ACTION OF A SUPER-SUPPRESSOR IN YEAST IN RELATION TO ALLELIC MAPPING AND COMPLEMENTATION[1,2]

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SUPPRESSOR mutations restore a function lost as the result of a mutation at another locus. Although most suppressors studied have been specific for a single allele, HAWTHORNE and MORTIMER (1963) described a class of suppressors in *Saccharomyces cerevisiae* that suppressed the phenotypes of about 25 percent of all the nutritional mutants they tested. The suppressible mutants represented 11 different phenotypes, and yet at any given locus these super-suppressors acted only on certain alleles and not on others. They compared three independently isolated suppressors of this type, finding no specificity differences among them. Two of the three were shown to be allelic.

Similar multiple-action suppressors—on a somewhat smaller scale—have been reported in several *Escherichia coli*-bacteriophage systems. CAMPBELL (1961) found suppressors in certain strains derived from *E. coli* K-12 that suppress a variety of mutations in the bacterial genome in addition to mutations in the bacteriophage λ, when it is grown in the suppressor-bearing bacterial strain. Similar suppressors in *E. coli* suppress mutants of bacteriophage T4. These suppressors act on numerous ambivalent *rII* mutants (BENZER and CHAMPE 1962), and also suppress certain mutants of the structural gene for alkaline phosphatase in the bacterium (GAREN and SIDDIQI 1962).

To investigate the properties of super-suppressible mutants of Saccharomyces a suitable gene-enzyme system had to be developed. In both *E. coli* and Neurospora, the tryptophan synthetase system has proved useful for studies of suppressor mechanisms (SUSKIND and KUREK 1959; BRODY and YANOFSKY 1963). The advantages offered by these systems, together with the availability of tryptophan synthetase-defective mutants (tr_5 locus) in yeast (HAWTHORNE and MORTIMER 1960) made this system attractive for studying super-suppressors.

This paper reports results of genetic studies on 36 UV induced tr_5 mutants. These have been characterized with respect to allelic complementation, accumulation and utilization of indole, suppression by a super-suppressor, and their position on an allele map has been determined by the use of X-ray-induced mitotic reversion (MANNEY and MORTIMER 1964).

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MATERIALS AND METHODS

Yeast strains: Heterothallic strains of *Saccharomyces cerevisiae* were studied. All were obtained from R. K. MORTIMER. Tryptophan synthetase-deficient mutants were isolated by replica plating (LEDERBERG and LEDERBERG 1952), following irradiation with ultraviolet light (UV) (MORTIMER, LERNER and BARR 1957). All mutants except $tr_{5\text{-}6}$ and $tr_{5\text{-}7}$ were derived from the standard-type haploid strain S288C, or from closely related strains. Complete phenotypes of the strains used in these studies have been described (MANNEY 1964).

Media: The following culture media were used:

YEPD: Complex medium for routine culturing and assaying viability: Difco yeast extract, 1 percent; Difco Bacto-Peptone, 2 percent; dextrose, 2 percent; and agar, 2 percent (omitted for liquid media).

GNA: High-glucose medium, used for presporulation growth (MILLER, COLVIN and TREMAINE 1955): dextrose, 5 percent; Difco yeast extract, 1 percent; Difco Bacto-nutrient agar, 2.3 percent; and additional agar, 0.5 percent.

RA: Sporulation medium (FOWELL 1952): raffinose, 0.022 percent; sodium acetate, 0.3 percent; agar, 2 percent.

MV: Synthetic minimal medium with vitamins: Difco Bacto-yeast nitrogen base, without amino acids (*Difco Manual* 1953), 0.67 percent; dextrose, 2 percent; and agar, 2 percent.

SC: Synthetic complete medium: MV supplemented with adenine, arginine, lysine, methionine, tryptophan, phenylalanine, tyrosine, and uracil, 20 mg/liter each; leucine, 30 mg/l; histidine, 10 mg/l; threonine, 350 mg/l; and serine, 375 mg/l.

—X: Omission media: SC with one of the supplements omitted. These media are designated by the omitted nutrient, e.g., —Tr = SC without tryptophan.

PET: A medium containing glycerol as the carbon source, used to score respiratory deficiency (petites): glycerol, 3 percent; dextrose, 0.025 percent; Difco yeast extract, 1 percent; Difco Bacto-Peptone, 2 percent; and agar, 2 percent.

Genetic methods: Allelism and allelic complementation were scored by a test employing replica plating. Overnight streak cultures were mixed on YEPD, incubated overnight to allow mating and growth of the diploid clones, and replica plated onto —Tr and SC. When allelic complementation was being scored the mixed cultures on YEPD were examined for zygotes under the microscope after 3 to 6 hours to ascertain that mating had occurred.

To establish diploid hybrid strains, haploids were mass mated on YEPD. Three hours later individual zygotes were isolated by micromanipulation, and allowed to form colonies (HAWTHORNE and MORTIMER 1960).

Super-suppressor action was determined by tetrad analysis. Hybrid strains, precultured two days on GNA slants, sporulated in RA. Asci were dissected by the method of JOHNSTON and MORTIMER (1959). Spore cultures were streaked on YEPD plates and their phenotypes scored by replica plating on appropriate media. Mating was scored, also with the aid of replica plating, on the basis of prototroph formation with suitably marked tester strains.

The methods used for allelic mapping with X rays have been described (MANNEY and MORTIMER 1964).

Accumulation tests: Mutant strains were grown in liquid SC with limiting tryptophan (4 mg/l). After incubation at 30°C for two days the medium was tested for indoleglycerol phosphate with ferric-chloride reagent (YANOFSKY 1956), and for indole with p-dimethylaminobenzaldehyde reagent (YANOFSKY 1955).

RESULTS

Accumulation and utilization tests: All of the tr_5 mutants accumulated indoleglycerol phosphate in the growth medium, as evidenced by the $FeCl_3$ test. Three of them—$tr_{5\text{-}6}$, $tr_{5\text{-}18}$, and $tr_{5\text{-}26}$—also accumulated indole. One mutant—$tr_{5\text{-}11}$—grew slowly on —Tr supplemented with indole (10 mg/l). Several grew slowly on unsupplemented —Tr.

Allelic complementation: Thirty-five tr_5 alleles were tested for complementation in diploids in all possible *trans* combinations, using the mutants and strains derived from them by dissection of hybrid asci. Most combinations were tested at least twice, and all positive tests were confirmed by retesting, in many cases by isolating zygotes and testing diploid clones. In all cases—positive and negative—formation of zygotes was confirmed by microscopic observation.

A complementation map, representing these results, is given in Figure 1. Each allele is represented by a line. If two alleles did not complement, their lines overlap. If they did complement, their lines do not overlap. The bottom line represents the alleles that did not complement any others. Since every allele can be represented by a continuous line, the map is linear.

Of the 35 alleles, 19 complement with at least one other while 16 do not. It is of interest to notice, however, that if merely three alleles—tr_{5-7}, tr_{5-11}, and tr_{5-29} (or tr_{5-18})—had been omitted, no complementation would have been found. Some of the 17 that gave only negative results in these tests may give positive results if tested with a sufficiently large number of alleles.

Allelic mapping: A method for allelic mapping in yeast using X-ray-induced mitotic reversion in heteroallelic diploids has been described (MANNEY and MORTIMER 1964). For this method the X-ray sensitivity for induction of heteroallelic reversion is assumed proportional to the distance between the two heteroalleles. This assumption is supported by the observation that it leads to precisely additive values for the map intervals. The sequence of alleles can therefore be determined, unambiguously, from two-point tests; in some instances sequences have been verified by three-point tests.

Figure 2 is a map of 29 tr_5 alleles constructed by this method. The units of the map (prototrophs per 10^6 survivors per 200 roentgens) must be considered as relative units; they have not yet been related to conventional recombination units. The order of some of the alleles that are close together has not been determined by the data. The uncertainty in the position of most of the alleles is about one unit, or about 1/25 of the length of the map, although in some cases it is much smaller. Four alleles near the left end of the map—tr_{5-2}, tr_{5-4}, tr_{5-21}, and

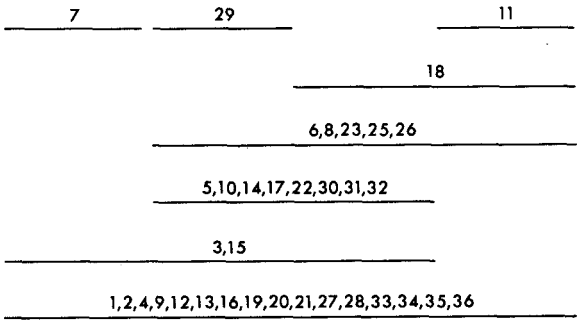

FIGURE 1.—Complementation map constructed from results of all possible complementation tests with 35 tr_5 mutants in heteroallelic diploids. Overlapping lines represent combinations that gave only negative results. Nonoverlapping lines represent combinations that gave positive results.

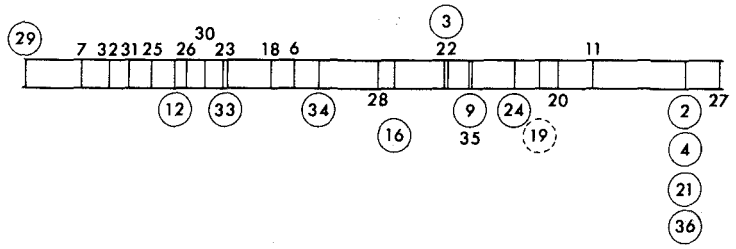

FIGURE 2.—Map of tr_5 mutants determined by X-ray method. Mutants numbered above the line are complementing, while those below are noncomplementing. The mutants circled are super-suppressible.

tr_{5-36}—appear to be repeat mutations at the same site (MANNEY and MORTIMER 1964). With the exception of these four, the alleles are distributed along the map at random. There is no indication of clustering, nor are there any appreciable regions of the map that are insensitive to UV mutagenesis.

The alleles numbered above the line in Figure 2 are complementing ones, while those numbered below are noncomplementing. Both types are distributed along most of the length of the map. The alleles numbered in circles will be discussed later.

Comparison of X-ray map with complementation map: Both the X-ray map and the complementation map assign sequences to the alleles. These are compared in Figure 3. The top part of the figure is a sequence map derived from the X-ray sensitivity data used to construct Figure 2. In this map only the sequences determined unambiguously are represented. The alleles are grouped in clusters; the sequence of the clusters has been established, but the sequence of the alleles within each cluster has not. The distance shown between each cluster is arbitrary. The bottom part of the figure is a complementation map of the same alleles.

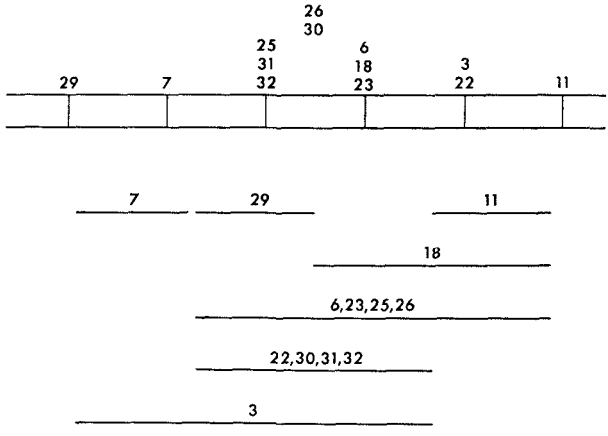

FIGURE 3.—X-ray sequence map of complementing tr_5 mutants compared with allelic complementation map.

For the most part, the sequences are colinear. There is one exception, however. The order of tr_{5-7} and tr_{5-29} relative to the rest of the alleles is different. This discrepancy will be seen to be of considerable theoretical importance with respect to a model for the action of super-suppressors.

Action of a super-suppressor: In their initial studies HAWTHORNE and MORTIMER (1963) investigated three super-suppressors which they designated S_a, S_b, and S_c. Although they found no differences in the spectra of mutants suppressed by them, the three suppressors represent at least two different loci. S_a and S_c are either very closely linked or are allelic, but show no linkage to S_b. A fourth super-suppressor, S_d, evidently represents an additional locus, because, unlike the first three, it is closely centromere linked. This suppressor, which suppresses many of the same alleles as the others, has been used to study the suppressibility of 32 tryptophan synthetase mutants.

The action of S_d was tested by tetrad analysis of hybrids heterozygous for the allele being tested and for the super-suppressor, and homozygous for ad_2 (adenine requiring). The homozygous ad_2 alleles were employed to "mark" the suppressor. Unfortunately, however, two different alleles at the ad_2 locus were inadvertantly used and one of them is not suppressible. Consequently, in some crosses the suppressor could not be scored in every spore. Nonetheless, if the tr_5 allele being tested was suppressible, there should have been an excess of tryptophan-independent phenotypes, consistent with the segregation of the suppressor; no suppressor-bearing spore could have the mutant phenotype of a suppressible allele. Accordingly, the occurrence of the mutant phenotype coincident with the suppressor in a spore culture was conclusive evidence that the allele responsible for that phenotype was not suppressed.

Thirty-two tr_5 alleles were tested for suppression by S_d. Thirteen were suppressed and 19 were not. The data for the 19 nonsuppressed ones are summarized in Table 1. Three types of evidence are tabulated: (1) segregation ratios of Tr^+:Tr^- phenotypes among four-spored asci, (2) segregation ratios of Tr^+:Tr^- phenotypes among suppressor-bearing spores, and (3) segregation ratios of Tr^+:Tr^- phenotypes among all spores examined. The tetrad ratios are based on data from complete asci. Those with inviable spores and those with irregular segregation of markers not suppressed by S_d were excluded. In each cross, several unsuppressed markers were included to detect "false tetrads" which occasionally arise from chance association of nonsister spores (JOHNSTON and MORTIMER 1959). Spores from incomplete and "false" asci were included in the spore ratios shown in the last two columns. Therefore, these data do not merely duplicate the tetrad ratios. In all cases where the suppressor could be scored, the strongest evidence that these alleles are not suppressed is the occurrence of suppressor-bearing spores that require tryptophan. In every case, at least two of the three criteria for nonsuppressibility are satisfied for these 19 alleles. In the two hybrids in which the suppressor could not be scored directly (XT317 and XT451), there is a significant absence of excess positive phenotypes.

Data for the 13 suppressible alleles are shown in Table 2. These results were confirmed by backcrossing a tryptophan-independent spore presumed to have

TABLE 1

Segregation data establishing nonsuppressibility by S_d *for 19* tr_5 *mutants*

Hybrid No.	tr_5 genotype	Tetrad ratios (+:− phenotypes)			Spore phenotypes	
					S_d bearing	All spores
		4:0	3:1	2:2	Tr+ : Tr−	Tr+ : Tr−
XT301	5–1	0	0	4	6:4	14:11
XT305	5–5	0	0	4	8:6	15:11
XT442	5–6	0	0	5	3:4	13:11
XT443	5–7	0	0	4	6:6	13:11
XT308	5–8	0	0	5	3:8	11:10
XT310	5–10	0	0	7	4:5	14:14
XT311	5–11	0	0	5	7:8	13:13
XT317	5–17	0	0	4	. . .	13:13
XT318	5–18	0	0	6	4:8	13:14
XT409	5–19	0	0	5	2:0	11:13
XT320	5–20	0	0	4	4:3	13:10
XT445	5–23	0	0	2	3:3	8:6
XT446	5–25	0	0	6	8:13	14:13
XT448	5–27	0	0	5	6:6	13:12
XT449	5–28	0	1	1	2:6	9:8
XT451	5–30	0	0	5	. . .	12:11
XT452	5–31	0	0	5	7:4	14:12
XT453	5–32	0	0	5	6:8	14:15
XT456	5–35	0	0	5	4:7	11:12
Totals		0	1	87	83:99	238:220

TABLE 2

Segregation data establishing suppressibility by S_d *for 13* tr_5 *mutants*

Hybrid No.	tr_5 genotype	Tetrad ratios (+:− phenotypes)			Spore phenotypes		Backcross	
					S_d bearing	All spores	Hybrid No.	Spore phenotypes
		4:0	3:1	2:2	Tr+ : Tr−	Tr+ : Tr−		Tr+ : Tr−
XT302	5–2	2	2	2	10:1	18:6	XT473	18:5
XT405	5–3	1	4	2	7:0	21:4	XT474	20:4
XT304	5–4	2	2	2	13:0	21:6	XT475	17:4
XT309	5–9	2	3	2	9:0	21:7	XT476	21:7
XT312	5–12	3	1	1	13:0	20:5	XT495	18:6
XT313	5–13	1	2	1	11:0	19:5	XT478	20:3
XT408	5–16	3	1	2	4:0	21:5		
XT321	5–21	2	0	1	6:0	18:6	XT481	21:6
XT458	5–24	2	0	2	13:0	21:5	XT482	19:4
XT450	5–29	3	2	1	14:0	23:5	XT483	24:3
XT454	5–33	3	3	0	12:0	25:7	XT497	17:7
XT455	5–34	3	0	1	13:0	24:2	XT485	16:7
XT457	5–36	2	2	1	15:0	24:5	XT486	20:5
Totals		29	22	18		276:68		231:61

the genotype tr_{5-x} S_d with a strain bearing several suppressible alleles at other loci. The phenotypic spore-ratios from the backcrosses are included in Table 2. Segregation of tryptophan-requiring spores from these hybrids confirms the presumed genotype of the spores that were backcrossed, and proves that they are suppressible. The backcross was not performed with tr_{5-16}, but the conclusion of suppressibility for this allele was confirmed by another method. A hybrid was made from a presumed tr_{5-16} S_d spore strain and an unsuppressed tr_{5-16} strain. The diploid required tryptophan. Therefore, it must have been homozygous for tr_{5-16}, and S_d must be a recessive suppressor of this allele.

The evidence for these 13 alleles clearly supports the conclusion that they are suppressible by S_d. The single suppressor-bearing, tryptophan-requiring spore in XT302 must be considered an error in light of the consistency of the other data for that allele.

The effect of S_d *on* tr_{5-19}: Although tr_{5-19} is not suppressible as judged by the tests described, it is affected by the super-suppressor. It appears to be "suppressed" to a different type of mutant, one that can utilize indole slowly. The spore cultures in many of the tests described above were scored on —Tr supplemented with indole (10 mg/l) in addition to unsupplemented —Tr. On the indole-supplemented medium segregation of tryptophan dependence in the hybrid bearing this allele (XT409) was characteristic of suppressibility. There were one 4:0, four 3:1, and one 2:2 asci. The ratio of positive to negative phenotypes among all spores was 18:6, and among spores known to bear the suppressor 2:0. A backcross of the suppressor-bearing, tr_{5-19} spore gave a ratio of positive to negative phenotypes on indole-supplemented —Tr of 18:2.

After a suggestion by B. D. MALING (personal communication) an attempt was made to isolate a super-suppressor that would suppress tr_{5-19} to prototrophy. Not only was the attempt unsuccessful, but this allele could not be made to revert at all. Even after UV irradiation (to about 20 percent survival) no prototrophs were recovered from more than 10^9 cells. However, when the cells were plated on indole-supplemented —Tr, and given the same UV dose, about five colonies were formed per 10^6 cells plated. When these indole-utilizing mutants were cultured and plated on —Tr, prototrophs were obtained.

It appears that tr_{5-19} reverts only by a two-step event. There are two tenable explanations. It could actually be a double tr_5 mutant, one alteration being suppressible and the other capable of utilizing indole. It could also be a small rearrangement, such as an inversion, that requires two base-changes for reversion. Experiments, employing S_d, designed to distinguish between these two possibilities are in progress.

The distribution of suppressible alleles on the X-ray allele map: The alleles suppressed by S_d are shown in circles on the map in Figure 2. They are distributed along the full length of the map, ruling out the possibility that the suppressor acts only on mutants at a specific site. There is, in fact, no discernable pattern. The outstanding feature is that the four mutants that appear to be repeat mutations at the same site are all suppressible. This adds further support to the hypothesis that they are identical. We may conclude that suppressible mutation

sites are randomly distributed along the map, and that at least one is significantly more sensitive to UV mutation induction than others. If it is assumed that there are no repeat mutations at other sites, then the 32 mutants tested for suppressibility are distributed among 29 sites. Of these, ten (or 35 percent) are suppressible. HAWTHORNE and MORTIMER (1963) tested 50 alleles at different loci and found 14 (or 28 percent) suppressible. Since their mutants were at many different loci, they could not have been repeats, and it is therefore appropriate to compare the number of mutation sites, rather than mutants. On this basis, the frequency of suppressible alleles among tr_5 mutation sites appears to be about the same as among nutritional mutation sites in general. This is consistent with the hypothesis that suppressible mutations can occur at every locus.

The distribution of suppressible alleles on the complementation map: In contrast to their nearly uniform distribution along the allelic map, super-suppressible mutants are almost completely restricted to one group of the complementation map: the group of noncomplementing alleles. There are two important exceptions: tr_{5-3} and tr_{5-29}. The distribution of 31 alleles with respect to suppressibility and complementation is summarized in Table 3. Of the noncomplementing alleles 11 of 16 are suppressible, whereas of the complementing ones, only two of 15 are. Only five of these 31 mutants fail to function either by complementation or by suppression.

The lower frequency of suppressibility among complementing mutants, compared with noncomplementing mutants is highly significant. The two exceptional alleles, however, cannot be ignored. An acceptable model must explain these mutants as well as the almost complete exclusion between complementation and suppressibility.

DISCUSSION

The characteristic properties of the class of mutants that are suppressed by S_1 may be summarized as follows: (1) They are distributed along the entire length of the mapped portion of the tr_5 locus, and apparently throughout the entire genome of Saccharomyces as well, perhaps at every locus. (2) Some of the sites at which these mutations occur have differing sensitivities to UV mutation induction (prototrophy to auxotrophy), as evidenced by the occurrence of four suppressible mutations at the same site. (3) Most of these super-suppressible mutants are noncomplementing, but there are exceptions.

TABLE 3

Summary of results of complementation and super-suppressibility tests for 31 tr_5 *mutants*

Suppressible	Complementing	Number	Alleles
+	−	11	2, 4, 9, 12, 13, 16, 19, 21, 33, 34, 36
+	+	2	3, 29
−	+	13	5, 6, 7, 8, 10, 11, 17, 18, 23, 25, 30, 31, 32
−	−	5	1, 20, 27, 28, 35
Total number		31	

Comparison with suppressible mutants in other organisms: These characteristics are similar to those of mutants in *E. coli* and bacteriophages that are suppressed by suppressors with multiple action. The ambivalent *rII* mutants of bacteriophage T4 are distributed among several sites in the *rII* A cistron (BENZER and CHAMPE 1962). The 15 suppressible alkaline phosphatase mutants studied by GAREN and SIDDIQI (1962) are distributed among at least seven sites. Further, they are noncomplementing. The suppressor-sensitive mutants of bacteriophage λ studied by CAMPBELL (1961) are similarly found at many sites, most of which he was able to group into nonoverlapping complementation groups. Complementation was not found within each group.

WITKIN (1963) has presented evidence that suggests that the frequency of this type of suppressible mutant among randomly selected UV-induced auxotrophs of *E. coli* is about the same as the frequency found by HAWTHORNE and MORTIMER (1963) for Saccharomyces. She studied a class of auxotrophs with particular reversion characteristics—a class comprising about one third of all auxotrophs. About half of all double-auxotrophic mutants (which she obtained by serial selection) from this class would revert to prototrophy by a single-step event. She proposed that multiple-acting suppressors account for these reversions. Her results indicate that about 20 percent of the randomly-selected UV-induced auxotrophs of *E. coli* (B/r and K-12) are "super-suppressible."

The similarities between these systems and Saccharomyces are striking. They motivate the working hypothesis that the mechanism of super-suppression in all of them is basically the same. The demonstration of these similarities is one of the significant results of these yeast studies. On the one hand, it demonstrates that this mechanism of gene action, whatever it is, is not merely a curiosity, unique to a single genetic system, for, genetically, yeast and the *E. coli*-bacteriophage systems are conspicuously dissimilar. On the other hand, if this working hypothesis is valid, the various systems—because of their differences—together provide a unique assemblage of genetic and biochemical approaches to the study of the mechanism of suppressor-gene action.

Models for super-suppressors: CAMPBELL (1961) proposed that suppressor-sensitive mutants of bacteriophage λ produce abnormally sensitive protein that is either stabilized or freed of inhibition, by the action of the suppressor. In support of this he had found that some of the suppressible mutants were also sensitive to pH or temperature extremes. BENZER and CHAMPE (1962) found evidence to support quite a different interpretation of the ambivalent-mutant suppressors. The ambivalent *rII* mutants behave as nonsense mutants: they appear unable to transcribe a complete protein. In agreement with this, GAREN and SIDDIQI (1962) found that the suppressible alkaline phosphatase mutants were noncomplementing and failed to produce detectable CRM (immunologically cross-reacting material) to the wild-type enzyme. BENZER and CHAMPE (1962) proposed that the suppressor changed the genetic code by altering either an amino-acid activating enzyme or an sRNA adaptor so that the nonsense sequence could be translated and a complete protein synthesized.

Another model that must be considered is the mechanism that SUSKIND and

KUREK (1959) demonstrated for suppression of the Neurospora mutant tr_{24}. Mutation may alter an enzyme making it sensitive to inhibition by something normally present in the cell. The suppressor could then act by removing or lowering the concentration of the inhibitor. In the case of super-suppressors the inhibitor could be a small organic molecule, and the suppressor loci control different steps in its biosynthesis. This would explain several different loci having the same suppressor action.

Still another possibility is that the super-suppressors are involved in regulation of the number of enzyme molecules produced by the cell, rather than in their structure. Since so little is known about the regulation of these enzymes in yeast, it will be difficult to rule out such possibilities until the properties of the enzyme produced by suppressed strains are compared with those of the wild-type enzyme.

Conclusions from present experiments: The experiments with tr_5 mutants reported in this paper do not conclusively eliminate any of these models from consideration. They do, however, make some appear less likely than others. The observed partial exclusion between complementation and suppressibility is a more likely property of nonsense mutants than of mutants that form enzymes sensitive to external conditions or to cytoplasmic inhibitors. This is not conclusive, of course. One could postulate a mechanism whereby protein hybrid formation, necessary for complementation, was inhibited, and such a mechanism would be difficult to distinguish from the nonsense model on the basis of present evidence.

The occurrence of the super-suppressible mutations along the entire length of the tr_5 locus would argue against them being primarily involved in regulating the amount of tryptophan synthetase formed. Further, $tr_{5\text{-}19}$ produces an altered form of the enzyme, and if it is not a double mutant, may provide direct evidence that the suppressor alters the structure of the enzyme. Further studies on this mutant are in progress.

The nonsense model, formulated by BENZER and CHAMPE (1962), states that these mutants synthesize only part of the normal enzyme. SARABHAI, STRETTON, BRENNER and BOLLE (1964) have recently provided direct experimental support for this model. They demonstrated that a class of suppressible amber mutants that affect the head protein of bacteriophage T4D produce fragments of the polypeptide chain. The fragments all appear to have a common origin, and their terminal ends correspond with the positions of the appropriate mutants on an allelic map.

The protein fragments that this model predicts for the super-suppressible tr_5 mutants are illustrated schematically in Figure 4. There is abundant evidence from *in vitro* studies that allelic complementation involves association of normally identical protein subunits to form an enzymatically active hybrid molecule (WOODWARD 1959; LOPER 1961; SUYAMA 1963; FINCHAM and CODDINGTON 1963; SCHLESINGER and LEVINTHAL 1963). This imposes severe restrictions on the complementing ability of super-suppressible mutants. Protein fragments such as those in Figure 4 could only complement with another mutant protein altered closer to the origin of translation. Therefore, this model requires that (1) a suppressible mutant can never complement with another suppressible mutant, and

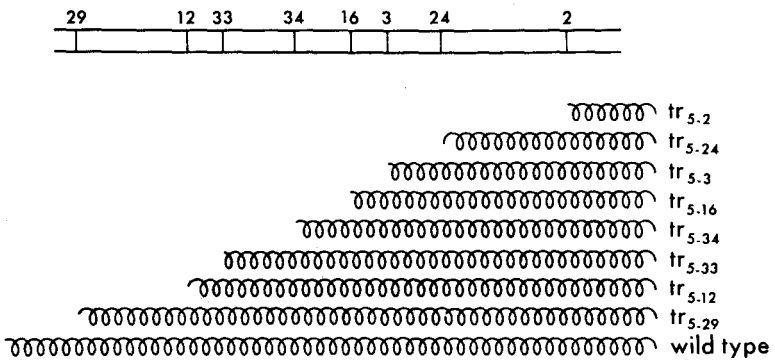

FIGURE 4.—Schematic representation of the proteins formed by super-suppressible mutants, predicted by the nonsense model.

(2) if, at any given locus, a suppressible mutant complements with a mutant, say to its right on the allele map, then neither that suppressible mutant nor any other suppressible mutant at that locus can complement with one to its left. If the model is correct, then, complementation involving super-suppressible mutants defines the direction of protein translation relative to the allelic map. These conditions provide a genetic test of the nonsense model. From knowledge of the frequency of complementing mutants in various regions of the locus one could calculate the expected frequency of mutants that complement mutants to both their right and to their left. It is then a matter of examining a sufficient number of mutants to either find a case incompatible with the model, or to demonstrate that such cases do not occur at the frequency expected if there were no such restriction.

The complementing, super-suppressible tr_5 mutants (tr_{5-3} and tr_{5-29}) are quite compatible with this model. They complement only with mutants that map to their right in Figure 2. Further, among all the mutants on the map, they are the only ones that complement only with mutants to their right; all others complement either to their left or to both right and left. This is strong circumstantial evidence favoring the nonsense model. The occurrence of noncomplementing suppressible mutants between tr_{5-3} and tr_{5-29} on the map is an apparent inconsistency that remains to be explained by the model.

The predictions of this model shown schematically in Figure 4 also suggest some biochemical tests. A particularly attractive approach is by the use of antisera against wild-type tryptophan synthetase to study the mutationally altered proteins (SUSKIND, YANOFSKY and BONNER 1955; SUSKIND 1957; MOHLER and SUSKIND 1960). The nonsense model applied to this system leads to the prediction that tr_{5-29} should produce a cross-reacting material (CRM), whereas the four mutants near the left end—tr_{5-2}, tr_{5-4}, tr_{5-21}, and tr_{5-36}—would not. It would be of further interest to examine the pattern of cross-reaction among the various intermediately situated mutants.

The final test of the model must come from demonstrations of the structure of

the protein produced by suppressible and suppressed mutants. The feasibility of using this protein for such studies, however, has not been assessed.

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SUMMARY

The tryptophan synthetase system of *Saccharomyces cerevisiae* has been investigated and used to study the action of a super-suppressor.

Thirty-six allelic tr_5 mutants were characterized. All accumulated indoleglycerol phosphate when grown on tryptophan-limited medium. Three also accumulated indole. One was able to utilize indole in place of tryptophan.

Of 35 mutants tested in all possible combinations in diploids, 19 complemented with at least one other. A linear complementation map was constructed from these results. A map of 29 mutants was constructed by the X-ray method. The mutants are randomly distributed along the length of this map with the exception of four that are repeats at the same site. This map is colinear with the complementation map with one exception.

Thirteen of 32 mutants tested were suppressed by the super-suppressor S_d. Of these, only two are of the complementing type. One mutant is altered by S_d to an indole-utilizing phenotype. This mutant has unusual reversion properties. The two mutants that are both suppressible and complementing are unique in that they complement only with alleles that are to their right on the X-ray map. The results are consistent with the model of super-suppressible mutants being nonsense mutants.

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