GENETIC CONTROL OF THE STRUCTURE AND ACTIVITY OF AN ENZYME AGGREGATE IN THE TRYPTOPHAN PATHWAY OF *NEUROSPORA CRASSA*[1]

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THE reactions specifically involved in tryptophan biosynthesis are under the control of four genes in *Neurospora crassa* (AHMAD and CATCHESIDE 1960). The complexity of the gene-enzyme relationships in this pathway was discovered by AHMAD and CATCHESIDE (1960) and AHMAD, KHALIL, KHAN and MOZMADAR (1964) who demonstrated that lesions at one of these genes, *tryp-1*, could prevent either the accumulation or the utilization of anthranilate, an intermediate in tryptophan biosynthesis. We have recently shown that this functional complexity has its basis in the fact that three of the reactions of tryptophan biosynthesis are catalyzed by an enzyme aggregate which is controlled genetically by two unlinked genes, *tryp-1* and *tryp-2* (DEMOSS and WEGMAN 1965). The three activities, the anthranilate synthetase, PRA isomerase, and InGP synthetase reactions, are associated with a single component in extracts of Neurospora. Purification procedures as well as several physical methods have failed to separate the apparent enzyme aggregate into subunits which are either enzymatically active or capable of reforming the active aggregate.

To define the role of the two genetic loci in controlling the structure and activity of the enzyme aggregate, we have carried out a detailed biochemical and genetic analysis of a series of *tryp-1* and *tryp-2* mutants. These studies permit a tentative assignment of the genetic control of various functions of the aggregate to specific regions of the *tryp-1* and *tryp-2* genes.

METHODS AND MATERIALS

Strains 74A, 74-OR-1-8a, 10575 (*tryp-1*, FGSC #558), 75001 (*tryp-2*, FGSC #511), and td 48R (*tryp-3*, FGSC #1002) were obtained from the stock culture collection of the late DR. DAVID BONNER. All the other mutants used in this study were selected from a collection of tryptophan auxotrophs derived from wild-type strains 74A or 74-OR-1-8a after treatment with X rays, ultraviolet radiation (UV) or nitrous acid in our laboratory and in the laboratory of DR. ANN LACY. The *tryp-1* and *tryp-2* mutants were identified by nutritional and genetic procedures previously described (DEMOSS and WEGMAN 1965).

All strains were maintained on slants of minimal medium (VOGEL 1954) or minimal medium plus 150 μg tryptophan per ml. For biochemical studies, mycelia were grown and crude extracts

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prepared as previously described (DeMoss 1965a). Each crude extract was passed over G-25 Sephadex to remove small molecules which interfered with the assay procedures. In certain cases, it was necessary to concentrate the activities by treating the crude extract with protamine sulfate, precipitating the activities with solid ammonium sulfate, dissolving the pellet in 0.1 the original volume of the crude extract and finally passing the concentrated fraction over a G-25 Sephadex column (DeMoss and Wegman 1965).

Anthranilate synthetase was determined fluorometrically (DeMoss 1965a) and InGP synthetase was assayed by the procedure of Wegman and DeMoss (1965). PRA isomerase was assayed by the following procedure. A reaction mixture containing 25 μmoles potassium phosphate buffer, pH 8.0, 2.5 μmoles $MgSO_4$, .075 μmoles anthranilate, 0.35 μmoles PRPP, and two enzyme units of anthranilate-PRPP phosphoribosyl transferase (purified about 100-fold from *N. crassa* and freed from anthranilate synthetase, PRA isomerase, and InGP synthetase) was incubated 2 min at 37°C to bring the PR-transferase reaction to equilibrium. An appropriate sample of extract containing PRA isomerase was added so that the final volume was 0.5 ml, the mixture incubated 10 min at 37°C, and 0.2 ml 1 M acetic acid was added. The acid conditions stopped the reaction and converted the residual PRA to anthranilate. The mixture was extracted with 5.0 ml of ethyl acetate and anthranilate was determined fluorometrically (activation: 340 mμ, emission: 400 mμ) in the ethyl acetate layer. The disappearance of anthranilate is a measure of the conversion of PRA to CDRP and InGP which are acid-stable. The reaction is linear to about 50% disappearance of the anthranilate. In this range the amount of activity is proportional to the amount of extract added.

In each assay an enzyme unit is defined as that amount of enzyme which converts 1 μmole of substrate, or forms 1 μmole of product, in one hour. Protein was determined by the procedure of Lowry, Rosebrough, Farr and Randall (1951).

Zone centrifugation studies were carried out as follows: A 0.3 ml sample of either a crude extract or a concentrated fraction and a 0.1 ml sample of dissolved crystalline catalase (Worthington) was layered on a 4.2 ml linear sucrose gradient (5 to 20% sucrose in 0.05 M potassium phosphate buffer, pH 7.0 plus 10^{-4} M EDTA). These tubes were centrifuged at 39,000 rpm for 11 hours in the SW 39 head in a Spinco Model L Centrifuge. The tubes were punctured at the bottom, and approximately 24 fractions of ten drops each were collected and diluted to 2.0 ml with .05 M phosphate buffer, pH 7.0 plus 10^{-4} M EDTA. Each fraction was assayed for catalase by measuring its absorbancy at 405 mμ in a spectrophotometer and for each of the pertinent enzyme activities as indicated above. The approximate sedimentation constant for each activity was estimated by comparing the position of the peak of the enzyme activity with the position of the peak of catalase. The S value was estimated by assuming a linear increase in S values from 0 to 11 between the top of the tube and the peak of catalase.

The two-point crosses between alleles were carried out according to the procedure of Kaplan, Suyama and Bonner (1964). All crosses were performed reciprocally and prototroph recombinants were scored after incubation of the ascospores for 48 hours on minimal medium. Prototroph frequencies were defined as number of prototroph recombinants per 100 ascospores plated. The total number of spores plated was determined from a count of two 0.25 cm^2 areas on each plate of the cross. Percent viability was estimated by counting the number of germinated spores among a total of 200 spores and was expressed as number of germinated spores per 100 plated spores.

To test each strain for its ability to revert to wild type, approximately 10^7 conidia were UV-irradiated at a dose estimated to kill 80% and plated on minimal medium. Any increase in the number of prototrophic colonies over those on control plates, layered with unirradiated conidia was scored as positive in this test.

RESULTS

To study the role of the *tryp-1* and *tryp-2* genes in the control of the enzyme aggregate, the effect of a series of mutations at each locus on the three activities associated with the aggregate has been investigated in detail. From a collection

of tryptophan auxotrophs, 45 random *tryp-1* mutants and nine random *tryp-2* mutants were selected for biochemical and genetic analyses. Crude extracts of each mutant were assayed for anthranilate synthetase, PRA isomerase, and InGP synthetase. In cases where partial activity of the aggregate was retained, crude or partially purified extracts were subjected to zone centrifugation in sucrose gradients to determine the approximate sedimentation constant of the active component and to establish whether any significant changes in the physical nature of the aggregate had occurred.

Biochemical and genetic analysis of tryp-2 *mutants.* Table 1 summarizes the biochemical analysis of the *tryp-2* mutants. As suggested by their nutritional characteristics, each lacked anthranilate synthetase but retained PRA isomerase and InGP synthetase at approximately wild-type levels. In extracts of each of the mutants, PRA isomerase and InGP synthetase exhibited identical profiles after zone centrifugation in sucrose gradients, indicating that the two reactions are catalyzed by a single component as in the wild type. However, in all cases except *tryp-2*-8 the active component had an approximate sedimentation constant of 7S (Table 1). The active component of *tryp-2*-8 had an approximate sedimentation constant of 10S similar to that of the wild-type aggregate. The zone centrifugation profiles obtained with partially purified extracts of *tryp-2*-6A and *tryp-2*-8A are shown with a wild-type profile in Figure 1A, B, and C.

TABLE 1

Biochemical properties of tryp-2 *and* tryp-1 *mutants*

Strains*	Enzyme specific activity†			Estimated sedimentation constant of active components‡
	Anthranilate synthetase	PRA isomerase	InGP synthetase	
Wild types:				
74A	.077	0.92	.096	10S
td 48R (*tryp-3*)	0.12	1.00	0.16	10S
tryp-2 mutants:				
2, 3, 6, 10, 11, 14, 15, 51	.000	0.86–2.82	0.19–0.30	7S
8	.000	1.74	0.39	10S
tryp-1C mutants:				
1, 2, 6, 12, 15, 20	.000	1.55–2.52	0.19–0.35	...
tryp-1 mutants:				
2, 3, 6, 7, 8, 9, 10, 11, 13, 14, 15, 18, 26, 31	.000	0.00	.000	...
4, 12, 16	.004–.010	0.00	.000	...
17, 19, 24	.014–.023	0.00	.000	7S
25	.041	0.00	.000	10S
10575, 21, 22, 27, 29	.086–0.52	0.99–2.7	.000	10S
23, 28	0.24, 0.16	2.2, 0.84	.000	...
20	.066	0.00	.030	...
5, 30	.000	0.00	.021, .013	...

* The *A* mating type of each strain was used for the preparation of extracts except for mutant *tryp-2*-15.
† μmoles/hr/mg protein in the crude extract.
‡ Estimated by comparing the activity peak of the enzyme activity to the position of crystalline catalase after centrifugation of both in a sucrose gradient as outlined in METHODS.

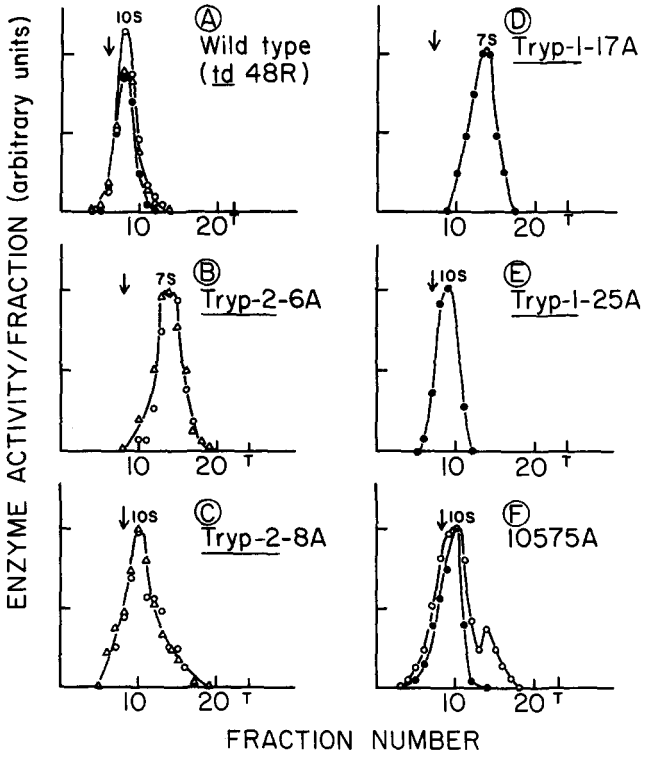

FIGURE 1.—Zone centrifugation profiles of enzyme activities in extracts of wild type and various tryp-1 and tryp-2 mutants (anthranilate synthetase -●-●-●-, PRA isomerase -O-O-O-, InGP synthetase -△-△-△-). Activities are plotted in arbitrary units for comparative purposes. The ratios of the absolute activities correspond to those shown for these strains in Table 1. In each case the peak of catalase activity is indicated by the arrow and the estimated sedimentation constant is indicated above each peak of enzyme activity.

These presumptive *tryp-2* mutants and a known *tryp-2* allele, 75001, were crossed in all combinations in a series of two-point crosses. The maximum prototroph frequency obtained in any cross was 0.17%.

Biochemical properties of tryp-1 *mutants.* As previously shown (AHMAD and CATCHESIDE 1960; AHMAD *et al.* 1964; WEGMAN 1964), the *tryp-1* mutants may be divided into two groups on the basis of nutritional characteristics. The *tryp-1*C mutants can grow on anthranilate as well as indole or tryptophan, while the remainder can grow only on indole or tryptophan.

Of the 45 *tryp-1* mutants collected, 14 corresponded to the *tryp-1*C type. Six of these were examined and, as expected from their nutritional characteristics, lacked anthranilate synthetase but retained PRA isomerase and InGP synthetase (Table 1). Neither of these activities was recovered after crude or partially purified extracts were centrifuged on sucrose gradients. The reason for this instability is not understood at present; it is surprising because the activities are stable over a 100-fold range of purification. As a result, no definite size can be assigned to the

active component(s), but it is clear that a significant change in the stability of the aggregate has occurred in these mutants.

Table 1 also summarizes the biochemical properties of the remaining 31 *tryp-1* mutants. Fourteen mutants were devoid of all three enzyme activities. Of these, all but mutants 3, 6, and 10 reverted to prototrophy when treated with UV. This class provides the basis for concluding that all three activities are dependent in some way on the *tryp-1* locus.

The remainder of the mutants exhibited at least one of the three activities. Seven mutants retained anthranilate synthetase but were lacking in both PRA isomerase and InGP synthetase. Because of the low activity, it was possible to examine the zone centrifugation pattern of only four of these mutants. In three, anthranilate synthetase had an estimated sedimentation constant of 7S while in the other mutant, *tryp-1*-25, the active component had an estimated sedimentation constant of 10S. Examples of the sucrose gradient profiles of the 7S type and the 10S type are shown in Figure 1D and E.

Another group of seven mutants retained both anthranilate synthetase and PRA isomerase but lacked InGP synthetase. In general, extracts from these mutants had specific activities for the retained activities which were as high as, or higher than, that of the wild-type extracts. These activities were examined by zone centrifugation in five of the seven mutants and in each case exhibited identical profiles with estimated sedimentation constants of 10S. An example of the zone centrifugation pattern obtained with this mutant type is shown in Figure 1F. Occasionally a separated minor peak of 7S was observed for the PRA isomerase activity.

The remaining three mutants retained InGP synthetase activity. Two of these lacked both anthranilate synthetase and PRA isomerase while the other, *tryp-1*-20A, retained anthranilate synthetase activity. Because of the relatively low activities found in the extracts and instability of the activities it was not possible to determine sedimentation constants for the active components.

All the mutants which retain some activity, except mutants 4, 5, 12, 20, and 30, reverted to prototrophy after treatment with UV.

Fine structure analysis of the tryp-1 *locus.* From the above biochemical analysis, it is apparent that mutations at the *tryp-1* locus may alter the activities of the three enzymes, anthranilate synthetase, PRA isomerase, and InGP synthetase, independently of one another or simultaneously. In addition, some of the mutations alter the apparent size of the component which catalyzes the reactions. In an attempt to understand how this locus exerts its control over the three activities we have carried out the following fine-structure analysis.

From a preliminary series of two-point crosses of *tryp-1* mutants, four alleles were selected which appeared to be well distributed within a map constructed from prototroph frequencies. The results of two-point crosses between these four alleles are shown in Table 2. From the prototroph frequencies, a map could be constructed with an unambiguous order and additive distances (Figure 2). These alleles were then used in a series of two-point crosses with each of the remaining *tryp-1* mutants in an attempt to localize each lesion in a fine-structure map of

TABLE 2

Two-point crosses between tryp-1 *mutants 9, 12, 14 and 25*

Cross (*tryp-1* × *tryp-1*)	Prototroph frequency*	Spores × 10^3	Prototroph colonies	Percent viability
12 × 9	.07	154	105	62
12 × 14	.13	116	152	83
12 × 25	.30	207	613	90
9 × 14	.07	50	37	. .
9 × 25	.22	236	513	94
14 × 25	.14	154	211	91

* Pooled results of reciprocal crosses repeated once.

the locus. In most of the studies, only three of the alleles (9, 12, 14) were used; in some of the later studies, *tryp-1*-25 was also used as a mapping allele.

The results of two-point crosses between the 14 *tryp-1*C mutants and the mapping alleles are shown in Table 3. Surprisingly, the prototroph frequencies obtained in all of these crosses were similar for a given mapping allele. In addition, the frequencies were generally consistent with the map derived from the two-point crosses between the mapping alleles (see Figure 2). Each of these *tryp-1*C mutants reverted to prototrophy when treated with UV. In a series of two-point crosses between the 14 *tryp-1*C mutants, very few prototroph recombinants were obtained although the crosses were highly fertile. These results indicate that the *tryp-1*C type lesions are point mutations which are localized in a very restricted region of the genetic map, and tend to confirm the relative position of the three mapping alleles in the fine-structure map of the *tryp-1* locus.

When the remaining 28 *tryp-1* mutants were crossed with the mapping alleles, more ambiguous results were obtained. A few of the crosses were infertile and a number of others yielded prototroph frequencies which could not be correlated in any way with the map derived from crosses between mapping alleles. Crosses of 16 of the mutants with the mapping alleles, however, yielded prototroph frequencies (Table 4) which could be used to assign a position relative to the mapping alleles in the map of the *tryp-1* locus (Figure 3). Although the absolute frequencies observed in the crosses varied over a large range, values from the crosses of any one mutant are consistent with the relative order and position of the mapping alleles shown at the top of Figure 3. All these mutants except 6, 10 and 30 reverted to prototrophy when irradiated with UV. Only *tryp-1*-10 mapped

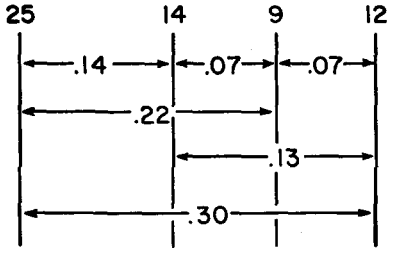

FIGURE 2.—A map of four *tryp-1* alleles derived from the prototroph frequencies of Table 2.

TABLE 3

Crosses between tryp-1*C mutants and* tryp-1 *mapping alleles*

Allele crossed	Allele × *tryp-1*–14			Allele × *tryp-1*–9			Allele × *tryp-1*–12		
	Prototroph frequency*	Spores† × 10^3	Wild-type recomb.	Prototroph frequency*	Spores† × 10^3	Wild-type recomb.	Prototroph frequency*	Spores† × 10^3	Wild-type recomb.
*tryp-1*C–1	.004	798	31	.10	795	808	.18	648	1188
–2	.008	115	9	.06	371	224	.12	319	369
–3	.002	94	2	.09	239	217	.13	83	107
–6	.002	233	4	.07	238	178	.12	303	360
–7	.013	470	60	.13	568	715	.20	564	1147
–12	.003	399	13	.08	387	323	.10	280	284
–15	.005	438	23	.07	465	334	.14	423	596
–16	.011	102	11	.04	162	70	.12	174	212
–17	.003	208	8	.07	228	162	.14	205	283
–18	.008	103	8	.21	171	353	.21	101	210
–19	0	254	0	.11	372	427	.21	402	840
–20	0	149	0	.12	143	170	.21	160	335
–21	.000	280	1	.10	370	362	.19	292	560
–23	.024	201	48	.07	169	123	.17	152	252

* Based on pooled results of reciprocal crosses.
† Viability ranged from 81 to 97%.

TABLE 4

Crosses between tryp-1 *mutants and* tryp-1 *mapping alleles*

Allele crossed	Allele × *tryp-1*–14			Allele × *tryp-1*–9			Allele × *tryp-1*–12			Allele × *tryp-1*–25		
	Prototroph frequency*	Spores† × 10^3	Wild-type recomb.	Prototroph frequency*	Spores† × 10^3	Wild-type recomb.	Prototroph frequency*	Spores† × 10^3	Wild-type recomb.	Prototroph frequency*	Spores† × 10^3	Wild-type recomb.
10575	.021	829	170	.010	985	83	<.001	775	2	.06	563	313
tryp-1–5	.24	115	272	.36	87	309	.44	176	768	.007	308	23
–6	<.01	23	1	.04	45	17	.06	37	21			
–7	.10	58	49	.30	77	235	.45	187	844			
–8	.11	154	164	.45	155	695	.55	198	1088			
–11	.06	28	18	.16	48	79	.36	55	198			
–15	.02	95	14	.20	45	89	.37	137	515			
–16	.07	310	211	.09	294	266	.22	220	480	.40	170	681
–17	.08	425	341	.11	249	269	.26	375	958	.31	174	538
–19	.08	249	188	.11	286	317	.27	248	672	.42	216	899
–21	.09	251	234	.07	298	210	.002	387	8	.15	160	246
–22	.11	30	34	.05	64	33	0	52	0			
–26	<.001	432	3	.07	482	339	.12	364	441			
–29	.12	133	153	.08	50	38	.05	108	60			
–30	.02	388	67	.30	381	1137	.36	433	1563			
–10	0	133	0	0	246	0	.007	231	16	.11	289	318

* Based on pooled results of reciprocal crosses.
† Viability ranged from 81 to 97%.

as an extended lesion mutant in the crosses. An interpretation of the fine-structure map is presented below.

DISCUSSION

The control by two unlinked genes of the enzyme aggregate which catalyzes the reactions of anthranilate synthetase, PRA isomerase, and InGP synthetase suggests that the active aggregate is formed by the interaction of the protein products of these two genes. Consistent with this view is the finding that mutations at either locus can alter the apparent size of the active component from 10S to 7S. Moreover, this finding demonstrates that mutations at either locus can prevent or alter the interaction between the two gene products as well as alter the three enzyme activities.

From a consideration of the biochemical properties of the mutant classes, the following deductions can be made: (1) The *tryp-2* gene product is probably the component of the aggregate which catalyzes anthranilate synthetase reaction since lesions at the *tryp-2* locus invariably lead to the loss of anthranilate synthetase but not PRA isomerase or InGP synthetase. (2) The *tryp-1* gene product is the component of the aggregate which catalyzes the PRA isomerase and InGP synthetase reactions. This is suggested by the finding that only lesions at the *tryp-1* locus will alter the ability of a strain to catalyze these reactions. (3) The *tryp-1* gene product would appear to be a single polypeptide chain. The fact that many revertible point mutations lead to the loss of both PRA isomerase and InGP synthetase would be compatible with both activities being associated with a single polypeptide chain or with each activity being associated with a distinct polypeptide chain, the two chains being specified by linked genes in an operon. However, the distribution of such lesions in the fine structure map of the *tryp-1* locus (see below) would appear to rule out any hypothesis involving polarity effects on linked cistrons; these lesions cluster in the central region of the map rather than extending to one end of the locus. (4) The precise role that the interaction between two components plays in the expression of the three activities is not clear from the results presented here. The fact that most *tryp-2* mutations result in a 7S form of InGP synthetase and PRA isomerase suggests that the *tryp-1* gene product is active without interacting with the *tryp-2* gene product. The observation that a 7S shoulder of PRA isomerase and InGP synthetase not associated with anthranilate synthetase is often seen in the sucrose gradients of extracts from wild type also supports this suggestion.

The data presented here provide conflicting evidence concerning the role of the interaction of the two components in the expression of anthranilate synthetase activity. On the one hand, weak anthranilate synthetase activity is found associated with a 7S component in certain *tryp-1* mutants which are lacking both InGP synthetase and PRA isomerase, suggesting that the *tryp-2* gene product is active by itself. On the other hand, the fact that many lesions at the *tryp-1* locus result in the loss of anthranilate synthetase demonstrates that the presence of wild-type *tryp-2* gene product is not sufficient for the expression of anthranilate synthetase activity but that the *tryp-1* product is also involved in some way.

Furthermore, when *full* anthranilate synthetase activity is retained by *tryp-1* mutants, it is invariably associated with a 10S component rather than a 7S component (see Table 1).

It is tempting to propose that two distinct 7S components, the products of the *tryp-1* and *tryp-2* genes, interact to form the active 10S component. If such an interaction does occur, it is essentially irreversible since it has not been possible to reconstitute wild type activities by mixing extracts of *tryp-1* and *tryp-2* mutants (DEMOSS and WEGMAN 1965). A precise determination of the number of distinct polypeptide components involved in formation of the aggregate and of the role of each component in the expression of the three activities must await a detailed physical and enzymological study on the isolated aggregate.

The availability of the various mutant classes among the *tryp-1* mutants has made it possible to study in some detail the functional organization of genetic information within the gene.

The fine structure map derived from the two-point crosses has to be regarded as provisional since it has been shown that the levels of recombination observed in intragenic two-point crosses can be affected by various genetic factors present in presumably closely related strains of Neurospora (CATCHESIDE, JESSOP and SMITH 1964). It can be seen in the crosses presented here, however, that by utilizing a set of mapping alleles which provide by themselves a reasonably unambiguous map, information concerning the positions of different alleles, relative to the mapping alleles, can be obtained. However, absolute order or map distances cannot be established with these data.

With the above limitation in mind, the distribution within the locus of various types of biochemical lesions can be examined in a fine structure map derived from Figure 3. Several significant features emerge from such an analysis (Figure 4):

(1) InGP synthetase and PRA isomerase appear to be controlled by opposite ends of the locus. Mutations leading to the loss of InGP synthetase but the retention of PRA isomerase (line D) are restricted to one end of the locus while mutations leading to the loss of PRA isomerase and the retention of InGP synthetase (line E) are restricted to the other end of the locus. Although the number of mutants analyzed is small, the results are unambiguous with no area of overlap existing.

(2) Mutations which alter the interaction of the *tryp-1* gene product with the *tryp-2* gene product tend to cluster in the center of the locus. Thus, *tryp-1*C mutations (line B) all cluster in a very restricted region towards the center of the map. Since these mutations leave the PRA isomerase and InGP synthetase activities unaltered but result in the absence of anthranilate synthetase activity (even though the normal protein specified by the *tryp-2* gene is presumably present), the region delineated by their location must be involved in the expression of anthranilate synthetase activity by the *tryp-2* gene product. Mutations which lead to the loss of all three activities (line A) are also clustered in the center of the map in an apparently less restricted region than the *tryp-1*C mutations. This result was surprising at first but suggests that such mutations alter the ability of

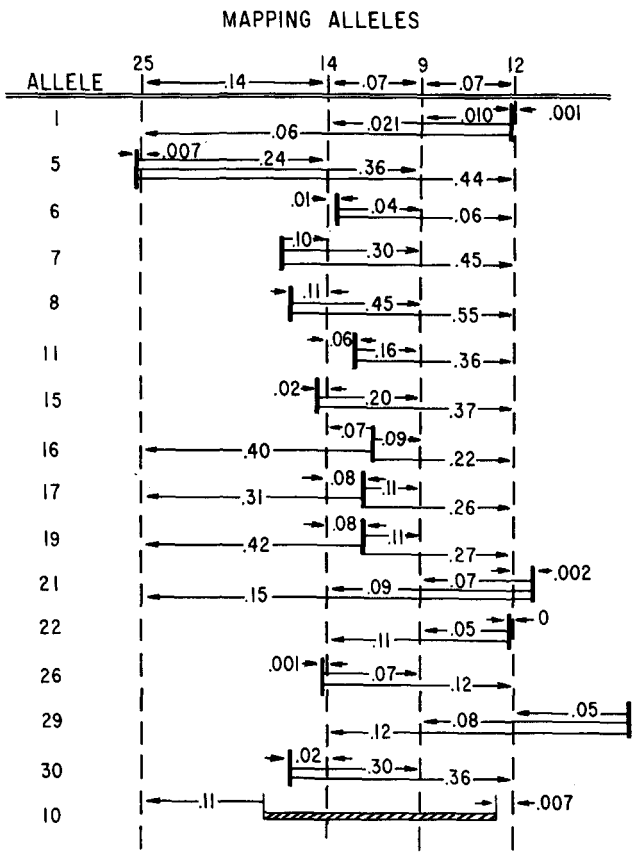

FIGURE 3.—The placement of various *tryp-1* alleles relative to the mapping alleles. Each allele is positioned in the map shown at the top by utilizing the prototroph frequencies shown in Table 3.

the protein product to catalyze both PRA isomerase and InGP synthetase (controlled by opposite ends of the locus) as well as its ability to interact with the *tryp-2* gene product to form active anthranilate synthetase (controlled by the center of the locus). Furthermore, while mutations which alter PRA isomerase or InGP synthetase, or both, but which result in active anthranilate synthetase appear to be more or less randomly distributed throughout the locus (line C), the retained anthranilate synthetase activity is carried by a 7S component when the lesion is located towards the center of the map and is carried by a 10S component when the lesion is located at either end of the map.

This tentative localization of functions within the map of the *tryp-1* locus is summarized diagrammatically in line F of Figure 4.

While a detailed complementation analysis of the *tryp-1* locus has been carried out (CATCHESIDE 1964), it is difficult to correlate it with the studies presented here, since the biochemical properties of the mutants used were not examined in that study. However, the complex nature of the complementation map presented

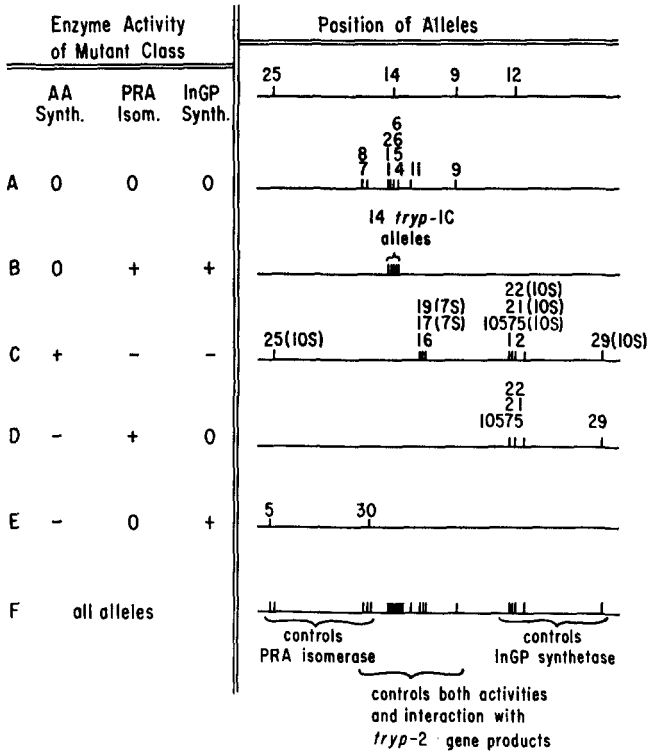

FIGURE 4.—The distribution of various *tryp-1* mutants in the fine structure map derived from Figure 3. The biochemical classes are shown on the left. A dash is used to indicate that mutants in this category may or may not lack the enzyme activity; all such mutants lack at least one, and in some cases both, of the activities indicated. The vertical marks on each line indicate the approximate position of the indicated alleles in the map shown at the top. In line C, the estimated sedimentation constant of anthranilate synthetase for various alleles is indicated in parentheses.

could very reasonably be explained by the possible subunit arrangements of the *tryp-1* gene-product in the enzyme aggregate.

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SUMMARY

Three enzymes of tryptophan biosynthesis, anthranilate synthetase, PRA isomerase, and InGP synthetase, are associated with a 10S component in extracts. Mutations at either the *tryp-1* or *tryp-2* loci can alter these three activities as well as the physical nature of the activities which are retained. The pattern of these alterations in various mutants shows (1) that mutations at either *tryp-1* or *tryp-2* may alter the wild-type 10S aggregate to a partially active 7S component, (2) that *tryp-1* specifies the component which catalyzes the PRA isomerase and InGP synthetase activities and that *tryp-2* specifies the component

which catalyzes the anthranilate synthetase reaction, and (3) that the interaction of the two components is not required for the expression of PRA isomerase and InGP synthetase activities, but is involved in some way in the expression of anthranilate synthetase activity.—A fine-structure map of the *tryp-1* gene has been derived from a series of two-point crosses. On the basis of this map, the genetic control of PRA isomerase, of InGP synthetase, and of the interaction of the *tryp-1* gene product with the *tryp-2* gene product can be tentatively assigned to specific regions of the locus.

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