unc-93(e1500): A BEHAVIORAL MUTANT OF *CAENORHABDITIS ELEGANS* THAT DEFINES A GENE WITH A WILD-TYPE NULL PHENOTYPE

IVA S. GREENWALD AND H. ROBERT HORVITZ

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

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ABSTRACT

The uncoordinated, egg-laying-defective mutation, *unc-93(e1500) III*, of the nematode *Caenorhabditis elegans* spontaneously reverts to a wild-type phenotype. We describe 102 spontaneous and mutagen-induced revertants that define three loci, two extragenic (*sup-9 II* and *sup-10 X*) and one intragenic. Genetic analysis suggests that *e1500* is a rare visible allele that generates a toxic product and that intragenic reversion, resulting from the generation of null alleles of the *unc-93* gene, eliminates the toxic product. We propose that the genetic properties of the *unc-93* locus, including the spontaneous reversion of the *e1500* mutation, indicate that *unc-93* may be a member of a multigene family. The extragenic suppressors also appear to arise as the result of elimination of gene activity; these genes may encode regulatory functions or products that interact with the *unc-93* gene product. Genes such as *unc-93*, *sup-9* and *sup-10* may be useful for genetic manipulations, including the generation of deficiencies and mutagen testing.

THE free-living soil nematode, *Caenorhabditis elegans*, is well suited for genetic studies of behavior (BRENNER 1973, 1974; WARD 1977; HERMAN and HORVITZ 1980). We have examined *e1500*, an egg-laying-defective uncoordinated mutant, as part of a general study of genes that affect the structure, function and development of the *C. elegans* egg-laying system. The *e1500* mutant has abnormal muscle structure and defines a new muscle gene, *unc-93 III*; many other behavioral mutants of *C. elegans* with abnormal muscle structure and function have been described (EPSTEIN, WATERSTON and BRENNER 1974; MACLEOD *et al.* 1977; WATERSTON, THOMSON and BRENNER 1980). Our interest in the *unc-93* locus was stimulated when we observed that wild-type revertants of *e1500* arose spontaneously at high frequency. Analysis of these revertants has revealed other novel genetic properties of the *unc-93* locus.

MATERIALS AND METHODS

General methods: General methods for the handling and culturing of nematodes have been described by BRENNER (1974). Most experiments were performed at 20°; experiments utilizing *sup-7(st5) X* were performed at 25°.

Strains and genetic nomenclature: Caenorhabditis elegans var. Bristol strain N2 (wild-type) and most of the mutant strains used for genetic mapping and other manipulations have been

previously described (BRENNER 1974; HERMAN and HORVITZ 1980). The *him-5* mutation (HODGKIN, HORVITZ and BRENNER 1979) was used in several experiments to generate homozygous males. P. MENEELY and R. HERMAN (1979) provided the *X*-chromosome deficiencies. R. WATERSTON (submitted for publication) provided *sup-7(st5) X*. N. TSUNG isolated *lin-8(n301) II*, a recessive multivulva mutation, in this laboratory. A partial genetic map of *C. elegans* is shown in Figure 1. The genes and alleles that were identified in these studies are presented in Table 1, and other mutations used in these studies are as follows: *LG I: dpy-5(e61)*; *LG II: lin-8(n301), unc-85(e1414), bli-2(e768), dpy-10(e128), unc-4(e120), rol-1(e91)*; *LG III: dpy-1(e1), daf-7(e1372); daf-2(e1370), unc-79(e1068), dpy-17(e164), lon-1(e185), sma-2(e502), unc-32(e189), unc-86(e1416), dpy-18(e364)*; *LG IV: unc-17(e245), dpy-13(e184)*; *LG V: dpy-11(e224), him-5(e1467), mnDp1*; and *LG X: lon-2(e678), sup-7(st5), unc-58(e665), unc-3(e151), mnDf1, mnDf4, mnDf8, mnDf11, mnDf19*.

This paper conforms to the standardized nomenclature for *C. elegans* genetics (HORVITZ *et al.* 1979). Intragenic revertants of *e1500* and *n200* are named as though they were double mutants, *e.g. e1500 n224*. We have adopted this nomenclature because (1) wild-type function is not restored (see RESULTS), indicating that a new allele has been generated, and (2) the original *e1500* mutation probably exists in at least some of the revertants (*e.g.*, suppression studies of *e1500 n234* (see RESULTS) suggest that *e1500* is present). However, not all revertants necessarily retain the *e1500* lesion: *e.g.*, some of the mutagens used can induce deletions, and small deletions within *unc-93* would result in viable revertants according to our model (see RESULTS).

Mutagenesis

Mutageneses: Reversion experiments were performed using a variety of mutagens. *Ethyl methanesulfonate (EMS):* To 3 ml of animals, 1 ml of EMS (Sigma) in M9 buffer was added so that the final concentration was 0.05 M (BRENNER 1974). Animals were incubated for 4 hr and washed 2 or 3 times in M9 buffer before plating. *Diethyl sulfate (DES):* D. RIDDLE (personal communication) has shown that DES is a mutagen for *C. elegans*. To 3 ml of animals in M9 buffer, 1 ml

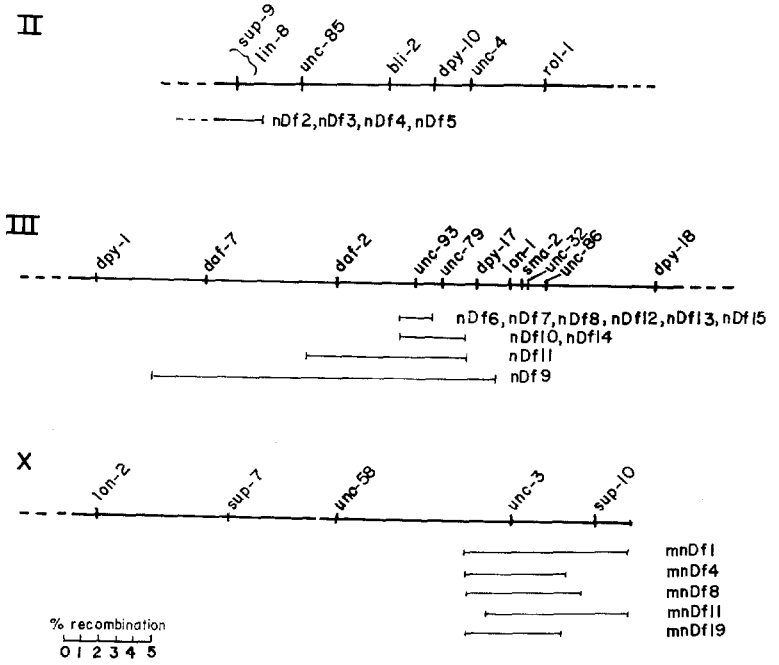

FIGURE 1.—Partial genetic map of *C. elegans*.

TABLE 1

Genes and alleles identified in these studies

Gene	Alleles	Source
sup-9 II	*n180, n222, n223, n292*	Spontaneous reversion of *e1500*
	n186, n188, n189, n190, n191, n213, n264, n266, n271	EMS reversion of *e1500*
	n229, n233, n238, n241	DES reversion of *e1500*
	n192, n219	UV reversion of *e1500*
	n260, n261	ALD reversion of *e1500*
	n345, n350	NTG reversion of *e1500*
	n353	EMS reversion of *n200*
unc-93 III	*e1500, n200*	EMS mutagenesis of N2
	n201, n202, n203, n204, n205, n206, n207, n220, n224, n225, n226, n291	Spontaneous reversion of *e1500*
	n208, n209, n210, n211, n212, n214, n267, n268, n270	EMS reversion of *e1500*
	n230, n231, n232, n234, n235, n236, n239	DES reversion of *e1500*
	n192, n215, n216, n217, n218, n337	UV reversion of *e1500*
	n243, n244, n246, n248, n254, n255,	GR reversion of *e1500*
	n256, n257, n259	ALD reversion of *e1500*
	n340, n341, n343, n344, n348, n349	NTG reversion of *e1500*
	n351, n352, n354	EMS reversion of *n200*
	n392, n393, n394, n395, n396	Complementation screening with *e1500*
sup-10 X	*n181, n182, n183, n184, n185, n221, n227*	Spontaneous reversion of *e1500*
	n262, n263, n265, n269	EMS reversion of *e1500*
	n237, n240	DES reversion of *e1500*
	n245, n247, n249, n250, n251, n252, n253	GR reversion of *e1500*
	n258	ALD reversion of *e1500*
	n342, n345, n346	NTG reversion of *e1500*

The uncoordinated, egg-laying-defective mutant *e1500* defined the gene *unc-93 III*. The *n200* mutation was isolated as an egg-laying-deficient mutant and shown to be linked to *unc-32* by N. TSUNG and to be an allele of *unc-93* by complementation testing: (1) *n180; e1500* males were crossed with *n200* hermaphrodites, and all cross-progeny were uncoordinated; (2) *e1500; n183* males were crossed with *n200* hermaphrodites, and all cross-progeny were uncoordinated; (3) *n234 e1500; him-5; lon-2* males were crossed with *n200 unc-32* hermaphrodites, and all cross-progeny were uncoordinated; (4) *n180; e1500* males were crossed with *n200 n352 unc-32* hermaphrodites, and all cross-progeny were uncoordinated. Revertants are discussed in the text; a few of the revertants included in Table 3 were lost and not given allele designations. Mutagen abbreviations are given in the text.

of DES (Aldrich) in M9 was added so that the final concentration was 10^{-3} M or 10^{-4} M. After 1 and 2 hr, animals were washed 2 or 3 times and plated. The most effective treatment (*i.e.*, the procedure yielding the highest frequency of revertants; see Table 3 legend) was 10^{-3} M for 2 hr; 10^{-2} M for 2 hr drastically reduced the brood size. *Ultraviolet light (UV):* L4 hermaphrodites on uncovered Petri dishes were irradiated with 1 W/m²/sec for 2, 10, 60, 300 and 1500 sec. The most effective treatment was 300 sec; no revertants were seen after a 2 sec treatment, and 1500 sec exposure resulted in massive sterility. *Gamma radiation (GR):* L4 hermaphrodites on Petri dishes were irradiated for 15 and 150 sec at a dose rate of approximately 50 rad/sec

in a Gammacell 220 ^{60}Co source (Atomic Energy of Canada, Ltd.), courtesy of A. J. SINSKEY. These 2 treatments yielded similar numbers of revertants. *Acetaldehyde (ALD):* Acetaldehyde has been shown to be a mutagen for *C. elegans* (HODGKIN, HORVITZ and BRENNER 1979). To 3 ml of animals in M9, 1 ml of acetaldehyde (Aldrich) in M9 was added so that the final concentration was 0.1% or 1.0%. Animals were incubated for 2 hr, then washed 2 or 3 times before plating. Revertants were obtained after treatment with 0.1% ALD; 1.0% greatly reduced the brood size. *Nitrosoguanidine (NTG):* To 3 ml of worms, 1 ml of NTG (Aldrich) was added so that the final concentration was 0.5 mg/ml (D. RIDDLE, personal communication). Animals were incubated for 1 hr and washed 2 or 3 times before plating.

Complementation tests: To characterize spontaneous and mutagen-induced revertants, 3 complementation tests were performed, using the canonical alleles *n180 (sup-9 II)*, *n183 (sup-10 X)* and *n224* (intragenic). (1) Revertant strains were crossed with homozygous *sup-9; 1500* males: all cross progeny were wild type if the unknown suppressor was an allele of *sup-9*; all cross progeny were uncoordinated if the unknown suppressor was autosomal but not an allele of *sup-9*; males were wild type, but hermaphrodites were uncoordinated if the unknown suppressor was sex-linked. (2) Revertant strains were crossed with homozygous *e1500; sup-10* males: all cross progeny were wild type if the unknown suppressor was an allele of *sup-10*, and all cross progeny were uncoordinated if it was autosomal. (3) Revertant strains were crossed with homozygous *e1500 n224* males: all cross progeny were wild-type if the unknown revertant was intragenic, and uncoordinated if it was autosomal but extragenic; males were wild type, but hermaphrodites were uncoordinated if the unknown suppressor was sex-linked. Dominant suppressors resulted in wild-type cross progeny in all of these tests; other recessive suppressors would have complemented all three revertants.

Generation of deficiencies: The strategy for the generation of deficiencies of *unc-93* and *sup-9* is described in RESULTS. L4 males on Petri dishes were irradiated with gamma rays for 150 sec, as described above, and then mated to untreated hermaphrodites of appropriate genotype, 10 of each sex per plate. The parents were transferred to fresh plates every 1 or 2 days to prevent starvation and to facilitate scoring of progeny. Deficiency candidates were detected as rare wild-type animals within a predominantly mutant population.

RESULTS

Visible mutations at the unc-93 *locus:* The mutation *e1500* confers several obvious phenotypic characteristics: uncoordinated movement, absolute egg-laying deficiency, slightly long body, abnormal body postures and an unusual response to touch (when stroked on the head, the animal quickly recoils and relaxes). Mild disorganization of the body-wall musculature is evident with polarized light microscopy (WATERSTON, FISHPOOL and BRENNER 1977), but there is an apparently normal level of birefringence. The *e1500* mutation is weakly semidominant, but we have treated this mutation as if it were recessive for most genetic manipulations. The *e1500* mutation was mapped (Table 2) and found to define a new gene, *unc-93 III*. The *n200* mutation confers the same spectrum of phenotypic characteristics as *e1500*, but is less severe; *n200* was shown to be an allele of *e1500* by complementation testing (see Table 1 legend).

Revertants of e1500: Several independent wild-type revertants arose spontaneously during routine maintenance of mutant stocks. The revertants were recognized because of their wild-type movement and egg-laying behavior; a single wild-type animal can easily be detected on a Petri plate containing 10^4 mutant individuals. Spontaneous reversion appears to be associated with the *e1500* mutation. We constructed double mutants of *e1500* and each of the

following mutations: *dpy-5(e61) I*, *dpy-10(e128) II*, *dpy-1(e1) III*, *dpy-17 (e164) III*, *dpy-13(e184) IV*, *dpy-11(e224) V* and *lon-2(e678) X*. To a first approximation, these strains represent the replacement of each of the chromosomes of the original *e1500* strain. Spontaneous reversion has occurred in all of these strains (and others), suggesting that this property is closely linked to *e1500*. A general elevation of mutation rate due to *e1500* is probably not responsible for spontaneous reversion because we have not seen other mutations appear in strains containing *e1500*.

We characterized the first spontaneous revertants by determining if *e1500* could be reisolated from the revertant strain; revertants were crossed with *lon-2(e678) X* males, and F_2 progeny were examined for the presence of Unc animals. The *lon-2* mutation was included to distinguish self- from cross-progeny: cross-progeny segregated Lon animals among their progeny. Both unlinked, recessive suppressors and tightly linked revertants were identified. When the revertant strain carried an extragenic, unlinked suppressor mutation, *e1500* was reisolated, and the proportion of F_2 Unc progeny indicated a recessive (3/16) or dominant (1/16) suppressor. When the reversion site was tightly linked to *e1500*, no Unc progeny were recovered. Complementation tests among the unlinked suppressors defined two genes, *sup-9 II* and *sup-10 X* (see Tables 2 and 4 for map data and below for complementation test results). The tightly linked revertants were recessive to *e1500* and appeared to be intragenic: *e1500 n234* hermaphrodites, which appear to retain the *e1500* mutation (see below), were crossed with *lon-2* males, and no Unc animals were detected among approximately 6,000 F_2 progeny, indicating that the recombination frequency (p, as in Table 2 legend) is less than 0.03%. This observation suggested that the linked revertants were intragenic, an interpretation confirmed by the complementation screening experiment described in the next section.

To characterize spontaneous and mutagen-induced revertants, three complementation tests were performed, using the canonical alleles *n180* (*sup-9 II*), *n183* (*sup-10 X*) and *n224* (intragenic; see MATERIALS AND METHODS for nomenclature and description of the complementation tests). Table 3 presents the results of these complementation tests, as well as approximate frequencies of reversion. Revertants of all classes were obtained after ethyl methanesulfonate (EMS) treatment with a frequency of approximately 4×10^{-4} per gene. This frequency is the same as the frequency with which mutations that eliminate gene function in an average *C. elegans* gene arise after the same EMS mutagenesis protocol (BRENNER 1974). That intragenic revertants arose at this frequency suggests that intragenic reversion is due to the elimination of *e1500* function. Two other observations support this interpretation. First, all intragenic revertants are recessive, indicating that wild-type function has not been restored. Second, visible alleles of *unc-93* other than *e1500* and *n200* have not been detected despite intensive screening for muscle mutants (WATERSTON, THOMSON and BRENNER 1980; H. EPSTEIN, personal communication) and egg-laying-defective mutants (N. TSUNG and H. R. HORVITZ, unpublished), suggesting that mutations in *unc-93* that result in uncoordination may be rare. These three con-

TABLE 2

Map data for unc-93 III, sup-9 II *and* sup-10 X

A. Two-factor crosses

Gene (allele)	Heterozygous parent	Segregants	Map distance (p)
unc-93(e1500) III	*unc-93 dpy-17/++*	720 wild type 238 Unc Dpy 22 Unc 13 Dpy	3.5%
	unc-93 unc-32/++	514 wild type 125 Unc-93 Unc-32 22 Unc-93 15 Unc-32	5.5%
sup-9(n180) II	*sup-9 dpy-10/++; e1500*	105 Unc(*e1500*) 33 Dpy 8 wild type 10 Dpy Unc	12.0%
sup-10(n183) X	*e1500; unc-3 +/+ sup-10*	3/20 wild type segregants were *e1500; unc-3 sup-10/+ sup-10*	7.5%

B. Three-factor crosses

Gene (allele)	Heterozygous parent	Recombinant phenotype	Recombinant genotype	Number
unc-93(e1500) III	*dpy-17 unc-32/unc-93*	Dpy	$\frac{+\ dpy\ +}{+\ dpy\ unc\text{-}32}$	8
		Unc	$\frac{unc\text{-}93\ +\ unc\text{-}32}{+\ \ dpy\ unc\text{-}32}$	4
	daf-2/unc-93 dpy-17	Dpy	$\frac{daf\ +\ dpy}{+\ unc\ dpy}$	7
		Unc	$\frac{+\ unc\ +}{+\ unc\ dpy}$	4
	daf-2/dpy-1 unc-93	Dpy	$\frac{dpy\ daf\ +}{dpy\ +\ unc}$	16
		Dpy	$\frac{dpy\ +\ +}{dpy\ +\ unc}$	4
		Unc	$\frac{+\ +\ unc}{dpy\ +\ unc}$	14
		Unc	$\frac{+\ daf\ unc}{dpy\ +\ unc}$	6
	unc-79/unc-93 dpy-17	Unc	$\frac{unc\text{-}93\ +\ +}{unc\text{-}93\ +\ dpy}$	5
		Unc	$\frac{unc\text{-}93\ unc\text{-}79\ +}{unc\text{-}93\ \ +\ \ dpy}$	6
sup-9(n180) II	*sup-9/dpy-10 rol-1; e1500/+*	Rol	$\frac{sup\ +\ rol\ \ e1500}{+\ dpy\ rol}; \frac{}{+\ \ +}$	7
	sup-9/lin-8 bli-2; e1500/+	Lin	$\frac{(+\ lin)\ +\ \ e1500}{(+\ lin)\ bli}; \frac{}{+\ \ +}$	11

TABLE 3

Spontaneous and mutagen-induced revertants of e1500

Mutagen	Frequency	*sup-9 II*	*sup-10 X*	Intragenic	Dominant	Total
Spont.	6×10^{-6}	4	7	14	0	25
GR	2×10^{-3}	0	7	7	1	15
EMS	1×10^{-3}	9	4	9	1	23
DES	5×10^{-4}	4	2	7	0	13
UV	2×10^{-4}	2	0	6	0	8
NTG	1×10^{-4}	2	3	6	0	11
ALD	1×10^{-4}	2	1	4	0	7
		23	24	53	2	102

Animals were mutagenized as in MATERIALS AND METHODS and put on 15 × 100 mm Petri plates (either three L4's per plate or approximately 100 L4's per plate). Plates were examined for F_1 and F_2 revertants, which were cloned and crossed with canonical revertants in complementation tests as described in the text.

The reversion rate, which represents the frequency of mutations appearing per haploid genome per generation, is calculated as $r = \frac{\#\text{ plates segregating revertants}}{2 \times \#\ F_1\text{ animals/plate} \times \#\text{ plates}}$. The number of plates segregating revertants is approximately the number of independent revertants generated if this number is small relative to the number of plates that do not contain revertants. Only one revertant clone was analyzed from each plate yielding revertants to ensure independence. The reversion rate for the most effective treatment (see MATERIALS AND METHODS) is given.

It was not possible to quantify accurately the rates of reversion of *n200* because the mutant phenotype is so subtle. However, four revertants were obtained by mutagenizing *n200 unc-32* (the *unc-32* mutation facilitated recognition of revertants). Three of the revertants were tightly linked to *n200* and failed to complement *e1500 n234* (see also legend to Table 1), and one revertant carried an allele of *sup-9*.

We are currently analyzing the rare dominant suppressors. They are not true revertants and appear to be unlinked to *unc-93*.

Spont. = spontaneous; other mutagen abbreviations are given in MATERIALS AND METHODS.

siderations suggested that the null phenotype — the phenotype due to elimination of gene function — of *unc-93* is essentially wild-type.

We performed a number of experiments to test this hypothesis: we isolated additional alleles of *unc-93* by complementation screening and found that all were phenotypically wild-type as homozygotes; we generated deficiencies of *unc-93* and showed that they exhibit the same properties in complementation tests as intragenic revertants; and we identified an intragenic revertant that is suppressible by a null allele-specific suppressor. These experiments are de-

Alleles of *sup-9* and *sup-10* have no associated visible phenotype and hence must be scored in a homozygous *e1500* background.

A. Two-factor crosses: Recombination frequency (p) for two-factor *cis* crosses ($ab/++$) was calculated from the total number of recombinant progeny (R) according to the formula $p = 1 - (1-2R)^{1/2}$; for two-factor *trans* crosses ($a +/+ b$), $2p$ is approximately the frequency of A animals heterozygous for b (*i.e.*, $ab/a+$) (BRENNER 1974).

B. Three-factor crosses: Three-factor crosses were performed as described in BRENNER (1974): from heterozygotes $a\ b/c$, A non-B and B non-A recombinants were picked and their genotypes determined by progeny testing. Because it is difficult to score some recombinant phenotypes in Unc(*e1500*) animals, non-Unc(*e1500*) recombinants were picked, and homozygous *e1500* segregants were then scored for subsequent segregation of suppressed animals. The map order of *sup-9* relative to *lin-8* has not been definitively established by these crosses.

scribed in the next three sections. The extragenic suppressors also appear to represent elimination of gene function and are considered in subsequent sections.

Generation of alleles of unc-93 *by complementation screening:* In Figure 2A, we have outlined the complementation screening protocol used to generate new alleles of *unc-93*. This strategy involved forming heterozygotes between *e1500* and marked mutagenized chromosomes, and screening for failure of complementation with new alleles of *unc-93* by the appearance of F_1 Unc(*e1500*) progeny. L4 *dpy-17* hermaphrodites were mutagenized with EMS and crossed with *sup-9*; *e1500* males. F_1 hermaphrodite cross-progeny were examined for rare Unc(*e1500*) animals, which were candidates for harboring new *unc-93* alleles. Five independent Unc hermaphrodites were detected among an estimated 10,000 cross-progeny hermaphrodites. Each Unc hermaphrodite segregated approximately ¼ Dpy non-Unc and no Dpy Unc animals among their progeny, suggesting that the mutagenized chromosome bearing the *dpy-17* mutation also carried an allele of *unc-93* that did not confer a visible phenotype. The Dpy non-Unc progeny had the following putative genotypes: *sup-9*; *unc-93(0) dpy-17*,

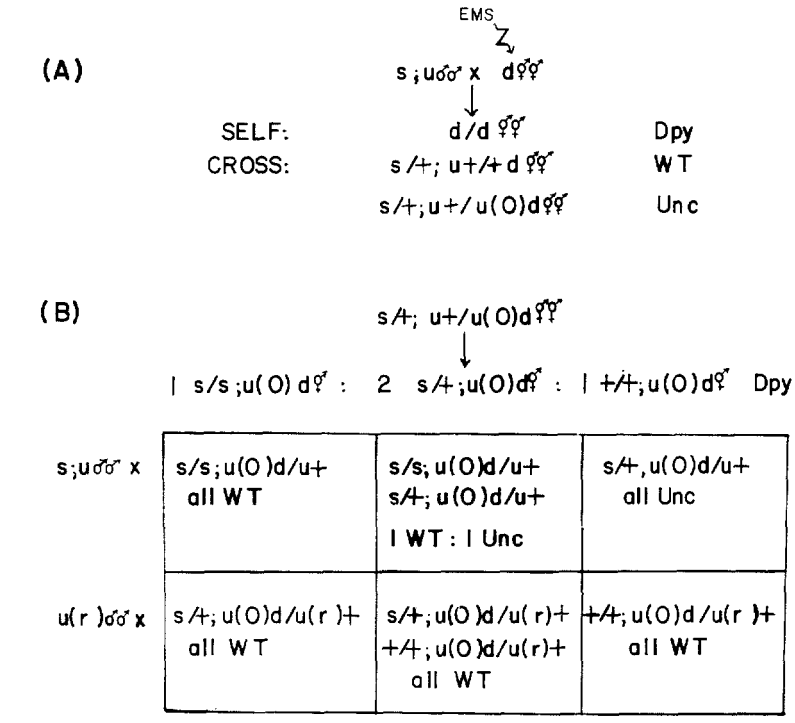

FIGURE 2.—Generation of alleles of *unc-93* by complementation screening. (A) Protocol for the isolation of alleles of *unc-93* by complementing screening. (B) Analysis of candidates: Dpy segregants from Unc candidates were individually mated with *sup-9; e1500* or *e1500 n234* (intragenic revertant) males in complementation tests.

Lower-case letters indicate genotypes: d = *dpy-17*, s = *sup-9*, u = *e1500*, u(0) = null allele of *unc-93* generated by this protocol, u(r) = *e1500 n234*. Upper-case letters indicate phenotypes: Dpy = Dpy-17, WT = wild type, Unc = Unc(*e1500*).

sup-9/+; *unc-93(0) dpy-17*, or +; *unc-93(0) dpy-17* in a 1:2:1 ratio. ("*unc-93(0)*" signifies a null allele of *unc-93*.) Complementation tests were then performed to confirm that alleles of *unc-93* were present (Figure 2B). Several Dpy non-Unc hermaphrodite progeny from each candidate were individually mated with *sup-9*; *e1500* males, and Unc animals were recovered from ¾ of these matings, indicating that an allele of *unc-93* was present; about ¼ of these matings yielded only Unc cross progeny [presumed parental genotype: +/+; *unc-93(0) dpy-17*]. Dpy non-Unc hermaphrodite progeny from each candidate were also mated with *e1500 n234* males, and the cross-progeny were all wild-type. Dpy non-Unc hermaphrodite progeny from some of the candidates were also individually mated with *e1500*; *sup-10* males, and only Unc(*e1500*) cross-progeny were seen. These complementation tests confirmed that alleles of *unc-93* that result in no visible phenotype were generated.

None of the five alleles of *unc-93* obtained in this experiment conferred a visible phenotype, establishing that the common phenotype of mutations in the *unc-93* locus is wild type, and that alleles similar to *e1500* and *n200* must be relatively rare. These new alleles appeared after EMS mutagenesis at a frequency of approximately 5×10^{-4}, again suggesting that elimination of *unc-93* gene function is involved. That these alleles were generated on a chromosome that did not have the *e1500* mutation demonstrates that the presence of the *e1500* mutation is not necessary for the wild-type phenotype of the alleles generated by reversion. Finally, the results from this experiment strongly support the contention that the tightly linked revertants described above are intragenic. Whereas elimination of activity at an extragenic locus would have resulted in a wild-type phenotype (*sup-9*/+; *e1500* + +/+ "*sup*" *dpy-17*), elimination of *unc-93* activity would have resulted in an Unc(*e1500*) phenotype (*sup-9*/+; *e1500* +/*unc-93(0) dpy-17*). Because Unc(*e1500*) animals were detected at a frequency of 5×10^{-4}, it is unlikely that elimination of suppressor gene activity is responsible for the wild-type null phenotype of the tightly linked revertants.

Generation of deficiencies spanning unc-93: The strategy we used to generate deficiencies of *unc-93* is diagrammed in Figure 3A. This approach assumes that *Df(unc-93)/intragenic revertant* is wild type, an assumption that was subsequently verified. Males of genotype *e1500*; *him-5*; *sup-10* were irradiated and crossed with *unc-4*; *e1500 n234* hermaphrodites (*unc-4* is easily distinguished from *e1500*, and *e1500 n234* is an intragenic revertant of *e1500*). Self-progeny were of the Unc-4 phenotype, and most cross-progeny were of the Unc(*e1500*) phenotype, because intragenic revertants are recessive to *e1500*. Rare cross-progeny were wild type and were candidates for carrying deficiencies of *unc-93*. However, there were two alternative possibilities: a new intragenic revertant allele or a dominant suppressor of *e1500*.

To determine if candidates were indeed deficiency heterozygotes, we first used the criterion of recessive lethality. Male candidates were crossed with *e1500 dpy-18* hermaphrodites (Figure 3B). At this point, if the candidate harbored a dominant suppressor, wild-type as well as Unc non-Dpy cross progeny would have been evident; no such candidates were obtained. Unc non-Dpy cross prog-

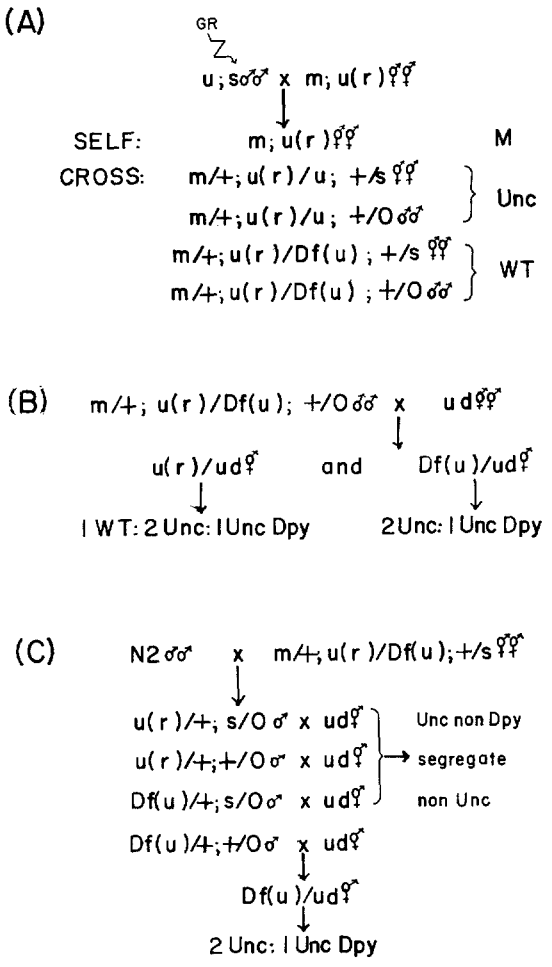

FIGURE 3.—Generation of deficiencies spanning *unc-93*. (A) Protocol for the generation of deficiencies of *unc-93*. Forty-eight wild-type candidates were obtained out of an estimated 39,000 cross-progeny; 32 of these were independent, *i.e.*, found as the progeny of separate matings. Of these, only 17 independent candidates were fertile and could be analyzed. (B) Analysis of male candidates: recessive lethality as a criterion for deficiencies of *unc-93*. Of 10 male candidates analyzed, seven harbored putative deficiencies. See text for further discussion. (C) Analysis of hermaphrodite candidates: recessive lethality as a criterion for deficiencies of *unc-93*. Wild-type (N2) males were crossed with hermaphrodite candidates, and twelve cross-progeny were individually mated with *e1500 dpy-18* hermaphrodites. Unc non-Dpy progeny from these crosses were picked, and their self-progeny were examined for the appearance of non-Unc (*i.e.*, wild-type) individuals. If any of the Unc non-Dpy hermaphrodites failed to segregate wild-type progeny, it suggested that *e1500* was not complemented by a recessive lethal mutation, presumably a deficiency. Note that the progeny of males hemizygous for *sup-10(n183) X* as well as heterozygous for *e1500 n234* segregated wild-type progeny, so that only 1/4 of the single male matings are diagnostic. Of seven hermaphrodite candidates, three harbored putative deficiencies.

Lower-case letters indicate genotypes: u = *e1500*, s = *n183*, *m* = *unc-4*, u(r) = *e1500 n234*, Df(u) = putative deficiency of *unc-93*, d = *dpy-18*. Upper-case letters indicate phenotypes: M = Unc-4, Unc = Unc(*e1500*), WT = wild type.

eny hermaphrodites were cloned. If all of the Unc non-Dpy hermaphrodites segregated non-Unc progeny, a new nonlethal intragenic revertant allele probably had been generated; if half of the Unc non-Dpy hermaphrodites failed to segregate non-Unc progeny, it suggested that *e1500* was not complemented by a recessive lethal mutation, presumably a deficiency. Hermaphrodite candidates were analyzed in an analogous manner (Figure 3C).

We obtained 10 independent gamma-ray-induced recessive lethal alleles that we considered to be deficiencies (*nDf6* through *nDf15*). We then tested these putative deficiencies for complementation with mutations in neighboring genes. Unc non-Dpy hermaphrodites [genotype *Df(unc-93)/e1500 dpy-18*] were crossed with *e1500 n234*; *him-5*; *lon-2* males and wild-type progeny males and hermaphrodites were produced; these males [*Df(unc-93)/e1500 n234*] were crossed with *unc-79 dpy-17* hermaphrodites in a complementation test. Three of the putative deficiencies, *nDf10*, *nDf11* and *nDf14*, failed to complement *unc-79*: Unc-79 non-Dpy cross-progeny were seen. The Unc-79 non-Dpy hermaphrodites were cloned and segregated 2 Unc-79: 1 Unc-79 Dpy: 1 inviable eggs. Deficiency heterozygotes were also crossed with *daf-2*/+ males in a complementation test at 25° (*daf-2* mutants are heat sensitive for constitutive dauer larva formation; RIDDLE 1977); *nDf11* failed to complement *daf-2(e1370)*, as cross-progeny dauer larvae were seen. Thus, *nDf11* is a deficiency that spans the *unc-93* locus. *nDf11* complemented *daf-7*. Another putative deficiency, *nDf9*, failed to complement *dpy-17*, *daf-2* and *daf-7*, indicating that *nDf9* is an extensive deficiency that spans *unc-93*; also, *nDf9* complemented *dpy-1*, *lon-1*, *sma-2* and *unc-86*. We assume that *nDf6*, *nDf7*, *nDf8*, *nDf12*, *nDf13* and *nDf15* are deficiencies within the interval from *daf-2* to *unc-79*.

These deficiencies of *unc-93* behave in a manner consistent with the hypothesis that the null phenotype of *unc-93* is wild type. First, the mutant phenotype of *e1500/Df(unc-93)* is the same as that of *e1500/e1500 n234* (or of heterozygotes between *e1500* and other intragenic revertants). Second, *Df(unc-93)/e1500 n234*, like *e1500 n234/e1500 n234*, and *Df(unc-93)*/+, like *e1500 n234*/+, are wild type. Thus, by these tests, intragenic revertants of *e1500* and deficiencies of *unc-93* are genetically equivalent.

Identification of a suppressible intragenic revertant of e1500: The genetic identification of null alleles has been facilitated by the isolation of two suppressors, *sup-5(e1464) III* (WATERSTON and BRENNER 1978) and *sup-7(st5) X* (WATERSTON, submitted for publication). Mutants in these genes appear to be allele-specific, gene-nonspecific suppressors of certain null alleles; *sup-5* and *sup-7* have the same spectrum of suppression (WATERSON, submitted for publication; HORVITZ and SULSTON 1980). We used *sup-7 X* to determine if any of the *unc-93* alleles are suppressible. We first confirmed that *e1500* is not suppressed: wild-type phenotype is not restored to *e1500*; *sup-7*. We then used *sup-7 X* to identify a suppressible intragenic revertant of *e1500*, *i.e.*, one that is restored to the Unc(*e1500*) mutant phenotype in the presence of the suppressor. Males of genotype *dpy-18*/+; *sup-7/0* were crossed with intragenic revertant hermaphrodites, and F_1 progeny were cloned and identified as cross-progeny by their subsequent segregation of Dpy animals in the F_2 generation. If the intragenic

revertant were suppressible by *sup-7*, uncoordinated animals would be seen in the F_2 generation, and the genotype of the suppressed animals could be inferred from the proportion of uncoordinated animals and the phenotype of the F_3 segregants. Twenty-four spontaneous and mutagen-induced revertants were tested: eight spontaneous, seven EMS, five DES, two UV and two gamma-ray. One revertant, *e1500 n234*, which was induced by diethyl sulfate, yielded approximately 1/16 F_2 uncoordinated progeny, and these segregated only uncoordinated progeny, suggesting that the genotype of these uncoordinated animals was *e1500 n234*; *sup-7*. We confirmed this result by crossing the presumed suppressed intragenic revertant with *dpy-18 III*; *him-5 V*; *lon-2 X* males to balance both *unc-93 III* and *sup-7 X*; all F_2 uncoordinated progeny failed to segregate Dpy and Lon. The presence of homozygous *sup-7* in the presumed suppressed intragenic revertant was confirmed by sterility at 15° (*sup-7* is a cold-sensitive recessive sterile; WATERSTON, submitted for publication). The phenotype of *e1500 n234*; *sup-7* is qualitatively similar to that of *e1500*, but is somewhat less severe, which is consistent with data that suggest that only a fraction of wild-type levels of gene activity is restored (WATERSTON, submitted for publication).

The extragenic suppressors of e1500: There are two extragenic suppressors of *e1500*: *sup-9 II* (canonical allele: *n180*) and *sup-10 X* (canonical allele: *n183*). They are essentially recessive suppressors of *e1500*, although *e1500*; *sup*/+ hermaphrodites may occasionally release a few eggs or larvae. Both suppressors also suppress *n200* (data not shown), so that they appear to be allele nonspecific, and an allele of *sup-9* was found among four EMS-induced revertants of *n200* (Table 3 legend). The canonical alleles of the suppressors were separated from *e1500*, using appropriate markers (*dpy-10 II* and *unc-3 X*): *e.g.*, from *sup-9/dpy-10*; *e1500*/+ wild-type animals were picked, and from those that did not segregate Unc(*e1500*) but did segregate Dpy (genotype *sup-9/dpy-10*; +/+), wild-type progeny were picked; ⅓ of these did not segregate Dpy and were *sup-9/sup-9*; +/+. This genotype was confirmed by showing suppressor activity and by complementation testing (data not shown). Isolated *sup-9* and *sup-10* homozygotes have no obvious phenotypic differences from wild type. In addition, four combinations of suppressor mutations have been constructed: (1) *sup-9*; *e1500 n234*, (2) *e1500 n234*; *sup-10*, (3) *sup-9*; *sup-10*, and (4) *sup-9*; *e1500*; *sup-10*. All of these combinations are wild type.

The extragenic suppressors, like the intragenic revertants, appear to occur as a result of elimination of suppressor gene activity; after EMS mutagenesis, they arise at a frequency of about 4×10^{-4} per locus. In both cases, we have shown that *sup/Df(sup)* has suppressor activity (see below), which is consistent with the hypothesis that null alleles of these genes have suppressor activity.

Deficiencies spanning the suppressors: Our tentative map position for *sup-10 X* (Table 2) indicated that it mapped in a region of the *X* chromosome that has a series of well-characterized deficiencies (MENEELY and HERMAN 1979). We used these deficiencies to show that *sup-10/Df(sup-10)* suppresses *e1500* and to map *sup-10* as described in the legend to Table 4.

TABLE 4

Deficiency mapping of sup-10 X

Deficiency	Number of F_1 animals segregating Unc(*e1500*)	Number of F_1 animals segregating non-Unc only
mnDf1	11	5
mnDf4	12	0
mnDf8	10	0
mnDf11	11	4
mnDf19	12	0

The *X*-linked suppressor, *sup-10(n183)*, was mapped by complementation testing with several deficiencies in the *unc-3 X* region (MENEELY and HERMAN 1979). Homozygous *e1500*; *lon-2 n183* males were crossed with *mnDp1/+*; *mnDf* hermaphrodites (*mnDp1* balances all the deficiencies used and is homozygous sterile). Putative F_1 cross progeny were picked and confirmed by segregation of Lon in the F_2 generation. The cross-progeny were of two possible genotypes, *e1500/+*; *+/+*; *lon-2 n183/Df* and *e1500/+*; *mnDp1/+*; *lon-2 n183/Df*. Animals of the former genotype failed to segregate Unc(*e1500*) progeny if *sup-10* was not complemented by the deficiency and segregated Unc(*e1500*) progeny if *sup-10* was complemented by the deficiency. Animals of the latter genotype always segregated Unc(*e1500*) progeny.

There were no deficiencies available in the region of *sup-9 II*. To generate deficiencies of *sup-9*, we used a strategy similar to that used to obtain deficiencies of *unc-93*. Figure 4 outlines this procedure. Homozygous *e1500 n234*; *him-5*; *lon-2 males* (note, the *lon-2* mutation is irrelevant) were irradiated and crossed with *sup-9*; *e1500*; *unc-17* hermaphrodites. Self-progeny were Unc-17, and most cross-progeny were Unc (*e1500*); rare cross-progeny were wild type and were candidates for carrying deficiencies of *sup-9*. We tested these candidates for complementation with neighboring markers. Hermaphrodite candidates were crossed with *lin-8 bli-2/+ +* males, and their progeny were examined for Lin and Bli. Four out of five hermaphrodite candidates that successfully mated had Lin non-Bli progeny. The Lin progeny were presumed to contain deficiencies of *sup-9* and *lin-8*. Cloned Lin non-Bli hermaphrodites segregated about 2 Lin:

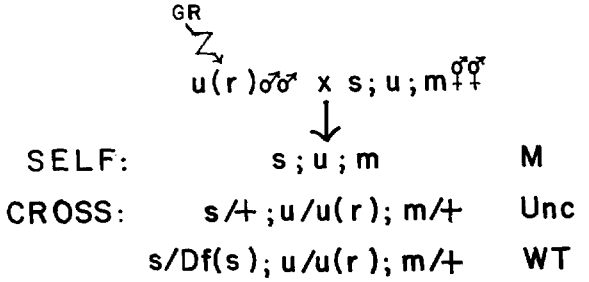

FIGURE 4.—Generation of deficiencies of *sup-9*. Nineteen wild-type candidates were obtained out of an estimated 5,000 cross progeny; 10 of these were independent. Only five of the 10 hermaphrodite candidates were fertile, and four carried putative deficiencies that failed to complement *lin-8(n301)*; none of the nine male candidates mated successfully.

Lower-case letters indicate genotypes: u(r) = *e1500 n234*, s = *n180*, u = *e1500*, m = *unc-17*, Df(s) = Df(*sup-9*). Upper-case letters indicate phenotypes: M = Unc-17, Unc = Unc(*e1500*), WT = wild type.

1 Lin Bli and produced inviable zygotes. All of the deficiencies complemented *unc-85*.

To confirm that these putative deficiencies (*nDf2* through *nDf5*) failed to complement *sup-9*, Lin non-Bli hermaphrodites were crossed with *sup-9*; *e1500* males. Non-Lin hermaphrodites were picked; half segregated Unc(*e1500*) and Lin Bli progeny, and half failed to segregate either Unc or Lin Bli progeny. Thus, the deficiencies failed to complement *sup-9* for suppressor activity. We also showed that *Df(sup-9)/+*, like *sup-9/+*, does not suppress *e1500* (data not shown).

DISCUSSION

Our work has demonstrated that, although rare mutations (*e1500*, *n200*) at the *unc-93* locus result in several aberrant phenotypic characteristics, mutations that eliminate *unc-93* function have no obvious effects. This conclusion is based on several genetic criteria. (1) Intragenic revertants of *e1500* and alleles of *unc-93* that do not confer a visible phenotype are common; they appear after EMS mutagenesis at a frequency of approximately 5×10^{-4}, which is the same frequency at which mutations that eliminate gene function in an average *C. elegans* gene arise after EMS mutagenesis (BRENNER 1974). (2) Mutations resulting in visible *unc-93* alleles are relatively rare; only two visible alleles of *unc-93* have been detected so far, and in the complementation screening experiment described above no visible alleles of *unc-93* were found. (3) Although *e1500/+* is only slightly uncoordinated, *e1500/intragenic revertant* is similar in phenotype to *e1500* homozygotes, suggesting that wild-type function is not restored. (4) Intragenic revertants and deficiencies of *unc-93* are equivalent in complementation tests: the mutant phenotype of *e1500/Df(unc-93)* is the same as that of *e1500/intragenic revertant*; *Df(unc-93)/intragenic revertant*, like intragenic revertant homozygotes, and *Df(unc-93)/+*, like *intragenic revertant/+*, are wild-type. (5) At least one intragenic revertant is suppressed to the mutant phenotype by *sup-7 X*, a suppressor that is apparently specific for null alleles (WATERSTON, submitted for publication).

The effects of mutations in *unc-93* that result in visible phenotypes are relieved by the elimination of gene function. Two classes of events may be envisaged that would behave in this manner: (1) mutations that structurally alter the gene product in such a way that it is toxic to the animal, or (2) mutations that alter the amount of the gene product in the animal, *e.g.*, resulting in elevation of wild-type gene product to toxic levels or in spatially or temporally inappropriate expression of gene product. A comparison of *e1500/null* animals, which are mutant, and *e1500/+* animals, which are essentially wild type, suggests that it is unlikely that *e1500* results in the dominant overproduction of the *unc-93* product because *e1500/+* would contain more product than *e1500/null* and hence should be at least as mutant. That *e1500* is only weakly semidominant suggests that wild-type gene activity can overcome the toxic effects of the mutation; the wild-type allele may do so by producing a product that efficiently replaces a poisonous gene product or by restoring proper regulation to a product that is autoregulated (GOLDBERGER 1974).

The data for *sup-7 X* suppression of the intragenic revertant *e1500 n234* are consistent with the interpretation that the *e1500* product is a poison. Under the conditions used, homozygous *sup-7* restores less than 20% of the wild-type levels of gene product for suppressible *unc-15* alleles (WATERSTON, submitted for publication). Since *e1500 n234*; *sup-7* is uncoordinated and egg-laying defective, it appears that only a fraction of the activity present in *e1500* homozygotes confers the mutant phenotype. Again, it seems unlikely that *e1500* results in a dominant overproduction of a wild-type product, since *e1500*/+ would have more activity than *e1500 n234*; *sup-7*. Also, the restoration of gene product to a null mutant by dose-dependent suppression may indicate stoichiometric (as opposed to catalytic) function (WATERSTON and BRENNER 1978; HORVITZ and SULSTON 1980). Thus, the need for two doses of *sup-7* to restore the mutant phenotype might be expected if the *e1500* product were a stoichiometric poison, and is consistent with the possibility that the *unc-93* gene encodes a muscle structural protein. If the *sup-7* product proves to act at or after translation, the suppression results suggest that *unc-93* encodes a polypeptide.

The apparently high rate (10^{-6}) of spontaneous reversion of *unc-93(e1500)* presumably reflects the fact that reversion can occur by many different mutational events leading to inactivation within the *unc-93* locus (as well as at the two extragenic suppressor loci), which contrasts with the specific event(s) generally necessary for the restoration of gene activity to mutants that confer a visible phenotype. We believe that the spontaneous reversion of *e1500* reflects the normal rate of spontaneous mutagenesis, which can be detected and quantified easily only in genetic backgrounds where the occurrence of a mutation is obvious, such as the conversion of an uncoordinated to a wild-type phenotype.

One possible explanation for the wild-type null phenotype of *unc-93* is that redundant biochemical and/or morphogenetic pathways exist; in the absence of the *unc-93* pathway, alternative pathways still function. A specific example of such redundancy is that another gene product may be able to substitute directly for the *unc-93* product: perhaps *unc-93* is a member of a multigene family, and the product of another family member is present in sufficient quantity to substitute for the missing *unc-93* product. Elimination of one member of a multigene family may not confer a visible phenotype, although elimination of all members of a family should be deleterious (*e.g.*, CULOTTI *et al.*, submitted for publication). The existence of multigene families of related muscle proteins in *C. elegans* (MACLEOD *et al.* 1977; SCHACHAT *et al.* 1978; D. HIRSH, personal communication) and in other organisms (*e.g.*, STARR and OFFER 1973; ROY, POTTER and SARKER 1976; WHALEN, BUTLER-BROWNE and GROS 1976; DHOOT and PERRY 1979) makes the possibility that *unc-93* is a member of a multigene family attractive. It is known that one member of a multigene family can overcome defects associated with the elimination of another member, such as in the hereditary persistence of fetal hemoglobin in beta-thalassemics (*e.g.*, WEATHERALL and CLEGG 1980) and possibly in the indirect suppression of *C. elegans unc-54* myosin mutants (RIDDLE and BRENNER 1978; R. WATERSTON, personal communication), but in these cases secondary mutations are required to enable elevated synthesis of the substituents.

Alternatively, *unc-93* need not be a member of a multigene family; the *unc-93* product may be irrelevant under normal laboratory conditions, or *unc-93* null mutations may have consequences too minimal to detect easily.

Since members of multigene families may have wild-type null phenotypes, they may well display genetic characteristics similar to those of the *unc-93* locus: rare visible alleles, many of which might be dominant or semidominant poisons that overcome the wild-type product of other family members; intragenic reversion of visible alleles at frequencies expected for the elimination of gene activity; and the recessive behavior of intragenic revertants. Spontaneous intragenic reversion may be a particularly useful indicator of members of multigene families, because wild-type individuals will be detected during routine maintenance of mutant stocks. Therefore, other mutants that revert spontaneously may be members of multigene families. For example, *e665* (*unc-58 X*), a strongly semidominant mutation that results in a severely uncoordinated "shaker" phenotype (BRENNER 1974), reverts spontaneously at a frequency of about 10^{-6} (HODGKIN 1974). We generated 19 revertants with EMS at a frequency of about 5×10^{-4}; 18 of these revertants were tightly linked to *e665* and recessive (*i.e.*, *e665/revertant* was more uncoordinated than *e665/+*). The semidominant muscle mutant, *unc-92(st15) V*, reverts spontaneously and appears to define another gene that has an essentially wild-type null phenotype (R. WATERSTON, personal communication).

Both *sup-9 II* and *sup-10 X*, the extragenic suppressors of *e1500*, arise as a result of elimination of gene activity; both are indirect suppressors (HARTMAN and ROTH 1973). Our data do not allow us to distinguish among three classes of models: (1) The suppressor(s) encodes an activator of *unc-93* expression; elimination of the activator decreases the expression of the *unc-93* locus. (2) The suppressor(s) encodes a negative regulator of a gene product that can substitute for or bypass the need for *unc-93* product; increased production of this alternative product overcomes the toxic effects of *unc-93* mutants. The suppressor mutations could therefore be analogous to the secondary mutations mentioned above that enable hereditary persistence of fetal hemoglobin and indirect suppression of *C. elegans* myosin mutants. (3) The suppressor(s) encodes a product that interacts with the *e1500* product either directly or indirectly to produce the toxin.

Our results indicate that some genes for which rare alleles confer a visible phenotype have a wild-type null phenotype. Such genes would not be included in estimates of gene number based on the frequency of occurrence of recessive lethal mutations (*e.g.*, HOCHMAN 1971; LEWIN 1974; BRENNER 1974). If there are many genes with properties similar to those of *unc-93*, as could well be the case if such genetic properties are typical of multigene families, current estimates of the number of genes in higher organisms may be significantly low.

Genes such as *unc-93* may provide useful genetic tools. For example, deficiencies can be readily generated by the methods described, because wild-type candidates are easily detected in a mutant background, and could supplement other strategies for generating deficiencies (*e.g.*, MENEELY and HERMAN 1979; P.

ANDERSON, personal communication). The protocol used for generating deficiencies of *unc-93* may be modified for genes that are not suppressed by extragenic suppressors: mutant males may be irradiated and directly mated with marked revertant hermaphrodites or, if mutant males are unable to mate, revertant males may be mated with irradiated mutant hermaphrodites, and the wild-type progeny would be candidates for carrying deficiencies. The protocol used for producing deficiencies of *sup-9* may be used with modifications for appropriate extragenic recessive suppressors: irradiated mutant males may be mated with marked suppressed hermaphrodites or suppressed males may be mated with irradiated mutant hermaphrodites. Because it is easy to detect wild-type animals among large numbers of mutant individuals, genes such as *unc-93* can be used to assess the potency of various mutagenic agents in assays that are more sensitive than measurement of mutation to visible phenotypes or of reversion involving a specific site or sites. In addition, because large numbers of alleles and deficiencies can be easily obtained, genes such as *unc-93* may be useful for fine-structure genetics and molecular biology.

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