SUPPRESSOR GENE ACTION IN THE TRYPTOPHAN SYNTHETASE SYSTEM OF NEUROSPORA CRASSA. I. GENETIC STUDIES[1]

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EXTRAGENIC suppressors which alleviate nutritional requirements of specific microbial auxotrophs appear to function by a variety of mechanisms. The suppressor may act indirectly in one of several ways to make available the required compound. In *Neurospora crassa* these mechanisms include the utilization of separate pathways of metabolism (LEIN and LEIN 1952; STRAUSS and PIEROG 1954), decompartmentalization of a metabolite which has accumulated before a partial block and is required in a separate pathway (DAVIS 1962; DAVIS and WOODWARD 1962) and the removal of an inhibitor to which the mutant enzyme is sensitive with resultant partial restoration of the biosynthetic step lacking in the mutant (SUSKIND and KUREK 1959; SUSKIND, LIGON and CARSIOTIS 1962).

Other suppressors appear to act by repair of the primary structure of the catalytically defective mutant enzyme. The existence of this type of suppressor mechanism in *N. crassa* was suggested initially by the work of YANOFSKY with one suppressed tryptophan synthetase mutant (1952). A number of theories involving effects on protein biosynthesis have been proposed to explain the mechanism of action of such suppressors. These include: (1) the "missense to sense" (YANOFSKY and ST. LAWRENCE 1960; YANOFSKY, HELINSKI and MALING 1961) and the "nonsense to sense" theories (GAREN and SIDDIQI 1962; BENZER and CHAMPE 1962), which invoke changes in the specificity of protein biosynthesis at the level of the activating enzymes and transfer RNAs, and (2) modifications in the ribosomal reading of the messenger RNA code (GORINI and KATAJA 1964; DAVIES, GILBERT and GORINI 1964).

Wild-type tryptophan synthetase (TSase) of *N. crassa* catalyzes the following reactions (YANOFSKY 1960; GARRICK, ELBERFELD and SUSKIND 1964):

1. indoleglycerolphosphate + L-serine → L-tryptophan + D-glyceraldehyde 3-phosphate + H_2O
2. indole + L-serine → L-tryptophan + H_2O
3. indoleglycerolphosphate $\underset{r}{\overset{f}{\rightleftarrows}}$ indole + D-glyceraldehyde 3-phosphate

Tryptophan synthetase mutant td_{201} (*td* locus, the TSase structural gene), an indoleglycerol accumulator, forms higher than normal levels of a mutationally

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altered enzyme or CRM (CRM, crossreacting material) with no indoleglycerol-phosphate utilizing activities (reactions 1 and 3f) but with residual reaction 2 activity. Consequently td_{201} can utilize either indole or L-tryptophan for growth (RACHMELER and YANOFSKY 1961). This paper describes the genetics of a new group of suppressed tryptophan synthetase mutants of *N. crassa* obtained from mutant td_{201}. Seven independently occurring suppressed td_{201} mutants have been isolated and each has been characterized nutritionally, genetically, and enzymologically. The td_{201} suppressors are site-specific and all map in a restricted region of linkage group VIIR. They appear to effect a change in the structure of the mutationally altered enzyme, thereby restoring wild-type TSase activity (YOURNO and SUSKIND 1964). A preliminary report of part of this material has been presented (YOURNO and SUSKIND 1963).

MATERIALS AND METHODS

Isolation of prototrophic strains from td_{201}: TSase mutant td_{201}, originally isolated by AHMAD and CATCHESIDE (1960), was obtained from DR. C. YANOFSKY. Tryptophan-independent strains were isolated from td_{201} by ultraviolet irradiation of macroconidia with a calibrated mineralight mercury lamp (2537 Å) at dosages of 2 to 20 $\times$ 10^{16} quanta/cm^2. Treated macroconidia were plated on minimal sorbose agar as described by RYAN (1950). The plates were incubated at 30°C for several days. Seven fast-growing colonies (visible after 48 hr incubation) and 11 slow-growing colonies (visible only after 72 to 120 hr incubation) were isolated from several plates. Control platings of untreated conidia on minimal sorbose agar established that no fast or slow-growing prototrophs were originally present. To insure that no repeat back mutants were selected from the same plates, colonies were picked after six days incubation, before conidiation and spreading had occurred. All isolates were maintained thereafter on minimal agar slants.

Growth studies: Growth rate estimates for tryptophan-independent strains were made visually on minimal medium agar slants (tryptophan-supplemented and unsupplemented) and by measurement of mycelial dry weight of aerated cultures grown either on minimal medium or minimal medium supplemented with tryptophan or indole.

Quantitative growth analyses were performed in duplicate in still culture as described by RYAN (1950). Minimal medium suspensions of fresh conidia were inoculated into 25 ml of medium in 125 ml De Long culture flasks equipped with metal stoppers and incubated at 30°C. Mycelial pads were harvested after specified times of incubation, oven dried, and weighed.

Genetic studies: Crosses and tetrad or random single-spore analyses were performed as described by RYAN (1950).

Genetic characterization of fast-growing and slow-growing prototrophs: Tryptophan-independent strains were crossed with wild-type 5297a or 74A protoperithecial cultures grown on minimal Westergaard agar slants. Tetrads were analyzed from crosses involving heterocaryotic strains originating directly from td_{201} macroconidia. Random spore analysis was used in scoring strains which previously had been crossed to 5297a and were considered homocaryotic. All spores were isolated on tryptophan-supplemented slants and cultures were tested at 30°C on minimal medium slants: td_{201} mutants do not grow on minimal agar (T$^-$), wild types show substantial growth within 24 hours (T$^+$), and suppressed td_{201} "slow-growing" strains require 48 to 72 hours to attain comparable growth levels (T$^{+/2}$).

td_{201} *suppressor allelism tests:* Recombination between independently occurring td_{201} suppressors was investigated by random single-spore analysis of crosses of suppressed strains to determine the frequency of unsuppressed td_{201} recombinants.

A td_{201} $su_{201\text{-}8}$ strain was used as a standard su_{201} marker to which all other independently occurring suppressed strains were crossed, either as td_{201} $su_{201\text{-}a}$ or td^+ $su_{201\text{-}a}$ (suppressed wild-

type) genotypes. To protect against loss of the suppressor in *td*+ su_{201} and td_{201} su_{201} parental stocks, inocula for the crosses were taken directly from ascospore-initiated cultures which had been characterized previously as to genotype. All cultures were grown on minimal medium agar, whether for conidial or protoperithecial parentage. Loss of the suppressor in either parent would have yielded td_{201} su_{201}+ spores which would have appeared spuriously as recombinants. The assumption was made that any doubly suppressed td_{201} recombinant would behave as a slow-grower on minimal medium.

Ascospores from the same suppressor crosses examined by random single-spore analysis were mass-plated on sorbose agar with varying concentrations of DL-tryptophan. Under these conditions td_{201} su_{201}+ ascospores from control crosses formed microcolonies on sorbose agar supplemented with 0.75 μg DL-tryptophan per ml. Spores were collected from the cross tube with a sterile spatula and suspended in soft 0.5 percent sorbose agar. An aliquot of the agar suspension was counted and samples of 200 to 250 spores were shocked at 60°C for 1 hr in 3 to 5 ml of soft 0.5 percent sorbose agar and plated. After 24 hr incubation at 30°C, 3 to 5 ml of 1.25 percent soft sorbose agar of the same nutritional composition were plated over the germinated spore-containing layer to suppress condiation which can obscure microcolonies. Colony growth was followed daily to insure detection of microcolonies during the 4 to 7 day incubation at 30°C. Colony density per plate was kept under 100, since with larger numbers competition between colonies occurred and "pseudo" microcolonies were formed.

td_{201} *suppressor specificity tests with various* td *alleles:* *td*+ su_{201} strains were grown as protoperithecial parents on minimal Westergaard slants and were crossed with several different *td* mutants. Random spore progeny from each cross were isolated on tryptophan-supplemented slants and were subsequently scored for growth rate on minimal medium slants. One protoperithecial culture of each *td*+ su_{201} group was crossed to td_{201} and slow-growing progeny were isolated to confirm the presence of the td_{201} suppressor in the *td*+ su_{201} inocula.

The indole-utilizing TSase mutants tested for td_{201} suppressor specificity were sent to us by DR. D. M. BONNER.

Mapping of the td_{201} *suppressor genes:* A td_{201} $su_{201\text{-}2}$ protoperithecial parent was crossed with strains marked on one of each of the seven linkage groups. The linkage of the suppressor to these markers was examined by random single-spore analysis. Subsequently all seven independently occurring td_{201} su_{201} isolates were crossed to selected strains containing linkage group VII markers: To select against loss of the suppressor in td_{201} su_{201} parents, the properithecial cultures, taken directly from ascospore-initiated stocks, were grown on minimal Westergaard agar. From each cross 48 to 200 random ascospore progeny were isolated on agar containing all necessary supplements. The nutritional and morphological phenotypes of the germinated progeny were scored on specifically supplemented agar slants. Supplements were added in the following concentrations: amino acids, 150 μg per ml, except L-arginine, 500 μg per ml; pyrimidines and adenine, 100 μg per ml; pantothenic acid, 10 μg per ml. The marked strains used for mapping the td_{201} suppressor mutations were furnished generously by the Fungal Genetics Stock Center at Darmouth College and by DR. D. D. PERKINS.

Preparation of TSase extracts: Small batches of mycelia for crude extract TSase assay were grown at 30°C on a New Brunswick shaker in 1 liter flasks which contained 200 ml minimal medium (supplemented when necessary with 150 μg DL-tryptophan or 40 μg indole per ml). Cultures of fast-growing prototrophs isolated from td_{201} and cultures of the wild type were harvested from supplemented or unsupplemented medium after 24 to 48 hr incubation. Suppressed and unsuppressed td_{201} mutant cultures were also harvested after 24 to 48 hr incubation when grown on supplemented medium. On minimal medium, however, suppressed td_{201} mutants, being slow-growers, were harvested after 72 to 240 hr incubation. The method of harvesting and lyophilizing the mycelia and preparing crude extracts was that of MOHLER and SUSKIND (1960).

Assay procedures: TSase reaction 1 activity was assayed by measuring the disappearance of indoleglycerolphosphate (InGP) and reaction 2 activity by the disappearance of indole. Reaction 3f was assayed by determining indole formation from InGP. Unless otherwise indicated all TSase assays followed published procedures (MOHLER and SUSKIND 1960; SMITH and YANOFSKY

1962) with the following modifications: reaction 3f assay mixtures contained 250 μmoles phosphate buffer, pH 6.2, and 4×10^{-3} M EDTA in a final volume of 1.2 ml; to measure the small amounts of indole produced in this reaction, only 2 ml of toluene were used to extract the indole and 1 ml of the toluene layer was added to 1 ml of 95 percent ethanol and 0.5 ml of Erlich's reagent for the standard indole colorimetric assay. Reactions 1 and 2 remain linear up to 80 percent and 95 percent conversion of substrate respectively, while reaction 3f activity becomes nonlinear after 15 to 20 percent conversion of substrate. One unit of enzyme catalyzes the utilization or formation of 0.1 μmole of the respective compound per hr at 37°C.

Protein was determined by the microbiuret method (ZAMENHOF 1962).

RESULTS

A. *General characterization of the* td_{201} *suppressor system:* Two classes of tryptophan-independent strains were isolated from td_{201} following UV treatment. One class (designated td_{201}R), represented by seven isolates, appeared on the minimal medium sorbose agar isolation plates within 48 hr incubation. Unlike the parental td_{201} these isolates did not accumulate indoleglycerol (InG) and they grew at a wild-type rate in minimal medium aerated culture. Quantitative studies of two of these strains on minimal medium confirmed their wild-type growth rate and revealed that growth inhibition caused by DL-tryptophan (or indole) is similar to that observed with wild-type strains (Figure 1).

A second class of tryptophan-independent strains, (designated td_{201} su_{201}), of which 11 were isolated, appeared on the minimal medium sorbose agar plates after 3 to 5 days incubation. In minimal medium, these culture accumulate InG and exhibit a growth lag and a lower growth rate. On minimal medium supplemented with DL-tryptophan (or indole) these strains, like td_{201}, grow at a wild-type rate (Figure 1).

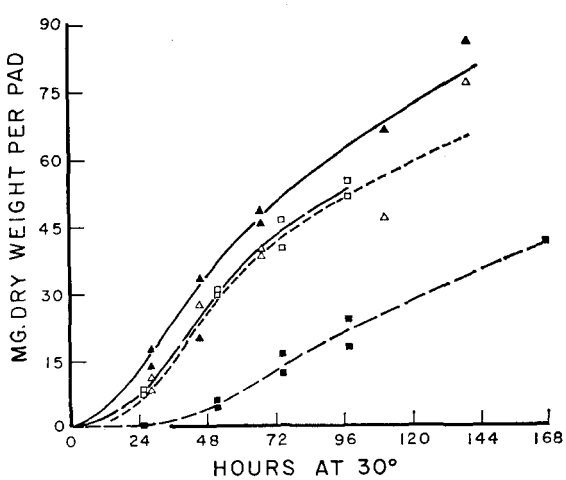

FIGURE 1.—Growth comparison of fast-growing isolate $td_{201}R_2$ and slow-growing isolate td_{201} $su_{201\text{-}8}$; ▲ $td_{201}R_2$ in minimal medium; △ $td_{201}R_2$ in minimal medium + 150 μg DL-tryptophan per ml; ■ $td_{201}su_{201\text{-}8}$ in minimal medium; □ $td_{201}su_{201\text{-}8}$ in minimal medium + 150 μg DL-tryptophan per ml. Another fast-growing isolate, $td_{201}R_7$, gave results similar to those with $td_{201}R_2$. Growth response of these strains in minimal medium plus indole (20 μg per ml) was similar to that in tryptophan-supplemented medium.

TABLE 1

Characterization of crude extract TSase of fast-growing and slow-growing prototrophs isolated from td_{201}

Strain	Specific activity			Ratio of activities		
	Reaction			Reaction		
	1	2	3f	1	2	3f
td_{201} R_7	0.37	0.70	0.0068	54	100	1.0
td_{201} $su_{201\text{-}8}$	0.13	7.00	<0.0005	1.7	100	0
5297a (wild type)	0.51	1.10	0.0120	46	100	1.1
td_{201}	<0.01	6.35	<0.0005	0	100	0

TSase specific activities of representative homocaryotic fast-growing and slow-growing prototrophs isolated from td_{201} are compared to those of the parent mutant and of the wild type. See METHODS for growth and extract preparation.

Crude extracts of all fast-growing tryptophan-independent isolates, unlike those of td_{201}, contain TSase reactions 1 and 3f activity in addition to the level of reaction 2 activity characteristic of the wild-type enzyme (Table 1). Crude extract TSase specific activities and CRM-levels of ascospore cultures of all fast growers were indistinguishable from those of wild-type controls.

In contrast, crude extracts of all slow-growing isolates retain the high TSase reaction 2 specific activity characteristic of td_{201} and in addition they possess 10 to 20 percent of wild-type levels of reaction 1 specific activity (Table 1). Reaction 3f activity was not detected in crude extracts of slow-growing strains, but it was found in partially purified preparations of the enzyme.

Tetrad analysis of crosses of two fast-growers, td_{201} R_4 and td_{201} R_7, by wild-type 5297a yielded only two segregant classes, 8 (T^+) and 4(T^+):4(T^-) (Table 2). Since the original isolates very likely were heterocaryotic, the presence of the 4(T^+):4(T^-) class is not surprising. Ascospore cultures of all fast-growers were crossed to wild-type strains and random spore progeny were plated on sorbose agar containing a low concentration of DL-tryptophan. No td_{201} recombinant microcolonies were observed among the 250 to 1000 progeny tested from each

TABLE 2

Tetrad analysis of slow-growing and fast-growing prototrophs isolated from td_{201}

Cross	Number of tetrads		
	4T+:4T+/2	4T+:2T+/2:2T-	4T+:4T-
td_{201} $su_{201\text{-}2}$ × 5297a	3	11	1
td_{201} $su_{201\text{-}4}$ × 5297a	1	6	5
td_{201} $su_{201\text{-}6}$ × 5297a	1	8	5
td_{201} $su_{201\text{-}7}$ × 5297a	0	10	2
td_{201} $su_{201\text{-}8}$ × 5297a	2	7	2
	8T+	6T+:2T-	4T+:4T-
td_{201} R_4 × 5297a	7	0	7
td_{201} R_7 × 5297a	13	0	0

Nutritional phenotypes of spore progeny: T+, wild-type growth rate in the absence of tryptophan at 30°C; T+/2, slow growth rate in the absence of tryptophan at 30°C; T−, no growth in the absence of tryptophan.

cross (cf. Section B below). Thus the combination of nutritional, enzymatic, and genetic data suggests that the fastgrowing prototrophs derived from td_{201} are reversions at the primary mutant site, although they could represent closely linked or intragenic suppressor mutations in which virtually complete phenotypic repair of the genetic damage has been effected.

In only five of 11 crosses of slow-grower by 5297a was germination sufficient to allow unequivocal tetrad analysis. The high incidence of $4(T^+) : 2(T^{+/2}) : (2T^-)$ tetrads in all five successful analyses indicates that these slow-growers are of the td_{201} genotype and carry an extragenic suppressor which is unlinked or distantly linked to the *td* locus (linkage group II) (Table 2). Ascospore cultures of these and two additional slow-growing strains were crossed again to wild-types 5297a or 74A. Recovery of unsuppressed td_{201} progeny in random spore analyses at expected frequencies confirmed the presence of unlinked or distantly linked td_{201} suppressor mutations.

B. *Genetic studies of the* td_{201} *suppressors: Allelism tests:* To determine whether the independently occurring td_{201} suppressor mutations represented alterations at the same locus or at different loci, crosses of $td_{201}\ su_{201\text{-a}}$ ($su_{201\text{-a}}$, any of the seven characterized td_{201} suppressors other than $su_{201\text{-8}}$) $\times$ $td_{201}\ su_{201\text{-8}}$ and $td^+\ su_{201\text{-a}} \times td_{201}\ su_{201\text{-8}}$ on minimal Westergaard agar were examined for frequencies of $td_{201}\ su^+{}_{201}$ recombinants. Were nonlinkage of individual suppressors to obtain, crosses of the first type would yield 25 percent $td_{201}\ su^+{}_{201}$ recombinants, while crosses of the second type would yield 12.5 percent $td_{201}\ su^+{}_{201}$ recombinants. Up to 200 random single-ascospore isolates were tested from each cross with no recovery of $td_{201}\ su^+{}_{201}$ recombinants (Table 3). To assess possible selection against $td_{201}\ su^+{}_{201}$ progeny in these crosses, a number of indirect control crosses of $td_{201}\ su_{201}$, $td_{201}\ su^+{}_{201}$, $td^+\ su^+{}_{201}$ and $td^+\ su_{201}$ strains in various combinations on minimal Westergaard agar were examined for expected and observed frequency of $td_{201}\ su^+{}_{201}$ progeny. These crosses indicated that an adverse selection of 60 percent may occur against $td_{201}\ su^+{}_{201}$ progeny in crosses of $td_{201}\ su_{201\text{-8}} \times td_{201}\ su_{201\text{-a}}$ and the data are corrected accordingly. On the other hand, little or no selection was indicated for those crosses involving a $td^+\ su_{201\text{-a}}$ parent.

The pooled data, corrected for selection against td_{201} progeny, localize $su_{201\text{-1}}$, $su_{201\text{-2}}$, $su_{201\text{-4}}$, $su_{201\text{-6}}$, $su_{201\text{-7}}$, and $su_{201\text{-8}}$ and $su_{201\text{-11}}$ on the same linkage group.

In order to obtain better resolution in the suppressor allelism tests, ascospores from crosses of $td_{201}\ su_{201\text{-a}} \times td_{201}\ su_{201\text{-8}}$ and $td^+\ su_{201\text{-a}} \times td_{201}\ su_{201\text{-8}}$ were plated on sorbose agar containing 0.75 μg DL-tryptophan per ml. The $td_{201}\ su^+{}_{201}$ progeny from control crosses ($td_{201}\ R_4 \times td_{201}$ and $td_{201}\ su_{201\text{-8}} \times td_{201}$) formed microcolonies which appeared at the frequency predicted by random single spore analysis of these crosses and which showed a graded growth response to concentrations of DL-tryptophan up to 10 μg per ml in the sorbose medium. At 0.75 μg DL-tryptophan per ml these microcolonies, if incubated approximately 72 hours or less at 30°C, were stimulated by the addition of crystals of DL-tryptophan or indole and could be isolated and characterized as td_{201} mutants. If incubated longer, however, the microcolonies failed to survive. Since the time required to distinguish putative microcolonies from suppressed mutants was four to five days at 30°C, direct

TABLE 3

Allelism test of independently isolated td_{201} suppressors by random single spore analysis for $td_{201}\ su^{+}_{201}$ *recombinants*

Cross	Number of spores T+/2	Number of spores T-	Percent germination	Cross	Number of spores T+ or T+/2	Number of spores T-	Percent germination	Maximum distance $su_{201\text{-}8}$ to $su_{201\text{-}a}$*
$td_{201}\ su_{201\text{-}8}$								
$\times\ td_{201}\ su_{201\text{-}1}$	119	0	82					35.0
				$td_{201}\ su_{201\text{-}8}$				
$\times\ td_{201}\ su_{201\text{-}2}$	154	0	55	$\times\ td^{+}\ su_{201\text{-}2}$	98	0	68	17.4
$\times\ td_{201}\ su_{201\text{-}4}$	126	0	62	$\times\ td^{+}\ su_{201\text{-}4}$	121	0	60	18.0
$\times\ td_{201}\ su_{201\text{-}6}$	189	0	87	$\times\ td^{+}\ su_{201\text{-}6}$	191	0	70	11.8
$\times\ td_{201}\ su_{201\text{-}7}$	140	0	79	$\times\ td^{+}\ su_{201\text{-}7}$	126	0	78	16.8
$\times\ td_{201}\ su_{201\text{-}11}$	134	0	93					31.2

See Table 2 footnote for nutritional phenotype designation of spore progeny.

* $su_{201\text{-}a}$–$su_{201\text{-}8}$ map distances (in standard map units) were calculated at the 95 percent confidence level by the chi-square method.

isolation and characterization was not possible. Therefore, ascospores from experimental crosses also were plated on sorbose agar containing 150 μg DL-tryptophan per ml in order to correct for the present tryptophan-independent microcolonies.

The corrected microcolony frequency on "low" tryptophan sorbose agar is zero for several hundred progeny tested in each experimental cross (Table 4). These results indicate that the seven independently occurring td_{201} suppressors all reside on the same linkage group in a region totalling no more than 5 map units (95 percent confidence). Thus the suppressor genes appear to be allelic or closely linked by this recombinational analysis.

Suppressor site-specificity: To determine the site-specificity of the td_{201} suppressor genes with respect to different TSase mutants, wild-type strains carrying either $su_{201\text{-}2}$, $su_{201\text{-}6}$, or $su_{201\text{-}7}$ were crossed to *td* mutants of the CRM negative and CRM positive phenotype. If cross-suppression had occurred with any of these TSase mutants, approximately 25 percent of the progeny ($td^-\ su^-$) from the respective cross should have grown, probably quite slowly, on minimal medium, while 25 percent of the progeny ($td^-\ su^+$) would have required tryptophan and 50 percent would have been wild type ($td^+\ su^\pm$). If the suppressor had no effect, a 1:1 segregation of wild type ($td^+\ su^\pm$) and tryptophan requiring ($td^-\ su^\pm$) progeny should have occurred. Random single spore analysis of each cross revealed no slow-growing progeny, with the exception of control crosses of $td^-\ su_{201}$

TABLE 4

Detection of td_{201} *recombinants by random spore plating on sorbose agar*

Cross	Number of spores in presence of 0.75 μg DL-tryp./ml: T+ or T+/2 colonies	0.75 μg DL-tryp./ml: Micro-colonies	150 μg DL-tryp/ml: T+ or T+/2 colonies	150 μg DL-tryp/ml: Micro-colonies	T- micro-colonies corrected	Maximum distance $su_{201\text{-}8}$ to $su_{201\text{-}a}$*
$td_{201}\ su_{201\text{-}8}$						
× $td_{201}\ su_{201\text{-}1}$	2291	5	1060	2	1	2.4
× $td_{201}\ su_{201\text{-}2}$	127	0				
× $td_{201}\ su_{201\text{-}4}$	940	0				
× $td_{201}\ su_{201\text{-}6}$	868	0				
× $td_{201}\ su_{201\text{-}7}$	880	0				
× $td_{201}\ su_{201\text{-}11}$	1605	1	92	0	0	2.2
			Totals 1152		2 (or 1.7 micro-/1000 normal colonies)	
$td_{201}\ su_{201\text{-}8}$						
× $td^+\ su_{201\text{-}2}$	2054	3	238	0	0	2.0
× $td^+\ su_{201\text{-}4}$	2380	2	857	2	0	1.6
× td^+-$su_{201\text{-}6}$	2344	3	1139	2	0	1.6
× $td^+\ su_{201\text{-}7}$	2666	2	2138	3	0	1.6
			Totals 4372		7 (or 1.6 micro-/1000 normal colonies)	

* See Table 2 and Table 3 footnotes.

TABLE 5

Site-specificity of su_{201-2} *tested by crosses of* $m^- td^+ su_{201-2} \times m^+ td^- su^+_{201}$ *strains (random single-spore analysis)*

Cross	CRM	*td* mutant type: residual enzyme activity	Phenotype: T^+m^-	T^+m^+	T^-m^-	T^-m^+	$T^{+/14}m^+$ or m^-	Percent germination
$td^+ su_{201-2}$								
$\times td_{201}$	+	reaction 2	8	2	0	4	7($T^{+/2}m^+$) 1($T^{+/2}m^-$)	92
$\times td_{141}$	+	reaction 2	16	5	2	16	0	81
$\times td_{37}$ R	+	reaction 2	21	1	3	18	0	90
$\times td_{138}$ R	+	reaction 2	5	2	5	20	0	67
$\times td_{71}$	+	reaction 3f	16	1	2	14	0	69
$\times td_{7}$	+	reaction 3f	16	0	1	17	0	69
$\times td_{6}$	+	—	19	0	0	21	0	56
$\times td_{1}$	—	—	22	1	1	21	0	92
$\times td_{16}$	—	—	1	0	2	9	0	25

See Table 2 footnote for nutritional phenotype of spore progeny. The designation $T^{+/14}$ indicates noticeable growth in the absence of tryptophan after 14 days incubation. Morphological phenotype of spore progeny: m^-, microconidial, due to marker m^- closely linked to *td*; m^+, macroconidial, due to wild-type allele of m^-. Crosses of td^+su_{201-6} and td^+su_{201-7} strains by the TSase mutants yielded similar results.

$\times td_{201}$ in each series (Table 5). Rather, a 1:1 segregation of mutant and wild-type progeny was generally observed. Had the suppressors elicited wild-type growth rates in *td* mutants other than td_{201}, such strains could have been distinguished from true td^+ progeny which carried a microconidial marker, m^-, resembling fluffy, closely linked to the *td* locus. Low frequencies of fast-growing m^+ (macroconidal) progeny can be attributed to crossover of *td* and *m* loci and possible modifier effects. Several indole-utilizing mutants, including td_{141}, which like td_{201} forms high levels of CRM with reaction 2 activity, are not affected by these td_{201} suppressors. Thus the td_{201} suppressors appear to be specific for the td_{201} mutant site.

Mapping of the td_{201} *suppressor genes:* su_{201-2} was tested for linkage to markers in all seven linkage groups, in a series of crosses between td_{201} su_{201-2} and strains containing the following markers: *aur*, *fr*: aurescent, frost (isolation numbers 34508, B110; IR, IL); *arg-5*, *fl*: arginine-5, fluffy (27947, IIC, IIR); *pyr-4*: pyrimidine-4 (36601, IIL); *sc*: scumbo (5801, IIIR); *vel*: velvet (B18, IIIR); *pyr-2*, col-4: pyrimidine-2, colonial-4 (38502, 70007; IVR); *pyr-1*, *mat:* pyrimidine-1, mat (H263, B57; IVC, IVR); *lys-1*, bis: lysine-1, biscuit (33933, B6; VC, VR); *ad-8*, *ylo*: adenine-8, yellow (Y152-M7, Y30539y; VIL); *pan-2*: pantothenate-2 (Y-153-M66, VIR); *sk*: skin (B106, VIIR); *arg-10*, *sk*: arginine-10, skin (B317, B106; VIIR); *me-7*: methionine-7 (4894, VIIC). (R = right arm, L = left arm, C = centromere region.)

Only with the marker *sk* in linkage group VII was recombination with the suppressor significantly less than 50 percent (13 recombinants/57 total number of progeny). Accordingly, crosses of all independently occurring td_{201} su_{201} strains by group VII marker strains were examined for evidence of suppressor-marker

linkage. The 2-point crosses with a *me-7* (VII centromere) marker strain (Table 6) indicated a su_{201}-*me-7* linkage of 13 to 28 map units (mean value 18.0). Three-point crosses of all td_{201} su_{201} strains by an *arg-10*, *sk* marker strain (VII right) were analyzed. The five successfully analyzed suppressors were all located 5 to 8 map units left of *arg-10* (mean 6.6) and 19 to 28 units left of *sk* (mean 21.6). For each cross the crossover values of the *su–arg-10* and *su–sk* intervals can be normalized using the ratio of these values to the crossover value of the *arg-10–sk* interval. Such normalization positions the suppressors even more closely to one another (Table 6). The slight variations in *su*–marker recombination values may represent sampling error, differential crossing-over behavior, or slight but real differences in suppressor location. The sum of the mean map distances of the me-7–*su* plus the *su–arg-10* interval (24.6 units) or *su–sk* interval (39.6 units) found here agrees well with the published values (ca. 19 units and 35 units [PERKINS 1959]). Also the *arg-10–sk* map distance (mean value 14.9 units) is quite close to published values (ca. 16 units, [PERKINS 1959]), and as might be expected with the small sample size, no double crossovers were detected within the *su–arg-10–sk* interval.

One 3-point cross with $su_{201\text{-}4}$ gave anomalous results. Several recombinant

TABLE 6

Analysis of recombination between various td_{201} *suppressors and markers on linkage group VII*

Genotype of progeny	Number of progeny from cross using specified suppressor											
	Cross: *arg-10 sk* × $td_{201}su_{201-x}$						Cross: *me-7* × $td_{201}su_{201-x}$					
	su_{201-1}	201–2	201–4	201–6	201–7	201–11	su_{201-2}	201–8	201–6	201–4	201–11	201–7
td⁻ *su*⁻ *a*⁺ *b*+	18	21	23	15	20	18	28	25	16	22	12	15
td⁻ *su*+ *a*⁻ *b*⁻	17	15	19	17	18	18						
td⁻ *su*⁻ *a*⁺ *b*⁻	2	6	0	3	2	2						
td⁻ *su*+ *a*⁻ *b*+	4	4	0	3	4	4	21	7	15	20	21	16
td⁻ *su*⁻ *a*⁻ *b*+	0	0	0	0	0	0	5	2	1	2	7	3
td⁻ *su*+ *a*⁺ *b*⁻	0	0	0	0	0	0						
td⁻ *su*+ *a*⁺ *b*+	2	2	0	1	2	2	8	3	5	4	6	2
td⁻ *su*⁻ *a*⁻ *b*⁻	1	2	2	1	1	1						
td⁺ *su*± *a*⁺ *b*+	19	22	21	26	24	22	34	19	25	17	26	19
td⁺ *su*± a⁻ *b*⁻	25	19	18	18	17	19						
td⁺ *su*± *a*⁺ *b*⁻	2	2	3	3	2	2						
td⁺ *su*± *a*⁻ *b*+	1	2	0	1	2	0	34	20	27	13	17	23
A. % recombination, *su–a*	6.8	8.0	4.6	5.0	6.4	6.7	21	14	16	13	28	14
B. % recombination, *su–b*	20.5	28.0	4.6	20.0	19.2	20.2						
C. % recombination, *a–b*	13.6	20.0	0.0	15.0	12.8	13.3						
Ratio A/C	0.50	0.40	..	0.33	0.50	0.51						
Ratio B/C	1.51	1.40	..	1.33	1.50	1.51						
Percent germination	95	99	90	92	96	92	69	79	94	81	92	81

a signifies *arg-10* or *me-7*; *b* signifies *sk*.

classes are absent, perhaps because of adverse selection. The remaining 3-point cross with $su_{201\text{-}8}$ was sterile. But $su_{201\text{-}8}$ and $su_{201\text{-}4}$ showed the predicted degree of linkage with *me-7* and are assumed to map in the same region as the remaining suppressors.

The su_{201} linkage data are mapped in Figure 2. These results, besides mapping the td_{201} suppressors on the right arm of linkage group VII, corroborate the findings on recombination between the td_{201} suppressors which suggested their allelism or close linkage.

No effect of the td_{201} suppressor was apparent on the nine biochemical and ten morphological markers examined. These results are additional evidence for the stringent site-specificity of the td_{201} suppressor mutations.

DISCUSSION

The data indicate that TSase mutant td_{201} is susceptible to the action of a group of unlinked suppressor mutations which restore a low level of wild-type TSase activity and thereby allow the mutant to grow slowly on unsupplemented medium. The suppressors appear to act by repair of the structure of the mutant enzyme (YOURNO and SUSKIND 1964).

The td_{201} suppressors were found to be specific for the td_{201} mutant site. They failed to suppress not only a wide variety of nutritional and morphological markers scattered throughout the genetic map, but also a number of different TSase mutants. Notably, td_{141}, which is phenotypically similar to td_{201}, is not affected by td_{201} suppressors. Suppressors which seem to act by repair of enzyme structure are site-specific in Neurospora (YANOFSKY 1952; YANOFSKY and BONNER 1955) and *E. coli* (GAREN and SIDDIQI 1962; YANOFSKY, HELINSKI, and MALING 1961). In contrast, suppressors which act indirectly (cf. Introduction; KAKAR 1963; HOWARTH 1958) may suppress all mutants lacking a common enzymatic function. The rigid site-specificity of suppressors which repair the primary structure of a mutant enzyme is a property predicted by either the mistake hypothesis or the nonsense-to-sense hypothesis of suppressor action

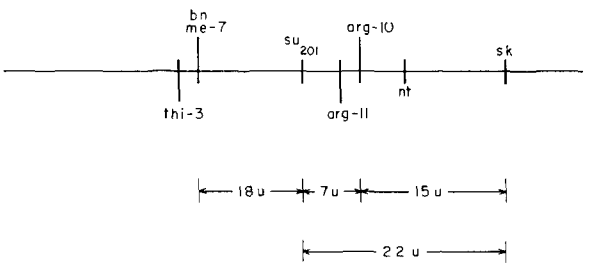

FIGURE 2.—Map of linkage group VIIR incorporating the su_{201} region. The map is taken from PERKINS (1959) with the exception of *me-7*, which shows no recombination with button (*bn*) (PERKINS, unpublished). The centromere is in the region of *thi-3*. The mean map distances between markers and the su_{201} mutations are shown. The *me-7*–su_{201} interval is approximately 6 units longer than predicted by the map scale. This discrepancy may represent sampling error or higher frequency of recombination in our crosses.

(YOURNO and SUSKIND 1964). On the other hand, these site-specific td_{201} suppressors might be expected to suppress any mutation, regardless of locus, which requires a suppressor mechanism and an amino acid switch identical to that of the td_{201} mutation. The absence of an effect of td_{201} suppressors on those mutants tested suggests that such conditions were not met.

It is of considerable interest that a limited number of cross-suppressible mutants has been found in the TSase system of Neurospora (YANOFSKY and BONNER 1955) as well as in the alkaline phosphatase system of *E. coli* (GAREN and SIDDIQI 1962). A high frequency of cross-suppression by site-specific super-suppressors has been observed in Saccharomyces (HAWTHORNE and MORTIMER 1963). The suppressors appear to affect many but not all mutants at several different loci.

The td_{201} suppressors described herein appear to be allelic or closely linked. However, among several newly isolated suppressed td_{201} mutants we have obtained one strain which shows markedly slower growth on minimal medium and which does not appear to be allelic with the linkage group VII td_{201} suppressors. ST. LAWRENCE (personal communication) has also found several td_{201} suppressor mutations at two unlinked loci; one group appears to be allelic with our suppressors on VII. YANOFSKY and BONNER have reported that suppressors of Neurospora TSase mutant td_2 occupy at least four widely separate loci (1955), and LACY (1962) reports td_2 suppressors on I and III. Suppressors specific for the same site in Saccharomyces appear to occupy several distinct loci (KAKAR 1963; HAWTHORNE and MORTIMER 1963). On the other hand, suppressors of *E. coli* CRM negative alkaline phosphatase mutants appear to reside in a common locus (GAREN and SIDDIQI 1962). DAWSON and SMITH-KEARY (1960) have reported that site-specific suppressors of leucineless mutants of *Salmonella typhimurium* reside in a single region closely linked to but distinct from the leucine locus. Such genetic data from diverse suppressor systems simply may reflect a multiplicity of mechanisms capable of affecting the translating or even the transcribing of genetic information.

SUMMARY

Tryptophan synthetase (TSase) mutant td_{201} requires either indole or L-tryptophan for growth. It produces high levels of crossreacting material (CRM) which retains indole + L-serine → tryptophan activity. Two classes of tryptophan-independent colonies were found following ultraviolet irradiation and plating of conidia on minimal sorbose agar: (a) isolates which exhibit the genetic, nutritional, and enzymatic properties of the wild type: (b) isolates which grow slowly on minimal medium and which are stimulated by DL-tryptophan or indole. Extracts of all slow-growing strains, while retaining the high indole + L-serine → tryptophan activity characteristic of td_{201}, contain about 10 percent of the wild-type level of indoleglycerolphosphate + L-serine → tryptophan activity. Seven independent isolates of type (b) carried an unlinked suppressor as judged by tetrad or random-spore analysis of the progeny from crosses of wild-type by slow-growers. The seven suppressors are allelic or closely linked, since no

td_{201} $su^{+}{}_{201}$ progeny were detected by random single-spore analysis or mass plating of spores from td_{201} $su_{201\text{-}8}$ × td_{201} $su_{201\text{-}a}$ or td_{201} $su_{201\text{-}8}$ × td^{+} $su_{201\text{-}a}$ crosses. Furthermore the suppressors show approximately the same degree of linkage with several markers in linkage group VII. The td_{201} suppressors exhibit stringent site-specificity. They do not affect a number of different biochemical and morphological mutants or several other TSase mutants.

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