A GENETIC MAP OF THE *td* LOCUS OF NEUROSPORA CRASSA[1]

Y. SUYAMA, A. M. LACY[2] AND D. M. BONNER

Department of Biology, University of California, San Diego, La Jolla, California

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DURING the past decade, a large number of *td* mutants have been isolated. These mutants represent alterations of the *td* locus, which controls the formation of tryptophan synthetase (TSase) in *Neurospora crassa.* Extensive biochemical and immunological studies have shown that these allelic mutants differ from each other in their ability to form protein (CRM, "crossreacting material") serologically related to tryptophan synthetase, and in the catalytic abilities and cofactor requirements of the CRM formed (cf. BONNER, SUYAMA, and DEMOSS 1960). The many biochemical differences detected among allelic *td* mutants make it possible to study the relationship between the location of mutational sites and the biological effects exerted by mutations at these sites.

The present paper is concerned with a genetic fine structural analysis of the *td* locus. Detailed genetic maps of the locus are presented (Figures 1 and 2).

MATERIALS AND METHODS

Mutants: The origins and properties of the mutants used in the present study are presented in Table 1. All mutants (unless noted otherwise in Table 1) were obtained from wild type 74A or 74-OR-1-8a after ultraviolet irradiation.

Mutants which form CRM are designated as CRM+; those not forming any detectable CRM are designated as CRM-. CRM+ mutants are further characterized by the activity of their respective CRM in catalyzing each of the three reactions of TSase.[3] CRM's of the "a" type have little or no detectable activity in any one of the three reactions (1^-, 2^-, 3^-). CRM's of the "b" type can catalyze reaction (2), but not reactions (1) or (3) (1^-, 2^+, 3^-); mutants belonging to this class utilize indole for growth (IU). Among this class, *td*201 and *td*141 are primary mutants obtained from wild type 5256A and wild type 74A, respectively. All other IU mutants were obtained as secondary mutations following ultraviolet irradiation of CRM- mutants (SUYAMA, unpublished data). CRM's of the "c" type retain the ability to catalyze reaction (3), but are unable to catalyze reaction (1) or (2) (1^-, 2^-, 3^+); mutants in this category accumulate indole during growth (IA). Among this last class of mutants, three subdivisions have been established based on cofactor requirements in reaction (3) (DEMOSS and BONNER 1959; SUYAMA 1960): requirement for pyridoxal phosphate (B_6P), requirement for pyridoxal phosphate and serine (Ser), and requirement for neither (W).

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[2] Present address: Department of Biology, Goucher College, Baltimore, Maryland.

[3] Wild-type TSase catalyzes three reactions (YANOFSKY and RACHMELER 1958; DEMOSS and BONNER 1959):

(1) Indole glycerol phosphate (InGP) + L-serine → tryptophan + triose phosphate
(2) Indole + L-serine → tryptophan
(3) InGP ⇆ indole + triose phosphate

TABLE 1

Origin and properties of td *mutants*

Classification by CRM§	Reaction¶	Mutants
CRM⁻	1⁻, 2⁻, 3⁻	*td*1*, *td*6, *td*16, *td*35†, *td*36†, *td*37†, *td*38†, *td*40†, *td*42†, *td*45†, *td*46†, *td*47†, *td*48†, *td*120, *td*121, *td*123, *td*128, *td*129, *td*139, *td*140
CRM⁺	(a) 1⁻, 2⁻, 3⁻	*td*3, *td*7, *td*135, *td*136
	(b) 1⁻, 2⁺, 3⁻	*td*141, *td*201* (A78), *td*37R, *td*48R, *td*132R, *td*133R, *td*138R
	(c) 1⁻, 2⁻, 3⁺ (Ser)	*td*71†, *td*97
	1⁻, 2⁻, 3⁺ (B_6P)	*td*2‡, *td*99, *td*100
	1⁻, 2⁻, 3⁺ (W)	*td*96, *td*98, *td*101, *td*102, *td*103, *td*104

* Mutants *td*1 (LEIN, MITCHELL and HOULAHAN 1948) and *td*201 (A78) (AHMAD and CATCHESIDE 1960) were obtained from Emerson wild-type derivatives.
† Mutants were obtained by X-irradiation.
‡ *td*2 was obtained from wild type 1A, a derivative of a Lindegren wild-type by methylcholanthrene (R. BARRATT, personal communication to A. LACY).
References: *td*1–*td*71 (LACY 1959); *td*96 ~ *td*141 (SUYAMA 1960; SUYAMA and KAPLAN, unpublished); *td*201(A78) (RACHMELER and YANOFSKY 1961).
§ CRM⁻: In the present classification, the limit of detection of CRM in crude extract is 1/20 of the activity of the wild type (specific activity = 0.15 μmole/hr/mg protein).
¶ See text footnote 3 for classification by reaction.
(a) Mutants neither utilize or accumulate indole.
(b) Mutants utilize indole for growth.
(c) Mutants accumulate indole during growth on tryptophan. (b) and (c) mutants are further designated as IU and IA, respectively.

Recombination analysis: Conidia of one mating type were inoculated onto slants of crossing medium consisting of 2 percent cornmeal agar supplemented with 0.1 percent sucrose and 500 μg L-tryptophan/ml. After five days incubation at 25°C, 0.5 ml of a conidial suspension of the opposite mating type was added. The tubes were reincubated at 25°C for four weeks. At this time, the spores were harvested, added to 25 ml aliquots of molten minimal sorbose medium (sorbose, 1 percent and glucose, 0.05 percent) and shocked at 60°C for 35 minutes. Then 5 ml aliquots were poured over each of five petri dishes already layered with the same medium. The plates were incubated at 30°C and examined daily for the appearance of prototrophic colonies. All true-wild-type colonies appeared within the first three days.

The percent frequency of "true wild type" colonies formed among the total number of spores plated was used as the basis for determining map distances. The total number of spores plated was estimated by averaging the counts of several 1 cm² areas per plate and multiplying this figure by the number of total cm²/plate. In order to determine the frequency of true wild-type progeny, it was necessary to detect and eliminate pseudowild types (MITCHELL, PITTENGER and MITCHELL 1952; PITTENGER 1954) which are frequently found among the progeny of crosses between complementing *td* mutants. These colonies are presumably formed by nondisjunction during meiosis with subsequent heterokaryon formation. In many cases it was possible to identify pseudowild-type colonies solely by their characteristic morphology and later time of appearance on the plates (LACY 1959). From crosses between mutants whose true and pseudowild-type progeny were not as readily distinguishable, the proportion of prototrophs giving rise to mutant progeny when crossed to wild type was determined and such prototrophs were eliminated from the data used to determine map distances.

RESULTS

CRM⁺ mutants: Eleven mutants were crossed with each other and determinations of the prototroph frequencies from each cross were made (see Table 2). The complete map (Figure 1) was obtained by analyzing the crosses of other

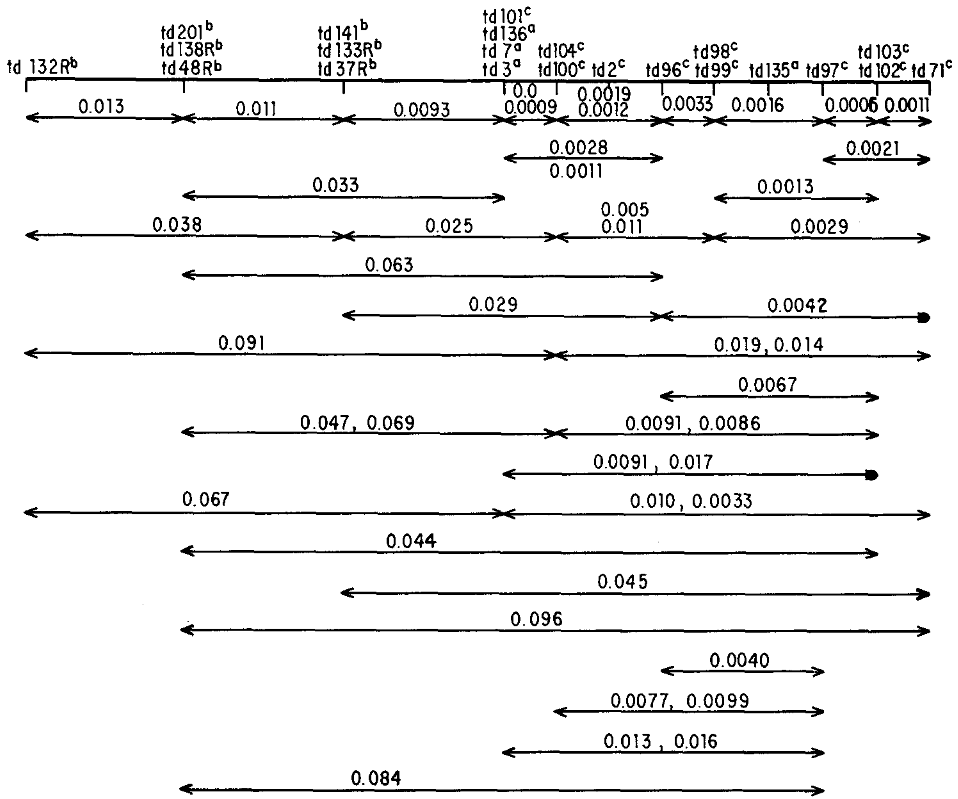

FIGURE 1.—Genetic map of CRM+ mutants at the *td* locus. Map distances (prototroph frequencies in percent) between mutant sites are represented by arrowed lines. (a) Mutants neither utilize nor accumulate indole (see Table 1 and text). (b) Mutants utilize indole for growth (IU). (c) Mutants accumulate indole (IA). (A schematic model of this map has been published elsewhere [BONNER, SUYAMA and DEMOSS 1960].)

mutants to the 11 mutants already mentioned (Table 3). Prototroph frequencies presented in Tables 2 and 3 serve only to determine proximity of mutational sites of these mutants on the map and they may not represent true physical distances or absolute relative position.

In the map, mutants which did not yield prototrophs from crosses by each other, are placed at the same mutational site. If the total number of spores analyzed for a given cross is less than 50,000, a map distance of 0.002 or less is unlikely to be detected. Because of this limit of resolution in the present recombination analysis, arbitrary decisions are necessary to position some mutants. For example, crosses of *td7* with either *td3* or *td100* yielded no prototrophic colonies. Since a larger number of spores was analyzed in the *td7* × *td3* cross than in the *td7* × *td100* cross, *td7* is placed at the same site as *td3* (Table 2).

Some mutants (e.g. *td2*, *td135* and *td132R*) recombined with all mutants thus far tested. These exceptional mutants were mapped between (or, in the case of

TABLE 2

Recombination data of 11 td *mutants (CRM$^+$)*

Crosses	Total no. spores	Number of prototrophs	Percent prototrophs	Number of experiments	Occurrence of pseudowild
*td*71 × *td*97	145,000	3	0.0021	2	..
*td*71 × *td*102	191,000	2	0.0011	2	..
*td*71 × *td*99	204,000	6	0.0029	2	..
*td*71 × *td*96	193,000	8	0.0042	2	..
*td*71 × *td*100	119,000	22	0.019	2	..
*td*71 × *td*101	116,000	16	0.014	2	..
*td*71 × *td*3	878,000	29	0.0033	6	..
*td*71 × *td*7	405,000	42	0.010	6	..
*td*71 × *td*48R	107,000	103	0.096	1	+
*td*71 × *td*37R	98,000	44	0.045	1	+
*td*97 × *td*102	177,000	1	0.0006	2	..
*td*97 × *td*99	310,000	5	0.0016	3	..
*td*97 × *td*96	372,000	15	0.0040	2	..
*td*97 × *td*100	313,000	31	0.0099	3	..
*td*97 × *td*101	222,000	17	0.0077	2	..
*td*97 × *td*3	195,000	26	0.013	2	..
*td*97 × *td*7	301,000	31	0.010	3	..
*td*97 × *td*48R	142,000	119	0.084	1	+
*td*102 × *td*99	239,000	3	0.0013	2	..
*td*102 × *td*96	178,000	12	0.0067	1	..
*td*102 × *td*100	197,000	18	0.0091	3	..
*td*102 × *td*101	327,000	28	0.0086	2	..
*td*102 × *td*3	320,000	29	0.0091	4	..
*td*102 × *td*7	377,000	64	0.017	4	..
*td*102 × *td*48R	16,000	7	0.044	1	..
*td*102 × *td*37R	82,000	33	0.040	1	..
*td*99 × *td*96	276,000	9	0.0033	2	..
*td*99 × *td*100	220,000	11	0.0050	2	..
*td*99 × *td*101	177,000	20	0.011	2	..
*td*99 × *td*3	114,000	11	0.0097	2	..
*td*96 × *td*100	162,000	3	0.0019	2	..
*td*96 × *td*101	116,000	2	0.0017	1	..
*td*96 × *td*3	72,000	2	0.0028	2	..
*td*96 × *td*7	92,000	1	0.0011	2	..
*td*100 × *td*101	63,000	0	0	2	..
*td*100 × *td*3	333,000	3	0.0009	4	+
*td*100 × *td*7	136,000	0	0	4	..
*td*100 × *td*37R	106,000	27	0.025	1	+
*td*101 × *td*3	141,000	0	0	2	..
*td*101 × *td*7	176,000	0	0	2	..
*td*3 × *td*7	398,000	0	0	4	..
*td*3 × *td*37R	139,000	13	0.0094	1	+
*td*7 × *td*48R	21,000	7	0.033	1	+
*td*48R × *td*48R	150,000	0	0	2	..
*td*37R × *td*37R	200,000	0	0	2	..
*td*3 × *td*3	75,000	0	0	1	..
*td*71 × *td*71	Infertile				
*td*102 × *td*102	121,000	0	0	1	..
*td*97 × *td*97	34,000	0	0	1	..
*td*100 × *td*100	103,000	0	0	1	..
*td*101 × *td*101	93,000	0	0	1	..

TABLE 3

Recombination data of 11 additional CRM⁺ mutants

Mutants crossed	Mutants in map order									
	*td*48R	*td*37R	*td*3	*td*7	*td*100	*td*96	*td*99	*td*97	*td*102	*td*71
*td*2	0.0053(p)	0.0037(p)	0.0009(p)	0.0009(p)	0.0007	0.0011	0.0014	0.0019	0.0026	0.0054
*td*98	....	....	0.011	0.032*	0.0060	....	0	0.0014	0.0014	0.0057
*td*103	....	....	....	....	0.0031	0.0036	0.0019	0.0036	0	0.0028*
*td*104	....	....	0*(p)	0*(p)	0	0.0018	0.0046	0.0041	0.0033	....
*td*135	0.092(p)	0.047	0.022*	0.023	0.032	0.014	0.0013	0.0030	0.0025	0.0022
*td*136	0.48*(p)	0.0083*	0	0*	0.0029*	0.0043	0.029	0.047*	0.033	....
*td*141	0.034*	0*	0.27*(p)	....	0.071*(p)	0.060*	....	0.068*	0.053*	0.053
*td*201	0	0.0013	0.017(p)	0.015(p)	0.016(p)	....	0.017(p)	0.026	....	0.024(p)
*td*132R	0.013	0.038	0.067(p)	0.091	....	....	....	....	....	....
*td*133R	0.011	0	0.010(p)	....	0.035(p)	....	....	....	0.065(p)	....
*td*138R	0	....	0.041(p)	....	0.056(p)	....	....	....	0.054(p)	....

Values are percent prototroph frequencies.
* Prototroph frequencies are based on less than 5×10^4 spores. Others without (*) are based on more than 5×10^4 spores.
(p) Occurrences of pseudowild-type colonies noted in this cross.

*td*132R, next to) the sites of those mutants with which they produced the fewest recombinants in crosses.

As seen in Table 3, the crosses *td*136 × *td*48R and *td*141 × *td*3 yielded 0.48 percent and 0.27 percent prototrophs, respectively. These values are very high as compared to the corresponding distances obtained from other crosses in the same intervals (see Figure 1). Since pseudowild types formed in these crosses were extremely small in colony size as compared to true wild type, it is unlikely that such higher frequencies are due to the formation of pseudowild types phenotypically indistinguishable from true wild type. Rather, reversion or some mechanism of selection of wild types during mating may account for such high prototroph frequencies. At present, no critical study has been made to clarify this point.

From the map (Figure 1), it can be seen that functionally similar mutations are clustered within the locus. For example, the seven indole utilizing mutants are clustered at the left side of the map, while the 11 indole accumulating mutants are clustered at the right side of the map, and those mutants forming CRM with little or no enzymatic activity (*td*3, *td*7, and *td*136) are clustered between the IA and IU regions. However, one mutant (*td*135) which forms inactive CRM, falls in the middle of the IA region.

It is also of interest that the (Ser)-type mutants (*td*97, *td*71) are separated from those designated as (B_6P)-type (*td*2, *td*100, *td*99) by the *td*135 site, while those of the (W)-type are distributed throughout the IA region.

CRM⁻ mutants: The recombination values obtained from crosses involving CRM⁻ mutants are presented in Table 4. As seen in the table, many of the CRM⁻ mutants exhibited aberrant recombination behavior. For example, no prototrophic progeny were produced from crosses of *td*45, *td*120, *td*121 or *td*134 with a series of mutants located linearly along a considerable length of the *td* locus; also, no prototrophic progeny were produced from crosses of *td*16, *td*38, *td*121 or *td*140 with several nonneighboring mutants, although prototrophs were produced from crosses with mutants located at intervening sites.

These general recombination aberrancies might be expected if the mutants arose from deletion or multisite mutation. In order to test these possibilities, they were irradiated with ultraviolet light (Table 4). No reversions have been obtained from *td*35, *td*38, *td*42 and *td*120. However, the other CRM⁻ mutants can be induced to mutate to prototrophy, indicating that deletion or multiple mutation of genetic material cannot account for the aberrancy of the observed recombination values. It has been noted (Table 4) that crosses involving CRM⁻ mutants were relatively infertile in terms of spore production. Therefore, the consequent reduction in resolving power may have contributed to the apparent aberrancy of their recombination behavior.

The map of CRM⁻ mutants (Figure 2) was prepared in the same manner as the map of CRM⁺ mutants. CRM⁻ mutants which did not recombine with mutants located along an extended region of the map, are represented by extended bars, although their ability to revert to prototrophy (except *td*120) would indicate that they do not represent true deletions. Some CRM⁻ mutants (*td*46, *td*123,

TABLE 4

Recombination data of 20 CRM⁻ mutants

Reversion†	CRM⁻ mutants	CRM⁺ mutants									
		*td*201 or *td*48R	*td*37R	*td*3	*td*7	*td*100	*td*96	*td*99	*td*97	*td*102	*td*71
+	*td*1	0.0035	0.007	0	0	0	....	0.0026	0.0045	0.0050	0.0011
+	*td*6	0.087	0.015	0	0	0	0.0027	0.016	0.017	0.015	0.012
+	*td*16	0.021	....	....	0	0.0021*	....	0*	0*	0.0052*	0.0038*
—	*td*35	0.0048	....	....	....	0.0019	....	0.0020	0*	0.0031	0.0037*
+	*td*36	0.015	....	....	....	0.029*	....	0.028*	0*	0*	0.020
+	*td*37	0.0017	0	....	....	0.0030	....	0.017	0.013	0.0090*	0.029
—	*td*38	0	....	....	....	0.0090*	....	0.033*	0*	0.011*	0.010*
+	*td*40	0.032	....	....	....	0.031	....	0	0.0029	0.0018	....
—	*td*42	0.015	....	....	....	0.019*	....	0.027	0*	0*	0.0040
+	*td*45	0*	....	....	....	0*	....	0*	0*	0*	0*
+	*td*46	0.023	....	....	....	0.018	....	0.014*	0.015	0.0064	0.056*
+	*td*48	0	....	....	....	0.0090*	....	0.012	0.017	0.023	0.020*
—	*td*120	0.013(p)‡	0*	0*	0	0	0	0	0	0	0
+	*td*121	0*	0*	0*	0*	0*	0.019*	0.026*	0.028*	0.029	0*
+	*td*123	0.11*	0.10*	0.043	....	0.057*	....	....	....	0.082*	....
+	*td*128	0.11	....	0.069	0.051	0.070	0.096	....	....	0.10	....
+	*td*129	....	....	0*	0*	0.0054*	....	....	....	0.030	....
+	*td*134	0.010*(p)‡	....	0.028*	0*	0	0	0.019*	0.015*	0.022*	0.028
+	*td*139	0.21	0.15(p)	0.042	....	0.11	0.043	0.017	0.041	....	0.040
+	*td*140	0.13(p)‡	0.089*	0	0.022*	0.033	0.014	0.0022	0.0028*	....	0*

Values are percent prototroph frequencies.
* Recombination frequencies are based on less than 5×10^4 spores.
† Ultraviolet induced reversion in 2×10^7 viable conidia; + indicates reversion, — no reversion.
(p) Occurrence of pseudowilds noted.
(p)‡ Pseudowilds were found in crosses with *td*48R but not with *td201*.

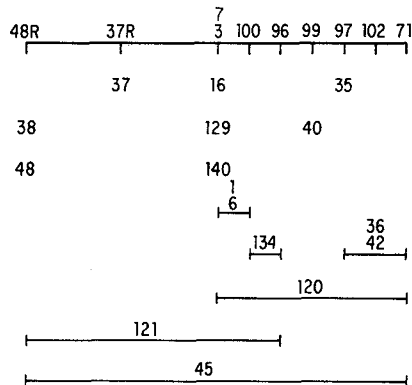

FIGURE 2.—Genetic map of CRM⁻ mutants. Numbers below the uppermost line represent possible mutational sites of CRM⁻ mutants in the genetic map of the *td* locus (see Figure 1). Mutants which did not recombine with more than one site, are represented by extended lines below each mutant number (see text).

*td*128 and *td*139) have not been included in the map, since their map positions are not clearly deducible from the available recombination data.

As seen from the map, it appears that CRM⁻ mutants are not truly clustered but are scattered throughout the locus. However, some concentration of CRM⁻ mutants can be noted in the *td*3–*td*7 region.

It is interesting to note (Table 4) that some CRM⁻ mutants are capable of forming pseudowild progeny in crosses and thus, presumably, of complementing each other.

DISCUSSION

The present recombination analysis of 42 *td* mutants has demonstrated the genetic divisibility of the *td* locus. Furthermore, it has been shown that these mutational sites can be arranged in a linear manner. Since the present analysis is based on two-point crosses only, proof of the one dimensional linearity of the map must await further studies.

The most interesting fact emerging from the present study is that mutants which form catalytically similar CRM are clustered on the map, while CRM⁻ mutants are rather scattered throughout the locus. These results have been discussed previously with reference to enzyme structure and function (BONNER *et al.* 1960).

It is well known that tryptophan synthetase from *E. coli* is easily separable into two components, proteins A and B (YANOFSKY 1960; CRAWFORD and YANOFSKY 1958), whereas all the available evidence points to the indivisibility of the Neurospora enzyme (SUSKIND, LIGON and CARSIOTIS 1962; ENSIGN personal communication). In view of this difference between TSase from these two organisms, it may be of interest to compare their *td* regions. The IU region and IA region in Neurospora correspond to the A and B cistrons, respectively, in *E. coli*. From the present map of the Neurospora *td* locus, it is estimated that the IU region is at

least two to three times as large as the IA region. On the other hand, in *E. coli*, as judged from the map distances of the two regions (YANOFSKY and CRAWFORD 1958; YANOFSKY 1960) and from the sizes of the corresponding proteins the A cistron appears to be smaller than the B cistron. (Estimated molecular weights of the A and B proteins of *E. coli* are 30,000 and 100,000, respectively [HATANAKA, WHITE, HORIBATA, and CRAWFORD 1962].)

In Neurospora, between the IA and IU regions, there is a "profound" region within which are located most of the CRM+ *td* mutants possessing little or no enzymatic activity. It is not yet possible to estimate the size of this region, since the three profound mutants mapped(*td*3, *td*7 and *td*136), have not been shown to be separable from each other or from some IA mutants. In *E. coli* there does not seem to be any genetically "profound region," nor are the two proteins of *E. coli* TSase connected by peptide bonding (YANOFSKY 1960). It may be suggested that the "profound region" controls the formation of a peptide necessary for the structural integrity of the Neurospora enzyme.

While the apparent clustering observed presents an attractive picture, it is not the only conceivable one. The *folding* of the tryptophan synthetase molecule could result in different portions of the polypeptide chain participating in identical functions, and thus, clustering of mutational sites controlling one function of the molecule would not be expected. It may be noted from the map (Figure 1), that one mutant (*td*135) which forms enzymatically inactive CRM falls in the middle of the IA region, although other CRM+ mutants of this type are located in the profound region. This indicates a second region in the locus in which a point mutation can lead to loss of all enzymatic activity. A detailed study of the CRM of this mutant is clearly of great interest.

As shown in the results (Table 4), some CRM- mutants behave abnormally in recombination. Reversion studies, however, show that the majority of CRM- mutants are unlikely to be the result of deletions. Further recombination analysis with much higher resolving power than obtained in the present study provides the necessary data for elucidating the nature of CRM- mutations (KAPLAN, SUYAMA and BONNER 1963).

SUMMARY

Two-point recombination data of 42 tryptophan synthetase mutants have been used to construct a genetic map of the *td* locus. Twenty-two mutants formed protein (CRM) serologically related to tryptophan synthetase; 20 were CRM negative. CRM positive mutants altered in similar functions appear to be clustered on the map. CRM negative mutants do not appear to be restricted to one region of the map.

LITERATURE CITED

AHMAD, M. and D. G. CATCHESIDE, 1960 Physiological diversity amongst tryptophan mutants in *Neurospora crassa*. Heredity **15**: 55–64.

BONNER, D. M., Y. SUYAMA, and J. A. DEMOSS, 1960 Genetic fine structure and enzyme formation. Federation Proc. **19**: 926–930.

CRAWFORD, I. P., and C. YANOFSKY, 1958 On the separation of the tryptophan synthetase of *Escherichia coli* into two protein components. Proc. Natl. Acad. Sci. U.S. **44**: 1161–1170.

DEMOSS, J. A., and D. M. BONNER, 1959 Studies on normal and genetically altered tryptophan synthetase from *Neurospora crassa.* Proc. Natl. Acad. Sci. U.S. **45**: 1405–1412.

HATANAKA, M., E. A. WHITE, K. HORIBATA, and I. P. CRAWFORD, 1962 A study of the catalytic properties of *Escherichia coli* tryptophan synthetase, a two-compound enzyme. Arch. Biochem. Biophys. **97**: 596–606.

KAPLAN, S., Y. SUYAMA, and D. M. BONNER, 1963 A genetic analysis of CRM negative mutants at the *td* locus of *Neurospora crassa.* Genet. Res. (in press).

LACY, A. M., 1959 A genetic analysis of the *td* locus in *Neurospora crassa.* Ph.D. thesis. Yale University, New Haven, Connecticut.

LEIN, J., H. K. MITCHELL, and M. B. HOULAHAN, 1948 A method for selection of biochemical mutants of Neurospora. Proc. Natl. Acad. Sci. U. S. **34**: 435–442.

MITCHELL, M. B., T. H. PITTENGER, and H. K. MITCHELL, 1952 Pseudowild types in *Neurospora crassa.* Proc. Natl. Acad. Sci. U. S. **38**: 569–580.

PITTENGER, T. H., 1954 The general incidence of pseudowild types in *Neurospora crassa.* Genetics **39**: 326–342.

RACHMELER, M., and C. YANOFSKY, 1961 Biochemical, immunological, and genetic studies with a new type of tryptophan synthetase mutant of *Neurospora crassa.* J. Bacteriol. **81**: 955–963.

SUSKIND, S., D. LIGON, and M. CARSIOTIS, 1962 Mutationally altered tryptophan synthetase in *Neurospora crassa.* pp. 307–321. *The Molecular Basis of Neoplasia.* 15th Annual Symposium on Fundamental Cancer Research, The University of Texas, M. D. Anderson Hospital and Tumor Institute. University of Texas Press, Austin.

SUYAMA, Y., 1960 Effects of pyridoxal phosphate and serine in conversion of indoleglycerolphosphate to indole by extracts from tryptophan mutants of *Neurospora crassa.* Biochem. Biophys. Res. Commun. **3**: 493–496.

YANOFSKY, C., and M. RACHMELER, 1958 The exclusion of free indole as an intermediate in the biosynthesis of tryptophan in *Neurospora crassa.* Biochim. Biophys. Acta **28**: 640–641.

YANOFSKY, C., and I. P. CRAWFORD, 1959 The effects of deletions, point mutations, reversions and suppressor mutations on the two components of the tryptophan synthetase of *Escherichia coli.* Proc. Natl. Acad. Sci. U. S. **45**: 1016–1026.

YANOFSKY, C., 1960 The tryptophan synthetase system. Bacteriol. Rev. **24**: 221–245.