DOMINANT SUPPRESSORS OF A MUSCLE MUTANT DEFINE AN ESSENTIAL GENE OF *CAENORHABDITIS ELEGANS*

IVA S. GREENWALD AND H. ROBERT HORVITZ

Department of Biology, Massachusetts Institute of Technology, Cambridge MA 02139

Manuscript received October 14, 1981
Revised copy accepted March 5, 1982

ABSTRACT

The *sup-11 I* locus of *C. elegans* was defined by rare dominant suppressors of *unc-93(e1500) III*, a mutation that affects muscle structure. All ten of these dominant suppressors have a recessive "scrawny" phenotype. Two additional classes of *sup-11* alleles were identified. One class, null alleles, was obtained by reversion of the dominant suppressor activity. These null alleles are recessive embryonic lethals, indicating that *sup-11* is an essential gene. Members of the second class, rare semidominant revertants of the "scrawny" phenotype, are partial suppressors of *unc-93(e1500)*. The genetic properties of the dominant suppressor mutations suggest that they are rare missense mutations that confer a novel activity to the *sup-11* protein. We consider some of the ways that *sup-11* alleles might suppress *unc-93(e1500)*, including the possibilities that the altered *sup-11* proteins restore function to a protein complex or are modified products of a gene that is a member of an *unc-93* gene family.

IN this paper, we describe the genetic analysis of the *sup-11 I* locus of *Caenorhabditis elegans*. This study comprises part of the continued characterization of the muscle mutant *unc-93(e1500) III*: the *sup-11* locus was defined by alleles that are dominant suppressors of *unc-93(e1500)*. We undertook the isolation and characterization of such dominant suppressors as an extension of our initial study of *unc-93(e1500)*, in which we defined two extragenic suppressor loci by recessive, apparently null, mutations (GREENWALD and HORVITZ 1980). We thought that dominant suppressor mutations might define new muscle genes. We also hoped that the study of dominant suppressors might lead to a better understanding of the *unc-93* locus.

We previously identified two visible *unc-93* alleles (*e1500*, *n200*) that confer a characteristic uncoordinated, egg-laying defective phenotype. We determined that the *e1500* and *n200* mutations are rare alleles that probably result in the synthesis of toxic *unc-93* proteins. Null alleles of *unc-93*, which eliminate the toxic products, can be generated as intragenic revertants of the two visible alleles. These null alleles confer no visible phenotype, *i.e.*, the null phenotype of the *unc-93* locus is wild-type. We suggested that a reasonable explanation for the wild-type null phenotype is that there exists a protein that can substitute for the missing *unc-93* protein; specifically, we proposed that *unc-93* may be a mem-

ber of a gene family and that the substituent function is provided by another member of the putative *unc-93* gene family. This proposal, which we discuss further below, has implications for the interpretation of our observations concerning the *sup-11* locus.

MATERIALS AND METHODS

General methods: General methods for the handling, culturing and ethyl methanesulfonate (EMS) mutagenesis of *C. elegans* have been described by BRENNER (1974). Experiments were performed at 20° unless otherwise indicated.

Strains and genetic nomenclature: *Caenorhabditis elegans* var. Bristol strain N2 is the wild-type parent of all strains used in this work.

The *sup-11 I*, *sup-9 II*, *unc-93 III*, and *sup-10 X* alleles studied are listed in Table 1. Other mutations used are listed below; most were isolated by BRENNER (1974). The informational suppressors *sup-5 III* and *sup-7 X* have been described by WATERSTON and BRENNER (1978) and WATERSTON (1980). Two mutants were isolated and characterized in our laboratory: the recessive bivulva mutation *lin-17(n677) I* maps approximately 12% to the left of *dpy-5*, and the semidominant multivulva mutation *lin-12(n137) III* is very tightly linked to *unc-32* (C. FERGUSON, personal communication).

The other mutations are:
LG*I*: *lin-17(n677)*, *unc-11(e47)*, *dpy-5(e61)*
LG*II*: *dpy-10(e128)*
LG*III*: *dpy-17(e164)*, *lon-1(e185)*, *sup-5(e1464)*, *lin-12(n137)*, *dpy-18(e364)*

TABLE 1

Some genes and alleles used in this study

Gene	Allele(s)	Reference allele	Symbol
sup-11 I	*n187*, *n401*, *n402*, *n403*, *n404*, *n405*, *n616*, *n628*, *n710*, *n711*	*n403*	*sup-11(d)*
	n403 n406, *n403 n425*, *n403 n426*, *n403 n525*, *n403 n526*, *n403 n712*, *n403 n713*, *n403 n714*	*n403 n406*	*sup-11(r)*
	n403 n681, *n403 n682*, *n403 n683*, *n403 n684*	*n403 n682*	*sup-11(0)*
sup-9 II	*n180*		
unc-93 III	*e1500**, *n200**		
	e1500 n234†		*unc-93(0)*
sup-10 X	*n183*		

The *sup-9*, *unc-93* and *sup-10* mutations, and *sup-11(n187)* were described previously (GREENWALD and HORVITZ 1980). All other *sup-11* mutations were generated as part of this study. The dominant suppressor alleles were generated after mutagenesis of *unc-93(e1500)* as described in MATERIALS AND METHODS. The other *sup-11* alleles were named as double mutants because they were generated from *sup-11(n403)* and, in most cases, the original *n403* mutation was probably retained.

* Visible *unc-93* alleles.
† *unc-93* null allele (no visible phenotype).

LG*IV*: *dpy-13(e184)*
LG*V*: *dpy-11(e224)*
LG*X*: *dpy-7(e1324), sup-7(st5)*

This paper conforms to the standard nomenclature for *C. elegans* genetics (HORVITZ *et al.* 1979).

Isolation of dominant suppressors of unc-93(e1500): Ten dominant suppressor alleles of *sup-11* ["*sup-11(d)*"] were independently isolated. Six of these alleles were obtained in a single experiment by distributing EMS-mutagenized *unc-93(e1500)* hermaphrodites of mixed ages to 33 100 mm Petri plates and screening 10^6–10^7 total progeny for animals with improved mobility after three, four and five days (*i.e.*, after one generation). Three other *sup-11(d)* alleles were identified in two additional reversion experiments. One *sup-11(d)* mutation *(n187)* was obtained in our initial set of revertants (GREENWALD and HORVITZ 1980).

All *sup-11(d)* alleles have a recessive scrawny (Scr) phenotype.

Mapping dominant suppressors of unc-93(e1500): The reference allele *sup-11(n403)* was shown to be autosomal, linked to *dpy-5 I* (approximate recombination frequency $p = 8\%$) and unlinked to the markers *dpy-10 II*, *unc-93(e1500) III*, *dpy-13 IV* and *dpy-11 V*; the other dominant suppressor mutations were also found to be linked to *dpy-5 I* (data not shown).

Standard three-factor crosses (BRENNER 1974) were performed utilizing the recessive scrawny (Scr) phenotype of *sup-11(n403)*. From the heterozyygote + *unc-11 dpy-5/sup-11* + +, 15/15 Dpy non-Unc recombinants segregated *sup-11*. From the heterozygote + *unc-11* +/*sup-11* + *dpy-5*, 7/18 Dpy non-Scr recombinants segregated *unc-11*. From the heterozygote *lin-17* + *dpy-5*/+ *sup-11* +, 7/13 Lin non-Dpy recombinants segregated *sup-11*. Thus, the map order in this region of LG*I* is *lin-17 sup-11 unc-11 dpy-5*. The *lin-17* and *unc-11* are the closest flanking genes identified to date.

Complementation tests: (a) We previously demonstrated that three classes of *unc-93(e1500)* revertants are relatively common afer EMS mutagenesis (GREENWALD and HORVITZ 1980); one class is comprised of null alleles of *unc-93*, and the two other classes are comprised of alleles of the extragenic suppressors *sup-9 II* and *sup-10 X*. These three classes are recessive, allowing rapid identification of other *sup-9*, *sup-10* and *unc-93* null alleles by complementation tests with three canonical revertants: (1) *unc-93(e1500 n234)*; (2) *sup-9*; *e1500*; and (3) *e1500*; *sup-10*. Males of each of these three genotypes are crossed with revertant hermaphrodites of unknown genotype. Most recessive suppressors fail to complement one of the tester strains (yielding only wild-type cross progeny) and complement the other two (yielding Unc*(e1500)* cross progeny). Dominant suppressors, such as *sup-11(d)*, yield only wild-type progeny from these three crosses. Recessive suppressors that are not *sup-9*, *sup-10* or *unc-93* null alleles, such as *sup-11(r)* (see below), complement all three tester strains and yield only Unc*(e1500)* cross progeny.

(b) All *sup-11(d)* mutations described in this study result in a recessive Scr phenotype. To demonstrate that the dominant suppressor mutations belong to a single complementation group, complementation tests for the Scr phenotype were performed with the reference *sup-11(d)* mutation *sup-11(n403)*. Males heterozygous for a dominant suppressor mutation were crossed with *sup-11(n403) dpy-5*; *unc-93(e1500)* hermaphrodites. The appearance of Scr non-Dpy cross progeny indicated that the dominant suppressor was an allele of *sup-11*.

(c) The recessive Scr phenotype of the *sup-11(d)* mutations enabled a complementation test with the recessive lethal allele *sup-11(n403 n682)*, which appears to be a null allele; this complementation test also demonstrated that the *sup-11(d)* mutations belong to a single complementation group. Males heterozygous for a *sup-11(d)* mutation were crossed with the balanced heterozygote *sup-11(n403 n682) dpy-5/unc-11*, and the mating animals were transferred to fresh Petri plates daily. The appearance of Scr non-Dpy cross progeny indicated that the dominant suppressor was an allele of *sup-11*.

(d) To demonstrate that all of the putative *sup-11* null alleles ("*sup-11(0)*") belong to a single complementation group, complementation tests for recessive lethality were performed. Balanced hermaphrodite heterozygotes of genotype *sup-11(0) dpy-5/unc-11* were crossed with wild-type males, and cross progeny males were individually mated with (1) *unc-11 dpy-5* hermaphrodites for 24 hr and then with (2) *sup-11(n403 n682) dpy-5/unc-11* hermaphrodites

for 24 hr. The appearance of cross progeny Dpy non-Unc males from mating (1) and the lack of appearance of Dpy males among the males produced from mating (2) for an individual male indicated that the *sup-11(0)* allele failed to complement *sup-11(n403 n682)* for recessive lethality.

(e) The "*sup-11(r)*" revertants of the Scr phenotype of *sup-11(n403)* are recessive partial suppressors of *e1500*: larvae are similar to Unc*(e1500)* larvae, but adult hermaphrodites lay eggs and have improved mobility. To demonstrate that the *sup-11(r)* revertant *n403 n406* fails to complement *sup-11(0)* alleles, *sup-11(0) dpy-5/++*; *lin-12/+* males were crossed with *sup-11(r) dpy-5; unc-93(e1500)* hermaphrodites and Dpy Lin hermaphrodites were picked [genotype *sup-11(0) dpy-5/sup-11(r) dpy-5*; *unc-93(e1500) +/+ lin-12*]. Several non-Lin progeny from these hermaphrodites were picked; all were of the partially suppressed phenotype. Approximately 2/3 of the partially suppressed hermaphrodites segregated 1/4 arrested embryos and were presumed to be *sup-11(0) dpy-5/sup-11(r) dpy-5*; *unc-93(e1500)*; in some cases this genotype was verified by crossing with *sup-11(n403)/+* males and observing Scr cross progeny. Approximately 1/3 of the partially suppressed hermaphrodites failed to segregate arrested embryos and were presumed to be *sup-11(r) dpy-5/sup-11(r) dpy-5*; *unc-93(e1500)*; in some cases this genotype was verified by crossing the hermaphrodites with *sup-11(n403)/+* males and observing that there were no Scr cross progeny. The *sup-11(0)* alleles used were *n403 n681* and *n403 n682*.

(f) To demonstrate that all *sup-11(r)* alleles fail to complement for the partial suppressor activity, the reference allele *sup-11(n403 n406)* was chosen. Males of genotype *sup-11(r)/+*; *unc-93(e1500)/+* were crossed with *sup-11(n403 n406) dpy-5*; *unc-93(e1500)* hermaphrodites and 12 cross progeny Unc*(e1500)* non-Dpy larvae were picked. That approximately 1/2 of these larvae developed into partially suppressed adults indicated that the *sup-11(r)* allele failed to complement *sup-11(n403 n406)* for the partial suppressor activity.

(g) The *sup-11(r)* alleles, all of which were derived from the *sup-11(d)* allele *n403*, are semidominant revertants of *n403*, *i.e.*, *n403/sup-11(r)* hermaphrodites are non-Scr although somewhat smaller than wild-type. To determine the phenotype of various *sup-11(d)/sup-11(r)* hermaphrodites, *sup-11(d)/+*; *e1500/+* males were crossed with *sup-11(r)*; *e1500*; *dpy-11* hermaphrodites. The lack of appearance of Scr non-Dpy hermaphrodites indicated that *sup-11(d)/sup-11(r)* was non-Scr, and non-Scr hermaphrodites that were smaller than wild type [cf. *n403/sup-11(r)*] were generally seen. Heterozygotes between three *sup-11(r)* alleles (*n403 n406*, *n403 n425*, and *n403 n426*) and all ten *sup-11(d)* alleles were constructed.

Suppression studies with sup-5 III *and* sup-7 X: The *sup-5(e1464) III* suppressor is apparently specific for a subclass of null alleles of many genes (WATERSTON and BRENNER 1978). We tested three recessive lethal alleles of *sup-11*, referred to as *sup-11(0)*, for suppression by *sup-5*. Males of genotype *lon-1 sup-5/++* were mated with hermaphrodites of genotype *sup-11(0) dpy-5/sup-11(n403) dpy-5*, and cross progeny hermaphrodites of genotype *sup-11(0) dpy-5/++*; *lon-1 sup-5/++* were recognized by segregation of Lon animals, arrested embryos, and occasional Dpy recombinants. At least 16 Lon progeny were picked onto individual Petri dishes: approximately 2/3 of these should have been *sup-11(0) dpy-5/++*; *lon-1 sup-5* and 1/3 should have been *++/++*; *lon-1 sup-5*. Two *sup-11(0)* alleles, *n403 n682* and *n403 n683* were not suppressed by *sup-5*: approximately 2/3 of the Lon hermaphrodites segregated 1/4 arrested embryos and occasional Dpy recombinants (*dpy-5* is epistatic to *lon-1*). One *sup-11(0)* allele, *n403 n681*, appeared to be partially suppressed by *sup-5*: virtually all of the Lon hermaphrodites segregated few or no arrested embryos, and approximately 2/3 of these Lon hermaphrodites segregated 1/4 arrested larvae. Based upon cell lineage criteria (SULSTON and HORVITZ 1977), these larvae arrested at the mid-L1 stage. The proportion of arrested embryos and arrested larvae indicated that two copies of *sup-5* were necessary to suppress the embryonic lethality of *sup-11(n403 n681)*: among 532 total progeny of *n403 n681 dpy-5/++*; *lon-1 sup-5/++* hermaphrodites, 96 were arrested embryos and 21 were arrested larvae, which is similar to the values expected (3/16 arrested embryos, 1/16 arrested L1's) if two copies of *sup-5* were necessary for development to proceed to the L1 stage.

Like *sup-5(e1464) III*, the suppressor *sup-7(st5) X* is apparently specific for null alleles (WATERSTON 1980); the same alleles are suppressed by both suppressors (WATERSTON 1980; HORVITZ and SULSTON 1980). We tested this suppressor for suppression of *sup-11(n403 n681)*. At 25°, males of genotype *sup-11(n403 n681) dpy-5/++* were crossed with *dpy-7 sup-7* hermaphrodites and cross progeny hermaphrodites of genotype *sup-11(n403 n681) dpy-5/++; dpy-7 sup-7/++* were recognized by the segregation of occasional Dpy-5 progeny. From these hermaphrodites, Dpy-7 self-progeny were picked, and the *sup-11(n403 n681) dpy-5/++; dpy-7 sup-7* animals were recognized by the segregation of occasional Dpy-5 progeny. These hermaphrodites were allowed to lay eggs at 25° and 15° (*sup-7* suppression may be stronger at 15° than at 25°; WATERSTON 1980). At both temperatures, approximately 1/4 arrested larvae were seen; these larvae arrested at the mid-L1 stage.

RESULTS

Dominant suppressors of unc-93(e1500): Ten dominant suppressors of *unc-93(e1500)* were isolated as F_1 progeny of EMS-mutagenized hermaphrodites as described in MATERIALS AND METHODS. These revertants, which were picked as motile hermaphrodites of relatively normal body size, were heterozygous for dominant suppressor mutations that result in a recessive "scrawny" (Scr) phenotype (Figure 1): the non-Scr non-Unc hermaphrodite revertants segregated approximately 2 non-Scr non-Unc:1 Scr non-Unc:1 Unc(*e1500*). The Scr non-Unc animals are homozygous for the suppressor, are smaller and thinner than wild-type, and have a reduced brood size, but are vigorous and lay eggs. The presence of dominant suppressor mutations was confirmed by appropriate crosses [complementation test (a), MATERIALS AND METHODS]. One dominant suppressor mutation, *n403*, was mapped and found to define a new gene, *sup-11 I* (see MATERIALS AND METHODS for data). The nine other dominant suppressor mutations appear to be alleles of *sup-11*: they are linked to *dpy-5 I* and fail to complement *n403* for the Scr phenotype [complementation test (b), MATERIALS AND METHODS]; they also fail to complement *sup-11(n403 n682)*, a null allele that will be discussed below, for the Scr phenotype [complementation test (c), MATERIALS AND METHODS]. The Scr phenotype of *sup-11(n403)* does not depend on the

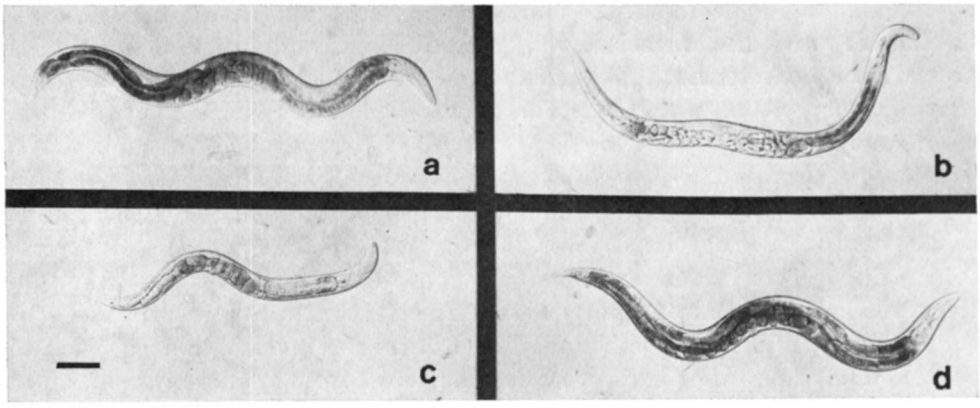

FIGURE 1.—Bright-field photomicrographs of some of the mutants described in this paper. (a) wild type, (b) *unc-93(e1500)*, (c) *sup-11(n403)*, (d) *sup-11(n403 n406)*. Bar = 0.1 mm.

unc-93 genotype, *i.e.*, *sup-11(n403); unc-93* animals are Scr for the *unc-93* alleles +, *e1500*, or *e1500 n234* (an *unc-93* null allele).

The *sup-11* alleles differ in their efficiency of suppression of *e1500*. Eight alleles, including the reference allele *n403*, are strong suppressors; two alleles (*n628*, *n710*) are weaker suppressors. The phenotype of *sup-11(n403)/+; unc-93(e1500)* animals resembles that of *e1500/+*: hermaphrodites are motile and lay eggs, although they are not completely wild-type, and males are more vigorous than *e1500* homozygotes but do not have wild-type movement and are generally unable to mate. Hermaphrodites of genotype *sup-11(n628)/+; unc-93 (e1500)* or *sup-11(n710)/+; unc-93(e1500)* are longer and more uncoordinated than are *sup-11(n403)/+; unc-93(e1500)* hermaphrodites.

The dominant suppressors ["*sup-11(d)*"] were tested for suppression of the other *unc-93* visible allele, *n200*, which has a phenotype similar to but less severe than that of *e1500*. Hermaphrodites of genotype *sup-11(d); unc-93(n200) dpy-17* were crossed with *unc-93(n200)/dpy-17* males and cross progeny non-Dpy hermaphrodites [genotype *sup-11(d)/+; unc-93(n200) dpy-17/unc-93(n200)* +] were examined for suppression. All ten *sup-11(d)* mutations suppressed *unc-93(n200)*.

Dominant suppressors of e1500 *are relatively rare*: The frequency at which dominant suppressors are generated after EMS mutagenesis might be an indication of the nature of the mutational event: a low frequency would suggest a relatively specific event; a high frequency would suggest a relatively nonspecific event, such as the generation of null alleles. Dominant suppressor mutations appeared to be relatively rare after EMS mutagenesis (GREENWALD and HORVITZ 1980; also see MATERIALS AND METHODS above).

We have compared directly the frequency at which dominant suppressors are generated to that of a class of mutations of known frequency. We previously determined that after EMS mutagenesis *unc-93* null alleles occur at a frequency of 5×10^{-4}/mutagenized gamete, which is the same as that for mutations in an average *C. elegans* gene (BRENNER 1974; MENEELY and HERMAN 1979). We have compared directly the frequency at which dominant suppressors and *unc-93* null alleles are generated. We found no dominant suppressors and 21 *unc-93* null alleles using the protocol shown in Figure 2, indicating that dominant suppressors are rare after EMS mutagenesis and occur at less than 1/20 of the average frequency of null mutations. This relatively low frequency suggested that the dominant suppressors of *unc-93(e1500)* do not result from null mutations.

The null phenotype of sup-11 *is recessive lethal*: Null alleles of genes have been obtained by reversion of dominant "neomorphic" mutations (which result in the acquisition of novel activities) and "hypermorphic" mutations (which result in the overproduction of gene activity) of those genes (*e.g.*, LIFSHYTZ and FALK 1969; LIFSHYTZ and GREEN 1979). Since dominant suppressor alleles of *sup-11* appeared likely not to be null alleles, it seemed possible that reversion of the dominant suppressor activity of *sup-11(n403)* would generate *sup-11* null alleles ("*sup-11(0)*"). We generated four revertants of the dominant suppressor activity of *sup-11(n403)* as detailed in Figure 3. All are recessive lethal muta-

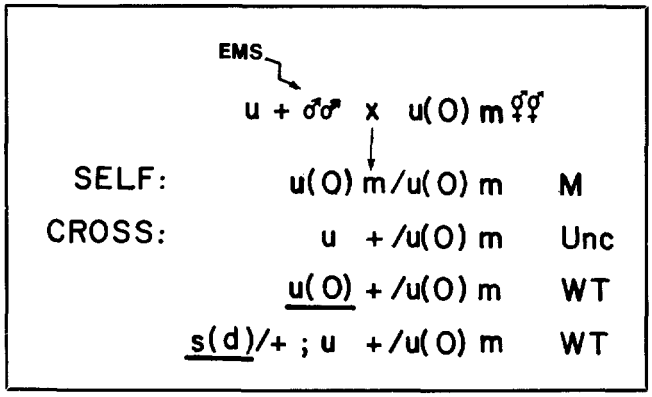

FIGURE 2.—Schematic representation of an experiment to determine the relative frequencies after EMS mutagenesis of dominant suppressors of *unc-93(e1500)* and of *unc-93* null alleles. Lower case letters indicate genotypes: u = *unc-93(e1500)*, u(0) = an *unc-93* null allele, m = a marker linked to *unc-93*, *s(d)* = a dominant suppressor of *unc-93(e1500)*. Underlining indicates mutations expected as a result of the mutagenesis. Upper case letters indicate phenotypes, *i.e.*, M = phenotype of *m*, Unc = uncoordinated, WT = wild type.

According to the above scheme, males homozygous for *unc-93(e1500)* [u] would be mutagenized with EMS and crossed to hermaphrodites homozygous for *unc-93(e1500 n234)* [u(0)] and a linked marker, such as *unc-32* [m]. Self progeny would be of the Unc-32 phenotype, and most cross progeny would be of the Unc(*e1500*) phenotype, because null alleles of *unc-93* are recessive to *e1500*. Rare non-Unc(*e1500*) cross progeny would be expected; these would be candidates for carrying either new *unc-93* null alleles [*s(0)*] or dominant suppressor mutations [*s(d)*]. Hermaphrodite candidates carrying new *unc-93* null alleles would not segregate Unc (*e1500*) progeny. Hermaphrodites carrying dominant suppressor mutations would segregate Unc(*e1500*) progeny, and *sup-11* heterozygotes might be expceted to segregate Scr as well.

This experiment was performed essentially as represented above. However, because *unc-93 (e1500)* males do not mate, a recessive suppressor of *e1500* (either *sup-9* or *sup-10*) was included to enable mating; because these suppressors are recessive, the phenotypes of the cross progeny as predicted by the above scheme were not affected. Twenty-three independent non-Unc(*e1500*) hermaphrodite candidates were obtained among an estimated 25,000 cross progeny hermaphrodites; of the 21 fertile candidates, none segregated Unc(*e1500*), and the appropriate complementation tests [complementation test (a), MATERIALS AND METHODS] confirmed that all contained *unc-93* null alleles.

tions tightly linked to *sup-11(n403)*. For the lethal mutation *n682*, no Scr recombinants appeared among the 926 total progeny of four *n403 n682 dpy-5/ unc-11* hermaphrodites (recombination frequency $p < 0.2\%$); in addition, Scr animals have not been seen during the maintenance of such balanced heterozygous stocks.

The recessive lethality of *sup-11(n403 n682)* is not complemented by the other three recessive lethal mutations generated by reverting *sup-11(n403)* [complementation test (d), MATERIALS AND METHODS], which indicates that these four mutations are allelic. All *sup-11* dominant suppressor alleles fail to complement the lethal mutation *n403 n682* for the Scr phenotype [complementation test (b), MATERIALS AND METHODS], suggesting that the lethal mutations are alleles of *sup-11*. The phenotype of the arrested embryos is similar for all alleles.

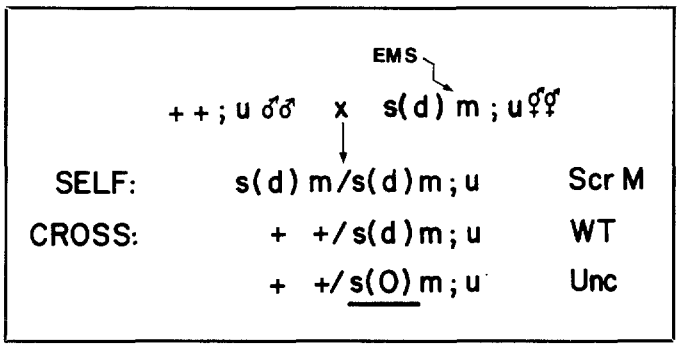

FIGURE 3.—Schematic representation of an experiment to revert the dominant suppressor activity of *sup-11(n403)*. Lower case letters indicate genotypes: u = *unc-93(e1500)*, s(d) = *sup-11(n403)*, m = a marker linked to *sup-11*, *s(0)* = a putative null allele of *sup-11* that would be generated by this experiment. Upper case letters indicate phenotypes as in Figure 2, with the addition of Scr = scrawny.

According to the above scheme, males homozygous for *unc-93(e1500)* would be mated to EMS-mutagenized hermaphrodites homozygous for *sup-11(n403)*, a linked marker such as *dpy-5*, and *unc-93(e1500)*. Self progeny would be Scr Dpy and most cross progeny would be wild type because of the dominant suppressor activity of *sup-11(n403)*. Rare cross progeny of the characteristic Unc(*e1500*) phenotype would be candidates for harboring heterozygous *sup-11* null alleles. The rare Unc(*e1500*) cross progeny hermaphrodites would segregate homozygotes for putative *sup-11* null alleles that could be recognized by the linked *dpy-5* marker.

This experiment was performed essentially as represented above; however, because *unc-93 (e1500)* males do not mate, *sup-10*, a recessive suppressor of *e1500*, was included (see Figure 2 legend). Four independent Unc(*e1500*) hermaphrodite candidates were obtained among approximately 9,000 cross progeny. Each of the four failed to segregate Scr or Scr Dpy and segregated no or very few *dpy-5* homozygotes, suggesting that a lethal mutation linked to *sup-11* and *dpy-5* had been generated (the few *dpy-5* homozygotes presumably were recombinants). Progeny from the candidates were mated with *lin-17 dpy-5*/++ or *unc-11 dpy-5*/++ males to recover the chromosome containing the putative recessive lethal mutation, and the Dpy hermaphrodites from these crosses were mated with *unc-11*/+ males to establish the balanced strain *sup-11(0) dpy-5/unc-11* (the other markers were eliminated by segregation). These strains segregated 1/4 arrested embryos and occasional Dpy-5 recombinants.

The arrest occurs after the onset of morphogenesis; the embryos are abnormal, but nonetheless pharyngeal, intestinal and tail structures are evident (Figure 4).

We tested three of these recessive lethal mutations for suppression by *sup-5 III* (see MATERIALS AND METHODS), which is an informational suppressor specific for null alleles (WATERSTON and BRENNER 1978) and encodes an amber suppressor tRNA (R. WATERSTON, N. WILLS, and R. GESTELAND, personal communication; J. KARN, personal communication). One allele, *sup-11(n403 n681)*, was partially suppressed in *sup-5* homozygotes: at 20°, animals of genotype *sup-11 (n403 n681) dpy-5; sup-5* arrest as L1 larvae (Figure 4). We also tested *sup-11 (n403 n681)* for suppression by *sup-7 X* (see MATERIALS AND METHODS), another informational suppressor (WATERSTON 1980) that suppresses the same alleles as *sup-5* (WATERSTON 1980; HORVITZ and SULSTON 1980; MENEELY and HERMAN 1981) and also encodes an amber suppressor tRNA (R. WATERSTON, N. WILLS and R. GESTELAND, personal communication; J. KARN, personal communication).

FIGURE 4.—Photomicrographs of arrested individuals homozygous for *sup-11* null alleles; NOMARSKI differential interference contrast optics. (a) Arrested embryo, *sup-11(n403 n681) dpy-5*, (b) Arrested L1 larva, *sup-11(n403 n681) dpy-5*; *sup-5* grown at 20°. Bar = 0.01 mm. Note: the phenotype for *sup-11(n403 n681) dpy-5*(+) is the same as the phenotype of *sup-11(n403 n481) dpy-5* (not shown).

The *sup-11(n403 n681) dpy-5; sup-7* animals arrest as L1 larvae at 25° and 15°. We also found that the *sup-11(d)* suppressor activity is only weakly restored in *sup-11(n403 n681) dpy-5/++; unc-93(e1500) sup-5* hermaphrodites, which move slightly better than *unc-93(e1500)* hermaphrodites but remain very egg-laying defective; in addition, *sup-11(n403 n681) dpy-5; unc-93 (e1500) sup-5*-arrested larvae are less motile than *sup-11(n403 n681) dpy-5; sup-5*-arrested larvae.

These recessive lethal mutations appear to be null alleles of *sup-11*: in *cis*, they abolish the dominant suppressor activity of *sup-11(n403)* and in *trans* they fail to complement *sup-11(n403)* for the recessive scrawny phenotype. In addition, they arise after EMS mutagenesis at a frequency of 4×10^{-4}, which is similar to the frequency of 5×10^{-4} expected for mutations that eliminate gene activity (BRENNER 1974; MENEELY and HERMAN 1979; GREENWALD and HORVITZ 1980). Finally, one of the alleles, *sup-11(n403 n681)*, is suppressible by the null allele-specific informational suppressors *sup-5* and *sup-7*.

Rare sup-11 *alleles are recessive partial suppressors of* unc-93(e1500): The F_1 and F_2 progeny of EMS-mutagenized *sup-11(n403); unc-93(e1500)* hermaphrodites were examined for non-Scr revertants. Five revertants were obtained; these revertants, at least three of which were detected as F_1 non-Scr motile individuals, were rare (estimated frequency less than 10^{-6}). Approximately ¼ of the progeny of these five revertants were of a new phenotypic class and proved to be homozygous for partial suppressors ["*sup-11(r)*"] of *unc-93 (e1500)*: young larvae have a phenotype similar to that of *unc-93(e1500)* larvae, but adults lay eggs and are motile. These partially suppressed hermaphrodites were crossed with wild-type males, and the reversion site proved to be tightly linked to *n403*: no Scr hermaphrodites were detected among at least 300 F_2 progeny for each revertant. For the canonical revertant *n403 n406*, no motile recombinant larvae were detected among approximately 68,000 progeny of *n403 n406* +/ + *dpy-5; e1500* (recombination frequency $p < 0.003\%$).

Three revertants of the Scr phenotype of *sup-11(n403); unc-93*(+) were also generated with EMS. As with the revertants described above, these revertants are semidominant suppressors of *n403* and partial suppressors of *e1500*, and the reversion site is linked to *n403*.

The tight linkage demonstrated between *n403* and *n406* suggested that these revertants are intragenic. A complementation test [(e), MATERIALS AND METHODS] between *n403 n406* and *sup-11(0)* supports this interpretation: hermaphrodites of genotypes *n403 n406/sup-11(0); unc-93(e1500)* are of a partially suppressed phenotype similar to *n403 n406; unc-93(e1500)* and distinct from the Unc*(1500)* phenotype of +/*sup-11(0); unc-93(e1500)* or *n403 n406*/+; *unc-93(e1500)*, *i.e.*, a null allele of *sup-11* fails to complement *n403 n406* for the recessive suppressor activity.

The other linked revertants generated by reversion of the Scr phenotype of *sup-11(n403); unc-93(e1500)* or *sup-11(n403);* + fail to complement *sup-11 (n403 n406)* for the partial suppressor activity and are thus also probably mutations in *sup-11* [complementation test (f), MATERIALS AND METHODS]. In addition, *sup-11(d)/sup-11(r)* heterozygotes were constructed between the ten *sup-11(d)* alleles, and three *sup-11(r)* alleles and all were non-Scr [complementation tests (g), MATERIALS AND METHODS].

The phenotype of *sup-11(n403 n406); unc-93*(+) is essentially wild type, indicating that the uncoordinated phenotype of *sup-11(n403 n406); unc-93 (e1500)* results from the *e1500* mutation. In addition, reversion of the partially uncoordinated phenotype of *sup-11(n403 n406); unc-93(e1500)* yields *sup-9*, *sup-10* and *unc-93* null alleles at an average frequency of 5×10^{-4}/mutagenized gamete, establishing that suppressors of *e1500* suppress the uncoordinated phenotype of *sup-11(n403 n406); unc-93(e1500)*.

DISCUSSION

We have identified three classes of *sup-11 I* alleles. The *sup-11* locus was defined by relatively rare dominant suppressors ["*sup-11(d)*"] of *unc-93(e1500)*, an uncoordinated and egg-laying defective muscle mutant that we described previously (GREENWALD and HORVITZ 1980). These dominant suppressors also result in a recessive "scrawny" (Scr) phenotype. Reversion of the Scr phenotype of *sup-11(n403)* generated a class of relatively rare *sup-11* alleles ["*sup-11(r)*"] that are recessive partial suppressors of *e1500*. Reversion of the dominant suppressor activity of *sup-11(n403)* generated a class of relatively common *sup-11* alleles ["*sup-11(0)*"] that are recessive lethal and appear to be null alleles. Table 2 summarizes the phenotypes of hermaphrodites of various *sup-11* genotypes. In this discussion, we first consider in more detail the classes of *sup-11* mutations. We then consider the nature of the interaction between *sup-11* and *unc-93*.

The *sup-11(d)* mutations are dominant suppressors of *unc-93(e1500)* and have a recessive Scr phenotype. A *sup-11(d)* mutation might alter either the level or structure of the *sup-11* gene product. Genetic considerations suggest that *sup-11(d)* does not simply alter the level of *sup-11* gene product. It is unlikely that *sup-11(d)* results in a *cis*-dominant overproduction of a gene product, because *sup-11(d)/sup-11(d)* would then contain more product than *sup-11 (d)*/+, which in turn would contain more product than *sup-11(d)/sup-11(0)*; thus, *sup-11(d)*/+ should be at least as mutant as *sup-11(d)/sup-11(0)*.

TABLE 2

Some phenotypic characteristics resulting from various sup-11 *genotypes*

Genotype	Morphology	Suppressor activity
sup-11(d)/sup-11(d)	Scr	strong
sup-11(0)/sup-11(0)	arrested embryo	.....
sup-11(r)sup-11(r)	WT	partial
sup-11(d)/+	WT	strong
sup-11(0)+	WT	none
sup-11(r)+	WT	none
sup-11(d)/sup-11(0)	Scr	strong
sup-11(r)/sup-11(0)	WT	partial
sup-11(d)/sup-11(r)	non-Scr, small	strong
sup-11(0); sup-5	arrested L1 larva*	weak
sup-11(0)/+; sup-5	ND	very weak

The genotype abbreviations are the same as those in the text and in Table 1. "Morphology" refers to the phenotype of animals of *unc-93*(+) genotype; the one exception, indicated by an asterisk (*), was of *unc-93(e1500)* genotype. "Suppressor activity" refers to the suppression of *unc-93(e1500)*. The phenotypes of "partial" and "weak" suppressors are described in the text; "partial" suppression is greater than "weak" suppression. The phenotypes in the presence of *sup-5* are for the *sup-11(0)* allele *sup-11(n403 n681)*. WT = wild type, Scr = Scrawny, ND = not done, — = unable to determine.

However, *sup-11(d)/+* is non-Scr whereas *sup-11(d)/sup-11(d)* and *sup-11(d)/sup-11(0)* are Scr. Similarly, it is unlikely that *sup-11(d)* merely lowers the level of *sup-11* product, because *sup-11(0)/+* would then contain less product than *sup-11(d)/+*; thus, *sup-11(0)/+* should be a better suppressor than is *sup-11(d)/+*. However, *sup-11(0)/+; e1500* is of the Unc*(e1500)* phenotype, whereas *sup-11(d)/+; e1500* is suppressed. These considerations suggest that the *sup-11(d)* mutation results in an altered *sup-11* product. The dominance of the suppressor activity and the relative rarity of dominant suppressor mutations after EMS mutagenesis are consistent with the interpretation that a novel function has been acquired by the *sup-11(d)* product. The Scr phenotype, however, may result either from the loss of a particular activity of the wild-type *sup-11* product or from a toxic effect of the altered product; because the Scr phenotype is recessive, the wild-type allele either provides the missing activity or overcomes the toxic effect.

Rare intragenic revertants of the Scr phenotype were obtained after EMS mutagenesis. These *sup-11(r)* mutations are recessive partial suppressors of *unc-93(e1500)*: hermaphrodites of genotype *sup-11(r); unc-93(e1500)* are essentially mutant as larvae but lay eggs and have improved motility as adults. Genetic considerations suggest that these *sup-11(r)* mutations, like the *sup-11(d)* mutations from which they were derived, result in altered *sup-11* products rather than in altered levels of *sup-11* [specifically, *sup-11(d)*] product. It is unlikely that *sup-11(r)* lowers the level of *sup-11(d)* product, because in this case *sup-11(d)/sup-11(r)*, which is non-Scr, should contain more product than *sup-11(d)/sup-11(0)*, which is Scr [as is *sup-11(d)/sup-11(d)*]. Similarly, it is unlikely that *sup-11(r)* elevates the level of *sup-11(d)* because *sup-11(r)/*

sup-11(r) would contain more product than *sup-11(r)/sup-11(d)*, which would contain more product than *sup-11(r)/sup-11(0)*; yet *sup-11(r)/sup-11(r)* and *sup-11(r)/sup-11(0)* only partially suppress *unc-93(e1500)* and are of wild-type size whereas *sup-11(r)/sup(d)* strongly suppresses *unc-93(e1500)* and is somewhat smaller than wild type. Thus, *sup-11(r)* alleles appear to result in novel *sup-11* products that are different from the *sup-11(d)* product; the *sup-11(r)* alleles are probably double mutants that retained the *sup-11(d)* mutation while acquiring a second-site mutation within the structural gene. It seems likely that the partial suppression of *e1500* by *sup-11(r)* results from residual *sup-11(d)* activity, although it is conceivable that a new suppressor activity is present in the double mutant. That *sup-11(d)/sup-11(r)* is non-Scr implies that the *sup-11(r)* mutations either restore the activity lost in *sup-11(d)* mutants or result in a product that can overcome the toxic effects of the *sup-11 (d)* product; in this respect, the *sup-11(r)* mutations are similar to the *sup-11 (+)* allele.

Reversion of the dominant suppressor activity of *sup-11(d)* generated null alleles [*sup-11(0)*] of *sup-11*. These null alleles are recessive lethal, and embryos homozygous for *sup-11* null alleles arrest after the onset of morphogenesis. The recessive embryonic lethal null phenotype demonstrates that there is a function of *sup-11* that is essential for development. One *sup-11(0)* allele is suppressible by the null allele-specific suppressors *sup-5* and *sup-7* (WATERSTON and BRENNER 1978; WATERSTON 1980). Recent results have indicated that the *sup-5* and *sup-7* suppressors are amber suppressor tRNA's (R. WATERSTON, N. WILLS and R. GESTELAND, personal communication; J. KARN, personal communication); therefore, *sup-11* has a polypeptide product. Although the embryonic lethality of *sup-11(0)* is suppressed by these informational suppressors, the animals arrest at the L1 stage. These arrested larvae exhibit abnormal pharyngeal pumping and gut characteristics of "starved" animals, suggesting that they may arrest because they do not obtain sufficient nutrition; this interpretation is consistent with our observation that wild-type animals arrest at the L1 stage when eggs hatch in the absence of bacteria. In addition, *sup-11(d)* suppressor activity is only weakly restored to *sup-11(0)/+*; *unc-93(e1500) sup-5* hermaphrodites. These results suggest that the *sup-11* product may be required in stoichiometric (as opposed to catalytic) quantities (see HORVITZ and SULSTON 1980).

We will now consider some of the ways that the *sup-11(d)* mutations might suppress *unc-93(e1500)*. Although other mechanisms are conceivable, we will discuss only those that seem to us most reasonable.

One possible way that the *sup-11(d)* mutations might suppress *unc-93(e1500)* is by acting as informational suppressors (HARTMAN and ROTH 1973). We do not think that this possibility is likely. Informational suppressors are allele-specific and gene-nonspecific. The *sup-11(d)* suppressors appear to be allele-nonspecific (see below) and gene-specific: during the course of this work, strains containing *sup-11(d)* and a variety of apparently unrelated *dpy* and *unc* mutations were constructed, and suppression of these mutations was not observed; in

addition, reversion studies of many mutations carried out in several laboratories have not generated *sup-11* alleles.

We will consider below two ways that *sup-11(d)* mutations might suppress *unc-93(e1500, n200)* based on the assumption that *sup-11* and *unc-93* are involved in muscle function. Because they disrupt muscle structure, mutations in *unc-93(e1500, n200)* and several other genes have been considered to define a set of genes responsible for muscle structure and function (WATERSTON, THOMSON and BRENNER 1980; ZENGEL and EPSTEIN 1980). That the *sup-11(d)* mutations correct the muscle defect of *unc-93(e1500)* implies that *sup-11* affects muscle function as well. However, even though the *sup-11* product is likely to function in muscle cells, it may also have nonmuscle cell activities: because proteins closely related to muscle structural proteins are found in nonmuscle cells (*e.g.*, CLARKE and SPUDICH 1977) it is plausible that the recessive lethality of *sup-11* null alleles results from the loss of a nonmuscle function of *sup-11 (+)* and not from the loss of a muscle function *per se*.

The *sup-11(d)* mutations might suppress *unc-93(e1500)* by restoring proper function to the *unc-93* product, *e.g.*, by restoring function to a complex of proteins consisting of at least the products of *sup-11* and *unc-93*. Ample precedent for the suppression of a missense mutation by the alteration of an interacting polypeptide exists (HARTMAN and ROTH 1973). However, such suppressors might be expected to be allele-specific. Because the visible *unc-93* alleles, *e1500* and *n200*, confer easily distinguishable phenotypes, they are likely to be different mutations; similarly, because the *sup-11(d)* alleles differ in their efficiencies of suppression of *e1500*, some of these are likely to be different mutations. All *sup-11(d)* mutations, which were isolated as suppressors of *e1500*, also suppress *n200* and therefore appear to be *unc-93* allele-nonspecific, subject to the caveat that there are only two visible alleles of *unc-93* to test and the products of these two alleles could have some shared structural similarity that is recognized by the *sup-11(d)* products.

Alternatively, the *sup-11(d)* mutations might suppress *unc-93(e1500)* by bypassing the defect caused by *unc-93(e1500)* without restoring function to the *e1500* product. A bypass mechanism would display allele-nonspecificity, which is consistent with our observations. We would like to consider one specific bypass mechanism in the context of our earlier studies of *unc-93*: we suggested (GREENWALD and HORVITZ 1980) that *unc-93* null mutants are wild type because there exists an alternative to the *unc-93* product, such as one provided by another member of an *unc-93* gene family; the rare semidominant mutation *e1500* may result in a toxic product that interferes with muscle structure and function even in the presence of putative related gene products. More generally, we proposed that some members of other gene families might have wild-type null phenotypes and be identified by rare semidominant mutations. Currently, nine *C. elegans* genes with these genetic properties have been identified in a number of laboratories, including our own, and preliminary results for several other genes suggest that this number is likely to increase. Recently, the proposal that such genes

are members of gene families has been supported by molecular studies of two of these genes: *unc-92 V*, which appears to be a member of an actin gene family (C. LANDEL and D. HIRSH, personal communication), and *sup-7 X*, a tRNA gene (R. WATERSTON, N. WILLS, and R. GESTELAND, personal communication).

If *unc-93* is a member of a gene family, then the modification of another family member so that its product is effective even in the presence of the *e1500* product is one way that a bypass could be accomplished. That *e1500/+* is much less mutant than *e1500/e1500* implies that the *unc-93* wild-type product can compete with and overcome the toxic effects of the *e1500* product; perhaps the *sup-11(d)* mutations, which appear to be missense mutations (see above), alter the *sup-11* product to confer an *unc-93*(+)-like activity. In other words, *sup-11* and *unc-93* may be nonidentical members of a gene family, with *sup-11(d)* mutations conferring an *unc-93*(+) activity to the *sup-11* product. Studies of genes *23* and *24* of bacteriophage T4 suggest that such mutations are possible: the wild-type products are structural components of the phage head, and null mutations of either gene are lethal; however, a missense mutant of gene *23* (which encodes the major capsid protein) has been isolated that suppresses *24*⁻ amber mutants, apparently by conferring a *24*⁺-like activity to the gene *23* product (MCNICOL, SIMON and BLACK 1974).

We thank P. STERNBERG for determining the stages of arrested larvae and for assistance with photomicrography. We also thank D. BOTSTEIN and J. HODGKIN for comments on this manuscript. This work was supported by research grants GM24663 and GM24943 and predoctoral training grant GM07287 from the Public Health Service.

LITERATURE CITED

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

CLARKE, M. and J. A. SPUDICH, 1977 Nonmuscle contractile proteins: the role of actin and myosin in cell motility and shape determination. Ann. Rev. Biochem. **46:** 797–822.

GREENWALD, I. and R. HORVITZ, 1980 *unc-93(e1500) III*: A behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype. Genetics **96:** 147–164.

HARTMAN, P. and J. ROTH, 1973 Mechanisms of suppression. Advan. Genet. **17:** 1–105.

HORVITZ, R., S. BRENNER, J. HODGKIN and R. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Molec. Gen. Genet. **175:** 129–133.

HORVITZ, R. and J. SULSTON, 1980 Isolation and genetic characterization of cell-lineage mutants of the nematode *Caenorhabditis elegans*. Genetics **96:** 435–454.

LIFSHYTZ, E. and R. FALK, 1969 A genetic analysis of the Killer-prune (*K-pn*) locus of *Drosophila melanogaster*. Genetics **62:** 353–358.

LIFSHYTZ, E. and M. GREEN, 1979 Genetic identification of dominant overproducing mutations: the Beadex gene. Molec. Gen. Genet. **171:** 153–159.

MCNICOL, L. A., L. D. SIMON and L. BLACK, 1974 A mutation which bypasses the requirement for p24 in bacteriophage T4 capsid morphogenesis. J. Mol. Biol. **116:** 261–283.

MENEELY, P. and R. HERMAN, 1979 Lethals, steriles and deficiencies in a region of the *X* chromosome of *Caenorhabditis elegans*. Genetics **92:** 99–115.

SULSTON, J. and R. HORVITZ, 1977 Postembryonic cell lineages of the nematode, *Caenorhabditis elegans*. Devel. Biol. **56:** 110–156.

WATERSTON, R. and S. BRENNER, 1978 A suppressor mutation in the nematode acting on specific alleles of many genes. Nature **275**: 715–719.

WATERSTON, R., 1980 A second informational suppressor, *sup-7 X*, in *C. elegans*. Genetics **97**: 307–325.

WATERSTON, R., N. THOMSON and S. BRENNER, 1980 Mutants with altered muscle structure in *Caenorhabditis elegans*. Dev. Biol. **77**: 271–302.

ZENGEL, J. and H. EPSTEIN, 1980 Identification of genetic elements associated with muscle structure in the nematode *Caenorhabditis elegans*. Cell Motility **1**: 73–97.

Corresponding editor. R. K. HERMAN