INDIRECT SUPPRESSION IN *CAENORHABDITIS ELEGANS*

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ABSTRACT

Two cases of indirect suppression have been characterized. One case involves suppressors compensating for defects in muscle structure. Nine independent suppressor mutations were judged to lie in a single suppressor gene, *sup-3*. Suppression is dominant, but dose dependent, and results in improved locomotion, as well as in an increase in the ability of mutant animals to lay eggs. Mutations in six genes known to affect muscle structure were tested for suppression by representative *sup-3* mutations. Alleles of three of the six genes are suppressed, two of which are known to code for thick filament proteins. One suppressor allele was identified as a deletion by genetic criteria. A second case of indirect suppression is not associated with muscle defects, but involves two mutant genes producing uncoordinated phenotypes very similar to one another. As in the first case, suppression is dominant but dose dependent and is not allele specific.

THERE are two general mechanisms for intergenic suppression. One type of suppressor corrects the amino acid sequence of a mutant protein by means of an alteration in the protein synthesizing system (SMITH 1972). A second type of suppressor allows continued synthesis of the gene product in mutant form, but compensates indirectly to allow expression of the wild-type phenotype by other means. For example, if a mutant protein fails to interact with a second protein, alteration of the second protein may in some cases restore normal interaction. Such situations have been encountered in the study of ribosome assembly and function (NOMURA 1970; BIRGE and KIRLAND 1970; BJARE and GORINI 1971; KREIDER and BROWNSTEIN 1972). Suppression may also result from substitute protein activity as in the case of a suppressor of *recB* and *recC* in *Escherichia coli* (BARBOUR *et al.* 1970). Such suppressors are gene-specific, rather than allele-specific. Mechanisms of genetic suppression have been reviewed by HARTMAN and ROTH (1973).

Where methods of genetic selection exist, indirect suppression is potentially useful in the study of development. In multicellular organisms, different cell types often produce related "isozymes" that are structurally distinct but functionally very similar. Regulatory mutations allowing expression of an isozyme to replace a missing function would be interesting cases of suppression from the

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standpoint of genetic control. Cases of suppression involving protein-protein interactions are potentially useful in the study of a variety of complex cell structures.

The work described here was initiated to determine if indirect suppression could be detected in mutants of *C. elegans*. Muscle mutants were used in this study in order that something might be learned about the functional interaction between muscle proteins or the control mechanisms governing expression of genes for muscle proteins. Also, genes for particular muscle proteins might be detected that are not detectable by other means.

Mutants severely defective in body musculature are paralyzed or dystrophic. Such mutants have been characterized using polarized light microscopy, electron microscopy, and biochemical analysis (EPSTEIN, WATERSTON and BRENNER 1974; MACLEOD *et al.* 1977). Paralyzed mutants of C. elegans are variable because reproduction is typically hermaphroditic and active males are not necessary for the production of offspring. It is also possible to obtain mutants paralyzed in the body that are not affected in pharyngeal function, so that feeding and growth are possible. Mutations in at least 12 genes are known to affect body musculature (WATERSTON, FISHPOOL and BRENNER 1977). Identification of the gene for a myosin heavy chain, *unc-54* (MACLEOD *et al.* 1977) and the gene for paramyosin, *unc-15* (WATERSTON, FISHPOOL and BRENNER 1977) provided the basis for a study of the genetic suppression of known muscle defects.

Two suppressor genes are described in this paper. The most thoroughly characterized gene suppresses alleles of *unc-15* and *unc-54*, both of which specify thick filament proteins. A second suppressor type is not associated with muscle defects, but involves two mutant genes, *unc-1* and *unc-24*, which produce uncoordinated phenotypes very similar to one another.

MATERIALS AND METHODS

Nematode strains and nomenclature: All mutants are derivatives of the wild-type N2 strain of *Caenorhabditis elegans* var. Bristol. Type alleles used for genetic mapping are those described by BRENNER (1974). Strains described in this work are listed in Table 1.

For Cambridge stocks, each independent homozygous mutant is assigned an isolation number with an "E" prefix after having been backcrossed with N2 males and resegregated to eliminate possible mutations in the genetic background. Allele numbers correspond to homozygous stock numbers, but carry an "*e*" prefix and are italicized. Multiple mutants are given catalogue numbers with a "D" prefix. The prefixes "R" and "H" refer to revertant stocks and heterozygous stocks, respectively. The same procedures are used for Missouri stocks, except that the prefixes M, M-M, M-R and M-H are used to designate single mutants, multiple mutants, revertants, and heterozygous stocks, respectively.

Gene designations are as previously described (BRENNER 1974) including uncoordinated (*unc*), dumpy (*dpy*), small (*sma*), long (*lon*), and roller (*rol*).

Hermaphrodite genetics: The basic methods of mutagenesis, complementation tests and genetic mapping in *C. elegans* have been described (BRENNER 1974). All matings were performed on 50 mm or 60 mm diameter petri plates with two to three young adult hermaphrodites and three to four males. When homozygous, *unc-54* mutant hermaphrodites were mated with males, 10 to 12 L4 hermaphrodites were placed on a plate with three to four males. The large number of hermaphrodites is used because *unc-54* broods are small and reproductive periods are short (see RESULTS).

TABLE 1

*Nematode strains**

Strain	Genotype	Derivative of
D454	*unc-54 sup-3*	E1390 ♂ × E190
D472	*unc-41 unc-42*	$\frac{e268}{+}$ ♂ × E270
E224	*dpy-11*	BRENNER 1974
E1390	*sup-3*	$\frac{e224}{+}$ ♂ × R188
E1407	*sup-3*	$\frac{e224}{+}$ ♂ R210
H89	$\frac{e224 \quad +}{+ \quad e1405}$	$\frac{e224}{+}$ ♂ × R189
M–H1	$\frac{e224 \quad +}{+ \quad e1405}$; $\frac{e73}{e73}$	H89 ♂ × M–M17
M–H3	$\frac{e30 \quad +}{+ \quad e1405}$	$\frac{e30}{+}$ ♂ × R189
M–H8	$\frac{e1405}{+}$; $\frac{e73}{e73}$	H89 ♂ × E73
M–M17	$\frac{e224}{e224}$; $\frac{e73}{e73}$	$\frac{e224}{+}$ ♂ × E73
M–M76	$\frac{e1407}{e1407}$; $\frac{e190}{e190}$	E1407 ♂ × E190
R188	$\frac{e1390}{e1390}$; $\frac{e1258}{e1258}$	EMS mutagenesis of E1258
R189	$\frac{e1405}{+}$; $\frac{e1258}{e1258}$	EMS mutagenesis of E1258
R206	$\frac{m48}{m48}$; $\frac{e138}{e138}$	EMS mutagenesis of E138
R210	$\frac{e1407}{e1407}$; $\frac{e1315}{e1315}$	EMS mutagenesis of E1315

* Additional strains are listed in Tables 2 and 3.

Heterozygous F_1 hermaphrodites whose progeny were to be scored according to phenotype were removed from mating plates as L4 larvae and isolated on fresh plates. Progeny were then scored on the fourth and fifth day of incubation. To avoid bias in scoring phenotypic ratios, all progeny were counted. In cases where brood sizes were measured, L4 hermaphrodites were isolated on 60 mm diameter petri plates and transferred to fresh plates daily during their egg-laying period. After three to four days of incubation, adult progeny were counted as they were removed from the plate through the drawn-out tip of a pasteur pipette connected to a vacuum line.

Reversion tests: Mutant stocks were grown on 100 mm diameter petri plates until bacteria were nearly exhausted. The animals were then washed from the plate in M9 buffer and treated with ethyl methanesulfonate (EMS) according to BRENNER (1974). Using a 5 ml pipette, a mutagenized population was distributed to forty 100 mm petri plates previously seeded with a uniform lawn of bacteria. About 100 worms per plate were obtained by dropping 0.1 ml of suspended animals at the edge of each plate. Since the mutants are paralyzed, or uncoordinated, the population advances across the surface of the agar very slowly. Revertants can be detected by their tracks as they migrate away from the densely populated portion of the plate. Populations were allowed to grow for seven days (the equivalent of two generations) so that both dominant and recessive revertants could be detected. In the case of E73 (*unc-15*), which has a very high EMS-induced reversion frequency, 20 mutagenized adult animals were placed at one edge of each of 25 plates. This permitted a quantitative estimate of the frequency of reversion since the average number of progeny per E73 hermaphrodite is known (see RESULTS).

Strain construction and cross-suppression tests: Once the *sup-3* locus had been mapped, it was possible to remove the suppressor alleles from their original *unc-54* or *unc-15* genetic background. To construct strain E1390, *dpy-11*/+ males were mated with R188 slow hermaphrodites. Wild-type F_2 progeny were isolated from a population segregating *dpy-11* homozygotes. The E1390 stock was chosen from those F_2 progenies which segregated neither dumpy nor slow animals. Spontaneous males were subsequently used to establish a male stock for use in cross-suppression tests. The males were first tested for the presence of *sup-3* by crossing with the parental mutant, E1258. Using the same procedure, an E1407 male stock was derived from R210. Strains E1390 and E1407 are wild type in morphology and behavior.

The lethal *sup-3* allele, *e1405* (see RESULTS), was removed from its original *unc-54* background by crossing *sma-1*/+ males with R189 hermaphrodites. Wild-type F_2 progeny were picked from a population containing both small segregants and slow (suppressed) segregants. One of those segregating small animals and abnormal L1 larvae (*e1405* homozygotes) was saved as a balanced stock, M-H3. Spontaneous M-H3 males were propagated for use in cross-suppression tests. For mapping *e1405* with respect to *dpy-11*, strain M-H1 (*dpy-11/e1405*; *unc-15*) was constructed in a way analogous to the construction of M-H3.

To test suppression of the various *unc* alleles, E1390, E1407, and M-H3 males were mated with homozygous mutant hermaphrodites. In the absence of suppression, wild-type F_1 hermaphrodites segregate wild-type and paralyzed progeny in a 3:1 ratio. If suppression occurs, the ratio of wild-type: slow (suppressed):paralyzed is 12:3:1. In crosses with M-H3 (*sma-1/e1405*) males, half the F_1 progeny receive the lethal *e1405* allele, while half receive the balancing *sma-1* allele. Thus F_1 animals segregating *sma-1* homozygotes were discarded, while populations containing abnormal L1 larvae (*e1405* homozygotes) were scored for suppression, as above. The *sma-1* gene is very close to *e1405*, and no recombination has been observed between these markers.

Microscopy: For polarized light microscopy, a Zeiss Universal Microscope was used with strain-free achromatic objectives. Animals were picked from growth plates and suspended in 10 μl of M9 buffer. No. 1 glass cover slips were placed over the suspended animals.

RESULTS

Revertants: Revertants of the *unc-54* (I) mutant, E1258, were obtained by treating a mixed population of worms with EMS (see MATERIALS AND METHODS) and five revertants were found (Table 2). Although sufficient time was allowed in these experiments to detect revertants as homozygotes, all were found to be heterozygous and were detected because the revertants were dominant. The paralyzed mutants do not lay eggs, but larvae hatch internally and consume the parent. This shortens the reproductive period and sharply reduces the number of progeny produced per worm. It is likely, therefore, that many of the more

TABLE 2

Summary of reversion experiments

Strain	Mutant gene	Mutagenic treatment*	No. of EMS-induced revertants	Approximate reversion frequency	Nature of revertants	Suppressor allele described
E1258	*unc-54*	DES	5	3×10^{-5}	3 intragenic	
					1 suppressed	*e1390*
					1 heterozygous	*e1405*
E1315	*unc-54*	EMS	1	10^{-5}	suppressed	*e1407*
E73	*unc-15*	EMS	10	3×10^{-4}	4 intragenic	
					6 suppressed	
E138	*unc-24*	EMS	10	4×10^{-5}	10 suppressed	*m48*
E1172	*unc-24*	ICR-191	10	7×10^{-5}	10 suppressed	

* Abbreviations are as follows: DES (diethyl sulfate), EMS (ethylmethane sulfonate), ICR-191 [2-methoxy-6-chloro-9-(3-(2-chloroethyl)aminopropylamino) acridine dihydrochloride)].

mature mutagenized animals die before mutant clones are generated, and that many of the mutants may arise from larvae placed on the plate. Even after seven days of growth (two generations) some adult revertants selected are likely to be F_1 animals. For the same reason, the estimated EMS-induced reversion frequency of the mutants (Table 2) is likely to be an underestimate.

With one exception, R189, the heterozygous revertants segregated homozygotes. The R189 stock is persistently heterozygous, segregating animals with the paralyzed phenotype of *unc-54* (*e1258*) and, in addition, severely uncoordinated larvae that die before completion of the L1 molt. These larvae are presumed to be homozygous. Restoration of the normal phenotype varied considerably among the revertants. Three were nearly wild-type, while the remaining two, carrying *e1390* and *e1405*, moved slowly but were distinctly improved.

In an experiment with E1315, another allele of *unc-54*, one partial revertant was found. In this case no animals were found to exhibit complete restoration of movement (Table 2).

In order to determine if any of the homozygous revertants carried unlinked suppressors, revertant hermaphrodites were crossed with wild-type (N2) males as described in MATERIALS AND METHODS. Heterozygous F_1 males were then crossed with the *unc-54* parent strain. In these crosses one-quarter of the male progeny are paralyzed, where reversion is due to an unlinked, dominant autosomal suppressor mutation. On the other hand, if the revertant site is closely linked to the parental mutation, all progeny are wild type. Owing to the necessity of distinguishing outcrossed from self-crossed progeny, only males could be scored. (Virtually all self-crossed progeny are hermaphrodites.)

Three of the five revertants of E1258 were linked to *unc-54*, ($< 1\%$) and were presumed to lie within the *unc-54* gene itself. All of these were revertants with good movement. The remaining E1258 revertants and the revertant of E1315 carried unlinked autosomal suppressor mutations.

Mapping suppressor mutations: The poor restoration of movement by the suppressors of *unc-54* made mapping experiments difficult. Fortunately, it was found that the suppressors of E1258 also suppressed the *unc-15* (I) mutant E73. Suppression of *e73* is strong and crosses can be easily scored. Therefore, all mapping experiments were performed using this mutant. Indeed, unlinked suppressors of the same type can be obtained by reversion of E73 (see below).

The suppressor alleles were segregated from the *unc-54* background and combined with *unc-15* (*e73*), as described in MATERIALS AND METHODS. One of the suppressor alleles, *e1390*, was tested for linkage with autosomal morphological markers. Heterozygous *e1390*/+; *unc-15*/+ males were mated with double mutants, each homozygous for *unc-15* and a recessive dumpy mutation. Slow (suppressed) hermaphrodite progeny were isolated and the phenotypes of their progeny were scored. In such crosses, phenotypic ratios of slow:paralyzed:slow-dumpy:paralyzed-dumpy approximating 9:3:3:1 indicate that *e1390* is unlinked to the tester marker. The crosses showed that *e1390* is closely linked to *dpy-11* on LG V. Animals of the genotype *unc-15*; *dpy-11*/*e1390* segregated only 16 paralyzed and 11 slow-dumpy recombinants among 706 progeny. Thus, the frequency of recombination between *e1390* and *dpy-11* is about 4% (Figure 1). In similar crosses with *dpy-11* and a second suppressor mutation, *e1407*, 12 paralyzed and 11 slow-dumpy recombinants were found among 712 progeny. All other suppressor alleles, including six directly selected from E73 (*unc-15*), were subsequently tested and found to be linked to *dpy-11*.

Since all of the suppressors are dominant, conventional complementation tests are not possible. However, based on similarity of phenotype and map location, we

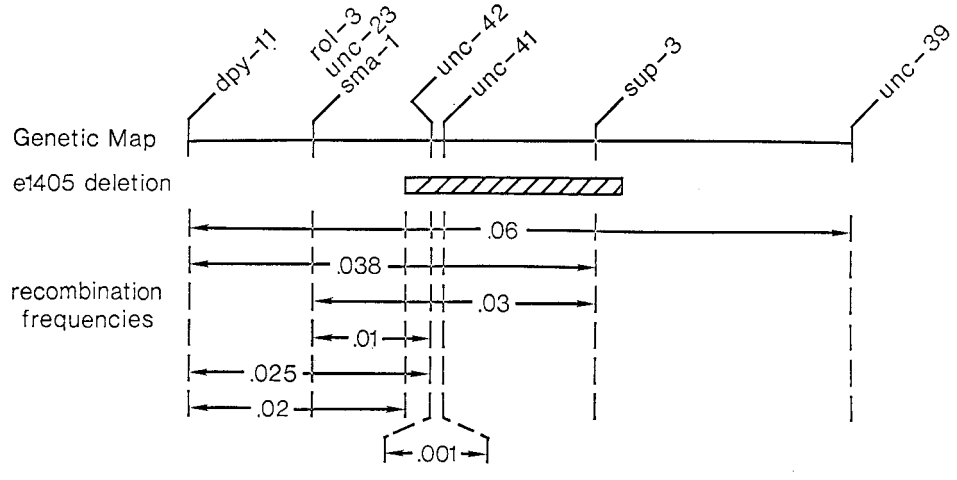

FIGURE 1.—Genetic map of the region between *dpy-11* and *unc-39* on LG V. Gene names are given above the line. Separate loci are shown only for mutant sites that have been unambiguously ordered. The recombination values shown were obtained from two-factor crosses. Mapping data for the *sup-3* and *unc-41 loci* are presented in this paper. The exact position of the right end of the deletion, e1405 (between *sup-3* and *unc-39*) has not been determined.

suspect that all of the independently isolated suppressor mutations are allelic. In all cases tested, suppression of *unc-15* (*e73*) is stronger than suppression of *unc-54* alleles, as judged on the basis of movement or egg-laying ability (see Table 4). This probably accounts for the observation that suppressor mutations are found more frequently in *unc-15* (*e73*) reversion experiments than among unc-54 revertants (Table 2). Also, homozygous suppressed animals are invariably more nearly wild-type than heterozygous suppressed animals. Thus, suppression is dominant, but dose dependent.

The dose dependence of suppression was used in crosses to order the suppressor locus, designated *sup-3*, with respect to the nearby markers, *unc-42* and *dpy-11*. Among the progeny of 12 animals of genotype *unc-15; dpy-11 sup-3 (e1390)/ unc-42* ten fast (homozygous suppressed) recombinants were found. Six of these recombinants were heterozygous for the *unc-42* allele, while four were not, indicating that *unc-42* lies between *dpy-11* and *sup-3*. The gene order, *dpy-11 ... unc-42 ... sup-3* is also consistent with linkage data from two-factor crosses.

The lethal revertant of E1258: The lethal revertant R189 carries a suppressor allele (*e1405*) corresponding to the *sup-3* map position. This mutant was mapped both on the basis of suppression and as a recessive lethal marker, without regard to its suppressor phenotype. For mapping on the basis of suppression, strain M-H1 (*dpy-11/sup-3* (*e1405*); *unc-15* was constructed from H89 (see MATERIALS AND METHODS). Strain M-H1 segregates primarily parental type, slow animals and paralyzed dumpies as well as *e1405* homozygotes that are severely uncoordinated and die prior to completion of the L1 molt. Recombinant types are either suppressed-dumpy or paralyzed. Among 1096 progeny scored, 14 paralyzed recombinants and 16 suppressed-dumpy recombinants were found. This places *e1405* about 2.7% from *dpy-11*.

A second cross was performed in order to map *e1405* on the basis of its recessive-lethal phenotype. Recombinants of genotype *dpy-11+/dpy-11 sup-3* (*e1405*); *unc-15* were derived from M-H1 and mated with wild-type males. Heterozygous F_2 segregants of genotype *dpy-11 sup-3* (*e1405*)/++ were identified by their segregation of abnormal *dpy-11 sup-3* (*e1405*) larvae and rare dumpy recombinants *dpy-11+/dpy-11 sup-3* (*e1405*). Among 15 populations containing abnormal L1 larvae, 33 dumpy recombinants were found among 1,926 mature progeny. From these data the frequency of recombination between *sup-3* (*e1405*) and *dpy-11* was estimated to be 2.5%. This value is in good agreement with the map distance obtained when suppression of *unc-15* (*e73*) was the phenotype scored. However, this recombination frequency with *dpy-11* differs from that obtained for the nonlethal suppressor alleles, *e1390* and *e1407*. The discrepancy is accounted for by the fact that *e1405* is a small deletion encompassing at least one map unit.

e1405 *is a deletion*: The *e1405* mutation was identified as a deletion on the basis of the following observations: (1) *e1405* fails to complement mutations in *unc-42* or *unc-41*, genes that are adjacent to *sup-3* on the genetic map, (2) *e1405* fails to recombine with *unc-41*, *unc-42* and *sup-3* alleles tested, and (3) the *unc-41*, *unc-42* and *sup-3* point mutations all recombine with one another.

It was discovered that *e1405* fails to complement *unc-42* when an attempt was made to perform a three-factor cross. Heterozygous *unc-42*/+ males were mated with *dpy-11 sup-3* (*e1405*)/++ hermaphrodites. One fourth of the F_1 progeny from this cross were uncoordinated, exhibiting the phenotype characteristic of *unc-42* homozygotes. Subsequent complementation tests were performed by crossing males heterozygous for the marker to be tested with either H89 (*dpy-11* +/+ *e1405*) or M-H8 (*e1405*/+; *unc-15*) suppressed hermaphrodites. All mutants mapping in the *sup-3* region complement *e1405*, with the exception of *unc-41* and *unc-42* mutants. Both *unc-41/e1405* and *unc-42/e1405* heterozygotes are uncoordinated. We conclude that *e1405* is a short deletion extending through these two genes.

Since the *unc-41* gene had not been precisely located on LG V (BRENNER 1974), the location of *unc-41* relative to *unc-42* and *sup-3* was determined in order to estimate the extent of the *e1405* deletion. Among 10,400 progeny of *unc-41 unc-42*/++ animals, ten recombinants were found, corresponding to 0.1 map unit. These two genes were ordered with respect to *dpy-11* by testing the recombinant progeny of a *dpy-11/unc-41 unc-42* heterozygote for segregation of the dumpy marker. None of five homozygous *unc-42* recombinants carried the dumpy marker, while one *unc-41* recombinant tested was found to segregate dumpies. Therefore, the gene order is *dpy-11* . . . *unc-42* . . . *unc-41*. These crosses, when taken together with those described above, demonstrate that recombination can be detected between *unc-41*, *unc-42* and *sup-3* point mutations.

If *e1405* is a deletion encompassing *unc-41*, *unc-42* and *sup-3*, it should not recombine with any of them. The occurrence of wild-type recombinants among progeny of *unc-41/e1405* or *unc-42/e1405* heterozygotes was tested using the methods developed for reversion tests (see MATERIALS AND METHODS). No wild-type recombinants were found among an estimated 1.6×10^5 *unc-41/e1405* animals. In an analogous experiment, no recombinants were found among an estimated 1.2×10^5 heterozygotes of genotype *unc-42/e1405*. The fact that wild-type animals can be detected if they arise in such an experiment was demonstrated by the isolation of revertants from progeny of EMS-mutagenized *unc-41* and *unc-42* alleles (unpublished results). When taken together, the genetic data show that *unc-41* and *unc-42* recombine with one another at a frequency of 10^{-3}, but fail to recombine with *e1405* even at the level of 10^{-5}.

Recombination between *e1405* and *sup-3* point mutations like *e1390* is more difficult to detect than with *unc-41* or *unc-42* mutations. This is because the parental *trans*-heterozygote (*e1390/e1405*; *unc-15*) is not uncoordinated. The only detectable recombinant classes are *e1405*/+ or *e1390*/+ heterozygotes. Such heterozygous suppressed animals are distinctly slower than the parental homozygous-suppressed strain due to the dose dependence of suppression. No "slow" recombinants were detected among 2000 progeny scored. Based on these results, we conclude that *e1405* is a deletion including the *unc-41*, *unc-42* and *sup-3* loci. We conclude that the suppressor phenotype of *e1405* results from a reduction in gene dosage of *sup-3*+ in *e1405*/+ heterozygotes. The recessive L1-

lethal phenotype may be due to the elimination of *sup-3*$^+$ and/or adjacent genes in *e1405* homozygotes.

The *e1405* deletion is diagrammed in Figure 1. As shown in the figure, both the *e1405* deletion and *unc-42* point mutations recombine with *dpy-11* at a frequency of about 2%. This indicates that (1) *e1405* does not extend far to the left of *unc-42*, and (2) *e1405* does not greatly suppress recombination between itself and *dpy-11*.

Of the nine suppressor mutants isolated, *e1405* is the only one having the characteristics of a deletion. In microorganisms, EMS induces primarily G to A substitutions in DNA (DRAKE 1970), not deletions. Whether *e1405* was induced by EMS or arose spontaneously in our reversion experiment is unknown. However, no revertants have arisen spontaneously in unmutagenized cultures of *unc-15* or *unc-54* mutants.

Specificity of suppression: The ability of three independent suppressor mutations to suppress *unc-15* and *unc-54* alleles was tested, as well as their ability to suppress mutations in other genes known to affect body musculature (see MATERIALS AND METHODS). The results of cross-suppression tests are shown in Table 3. Mutations in *unc-54*, *unc-15* and *unc-87* are suppressed, while mutations in three other genes are not suppressed. Not all *unc-54* alleles are suppressed. The *unc-54* mutants that are suppressed best are those containing reduced or

TABLE 3

Suppression of mutants defective in body musculature

Muscle mutants							
Gene	Gene product	Allele	Dominance	Amount of gene product recoverable from mutant†	Suppression by *sup-3* alleles		
					e1390	*e1407*	*e1405*
unc-54	Myosin*	*e190*	Recessive	none	+	+	+
	Heavy	*e1009*	Recessive	none	+	+	+
	Chain	*e1168*	Recessive	none	+	+	+
		e1315	Recessive	none	+	+	+
		e1092	Recessive	none	+	+	+
		e1258	Recessive	none	+	+	+
		e675	Semi-dominant	full	—	+	+
		e1301	ts recessive	full	—	NT‡	NT‡
		e1152	Dominant	full	—	NT	NT
unc-15	Paramyosin†	*e73*	Semi-dominant	full	++	++	++
		e1214	Recessive	none	±	NT	NT
unc-52	?	*e444*	Recessive	?	—	—	—
unc-60	?	*e677*	Recessive	?	—	—	—
unc-22	?	*e1179*	Recessive	?	—	—	—
unc-87	?	*e843*	Recessive	?	—	+	+

* EPSTEIN, WATERSTON and BRENNER 1974.
† WATERSTON, FISHPOOL and BRENNER 1977.
‡ Not tested.

undetectable amounts of gene product, as determined by gel electrophoresis and column chromatography of actomyosin preparations (MACLEOD, *et al.* 1977). The *unc-54* mutants that are suppressed poorly or not at all are those mutants containing a wild-type level of *unc-54* protein or those mutants that are dominant. This suggests that the suppressor permits body wall muscles to function in the absence of *unc-54* protein, while the presence of a large amount of mutationally altered protein interferes with suppressor activity.

In contrast to the *unc-54* mutants, the null allele of *unc-15*, *e1214*, was judged to be suppressed poorly, while the allele producing the full amount of protein, *e73*, is suppressed best. Suppression of *e73*, is stronger than suppression of any other mutant tested.

The three suppressor alleles listed in Table 3 exhibit similar patterns of cross suppression. The only differences observed are that *e1407* and *e1405* suppress *e843* and marginally suppress *e675*, while *e1390* does not suppress these mutations. These differences may reflect a quantitative difference in efficiency of suppression.

Efficiency of suppression: Brood sizes of suppressor-carrying hermaphrodites were determined in order to quantitate suppression (see MATERIALS AND METHODS). Suppression of *unc-54* and *unc-15* mutants by *e1390*, *e1407* and *e1405* was measured (Table 4).

The *unc-54* mutants cannot lay eggs, since egg laying depends upon contraction of vulval muscles. Progeny hatch within the parental hermaphrodite and consume it. The number of offspring is therefore limited to the number of fertilized eggs the mutant can produce and hold prior to destruction by internally hatched larvae. The *unc-54* mutants produce about 30 progeny per hermaphrodite as compared to nearly 350 for wild type (Table 4). Strain E73 (*unc-15*) is capable of laying a few eggs prior to internal hatching, and this is reflected in its larger broods relative to the *unc-54* mutants. Production of larger broods by

TABLE 4

Suppression of unc-54 *and* unc-15 *as measured by brood size**

Suppressor genotype	*unc* genotype: Wild	*unc-54*		*unc-15*
	+/+	*e190/e190*	*e1258/e1258*	*e73/e73*
+/+	347±15	32±2	27±2	67±8
+/*e1390*	———	40±2	38±2	211±19
e1390/e1390	329±15	152±27	99±12	259±18
+/*e1407*	———	100±10	83±10	241±7
e1407/e1407	283±10	149±17	131±17	206±8
+/*e1405*†	———	48±2	57±8	155±3
		(64)	(76)	(206)

* The numbers presented are the mean brood sizes of 10–12 animals, together with the estimated standard error of the mean.

† Strains heterozygous for *e1405* segregate severely defective progeny which fail to mature beyond the L1 molt. These individuals are not included in the progeny count; corrected figures are shown in parentheses as an estimate of the number of eggs actually laid.

suppressed strains relative to the parent mutants reflects an increased ability to lay eggs.

We interpret the data in Table 4 as follows: (1) Larger broods produced by homozygous suppressed animals (rows 3 and 5) relative to heterozygous suppressed animals (rows 2 and 4) reflect the dose dependence of suppression. (2) *e73* (column 4) is suppressed more efficiently than either *unc-54* allele tested. (3) Quantitative differences between different suppressor alleles are apparent. The *e1407* allele is a stronger suppressor than *e1390*, particularly when heterozygous. All the mutants heterozygous for *e1407* have larger broods than corresponding *e1390*/+ heterozygotes. This is consistent with the observation that *unc-54* or *unc-15* mutants carrying the *e1407* suppressor are more nearly wild type in movement than mutants carrying *e1390*. When heterozygous-suppressed *unc-54* mutants are compared, suppression by *e1407* is at least as strong as by the deletion mutant, *e1405*. (4) Suppressor mutations seem to affect fertility in a wild-type genetic background. Column 1 of Table 4 shows brood sizes of strains that carrying suppressor mutations, but are otherwise wild type. Strain E1407 has about 20% fewer progeny than wild type, although strain E1407 is normal in behavior and morphology. The effect of *e1407* on fertility is again apparent in the *unc-15* background, where broods of homozygous-suppressed (*e1407/e1407*) animals are, on the average, smaller than broods of *e1407*/+ animals. The possibility that decreased fertility might be due to closely linked secondary mutations has not been ruled out.

Muscle organization in suppressor mutants: In order to study suppressor phenotypes microscopically, strain M-M76, a suppressed derivative of E190 (*unc-54*), and E1407 were chosen for examination. The *unc-54* gene codes for the major body wall myosin heavy chain in *C. elegans*, and the E190 mutant is a null allele containing no detectable *unc-54* myosin (MacLeod *et al.* 1977). This genetic background offers the best chance of detecting changes in protein content resulting from suppressor activity.

Polarized light microscopy of mutant and suppressed animals is shown in Figure 2. The body wall musculature of *C. elegans* consists of four strips of muscle cells arranged in quadrants extending the entire length of the animal. The organization of sarcomeres is visible as discrete patterns of birefringence when viewed in the polarized-light microscope (Epstein, Waterston and Brenner 1974). Distinct, repeating anisotropic and isotropic zones are arranged longitudinally in wild-type animals (Figure 2f).

In Figure 2, an improvement in muscle organization and a stepwise increase in birefringence is apparent when comparing strains from left to right. The low level of birefringence seen in E190 (Figure 2a, 2b) is presumably associated with residual filaments present in all *unc-54* mutants. Mutants such as E190, which contain no detectable *unc-54* myosin, have very disorganized muscle but still have about 20% of the normal number of thick filaments, as shown in electron micrographs of body wall muscle cells. Such mutants produce at least one "residual" myosin (MacLeod *et al.* 1977; Schachat, Harris and Epstein 1977) in addition to pharyngeal myosin (Waterston, Epstein and Brenner 1974).

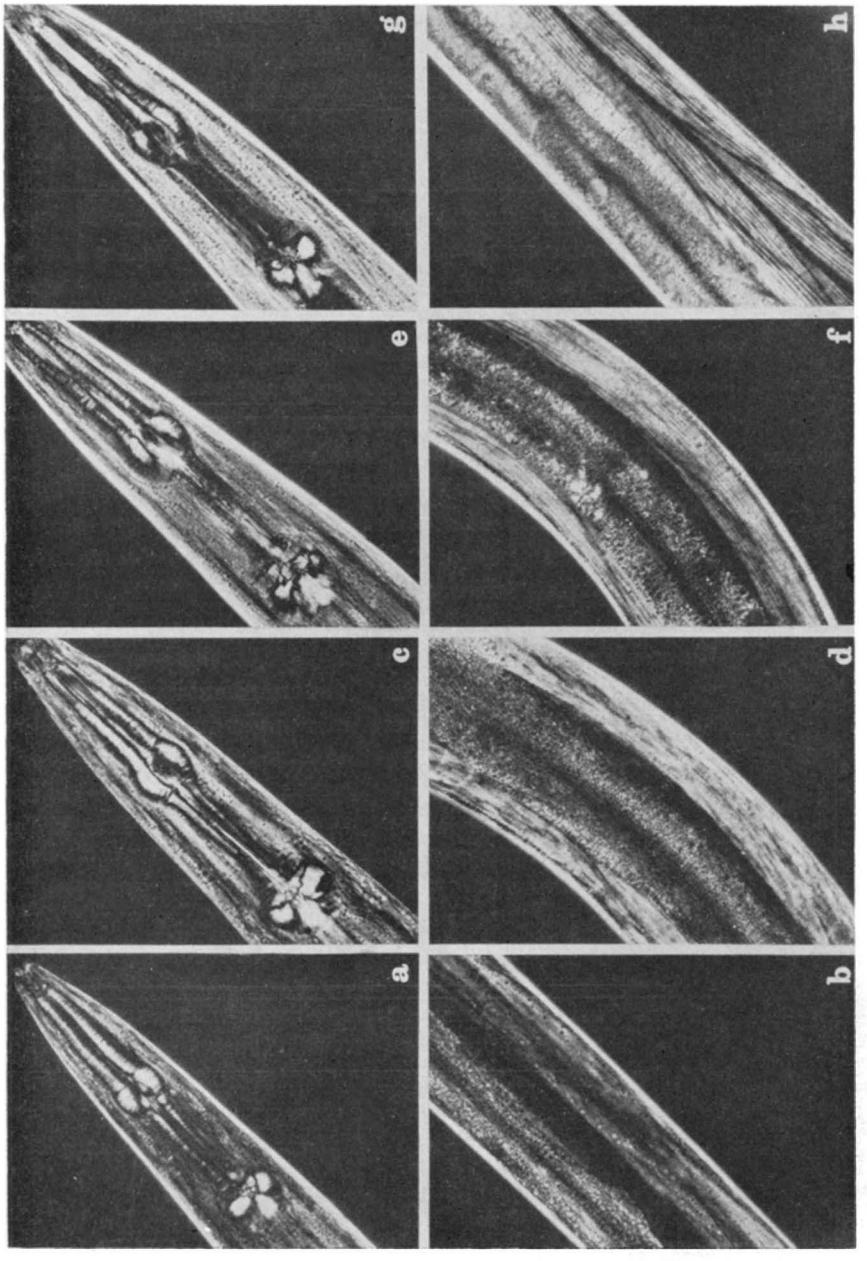

FIGURE 2.—Birefringence of nematode body musculature seen in polarized light. Young adult hermaphrodites are shown at 500×. One dorsal and one ventral muscle strip is visible except in (b) and (h) where the orientation of the animals reveals the two ventral muscle strips. The granular regions in the center of the animals in (d) and (f) and on the upper side in (b) and (h) are intestine. (a,b) E190, *unc-54*; (c,d) M-M76, *unc-54*, *sup-3*; (e,f) N2, wild type; (g,h) E1407, *sup-3*.

A comparison of E190 and M-M76 animals is shown in Figure 2a, b, c, and d. The comparison suggests that muscle organization is enhanced in suppressed strains. Total birefringence is increased, and the fibrillar organization in M-M76 is much more pronounced than in the E190 parent strain, although the musculature is not as organized as that in wild-type N2 (Figure 2e, 2f). Strain E1407 (Figure 2g, 2h) is homozygous for the *sup-3* mutation, but is otherwise wild

type. This strain exhibits extraordinarily intense birefringence and by this criterion has a musculature somewhat superior to wild type. Photographs 2a, c, e and g show pharyngeal musculature as well as body wall muscle in the head. Pharyngeal birefringence remains constant in all of these strains, while the relative birefringence of the body wall musculature increases from left to right.

A suppressor of unc-24 *and* unc-1 *mutants*: A second case of indirect suppression involves genes not associated with muscle structure. In reversion experiments similar to those described above, suppressed derivatives of *unc-24* mutants were obtained after EMS mutagenesis (Table 2). No closely linked revertants were found. Backcrosses showed that all revertants carried unlinked, dominant autosomal suppressors. All revertants are viable as homozygotes and a male stock of one revertant, R206, was used for cross-suppression tests. In the first test, R206 (*m48/m48*; *e138/e138*) males were crossed with the *unc-24* mutant, E927, to produce *m48*/+; *e138/e927* heterozygotes. If *e927* were suppressed, this heterozygote would produce a ratio of suppressed to uncoordinated progeny of 3.0 (3:1 segregation). If *e927* were not suppressed, the ratio would be 1.3 (9:7 segregation). Such crosses showed that the *e927* mutation and all other alleles of *unc-24* are suppressed by *m48* (Table 5). The *sup-3* mutations, however, do not suppress *unc-24* alleles.

To determine if the *m48* suppressor is gene specific, a series of *X*-linked *unc* mutants was screened for suppression. Mutant hermaphrodites were crossed with R206 males. In these crosses all male progeny are hemizygous for the *X*-linked mutation from the mother and heterozygous for the autosomal suppressor from the father. These males will exhibit the maternal mutant phenotype, unless suppressed. In these crosses, the two *unc-1* alleles tested, *e94* and *m6*, were suppressed, while the following mutants were not suppressed: E5 (*unc-7*), E101 (*unc-9*), E151 (*unc-3*), E78 (*unc-6*), E139 (*unc-12*), and E1363 (*unc-58*). Since no autosomal mutants outside the *unc-24* gene were tested, it is possible that other mutants are suppressible by *m48*. However, it may be sig-

TABLE 5

Suppression of unc-24 *alleles by the* m48 *mutation*

Parent	Progeny		Ratio	Alleles suppressed
	Suppressed	Mutant		
$\frac{m48}{+}$; $\frac{e138}{e927}$	104	37	2.8	yes
$\frac{m48}{+}$; $\frac{e138}{e448}$	156	54	2.9	yes
$\frac{m48}{+}$; $\frac{e138}{e1172}$	171	63	2.7	yes
$\frac{m48}{+}$; $\frac{e138}{e138}$	124	42	3.0	yes

nificant that the only *X*-linked mutants to be suppressed exhibit an uncoordinated phenotype virtually indistinguishable from *unc-24* mutants. No autosomal mutants other than *unc-24* have such a phenotype.

DISCUSSION

In the case of suppression of muscle defects we have characterized a number of independent suppressor mutations that are judged to be alleles of *sup-3* on the following basis: (1) Suppressor mutations are indistinguishable in map position. (2) The three suppressor alleles tested exhibit similar specificities of suppression. (3) All suppressor mutants have a similar "slow" phenotype.

The high EMS-induced *sup-3* mutation frequency (approximately 10^{-4}) is comparable to the average foreward induced mutation frequency per gene under our conditions of mutagenesis. This suggests that suppression may result from any mutation that decreases the activity or the amount of the *sup-3* gene product. The finding that one *sup-3* allele, *e1405*, is a deletion, supports this hypothesis. Any mechanism of suppression that requires qualitative alteration of suppressor gene activity is unlikely.

Suppressor activity previously has been associated with deletion mutations in the case of *sup-x* in *Salmonella typhimurium* (DABNAU and MARGOLIN 1972) and the suppressor of Hairless in *Drosophila melanogaster* (NASH 1970). In Drosophila, several suppressor genes have been studied in detail, including suppressors of Bar (STEINBERG 1941), lozenge (BENDER and GREEN 1960), sable (WHITE *et al.* 1973) and vermilion (TWARDZIC, GRELL and JACOBSON 1971). In no case has the molecular basis of suppression been clearly determined. Although several mechanisms of suppression by *sup-3* have been eliminated in this study, the exact mechanism of suppression remains unknown, and its elucidation will require ultrastructural and biochemical analysis.

Suppression of E190, an *unc-54* mutant producing no detectable *unc-54* myosin, shows that suppression does not occur via interactions with altered *unc-54* polypeptides. Instead, suppression may involve a regulatory alteration increasing the amount of "residual" myosin normally present in small amounts relative to *unc-54* myosin. Alternatively, a myosin not normally present in body wall cells, such as pharyngeal myosin, may be produced as a substitute protein in suppressed mutants. Suppression of E73 (*unc-15*), the paramyosin mutant, could be explained if the substitute myosin is able to assemble and function with the altered E73 protein. Increased muscle birefringence in the *sup-3* strains, M-M76 and E1407, is consistent with the idea that the amount of muscle protein is increased in these mutants. The *unc-54* mutants that are suppressed poorly or not at all are those containing a wild-type level of *unc-54* protein or those that are dominant. This suggests that the suppressor permits body wall muscles to function in the absence of *unc-54* protein, while the presence of a large amount of mutationally altered protein interferes with suppressor activity.

It is conceivable that improved organization and function of muscle filaments could result indirectly from changes in cell structure or metabolism. In this way,

sup-3 mutations might enhance the function of the residual muscle filaments present in *unc-54* or *unc-15* mutants.

The *sup-3* mutations may be useful aids in further study of muscle organization in *C. elegans* even though the mechanism of suppression is not understood. For example, isolation of muscle mutants in a *sup-3* genetic background might reveal new classes of mutants not detected in a wild-type background. Suppression also provides a potential basis for a positive selection for deletions in the *sup-3* region of LG V. A collection of deletions of varying lengths could be used for investigation of genetic fine structure on LG V. Finally, if other types of suppressors of muscle defects exist, their detection may be made difficult by the frequent occurrence of *sup-3* mutations. This difficulty may be avoided by concentrating a further search for suppressors on the mutants that are not suppressed by *sup-3* mutations.

Interpretation of suppressor data on the *unc-1* and *unc-24* genes is limited by the fact that the products of these genes have not been identified. However, *unc-1* and *unc-24* mutants exhibit strikingly similar uncoordinated phenotypes, suggesting that these mutants suffer from similar defects. The suppressor data provide genetic evidence that these two genes are functionally related. The absence of allele specificity in suppression of *unc-1* and *unc-24* suggests that suppression is indirect rather than informational.

Our results show that indirect suppression does occur in *C. elegans*, and it may, in fact, be a common mode of reversion. Since this work was completed, indirect suppression has been used to order genes involved in the process of dauer larva formation in *C. elegans* (RIDDLE 1977). Recently, allele-specific suppression in *C. elegans* also has been demonstrated (WATERSTON and BRENNER, in preparation).

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