GENE INTERACTION IN TRYPTOPHAN SYNTHETASE FORMATION[1]

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IN a previous study (YANOFSKY 1952) of two biochemically similar tryptophan-requiring mutants of *Neurospora crassa* it was found that each strain differed from the wild type in that it was unable to carry out the tryptophan synthetase reaction (the coupling of indole and serine to form tryptophan). In both mutants the genetic change which resulted in a requirement for exogenous tryptophan seemed to be at the same locus (termed the *td* locus) and thus, on the basis of the initial biochemical and genetic criteria that were applied, the two strains appeared to be identical. The occurrence of a suppressor mutation (restoring the ability to grow in the absence of tryptophan) in one of the strains subsequently permitted the demonstration that the two mutants differed. Only one of the mutant strains responded to the presence of the suppressor gene by forming tryptophan synthetase and growing in the absence of a tryptophan supplement; the second strain, when it contained the suppressor gene, showed neither of these effects.

Since these observations indicate that suppressor gene specificity can be used to detect differences at the locus controlling the formation of tryptophan synthetase, additional mutants and suppressors have been obtained and examined. The behavior of these new mutants and suppressors is the subject of this paper.

TERMINOLOGY

Mutant-suppressor combinations that grow in the absence of an exogenous supply of tryptophan are called suppressed mutants; mutants which are capable of responding to specific suppressor genes are called suppressable mutants. Mutants which do not carry suppressor genes or are unaffected by the suppressor genes they carry are termed unsuppressed mutants; mutants that do not respond to any of the known suppressor genes are tentatively classified as unsuppressable mutants.

DESCRIPTION OF MUTANTS

Twenty-five tryptophan-requiring (indole non-utilizing) mutant strains, designated td_1 through td_{25}, (*td* strains are *tryp-3* strains in the terminology of BARRATT, NEWMEYER, PERKINS and GARNJOBST 1954) were employed in this investigation. Of these, td_1 (C-83) was supplied by DR. H. K. MITCHELL of the California Institute of Technology and td_2 (S-1952) by DR. E. L. TATUM of Stanford University. Strains td_3 through td_{25} were obtained in this laboratory from ultraviolet irradiated macro-

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conidia of a single stock, the ST. LAWRENCE wild type strain 74 A. Strain td_{24} was obtained by J. WEIJER. The filtration method of WOODWARD, DEZEEUW and SRB (1952) was employed in the isolation of the twenty-three mutants. Since these strains originated from a single stock, they are presumably isogenic except for the genetic change which resulted in a requirement for tryptophan. Mutant strains td_3 through td_{25} represent the yield from four separate searches.

The tryptophan requirement characteristic of each of the twenty-five *td* mutants cannot be satisfied by indole or anthranilic acid. All the strains respond approximately equally well to added tryptophan and, except for strains td_3 and td_{24}, they do not grow in the absence of an exogenous supply of this amino acid. Strain td_3 grows very slightly in the absence of tryptophan (in a two-week period) and strain td_{24}, a temperature sensitive mutant, will grow without added tryptophan at temperatures of 30°C or above. Extracts of the latter strain, as might be expected, exhibit tryptophan synthetase activity though the content of this enzyme is very low. It has not as yet been possible to detect tryptophan synthetase activity in extracts of any of the other twenty-four mutant strains. Details of the examination of extracts of strains td_1 and td_2 have been reported previously (YANOFSKY 1952).

Allelism tests of the various *td* strains suggest that all of these strains represent alterations of the same locus. Extensive evidence for the allelism of the mutant loci in strains td_1 and td_2 has been presented previously (NEWMEYER 1954; YANOFSKY 1952). Other members of the *td* series have also been examined, though in less detail (WEIJER 1954), and again the evidence suggests they are all allelic. In many of the interallele crosses that have been examined, tryptophan independent strains have been found among the progeny of the crosses. Such tryptophan independent strains occur infrequently. These *td* strains thus appear to resemble *Q* locus mutants (BONNER 1951; ST. LAWRENCE 1955). Crosses of $td_1 \times td_1$ have also yielded infrequent tryptophan independent progeny (ca. $1/10^4$ ascospores). The origin of such tryptophan independent progeny has not been established. In any event, however, it appears that the *td* strains represent either mutations of a common locus or mutations of closely linked interdependent units, with a common function.

ORIGIN OF THE SUPPRESSORS

Tryptophan independent (T^+) cultures could arise from tryptophan dependent (T^-) *td* mutants as a result of either reversion or suppression. To distinguish between these possibilities it is necessary to perform a cross. If, for example, a T^+ culture were crossed to wild type only $8T^+:0T^-$ asci would be formed if tryptophan independence resulted from reversion. If, however, suppression was responsible for tryptophan independence, $4T^+:4T^-$ and $6T^+:2T^-$ asci would be obtained in addition to the $8T^+:0T^-$ class. In such crosses the appearance of a $6T^+:2T^-$ ascus would be particularly significant for two reasons. First, since T^+ colonies derived from T^- macroconidia are frequently heterocaryons (with the T^- strain), $4T^+:4T^-$ asci would also be expected from a cross of wild type by a T^+ revertant colony. This leaves the 6:2 ascus as our only initial means of distinguishing between suppression and reversion. Secondly, a $6T^+:2T^-$ ascus is itself proof of suppression since in an ascus of this type one mutant spore pair must carry a suppressor while the second mutant spore pair does not.

It was reported previously (YANOFSKY 1952) that td_2 su_2 (each suppressor gene, i.e. su_2, is assigned the number of the *td* mutant in which it was discovered) exhibits a pronounced growth lag on a medium lacking tryptophan. This lag is especially noticeable on sorbose-minimal agar (TATUM *et al.* 1949). On this medium td_2 su_2 colonies derived from plated macroconidia become visible 3–4 days later than wild type colonies and are classified as delayed colonies. The td_2 su_2 colonies are morphologically distinguishable from the wild type colonies in that they consist of relatively few mycelial filaments and never give the thick, sharp-bordered appearance characteristic of wild type colonies. These differences between wild type and td_2 su_2 colonies, as it turned out, were helpful in the isolation of new suppressors.

A typical experiment designed to detect suppressor mutations was performed as follows: Portions of a suspension of macroconidia of a *td* mutant were plated on sorbose-minimal agar before and after ultraviolet irradiation (to obtain both spontaneous and induced suppressor mutations). Tryptophan independent colonies usually appeared on the plates after 2–3 days of incubation at 30°. These colonies were picked and transferred to minimal agar slants. The plates were reincubated for 4–5 days and if a second crop of colonies appeared they were also picked. This second crop usually contained a large proportion of delayed colonies. All the colonies which were picked were then crossed to wild type to distinguish between reversion at the *td* locus or suppressor mutations at other loci. From each cross asci were dissected in order and the cultures derived from the ascospores were tested to determine whether or not they required tryptophan.

The predominant ascus type encountered in most of the crosses contained four T^+ and four T^- spores. This was anticipated since the colonies obtained by the method employed were almost invariably heterocaryotic. Some crosses gave only $8T^+:0T^-$ asci in addition to the 4:4 type. This was taken as in indication that the tryptophan independence of the picked colony employed in the cross was the result of reversion at the *td* locus. An occasional cross gave $6T^+:2T^-$ asci in addition to the other two types. As mentioned previously, the 6:2 ascus establishes that an independently segregating gene, distinct from the *td* locus, is responsible for the tryptophan independence of two of the spores. Of the six T^+ cultures two usually showed a 3–4 day lag (in liquid minimal medium) and grew much slower than the other T^+ cultures. These slow growing T^+ cultures in 6:2 asci were presumably the suppressed mutants. To prove that they actually were suppressed mutants one spore of each spore pair was crossed to wild type (see crosses 1, 3, 5 and 6 in table 1). The recovery of mutant spore pairs from these crosses indicated that the slow growing parental strain was a suppressed mutant. In a number of cases the presence of a suppressor gene was confirmed by showing that the T^+ cultures derived from 4:4 asci were wild type strains carrying suppressors (see crosses 2, 4 and 7 in table 1).

It was mentioned previously that both fast growing colonies and delayed colonies were encountered in hunts for new suppressors. In our experiences the tryptophan independence of the fast growing colonies is inseparable from the *td* locus suggesting that in these colonies reversion is the cause of tryptophan independence. To date, well over 50 fast growing colonies derived from a number of different *td* mutants have been analyzed and, without exception, tryptophan independence has been inseparable from the *td* locus. The tryptophan independence of delayed colonies,

TABLE 1

Detection of suppressors in strains td_2, td_3, td_6 *and* td_{24}

	Cross	Ascus type		
		$8T^+$	$6T^+:2T^-$	$4T^+:4T^-$
1	$td_2\ su_2 \times$ wild type	4	17	5
2	$su_2 \times td_2$	13	42	19
3	$td_6\ su_6 \times$ wild type	2	9	3
4	$su_6 \times td_6$	5	11	3
5	$td_3\ su_3 \times$ wild type	1	12	0
6	$td_{24}\ su_{24} \times$ wild type	1	7	2
7	$su_{24} \times td_{24}$	2	8	0

T^+ = tryptophan independent T^- = tryptophan dependent

on the other hand, was frequently due to suppressor mutations. All suppressors obtained to date have been isolated from delayed colonies. Delayed colonies have not been obtained from all *td* mutants; some strains give only the fast growing type. In this connection it is interesting to note that td_1, a strain in which all attempts to isolate a suppressor have been unsuccessful, does not give delayed colonies.

SUPPRESSOR GENE SPECIFICITY

The specificity of the four suppressor genes was examined by crossing wild type strains carrying each of the suppressors to the four suppressable strains and to td_1, which was previously found to be unsuppressable by su_2 (YANOFSKY 1952). The results of these crosses are given in table 2. It can be seen that su_2 suppresses td_2 but does not suppress the mutant allele in any of the other strains. Suppressor$_6$ suppresses td_2 as well as td_6. Both td_3 and td_{24} are suppressed by su_3 and su_{24} but neither of these suppressors affects td_1, td_2 or td_6.

Several crosses were performed with two suppressors present to determine whether any of the suppressor genes are allelic and whether the presence of one suppressor gene would affect suppression by a second suppressor gene. The results of these crosses are given in table 3. In no case was there any indication that one suppressor gene inhibits the effect of a second suppressor gene. In the cross $su_6 \times td_2\ su_2$, as can be seen in table 3, the $8T^+:0T^-$ ascus class was very large. This was expected since both su_2 and su_6 suppress td_2. The occurrence of 6:2 and 4:4 asci in this cross indicates that su_2 and su_6 are not allelic. The absence of 6:2 and 4:4 asci from crosses involving both su_3 and su_{24} would suggest that these suppressors are allelic or very closely linked. The similarity of su_3 and su_{24} is also evident from growth studies performed with strains carrying these suppressor genes. These studies have shown that both $td_3\ su_3$ and $td_{24}\ su_{24}$, in contrast to other suppressed mutants, are temperature sensitive (in the absence of tryptophan they grow best at high temperatures, as does td_{24}).

Two suppressor genes, su_2 and su_6, were crossed into all 25 of the *td* mutants to determine the extent of the specificity of these two suppressors. None of the *td* mutant alleles except td_2 (suppressed by su_2 and su_6) and td_6 (suppressed by su_6) was suppressed by either su_2 or su_6.

TABLE 2

Suppressor specificity crosses with strains td_1, td_2, td_3, td_6 *and* td_{24}

Cross	Ascus type		
	$8T^+$	$6T^+:2T^-$	$4T^+:4T^-$
$su_2 \times td_1^*$	0	0	59
$su_2 \times td_3$	0	0	5
$su_2 \times td_6^*$	0	0	19
$su_2 \times td_{24}$	0	0	12
$su_6 \times td_1^*$	0	0	26
$su_6 \times td_2$	1	5	0
$su_6 \times td_3^*$	0	0	5
$su_6 \times td_{24}$	0	0	11
$su_3 \times td_1$	0	0	10
$su_3 \times td_2$	0	0	12
$su_3 \times td_6$	0	0	5
$su_3 \times td_{24}$	1	2	2
$su_{24} \times td_1$	0	0	6
$su_{24} \times td_2$	0	0	10
$su_{24} \times td_3$	3	3	3
$su_{24} \times td_6$	0	0	17

T^+ = tryptophan independent T^- = tryptophan dependent

* The presence of the expected suppressor gene in the progeny of this cross was confirmed by an additional cross to a mutant affected by the suppressor.

TABLE 3

Crosses involving two suppressors

Cross	Ascus type		
	$8T^+$	$6T^+:2T^-$	$4T^+:4T^-$
$su_2 \times td_6\ su_6$	3	12	5
$su_6 \times td_2\ su_2$	10	9	3
$su_2 \times td_{24}\ su_{24}$	1	9	0
$su_6 \times td_{24}\ su_{24}$	0	16	2
$su_3 \times td_{24}\ su_{24}$	20	0	0
$su_{24} \times td_3\ su_3$	13	0	0

T^+ = tryptophan independent T^- = tryptophan dependent

Crosses were also carried out to examine the possibility that an unsuppressable mutant, such as td_1, does not respond to su_2 because it does not carry the proper modifier genes. A revertant derived from a suppressable td_2 stock and presumably carrying any and all modifiers necessary for suppression by su_2 was employed in these crosses. This revertant was crossed to td_1 and a td_1 stock obtained from the progeny. This td_1 was crossed to the revertant and a td_1 stock was again isolated from the progeny. The revertant was crossed to this new td_1 stock and a td_1 was selected from the progeny of this cross. This td_1 stock which was approaching isogenicity with the td_2 revertant, was then crossed to wt su_2. This new td_1, like the original parental td_1, was unaffected by su_2. Thus the inability of td_1 to respond to su_2 appears to be associated with the *td* locus.

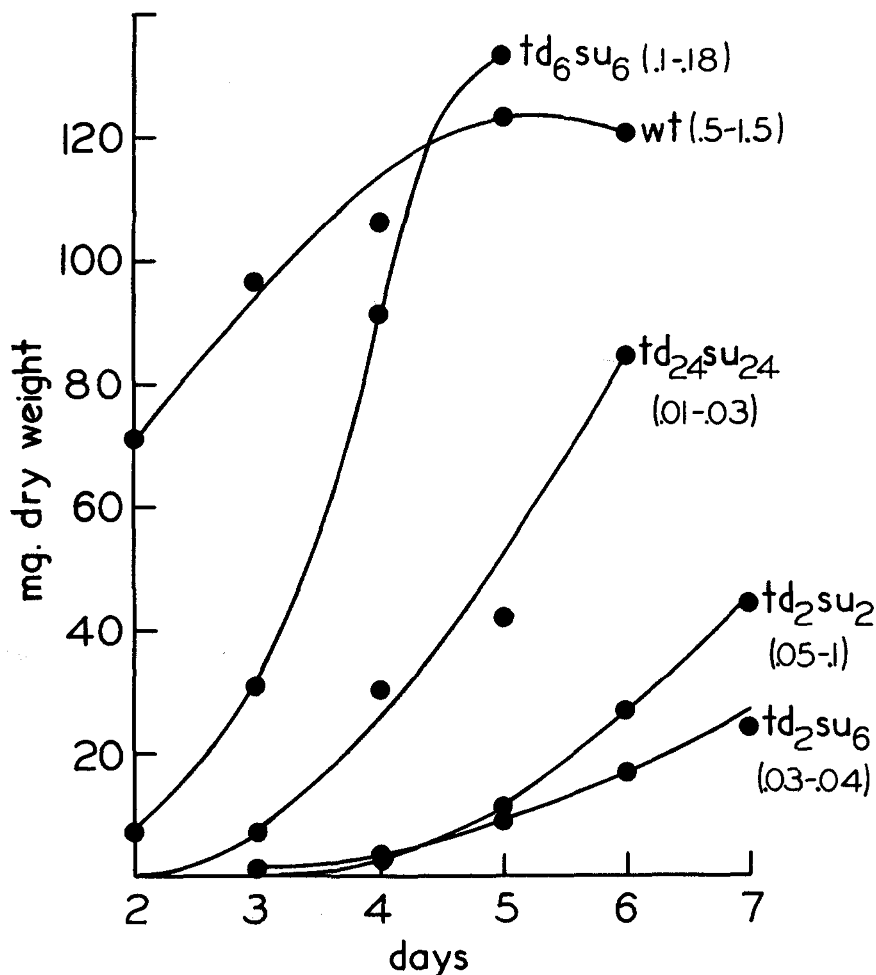

FIGURE 1.—Typical growth curves on minimal medium of several suppressed mutants and wild type. Specific tryptophan synthetase activities of the various strains are given in the parenthese after each curve.

CHARACTERISTICS OF SUPPRESSED MUTANTS

Although all suppressed mutants grow in the absence of tryptophan, each is stimulated markedly by this amino acid. In contrast to the mutants, however, low levels of tryptophan give maximal growth responses. A comparison of the growth curves on minimal medium of several of the suppressed mutants and of a typical wild type strain is shown in figure 1. From this figure it can be seen that there is a pronounced lag in the growth of suppressed mutants. The length of this lag is critically affected by inoculum size. In the presence of tryptophan, however, inoculum size is not a limiting factor.

Extracts of a number of the suppressed mutants have been examined for trypto-

TABLE 4

Comparison of the properties of tryptophan synthetase preparations from td_2 su_2, td_6 su_6 and wild type

	Wild type	td_2 su_2	td_6 su_6
pH optimum	7.8	7.8	7.6
Concentration required for half maximum velocity			
indole	5.6×10^{-5}M	4.8×10^{-5}M	5.3×10^{-5}M
L-serine	3.4×10^{-3}M	3.4×10^{-3}M	3.2×10^{-3}M
B_6alP	1.3×10^{-6}M	1.8×10^{-6}M	1.1×10^{-6}M
stability			
loss of activity at 2°C	over 50% in 24 hours	over 50% in 24 hours	over 50% in 24 hours
protected by	B_6alP+GSH	B_6alP+GSH	B_6alP+GSH
% inhibition by			
NH_2OH 10^{-3}M	92	100	100
CN^- 10^{3}M	54	40	51
Co^{++} 10^{-3}M	76	73	87
Zn^{++} 10^{-3}M	81	78	61
L-tryptophan 2×10^{-3}M	29	36	29
Energy of activation	ca. 12,000 cal/mole	ca. 12,000 cal/mole	—
Fractionation behavior		same as wild type	same as wild type

B_6alP = pyridoxal phosphate
GSH = glutathione

phan synthetase activity and in every case the presence of the specific suppressor gene restored the ability to form this enzyme. However, in no case did the tryptophan synthetase content of a suppressed-mutant extract reach the wild type level. Typical tryptophan synthetase levels (specific activities) of several of the suppressed mutants are given in parentheses in figure 1. Partially purified tryptophan synthetase preparations from two of these suppressed mutants, td_2 su_2 and td_6 su_6, have been compared with similar preparations from a wild type strain. A summary of the properties examined and the results obtained in these comparisons is given in table 4. It is apparent that no major differences were detected.

DISCUSSION

The data presented emphasize the biochemical similarities of the 25 *td* mutants. The genetic change in each of these mutants is expressed as a tryptophan requirement which cannot be satisfied by indole, and as an inability or limited ability to form tryptophan synthetase. Each of the *td* mutants presumably represents an independent occurrence of a mutation affecting tryptophan synthetase formation. The fact that so many independent occurrences of mutations affecting one enzyme appear to be at or near the same chromosomal area probably indicates that this area is most critically concerned with tryptophan synthetase formation. Mutations at other loci, if they also affect tryptophan synthetase formation, do not appear to be capable of reducing the level of this enzyme to a point at which it limits growth.

TABLE 5

Suppressor specificity

td	Suppressor			
	su_2	su_3	su_6	su_{24}
td_1	−	−	−	−
td_2	+	−	+	−
td_3	−	+	−	+
td_6	−	−	+	−
td_{24}	−	+	−	+

+ indicates suppression.

In spite of the apparent biochemical and genetic similarities of the *td* mutants, studies employing suppressor genes have permitted the detection of distinct differences between several of the *td* mutants. The suppressor genes were found to be highly specific (a summary of suppressor specificity is presented in table 5) and to suppress only certain members of the *td* series. Thus, although our present biochemical techniques do not enable us to demonstrate differences in the various *td* mutants, the suppressor studies show that these differences do exist. The conclusion is inescapable that the chromosomal area we call the *td* locus has been affected differently in different members of the *td* series.

In attempting to account for the results obtained two hypotheses are being considered. The first hypothesis assumes 1) the *td* locus, either directly or indirectly controls the synthesis of one enzyme, tryptophan synthetase, 2) many of the *td* mutants are blocked in different steps or different phases of tryptophan synthetase formation and 3) the suppressors provide specific substances which are capable of partially overcoming the defect in tryptophan synthetase formation in specific *td* mutants. The second hypothesis assumes that each *td* type produces a substance which specifically inhibits the formation of tryptophan synthetase. The suppressors would then act by blocking the synthesis of specific inhibitors. This hypothesis requires that the *td* locus control the formation of several different inhibitors, each specific in its action on tryptophan synthetase formation.

If the defects in the *td* mutants do affect tryptophan synthetase formation, as our results suggest, suppressable mutants might be expected to accumulate "unfinished enzyme". Some preliminary serological studies (SUSKIND, YANOFSKY and BONNER 1955) aimed at examining this possibility have shown that suppressable mutants do, in fact, form an antigen or antigens closely related to tryptophan synthetase while an unsuppressable strain, such as td_1, does not. Although the actual relationship of the antigen to tryptophan synthetase formation is at present unknown, the serological approach seems promising and may, in time, permit us to distinguish between the two hypotheses which are being considered.

SUMMARY

Twenty-five biochemically similar tryptophan requiring-strains have been studied in respect to their response to specific suppressor genes.

These mutant strains appear to be allelic and are altered in that each has a tryptophan requirement which cannot be satisfied by indole, and each is unable or has limited ability to form tryptophan synthetase.

Despite the apparent biochemical and genetic similarities of these strains, studies employing suppressor genes permit the detection of distinct differences between some of the strains. Thus the chromosomal area, termed the *td* locus, has been affected differently in different members of this allelic series.

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