[11] Pratt, David, and Gunther S. Stent, these PROCEEDINGS, **45**, 1507 (1959).

[12] The authors gratefully acknowledge the contributions of Dr. A. W. Kimball of the Oak Ridge National Laboratory Mathematics Panel in the analysis of this model.

[13] Watson, J. D., and F. H. C. Crick, *Cold Spring Harbor Symposia on Quant. Biol.*, **18**, 123 (1953).

---

*COMPLEMENTATION BETWEEN ALLELES OF THE Td LOCUS IN NEUROSPORA CRASSA**

BY ANN M. LACY† AND DAVID M. BONNER‡

DEPARTMENT OF MICROBIOLOGY, YALE UNIVERSITY

Communicated November 10, 1960

During the past ten years, a large number of mutants of *Neurospora crassa* have been isolated which are unable to catalyze the final step in tryptophan synthesis.[1, 2] Despite the similarity of these mutants as judged in terms of their nutritional requirements, loss of enzymatic activity, and location of altered genetic sites,[1] they differ from each other when judged in terms of temperature sensitivity,[1] accumulation of indole,[2] response to specific suppressor genes,[1] and ability to form CRM (cross-reacting material), a protein immunologically related to the enzyme.[3] These differences clearly indicate that the locus involved is mutationally highly complex. In fact, it would appear that none of the more than 100 mutants now isolated is truly identical with any of the others.

In view of the detailed information available concerning the functional differences among these allelic strains, it was desirable to determine whether or not these strains could exhibit complementation when combined in heterocaryons. If such proved to be the case, then it should be possible to construct a complementation map[4] of the locus and to observe any correlations existing between the grouping of the mutants by complementation responses and their already known functional differences.

The present paper will deal with the complementation pattern of this locus—which controls the formation of the enzyme tryptophan synthetase in *Neurospora crassa*. This enzyme catalyzes the terminal step in tryptophan synthesis: an exchange reaction of indole-3-glycerol phosphate (IGP) with serine to form tryptophan.[5, 14] It has also been shown that this enzyme can catalyze a reaction involving the conversion of IGP to indole and a reaction of indole with serine to form tryptophan.[5, 14]

Materials and Methods.—All of the *Td* mutants used in these experiments, with the exception of *td* 1 and *td* 2, were isolated from the St. Lawrence wild type strain 74*A*, or its derivatives, by the filtration technique of Woodward *et al.*,[4] following ultraviolet or X irradiation of macroconidia.[2] Many of the mutants were extensively back-crossed to wild type 74*A*, and isolates were selected which exhibited both heterocaryon compatibility and fertility in interallelic crosses.[2]

Tests for complementation in heterocaryons were carried out by a method similar to that of de Serres.[7] Drops of conidial suspensions of each of two mutant strains were pipetted onto a minimal agar plate in such a way as to have control spots of

the two strains as well as a mixed spot containing both strains. Combinations were tested in duplicate and incubated at 30°C (25°C if a temperature-sensitive mutant was involved). Any combination which did not exhibit more growth after 14 days incubation than did the control spots was considered to be negative in terms of complementing ability. Minimal Medium *N* (Vogel)[8] was used throughout this investigation.

In crosses between *Td* mutants, pseudo-wild type progeny[9] were distinguished from true wild type progeny by a difference in colony morphology and a later time of colony appearance. The validity of this method had been previously established by crossing such progeny by a known wild type strain and recovering both mutant and wild type progeny.

The crude extracts of *Neurospora* tested for tryptophan synthetase activity were prepared as described by Suskind[14] and the enzyme was assayed by the method of Yanofsky.[11]

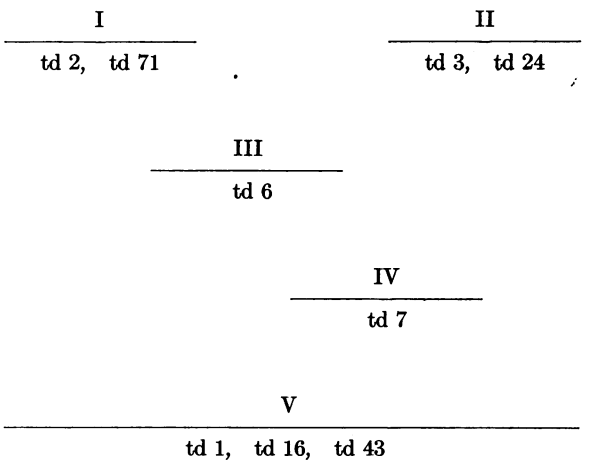

FIG. 1.—Complementation Map of the *Td* Locus. *Td* mutants can be divided into five groups on the basis of complementation responses in heterocaryons. The relationship between these groups is expressed above in terms of a complementation map in which overlapping bars indicate a lack of complementarity. Mutants typical of each group are indicated below each bar.

Results.—In this study, 75 *Td* mutants were tested for complementing ability. While 62 were tested in all combinations, 13 others were tested only with selected strains. Of the 75 mutants, 18 were able to complement at least one other *Td* mutant. Of these, 12 mutants can be divided into four different complementing groups on the basis of their responses in heterocaryons. The other 6 complementing mutants cannot yet be classified since their ability to complement is sporadic. A large fifth group is composed of the 57 apparently noncomplementing mutants. In Fig. 1, these five groups are arranged in a diagrammatic way to form a complementation map similar to that designed for the *ad-4* locus by Woodward *et al.*[4]

In crosses between many of these *Td* mutants (the detailed results of which will be presented elsewhere[12]), some pseudo-wild type progeny as well as true wild type progeny were obtained. As might be expected, there was a close correlation between the occurrence of pseudo-wild type progeny and the complementing ability of the two parents when combined in a heterocaryon.

A cross of *td* 7 × *td* 71 was the only cross between mutually complementing mutants which did *not* appear to yield any pseudo-wild type progeny. In this case, the nonappearance of such progeny may be attributable to the very slow growth of the *td* 7-*td* 71 heterocaryons formed from pseudo-wild progeny, which prevented the detection of the colonies before termination of the experiment.

Characteristic of all the complementing *Td* mutant strains is the fact that functional heterocaryons between them form slowly and that the growth of these heterocaryons on minimal medium is far slower than that of the wild type strain. Forced heterocaryons were formed (on tryptophan supplemented medium) be tween complementing *Td* mutants by the use of additional biochemically unrelated markers. This technique did not result in an increased growth rate of the heterocaryons following transfer to minimal medium.

At the present time, only two interallelic *Td* heterocaryons have been examined for the presence of tryptophan synthetase activity measured in terms of the ability to catalyze the reaction of indole and serine to form tryptophan. A *td* 6–*td* 3 heterocaryon was found to exhibit about 5 per cent of the tryptophan synthetase activity characteristic of the wild type strain grown under the same conditions, while a *td* 71–*td* 24 heterocaryon (grown at 25°C) yielded about 18 per cent of that of the wild type. It was found that the enzyme formed by the *td* 71-*td* 24 heterocaryon is more sensitive to zinc than is that formed by the wild type strain.[2]

Discussion.—Previous studies of the *Td* locus in *Neurospora crassa* have clearly indicated that this genetic region which controls the formation of tryptophan synthetase is highly complex in terms of mutation, recombination, and function.[1, 2] This complexity has permitted inquiry into the question of whether or not the phenotypic differences found among allelic *Td* mutants are reflected in the complementation pattern of the locus.

It is immediately apparent that those mutants which are able to complement exhibit other interesting characteristics (Table 1). One trait shared by all of the

TABLE 1

FUNCTIONAL CHARACTERISTICS OF TD MUTANTS

Characteristics	Groups I	II	III	IV	V
Temperature sensitivity	−	+	−	−	−
Indole accumulation	+	−	−	−	−
Suppressibility	+	+	+	−	−
CRM formation	+	+	+	+	−
Representative Td mutants	td 2, td 71	td 3, td 24	td 6	td 7	td 1, td 16
			td 43		

Td mutants can be divided into five major groups on the basis of the four criteria listed above. Mutants representative of each group are listed on the bottom lines. A number of the *Td* mutants have not yet been classified as to whether they belong in groups III, IV, or V. *Td* 43, for example, has not been tested for response to known suppressor genes.

complementing mutants tested is the ability to form CRM, a protein immunologically related to the tryptophan synthetase formed by the wild type organism.[13] Although not all CRM-forming *Td* mutants are able to complement, no case has yet been observed in which a non-CRM-forming *Td* mutant is capable of complementation. Perhaps a prerequisite for complementation at this locus is the ability of each of the mutants involved to form a protein which is closely related to the wild type enzyme.

A consideration of other characteristics of the complementing mutants seems to indicate that, in general, those mutants which are closely related functionally cannot complement each other, while those mutants having different types of functional alterations are often capable of complementation. For example, mutant strains *td* 2 and *td* 71 both accumulate indole[2] and have been shown to form CRM's which are enzymatically active in catalyzing the formation of indole from IGP.[14] These two strains cannot complement each other, but either strain will complement *td* 3 or *td* 24, neither of which accumulates indole.[2] Strain *td* 24 is temperature-sensitive and suppressible.[1] *Td* 3 is suppressed by the same suppressor as *td* 24, and in this condition, it is also temperature-sensitive.[1] Both *td* 24 and *td* 3 *su* 3 form tryptophan synthetase which is more zinc-sensitive than is that of the wild type organism.[14] Thus, the mutational alterations of *td* 24 and *td* 3 would appear to be similar and the two strains, as one might expect, do not complement each other but do complement *td* 2 and *td* 71.

Strain *td* 6 is not temperature-sensitive[1] and does not accumulate indole[2] but would appear to be related to strain *td* 2, since it can be suppressed by one of the same suppressors.[1] *Td* 7 does not accumulate indole,[2] is not temperature-sensitive,[1] and is not suppressed by any known suppressor;[1] thus, its functional relationship to the other mutants is unknown. Of these 6 mutants, then, *td* 2 and *td* 71 will complement *td* 7, *td* 3, and *td* 24, but not *td* 6. *Td* 3 and *td* 24 will complement *td* 2, *td* 71, and *td* 6, but not *td* 7. The other 6 complementing mutants which can be classified would appear to belong in the same group as *td* 6 on the basis of their complementation responses. It has been shown that these mutants, also, do not accumulate indole[2] and are not temperature-sensitive.[2] (Their other characteristics are currently under investigation.) All these observations suggest that mutants exhibiting similar functional characteristics tend to have similar complementation responses.

Conventional heterocaryon tests indicate the existence of 4 complementing groups. However, the recovery, in preliminary studies, of what appear to be rare pseudo-wild type progeny from crosses of *td* 6 × *td* 7 suggests that the *Td* locus may comprise only 2 *major* functional regions; i.e. it appears possible that only two major complementing groups may be found.

The possible division of these complementing *Td* mutants into 2 major groups is of particular interest in view of the observation of Crawford and Yanofsky[16] that the tryptophan synthetase of *E. coli* is dissociable into two protein components. The suggestion might be made that complementation phenomena in the case of the *Td* locus of Neurospora reflect a bipartite nature of the enzyme. However, present evidence does not support the view that the tryptophan synthetase of *Neurospora crassa* is dissociable.[14] It would appear unlikely, then, that complementation in this instance can be accounted for simply as enzymatic dissociation and recombination of the type observed by Singer *et al.*,[14] to occur in the hemoglobin molecule.

Co-polymerization has also been suggested as a possible mechanism of complementation.[19] However, this explanation does not appear to be favored by the evidence accumulated in the study of the *Td* locus.

Perhaps complementation between these *Td* mutant strains does not always involve interaction at the level of protein synthesis. A reactivation process might

be involved in some cases. We have already mentioned the temperature-sensitive mutant, *td* 24. This mutant when grown at 25°C (a temperature at which it exhibits its mutant phenotype) forms a CRM which can function as active enzyme after fractionation or dialysis.[15] This purified active enzyme can be shown to be more zinc sensitive than tryptophan synthetase formed by the wild type organism grown under the same conditions.[15] When *td* 24 is grown at 35°C or when *su* 24 is introduced into its genome, this strain can grow on a minimal medium as a result of the formation of active enzyme. When extracted and tested, this enzyme is found to be as sensitive to zinc as that formed when *td* 24 is incubated under conditions which will not permit its growth on minimal medium. Thus, *td* 24 appears to form a zinc-sensitive tryptophan synthetase, which under certain cellular conditions may remain enzymatically active, while under other conditions this enzyme is altered to CRM.[14] It could be postulated that, in complementing heterocaryons involving strain *td* 24, the functional enzyme is formed by the *td* 24 component, while the CRM formed by the other component strain would act in some way to free the *td* 24 enzyme from intracellular inhibition, perhaps through a greater affinity for zinc. A preliminary test of the enzyme formed by a *td* 71–*td* 24 heterocaryon seems to indicate that this enzyme is, indeed, more sensitive to zinc than that formed by the wild type organism grown under the same conditions.[2]

At present, the mechanism of complementation is not known and the explanation awaits a better understanding of the nature of the active enzymes formed through complementation in heterocaryons. The recent work of Fincham on a hybrid enzyme formed by a heterocaryon composed of two different allelic *am* mutants is a start in this direction.[24]

An additional point of interest is the possible relationship between the complementation map presented here and a recombination map of the same locus.[12] Since in both of these maps mutant strains can be arranged in a linear sequence, it is of interest to determine how close a correlation exists between the placement of alleles on each of them. This problem has been examined in considerable detail at the *pan-2* locus of *Neurospora crassa* by Case and Giles.[21] They conclude that there is good over-all correspondence between the two maps although there are instances on their maps in which the placement of alleles is not identical. A similar situation exists for the *Td* locus, although here instances have been noted in which complementation can occur between two alleles and yet no genetic recombination between these alleles has been detected.[12] This may indicate that the complementation map is not necessarily a reflection of the genetic map.

Summary.—The complementation characteristics of 75 *Td* mutants of *Neurospora crassa* have been examined. These mutants were found to comprise five complementation groups.** These mutants may also be divided into five generally corresponding groups on the basis of previously determined functional characteristics, suggesting a relationship between functional alterations and ability to complement in a heterocaryon. It was also noted that the ability to form a CRM, a protein immunologically related to the enzyme, appears to be a prerequisite for complementation in the *Td* system.

The significance of these observations was discussed in terms of possible mechanisms of complementation and of the relationship between structure and function of the region controlling the formation of tryptophan synthetase in *N. crassa*.

* This work was presented by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Yale University. It was supported in part by a grant from the Atomic Energy Commission (At (30-1) 1017).

† Public Health Service Pre-doctoral Fellow of the National Cancer Institute (CF-5510 and CF-5510-C3). Present address: Department of Biological Sciences, Goucher College, Towson, Baltimore 4, Maryland.

‡ Present address: Biology Department, University of California, La Jolla, Calif.

** Catcheside and associates,[22] studying *Td* mutants isolated from a different wild type strain of Neurospora, have recently determined six complementation groups. One of these groups is represented by a mutant which can form tryptophan from serine and indole, a type of mutant not considered in this paper. The precise relationship of their mutants to those used in the present study is being investigated.

[1] Yanofsky, C., and D. M. Bonner, *Genetics*, **40**, 761 (1955).

[2] Lacy, A. M., Ph.D. Thesis, Yale University (1959).

[3] Suskind, S. R., C. Yanofsky, and D. M. Bonner, these PROCEEDINGS, **41**, 577 (1955).

[4] Woodward, D. O., C. W. H. Partridge, and N. H. Giles, these PROCEEDINGS, **44**, 1237 (1958).

[5] Yanofsky, C., and M. Rachmeler, *Biochem. et Biophys. Acta*, **28**, 640 (1958).

[6] Woodward, V. W., J. R. de Zeeuw, and A. M. Srb, these PROCEEDINGS, **40**, 192 (1954).

[7] deSerres, F. J., *Genetics*, **41**, 688 (1956).

[8] Vogel, H. J., *Microb. Gen. Bull.*, **13**, 42 (1956).

[9] Mitchell, M. B., T. H. Pittenger, and H. K. Mitchell, these PROCEEDINGS, **38**, 569 (1952).

[10] Suskind, S. R., *J. Bacteriol.*, **74**, 308 (1957).

[11] Yanofsky, C., in *Methods in Enzymology*, ed. S. P. Colowick and N. O. Kaplan, (New York: Academic Press Inc., 1955), p. 233.

[12] Suyama, Y., D. M. Bonner, and A. M. Lacy (in preparation).

[13] Lacy, A. M., and D. M. Bonner, *Proc. X Intern. Cong. Genetics*, **2**, 157 (1958).

[14] DeMoss, J. A., and D. M. Bonner, these PROCEEDINGS, **45**, 1405 (1959).

[15] Suskind, S. R., *Proc. X Intern. Cong. Genetics*, **2**, 284 (1958).

[16] Crawford, I. P., and C. Yanofsky, these PROCEEDINGS, **44**, 1161 (1958).

[17] Suskind, S. R., W. C. Mohler, C. Yanofsky, and J. A. DeMoss, personal communication.

[18] Singer, S. J., and H. A. Itano, these PROCEEDINGS, **45**, 174 (1959).

[19] Brenner, S., in *Biochemistry of Human Genetics*, ed. Wolstenholm and O'Conner (Boston: Little Brown & Co., 1959), p. 304.

[20] Fincham, J. R. S., *J. Gen. Microbiol.*, **21**, 600 (1959).

[21] Case, M., and N. H. Giles, these PROCEEDINGS, **46**, 659 (1960).

[22] Catcheside, D. G., *Symposium Soc. Gen. Microbiol.*, **10**, 181 (1960).