45*	*34*	*38*	*26*	*41*	*58*
a)	*90*	7.9	6.3	20.1	24.5	29.9	22.3	57.4	96.5	36.8	36.3
	103	16.8	10.0	18.3	14.5	25.0	25.8	23.9	40.2	30.2	38.1
	109	2.0	2.5	4.8	4.5	9.0	26.0	22.6	26.9	32.8	28.4
	110	3.3	3.1	11.8	7.0	16.7	26.4	25.4	42.5	43.8	26.7
	114	3.4	4.1	7.9	10.9	25.4	27.0	24.4	18.8	30.2	32.0
	121	13.2	17.6	16.6	16.0	28.7	27.0	17.2	36.5	24.9	23.6
	123	29.6	27.4	27.4	32.6	36.3	45.9	23.3	49.0	47.6	36.3
	125	3.4	3.4	11.1	8.7	17.2	26.1	39.8	31.5	30.6	34.6
c)	*97*	3.1	4.6	0.68	1.0	11.9	10.3	22.3	36.1	33.4	16.4
	104	8.1		1.1	2.2	14.4	17.7	14.2	39.1	35.2	33.8
	116	3.0	2.3	0.16	2.5	11.5	11.3	20.6	31.8	31.4	20.9
	119		0	9.3	3.8	32.1	27.9	33.0	47.3	45.5	45.7
	132	0.68	0.63	8.2	6.4	10.7	17.5	26.3	30.0	26.1	25.0
	133	3.7	3.6	0.21	0.42	7.9	11.8	25.1	25.9	27.4	22.6
d)	*99*	11.3	7.4	0.53	0.01	8.3	7.9	14.0	20.5	27.2	16.3
	111	9.3	7.3	1.7	0.23	6.6	7.5	10.8	12.9	19.5	41.2
	124	3.3	11.8	0	1.3	4.6		16.7	44.6	36.4	
e)	*127*	23.8	16.3	4.1	2.5	1.5	3.5	4.3	27.1	24.0	24.6
f)	*105*	21.2		8.7	8.3	0.98	0.96	3.3	18.0	33.7	15.0
	106	20.8	13.9	11.6	6.3	0.28	0	1.2	10.1	16.5	9.5
	113	39.9	12.0	26.1	10.9	0.48	0.68	0.28	7.9	28.4	7.0
g)	*96*	26.4	25.1	11.6	19.0	5.3	2.5	0.95	5.4	17.0	6.6
	100	17.6	23.7	15.5	13.7	6.1	4.7	2.5	1.8	9.1	4.1
	131	20.4	23.3	13.2	13.2	4.3	2.4	1.5	6.5	13.9	5.4
h)	*101*	10.5	8.7	8.2	4.9	2.9	3.0	2.4	10.7	10.7	10.3
i)	*89*					16.1			0	3.05	8.7
	95		47.6	45.3	27.2	17.8	13.0	7.8	2.2	13.7	2.7
	115	54.6	41.0	69.7	28.1	15.9	28.3	12.6	3.9	0	0.003
	117		42.1		30.9	31.9	40.9	37.8	0.08	0	0.009
	128	33.4	30.8	15.5	22.9	9.8	10.2	7.2	0	0.61	0.45
	129	36.0	32.3	22.1	26.0	10.0	8.2	11.0	0.009	0.81	0.49
k)	*107*	43.2	27.3	72.0	21.3		15.6	8.4	1.5		2.3

* Tester alleles are arranged in map order. New *ade3* alleles are grouped by region as determined by crosses to deletions and to tester alleles. Frequencies are expressed as recombinants/10^3 asci.

the basis of crosses involving deletions, whereas frequencies of recombination for heteroallelic diploids would place it much closer to *26*. Where conflicting evidence of this sort was observed, the assignment derived from deletion analysis has been accepted. This decision was based on the *cyc1* precedent (MOORE and SHERMAN 1975; SHERMAN *et al.* 1975) where it has been found that deletion mapping generates an internally consistent map, whereas the map based on recombination frequencies is internally inconsistent and may even have alleles out of their proper order. Two of the 34 alleles could not be mapped. In crosses to the deletions allele *126* showed the pattern of recombination expected for a mutation mapping in region c or g, yet it gives high frequencies of recombination in crosses to all tester alleles. (The lowest frequency was 9.3 units.) Mutant *112* fails to recombine with allele *41* and fails to recombine with deletion *10*; possibly *112* carries two mutations. These two mutant strains have been ignored in these and subsequent analyses.

The assignments for the 32 emplaced alleles are given in Figure 2. Tester

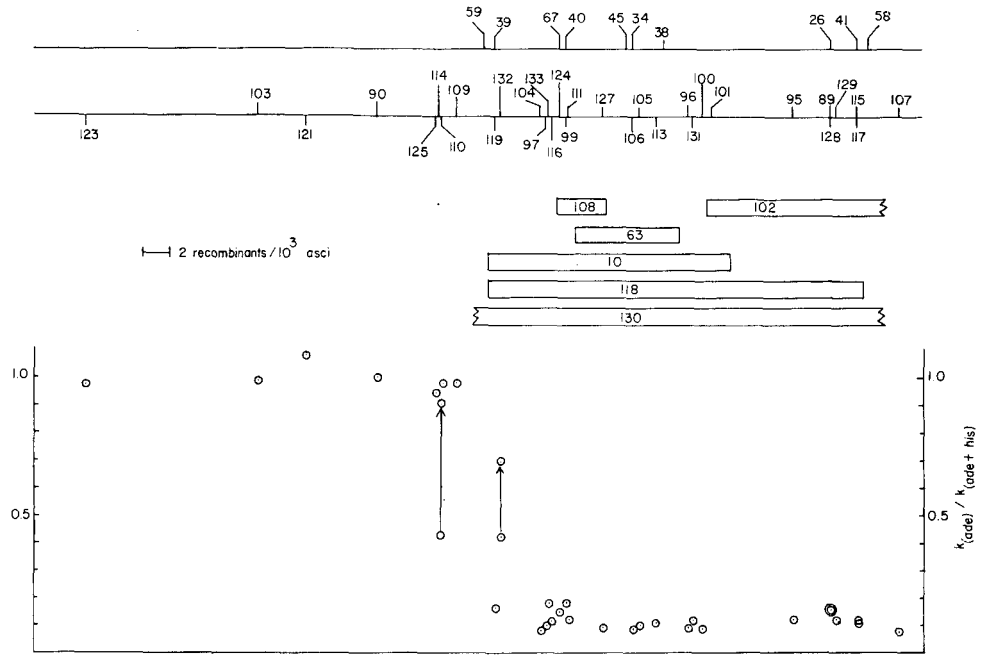

FIGURE 2.—Relation of mutant phenotype of heteroallelic diploids to map position of the new mutant allele. Alleles in the top line are tester alleles previously mapped. Alleles in the second line are the new mutations. They have been positioned within the regions defined in Figure 1 on the basis of recombination frequencies in crosses to tester alleles. Open bars represent deletions. $k_{(+\text{ade})}/k_{(\text{ade}+\text{his})}$ is the relative growth rate constant for growth in the absence of histidine and is the ratio: (1/doubling time in hours on minimal medium supplemented with adenine)/(1/doubling time in hours on minimal medium supplemented with adenine and histidine). A relative growth rate constant of 1.0 signifies that the heteroallelic diploid, *ade3-10/ade3-x*, does not require histidine at all. The double circle indicates that the two alleles *89* and *128* showed the same relative growth rate constants.

alleles are given in the top line, the 32 newly mapped alleles in the second line. No significance attaches to placement above or below the second line.

Growth characteristics of the mutants: As mentioned above, mutations in the *ade3* locus may result in a requirement for adenine and histidine, for adenine alone, or for an alternate requirement for one nutrient or the other (ROMAN 1956; LOMAX, GROSS and WOODS 1971; LAM and JONES 1973). In order to quantify the dependence of the cells on one or the other of these nutrients, growth rate constants were determined. The extent of the adenine requirement could not be determined on the newly isolated haploid strains, for all strains bear *ade2–1*, which itself causes an adenine requirement. Outcrossing to recover the *ade3* allele free of *ade2–1* could well result in variations in growth rate attributable to genes other than the *ade3* allele. In order to eliminate differences in growth rate attributable to differences in genetic background, the growth rates were determined in the following fashion. The *ade2–1 ade3–x* strains were each crossed to a strain bearing the deletion *ade3–10*, diploids were isolated, and doubling times were determined for the resultant diploids on each of four media. These four media contained, respectively: no additions, histidine only, adenine only, or adenine and histidine. The growth rate constant, k, (1/doubling time in hours) for growth on adenine and histidine is given in column 4 of Table 5 for these diploids. The growth rate constants for growth on the other three media were also determined. However, to facilitate comparisons, only the relative growth rate constants are given in Table 5. The relative growth rate constant is the ratio of the growth rate constant on the stipulated medium to the growth rate constant on the fully supplemented medium. As an example, in Table 5, the diploid *ade3–10/ade3–90* shows 0.56 doublings/hr (k). When neither adenine nor histidine is supplied, the ratio of the two growth rate constants is 0.60, and hence this diploid grows 60% as well if no nutrient is supplied as it does if both are supplied. The growth rate constant, k, for 10/90 on unsupplemented medium is (0.60) (0.56) = 0.34 doublings/hr. If histidine is supplied, the relative growth rate constant is increased somewhat to 67% of the fully supplemented rate constant. If adenine only is supplied, the diploid grows as well as it does if both nutrients are supplied. In fact, this diploid does not require histidine at all. With one exception, all diploids involving *ade3–10* and an allele from region a show no histidine requirement at all. The exception, the diploid involving allele *114*, grows better when histidine is supplied in addition to adenine. When one determines the extent of the histidine requirement for the haploid *ade2 ade3–114* (column 5), one finds a relative growth rate constant of 0.91. Whether this difference between the haploid ratio and the diploid ratio arises due to negative interaction between altered gene products, or rather reflects a difference in the histidine requirement of a haploid as compared to a diploid cell, is unknown.

With one exception diploids involving alleles mapping in regions other than region a show strict histidine requirements. Thus the diploid *ade3–10/ade3–97* grows at 10% of the rate in the absence of histidine as it does in its presence (line 9, column 3). The relative growth rate constants for growth in the absence of

TABLE 5

Relative growth rate constants for haploids and diploids involving newly mapped ade3 *alleles**

	Allele	Relative growth rate constants for diploids Additions to minimal medium† (1) —	(2) His	(3) Ade	Diploid growth rate constant Min+ade+his (4) k	Relative growth rate constant for haploid on Min+ade (5) —	Haploid growth rate constant Min+ade+his (6) k
a)	*90*	0.60	0.67	1.00	0.56	0.98	0.50
	103	0.30	0.39	0.99	0.54	1.00	0.50
	109	0.85	0.88	0.98	0.57	0.97	0.52
	110	0.36	0.46	0.98	0.63	0.98	0.51
	114	0.18	0.25	0.43	0.58	0.91	0.50
	121	0.30	0.41	1.08	0.62	0.94	0.60
	123	0.13	0.35	0.98	0.63	1.04	0.55
	125	0.31	0.36	0.95	0.57	0.91	0.58
c)	*97*	0.13	0.13	0.10	0.57	0.11	0.54
	104	0.07	0.15	0.08	0.57	0.11	0.47
	116	0.07	0.17	0.12	0.53	0.09	0.50
	119	0.08	0.15	0.16	0.56	nd	nd
	132	0.21	0.23	0.42	0.58	0.70	0.47
	133	0.14	0.17	0.18	0.53	0.16	0.52
d)	*99*	0.09	0.13	0.18	0.58	0.12	0.54
	111	<0.07	<0.07	0.12	0.63	0.11	0.49
	124	0.08	0.13	0.15	0.55	nd	nd
e)	*127*	<0.07	0.13	0.09	0.57	0.14	0.50
f)	*105*	0.08	0.09	0.10	0.54	0.11	0.49
	106	<0.07	0.09	0.09	0.56	0.08	0.46
	113	<0.07	0.14	0.11	0.54	0.11	0.49
g)	*96*	0.07	0.08	0.09	0.56	nd	nd
	100	<0.07	0.10	0.09	0.56	0.15	0.55
	131	<0.07	0.10	0.12	0.54	0.13	0.55
h)	*101*	nd	nd	nd	nd	nd	nd
i)	*89*	<0.07	0.13	0.16	0.56	nd	nd
	95	<0.07	0.14	0.12	0.56	nd	nd
	115	<0.07	0.11	0.12	0.56	nd	nd
	117	<0.07	0.08	0.11	0.58	nd	nd
	128	0.11	0.15	0.16	0.53	nd	nd
	129	0.07	0.14	0.12	0.58	0.11	0.53
k)	*107*	<0.07	0.08	0.08	0.54	0.09	0.55

* Growth rate constants were determined for diploids on each of four media: minimal, minimal + his, minimal + ade, and minimal + ade + his. The growth rate constant k is doublings per hr and is 1/doubling time (in hours). In column 4 are given doublings/hr for the diploids growing on test medium/growth rate constant on MV+ade+his. Relative growth rate constants for growth of the diploids in unsupplemented minimal are given in column 1, in minimal supplemented with histidine in column 2, and in minimal supplemented with adenine in column 3. In column 6 the growth rate constant for the haploid strain in minimal supplemented with adenine and histidine is given. The relative growth rate constant for growth of the haploid on minimal supplemented with adenine (calculated as given above) is given in column 5. All diploids are heteroallelic and contain deletion *ade3–10* and the new *ade3* allele. Haploid strains are *ade2–1 ade3–x*. n.d. signifies not determined.

† Minimal medium contains lysine, tryptophan, and uracil.

histidine range from 0.08 to 0.18 with the exception of *ade3–10/ade3–132*, where the ratio is 0.42.

The relative growth rate constants for growth in the absence of histidine have been plotted as a function of map position in the bottom part of Figure 2. The pattern seems quite clear. Diploids involving alleles to the left side of the locus (anything to the left of the left end of deletion *130*) do not require histidine. Two entries have been made for the diploid involving *ade3–114*. An arrow connects the two entries. That at the head is the ratio for the haploid, at the tail for the diploid. Similarly, a double entry for *ade3–10/ade3–132* has been made.

The ratios for diploids involving alleles to the right are all low. In the region near the left end of deletions *10*, *118* and *130*, the pattern is confused. Allele *119* appears to map to the left of allele *132*. Yet the diploid involving *119* has a strict histidine requirement, whereas that involving *132* has only a partial requirement. Whether this inconsistency is real or reflects improper map positions can only be resolved by further analysis. If one examines the strictness of the adenine requirement, a similar pattern is obtained. Diploids involving alleles to the right end of the locus (regions c through k) show growth rates about 10% as great in the absence of adenine as compared to its presence. Diploids involving alleles to the left end (region a) show only partial requirements for adenine. (The ratios range from 0.25 to 0.88). These diploids are the bradytrophic diploids referred to in MATERIALS AND METHODS. Recombination frequencies for these diploids are only approximate, for the background growth due to the unsporulated diploids and presumably the haploid *ade3-x* is extensive.

There is one diploid, *ade3–10/ade3–109*, which can hardly be said to require any nutrient at all, for its grows at 85% the normal rate in the absence of supplement. This diploid was made several times with similar results each time. Obviously a recombination frequency for this diploid could not be obtained. Yet recombination frequencies for *ade3–109* in crosses to other alleles could be and were obtained (see Table 4). One possible explanation for this anomaly is that *ade3–10* is an in-frame deletion and that complementation between the shortened *ade3–10* gene product and the *ade3–109* gene product can occur (either metabolically or through physical interaction).

The apparent complementation between *ade3–10* and *ade3–109* raised the possibility that the relative growth rate constants reported for diploids might have been distorted through allelic interactions. For this reason the relative growth rate constants for growth in the absence of histidine were determined for selected haploid *ade2–1 ade3–x* strains. All haploids from region a, nearly all from b, and selected haploids from the other regions were analyzed. With the exception of alleles *114* and *132*, the ratios obtained for haploids and diploids are quite comparable (compare entries in column 3 and column 5). Where the haploid and diploid ratios differ (alleles *114* and *132*), the diploid requirement is more strict than the haploid requirement.

One feature of the results presented in Table 5 needs emphasizing. Diploids involving alleles in region a do not require histidine. They can also grow relatively well in the absence of adenine. In every case, however, the requirement

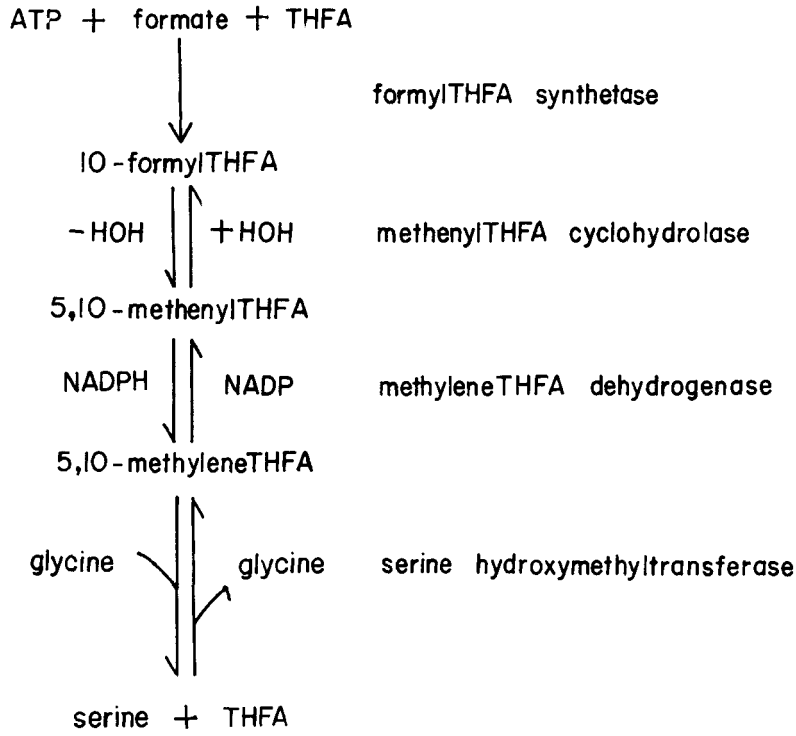

FIGURE 3.—Pathway and enzymes involved in interconversion of selected coenzymes of tetrahydrofolic acid.

for adenine is more stringent than that for histidine (compare entries in column 2 and column 3 for a given diploid). Whatever the basis of the relief of the adenine and histidine requirements is in these strains, it is clear that the histidine requirement is relieved to a greater extent than the adenine requirement for the same metabolic condition.

The *ade3* locus is involved in synthesis of three enzymes of THFA metabolism. The pathway and enzymes involved are depicted in Figure 3. The synthetase, cyclohydrolase, and one of two isoenzymes of the dehydrogenase are deficient in *ade3* mutants (JONES and MAGASANIK 1967a; LAZOWSKA and LUZZATTI 1970a,b; LOMAX, GROSS and WOODS 1971; LAM and JONES 1973). This results in a deficiency for 10-formylTHFA and 5,10-methenylTHFA. This reduction in level of 10-formylTHFA and 5,10-methenylTHFA leads to the inability of the cells to carry out the reactions which convert glycinamide ribonucleotide to α-N-formylglycinamide ribonucleotide and 5-amino-4-imidazole carboxamide ribonucleotide to 5-formamidoimidazole-4-carboxamide (JONES and MAGASANIK 1967b) and provides an explanation for the adenine requirement.

Accumulation studies indicate that intact cells of *ade3* mutants lack function for N-(5′-phospho-D-ribosylformimino)-5-amino-1-(5″-phosphoribosyl)-4-imidazolecarboxamide isomerase (EC5.3.1.16), the fourth enzyme of histidine bio-

synthesis, for the substrate of this enzyme accumulates intracellularly (JONES 1970). This malfunction is not due to deficiency for the isomerase, for extracts of *ade3* mutants contain the full complement of this activity. The isomerase has been partially purified and some of its properties examined. The enzyme is completely inhibited by 1.2×10^{-4} M 5,10-methyleneTHFA (JONES 1970), a derivative which accumulates in the *ade3* mutants (LUZZATI 1975). In addition, 5,10-methenylTHFA, a derivative present in reduced amounts in *ade3* mutants, appears to be an activator of the isomerase (JONES 1970). These two phenomena, the inhibition by 5,10-methyleneTHFA and the absence of activation due to reduced levels of 5,10-methenylTHFA, may be sufficient to cause malfunction of the isomerase and hence result in a histidine requirement in *ade3* mutants.

How can these observations be related to the finding of the bipartite nature of the *ade3* locus described above? One hypothesis is as follows: The *ade3* locus encodes the three enzymatic activities; transcription is from right to left, the dehydrogenase is encoded in the right side of the locus, the synthetase in the left part of the locus. Polar mutations in the right half of the locus result in absence of the dehydrogenase and synthetase activities and in requirements for adenine and histidine (more than 70% of *ade3* mutations are polar) (JONES 1972b). Polar mutations in the left half of the locus should result in absence of synthetase activity, and possibly dehydrogenase, depending on whether or not an enzyme complex or a polycephalic protein exists. Retention of partial or full activity for the dehydrogenase would be expected to relieve the histidine requirement by lowering the level of 5,10-methyleneTHFA and raising the level of 5,10-methenylTHFA, and would not be expected to relieve the adenine requirement to the same extent since 10-formylTHFA would still be required for synthesis of purine nucleotides. This hypothesis would explain the bipartite nature of the locus and the greater stringency of the adenine requirement as compared to the histidine requirement. It would accord with the observation that *ADE15*, a dominant mutation which results in the absence of synthetase activity, maps to the left of *ade3–59* (JONES and LAM 1973; LAM 1973).

We sought confirmation of this hypothesis by determining the levels of the three THFA interconversion enzymes in selected *ade3* mutants. Strains bearing mutations which mapped in the various regions of the locus were examined. Wherever possible, the strains chosen bore nonsense mutations. As indicated in Table 6, some of the mutations had been previously identified as bearing nonsense mutations (JONES 1972b). Identification of mutations *121*, *123*, *132*, *111*, and *131* as ochre mutations is based on a test described earlier (JONES 1972b). The *ade2–1 ade3–x* strains were examined for reversion to adenine independence in a single step. *ade2–1* is a known ochre mutation. Reversion of the *ade2–1 ade3–x* strain to adenine independence in one step occurs when an ochre-suppressor tRNA arises by mutation. For alleles *121*, *123 and 132* the ochre assignment must remain tentative, for the strains bearing these mutations are themselves bradytrophs. Suppression of *ade2–1* without suppression of *ade3–121*, for example, might give an apparent adenine-independent clone.

The levels of the three interconversion enzymes in the *ade3* mutants are

TABLE 6

Specific activities of tetrahydrofolate interconversion enzymes in S. cerevisiae *strains (nmoles/min/mg protein)*

	ade3 allele	Dehydrogenase	Cyclohydrolase	Synthetase	Mutational class
	+	12.2	7.4	44.7	
a)	*90*	1.73	0.60	3.77	ochre (JONES 1972b)
	121	0.27	0.24	3.04	ochre
	123	1.0	0.26	3.75	ochre
c)	*97*	1.91	0.53	3.64	amber (JONES 1972b)
	132	0.86	0.46	3.80	ochre
d)	*99*	1.64	0.54	5.45	ochre (JONES 1972b)
	111	0.76	0.21	2.81	ochre
f)	*113*	0.78	0.35	3.84	
g)	*96*	1.27	0.79	3.91	ochre (JONES 1972b)
	100	1.41	0.43	3.45	ochre (JONES 1972b)
	131	0.55	0.65	3.08	ochre
h)	*101*	1.55	0.48	4.05	ochre (JONES 1972b)
i)	*89*	1.86	0.63	3.00	amber (JONES 1972b)
	128	0.73	0.40	3.84	

reported in Table 6. It is immediately apparent that all mutations have greatly reduced levels of all three enzymatic activities. The enzyme levels for mutations mapping in region a are not distinguishable from those of mutations mapping in other regions. Thus these data lend no support to the hypothesis set forth above. They do not, however, vitiate the hypothesis, for it is certainly possible that the altered enzymes are unstable and become inactive or are destroyed by protease action after the cells are broken.

DISCUSSION

It is clear that earlier studies of the *ade3* locus (JONES 1972a), employing mutations chosen for low reversion rates and stringent requirements, resulted in a false picture of the locus. They failed to reveal the bipartite nature of the locus and led to an underestimate of the size of the locus. The current estimate of size is likely to be an overestimate, for map expansion is known to occur at the locus (JONES 1972a).

A specific proposal as to the encoding order of enzymatic activities within the locus was made, with synthetase encoded in the left end of the locus, and dehydrogenase in the right end. This proposal was derived from a consideration of the growth properties of the strains and a consideration of the metabolic pathways involved. This hypothesis was not substantiated by the data presented in this paper.

Several lines of evidence suggest that the *ade3* locus is the structural locus for these three enzymatic activities and that these enzymatic activities comprise an enzyme complex. If extracts of the wild type are chromatographed, all three enzymatic activities elute together. (MCKENZIE and JONES 1977). As the enzymes are purified further, the three activities remain together (JONES, unpublished

observations). One can isolate mutants which lack only dehydrogenase or only synthetase or dehydrogenase and cyclohydrolase (McKENZIE and JONES 1973, McKENZIE and JONES 1977). Mutant *1050* lacks synthetase activity but retains activity for dehydrogenase and cyclohydrolase. However, when extracts of mutant *1050* are chromatographed, the dehydrogenase and cyclohydrolase activities elute *together* at an atypical salt concentration. (McKENZIE and JONES 1977). It would appear that the mutation which eliminates synthetase activity has affected the chromatographic behavior and, by inference, the structure of the protein(s) containing the other two activities.

The mutations, including *1050*, which eliminate only one of the activities map within the *ade3* locus, interspersed with nonsense mutations which eliminate all three activities (JONES and McKENZIE, unpublished observations). These mutations map in the regions proposed, with synthetase mutations in the left half of the locus and dehydrogenase mutations in the right half. Moreover, *ADE15*, a dominant mutation which causes loss of synthetase activity (JONES and LAM 1970; LAM and JONES 1973) maps to the left of *ade3–59* (LAM 1973; JONES and LAM 1973) in the region expected for synthetase activity. The final line of evidence that implicates *ade3* as the structural locus for these activities is the finding that the dehydrogenase activity in extracts of *ade3–88* is labile (DRACKETT and JONES, unpublished observations).

LOMAX, GROSS and WOODS (1971) have described *ade3* mutations which have some of the growth properties observed for mutations which map in region a. These mutations require adenine but do not require histidine. However, growth of some of these mutants was inhibited by methionine or histidine. No evidence for inhibition of growth of our mutants by these two nutrients was found, nor would we have expected to isolate these classes of mutants, for the mutants described in this paper were grown in YEPD, which contains both histidine and methionine. The mutants isolated by LOMAX, GROSS and WOODS (1971) were grown on a minimal medium containing only adenine.

The mutants described by LOMAX, GROSS and WOODS (1971) do not fit easily into the hypothesis presented above. Since they have greatly reduced levels of dehydrogenase but substantial levels of synthetase, one would expect them to map in the right end of the locus (that encoded by dehydrogenase). Mutants mapping in the right end should have a requirement for histidine which these mutants do not. Resolution of these discrepancies must await further work.

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