SUPER SUPPRESSORS IN *NEUROSPORA CRASSA* I. INDUCTION, GENETIC LOCALIZATION AND RELATIONSHIP TO A MISSENSE SUPPRESSOR

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ABSTRACT

Genetic analyses have been made to test the feasibility of using coincident reversions to prototrophy of multiple mutants to select super suppressors (*ssu*) in *Neurospora crassa*. Of five double-mutant strains examined, only those mutant combinations in which both members had the properties of nonsense mutations did revert coincidently. Forty-eight genetically purified coincident revertants were crossed to the wild type, and each was shown to contain a suppressor mutation. Five super suppressors were examined more thoroughly. Tetrad and random spore analysis was used to demonstrate that each behaved as a single gene in crosses. Two super suppressors, *ssu*-1 and *ssu*-4 were localized respectively on the right and left arm of linkage group 7. Two others, *ssu*-2 and *ssu*-3, appear to map on the right arm of linkage group 1. The fifth super suppressor mapped, *ssu*-7, lies between *ad*-8 and *ylo*-1 on linkage group 6. One super suppressor, *ssu*-1, was interesting because it mapped near the location reported for the suppressor of the missense mutant *tryp*-3 (*td*201) (YOURNO and SUSKIND 1964a). However, no overlap was found in action spectrum of the two suppressors. Tetrad analysis showed the two suppressors were located about 10 map units apart, the missense suppressor being the more distal to the centromere.

RECENTLY a class of suppressors possessing unique properties has been isolated in Neurospora. Designated *super suppressors* (*ssu*), these mutations have the capacity to suppress mutants in physiologically unrelated genes, are allele specific yet locus non-specific (SEALE 1968; CHALMERS and SEALE 1971), and may result in the production of a qualitatively altered suppressed gene product (SEALE 1968; SEALE and KINNIBURGH, manuscript in preparation). That super suppressors affect mutants which produce profoundly altered polypeptide chains is indicated by the properties of the suppressible mutants. Most of these exhibit the complete loss of an enzymatic function (SEALE 1968; CHALMERS and SEALE 1971), many are non-complementing or lacking in serological cross-reacting material (CRM^-) (CASE and GILES 1968; SEALE 1968; TERRY 1966), and some result in pleiotropic defects (CASE and GILES 1968; CHALMERS and SEALE 1971). These mutants are probably of the nucleotide substitution type because many of them were induced and/or reverted by mutagens which act predominantly to cause such changes (e.g. nitrous acid, ethylmethane sulfonate). The

characteristics of these suppressor-sensitive mutants and of their super suppressors suggests an analogy to nonsense mutation and its suppression in *Escherichia coli* (GAREN 1968) and in *Saccharomyces cerevisiae* (HAWTHORNE 1969).

To investigate the hypothesis that super suppressors in Neurospora are indeed nonsense suppressors acting at the translation level, it would be valuable to have a number of genetically well-characterized strains carrying different super suppressors. This paper describes the induction of super suppressors in a double-mutant strain by ultraviolet light and shows that they constitute a significant fraction of all revertants from this strain. In addition five different super suppressor loci have been mapped on three linkage groups. The first super suppressor we localized was found to be near the reported location of the missense suppressor of the mutant *tryp*-3(*td*201) (YOURNO and SUSKIND 1964a). Because mutationally altered t-RNA species have been implicated in nonsense suppression (CAPECCHI and GUSSIN 1965) as well as in missense suppression in *E. coli* (CARBON, BERG and YANOFSKY 1966), a preliminary investigation of the relationship of missense suppression to nonsense suppression was made. Certain portions of these studies have been briefly reported (SEALE 1970).

MATERIALS AND METHODS

Strains: The super suppressible mutants *am*(17) and *tryp*-3(*td*140) were received respectively from DAVID R. STADLER and the Fungal Genetics Stock Center. These strains were crossed and the double mutant *am* (17) *tryp*-3(*td*140) was isolated from a random ascospore sample. Super suppressors, designated *ssu*, were assigned allele numbers that indicate the order in which they were isolated and the mutagen by which they were induced, e.g. *ssu*-1(WRN33), the first locus identified was the allele (W)ashington (R)evertant by (N)itrous acid number 33. WRU indicated an allele induced by ultraviolet light. The functionally detectable allele of the super suppressor will be designated *ssu*$^-$; *ssu*$^+$ designates the wild-type (non-suppressing) allele. To determine the genetic linkage of a super suppressor to its centromere and to linkage group (L.G.) markers, tester strains carrying *am*(17) together with markers for individual L.G.'s were employed. *am*(17) is an amination-deficient mutant located on the right arm of L.G. 5 which is super suppressible (SEALE 1968). The markers used were: m.t. and *al*-2(15300) (L.G. 1); *ad*-4(F2) (L.G. 3); *col*-4(70007) *uvs* (L.G. 4); *act*-2 (L.G. 5); *ylo*-1 *tryp*-2, *ad*-8(Y52M7) *ylo*-1(Y30539y) (L.G. 6); *nic*-3(431881), *me*-7(4894), *nt*(65001) (L.G. 7). Wild-type (3.1a) and most of the single mutants used in these crosses were obtained from the Fungal Genetics Stock Center as was the suppressor $Su\text{-}1_{td201}$ of *tryp*-3(*td*201). (The latter strain will be referred to merely as Su-1.) The *tryp*-3 allele *td*201 and its suppressors S_4, S_6, S_7, S_8 were kindly supplied by M. RACHMELER. The strains used to analyze the linkage relationship between the super suppressor *ssu*-1 and the missense-suppressor *Su*-1 were *tryp*-3$^-$ (*td*201) *am*$^-$(17) *ssu*-1$^-$ (WRN33) *Su*-1$^+$ and *tryp*-3$^-$ (*td*201) *am*$^-$(17) *ssu*-1$^+$ *Su*-1$^-$. These were obtained from asci dissected in order from the cross *am*(17)*ssu*-1 (WRN33) $\times$ *tryp*-3(*td*201) *Su*-1. The genotypes of these strains were confirmed by backcrossing them to wild type and analyzing random spores for the segregation of *td*201 and *am*(17).

Media: Stocks were grown and maintained on slants of minimal medium (VOGEL 1964), solidified with 1.5% Difco Agar and supplemented as needed with nutrilites at a concentration of 50μg/ml for nucleotides and amino acids except for tryptophan (150μg/ml) and alanine (450μg/ml). Strains having an *am* mutation were grown on alanine-supplemented medium and are conveniently scored on minimal medium supplemented with 1500 μg/ml glycine. Vitamins were supplied at a concentration of 5μg/ml. Crosses were performed on slants of synthetic crossing medium (WESTERGAARD and MITCHELL 1947) supplemented for the nutritional requirements of the protoperithecial parent.

Crossing and genetic procedures: Strains lacking the super suppressor were always used as protoperithecial parents because those carrying a super suppressor did not cross consistently when used as the female parent. Often they formed no perithecia or empty ones in contrast to crosses made in the opposite direction which generally produced a good ascospore yield. Following growth of the female parent for 4 to 5 days in the dark at 25°C, crosses were initiated by depositing conidia from a fresh slant of the fertilizing parent onto the surface of the maternal culture. Super suppressor crosses incubated at 25°C generally began to discharge ascospores two weeks after fertilization. Those which had been discharging spores for about 5 days were good sources of asci for dissection.

Genetic analysis of the crosses was made by dissecting asci in order, by individual random ascospore isolations, and by random ascospore platings on appropriately supplemented minimal medium containing sorbose (LESTER and GROSS 1958).

Induction and selection of revertants: The double-mutant strains were grown on alanine–tryptophan slants for five to seven days. Conidia from these slants were suspended in sterile distilled water, filtered through gauze to remove hyphal fragments and irradiated with light from a low pressure 15 watt General Electric germicidal lamp for 60 sec at a distance of 13 cm. An aliquot of the conidial suspension was added to molten sorbose medium selective for *am* reversion (supplemented with glycine and tryptophan), for *tryp* reversion (supplemented with alanine) and for *am*, *tryp* coincident reversion (supplemented with glycine). (Glycine at a concentration of 1500μg/ml is added to retard the slow growth of *am* mutants on minimal medium.)

RESULTS AND DISCUSSION

Induction of super suppressors in double-mutant strains by ultraviolet light: The initial objective of this study was the attempt to develop a strain of Neurospora useful for the production and isolation of a broad spectrum of super suppressors. Coincident reversion of physiologically unrelated mutants in multiple-mutant strains offers a means of direct selection of such suppressors and has been successfully employed for the isolation of nonsense suppressors in yeast (HAWTHORNE and MORTIMER 1963; GILMORE 1967) and in bacteria (GORINI and BECKWITH 1966; BERKOWITZ *et al.* 1968). The experiments now described test the efficiency of coincident reversion as an approach to the selection of super suppressors in Neurospora.

In Table 1 the reversion characteristics of five *am*$^-$*tryp*$^-$ double-mutant strains have been presented. Three of the mutations carried by these strains, *am*(17),

TABLE 1

Ultraviolet light-induced reversion of double mutants

Strain	Percent survival	Reversion frequency per 10^6 surviving conidia: UV treated *am*	UV treated *tryp*	UV treated *am; tryp**	Spontaneous *am*	Spontaneous *tryp*	Spontaneous *am; tryp**
am(17); *tryp*-3 (*td*140)	38	35	45	17	3.4	0.8	1.7
	53	32	44	18	<0.2	0.2	<0.2
am(17); *tryp*-3 (*td*2)	63	8.7	10	<0.1	<0.2	0.25	<0.2
am(17); *tryp*-3 (*td*201)	70	16.1	3.9	<0.1	<0.1	<0.1	<0.1
am(14); *tryp*-3 (*td*140)	73	6.8	38	<0.05	1.6	0.1	<0.05
am(17); *tryp*-2(10)	45	21	6.6	1.0	<0.07	<0.07	<0.07

Reversion frequencies were determined separately for each locus and for the double reversion event. Nutritional supplements were added for the unselected locus.

* This class represents coincident reversion of both loci.

tryp-3(*td*140) and *tryp*-2(10), have been shown to possess the properties of nonsense mutants and to be suppressible by the first super suppressor isolated, *ssu*-1(WRN33) (SEALE 1968; CHALMERS and SEALE 1971). Of the three remaining mutants, *am*(14), is a nitrous acid-induced mutant which exhibits the characteristics of missense mutants (STADLER 1966; SEALE 1968). *Tryp*-3(*td*201) and *tryp*-3(*td*2) are of special interest because each appears to be a missense mutant suppressible by extragenic suppressors (YOURNO and SUSKIND 1964a; KAPLAN *et al.* 1964; YANKOFSKY and BONNER 1955). Two strains, *am*(17)*tryp*-3(*td*140) and *am*(17)*tryp*-2(10), produced coincident revertants ten thousand times more frequently than could be accounted for by simultaneous independent mutational events leading to reversion. No other combination of mutants produced any detectable coincident revertants even when selective plates were held for seven days. From the data in Table 1, several important features of coincident reversion in these strains of Neurospora emerge. The first is that coincident reversion exhibits both allele specificity and the lack of locus specificity. This type of specificity excludes the possibility that coincident revertants can be accounted for by a special class of nuclei present in significant frequency which undergo non-random multiple mutations when mutagenized. A second feature of the data is that only those mutants whose properties were suggestive of nonsense mutations, *i.e.* *am*(17); *tryp*-3(*td*140); *tryp*-2(10), coincidentally reverted. Allele specificity and lack of locus specificity in the simultaneous reversion of physiologically unrelated loci combined with the properties of the mutants which responded to mutagenic treatment suggest that the coincident revertants are super suppressors, probably equivalent to nonsense suppressors. In Saccharomyces, coincident revertants obtained by HAWTHORNE and MORTIMER (1963) and by GILMORE (1967) always arose as the result of suppressor mutations. Further analysis has shown that such suppressors definitely suppress nonsense mutants (MANNEY 1968; GILMORE, STEWART and SHERMAN 1968; HAWTHORNE 1969). In addition, the specificity displayed was interesting because two mutants which failed to revert with *am*, *i.e.*, *td*201 and *td*2, appear to be missense and are known to be suppressible by extragenic suppressors. It would appear that, at least for these three alleles, there is no overlap of the action spectrum of missense suppressors and super suppressors. In *E. coli* mutationally modified t-RNAs can mediate both types of informational suppression but there is a similar lack of overlap in specificity (CARBON, BERG and YANOFSKY 1966). Finally, the strain *am*(17)*tryp*-3(*td*140) coincidently reverted about seventeen times more frequently than did *am*(17)*tryp*-2(10). This may suggest that the former strain can be restored to prototrophy by a less restricted class of super suppressors that can the latter. GILMORE (1967) has obtained similar results in his studies of yeast revertants. In *E. coli* different nonsense suppressors are known to cause the translation of nonsense condons as several different amino acids (GAREN, 1968; GORINI, 1970). While it may be possible to substitute any of a number of amino acids at a particular mutant site in a polypeptide chain, all of the substitutions may not lead to the restoration of the function of this polypeptide. Such functional restriction of acceptable amino acid substitutions

TABLE 2

Phenotypic and genotypic characterization of selected revertant classes from the double-mutant strain am*(17)* tryp-3 (td*140*)

Treatment	Analysis of phenotype* Locus selected	Number of isolates tested	Percent prototrophic for both loci
none			
	am(17)	25	100
	*td*140	6	100
UV			
	am(17)	40	74
	*td*140	57	18
	am(17); *td*140	35	100

Treatment	Analysis of genotype† Locus reverted‡	Number of strains tested	Percent suppressed
none			
	am(17)	17	100
	*td*140	9	78
	am(17); *td*140	13	100
UV			
	am(17)	10	30
	*td*140	26	0
	am(17); *td*140	35	100

* Prototrophs at either the *am* locus or the *tryp* locus on both were selected and subsequently tested for nutritional requirements.

† Genetically purified revertant strains were crossed to wild-type 3.1a and progeny ascospores examined for mutant segregants. Those strains yielding any mutant segregants were designated as suppressed.

‡ This class phenotypically confirmed by growth tests prior to genetic purification cross (e.g., *am* revertants constituted the class of revertants prototrophic for *am* but not for *td*).

could account for the differences in coincident reversion frequency observed between *am*(17)*tryp*-3(*td*140) and other suppressible *am*(17)*tryp* combinations. Since the broadest possible spectrum of super suppressors was wanted, further attention was confined to those isolated from *am*(17)*tryp*-3(*td*140).

Phenotypic and genotypic analyses of revertants from *am*(17)*tryp*-3(*td*140) are shown in Table 2. Phenotypic characterization of revertants selected for prototrophy at one locus but unselected at the second locus was carried out to determine the fraction of simultaneous revertants that occurred under conditions not specifically selective for them. Revertant colonies were picked and transferred to slant supplemented with alanine and tryptophan. Conidia from these slants were tested on medium selective for the *am* locus, the *td* locus, or both. These tests showed that coincident revertants (suppressors) occurred with appreciable frequency in both untreated and UV-treated material unselected for the second locus. Therefore, super suppressors contribute significantly to the reversion of *am*(17) and *tryp*-3(*td*140) even under conditions expected to be much less selective for them than those demanding prototrophy at both loci. There is, however, some discrepancy between the frequency of simultaneous

revertants expected on the basis of those detected among revertants selected for prototrophy at only one locus and that which is observed upon direct selection.

In the lower part of Table 2, recombinational evidence is presented to demonstrate that coincident revertants are indeed suppressed by super suppressors unlinked or loosely linked to the *am* and *tryp*-3 loci. The data in this part of the table were taken from a single UV reversion experiment. Revertant strains were genetically purified by crossing them back to their mutant parent of the opposite mating type. A single ascospore prototrophic for the selected locus was isolated and crossed back to a wild-type strain (3.1a) and random ascospores screened on solid medium in plates for the segregation of the appropriate mutants. A fre-

TABLE 3

Linkage of super suppressors to their centromeres and to linkage group markers

Suppressor locus	Gene combination* examined	Number of random ascospores: Parental genotype	Number of random ascospores: Recombinant genotype	Percent recombination	Linkage relationship
ssu-1 (WRN33)					
	ssu-1; *meth-7*	320	56	15	linked, L.G. VII RC†
	ssu-1; *nt*	129	38	23	linked, L.G. VII R
	meth-7; *nt*	126	60	32	linked, L.G. VII R
	ssu-1; *nic*-3	60	63	52	unlinked, L.G. VII L
	ssu-1; centromere‡			14	
ssu-2 (WRU35)					
	ssu-2; *al*-2	140	48	26	linked, L.G. I L
	ssu-2; M.T.	116	26	22	linked, L.G. I R
	al-2; M.T.	150	98	40	loosely linked, L.G. I
	ssu-2; centromere			7	
ssu-3 (WRU118)					
	ssu-3; *al*-2	155	76	33	loosely linked, L.G. I R
	ssu-3; M.T.	180	51	22	linked, L.G. I L
	ssu-3; centromere			10	
ssu-4 (WRU18)					
	ssu-4; *nic*-3	120	46	28	linked, L.G. VII L
	ssu-4; *meth-7*	68	17	20	linked, L.G. VII RC
	ssu-4; centromere			10	
ssu-7 (WRU7)					
	ssu-7; *ylo*-1	166	28	14	linked, L.G. VI L
	ssu-7; *ad*-8	122	10	8	linked, L.G. VI L
	ylo-1; *ad*-8	106	26	20	linked, L.G. VI L
	ssu-7; centromere			20	

* Crosses of suppressed *am* strains to linkage group tester also carrying the same *am* allele (e.g., *am*(17) *meth-7*+ *ssu*$^-$ × *am*(17) *meth-7*$^-$ *ssu*+) were used throughout so that all progeny could be scored directly for the presence or absence of the suppressor. Some testers carried two linkage group markers which allowed the ordering of loci by the consideration of the frequency of the double-recombinant class. Only those crosses which showed linkage of a *ssu* to a L.G. marker were included in this table.

† (L.G.) linkage group; (R,L,C) right arm, left arm, centromere region.

‡ Centromere distances were determined from *ssu* segregation patterns in asci dissected in order. At least 40 asci were dissected in each case.

quency of 25% mutant segregants was taken as evidence for suppression by an unlinked suppressor. Most strains examined in this manner produced 15–27% mutant spores which were confirmed to carry the original mutant allele. The data confirm the genetic expectations based on the phenotypic classification in all cases. As stated, all double revertants were clearly suppressed in both the treated and the untreated material. Revertants specifically prototrophic for *td*140 occurred only at or very closely linked to the *td* locus. About 30% of the *am* revertants not prototrophic for *td* were also suppressed.

Segregation of five super suppressors and determination of genetic linkage to their centromeres: Four ultraviolet light-induced super suppressors derived from *am*(17)*tryp*-3(*td*140) were chosen at random for further characterization from among those genetically purified. In addition to these four strains, *am*(17)*ssu* (WRU7), (WRU18), (WRU35) and (WRU118), one of the first super-suppressor carrying strains to be isolated, *am*(17)*ssu*(WRN33), (SEALE 1968), also was investigated. Segregation of *ssu*'s in crosses of the type *am*(17)*ssu*$^-$ ×*am*(17) *ssu*$^+$ could be followed directly by scoring for *am* prototrophy. (Throughout the remainder of this paper, *ssu*$^-$ will refer to the functionally detectable allele of the suppressor; *ssu*$^+$ will designate the wild-type (non-suppressing) allele.) Three hundred and thirty-six asci were dissected in order and each spore pair was tested for the presence of the super suppressor (Table 3). In every case the suppressor segregated 2$^+$:2$^-$ as expected for a single gene. In no case was there any indication of the selective loss of the functionally detectable allele of the suppressor. Each super-suppressor bearing strain appeared to be quite stable and exhibited an otherwise wild-type-like phenotype with the exception of its consistent inability to give rise to fertile crosses when used as the protoperithecial parent. Each of these super suppressors is clearly linked to its respective centromere.

Genetic mapping: An attempt was made to detect linkage among the five super suppressors by analyzing *ssu*$^-$ × *ssu*$^-$ crosses; however, except for some crosses involving *ssu* (WRU118), these were sterile. This approach was abandoned in favor of analyzing progeny from crosses of suppressed *am*(17) strains to linkage group (L.G.) tester stocks also carrying the *am*(17) allele. Such an arrangement allows the detection of either allele of the super suppressor in all progeny of the cross, a convenience in view of the difficulty, if not impossibility, of distinguishing between *am*$^+$ *ssu*$^+$, *am*$^+$ *ssu*$^-$ and *am*$^-$ *ssu*$^-$ in most conventional nutritional omission tests. Each *ssu* was crossed to all L.G. tester stocks and progeny analyzed from each cross. Table 3 shows the utility of this approach; the five super suppressors have been localized on three linkage groups with respect both to their centromeres and to at least one linkage group marker. The order of the genes shown in Figure 1 has been derived in each case by a combination of data from ordered asci and random spores.

Two super suppressors, *ssu*-1 (WRN33) and *ssu*-4 (WRU18), have been located on linkage group VII. Table 3 shows that the former is about 14 map units from the centromere, the latter about 10 units. Linkage to L.G. VII markers was first indicated in a cross of *am*(17)*me*-7 *ssu*-1$^+$ × *am*(17)*ssu*-1$^-$. One non-parental ditype ascus and nine tetratype asci were observed among 34 asci

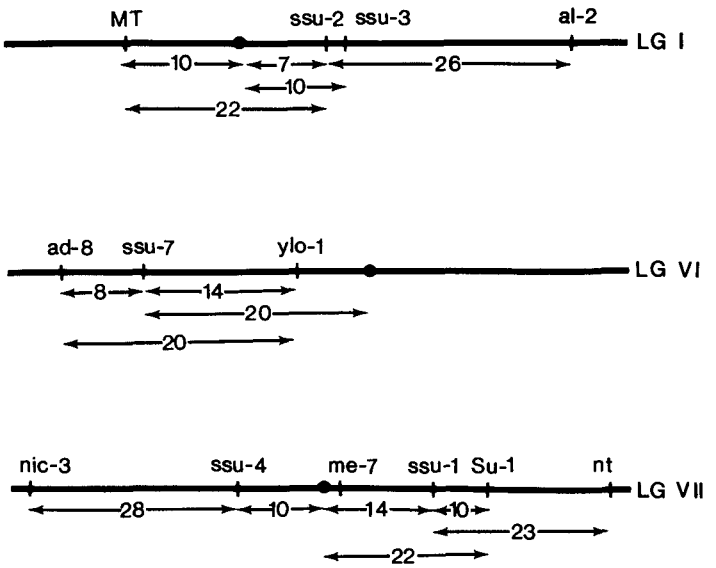

FIGURE 1.—Genetic map showing the location of five super suppressors in Neurospora. The values below the lines indicate map units. L.G. (linkage group)

examined, a clear indication of linkage to *me-7*. Random spore data (Table 3) show clearly that *ssu*-1 is located on the right arm of L.G. VII between *me-7* and the more distal marker *nt*. The distance of *nic*-3 (which marks the left arm of L.G. VII) from its centromere was determined to be about 30 units based on 40 asci dissected in order. While *ssu*-1 appeared to be unlinked to this marker. *ssu*-4 was loosely linked (Table 3). A consideration of centromere linkages of *ssu*-4 and *nic*-3 and recombination values with markers determined from random spores places *ssu*-4 on the left arm of L.G. VII more proximal to the centromere than *nic*-3. Map positions for *nic*-3, *me-7* and *nt* are very similar to those summarized by DAVIS and DE SERRES (1970).

Two super suppressors, *ssu*-2 and *ssu*-3, were located on L.G. I R. Based on a limited number of asci *ssu*-2 and *ssu*-3 are located respectively about 7 and 10 map units from the centromere. Since the mating type (M.T.) locus is positioned about ten map units from its centromere, on the left arm of L.G.I., both *ssu*'s, if linked to M.T., should be very tightly linked to this locus or be located on the right arm of this linkage group. The random spore data in Table 3 suggest both suppressor loci are located on the right arm of this linkage group. Segregation patterns in ordered asci (not shown) and random spore data lead to the placement of both *ssu*'s more proximal to the centromere than the *al*-2 marker, *ssu*-3 probably the closer of the two. Attempts to map these two super suppressors against several other markers on L.G. I R have been frustrated by problems of severe infertility of crosses. In spite of ascospore viability problems, these two super suppressors were shown to be non-allelic. From a cross of *am*(17)*ssu*-3 (WRU118) ♀ × *am*(17)*ssu*-2 ♂, 20*am*$^-$ ascospores: 170*am*$^+$ (suppressed): 293

aborted spores were detected. The recovery of a significant number of *am*$^-$ recombinants indicates that these suppressors are due to mutations in different genes. Aborted ascospores (light in color, often small or misshapen) are not seen in crosses of *ssu*$^+$ × *ssu*$^-$. The qualitative properties of the suppressed *am* gene product (glutamate dehydrogenase) in *am*(17)*ssu*-2 strains are also demonstrably different from those in *am*(17) strains suppressed by *ssu*-3 (SEALE and KINNIBURGH, manuscript in preparation). This is consistent with *ssu*-2(WRU35) and *ssu*-3(WRU118) resulting from mutations in different components of the translation system which lead to the substitution of different amino acids, e.g. different t-RNAs.

Finally, *ssu-7* has been mapped on the left arm of L.G. VI as shown in the lower part of Table 3. These data, when combined with segregation patterns in ordered asci, clearly place *ssu-7* between *ylo*-1 and *ad*-8, about 8 units from the latter marker. Again the map values are consistent with those summarized by DAVIS and DE SERRES (1970).

In the present study five super suppressors, selected at random from among some forty suppressor-bearing strains, were found to map at five genetically distinct loci. A sixth locus, *ssu*-6, has been identified on linkage group V by M. CASE (SEALE, CASE and BARRATT 1969). Preliminary analysis of other UV-induced *ssu*'s derived from the *am*(17)*tryp*-3(*td*140) strain indicates that several other super-suppressor genes exist in addition to those already detected (SEALE, unpublished observations). In yeast, HAWTHORNE and MORTIMER (1968) were able to locate sixteen genes involved in nonsense suppression dispersed on eleven different linkage groups. Eight of these nonsense suppressors promote the insertion of one amino acid, tyrosine, at the site of the nonsense cordon and thus, if the analogy to bacterial systems holds, appear to be redundant copies of the tyrosine t-RNA structural gene(s) (GILMORE, STEWART and SHERMAN 1971). It will be interesting to learn whether the suppressors of *am*(17)*td*140 can be differentiated on the basis of their ability to suppress different mutant alleles and to restore qualitatively different suppressed gene products or whether they may constitute a redundant set of t-RNA genes. SCHWEIZER, MACKECHNIE and HALVORSON (1969) report that about 320 to 400 t-RNA cistrons are present in the haploid genome. Thus, the detection of a large number of nonsense suppressors in yeast is not surprising if they result from mutationally altered t-RNAs. A similarly large number of distinct super-suppressor loci might be expected in Neurospora because of its phylogenetic relatedness to Saccharomyces. In *E. coli* mutations in at least nine distinct gene loci lead to ability to suppress nonsense (GORINI 1970).

The relationship of super suppressors to missense suppressors. Suppressors of the missense mutant *tryp*-3(*td*201) result in the production of a small amount of wild-type-like tryptophan synthetase activity in addition to the altered enzyme (and CRM) normally formed in the *td*201 mutant (YOURNO and SUSKIND 1964b; RACHMELER 1967). The first such suppressor described, *Su*-1$_{td201}$, was localized on the right arm of linkage group VII (YOURNO and SUSKIND 1964a). In *E. coli* both missense and nonsense suppression can be mediated by mutationally altered t-RNAs (CARBON, BERG and YANOFSKY 1966; CAPECCHI and GUSSIN 1965). This

TABLE 4

Effect of tryp-3 (td*201*) *missense suppressors on super-suppressible* am *mutants*

Cross	Ascus type for *am*			Ascus type for *tryp*-3 (*td*201)		
	8+ : 0-	6+ : 2-	4+ : 4-	8+ : 0-	6+ : 2-	4+ : 4-
am(17) × *td*201 Su-1	0	0	8	2	6	1
× *td*201 S_4	0	0	7	1	4	2
× *td*201 S_6	0	0	6	0	4	2
× *td*201 S_7	0	0	2	0	1	1
× *td*201 S_8	0	0	6	3	1	2
am(24) × *td*201 S_6	0	0	4	0	0	2
× *td*201 S_7	0	0	2	0	0	1
*td*201 × *am*(17) *ssu*-1 (WRN33)	1	3	1	0	0	5

The numbers in this table refer to asci with the segregation pattern specified for each mutant. S designations refer to independently isolated suppressors of *tryp*-3 (*td*201).

fact and the finding that *ssu*-1 also mapped on the right arm of linkage group VII called for an investigation of the relationships of super suppressors to missense suppressors. Table 4 shows that there was no detectable overlap in specificity of these two classes of suppressors. Crosses were made to ascertain whether the suppressors of *td*201 (Su-1, S_4, S_6, S_7, S_8) affected *am*(17) or another super-suppressible mutant, *am*(24), which displays a somewhat different suppressor response pattern (SEALE, unpublished results). Asci dissected in order from these crosses exhibited mutant segregation patterns consistent only with the failure of Su_{td201} to restore *am* function. Two of these suppressors, S_6 and S_7, were tested against the super-suppressible mutant *tryp*-3(*td*140) and failed to suppress it. Similarly, *td*201 is not suppressed by *ssu*-1. In each cross analyzed in Table 4 at least one ascus clearly contained a spore pair of the phenotype indicating the presence of the unsuppressed test allele and the heterologous suppressor (e.g., *am*$^-$ slow-growing *tryp*$^+$; expected genotype *am*(17)$^-$*td*201$^-$*Su*-1$^-$). This expected genotype was confirmed in each case by random spore analysis from the appropriate test cross. As might be expected, *am*(17) and *tryp*-3(*td*201) do not coincidently revert (Table 1). Evidently the alterations of translation fidelity which probably account for super suppression and suppression of *td*201 involve lesions in different components of the translation system or ones with non-overlapping specificities. As mentioned previously, another suppressible *tryp*-3 missense mutant, *td*2 (YANOFSKY and BONNER 1955), also failed to co-revert.

The genetic non-identity of *ssu*-1 and *Su*-1 was confirmed by mapping data shown in Table 5. Both random spore data and those from asci dissected in order indicate that the two are separated on L.G. VII by about 10 map units. Segregation of the suppressors in ordered asci demonstrates that *ssu*-1 is the more proximal to the centromere, and the comparison of centromere distances between the two suppressors is in good agreement with map distance between them based on random spore analysis. The location of *Su*-1 shown in Figure 1 is in good agreement with the mapping data of YOURNO and SUSKIND (1964a).

TABLE 5

Analysis of linkage relationship between a super suppressor (ssu*1*) *and the missense suppressor* Su-*1 of* tryp-3 (td*201*)

tryp-3 (*td*-201) *am*(17) *ssu*-1 (WRN33) × *tryp*-3 (*td*201) *am*(17) *Su*-1

ASSORTMENT OF SUPPRESSORS

ORDERED ASCI

Parental ditype	Non-parental ditype	Tetratype
54	2	14

$$\text{Percent recombination} = \frac{\text{NPD}+\frac{1}{2}\text{T}}{\text{total}} = \frac{2+7}{70} = 13\%$$

RANDOM SPORES

Parental types	Recombinant types	Percent recombination
479	56	10

SEGREGATION OF SUPPRESSORS

	Ascus type		
Suppressor	1st division	2nd division	Percent 2nd division
ssu-1 (WRN33)	123	48	28
Su-1	47	35	43

Assortment and segregation of suppressors was detected by scoring for prototrophy at the *am* locus (*ssu*-1) and the *tryp*-3 (*Su*-1).

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