EVIDENCE FOR CHAIN TERMINATION BY SUPER-SUPPRESSIBLE MUTANTS IN YEAST

THOMAS R. MANNEY

*Department of Microbiology, Case Western Reserve University School of Medicine
Cleveland, Ohio*

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MUTANTS of *Saccharomyces cerevisiae* that are suppressible by super-suppressors (HAWTHORNE and MORTIMER 1963) are formally analogous to *amber* and *ochre* nonsense mutants of *Escherichia coli* and its bacteriophages, with respect to several genetic characteristics (MANNEY 1964; MORTIMER and MANNEY 1964; HAWTHORNE and FRIIS 1964; FINK 1966; and MAGNI and PUGLISI 1967). SARABHAI, STRETTON, BRENNER and BOLLE have shown that *amber* mutations in bacteriophage T4D cause chain termination which results in formation of a fragment of the normal protein. The experimental results described in this paper provide biochemical evidence that super-suppressible mutations cause chain termination.

The nonsense-like character of super-suppressible mutations has been illustrated by a study of *tr_s* mutants, which affect tryptophan synthetase (MANNEY 1964). Most *tr_s* mutants are either suppressible by super-suppressors or are capable of participating in allelic complementation, but very few have both characteristics. Those few that are both suppressible and able to complement have a polarized pattern of complementation with respect to their position on the genetic map, as would be expected of nonsense mutants. One of these suppressible complementing mutants maps near one end of the locus and is slightly leaky; the other two map close together, near the middle. The rest of the super-suppressible mutants, which are noncomplementing and nonleaky, are distributed more or less uniformly throughout the map of the locus.

If super-suppressible mutants are nonsense mutants, a leaky, complementing one could only occur near the distal end of the locus, with respect to translation, where it would make a nearly complete protein. This type of mutant, accordingly defines the direction of translation of a gene (MORTIMER and MANNEY 1964). However, the complementing activity in mutants that terminate in the proximal half of the locus must reflect a special property of the tryptophan synthetase enzyme.

Tryptophan synthetase, both in *E. coli* and in *Neurospora crassa*, has been shown to catalyze three reactions (YANOFSKY 1960).

The enzymes from both of these organisms have two active sites, one with catalytic activity for reaction A, and the other with activity for reaction B. Reaction C results from an interaction between the two sites (CRAWFORD and YANOFSKY 1958; BONNER, SUYAMA, and DEMOSS 1960; and DEMOSS 1962). *E. coli* tryptophan synthetase readily dissociates into two subunits, the A protein and the B protein, which have the active sites for reactions A and B, respectively. Dissociation of the *Neurospora* enzyme into active subunits has not been reported.

The tryptophan synthetase system in yeast is most similar to the *Neurospora crassa* system. Most mutants, selected as tryptophan auxotrophs, lack all three reactions, and the few exceptional ones that either accumulate indole or can utilize it are clustered in two separate regions of the genetic map of the *tr*₅ locus (MANNEY 1964). The yeast enzyme also resists dissociation into active subunits (MANNEY 1967; BARBEN and MANNEY, unpublished). The possibility that termination of translation in the middle of this enzyme might yield a fragment that retained one of the catalytic sites, analogous to one of the subunits of *E. coli* tryptophan synthetase, provided the rationale for the experiments reported in this paper.

MATERIALS AND METHODS

Mutants, media, and genetic methods: The mutants, the media on which they were grown, and the genetic methods used to study them have been described previously (MANNEY 1964; and MANNEY and MORTIMER 1964).

A double mutant, bearing the suppressible mutations from mutants 35 and 48 was isolated from a random ascospore sample prepared from the following heteroallelic diploid strain:

$$\text{XT796: } \frac{a \quad tr_{5-35} + \quad + \quad + \quad + \quad ad_{2-1} \quad me_1}{\alpha \quad tr_{5-48} le_{1-12} \quad ar_{4-17} \quad hi_{5-2} \quad ly_{1-1} \quad ad_{2-1} \quad me_1}$$

The spore XT796-S1 was isolated as a suspected 35-48 double mutant because it did not recombine with either of the parent mutants, and because its reversion rate was less than 0.1% of that of either parent. From 2×10^9 UV survivors (374 ergs/mm^2) of this strain, no wild-type prototrophs were recovered, whereas this number of cells of either parent, under the same conditions, would yield about 10^4 prototrophs. After prolonged incubation of the plates, however, a few very slow-growing colonies appeared. Several of these were crossed with a wild-type strain and tested for the presence of a suppressor, by tetrad analysis of unordered asci. The results in Table 1 show that one of the slow growing revertants, XT796-S1-R2, did contain a suppressor.

TABLE 1

Segregation of a suppressor of the double mutant $tr_{5-35, 48}$
Cross: $tr_{5-35, 48} S_{35, 48} \times tr^+ S^+$

Ascospore phenotypes*	Number of Asci	Ascus type
++ ± ±	8	Parental ditype
++ --	2	Nonparental ditype
++ ± --	6	Tetratype

* ++ = Normal growth on tryptophanless medium.
± = Slow growth on tryptophanless medium.
-- = No growth on tryptophanless medium.

Although the parental ditype: nonparental ditype ratio of 9:2 would indicate that the suppressor is linked to tr_5 , this conclusion has not been supported by subsequent crosses. When this suppressed strain was crossed with the single mutants, 35 and 48, and subjected to tetrad analysis, the suppressor, $S_{35,48}$, proved to be a strong suppressor of each of these alleles independently. These results demonstrate that the tr_5 allele in XT796-S1 is doubly mutant, containing both of the suppressible alterations from 35 and 48.

Further tetrad analyses failed to reveal any detectable suppression of the following alleles by $S_{35,48}$: tr_{5-3} , tr_{5-9} , tr_{5-12} , tr_{5-21} , tr_{5-29} , tr_{5-33} , tr_{5-34} , ad_{2-1} , ar_{4-17} , ly_{1-1} , and hi_{5-2} . All of the above alleles, except tr_{5-35} , are suppressible by the super-suppressor S_d . These results demonstrate that $S_{35,48}$ is allele specific at the tr_5 locus, but locus nonspecificity is not demonstrated. On the basis of its suppression of the super-suppressible mutation tr_{5-48} and the finding by GILMORE and MORTIMER of classes of super-suppressors that suppress very limited subsets of the known super-suppressible mutants, it is reasonable to consider $S_{35,48}$ to be a super-suppressor with highly restricted specificity. Accordingly, the classification of tr_{5-35} as a nonsuppressible allele (MANNEY 1964) must be revised. In this paper tr_{5-35} will be considered as a super-suppressible mutation.

Preparation of cell extracts: Cells for extraction were grown from inocula taken from single-cell isolates. They were grown to the plateau of the growth curve overnight in 1000 ml of medium in a 4000 ml Erlenmeyer flask on a rotary shaker at 30°C. The medium was YEPD supplemented with L-tryptophan and adenine, 80 mg/liter each, to prevent enrichment for spontaneously occurring prototrophs. The cultures were centrifuged, and the cells washed with cold water, and resuspended in one or two ml of buffer for each gram (wet weight) of cells. The buffer contained 0.1 M K-PO₄ and 0.01 M K-EDTA, at pH 7.2. Whenever wild-type activity was to be assayed, the buffer also contained 10⁻⁵ to 10⁻⁴M pyridoxal phosphate, to stabilize the enzyme.

All operations after the cells were washed were performed in an ice bath or cold room. The suspensions were homogenized by passing them through an Aminco French pressure cell at 20,000 psi. The homogenates were centrifuged at 13,000 × *g* for 20 min and the pellet was discarded. The supernatants, which usually contained from 20 to 40 mg of protein per ml, were assayed for specific activity, and were used without further treatment for the experiments with Sephadex G100. For the experiments with G200 the crude extracts were treated with protamine sulfate to precipitate the nucleic acids, and then concentrated 5 to 10-fold. For each 100 ml of crude extract 16 ml of freshly prepared 1.5% protamine sulfate (Nutritional Biochemicals, Inc.) solution was added with stirring. After 20 min the mixture was centrifuged for 20 min at 13,000 × *g*. The supernatant was placed in a dialysis bag and buried in Aquacid (BioRad) overnight. The concentrated solution was again centrifuged and any precipitate discarded.

Assays: Reaction B was assayed by a modified version of the method used for the *Neurospora* enzyme (YANOFSKY 1955). Each 1 ml assay contained, in addition to the enzyme preparation, 0.4 micromoles of indole, 100 micromoles of K-PO₄, and 10 micromoles of K-EDTA, 60 micromoles of DL-serine, and 1.5 micromoles of pyridoxal phosphate. The pH of the reaction mixture was 7.2, which is optimal for the yeast enzyme. Indole disappearance was followed colorimetrically. Equivalence of indole removed and tryptophan formed was established by the tryptophanase method described by DEMOSS (1962).

Reactions A and C were assayed with the aid of InGP labeled in the 2 position of the indole ring with C¹⁴ (HARDMAN and YANOFSKY 1965). For reaction C the assay mixture was the same as described above for reaction B, except the indole was replaced by 0.3 micromoles of InGP-2-C¹⁴ at a specific activity of 2.8 × 10⁶ cpm/micromole. After incubation for 30 min at 37°C the tubes were placed in boiling water for two minutes, cooled, and repeatedly extracted with toluene until the extractable activity was negligible. Tryptophan in the reaction mixture was determined by adding 0.2 micromoles of carrier tryptophan and converting all the tryptophan to indole with tryptophanase from *E. coli*. The indole formed from the tryptophan was extracted with 2.0 ml of toluene and 1.0 ml of the toluene layer was added to 5 ml of a toluene-solvent scintillation fluid. The activity was counted in a Packard liquid-scintillation spectrometer at an efficiency of about 70%.

For reaction A the assay mixture contained 0.3 micromoles of InGP-2-C¹⁴, at the above specific activity, 300 micromoles of K-PO₄, pH 7.2, and the enzyme preparation. The volume

was adjusted to 1.0 ml with 0.1 M K-PO_4 , pH 7.2 buffer. After 30 min at 37°C the reaction was stopped with 0.2 ml of 1 N NaOH, and the indole was extracted with 2.0 ml of toluene. 1.0 ml of the toluene layer was counted as described above. The background was determined from a blank that contained no enzyme, but received the same incubation. The substrate was extracted repeatedly with toluene just before it was used. Extractable radioactivity increased slowly during incubation in the absence of any enzyme, at a rate that varied with the concentration of the buffer. This rate was minimal at the buffer concentration used.

Specific activities in crude extracts were determined from assays containing up to 0.4 ml of extract (20 to 40 mg of protein per ml) for reactions B and C and up to 0.8 ml for reaction A. Activities in column fractions were determined from assays containing 0.8 ml of the fraction for reaction A and 0.4 ml for reaction B.

Protein concentration was estimated by the method of LOWRY, ROSEBROUGH, FARR, and RANDALL (1951). Crystalline bovine plasma albumin (Armour) was used as a standard.

InGP was prepared enzymatically from indole by the method of HARDMAN and YANOFSKY (1965) using tryptophan synthetase from *E. coli* mutant T-3. InGP-2- C^{14} was prepared from In-2- C^{14} obtained from Nuclear Research Chemicals, Inc. at a specific activity of 16.3 mc/mmmole. The complete reaction mixture contained 14.4 mg of indole, 200 micromoles K-PO_4 , pH 7.8, 2 mg crystalline aldolase, 200 micromoles fructose-1, 6-diphosphate, and 900 units of tryptophan synthetase (1 unit = 1 micromole indole converted to tryptophan per hr, under the conditions described above). The total volume was 15.8 ml. The reaction was incubated for 2 hrs at 37°C, at which time only 30–40% of the initial activity was still extractable as indole, and no further conversion was occurring. InGP was followed throughout the procedure by the ferric chloride test, and was quantitated by the periodate method (YANOFSKY 1956).

The reaction mixture was centrifuged at $13,000 \times g$ for 10 min to remove the precipitate that formed during the incubation. The clarified supernatant was applied to a 2.5×100 cm column of Sephadex G25 (coarse) in 0.01M NH_4HCO_3 , and was eluted with the same solution. The InGP comes off the column after the proteins, and before the unconverted indole, which sticks to the gel. An additional volume equal to the total volume of the column is required to remove the indole. The fractions containing InGP were applied to a column of Dowex 1-X2, that had been equilibrated first with 0.2M Na_2CO_3 , and then with 0.01M NH_4HCO_3 . The column was washed with several volumes of the latter solution, and then eluted with 0.5M NH_4HCO_3 . Fractions containing InGP were combined and evaporated to dryness in a flash evaporator at 40°C and 15–20 mm of Hg. The residue was redissolved in 0.05% NH_4OH and reevaporated several times to remove the NH_4HCO_3 . The InGP was finally dissolved in 0.05% NH_4OH . This preparation was diluted with unlabeled InGP, prepared by the same method, to obtain the specific activity desired.

Sephadex G100 columns were prepared by first rehydrating the gel in boiled (hot) water. After several days the gel was washed with 0.5M acetic acid and washed repeatedly with water and finally with buffer (the same as used for extracts). The gel suspension was poured into a 2.5 cm column (Pharmacia) to a height of 40 cm at room temperature and then placed in the cold room with buffer flowing through the column while it was cooling. Samples were prepared for application to the column by mixing 7.5 ml of crude extract with 2.5 ml of 2M sucrose. This relatively dense solution was layered beneath the buffer at the top of the column on top of a nylon net applicator resting on the surface of the gel. The column was eluted at a flow rate of 30 ml/hr and fractions of approximately 10 ml were collected.

The Sephadex G200 column was prepared in the same manner. In this case the column was 2.5×100 cm. Concentrated extracts (1.5 ml of extract with 0.5 ml of 2M sucrose) were applied as described. The column was eluted at a flow rate of 17.5 ml/hr and fractions of 3.2 ml were collected.

RESULTS

Wild-type tryptophan synthetase: Crude extracts from a wild-type haploid strain of *S. cerevisiae*, S288C, catalyze the same three reactions characteristic

TABLE 2

Specific activities of a haploid wild-type strain of Saccharomyces cerevisiae (S288C) in tryptophan synthetase reactions

Reaction	$\mu\text{mole/hr/mg protein}$
A. InGP $\rightarrow$ In	0.010
B. In $\rightarrow$ T	0.29
C. InGP $\rightarrow$ T	0.12

Protein concentration = 12 mg/ml.

of tryptophan synthetase in other microorganisms. The specific activities for these reactions are given in Table 2. These specific activities were found to be nearly independent of the composition of the growth medium, under the conditions used. Neither repression by tryptophan, nor derepression in minimal medium could be demonstrated.

Mutations at the tr_5 locus: Crude extracts from nonleaky tr_5 mutants all lack activity for reaction C and most lack activity for reactions A and B as well or have greatly reduced levels. The variety of enzymatically altered forms of the enzyme produced by these mutants indicates that the tr_5 locus is the structural gene for this enzyme. The characteristics of 36 tr_5 mutants and a map of some of them have been published previously (MANNEY 1964). A revised map of some of the mutations at this locus is given in Figure 1A, and a complementation map is shown in Figure 2. All the mutants numbered below the line in Figure 1, except 35, are suppressible by the super-suppressor S_d , while the ones numbered above the line are not.

Among approximately 75 tr_5 mutants that have been tested in this laboratory, only two (66 and 67) have been found that are able to utilize indole efficiently in place of tryptophan for growth. Mutant 11 can utilize indole, but grows very slowly on it, and three mutants (6, 18, and 26) accumulate appreciable amounts of indole in their medium. These two classes of partially active mutants are located in two separate regions of the map, as in *Neurospora* and *E. coli*. Accordingly, they define two regions of the map that in turn correspond to two regions of the enzyme, analogous to the α and β subunits of *E. coli* tryptophan synthetase. The right-hand side of the map in Figure 1A corresponds to the α region, which contains the catalytic site for reaction A, and the left-hand side corresponds to the β region, which contains the catalytic site for reaction B. The activity for the wild-type reaction C is probably a consequence of an interaction between the two regions, as in the *E. coli* enzyme.

The suppressible-complementing mutants (3, 9 and 29) show a polarized pattern of complementation; they complement only with mutants to their right on the genetic map. If they are nonsense mutants, then the direction of translation of the locus must be from right to left as the map is drawn in Figure 1 (MANNEY 1964). Mutants 3 and 9 complement with the weak indole-utilizing mutant 11. (The complementation between 9 and 11 is weak, and was overlooked in the previous experiments). If 3 and 9 are nonsense mutants, however, they can only

FIGURE 1.—A. Genetic map of *tr_s* locus, constructed from frequencies of radiation-induced mitotic recombination (MANNEY and MORTIMER 1964). Mutants numbered below the line are suppressible. Those above the line are non-suppressible, and all except 27 are complementing. Mutants 66 and 67 can utilize indole, and 6, 18, and 26 accumulate indole.—B. InGP → In specific activity of super-suppressible mutants, plotted at their relative positions on the map.

make a fragment of the tryptophan synthetase protein corresponding to less than half of the map. Complementation between such a fragment, which in this case would include the catalytic site for reaction A, and the weak indole-utilizing mutant, which must have an active catalytic site for reaction B, is somewhat

FIGURE 2.—Complementation map constructed from results of all possible complementation tests with these *tr_s* mutant pairs in heteroallelic diploids. Overlapping lines represent combinations that gave only negative results. Nonoverlapping lines represent combinations that gave positive results.

analogous to the interaction between the α and β subunits of *E. coli* tryptophan synthetase.

Enzymatic activities in suppressible mutants: Crude extracts of suppressible mutants were examined for activity in reaction A corresponding to their ability to complement. These mutants do not accumulate detectable amounts of indole in their medium, so at most only very low levels of activity could be expected. In Figure 1B the reaction A specific activities of crude extracts of suppressible mutants are plotted at their relative positions on the genetic map. All complementing, suppressible mutants (3, 9 and 29) have significant activity. In addition, the noncomplementing, suppressible mutant 35 which maps very close to 3 and 9, is also active. Mutants 48 and 24 also appear to have significant, but somewhat lower activity, and the rest have insignificant levels. Comparison with Table 2 shows that the specific activities found in all of these mutants, except 29, are less than 1% of the wild-type specific activity for the same reaction. The specific activity of 29 is about 10% of wild type for this reaction.

To determine whether the activity in any of these mutants is associated with a fragment as small as the *E. coli* α subunit, crude extracts were analyzed on a Sephadex G100 column. Wild-type tryptophan synthetase elutes from G100 in the void volume, whereas the α subunit is appreciably retarded, as shown in Figure 3. The elution profiles of the reaction A activity of several suppressible mutants are also shown in Figure 3. Only mutants 3, 9, and 35 have activity that is significantly retarded, and these have identical profiles, with a major peak of activity at the position of the α subunit, and a trace of activity at the wild-type position. All the other mutants have activity only at the wild-type position.

Comparison with Figure 1 and Table 2 illustrates that the amount of activity at the wild-type position in these mutants is an extremely small fraction of the wild-type specific activity. In most cases the amount is less than 0.1% of the wild type and near the detection limit of the assay. The results do not demonstrate that this trace of activity is authentic tryptophan synthetase, as it is found in all mutants tested. This trace of activity, however, could be authentic; it could result from the presence of a very weak suppressor in the parent strain from which the mutants were derived; it could also result from occasional chain completion by a low-level mistake mechanism which would allow a charged t-RNA to recognize the nonsense triplet. The possibility of such a mistake mechanism operating non-specifically with an efficiency of 0.1% is difficult to rule out on general grounds.

If super-suppressible mutants are nonsense mutants, then the retarded activity would correspond to the enzyme fragment resulting from termination of translation at the site of the mutation in the messenger. Accordingly, under the action of a super-suppressor at least some of the fragment should be converted to enzyme with the size and activities of the wild-type enzyme. This result for mutant 3 suppressed by the super-suppressor S_d is shown in Figure 4. In this experiment the results are only qualitative because the efficiency of recovery of the activity applied to the column cannot be estimated with sufficient accuracy. Nonetheless, the strain containing the suppressor contains a significant increase of activity at the wild-type position, and appears to have less of the retarded activity. Further-

FIGURE 3.—Sephadex G100 elution profiles showing the relative positions of wild-type and mutant enzyme activities. The first panel shows the relative positions at which the wild-type yeast tryptophan synthetase, assayed for reactions A and B, elutes from the column. The position at which *E. coli* A protein elutes is included for comparison. The A protein, from crude extract of mutant B-8, was assayed with excess B protein, from crude extract of mutant A-2 for reaction B activity (HARDMAN and YANOFSKY 1965). These fractions were not assayed under the same conditions as the much less active mutant extracts, and are included only to illustrate the positions at which they elute from the column. Mutant profiles should be compared with values in Figure 1 for comparison of specific activities.

more, there is activity for reaction B at the wild-type position, which was not found under any circumstances at the retarded positions.

These experiments demonstrate the presence of an activity, found only in this small cluster of super-suppressible mutants, that resembles the α subunit of *E. coli* tryptophan synthetase in size and enzymatic activity. It is unique to this group of mutants, and its absence from extracts of the other mutants proves that it is a product of the tr_5 alleles of these mutants.

Estimation of the sizes of the active fragments: A longer column of Sephadex G200, and more concentrated samples of the extracts were employed to improve the resolution and, therefore, the precision of the molecular weight estimates for the fragments from mutants 3, 9, and 35. The column was calibrated by determining the elution volume for several proteins of known molecular weight and the void volume was determined with Blue Dextran 2000 (m.w. = 2×10^6 , Pharmacia). The elution volumes for the reaction A activity from concentrated ex-

FIGURE 4.—Action of super-suppressor S_d on mutant tr_{5-3} . Sephadex G100 profiles on activities from mutant tr_{5-3} and from tr_{5-3} suppressed by super-suppressor S_d . ●, InGP → In activity; ○, In → T activity. Fraction 15 was not assayed for InGP → In.

tracts of mutants 3, 9, and 35 were identical and are plotted on the column calibration curve in Figure 5. The elution volume for wild-type enzyme is also plotted on the curve. The molecular weight estimates, from these results, are approximately 160,000 for the wild-type enzyme, and 35,000 for the mutants. These values are not significantly different from the values of 166,000 and 29,000 reported for the complete *E. coli* tryptophan synthetase ($\alpha_2\beta_2$) and the α subunit, respectively (HENNING, HELINSKI, CHAO, and YANOFSKY 1962; and WILSON and CRAWFORD 1965).

On the basis of genetic mapping data, mutants 3, 9, and 35 would be expected to differ in size by no more than 10% (CAPALDO and MANNEY, unpublished). Since the G200 elution volume is relatively insensitive to small differences in molecular weight, the fact that no differences were detected in these experiments is probably not significant.

The direction of translation of the tr_5 locus: The rationale for the experiments described above was based on the direction of translation inferred from the polarized complementation of the suppressible, complementing mutants, and on the map positions of the indole utilizing and indole accumulating mutants. Unique properties of the mutant 35, however, provide an independent way to verify these conclusions from the properties of super-suppressible mutants alone.

FIGURE 5.—Molecular weight estimates of wild-type and mutant tryptophan synthetase activities on Sephadex G200. ●, standard protein markers: (1) Ribonuclease, 13,700 (2) Lysozyme, 14,400 (3) Bovine plasma albumin, 65,000 (4) Rabbit hemoglobin, 67,000 (5) Lactic dehydrogenase, 140,000 (6) Gamma globulin, 160,000. (SCHACHMAN 1963). ○, tr_{5-3} , tr_{5-9} , and tr_{5-35} ; ⊕, wild type.

Mutant 35 is different from all other known super-suppressible tr_5 mutants in that it is not suppressed by the suppressor S_d (MANNEY 1964). The plan of the experiment was to isolate a haploid double mutant from mutants 35 and 48, and study its enzymatic activities. If the 48 site is translated before the 35 site, then the double mutant should produce the same (inactive) product that 48 alone produces. That the absence of the active 35 product is the result of termination by the 48 nonsense codon, and not the result of some other extraneous alteration introduced in the construction of the double mutant, would be demonstrated if suppression by S_d of the 48 alteration in the double mutant resulted in restoration of the active 35 product.

The properties of this double mutant, with and without S_d , which suppresses 48, but not 35, are shown in Figure 6. The double mutant by itself has no detectable activity characteristic of 35, but when the 48 alteration is suppressed by S_d , the activity of 35 is restored. These results provide independent evidence that translation proceeds from right to left in Figure 1, and that the right-hand side of the map corresponds to the catalytic site for reaction A.

DISCUSSION

The simplest interpretation of these results follows from the assumption that

FIGURE 6.—Action of super-suppressor S_d on double mutant $tr_{5-35, 48}$. Sephadex G100 profiles of $InGP \rightarrow In$ activity of $tr_{5-35, 48}$ suppressed by super-suppressor S_d .

yeast tryptophan synthetase consists of two regions analogous to the α and β subunits of the *E. coli* enzyme, and that the position of the suppressible mutants that make active fragments is near the point where these two regions join. The types of tr_5 mutants that occur, and their complementation behavior would indicate that the enzyme consists of only a single kind of polypeptide chain, but there is no direct evidence to exclude the possibility that it consists of strongly interacting, nonidentical subunits.

A model for the gene-enzyme system, and for the various kinds of mutants is diagrammed in Figure 7. It is assumed that the position of a mutation on the map of the gene corresponds to position of the alteration in the polypeptide chain specified by the gene. Colinearity of mutation sites on the genetic map and the sites of alterations in the polypeptide chain have been demonstrated for missense mutations by YANOFSKY, CARLTON, GUEST, HELINSKI, and HENNING, and for nonsense mutations by SARABHAI, STRETTON, BRENNER, and BOLLE (1964). On the basis of their complementation, the indole-utilizing mutants and the indole-accumulating mutants are assumed to make complete enzyme molecules that have lost only the activity of one catalytic site or the other. The positions of these mutations on the map are assumed to correspond approximately to the positions of the inactivated catalytic sites on the enzyme, and consequently, define the functional regions of the wild-type enzyme.

FIGURE 7.—A model for the yeast tryptophan synthetase gene-enzyme system. *, *missense* amino acid substitution. ● *sense* amino acid substitution resulting from suppressor.

The three super-suppressible mutants 3, 9, and 35, which are clustered about one-third of the way from one end of the locus, produce fragments of the enzyme that retain a trace of enzymatic activity for reaction A ($\text{InGP} \rightarrow \text{In}$). The fact

that in the double mutant, 35–48, the 48 alteration prevents completion of the fragment normally made by 35, shows that this fragment corresponds to the portion of the enzyme coded for by the region to the right of 35 in Figure 1. These results provide evidence for the idea that these super-suppressible mutants contain chain-terminating nonsense mutations.

The experiments show that under the action of the super-suppressor S_d both wild-type-like enzyme molecules (probably containing a single amino acid substitution), and mutant fragments are formed. The suppressor, therefore, overcomes the chain-terminating effect of the mutation, but with less than 100% efficiency. The presence of active fragment in the suppressed double mutant (only the 48 site is suppressed) shows that the suppressor acts by altering the translation or transcription of the mutant gene, and not by any indirect mechanism.

The experiments do not establish that the active fragments found in these mutants terminate at the exact site of the mutation. If this is the case, however, the three mutants must make three slightly different fragments, since they are mutated at different sites in the gene. The activities in the mutants are strikingly similar; in fact, no significant differences have been detected. The mutations, however, are close together and the differences could be small. The question is unresolved.

If the enzyme consists of only one kind of polypeptide, containing both of the catalytic regions, then the active fragment in each of these mutants probably terminates at the site of the nonsense mutation. BONNER (1964) proposed that the similar *Neurospora* enzyme is composed of a single kind of polypeptide chain, which evolved from a form with two kinds of subunits (as in *E. coli*) by a genetic event that joined together the two corresponding genes into one. A chain-terminating mutation near the point of joining would then lead to the liberation of the ancestral subunit. BONNER's suggestion, therefore, provides a rationalization for why chain terminating mutations in a very small segment could result in active fragments. In return, these results provide circumstantial evidence in support of this idea.

If on the other hand, the yeast enzyme contains two kinds of subunits, these mutants could make either fragments of the α subunit, or complete α subunits and very small fragments of the β subunit, which would likely be too small to be bound to the α subunit and would not be recovered.

In either case, the absence of active fragments in the suppressible mutants that map between 35 and 29 can be understood by the postulated interaction between the two halves of the enzyme. These mutants would be expected to make fragments that include enough of the distal portion of the enzyme to form a specific, but incomplete, association with the proximal portion (analogous to the α - β interaction in *E. coli* tryptophan synthetase). This incomplete association could interfere with the active site for reaction A, rendering the fragment inactive. Mutant 29, then, probably makes enough of the chain to allow an active association of the two regions.

However the detailed explanation turns out of why these particular fragments

are active, the results leave little room for doubt that these super-suppressible mutants of yeast, and their suppressors, have the same molecular basis as the *amber* and *ochre* nonsense mutants of *E. coli* and their suppressors.

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SUMMARY

Enzymatically active fragments of tryptophan synthetase are produced by three suppressible mutants of *Saccharomyces cerevisiae*. These mutations are clustered near the middle of the structural gene (*tr₅*). The fragments catalyze conversion of indole-3-glycerol phosphate to indole. Their molecular weights, estimated by gel filtration, are approximately 35,000; the wild-type enzyme has a molecular weight of approximately 160,000. The fragments are similar in several properties to the α subunit of *E. coli* tryptophan synthetase. Thus, there are close functional analogies between the enzymes from *S. cerevisiae* and *E. coli*, although their gross structural properties are quite different.—When an inactive, suppressible *tr₅* mutation is coupled proximal (with respect to translation) to one that produces an active fragment, the activity is eliminated; suppression of the proximal mutation restores the activity. When a mutation that produces an active fragment is suppressed, both wild-type and fragment activities are produced. These results provide evidence that super-suppressible mutations cause chain termination.

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