STRUCTURAL AND FUNCTIONAL COMPLEXITY AT THE TRYPTOPHAN-1 LOCUS IN NEUROSPORA CRASSA

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THE tryptophan-1 (*tryp-1*) locus is located in linkage group III in *Neurospora crassa*. TATUM and BONNER (1944) and BEADLE and TATUM (1945) reported *tryp-1* mutant 10575, which could utilise either indole or tryptophan. AHMAD and CATCHESIDE (1960) brought forward evidence that a new group of five anthranilic acid mutants were allelic to 10575. The present paper describes complementation studies on 21 ultraviolet induced *tryp-1* mutants and 10575. It also includes studies on the relative positions of the five anthranilic acid mutants with respect to one another and to 10575.

MATERIALS AND METHODS

With the exception of A92, the mutants employed in this study were described in an earlier paper (AHMAD and CATCHESIDE 1960). They were originally classified into three subgroups, A, B, and C. Of the 25 mutants reported for this locus in 1960, five, A49, A51, A52, A62 and A110, have not been included in the present studies as they appear to be contaminated. The remaining 20 mutants which include the five anthranilic acid mutants (A10, A65, A67, A82, and A106) and the 15 indole mutants (A9, A20, A28, A38, A48, A50, A56, A63, A93, A94, A105, A107, A117, A121, and A123) have been studied further for complementation, along with the indole mutant A92 and the *tryp-1* mutant 10575. A92 was isolated along with the other mutants but was somehow left out from the previous studies. A heterocaryon compatible isolate of 10575 was used in these experiments. It was obtained by outcrossing 10575 thrice with the wild type.

The anthranilic acid mutants utilise anthranilic acid, indole, or tryptophan while the indole mutants fail to grow on anthranilic acid but grow on indole or tryptophan. That the 16 new indole mutants belonged to *tryp-1* locus was first inferred from their complementation behaviour. Recombinational tests undertaken later for nine of them (A28, A38, A63, A93, A105, A107, A117, A121 and A123) have supported the above inference. *leu-1* (33757), which was kindly sent to us by PROFESSOR D. G. CATCHESIDE, was used as a marker.

Heterocaryon tests were made in tubes on freshly prepared Vogel's minimal medium (VOGEL 1956) solidified with agar. The medium had normal amount of sucrose but no sorbose. Conidia from four to six day old cultures were used in these tests. Observations were taken up to 21 days. Crosses were made in 15 $\times$ 1.8 cm test tubes containing Westergaard's medium (WESTERGAARD and MITCHELL 1947) in which corrugated filter paper was embedded. The medium was supplemented with 30 mg tryptophan and 20 mg leucine per 100 ml. Where crosses failed on WESTERGAARD's medium, SUYAMA's crossing medium was utilised in which sucrose was reduced to 0.2 gm and tryptophan was increased to 50 mg per 100 ml of the medium (SUYAMA, WOODWARD, and SARACHEK 1958).

Spores from three-point crosses, where leucine-1 was used as a marker, were plated on minimal medium supplemented with 20 mg leucine per 100 ml. Plates were first kept at 58°C for 50 minutes and then incubated at 25°C for about 12 hours before a count of the spores was made.

For estimation of growth of heterocaryons in liquid medium, 100 ml flasks containing 25 ml of medium were inoculated with conidia to study the relative growth of heterocaryons and of the parental wild-type stock. All inoculations were done in duplicate. After inoculation the flasks were rotated and shaken so that the conidia got well mixed and evenly distributed. The flasks were incubated at 25°C for 72 hours and then boiled to kill the mycelium. The mycelium was next filtered off and dried. The dried mycelia were weighed and the average weight of each category was calculated and tabulated.

The time of initiation of heterocaryotic growth and the linear growth rates of heterocaryons were determined by inoculating conidia from cultures under study in 30 cm long growth tubes with an internal diameter of 1.3 cm (RYAN, BEADLE, and TATUM 1943). These tubes contained minimal medium solidified with 1.5 percent agar. All inoculations were done in duplicate and the tubes were incubated at 25°C.

EXPERIMENTS AND RESULTS

The five anthranilic acid and 17 indole mutants apparently belonging to the *tryp-1* locus were tested for complementation in all possible pairwise combinations. The data have been presented in the form of a two dimensional matrix in Figure 1. There are a number of variations both in the time of initiation of heterocaryons and in the degree of growth of the heterocaryons formed by different pairs of mutants and they will be reported elsewhere. Reactions have been recorded only as positive or negative.

All the 22 mutants complemented at least one other *tryp-1* mutant, and the mutants fell into nine groups (Figure 1). Mutants within a group failed to form heterocaryons amongst themselves. Of the nine groups, one (Group VII) was formed by the five anthranilic mutants, while the remaining eight groups were formed by the indole utilising mutants.

A one-dimensional complementation map was made from the above data (Figure 2) assuming that mutants having common defects do not complement. This showed that the locus *tryp-1* comprises at least five functionally distinct subunits (A, B, C, D, and E) arranged in a linear manner. Mutants falling in Groups V, VI, VII, and IX were each defective in single subunits, while mutants

	I	II	III	IV	V	VI	VII	VIII	IX
A948,56,93,94, 105,121 I	−	−	−	−	−	−	−	−	+
10575 II	−	−	−	−	−	−	+	−	−
A38,63 III	−	−	−	−	−	−	+	−	+
A107,117 IV	−	−	−	−	−	−	+	+	+
A28,123 V	−	−	−	−	+	+	+	+	+
A50 VI	−	−	−	−	+	+	+	+	+
A10,65,67,82,106 VII	−	−	−	−	+	+	+	+	+
A20 VIII	−	−	−	−	+	+	+	−	−
A92 IX	−	−	−	−	+	+	+	−	−

FIGURE 1.—Complementation matrix of 22 *tryp-1* mutants. Heterocaryon positive, +; heterocaryon negative, —. Mutants falling under each group are given on the left.

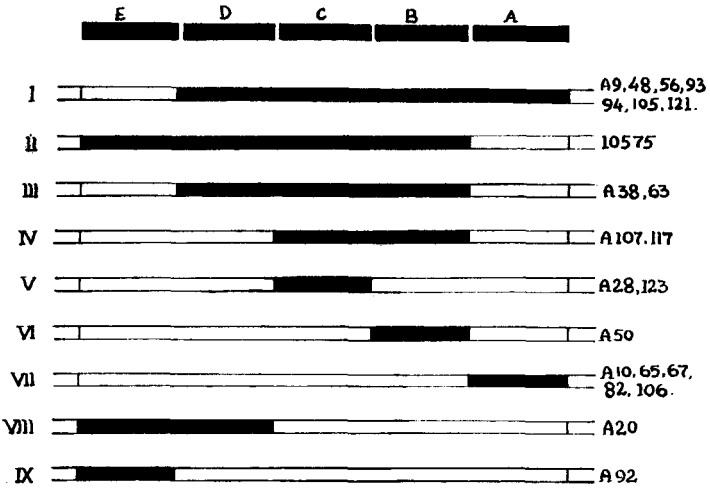

FIGURE 2.—Complementation map of the *tryp-1* locus. Short bars A, B, C, D, and E at the top of the figure indicate five physiological subunits of the locus. Functionally defective regions in each group of mutants have been represented by solid bars. Mutants falling under each group are given on the right side of the figure.

falling in Groups I, II, III, and VIII, were defective in two to four subunits. None of these multiple defects were however discontinuous.

Location of the five anthranilic acid mutants with respect to leu-1 *and 10575 in linkage group III:* The five anthranilic mutants and 10575 were crossed with *leu-1*. At least 100 spores from each cross were isolated and classified. As shown in Table 1, all five anthranilic acid mutants were found to be linked to *leu-1* and were located distal to 10575. Next, the order and distances of the five anthranilic acid mutants and 10575 were determined more precisely by three-point linkage tests using *leu-1* as a marker. The data from these crosses are pre-

TABLE 1

Linkage values of the five anthranilic acid mutants and 10575 with respect to leu-1

tryp-1 allele × *leu-1*	Number of spores isolated	Classification of spores				Linkage value (centimorgans)
		Leucine-mutants	Anthranilic mutants	Double mutant	Wild type	
A82 × *leu-1*	110	49	47	6	8	12.7
A10 × *leu-1*	102	48	41	4	9	12.8
A106 × *leu-1*	107	56	37	7	7	13.1
A65 × *leu-1*	106	50	42	9	5	13.2
A67 × *leu-1*	105	60	29	4	12	15.2
		Indole mutants				
10575 × *leu-1*	105	60	33	5	7	11.4

TABLE 2

Order and distances of the five anthranilic acid mutants from one another and of 10575 from A65

Cross	Number of ascospores: germinated	growing	*tryp*$^+$ *leu-1*$^+$	*tryp*$^+$ *leu-1*$^-$	Order and map distance	Mean map distance (centimorgans)
leu-1 A106 × A10	61,932	59	12	47	A10 .191 A106	.198
leu-1 A10 × A106	65,289	67	36	31	A10 .205 A106	
leu-1 A67 × A65	24,769	7	1	6	A65 .057 A67	.064
leu-1 A65 × A67	72,110	24	18	6	A65 .066 A67	
leu-1 A65 × A10	92,269	76	50	26	A65 .165 A10	.175
leu-1 A10 × A65	30,053	31	6	25	A65 .266 A10	
leu-1 A10 × A67	138,465	29	13	16	A67 .042 A10	.043
leu-1 A67 × A10	56,231	13	4	9	A10 .046 A67	
leu-1 A82 × A67	50,814	0	0	0		.002
leu-1 A67 × A82	73,034	1	1	0	A67 .003 A82	
leu-1 A82 × A65	28,720	0	0	0		.012
leu-1 A65 × A82	56,060	5	3	2	A65 .078 A82	
leu-1 A65 × A106	49,251	37	23	14	A65 .150 A106	
leu-1 A82 × A10	49,213	15	10	5	A82 .061 A10	
leu-1 10575 × A65	10,502	35	24	11	10575 .667 A65	

sented in Table 2 and Figure 3. Where data were available from reciprocal crosses, the map distances were obtained from weighted means.

Detailed studies on the heterocaryons formed by 10575 with the five anthranilic mutants: Estimates of degree of complementation between 10575 and the five anthranilic acid mutants were obtained in two ways. (a) *Growth of heterocaryons in liquid minimal medium:* Conidia from 10575 and one of the anthranilic acid mutants were inoculated in duplicate. Conidia from the wild-type stock were also inoculated in duplicate. Weights of 72-hour old mycelia are shown in

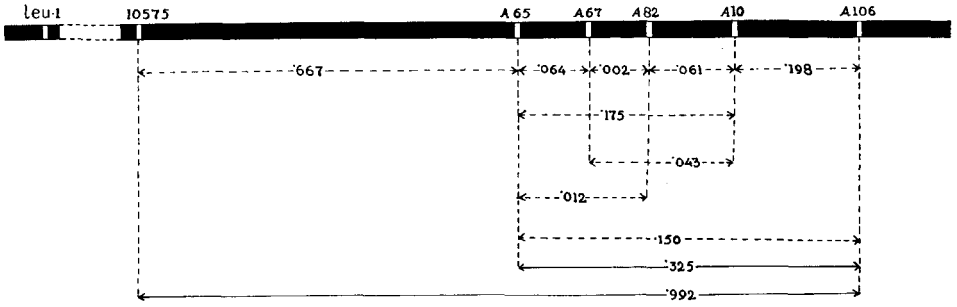

FIGURE 3.—Genetic map of *tryp-1* locus. The distances obtained directly from the experimental data have been indicated by dotted lines. The distances obtained by adding individual linear distances between the mutants have been indicated by continuous lines.

Table 3. No heterocaryon attained a weight more than one fourth of the wild type (Table 3), and weights of heterocaryons of 10575 with A10 and A106 were lower than the weights of heterocaryons of 10575 with A65, A82, and A67. (b) *Time of initiation of heterocaryotic growth, and growth rates of the heterocaryons and the parental wild-type strain:* These were investigated by inoculating in growth tubes (RYAN, BEADLE and TATUM 1943) conidia from 10575, wild type, A65 and 10575, A67 and 10575, A82 and 10575, A10 and 10575, and A106 and 10575. Readings were taken at an interval of 2 hours durings the first 12 hours, at 4 hr intervals from 12 to 40 hours, and 8 hr intervals from 40 to 220 hours.

There was no initiation of growth up to 2 hours in either wild type Em*a* or any of the heterocaryons. After 4 hours, while growth was initiated in wild type and in heterocaryons of A65, A67, and A82 with 10575, no initiation of growth was seen in the heterocaryons of A106 and A10 with 10575. After 6 hours, the A106 plus 10575 heterocaryon showed initiation of growth in both the growth tubes, but the A10 plus 10575 heterocaryon showed the initiation of growth only in one tube. After 8 hours, however, growth was initiated in the second growth tube of A10 plus 10575.

Strain 10575 alone did not show any growth on minimal medium in the growth tubes. The growth of mycelium of wild type and of the other five heterocaryons was measured 16 hours after inoculation. Up to 20 hours, the mycelial fronts of wild type and of A65 plus 10575, A67 plus 10575, and A82

TABLE 3

*Dry weights (mg) of 72-hour old heterocaryons and wild-type Em*a

	Heterocaryons					
	A65+10575	A67+10575	A82+10575	A10+10575	A106+10575	Wild type Em*a*
	5.0	4.6	5.0	3.7	2.3	24.8
	5.2	4.1	4.9	3.2	(contaminated)	24.45
Average	5.1	4.4	5.0	3.5	2.3	24.6

TABLE 4

Growth rates of five heterocaryons and parental wild-type culture in millimeters per hour on the basis of growth during the first 96 hours

Heterocaryons					Parental
A65+10575	A67+10575	A82+10575	A10+10575	A106+10575	wild type Em*a*
1.3	1.2	1.3	0.8	1.2	2.7

plus 10575 heterocaryons showed more or less similar growth. Heterocaryons of A106 plus 10575 and A10 plus 10575 showed considerably less growth than the mycelia from Em*a* and the other three heterocaryons. After 24 hours, wild type took a lead over all five heterocaryons and covered the entire 30 cm length of the growth tube in 104 hours.

The data obtained from this experiment showed that the growth rates in all cases were linear. When the growth rates of the wild type and the five heterocaryons were calculated for the first 96 hours (Table 4), it was observed that none of the heterocaryons attained even half the growth rate of the parental wild type. It was further seen that heterocaryons of A65, A67, A82, and A106 with 10575 had nearly the same growth rate. The growth rate of the heterocaryon of A10 with 10575 was significantly lower.

DISCUSSION

Except for the two mutants, A92 and 10575, all the other mutants reported in this paper were studied for complementation by AHMAD and CATCHESIDE (1960) and were found to fall into three groups. The present studies have revealed that 22 mutants belonging to the *tryp-1* locus fall into nine groups. The previous Group B has now been divided into five groups, and A107 and A123 have shown their capacity to complement. These differences are due to the inclusion of two additional mutants A92 and 10575, the use of fresh minimal medium without sorbose, and the use of cultures four to six days old.

An interesting point which has emerged from these studies is the lack of a noncomplementing class of mutants at this locus. This finding differs from the investigations of mutants for the other loci where noncomplementing mutants form the largest group (e.g. *ad-4*, WOODWARD, PARTRIDGE, and GILES 1958; *tryp-3*, LACY and BONNER 1961, AHMAD and CATCHESIDE 1960; *pan-2*, CASE and GILES 1960; *arg-1*, *arg-10*, *orn-2 (arg-6)*, CATCHESIDE and OVERTON 1958; *his-1*, *his-2*, *his-3*, *his-5*, CATCHESIDE 1960; *me-2*, MURRAY 1960). DE SERRES (1956) came across a similar situation during his studies of the mutants for the *ad-3* locus, but he subsequently found them to fall into two loci, *ad-3A* and *ad-3B*. The subdivision of the *tryp-1* locus into two independent units does not seem likely for the present, as is evident from the complementation map presented in Figure 2. Besides *tryp-1*, the only locus which has been reported to lack a noncomplementing class of mutants is *pyr-3* (WOODWARD 1962).

The complementation map for this locus is linear whereas the complementa-

tion maps for *tryp-3* (M. AHMAD, M. KHALIL, A. AHMAD, and M. ISLAM, unpublished observations), and *ad-8* (KAPULER and BERNSTEIN 1963) show discontinuous defects in the case of a few mutants. These differences in the complementation maps for *tryp-1* as compared to *tryp-3* and *ad-8* may follow from differences in the folding of the respective enzyme molecules, the enzyme specified by *tryp-3* and *ad-8* being possibly folded in a more complex manner than the enzyme synthesised by *tryp-1*.

The complementation map shows that the locus is divisible into five subunits or complons. According to the current concepts of the biosynthesis of tryptophan (BONNER, SUYAMA, and DEMOSS 1960; SMITH and YANOFSKY 1960; RIVERA and SRINIVASAN 1962), shikimic-5-phosphate is metabolised to indoleglycerol phosphate (IGP) in five steps. This suggests that there may be some relationship between complons and active sites of an enzyme.

Of the five complons A, B, C, D, and E, single complon mutants have been found for complons A, B, C, and E. No mutant has been found for complon D.

Linkage studies using *leu-1* as a marker have shown that the *tryp-1* locus occupies a length of about 0.992 centimorgan; of this the anthranilic acid utilising segment covers about 0.325 and is situated on the right of the indole utilising mutant 10575. Hence with regard to the position of the anthranilic acid synthesising segment, the complementation and the genetic maps seem to be colinear. Distance between mutants showing a physiological similarity has been found to be much less than the distance between mutants showing physiological dissimilarity. Thus the maximum distance between any two adjacent anthranilic mutants comes to 0.198 while the distance between the indole utilising mutant 10575 and the nearest anthranilic acid utilising mutant A65 is 0.667.

Considering the map length occupied by some other loci in *Neurospora crassa* it is seen that pyrimidine-3 comprises 0.2 map units (WOODWARD 1962), pantothenic-2 comprises about 0.338 map units (CASE and GILES 1960) and three isoleucine-valine *(iv)* loci together occupy a segment of not more than four map units (WAGNER, SOMERS, and BERGQUIST 1960). The length occupied by *tryp-1* therefore compares favourably with that reported for the *iv* loci.

In their studies on some *ad-4* mutants, WOODWARD, PARTRIDGE, and GILES (1958) found that the vigour of growth of a heterocaryon between two mutants was positively correlated to the distance between the two allelic mutants on the complementation map. Complementation studies on mutants for the *pan-2* locus (CASE and GILES 1960) and the loci *iv-2* and *iv-3* (BERNSTEIN and MILLER 1961) in Neurospora are similar. No correlation between the vigour of the heterocaryon formed by two mutants and the distance between them was noted in the present investigation. Thus heterocaryons of A65, A67, and A82 with 10575 gave more or less similar growth rates. Moreover, the growth attained by the heterocaryons of these three mutants was in excess of the growth attained by the heterocaryons of A10 within 72 to 96 hours, although A10 is located farther away from 10575 than A65, A67 or A82. It has therefore to be concluded that the degree of restoration of the functional activity of the enzyme

elaborated by a heterocaryon depends not only on the precise location of the two mutant sites with respect to one another but also on the nature of the genetic change in the two mutants. These results are in keeping with the findings of MURRAY (1960) in the case of methionine-2 locus in Neurospora and those of CARLSON (1959) on the dumpy locus in Drosophila.

Lastly one can consider whether the portions of the linkage group occupied by the five anthranilic acid mutants and the 17 indole mutants are to be regarded as two separate loci or a single locus. BEADLE and COONRADT (1944) formulated the hypothesis that mutants which formed a heterocaryon should be regarded as occupying separate loci, while two mutants which did not form a heterocaryon should be regarded as belonging to the same locus. Since the five anthranilic acid mutants are heterocaryon negative with 11 indole mutants, the two sets of mutants seem to form parts of the same locus. This conclusion is supported by the fact that even when heterocaryons are formed between the two sets of mutants, the growth rates of heterocaryons are much lower than the growth rate of the parental wild type. This shows that the two sets of sites are not able to produce normal enzyme separately when one is defective. It argues against the anthranilic acid utilising mutants and indole utilising mutants of linkage group III occupying two separate loci. It also does not seem that the locus *tryp-1* controls a common precursor, for mutations at the locus can impair the capacity of either the synthesis of anthranilic acid or the synthesis of a metabolic product higher up in the metabolic chain leaving the synthesis of anthranilic acid intact. Thus AHMAD and CATCHESIDE (1960) found that while the anthranilic acid mutant A10 did not accumulate any anthranilic acid, the indole utilising mutants A20 and 10575 both formed anthranilic acid and accumulated it.

SUMMARY

The *tryp-1* locus in *Neurospora crassa* has been found to have two classes of mutants, one utilising anthranilic acid and the other utilising indole. All the five anthranilic acid mutants are situated to the right-hand side of the indole mutant 10575. Complementation studies have shown the lack of a noncomplementing class of mutants. The mutants for the locus fall into nine complementing groups, while the remaining eight groups are formed by the 17 indole utilising mutants. Complemention between mutants is incomplete, growth on minimal being less than one fourth the normal wild-type rate. The vigour of heterocaryons has not been found to be related to the map distance between the two mutants.

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