# SUPERSUPPRESSIBLE MUTANTS IN NEUROSPORA: MUTANTS AT THE *tryp-1* AND *tryp-2* LOCI AFFECTING THE STRUCTURE OF THE MULTIENZYME COMPLEX IN THE TRYPTOPHAN PATHWAY

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IN Neurospora the enzymes of tryptophan biosynthesis, anthranilate synthetase, N-(5′-phosphoribosyl) anthranilate (PRA) isomerase, and indole-3-glycerol-phosphate (InGP) synthetase are organized in a multienzyme complex (DeMoss and Wegman 1965). This complex is controlled by two unlinked genes, *tryp-1* and *tryp-2*; it is a hexamer with a sedimentation constant of 10 S, composed of four protein subunits coded by the *tryp-1* gene and two by the *tryp-2* (DeMoss, Jackson and Chalmers 1967; Gaertner and DeMoss 1969; Arroyo-Begovich and DeMoss 1969). Mutation in the *tryp-2* gene causes the loss of anthranilate synthetase and in most cases the accumulation of a novel form of PRA isomerase and InGP synthetase with a sedimentation constant of 7 S (DeMoss, Jackson and Chalmers 1967; Chalmers and DeMoss 1970). This form has been identified as a tetramer of the *tryp-1* gene product (Arroyo-Begovich and DeMoss 1969; Gaertner and DeMoss 1969). In contrast, mutations at the *tryp-1* locus exhibit a variety of phenotypes with alterations in any or all of the activities of the complex (DeMoss, Jackson and Chalmers 1967). A major class and DeMoss 1970). When extracts of these *tryp-1* and *tryp-2* mutants are mixed, an active wild-type complex is generated *in vitro*, indicating that the genetic change in these strains leads to the production of completely inactive gene products (Arroyo-Begovich and DeMoss 1969; Chalmers and DeMoss 1970).

A number of observations have been made in Neurospora that mutations which cause complete loss of enzyme function (Seale 1968), or loss of antigenic cross-reaction (Terry 1966), or pleiotropic defects in multienzyme complexes (Case of these mutants lacks all three activities and accumulates the catalytically inactive *tryp-2* gene product (Arroyo-Begovich and DeMoss 1969; Chalmers and Giles 1968) are often suppressed by super suppressors analogous to the nonsense suppressors described in bacteria (Garen 1968) and in yeast (Hawthorne 1969). It may be assumed that these phenotypes are due to the premature termination of the protein product of the genes in question, resulting in peptide fragments with little or no biological activity. As the specific association of the products of the *tryp-1* and *tryp-2* genes is obligatory for the expression of anthranilate

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synthetase (CHALMERS and DEMOSS 1970), the extreme phenotypes of the totally inactive *tryp-1* mutants and the 7 S class of *tryp-2* mutants may be due to mutations of this type. The genetic and biochemical data reported here suggest that indeed such mutants do occur at these loci and that the disruption of the complex and the loss of its activity may be explained by this mechanism.

## MATERIALS AND METHODS

The majority of the tryptophan-requiring (tryp$^-$) mutants used in this investigation are from the laboratory of Dr. J. A. DEMOSS and were induced in the Oak Ridge derivatives of the ST. LAWRENCE wild types or crossed into this background. For the most part, these mutants were induced by ultraviolet light or nitrous acid, and all revert to prototrophy by irradiation with ultraviolet light (CHAMBERS 1968). Certain strains with the suffix *TB* were isolated in a *cot-1(C102t)* background by Dr. THOMAS BAKER. These mutants have been crossed into the Oak Ridge background. The mutant *tryp-2(1151)* was obtained from the Fungal Genetics Stock Center. The *am17* mutant was induced in the ST. LAWRENCE background (STADLER 1966). The super suppressors *am17;ssu-1(WRN33)*, *am17;ssu-2(WRN35);al-2*, *am17;ssu(WRU85)* and *am17;ssu-3 (WRU118);al-2* were also induced in this background (SEALE 1968).

Stocks were grown and maintained on slants of minimal medium (VOGEL 1964), solidified with 1.5% Difco Agar and supplemented as needed with 150 μg/ml L-tryptophan and 450 μg/ml L-alanine. For enzyme studies, mycelium was grown on a rotary shaker at 30°C in 3-liter Fernbach flasks containing one liter of liquid VOGEL's medium supplemented as above when appropriate. Crosses were performed on synthetic crossing medium (WESTERGAARD and MITCHELL 1947) containing 500 μg/ml L-tryptophan and 450 μg/ml L-alanine. In refractory cases, crosses were carried out on Difco cornmeal agar with dextrose, with the same supplementations (KAPLAN, SUYAMA and BONNER 1964).

The suppressibility of *tryp*$^-$ mutants could be assessed when ascospores from crosses of *tryp*$^-$ mutants to suppressed *am17* strains were plated on sorbose medium (LESTER and GROSS 1958) supplemented with alanine. Seventy-five percent of the ascospores were expected to be tryptophan-independent if the strain was suppressed, provided that the *tryp*$^-$ mutant and the super suppressor assort independently. The super suppressors utilized in these experiments were known to be unlinked to either the *tryp-1* or the *tryp-2* locus.

When the fertility of the crosses permitted, asci were analyzed. Prototroph to auxotroph patterns of 6$^+$:2$^-$ are diagnostic for suppression. From these same crosses, *am17;tryp*$^-$ double mutants were isolated and irradiated with ultraviolet light to determine the coincident reversion frequency for the *am17* and the *tryp*$^-$ alleles (SEALE 1968). In these experiments 0.02M glycine was added to the medium to inhibit the residual growth of *am17*.

Crude extracts of 50% ammonium sulfate fractions were made from lyophilized mycelial powder in .05 M potassium phosphate buffer at pH 7.0 containing $10^{-4}$ M EDTA and $2 \times 10^{-4}$ M dithiothreitol according to the methods of DEMOSS (1965) and CHALMERS and DEMOSS (1970). Anthranilate synthetase was measured by the fluorometric method of DEMOSS (1965); InGP synthetase was determined according to the method of WEGMAN and DEMOSS (1965). Specific activity is expressed as μmole product produced per mg of protein per hour. Protein was estimated by the LOWRY procedure (LOWRY *et al.* 1951).

Zone centrifugation in 5–20% sucrose gradients followed the procedure in DEMOSS, JACKSON and CHALMERS (1967) using crystalline catalase (CalBiochem) as an internal standard. Ten-drop fractions were collected, diluted to 2 ml and assayed. For assays of both anthranilate synthetase and InGP synthetase, 0.3 ml of the diluted fractions were used.

The *in vitro* complex formation assay was carried out according to the methods of ARROYO-BEGOVICH and DEMOSS (1969). The mutant *tryp-2(6)*A was used as the source of the *tryp-1* gene product.

## RESULTS

*Suppression of mutants: recombination and reversion tests:* To test the effect of the *am17* super suppressors on the tryptophan mutants, 36 *tryp-2* mutants were crossed to *am17; ssu-1(WRN33)*. Of these, 28 were of the 7 S class, a class which completely lacks anthranilate synthetase and has no biologically active *trp-2* gene product (DeMoss, Jackson and Chalmers 1967; Chalmers and DeMoss 1970). The remainder included several unclassified *tryp-2* mutants and one mutant, *tryp-2(8)*, which, while lacking anthranilate synthetase, retained PRA isomerase and InGP synthetase in a complex with a sedimentation constant like that of wild type (10 S). Some 20 *tryp-1* mutants were similarly crossed to the suppressed *am17* strain. With the exception of one unclassified mutant, these fell into two categories: one, composed of 13 strains, lacking all three activities of the complex; and another, exhibiting one or more of the activities of the complex, but in an abnormal form. For the most part these altered enzymes have lower sedimentation constants and reduced stabilities compared to the wild-type multienzyme complex. A compilation of the *tryp*$^-$ strains examined and their properties is given in Table 1.

Randomly collected shot ascospores from these crosses were plated at low density on alanine-supplemented medium at 30°C to determine the percentage of tryptophan prototrophs. Those strains which yielded 60% or more prototrophs were selected for further testing (Table 2). When the quality of the crosses permitted, asci were dissected and analyzed. Because of their slower growth rate when compared to wild type, suppressed *tryp*$^-$ mutants could be recognized and were backcrossed to *am17* or to wild type to confirm the presence of the *tryp*$^-$

TABLE 1

tryp-1 *and* tryp-2 *mutants examined*

| |
|---|
| Inactive *tryp-1 mutants**: |
| *tryp-1(2)*, *(3)*, *(6)*, *(7)*, *(8)*, *(9)*, *(10)*, *(11)*, *(13)*, *(14)*, *(15)*, *(18)*, *(31)* |
| Partially active *tryp-1* mutants†: |
| *tryp-1(5)*, *(16)*, *(17)*, *(25)*, *(30)*, *(10575)* |
| Unclassified *tryp-1* mutant: |
| *tryp-1(1151)* |
| *tryp-2* 7 S mutants‡: |
| *tryp-2(2)*, *(3)*, *(6)*, *(10)*, *(11)*, *(13)*, *(14)*, *(15)*, *(17)*, *(19)*, *(22)*, *(26)*, *(29)*, *(30)*, *(33)*, *(36)*, *(37)*, *(38)*, *(40)*, *(41)*, *(43)*, *(44)*, *(46)*, *(48)*, *(50)*, *(51)*, *(55)*, *(31TB)* |
| *tryp-2* 10 S mutant§: |
| *tryp-2(8)* |
| Unclassified *tryp-2* mutants: |
| *cot-1;tryp-2(23TB)*, *cot-1;tryp-2(26TB)* |
| *tryp-2(27)*, *(32)*, *(35)*, *(45)*, *(54)* |

* This class lacks anthranilate synthetase, PRA isomerase, and InGP synthetase.
† This class retains variously anthranilate synthetase, PRA isomerase, or InGP synthetase in abnormal forms.
‡ This class has PRA isomerase and InGP synthetase with a 7 S sedimentation constant.
§ This class has PRA isomerase and InGP synthetase with a 10 S sedimentation constant.

TABLE 2

*Crosses of the* tryp⁻ *mutants by the* am17 *suppressors*

| Strain | Prototrophs* | *tryp*⁻ | Total ascospores | Prototrophs (percent) | Ascus types† |
|---|---|---|---|---|---|
| | | *am17;ssu-1(WRN33)* | | | |
| *tryp-2(10)* | 278 | 134 | 412 | 67% | 6:2 |
| *tryp-2(22)* | 220 | 96 | 316 | 70% | —§ |
| *tryp-2(32)* | 145 | 97 | 242 | 60% | — |
| *tryp-2(41)* | 174 | 65 | 239 | 73% | 6:2, 8:0 |
| *tryp-2(50)* | 66 | 17 | 83 | 79% | — |
| *tryp-2(51)* | 240 | 134 | 374 | 65% | — |
| *tryp-2(31TB)* | 318 | 102 | 420 | 76% | 6:2, 8:0 |
| *tryp-1(3)* | 202 | 125 | 327 | 62% | — |
| *tryp-1(15)* | 317 | 117 | 431 | 73% | 6:2, 8:0 |
| *tryp-1(18)* | 235 | 139 | 374 | 63% | — |
| | | *am17;ssu-2(WRU35);al-2* | | | |
| *tryp-2(10)* | 79 | 32 | 111 | 71%‡ | — |
| *tryp-2(41)* | —§ | — | — | — | 6:2 |
| *tryp-2(31TB)* | 72 | 35 | 107 | 67% | 6:2 |
| | | *am17;ssu(WRU85)* | | | |
| *tryp-2(10)* | 74 | 29 | 103 | 72% | — |
| *tryp-2(41)* | 80 | 28 | 108 | 74%‡ | 6:2 |
| *tryp-2(31TB)* | 84 | 22 | 106 | 79%‡ | 6:2 |

* Tryptophan-independent colonies on alanine medium.
† Asci were analyzed when quality of crosses permitted.
‡ These are backcrosses of putatively suppressed *tryp*⁻ mutants.
§ Not determined or obtained.

allele. From these asci and from the original plates, *am17;tryp*⁻ double mutants were isolated. These double mutants were grown on alanine–tryptophan slants for five days. Conidia from these slants were irradiated with ultraviolet light and plated in minimal medium supplemented with 0.02 M glycine and incubated at 25°C. Since the single reversion frequency of the *am17* and *tryp*⁻ mutants is about

TABLE 3

*Coincident reversion frequencies of* am17 *and* tryp⁻ *mutants*

| Strain | Total conidia $\times 10^{-6}$ | Survival (percent) | Prototrophs* | Prototrophs/$10^6$ surviving conidia |
|---|---|---|---|---|
| *am17;tryp-2(10)* | 25.6 | 46% | 36 | 3.1 |
| *am17;tryp-2(22)* | 73.0 | 61% | 18 | 0.40 |
| *am17;tryp-2(32)* | 16.5 | 39% | 0 | 0.0 |
| *am17;tryp-2(50)* | 9.1 | 29% | 9 | 3.4 |
| *am17;tryp-2(51)* | 22.8 | 57% | 10 | 0.77 |
| *am17;tryp-2(31TB)* | + | + | + | ++ |
| *am17;tryp-1(18)* | 16.5 | 43% | 19 | 2.7 |
| *am17;tryp-1(3)* | 27.2 | 47% | 0 | 0.0 |

* Colonies growing on minimal sorbose + .02 M glycine at 25°C.
† Qualitative data of T. LAFFLER (unpublished experiments).

$10^{-5}$ prototrophs per surviving conidia (SEALE 1968), the coincident reversion frequency, if both mutants reverted by independent mechanisms would be in the neighborhood of $10^{-10}$. Hence, coincident reversion frequencies significantly greater than that expected for independent events indicate suppression. This interpretation is supported by the observation that the coincident reversion of *am17*; *tryp-3 (td140)* double mutants is solely by means of super suppressors (SEALE 1970). Table 3 gives the reversion data for all strains yielding 60% or more prototrophs when crossed to the *ssu-1(WRN33)* suppressor, except *tryp-2(41)* and *tryp-1(15)* which were not tested in this manner. Of those strains selected in the preliminary screening, all except *tryp-2(32)* and *tryp-1(3)* reverted coincidentally with *am17* in this experiment.

Interestingly enough, during a search for new super suppressors, 19 *am17;tryp* double mutants, derived from mutants not suppressed by *ssu-1(WRN33)* in the preliminary screening, were irradiated with ultraviolet light. In none of the cases examined were coincident revertants observed.

Those mutants which gave evidence of suppression by the above tests were crossed to three other suppressed *am17* stocks. Ascospores from these crosses were plated on minimal medium supplemented with alanine and scored for tryptophan independence. The results with the suppressors *ssu-2(WRU35)* and *ssu(WRU85)* are given in Table 2. A fourth suppressor *ssu-3(WRU118)* restored no activity to any of the *tryp*$^-$ alleles tested. In the case of *tryp-2(41)*, strains carrying the suppressor could be unambiguously isolated from 6:2 asci because of the simultaneous segregation of *am17* and *al-2* which is linked to *ssu-2* and *ssu-3*. In certain other cases too, putatively suppressed *tryp*$^-$ mutants were selected and backcrossed to *am17* to confirm the presence of the *tryp*$^-$ allele and the suppressor. These data rather than those of the original plating are tabulated, as the original crosses were quite infertile.

*Restoration of enzyme activities in suppressed mutants:* The restoration of anthranilate synthetase activity in suppressed *tryp-2* and *tryp-1* mutants is shown in Table 4. These data were obtained from concentrated ammonium sulfate fractions prepared for sucrose gradient analysis and diluted 20-fold with buffer. In order to equalize the growth rates of suppressed *tryp*$^-$ and wild-type strains, alanine and tryptophan were routinely added to the growth medium. These conditions apparently repressed the formation of InGP synthetase (LESTER 1968) as the suppressed *am17;tryp*$^+$ strains have normal levels of this enzyme when grown without tryptophan. This effect was also seen when *am17;tryp*$^-$ double mutants were grown in the same medium, although most isolates of *tryp-2(41)* have low specific activity anyway (CHALMERS, unpublished experiments). These enzyme preparations were mixed with catalase and layered on a 5–20% sucrose gradient. The results of these experiments demonstrated the reappearance of anthranilate synthetase in the region of the gradient corresponding to the wild-type multienzyme complex. Furthermore, comparably small amounts of InGP synthetase activity were transferred from the 7 S to the 10 S regions of the gradient. Typical gradients are shown in Figure 1.

Since the specific activity of anthranilate synthetase was lower than that of

TABLE 4

*Specific activities* of mutant and suppressed strains*

| Strain | Anthranilate synthetase | InGP synthetase |
|---|---|---|
| *am17* and suppressors | | |
| *am17* | .25 | .37 |
| *am17;ssu-1(WRN33)* | .33 | .34 |
| *am17;ssu-2(WRU35);al-2* | .17 | .26 |
| *am17;ssu(WRU85)* | .17 | .34 |
| *tryp-2* mutants | | |
| *am17;tryp-2(10)* | 0 | .15 |
| *tryp-2(22)* | 0 | .18 |
| *tryp-2(41)* | 0 | .07 |
| *am17;tryp-2(41)* | 0 | .05 |
| *tryp-2(50)* | 0 | .12 |
| *tryp-2(51)* | 0 | .15 |
| *am17;tryp-2(31TB)* | 0 | .04 |
| *tryp-2(31TB)* | 0 | .08 |
| *tryp-1* mutants | | |
| *tryp-1(15)* | 0 | 0 |
| *tryp-1(18)* | 0 | 0 |
| *ssu-1(WRN33)* | | |
| *tryp-2(10);ssu-1(WRN33)* | .003 | .017 |
| *tryp-2(41);ssu-1(WRN33)* | .005 | .025 |
| *tryp-2(31TB);ssu-1(WRN33)* | .022 | .023 |
| *tryp-1(15);ssu-1(WRN33)* | .064 | .018 |
| *ssu-2(WRU35)* | | |
| *tryp-2(10);ssu-2(WRU35); al-2* | .010 | .029 |
| *tryp-2(41);ssu-2(WRU35); al-2* | .024 | .049 |
| *tryp-2(31TB);ssu-2(WRU35); al-2* | .039 | — |
| *ssu(WRU85)* | | |
| *tryp-2(41);ssu(WRU85)* | .010 | .017 |
| *tryp-2(31TB);ssu(WRU85)* | .014 | .022 |

* Expressed as μmole product/mg protein/hr.
— indicates no measurement.

InGP synthetase, the 4–5 S region of a gradient of *tryp-2(41); ssu-2(WRU35); al-2* was titrated with an excess of *tryp-1* gene product from *tryp-2(6)*, a non-revertible 7 S *tryp-2* mutant. No anthranilate synthetase was generated in this assay (ARROYO-BEGOVICH and DEMOSS 1969), suggesting that no free pool of *tryp-2* gene product with a lowered affinity for the *tryp-1* product was present in the suppressed strain. However, when an extract of this strain was titrated with an excess of free *tryp-2* gene product, anthranilate synthetase was generated showing that the 7 S *tryp-1* product (InGP synthetase) in this strain is unassociated and available for complex formation with wild-type *tryp-2* gene product.

Similarly, the one *tryp-1* strain unambiguously suppressed by *ssu-1* was assayed for the restoration of both anthranilate synthetase and InGP synthetase. These results are also given in Table 4. Although in the wild-type complex anthranilate synthetase and InGP synthetase have similar specific activities, the InGP synthetase activity in this strain is relatively low. When subjected to su-

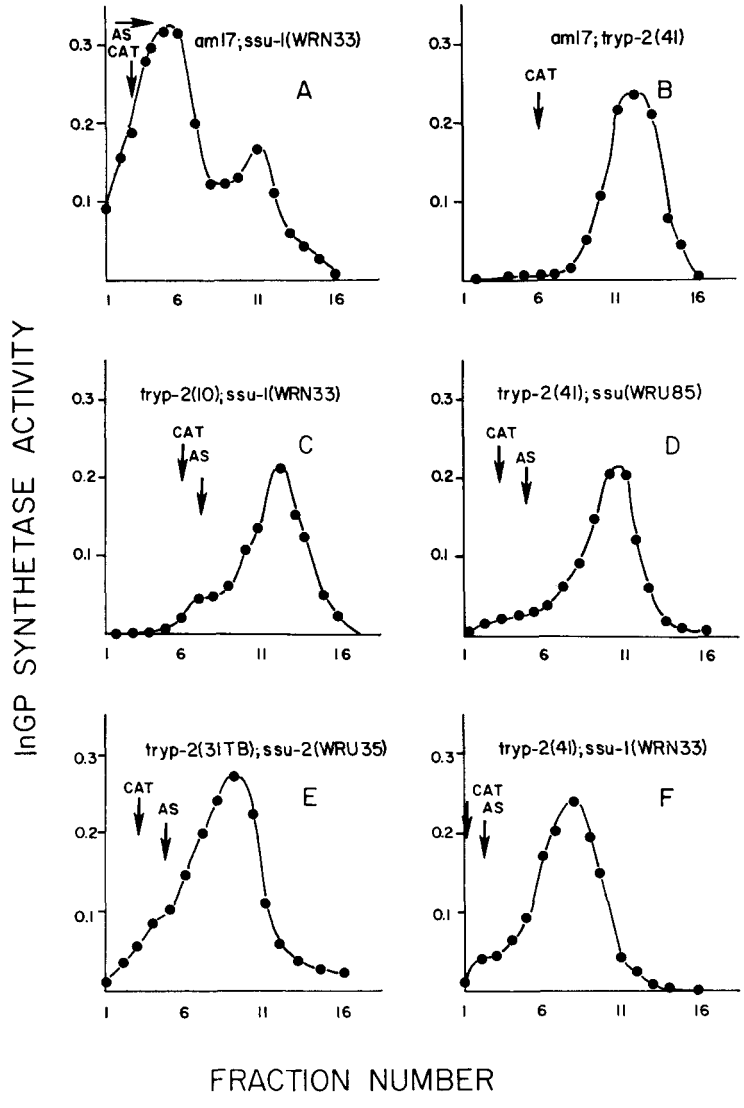


FIGURE 1.—Zone centrifugation patterns in sucrose gradients of a *tryp-2* mutant, a suppressed *am17* stock, and suppressed *tryp-2* mutant strains. The arrows show the positions of the 11.7 S catalase marker and the 10.3 S anthranilate synthetase activity of the intact multienzyme complex. InGP synthetase (●) activity is plotted in units of 0.1 μmoles of product generated in a 45 minute assay as described in MATERIALS AND METHODS. Gradient A is identical to wild type (CHALMERS 1968). Gradient B is typical of the suppressible *tryp-2* mutants. Gradients C, D, E, F show the restoration of the multienzyme complex by the shift of InGP synthetase activity to the 10 S region of the gradients and the reappearance of anthranilate synthetase.

crose gradient analysis on two occasions, a smear of this activity was seen spanning the region from about 7 S to 10 S. On the other hand, the anthranilate synthetase activity was located exclusively in the 10 S region.

Finally segregants from the crosses of *am17;ssu-1(WRN33)* by *tryp-1(3)* and other *tryp-1* mutants not suppressed by this allele were grown in liquid minimal medium containing 10 μgm/ml of indole. Both wild type and *tryp*⁻ mutants grow on this medium, but only those mutants with active anthranilate synthetase and another defect in the pathway accumulate anthranilic acid. None of the segregants from these crosses accumulated enough anthranilic acid to be visible when illuminated with a long-wave UV lamp. However, *tryp-1* mutants having abnormal complexes with some anthranilate synthetase activity fluoresce brightly under these conditions. Surprisingly, *tryp-1(18)* after a fairly long time, produced a small amount of fluorescent material. This fluorescence is probably due to anthranilate produced by the tryptophan–anthranilate cycle (MATCHETT and DEMOSS 1963) as no anthranilate synthetase could be detected in crude extracts of this mutant. In addition, these same strains were tested for growth on a medium containing 20 μg/ml of anthranilic acid. None of them grew, showing that the suppressor did not restore PRA isomerase and InGP synthetase.

## DISCUSSION

These results demonstrate that the mutant classes exhibiting extreme effects on the structure and the activity of the multienzyme complex include supersuppressible mutants. Eight of 41 profoundly altered but revertible mutants could be suppressed by at least one super suppressor. This observation suggests that the phenotypes exhibited by the suppressible strains result from the premature termination of the polypeptide chain products of mutant genes. Missense mutants, identified by their partial activity and aggregated enzyme complex, are not suppressed. Protein fragments would be expected to have little or no catalytic or complex-forming activity, and to allow the accumulation of the free uncomplexed product of the remaining wild-type locus. Two of the supersuppressible *tryp-2* mutants, *tryp-2(10)* and *tryp-2(41)* have been shown to accumulate free wild-type *tryp-1* gene product, in support of this contention (CHALMERS and DEMOSS 1970). The suppressors of chain-terminating mutants also should be allele specific, i.e., a given suppressor should not suppress all mutants at a locus nor is it expected to restore activity to all nonsense mutants. This too is observed. These data are all consistent with the chain-termination hypothesis, although, for technical reasons, no feasible method of directly identifying polypeptide fragments of the products of these genes has been developed.

Low but easily demonstrable levels of the tryptophan enzyme activities are found in suppressed mutant strains. The low specific activity of InGP synthetase in the suppressed mutants is surprising, but should not imply that the low activity of anthranilate synthetase in these strains is due to a limitation on the availability of InGP synthetase for complex formation with the suppressed *tryp-2* gene product. The association of InGP synthetase with wild-type *tryp-2* gene product is specific, rapid, and irreversible (ARROYO-BEGOVICH and DEMOSS 1969; CHALMERS and DEMOSS 1970). The excess of InGP synthetase, as determined by specific activity in extracts, and the preponderance of this activity in the unassoci-

ated 7 S form as shown by the sucrose gradients, indicates that sufficient InGP synthetase is produced to bind all suppressed *tryp-2* gene product despite the apparent repression. No pool of suppressed *tryp-2* gene product having a decreased affinity for InGP synthetase could be demonstrated. This observation and the fact that the excess InGP synthetase is unbound support our hypothesis that the variation in anthranilate synthetase specific activity may be a reflection of the efficiency of the super suppressors or possibly of the differences in the amino acid inserted. While only one isolate of each *tryp*$^-$ mutant and suppressor combination has been examined, these stocks are all closely related and grown under similar conditions.

The variation in the efficiency of suppression by different suppressors with a single allele and by the same suppressor with different alleles is to be expected. If different suppressors insert different amino acids, the inserted amino acids might well lead to proteins with different catalytic properties. Similarly, the same amino acid at different positions of a protein might result in enzymes with altered activity. On this basis some suppressed *tryp-1* mutants were expected to show only partial restoration of activity, that is, to possess either anthranilate synthetase or PRA isomerase–InGP synthetase but not both. No such suppressed mutants were detected among the progeny of *ssu* × *tryp-1* crosses, although mutants with these phenotypes are common at the *tryp-1* locus and are very closely clustered with the inactive alleles (DeMoss, Jackson and Chalmers 1967). Perhaps the right combinations were not tried, or more likely, the restrictions on allowable amino acid replacements at these sites are too stringent to permit partial restoration of activity. The failure of the *ssu-3(WRU118)* suppressor (known to suppress *am17*) to affect any of the *tryp*$^-$ alleles tested may be due to a similar type of restriction.

The majority of mutants at both loci are not suppressed by the four super suppressors. While this situation may be the result of incompatibility of the inserted amino acids and catalytic activity, it is also possible that more than one type of nonsense codon is present in this collection of phenotypically extreme mutants. By observing the number of revertant classes following mutagenic treatment of *am17*, Seale (1968) inferred that the codon present in *am17* was either UAG or UGA. It is likely, however, that UAA is also a chain-terminating codon in Neurospora, and so one must expect several classes of suppressors with distinct codon preferences (Hawthorne 1969). Since the association of the products of both genes is necessary for the expression of anthranilate synthetase (Chalmers and DeMoss 1970), any type of genetic change which results in gross conformational changes in the products of these genes will have the extreme phenotypes noted. These changes, in addition to nonsense mutants, include reading-frame shifts, deletions, and certain kinds of missense mutation where the amino acid replacement leads to a generalized misfolding of the protein product. A possible prototype of such a protein is afforded by *tryp-2(41)* in conjunction with *ssu-3 (WRU118)*, if this suppressor actually can translate the *tryp-2(41)* codon. Further experiments are in progress to determine whether the group of mutants which were unaffected by this limited sample of super suppressors does in fact

contain a new class of supersuppressible alleles. The experiments reported here establish that a significant fraction (20%) of profoundly altered revertible mutants in the *tryp-1* and *tryp-2* genes are supersuppressible and, therefore, probably nonsense mutants.

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Twenty-eight *tryp-2* and thirteen *tryp-1* mutants which affect both the activity and the structure of the multienzyme complex in the Neurospora tryptophan biosynthetic pathway have been examined for suppressibility by four super suppressors. Six of the *tryp-2* mutants and two of the *tryp-1* mutants were shown to be suppressed by both genetic and biochemical criteria. The genetic tests included tetrad analysis, prototroph frequency in mass platings of shot ascospores, and coincident reversion with a known supersuppressible allele. The reappearance of enzyme activity was measured in extracts, and the restoration of multienzyme complex with a sedimentation constant like that of the wild type was demonstrated by zone centrifugation in sucrose gradients. It is proposed that the extreme phenotypes exhibited by these classes of *tryp-1* and *tryp-2* mutants are due to premature chain termination by nonsense codons.

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