ALLELIC COMPLEMENTATION AT THE ADENYLOSUCCINASE LOCUS IN SCHIZOSACCHAROMYCES POMBE[1]

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COMPLEMENTATION between allelic mutants at the *ad-4* (adenine-4) locus, deficient for the enzyme adenylosuccinase (adenylosuccinate AMP-lyase, C.E. 4322), was first reported in Neurospora (GILES, PARTRIDGE and NELSON 1957; WOODWARD, PARTRIDGE and GILES 1958). It was found that certain pairs of these mutants, which as a result of the absence of adenylosuccinase activity require adenine for growth, can form heterocaryons which are independent of adenine and have restored activity for the enzyme.

This report concerns the genetics and complementation pattern of analogous mutants at the *ad*-8 locus (LEUPOLD 1955) of the yeast *Schizosaccharomyces pombe* strain Liquefaciens. Some results of this study have been briefly described previously (MEGNET 1959a; GILES 1964).

MATERIALS AND METHODS

The mutants used in this study are derived from the 975 wild-type strain (LEUPOLD 1955). They are either induced by ultraviolet light (UV) (L 106, U 71, U 72, U 80, U 83, Y 177, Y 228, Y 313, Y 321, Y 333) or of spontaneous origin (R 107, R 420, R 442, R 482, R 532, R 540). The UV mutants Y 177, Y 228, Y 313, Y 321, Y 333 were obtained by spreading a UV-irradiated suspension of wild-type cells on synthetic medium (MEGNET 1959b) supplemented with 50 μg/ml adenine, followed by incubation and subsequent replica plating on to medium containing hypoxanthine. The mutant L 106 was kindly given to us by DR. U. LEUPOLD. The mutants of spontaneous origin were isolated by a mutant concentration method involving inositolless death of prototrophic cells (MEGNET 1964). The mutants isolated were backcrossed to the wild-type strain 972 to secure a homogenous genetic background of the isolates and to obtain them in both mating types.

Qualitative crosses were made by streaking the strains to be tested on the surface of a malt extract plate which was seeded, on top, with cells of the tester strain of opposite mating type. These plates were incubated for 4 days at 25°C and subsequently replica plated onto minimal medium plates. Quantitative crosses between *ad-8* mutants were made on malt extract medium (LEUPOLD 1957), supplemented with 75μg/ml adenine. Prototroph frequencies from such crosses were assayed by spreading an aliquot of an ascospore suspension onto the surface of minimal medium plates, supplemented with 5 μg/ml eosin Y. The concentration of total spores in the suspension was determined by hemacytometer counts.

Adenylosuccinase activity in crude extracts of cells, disrupted in a Hughes Press, was determined as described for Neurospora by WOODWARD (1959).

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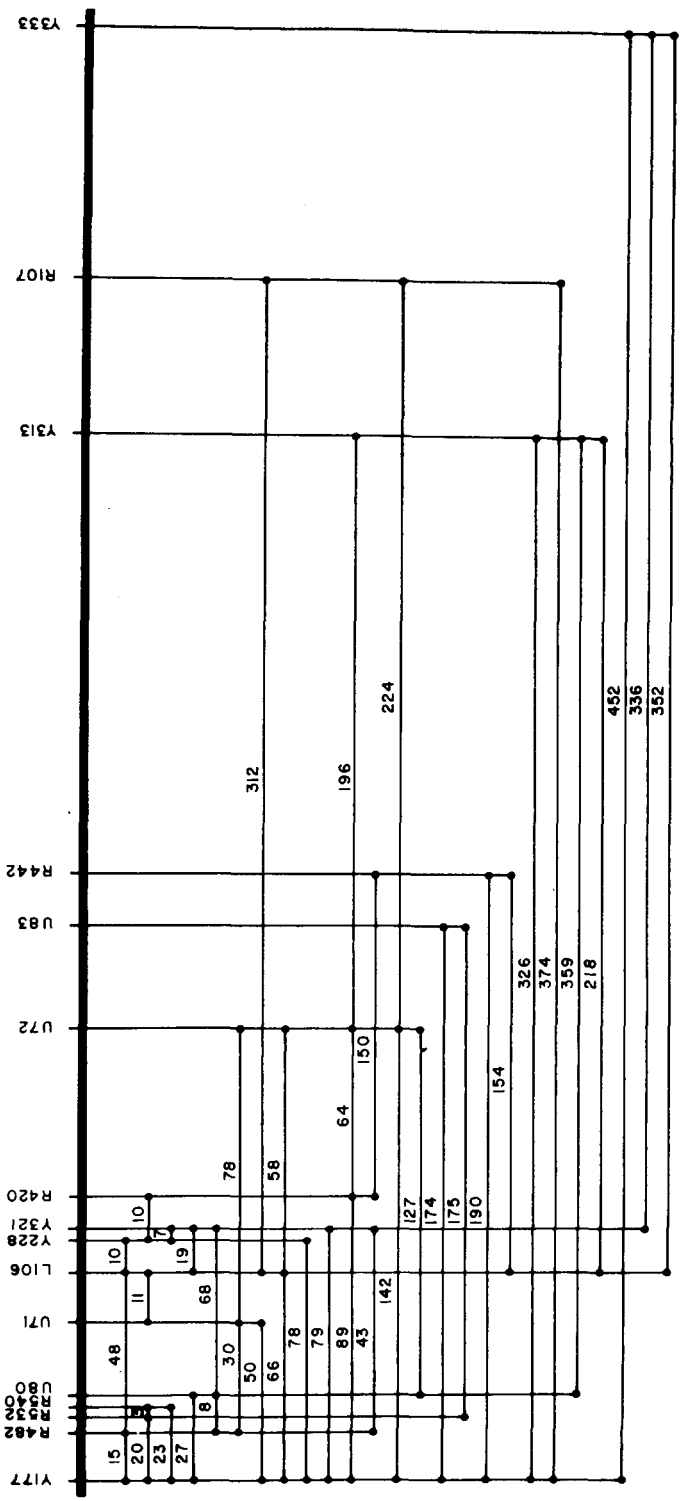

FIGURE 1.—Genetic map of the *ad-8* locus. Numbers refer to individual *ad-8* mutants. Distances are given as the number of prototrophs per 10^7 ascospores.

RESULTS

Biochemical and genetic characterization of ad-8 *mutants:* The *ad-8* mutants require adenine for growth. Hypoxanthine or inosine cannot be substituted for adenine. The mutants do not accumulate hypoxanthine or inosine as do mutants (*ad-2*) blocked immediately prior to the adenylosuccinase reaction. Adenylosuccinase activity could not be detected in any of the mutants used in this study. This result applies for the two known substrates of adenylosuccinase—adenosine monophosphate succinate (AMP-S) and N-(5-amino-1-ribosyl-4-imidazole-carbonyl)-L-aspartic acid-5′-phosphate (SAICAR) (BUCHANAN and HARTMAN 1959). On the other hand, activity of the enzyme could easily be demonstrated in the wild type.

The results of qualitative crosses between the *ad-8* mutants and the strain Y 333 and between a nonallelic *ad-2* mutant (R 2) gave a clear indication that all *ad-8* mutants are alleles at the same locus.

Interallelic crosses: Crosses between *ad-8* mutants give rise to ascospores of low viability. For this reason prototroph frequencies have been based on the total number of spores plated. Figure 1 gives the genetic map of the *ad-8* locus and summarizes the crossing data used for the construction of the map. Distances are given as the number of prototrophic ascospores per 10^7 spores plated. The order of the sites was established by the additivity relationships.

Interallelic complementation: The *ad-8* mutants can unequivocally be assigned to eight complementation classes. Complementation between a pair of mutants is detected by the appearance of a relatively large number of small, partially adenine-independent colonies on platings of ascospores from a cross of the two mutants onto eosin-containing minimal medium. These small colonies, consisting of diploid cells, arise from occasional diploid ascospores (LEUPOLD 1956). The color of these colonies, which differentiates them from the white, true prototrophic, recombinant colonies, is due to the presence of sporulating cells. Sporulating cells take up the dye (eosin) and turn pink, whereas nonsporulating, living cells do not take up any dye.

The results of the complementation tests performed with all possible pairwise combinations are summarized on the complementation map in Figure 2. The rules for the drawing of this complementation map are the ones used by WOODWARD *et al.* (1958). The complementation classes in which the mutants fall, represented on the map as short horizontal bars, are associated graphically with

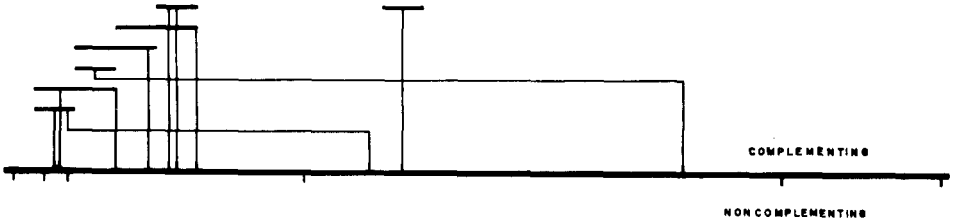

FIGURE 2.—Comparative complementation and genetic maps of the *ad-8* locus. Sites above the heavy line correspond to complementing mutants, those below the line to noncomplementing mutants.

the corresponding sites on the genetic map. The class of noncomplementing mutants is not represented on the complementation map, but the sites of such mutants are indicated below the line of the genetic map.

DISCUSSION

The nutritional, enzymatic, and complementational properties of the *ad-8* mutants are compatible with the assumption that the *ad-4* locus of Neurospora and the *ad-8* locus in Schizosaccharomyces code the same enzyme.

The similarity of the adenylosuccinase-less mutants in both organisms also includes the observation of low ascospore viability (WOODWARD, PARTRIDGE, and GILES 1958). This marginal viability of spores is probably due to growth-inhibitory compounds derived from accumulating purine precursors. By basing prototroph frequencies on the total number of spores and not, as is customarily done, on the number of viable ascospores, it has been possible to construct a consistent genetic map of the locus.

Interallelic complementation at the *ad-8* locus in Schizosaccharomyces was expected from its relatively close taxonomic relationship to Neurospora. In both cases a number of complementation classes have been found which can be ordered on a complementation map. Both the maps are linear. A comparison of the complementation map with the genetic map of the *ad-8* locus shows, with two exceptions (mutants U 83 and Y 313), that the order of the mutants on the two maps is identical.

The known facts about the mechanism of complementation in Neurospora (WOODWARD 1959; FINCHAM and CODDINGTON 1963) i.e., that it involves the formation of active enzyme molecules from inactive mutant molecules, and that so-called colinearity is observed between the complementation and genetic maps, have led to the construction of a number of models relating to the structure of the enzymes involved (CASE and GILES 1960; FINCHAM and CODDINGTON 1963; KAPULER and BERNSTEIN 1963). However, the lack of detailed information about the molecular structure of the adenylosuccinase molecule in Schizosaccharomyces prevents a profitable discussion of the applicability of such models to this enzyme.

It is hoped that future comparative studies of mutants at the *ad-8* locus in *Schizosaccharomyces pombe* and at the *ad-4* locus in *Neurospora crassa* will provide more information about the mechanism of complementation in these organisms. One practical result of such studies might be the possibility of a direct comparison of mutant enzyme proteins from these two organisms with one another by the *in vitro* formation of interspecific hybrid molecules.

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SUMMARY

Sixteen mutants were studied at the *ad-8* locus of the yeast *Schizosaccharomyces pombe*. This locus, which corresponds to the *ad-4* locus of *Neurospora*

crassa, controls the enzyme adenylosuccinase (adenylosuccinate AMP-lyase) which catalyzes the ultimate reaction in purine biosynthesis leading to adenosine-5′-phosphate. All mutants were tested in all possible pairwise combinations for their ability to complement in diploids. Ten of the mutants do complement, and the results of these tests can be summarized in a linear complementation map. The order of the mutants on the complementation map agrees, with two exceptions, with the order on the recombination map.

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