ALLELIC COMPLEMENTATION AT THE $ad_{5/7}$ LOCUS IN YEAST[1,2]

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THE mounting evidence of functional complementation between independently derived mutants at specific loci of various organisms indicates that allelic complementation is a widespread phenomenon. First described for heterocaryons of the *pyr-3* locus (MITCHELL and MITCHELL 1956) in Neurospora, allelic complementation has also been observed in such diverse organisms as bacteriophage (CHAMPE and BENZER 1962), bacteria (PERRIN 1963), yeast (LEUPOLD and GUTZ 1963), and Drosophila (CARLSON 1959). Comparative studies of allelic complementation and recombination constitute a tool for the investigation of gene action by relating the genetic structure of a locus to the functional interactions of its modified protein products. On the basis of correlated complementation and fine structure analyses of the *ad-8* locus of Neurospora (ISHIKAWA 1962a, b), KAPULER and BERNSTEIN (1963) have proposed a model for the affected enzyme, adenylosuccinate synthetase, in which the active enzyme is a layered stack of monomeric units, each shaped approximately as a two-turned spiral. An alternate model for complementation, proposed by CRICK and ORGEL (1964), suggests that complementation occurs as a result of localized corrections of mutation-caused alterations in the folding of monomeric polypeptide units by nondefective homologous stretches of complementary monomers about axes of symmetry.

Much of the work on complementation has hitherto been done with Neurospora heterocaryons. It was therefore considered of value to extend this kind of investigation of gene-protein relations to an organism in which haploid nuclei are fused as the diploid phase is established. *Saccharomyces cerevisiae* is especially convenient in having stable haploid and diploid vegetative phases and a controllable meiosis producing four-spored asci which can be analyzed by dissection as tetrads or by random methods. Spontaneous mutants at each of several loci controlling adenine biosynthesis were surveyed for allelic complementation (DORFMAN 1963). Results obtained with $ad_{5/7}$ alleles justified concentration on this locus to the exclusion of other loci. Allelic complementation in *S. cerevisiae* has been reported by BEVAN and WOODS (1962 abstract) for the ad_2 locus. Comparative studies of complementation and recombination have been made at the ad_6 locus (LEUPOLD and GUTZ 1963) in the yeast *Schizosaccharomyces pombe.*

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The present study of complementation among $ad_{5/7}$ alleles has produced a new complementation map in the form of a circle or helix with two "tails." Genetic mapping indicates the general colinearity of mutant sites with the complementation map, and also supports the division of the locus into two regions corresponding to the functional grouping as ad_5 and ad_7 alleles.

MATERIALS AND METHODS

Stocks: Yeasts for this study were kindly supplied by DR. S. FOGEL and DR. R. K. MORTIMER. Marker designations follow the conventions of the 1961 Carbondale Yeast Genetics Conference (VON BORSTEL 1963). Mating types are denoted by a and α. Adenine-requiring (ad^-) mutants carrying the recessive markers ad_1 and ad_2 accumulate a cell-limited red pigment. Other recessive markers used were tr_1 (tryptophan dependent), ur_3 (uracil dependent) and $ad_{5/7}$ (adenine-dependent), all unlinked.

Terminology: The symbols ad_5 and ad_7 are retained in their original sense to denote specific groups of ad^- mutants distinguished by having total positive intergroup, but not intragroup, complementation. The close linkage between ad_5 and ad_7, and the occurrence of mutants which complement neither group (ROMAN 1956) warrant their inclusion within a common framework. Accordingly, the symbol ad_5ad_7 will denote those mutants which do not complement either ad_5 or ad_7 mutants, but general reference to all three types will be subsumed under the symbol $ad_{5/7}$. For simplicity, marker references will be made in the singular form as "the $ad_{5/7}$ locus," without thereby implying any specific conclusions regarding the nature of the association involved. The system of numbering independently obtained alleles is given in the legend to Figure 4.

Media: Yeast-extract peptone (YEP) (ROMAN 1956) was used for general growth purposes and for storage slants. WICKERHAM's (1946) synthetic minimal served as a defined medium, supplemented, when necessary, with the following, per liter: DL-tryptophan, 20 mg; uracil, 10 mg; adenine, 20 mg.

All incubations were at 30°C. Crosses were made by the method of POMPER and BURKHOLDER (1949). Presporulation diploid populations were grown in 10 ml of GNA (FOGEL, personal communication)—8 g Difco nutrient broth, 3 g yeast extract, 100 g glucose per liter, on a rotary tube rack for two days. The cells were washed and sporulated on the medium of MCCLARY, NULTY and MILLER (1959). Petite strains were identified by their failure to grow on the sporulation medium of FOGEL and HURST (1963), designated GYP. All media were solidified, as needed, with 15 g agar per liter. Asci were dissected (JOHNSTON and MORTIMER 1959) after 1-hr digestion with commercial snail juice (prepared from *Helix pomatia* by L'Industrie Biologique Francaise, Génévilliers, France) diluted 1:20 in distilled water with 1 mg/ml of cysteine-HCl added (SHERMAN 1963).

Spontaneous mutants were collected by ROMAN's (1956) method as white sectors in old colonies of red ad_1 a, and ad_2 α strains. The white isolates have a second genetic defect in adenine biosynthesis at one of the loci ad_3 to ad_8. Additional ad^- mutants were induced by treatment with ethyl methanesulfonate (EMS). For complementation testing, mutants were cross-replicated for a day's growth on YEP from two master plates, each inoculated with eight parallel stripes of the same mating type. In the intergenic tests, the unknowns were crossed with eight tester strains, each bearing one of the ad^- markers. After identification, mutants at a given locus were crossed to produce diploids in allelic complementation tests. Upon replication to minimal medium, tests for nonallelism were scored for growth at the diploid intersection areas after one day, while allelic tests were scored after 1, 2, 4 and 7 days, in four semi-quantitative degrees: negative, barely perceptible growth, weak, and full growth. Diploid areas which had not progressed beyond barely perceptible growth by seven days were classed as negative for mapping purposes.

RESULTS

The ad_5 and ad_7 mutants are closely linked. ROMAN (1956) found no re-

combinant progeny in 163 asci dissected from a cross between two of them. The most frequent class, ad_5ad_7 usually fails to complement either ad_5 or ad_7, indicating that the two groups are functionally associated, although it is not certain whether one or two reactions are involved. The spontaneous appearance of revertants in lawns on minimal medium suggests that at least some of the ad_5ad_7's are point mutations, although the possibility that the revertants are due to suppressor mutations has not been excluded.

The choice of a particular mutant to serve as a tester strain is clearly important for a locus at which allelic complementation is common. Use of a freely complementing allele for testing would be expected to result in the exclusion from its group of some mutants properly belonging to the locus. In practice, the freedom from ambiguity is due in general to the greater intensity of nonallelic complementation on the first day in contrast with even the strongest allelic complementation. The final complementation tests with mutants at the $ad_{5/7}$ locus were made with their progeny in the form $ad_{5/7\text{-}x}$, a, tr_1 and $ad_{5/7\text{-}x}$, α, ur_3, isolated by ascus dissection. The matrices were picked up on minimal medium and on minimal plus adenine. On the latter plates the growth of all the diploid intersection areas, but not the haploid stripes, demonstrated that there were no cases in which mating failed to occur. A representative sample of 30 plates was further replicated to minimal medium plus tryptophan, lysine and uracil. Complementation here matched that on minimal in time and amount for each pairwise test, showing that the heterozygous forcing markers had no measurable effect on complementation between different alleles of ad_7. Failure to complement was not due to loss of viability. Cells were picked from 50 noncomplementing diploid areas after 30 days incubation on the minimal plates into tubes of YEP broth. Only three of the tubes required more than two days to develop turbidity.

Results of 6,484 different pairwise complementation tests between 115 complementing ad_7's are presented as a matrix of test scores in Figure 1, and by the derived complementation map shown in Figure 2. Of these tests 1,890 are repetitions, using progeny isolates of strains previously tested as obtained. In 3,916 cases, covering 89 of the mutants, the reciprocal relation of mating type and forcing-marker combinations was also tested. Agreement of the reciprocal tests exceeded 96 percent. In mapping the results for those cases where reciprocal tests disagreed, positive complementation was favored over noncomplementation. The usual convention of displaying complementation by a system of overlapping line segments is followed (CATCHESIDE and OVERTON 1958; GILES 1958).

By repeating unit 33 of the map at both ends, it is possible, as in Figure 3, to present the map in a straight line form for easier inspection of detail. The 168 noncomplementing ad_5ad_7's overlap all the ad_5's and ad_7's. The 59 ad_5's showing almost no interaction among themselves, occupy a unit at an extremity of the map, placed to the right of the ad_7 group in keeping with their relative position by meiotic recombination mapping (DORFMAN 1964). The other tail, in this helical or circular representation, is dictated by the behavior of several highly reactive ad_7 mutants which tend to complement most other ad_7 alleles. COSTELLO and BEVAN (1964) with similar material found the same general arrangement.

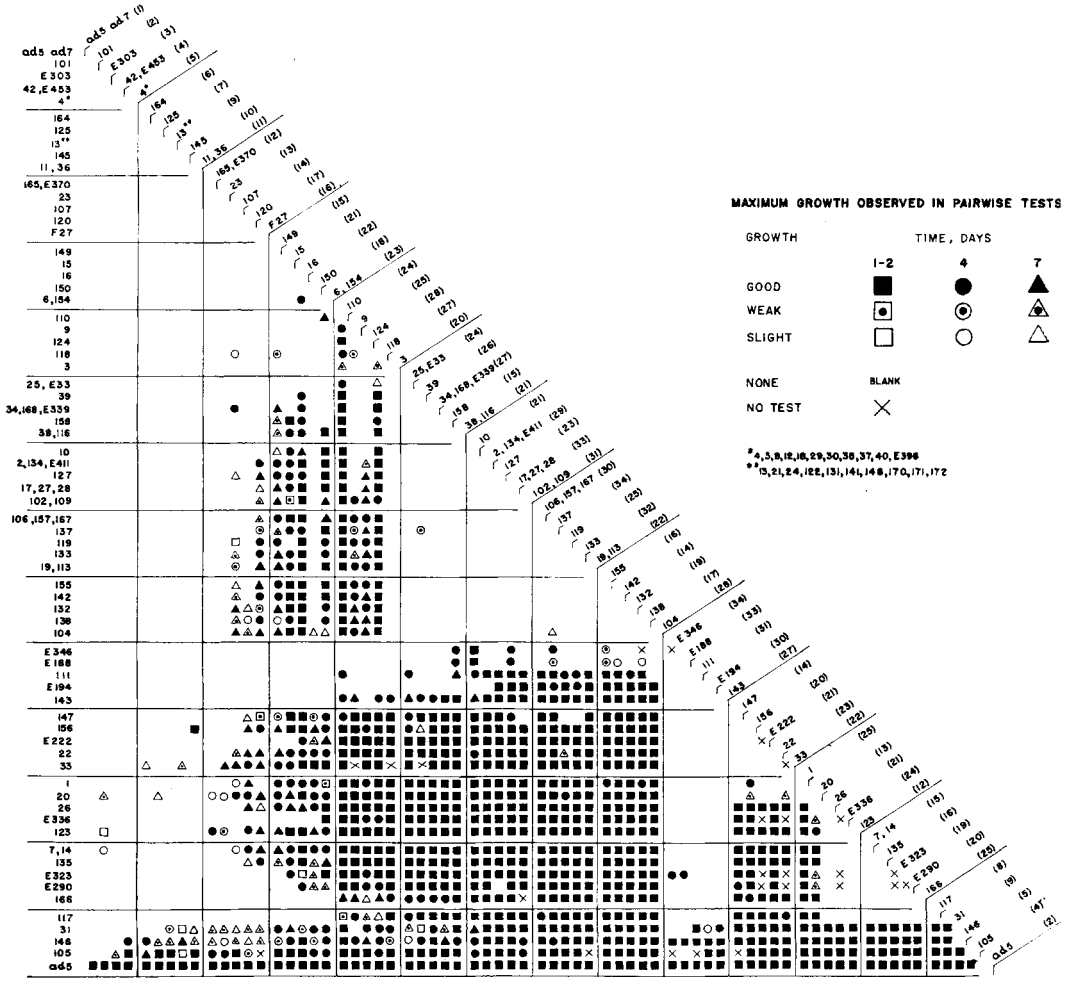

FIGURE 1.—Results of pairwise complementation tests between $ad_{5/7}$ mutants. Numbers in brackets refer to line numbers of Figure 3 on which the map segments representing the various alleles are drawn.

The present map has 34 complementation units, of which 30 are in the circular portion. Table 1 lists the pairwise reactions which are exceptional to the map as drawn.

According to CARLSON (1961), a circular map of n complementation units can have a maximum of $n\ (n-1)+1$ different line segments. For a map of 30 units this comes to 871 different possibilities. Only 68, 1/13th as many, are required by the 115 ad_7 mutants in this study; 16 segments describe multiple occurrences of mutants of identical pattern. The segments may be grouped for simplification by common characteristics which conceivably reflect structural properties of the affected protein. (For convenience in the following, the line-number on which named segments appear in Figure 3 will be bracketed after mutant identification

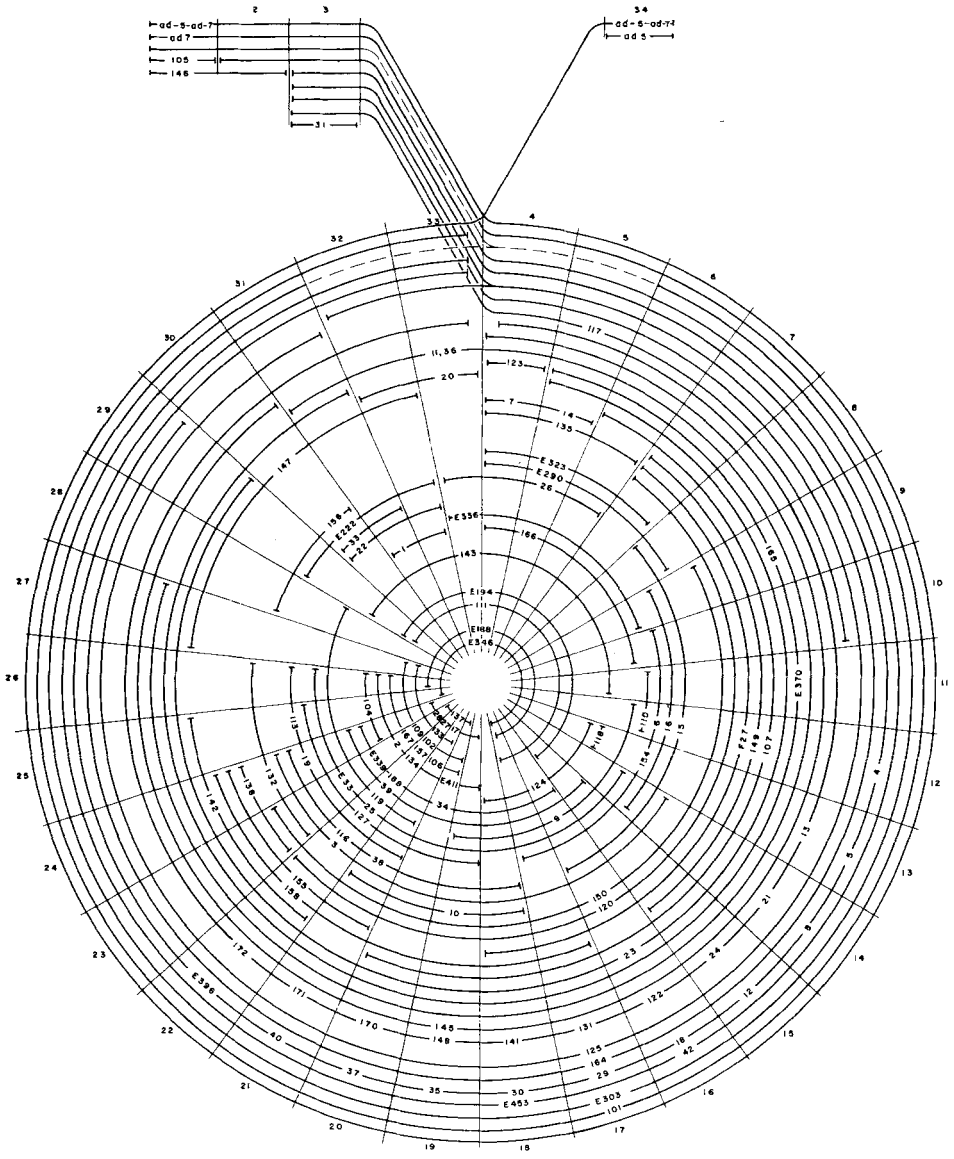

FIGURE 2.—Map of $ad_{5/7}$ complementation in *S. cerevisiae*.

numbers.) There is a group of mutants at the left end, Figure 3, showing a nearly common terminus and a stepwise progression toward less reactivity, mutants 123 [12], 7, 14 [15], 135 [16], E323 [19], E290 [20] and 166 [25]. Several mutants are shown by very small segments, e.g. 110 [24] and 118 [27]. These are very reactive and are responsible for some of the exceptions to consistency with linearity of the map; 118 [27] complements 149 [15]; E370 [12] and 172 [9]; 110 [24] complements 143 [27] and 6 [23]. The middle region evidently covers

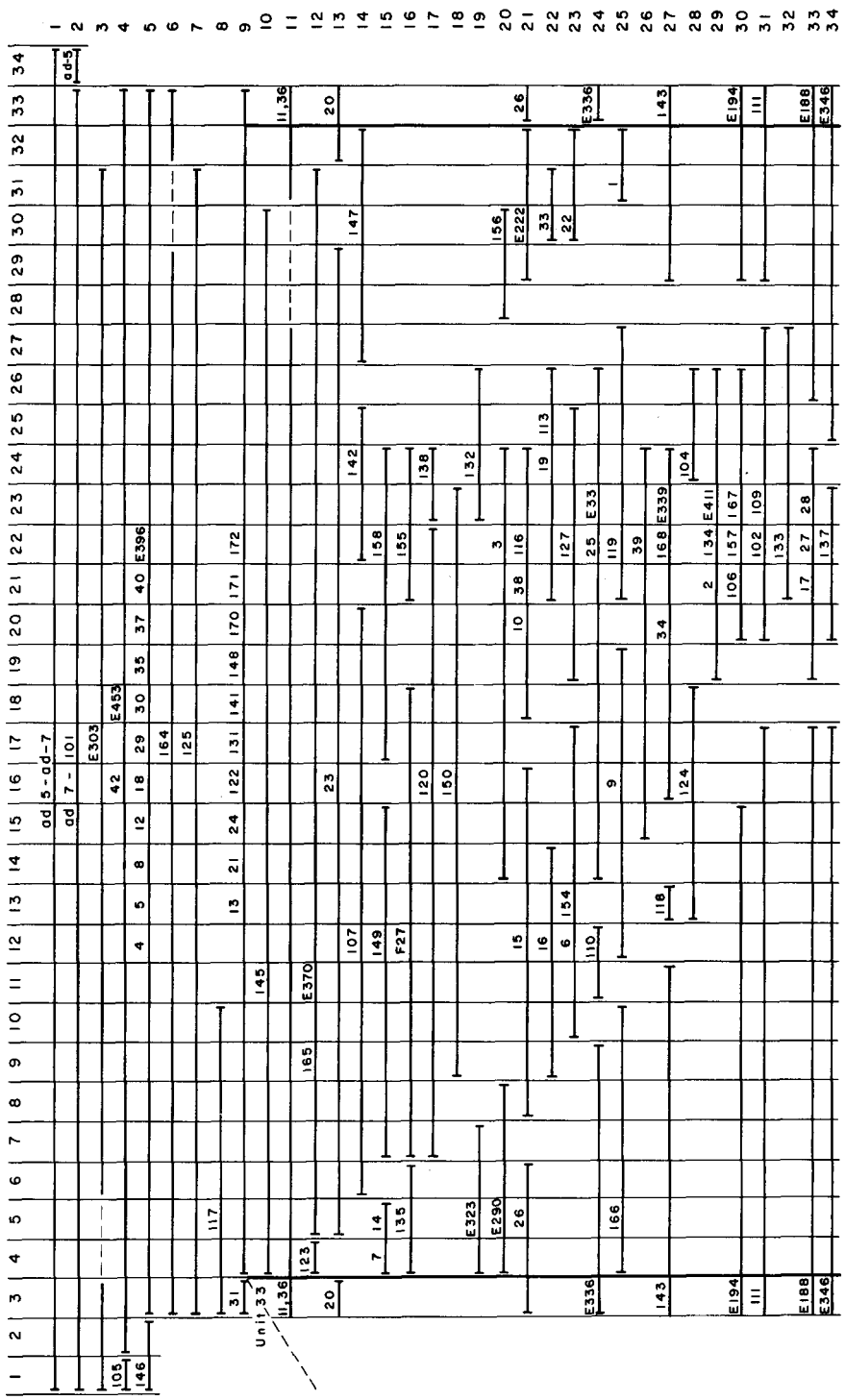

FIGURE 3.—Straight line form of the $ad_{5/7}$ complementation map.

TABLE 1

*Exceptions to continuity of segments on the complementation map**

Positive exceptions			Negative exceptions	
Allele-pair	No. of trials No. +	Total	Allele-pair	No. of trials
6– 16	1	2	1– 23	2
15–E336	1	1	1– F27	3
36– 137	1	2	119– 15	3
39– 137	1	2	E222– 15	1
25– 111	1	2	E222– F27	1
110– 111	1	2	E222– 120	1
110– 148	1	2	E222– 158	1
110– 154	1	2	E222– 149	1
110–E194	1	2	E411– 149	1
118– 149	1	2	10–E194	2
118– 172	1	1	31– 6	3
118–E370	1	1	31– 9	2
124– 168	2	2	31–E188	2
132– E33	1	1	31–E346	1
142– E33	1	1	146–E346	1
E453– E33	1	1	146– 6	1
147– 170	1	2	105– 133	1
147– 171	1	2		
E188–E323	1	1		

* The unconventional map segments E303, 164, 11 and 36 are not included in this list.

a mutational "hot stretch," yielding many very similarly placed mutants tending to terminate in units 24 and 26. Mutants at the right are again very reactive. One of these, 20, fails to complement 26 at the left, and would establish circularity even in the absence of the long overlapping mutants. The latter, a group of eight mutants (11, 36 [11], E336 [24], 143 [27], E194 [30], 111 [31], E188 [33], and E346 [34]) complement only those in the middle region, firmly forcing the map into its circular form. A disproportionate number of these are EMS-induced.

About 45 $ad_{5/7}$ alleles have been ordered by meiotic recombination in two-point crosses. The resulting map is shown schematically in Figure 4. Details of this study will be presented in a later report. On the genetic map the nine ad_5 mutants tested so far occupy sites exterior to and at one side of the region which includes

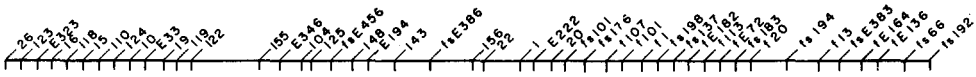

FIGURE 4.—Meiotic recombination among $ad_{5/7}$ mutants. The map is not drawn to scale. Symbols used here and in the text indicate mutant group and origin of each allele: an f identifies ad_5 mutants, fs signifies ad_5ad_7; ad_7 mutants are named by number only. EMS-induced alleles have an E preceding their isolation number. All others are spontaneous, and are distinguished in terms of their respective origin: mutants of ad_1 *a* backbround are numbered in the first hundred; those of ad_2 *α* origin have numbers starting from 101. F27 was received from DR. S. FOGEL.

the now mapped ad_7 alleles. Present preliminary evidence from nine of the 11 ad_5ad_7 alleles mapped indicates that the ad_5ad_7 group is confined to the ad_5 region. (The data from which fsE456 and fsE386 are tentatively placed in the ad_7 region are not convincing enough to challenge the general statement regarding the group.)

A detailed comparison of complementation and genetic maps here is possibly only for the ad_7 group, the one which exhibits complex, mappable complementation. The resolution of such a comparison is determined by the length and position of the complementation segments representing the alleles whose mutational sites are genetically mapped, since long-segment mutants cannot be localized in the sense of showing linear sequence. Even segments of restricted length allow considerable discretion in ascribing an order. Twenty-five of the 115 complementing ad_7 mutants have been mapped genetically. Of these, 19 are represented by relatively short segments, the longest of which, E33 [24], covers 13 of the 30 units in the circular portion of the map. While available data by meiotic recombination are not yet sufficient rigorously to order all of these mutants, they are consistent with the order presented in Figure 4, and also consistent with the sequence displayed by the short segments on the complementation map i.e., the two maps are colinear. The one apparent exception to colinearity is 110 [24], whose leakiness and other exceptional properties also set it apart from the others.

DISCUSSION

From the evidence now available the correlation between complementation and genetic maps of the $ad_{5/7}$ locus is reasonably straightforward. The locus is divided functionally and genetically into two discrete parts, corresponding directly to the ad_5 and ad_7 groups originally designated (ROMAN 1956) by complementation alone. Except for a few specific mutants of slower general response in this regard, all ad_7 alleles complement all ad_5's in amount and speed of growth to a degree that is roughly comparable with their nonallelic complementation in tests with ad^- mutants at other loci. The lack of complementation among the ad_5's is indeed nearly complete. In some instances, however, notably with fE164, some low level complementation was observed in tests with other ad_5 mutants. A few cases of similarly low level complementation between ad_5's and ad_5ad_7's have also occurred.

Although the exceptions to continuous linearity of the complementation map constitute only 0.5 percent of the total number of tests made, many of them are reproducible and seem to be valid evidence as to the complementation relation between the relevant alleles. On a circular complementation map, exceptional positive responses can be plotted by radial connectors to the respective line segments. Exceptional negative tests must be shown as circumferential connectors. The exceptions listed in Table 1 are diagrammed in Figure 5. The striking feature that emerges is the preponderance of positive exceptions on one half of the map, from unit 9 to unit 27, and negative exceptions in the other half from unit 24 around to unit 8. Closely related to this is the fact that the long segment mutants E346 [34], 125 [7], 148 [9], E194 [30], and 143 [27] map genetically in

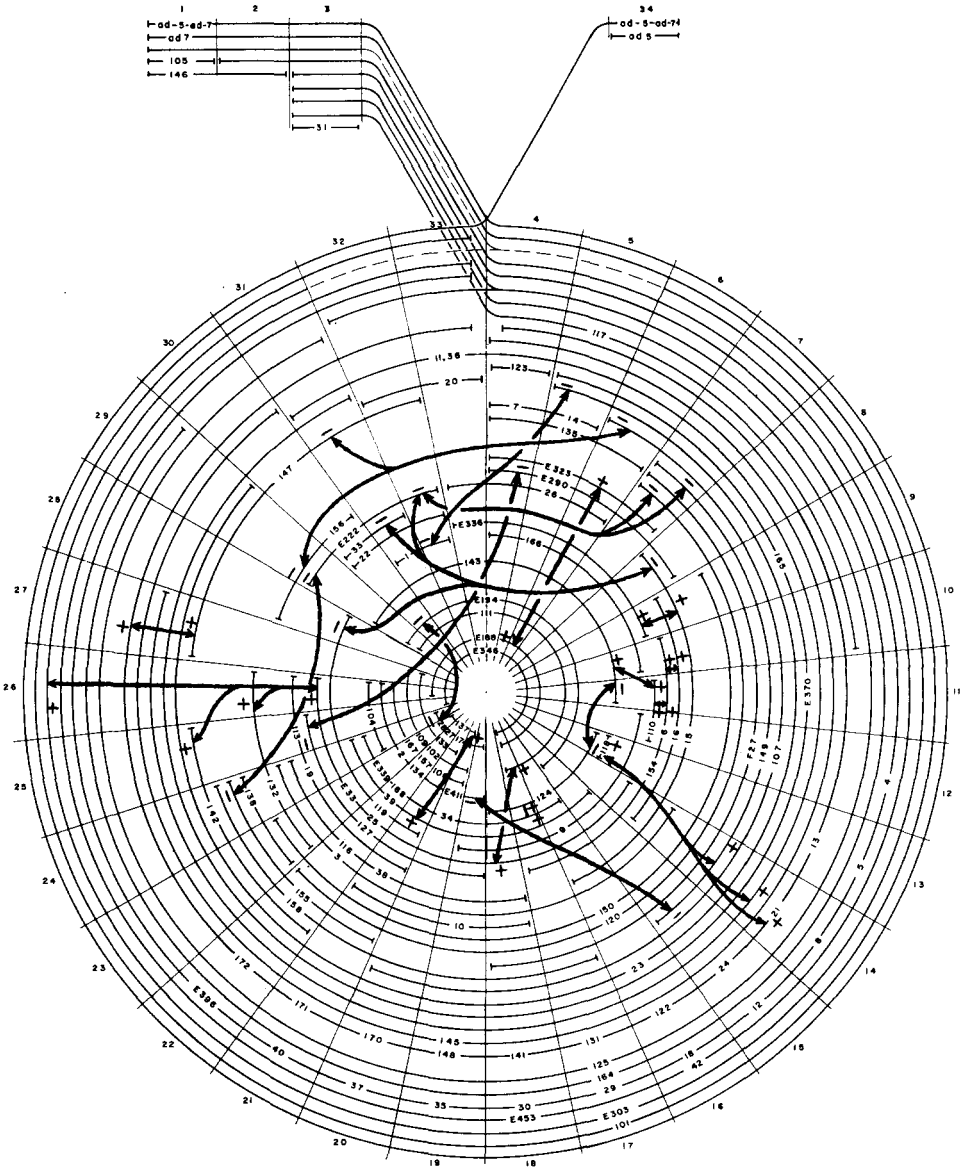

FIGURE 5.—Exceptions to continuity of the $ad_{5/7}$ complementation map.

a length corresponding to the comparatively uncluttered portion of the complementation map, from units 25 or 26 through 29. The mutational "hot stretch" alluded to earlier thus covers two adjacent lengths with opposite consequences for complementation behavior of mutants produced within their respective extents.

If recombinational map distance is assumed to reflect genetic, hence, polypeptide, length, the ad_5-controlled polypeptide is equally long or longer than that controlled by the ad_7 region. Assuming a single polypeptide product for the en-

tire locus implies association of monomeric units into a multimer (CRICK and ORGEL 1964) with the condition that, although any one undefective ad_5 product suffices to produce activity in a diploid cell, no two ad_5-defective products (with some rare exceptions) can accomplish a mutual correction. By contrast, almost all defective ad_7 products are subject to mutual correction in association with at least some others. Various hypotheses about folding and aggregation could be put forward to account for this distinction within the framework of a unitary protein. The necessary conditions could be met, for example, if the enzymatically active site of the protein was controlled by ad_5, while the ability to aggregate into the active form was the exclusive function of the ad_7 controlled portion. *In vitro* studies by PERRIN (1963) suggest that inactive mutant proteins occur at lower molecular weights than enzymaticaly active complementing aggregates, i.e., a possible reason for failure of complementation is the inability of the monomers properly to aggregate.

In the present case, however, it is simpler to assume, as a working hypothesis, that the ad_5 and ad_7 regions encode two separate proteins. On this view, the ad_5ad_7 mutants would constitute a class containing nonsense (BENZER and CHAMPE 1962) and/or reading frame mutants (CRICK, BARNETT, BRENNER and WATTS-TOBIN 1961). They might be expected to produce either polypeptide fragments or inactive protein products (BENZER and CHAMPE 1962). All of the ad_5ad_7's which were tested for reversion tentatively qualified as point mutations rather than deletions by the spontaneous appearance of revertants, and by generating prototrophs in heteroallelic crosses at frequencies commensurate with those of ad_5 or ad_7 alleles. These facts and the preliminary genetic localization of substantially all ad_5ad_7's in one of the two regions, the ad_5, suggest that the complex as a whole acts as a single unit of transcription. So interpreted, the locus has the attributes of an operon (JACOB and MONOD 1961). Operon-type complex loci have recently been shown to occur in the pathway of galactose utilization in yeast (DOUGLAS and HAWTHORNE 1964) and in histidine biosynthesis, at the *hist-3* region of Neurospora (AHMED, CASE and GILES 1964; GILES 1963) and at the hi_{4-9} locus of yeast (FINK, in preparation). The direction of transcription is assumed to be from the ad_5 to the ad_7 region. Missense mutational changes presumably leading to amino acid substitutions would be expressed as ad_5 or ad_7 mutants, depending on the site of mutation. Mutations in the ad_5 region which result in extensively changed or incomplete proteins prevent complementation with other mutants in either region. The critical test here lies in the precise localization of the ad_5ad_7 mutants. Three-point recombinational tests now in progress with intragenic double mutant strains should contribute to a decision as to their position.

Nonsense and reading frame mutations occurring in the ad_7 region might be expected to produce noncomplementing ad_7 alleles. In fact, the class of noncomplementing ad_7's is conspicuous by its near absence, being represented at present only by 101 [2]; all other alleles complement the "tail" mutants, as a minimum. None of the latter has been mapped genetically. Assuming, however, that the "tail" mutants conform to the observed colinearity they would be placed at the left extreme of the ad_7 region. Such an interpretation invites the further specula-

tion that the "tail" mutants produce proteins defective near the end of the polypeptide chain, with a barely marginal loss of correct folding.

How much information on protein conformation can be deduced from correlating complementation and genetic maps? According to present ideas on protein synthesis, the elements of gene structure, as DNA base-pair sequences are colinear with the primary structure of the proteins they encode. Gene alterations can result in alterations of the primary protein structure of the controlled proteins at corresponding linear positions. If we assume that allelic complementation occurs as an interaction of protein submits, then the complementation behavior of an allelic series of mutants is a measure of the effect on enzymatic function of the respective changes in protein primary structure at relative positions which can be approximated by recombination analysis of the same mutant series. CRICK and ORGEL (1964) maintain that there is no simple way to deduce details of protein conformation from such correlations. In view of the complexity of protein structure (cf. SHACHMAN 1963), this view occasions no surprise. On the other hand, self-consistent circular complementation maps and others of more eccentric pattern in association with a colinear order of genetic sites strongly encourage the belief that complementation maps do reflect at least some features of protein conformation.

The present difficulty in comparing complementation and genetic mapping with details of protein structure stems from two basic deficiencies. One of these is that the information content of the combined maps has not yet reached a scale of resolution commensurate with the detail of protein complexity. In cases where all mutants tested for allelic complementation yield results which conform to a linear map of few complementation units, a significant increase in information content of this kind may not be attainable. Where intricacy has been shown, however, there is no need to set arbitrary *a priori* limits to the degree of complexity that may ultimately be revealed.

The other, more serious, deficiency is that in no single case have the details of complementation and recombination mapping been compared with the independently determined structure of the affected protein. In the absence of such a comparison for at least a few illustrative cases there can be no certainty that structures proposed on the basis of mapping data resemble real ones.

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SUMMARY

Allelic complementation has been studied among spontaneous and ethyl methanesulfonate-induced mutants at the $ad_{5/7}$ locus controlling adenine biosynthesis in Saccharomyces. The mutually complementing groups ad_5 and ad_7 are closely linked. Functional association between them is indicated by noncomplementation of either ad_5 or ad_7 with the most frequent class of mutants found at the locus, the ad_5ad_7's; some of these undergo spontaneous reversion and therefore are judged to be point mutations rather than deletions. The ad_5's do not complement

each other. Results of 6,484 different pairwise complementation tests among 115 complementing ad_7 alleles are presented in the form of a circular or helical map of 34 complementation units with two "tails."

Preliminary fine structure analysis of the locus by meiotic prototroph frequencies in two-point crosses confirms the structural division of the locus into two adjacent parts corresponding to ad_5 and ad_7 regions. Substantially all of the ad_5ad_7's tested map in ad_5 region. For 19 of the ad_7 alleles the order of mutant sites by recombination is consistent with colinearity to the order derived by complementation. Complementation and genetic data suggest that the $ad_{5/7}$ locus may control the synthesis of two separate enzymes in adenine biosynthesis.

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