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Three New Classes of Mutations in the *Caenorhabditis elegans* Muscle Gene *sup-9*

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ABSTRACT

We are studying five interacting genes involved in the regulation or coordination of muscle contraction in *Caenorhabditis elegans.* A distinctive "rubber-band" muscle-defective phenotype was previously shown to result from rare altered-function mutations in either of two of these genes, *unc-93* and *sup-10*. Null mutations in *sup-9, sup-10, sup-18* or *unc-93* act as essentially recessive suppressors of these rubber-band mutations. In this work, we identify three new classes of *sup-9* alleles: altered-function rubber-band, partial loss-of-function and dominant-suppressor. The existence of rubber-band mutations in *sup-9, sup-10* and *unc-93* and the suppression of these mutations by null mutations in any of these three genes suggest that these proteins are required at the same step in muscle contraction. Moreover, allele-specific interactions shown by the partial loss-of-function mutations indicate that the products of these interacting genes may physically contact each other in a multiple subunit protein complex. Finally, the phenotypes of double rubber-band mutant combinations suggest that the rubber-band mutations affect a neurogenic rather than a myogenic input in excitation-contraction coupling in muscle.

MORE than 30 genes have been identified by mutations that affect muscle structure and function in *Caenorhabditis elegans* (reviewed by WATERSTON 1988). Such mutations often confer severe defects in movement due to the disruption of body-wall muscle function. These muscle genes can be classified as structural or regulatory based on their effects on muscle structure and function. Mutations in structural genes cause defects in myofilament assembly and structure. By contrast, mutations in regulatory genes cause severe defects in movement with comparatively minor structural defects; such mutations might disrupt excitation-contraction coupling, the process in a muscle cell by which an excitatory stimulus from a neuron or from the muscle cell itself leads to the sliding of the myosin and actin filaments to cause contraction (SHEPHERD 1988; RIOS and PIZARRO 1991).

A set of five interacting genes has been found to be involved in the regulation or coordination of *C. elegans* muscle contraction: *unc-93 III, sup-9 II, sup-10 X, sup-11 I* and *sup-18 III* (GREENWALD and HORVITZ 1980, 1982, 1986). *unc-93(e1500), unc-93(n200)* and *sup-10(n983)* are rare altered-function mutations that confer a distinctive defect in the regulation of muscle contraction termed "rubber-band" (GREENWALD and HORVITZ 1980, 1986). When a rubber-band mutant is prodded on its head, the worm contracts and then quickly relaxes without moving backward, whereas a wild-type worm simply moves backward. This phenotype indicates that rubber-band mutants can contract their body-wall muscles but are defective in the regulation or coordination of the contraction. In addition, rubber-band mutants show (1) sluggish and flaccid movement, like that of many *C. elegans* muscle mutants (WATERSTON, THOMSON and BRENNER 1980); (2) only minor structural defects in the body-wall muscles (GREENWALD and HORVITZ 1980; WATERSTON 1988); (3) a long, thin body shape; and (4) an egg-laying defect that reflects a dysfunction of the vulval and uterine muscles (GREENWALD and HORVITZ 1980, 1986). Genetic mosaic analysis has shown that *sup-10* functions within muscle cells (HERMAN 1984).

Reversion of the rubber-band phenotype caused by the *unc-93(e1500)* and *sup-10(n983)* mutations led to the identification of null mutations in *sup-9, sup-10, sup-18* and *unc-93* as recessive suppressors of these mutations (GREENWALD and HORVITZ 1980, 1986). *sup-18(0)* mutations (*i.e.*, null mutations of *sup-18*) are complete suppressors of *sup-10(n983)* but only partial suppressors of *unc-93(e1500)* (GREENWALD and HORVITZ 1986). All of these null mutations alone confer no visibly abnormal phenotype, possibly because each of these genes is functionally redundant with another gene (or set of genes) that has sufficient overlap in regulating muscle contraction with these genes to maintain normal muscle function. *sup-9, sup-10, sup-18* and *unc-93* function together to regulate muscle contraction, and based on genetic studies their prod-

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ucts have been suggested to interact as a protein complex.

The *sup-11(d)* class of mutations (*i.e.*, dominant alleles of *sup-11*) consists of rare altered-function alleles that were isolated as dominant suppressors of *unc-93(e1500)* and that also are recessive suppressors of *sup-10(n983)* (GREENWALD and HORVITZ 1982, 1986). *sup-11(0)* mutations result in embryonic lethality and do not act as suppressors of the rubber-band phenotype (GREENWALD and HORVITZ 1982). The genetic analysis of *sup-11* led to the suggestion that *sup-11* might not normally interact with these four genes and that the *sup-11(d)* altered-function gene product restores normal muscle function by bypassing the defect caused by the rubber-band mutations.

The molecular characteristics of the *unc-93* suggest that this gene encodes a novel membrane-associated protein (LEVIN and HORVITZ 1992). The role of *unc-93* and interacting genes in *C. elegans* muscle contraction remains unclear, but the rubber-band phenotype and the putative membrane localization of the Unc-93 protein suggest some possibilities. Specifically, the Unc-93 protein might act either as a component of an ion transport system involved in excitation-contraction coupling in muscle or in the coordination of muscle contraction between muscle cells by affecting the functioning of gap junctions. *unc-93*, *sup-9*, *sup-10* and *sup-18* might encode interacting proteins that function within the membranes of muscle cells.

In this work, we describe three new classes of *sup-9* mutations: rubber-band, partial loss-of-function, and dominant suppressor. These *sup-9* mutations reveal new facets of the genetic interactions among *unc-93*, *sup-9*, *sup-10* and *sup-18* and provide additional support for the hypothesis that their products directly interact.

MATERIALS AND METHODS

General methods and nomenclature: General methods for the handling and culturing of *C. elegans* strains have been described by BRENNER (1974). Unless otherwise indicated, *C. elegans* was grown at 20°. Mutageneses with ethyl methanesulfonate (EMS) were done as described by BRENNER (1974). *C. elegans* genetic nomenclature is described by HORVITZ *et al.* (1979).

Strains used: *C. elegans* variety Bristol strain N2 is the wild-type parent of all strains used in this work, except for the original *unc-93(e1500 n1415)* and *sup-10(e2127)* strains, which were isolated from a *mut-2* genetic background, as described by LEVIN and HORVITZ (1992). The previously identified *sup-9, sup-10, sup-11, sup-18* and *unc-93* mutations used in this work are listed in Table 1. The alleles generated in this study are listed in Table 2. The *n1550* mutation was isolated in a heterozygote by M. HERMAN (unpublished data) on the basis of its rubber-band phenotype. The following mutations were used as genetic markers in this study and, unless otherwise noted, were described by HODGKIN *et al.* (1988): LGII: *lin-42(n1089)* (LIU 1990), *lin-31(n301)*, *bli-2(e768)*, *dpy-10(e128)*; LGIII: *dpy-17(e164)*, *unc-32(e189)*; LGIV: *dpy-9(e12)*; LGV: *dpy-11(e224)*, *him-5(e1490)*; LGX: *lin-15(n765)*.

Scoring the rubber-band phenotype: The rubber-band phenotype, which is named for the distinctive response of a rubber-band mutant when prodded on its head, includes defects in movement, body shape and egg laying (GREENWALD and HORVITZ 1980, 1986). In this paper, we use a classification system to describe the severity of rubber-band phenotypes based on the phenotypes of the four rubber-band mutants (see RESULTS). We generally compared rubber-band phenotypes by direct observation of the worms. To distinguish subtle differences among rubber-band phenotypes, we compared worms of differing genotypes in a "blind" experiment in which we did not know the genotype of worms on a particular Petri dish. We grew worms at 25° for experiments with strains of genotypes that cause weak rubber-band phenotypes, such as *unc-93(e1500)/+* and *unc-93(n200)*, because rubber-band phenotypes are slightly stronger at 25°. For the brood size measurements in Table 6, we compared strains grown at the same time under identical conditions.

Isolation of new mutants: *Screen for F_1 revertants of sup-9(n1550)/+:* We mutagenized *n1550; unc-93(e1500 n1415) dpy-17* hermaphrodites with EMS, crossed them with *lin-42/+* males and screened the F_1 progeny for non-Unc non-Dpy revertants. Normal self progeny were Dpy non-Unc animals, since *unc-93(e1500 n1415)*, which is an *unc-93* null mutation, acts as a recessive suppressor of the dominant rubber-band phenotype of *n1550*. Normal cross progeny were Unc non-Dpy animals. If *n1550* were a *sup-9* allele, the induction of a *sup-9(0)* mutation in *cis* to *n1550* would produce a rare non-Unc, non-Dpy worm. A worm of this phenotype could also result from the induction of any other dominant suppressor of *n1550*. The *lin-42* mutation, which is about 1.5 map units from *sup-9* (EDGLEY and RIDDLE 1990), was included as a balancer to allow the recovery of *sup-9(0)* mutations, which result in no visibly abnormal phenotype alone. The *dpy-17* mutation, which is about 3.4 map units from *unc-93* (EDGLEY and RIDDLE 1990), was used both to distinguish cross progeny from self progeny and as a marker to help identify worms lacking the *unc-93(e1500 n1415)* mutation. We isolated three independent non-Unc, non-Dpy F_1 progeny in this way, two hermaphrodites and one male, from 8,868 F_1 progeny screened (3/8,868 haploid genomes).

To recover *sup-9(0)* homozygotes without the *unc-93(0)* mutation from the F_1 hermaphrodites, we picked phenotypically wild-type F_2 progeny that segregated no Dpy or Lin F_3 progeny. An F_1 hermaphrodite that failed to segregate Lin progeny would have been discarded because the *sup-9(0)* mutation could not have been easily recovered; this situation did not occur. We picked phenotypically wild-type F_3 progeny as gravid adults and allowed each to lay eggs on a single Petri dish for about 48 hr at 15°. We then processed each such adult individually for a single worm polymerase chain reaction (PCR) (BARSTEAD and WATERSTON 1991; SAIKI *et al.* 1988) to confirm that the worms were homozygous for *unc-93(+)*. Specifically, PCR amplification of DNA from worms carrying the Tc1 insertion allele *unc-93(e1500 n1415)* generates a 523-bp fragment with a Tc1 primer (positions 105–83) (ROSENZWEIG, LIAO and HIRSH 1983) and an *unc-93* primer (positions 2355–2379) (LEVIN and HORVITZ 1992). By contrast, PCR amplification of DNA from worms with an *unc-93* wild-type allele does not generate the 523-bp product with these primers. As a positive control, worm DNA samples were checked in parallel for PCR amplification with a pair of *unc-93* primers (positions 2355-2379 and 5055-5036) that generate a 2717-bp frag-

TABLE 1

Previously described rubber-band and suppressor mutations used in this study

Gene	Alleles	Symbol	Reference(s)
sup-9	*n180, n1009*	*sup-9(0)*	GREENWALD and HORVITZ (1980, 1986)
sup-10	*n983*	*sup-10(rubber-band)*	GREENWALD and HORVITZ (1986)
	n183, e2127	*sup-10(0)*	GREENWALD and HORVITZ (1980); LEVIN and HORVITZ (1992)
sup-11	*n187, n401, n402, n403, n404, n405, n616, n710, n711*	*sup-11(d)*	GREENWALD and HORVITZ (1982)
sup-18	*n1010, n1014*	*sup-18(0)*	GREENWALD and HORVITZ (1986)
unc-93	*e1500, n200*	*unc-93(rubber-band)*	GREENWALD and HORVITZ (1980)
	e1500 n234, e1500 n1415	*unc-93(0)*	GREENWALD and HORVITZ (1980); LEVIN and HORVITZ (1992)

sup-9(0), sup-10(0), sup-18(0) and *unc-93(0)* are putative null alleles.
sup-11(d) is a dominant suppressor of *unc-93(e1500).*
sup-10(rubber-band) and *unc-93(rubber-band)* are altered-function alleles that cause a rubber-band phenotype.

TABLE 2

Mutations first described in this study

Gene	Mutations	Symbol	Genotype screened [Generation]
sup-9	*n1550*	*sup-9(rubber-band)*	*him-5(e1490)* [F_2]
	n242[a]	*sup-9(dsp)*	*unc-93(e1500)* [F_1]
	n1435	*sup-9(dsp)*	*sup-10(n983)* [F_1]
	n1553[b]	*sup-9(0)*	*sup-10(n983)* [F_2]
	n2174, n2175, n2176[c]	*sup-9(0)*	*sup-9(n1550)/+* [F_1]
	n2276, n2278, n2279, n2281, n2282, n2283, n2284[d]	*sup-9(0)*	*sup-9(n1435); unc-93(e1500)* [F_2]
	n2285, n2286, n2287, n2291, n2292, n2294, n2296[c]	*sup-9(0)*	*sup-9(n1550); sup-18(n1014)* [F_2]
	n2343, n2344, n2345, n2346, n2347, n2348, n2349, n2350, n2351, n2352, n2353, n2354, n2355, n2356, n2357, n2358[c]	*sup-9(0)*	*sup-9(n1550); sup-18(n1014)* [F_1]
	n2359, n2360, n2361[c]	*sup-9(p)*	*sup-9(n1550); sup-18(n1014)* [F_1]
	n2288[c,e]	*sup-9(p)*	*sup-9(n1550); sup-18(n1014)* [F_2]
sup-10	*n2290, n2295, n2297*	*sup-10(0)*	*sup-9(n1550); sup-18(n1014)* [F_2]
	n2335, n2337	*sup-10(0)*	*sup-9(n1550); sup-18(n1014)* [F_1]
sup-11	*n2298*	*sup-11(d)*	*sup-9(n1550); sup-18(n1014)* [F_2]
unc-93	*n2275, n2277, n2280*	*unc-93(0)*	*sup-9(n1435); unc-93(e1500)* [F_2]
	n2289, n2293, n2299	*unc-93(0)*	*sup-9(n1550); sup-18(n1014)* [F_2]
	n2336, n2338, n2339, n2340	*unc-93(0)*	*sup-9(n1550); sup-18(n1014)* [F_1]

All of these mutations were isolated after EMS mutagenesis, except *n242* was isolated after gamma ray irradiation. [F_1], the revertants were picked in the F_1 generation. [F_2], the revertants were picked in the F_2 generation. *sup-9(0), sup-10(0)* and *unc-93(0)* are putative null alleles. *sup-9(dsp)* is a dominant-suppressor allele. *sup-9(p)* is a partial loss-of-function allele. *sup-11(d)* is described by GREENWALD and HORVITZ (1982).

[a] This allele was generated by GREENWALD and HORVITZ (1980 and unpublished data).
[b] We isolated *sup-9(n1553)* as a recessive suppressor of *sup-10(n983)* (our unpublished data).
[c] Because these mutations were generated in a *sup-9(n1550)* background, these alleles are actually *sup-9(n1550 n2174)*, etc.
[d] Because these mutations were generated in a *sup-9(n1435)* background, these alleles are actually *sup-9(n1435 n2276)*, etc.
[e] *n2288* is likely to be a *sup-9(p)* mutation (see text for details).

ment (LEVIN and HORVITZ 1992). PCR conditions were as described by LEVIN and HORVITZ (1992). To construct *sup-9(0); unc-93(e1500)* strains, we crossed F_4 progeny of the F_3 worms that did not contain the *unc-93(e1500 n1415)* mutation with wild-type males and then crossed the F_5 cross progeny males (genotype of *sup-9(0)/+*) with *unc-93(e1500)* hermaphrodites. We picked essentially wild-type F_6 cross progeny hermaphrodites, half of which were expected to be of the genotype *sup-9(0)/+; unc-93(e1500)/+*. From each F_6 hermaphrodite, we picked Unc F_7 progeny, 2/3 of which should be of the genotype *sup-9(0)/+; unc-93(e1500)* if the F_6 parent were heterozygous for *sup-9(0)*. We picked phenotypically wild-type F_8 self progeny and showed these animals were homozygous for a *sup-9(0)* allele by a complementation test (see below).

The one F_1 wild-type male revertant was crossed with *lin-42; sup-10(n983)* hermaphrodites. We identified phenotypically wild-type cross progeny F_2 hermaphrodites that segregated no Dpy F_3 progeny (putative F_2 genotype *lin-42 +/+ sup-9(0); unc-93(+)dpy-17(+); sup-10(n983)/+*). From the progeny of F_3 Unc hermaphrodites that segregated wild-type F_4 progeny (genotype *sup-9(0); sup-10(n983)* or rarely *unc-93(0); sup-10(n983)*), we picked phenotypically wild-type F_4 progeny as gravid adults and allowed them to lay eggs for about 24 hr at 20°. These adults were removed from the plates and processed for single worm PCR to check that

the worms were homozygous for *unc-93(+)* as described above. This revertant was shown to contain a *sup-9(0)* allele by a complementation test.

Screen for F_2 revertants of sup-9(n1550); sup-18(n1014): We mutagenized *sup-9(n1550); sup-18(n1014)* L4 rubber-band worms with EMS and screened the F_2 progeny for animals with improved motility. We isolated 15 independent wild-type revertants from the progeny of about 7,320 F_1 progeny screened, which corresponds to 14,640 haploid genomes, since each F_1 animal had two haploid genomes. Complementation tests with these new mutations were done as described below.

Screen for F_1 revertants of sup-9(n1550); sup-18(n1014): We mutagenized a population of *sup-9(n1550); sup-18(n1014)* worms containing about 55% L4 and young adult hermaphrodites with EMS and screened the F_1 progeny for animals with improved motility. We isolated 25 wild-type revertants by screening about 1.65×10^6 F_1 progeny (of L4 and young adult P0 hermaphrodites). The number of haploid genomes screened is difficult to estimate. Animals younger than L4 larvae have fewer germ-line precursor cells than do L4 larvae and young adults (KIMBLE and HIRSH 1979). Older adult animals have germ-line cells that are generated before the EMS mutagenesis treatment. We screened a minimum of about 3.3×10^6 haploid genomes based on the progeny of only the L4 and young adult EMS-mutagenized animals. We divided the worm population used in this experiment into four separate pools one generation before the P0 generation, so that a spontaneous recessive suppressor mutation occurring in the parents of the P0 generation would be limited to one of the four pools. We used this strategy because it is difficult to avoid having animals either homozygous or heterozygous for a spontaneous recessive suppressor within a large population of worms. Because the mutations that were isolated are recessive suppressors of *sup-9(n1550)*, the independence of mutations from the same pool is uncertain. Worms homozygous for recessive mutations might not be expected to be recovered in the F_1 generation following a mutagenesis; thus, these mutations might have been present in the P0 generation before the mutagenesis. The following sets of mutations isolated in this experiment might not be independent: *unc-93(n2338* and *n2339)*, *sup-9(n2343, n2349,* and *n2350)*, *sup-9(n2344, n2345, n2357,* and *n2358)*, *sup-9(n2346, n2347, n2348, n2355,* and *n2356)*, *sup-9(n2351, n2352, n2353,* and *n2354)* and *sup-9(n2360* and *n2361)*. In particular, we tested the *sup-9* partial loss-of-function alleles, *n1550 n2360* and *n1550 n2361*, in every construction done and in every case they behaved identically (see RESULTS). In some sections of this paper, we report the results only for *n1550 n2360*.

Screen for F_1 revertants of sup-10(n983): We mutagenized a population of *sup-10(n983)* worms containing mostly L4 hermaphrodites with EMS and screened the F_1 progeny for animals with improved motility. From four experiments, we isolated only one dominant suppressor, *n1435*. A total of about 1.05×10^6 F_1 progeny were screened, which corresponds to about 2.1×10^6 haploid genomes.

Screen for F_2 revertants of sup-9(n1435); unc-93(e1500): We mutagenized rubber-band *sup-9(n1435); unc-93(e1500)* L4 worms with EMS and screened the F_2 progeny for wild-type revertants. We isolated 10 independent wild-type revertants from the progeny of about 5,880 F_1 worms screened, which corresponds to about 11,760 haploid genomes. Seven of the 10 were *sup-9(0)* mutations based on complementation tests. To determine whether the activity of *n1435* as a dominant suppressor of *sup-10(n983)* had been eliminated, we tested three of these *sup-9(0)* alleles, *sup-9(n1435 n2276)*, *sup-9(n1435 n2279)* and *sup-9(n1435 n2281)*. We crossed wild-type males with *sup-9(0); unc-93(e1500)* revertant hermaphrodites to generate *sup-9(0)/+; unc-93(e1500)/+* males. We crossed these males with *sup-10(n983)* hermaphrodites and showed that all the male progeny had a moderate rubber-band phenotype, like that of *sup-10(n983)* worms, and none of the male progeny had a weak rubber-band phenotype, like that of *sup-9(n1435)/+; sup-10(n983)* worms. Thus, the *sup-9(0)* mutations in all three revertants abolished the *n1435* dominant suppressor activity.

Complementation tests: *Complementation tests for revertants of sup-9(n1550)/+:* For the two hermaphrodite revertants, hermaphrodites of putative genotype *sup-9(0); unc-93(e1500)* were crossed with *sup-9(n180); unc-93(e1500)* males, and only phenotypically wild-type progeny were produced. For the male revertant, hermaphrodites of putative genotype *sup-9(0); sup-10(n983)* were crossed with *sup-9(n180); unc-93(e1500)* males, and all males produced were wild-type in phenotype.

Complementation tests for revertants of sup-9(n1550); sup-18(n1014): We crossed revertant hermaphrodites with males of the following three tester strains: (1) *sup-10(n183)*, (2) *unc-93(e1500 n234); him-5* and (3) wild-type. Revertants containing a *sup-10(0)* mutation generated phenotypically wild-type male progeny from all three crosses (because *sup-10* is *X*-linked), wild-type hermaphrodite cross progeny from the first cross and rubber-band progeny from the second and third crosses. Revertants containing an *unc-93(0)* mutation generated phenotypically wild-type progeny from the second cross and rubber-band progeny from the first and third crosses. Revertants containing a *sup-9(0)* or *sup-9(p)* mutation (p indicates a partial loss-of-function mutation; see below) generated phenotypically wild-type progeny from all three crosses. To show the *sup-9(0)* revertants were *sup-9* alleles, we constructed *sup-9(0); unc-93(e1500)* double mutants. (The presence of *sup-18(n1014)* in these double mutants was not determined and is not relevant to these experiments, because *sup-18(0)* mutations are only weak suppressors of *unc-93(e1500)*.) We crossed these *sup-9(0); unc-93(e1500)* hermaphrodites with *sup-9(n180); unc-93(e1500)* males and with *unc-93(e1500); sup-10(n183)* males. The first cross produced phenotypically wild-type cross progeny, and the second cross produced rubber-band cross progeny.

Three *sup-9(p)* mutations generated in our screen for wild-type revertants in the F_1 progeny of EMS-mutagenized *sup-9(n1550); sup-18(n1014)* worms behaved abnormally in the complementation test described above. When we crossed wild-type males with these revertant hermaphrodites, the cross progeny had a wild-type phenotype, suggesting that these revertants contained either an intragenic *sup-9* mutation that inactivates *n1550* or a dominant extragenic suppressor. To distinguish between these possibilities, we crossed *dpy-9/+* males with revertant hermaphrodites and looked for Unc worms among the progeny of F_1 cross progeny. (F_1 cross progeny were identified as animals that segregated Dpy progeny.) In the case of an unlinked dominant suppressor, some of the F_2 progeny would be Unc, whereas in the case of an intragenic *sup-9* mutation, none of the F_2 progeny would be Unc. We found no Unc progeny among 564, 1,497 and 1,574 F_2 progeny for *n2359*, *n2360* and *n2361*, respectively. Thus, these three mutations are tightly linked to *n1550* and are likely to be *sup-9* intragenic revertants.

To show the *sup-9(p)* revertants, *n1550 n2359*, *n1550 n2360* and *n1550 n2361*, contained *sup-9* intragenic mutations, we constructed *sup-9(p); sup-10(n983)* double mutants. We crossed these hermaphrodites with *sup-9(n180)* males and found that all the males produced had a wild-type phenotype. For *n2288*, we crossed *sup-9(n1550 n2288); sup-*

18(n1014) worms with *sup-9(n180)* males to generate *sup-9(n1550 n2288)/sup-9(n180); sup-18(n1014)/+* males. We crossed these males with *sup-9(n1009); sup-10(n983)* hermaphrodites and found that all the males produced had a wild-type phenotype, indicating that *n2288* is a *sup-9* allele.

For *sup-11(n2298)*, we noticed a scrawny phenotype similar to that seen for worms homozygous for *sup-11(d)* mutations (GREENWALD and HORVITZ 1982) and tested for failure to complement a *sup-11(d)* mutation for this recessive phenotype. We crossed *sup-11(n711)/+* males with revertant hermaphrodites of the putative genotype *sup-11(n2298); sup-9(n1550); sup-18(n1014)* and found that about one half of the cross progeny males were scrawny.

Complementation tests for revertants of sup-9(n1435); unc-93(e1500): We crossed revertant hermaphrodites with males of the following three tester strains: (1) *sup-9(n180); unc-93(e1500)*, (2) *unc-93(e1500); sup-10(n183)* and (3) *unc-93(e1500 n234); him-5*, which contain *sup-9(0)*, *sup-10(0)* and *unc-93(0)* mutations, respectively. Revertants containing a *sup-9(0)* mutation generated phenotypically wild-type progeny from the first cross and rubber-band progeny from the second and third crosses. Revertants containing an *unc-93(0)* mutation generated phenotypically wild-type progeny from the third cross and rubber-band progeny from the first and second crosses. Although no *sup-10(0)* mutations were isolated, revertants containing a *sup-10(0)* mutation would have generated phenotypically wild-type hermaphrodite progeny from the second cross and rubber-band hermaphrodite progeny from the first and third crosses; male progeny generated from all three crosses would have been phenotypically wild-type because *sup-10* is *X*-linked.

Genetic mapping: *Mapping of sup-9(n1550):* We used two-factor mapping to show that *n1550* maps to the same region of LG II as *sup-9*. From heterozygotes of the genotype *n1550 +/+ lin-31*, we picked 114 rubber-band non-Lin animals and nine of the 114 animals segregated only non-Lin animals (genotype *n1550 +/+ +*). The recombination frequency between *n1550* and *lin-31* is calculated as $P = 9/114 = 7.9\%$. From heterozygotes of the genotype *n1550 +/+ lin-31*, we picked all the nonrubber-band progeny and found 63 Lin animals (genotype *+ lin-31/+ lin-31*) and 15 non-Lin animals (genotype *+ +/+ lin-31*). The recombination frequency between *n1550* and *lin-31* is calculated as $P = 15/(2 \times 78) = 9.6\%$.

To further map *n1550*, we constructed *sup-9(n1550)/nDf3* heterozygotes. The deficiency *nDf3* deletes part of LG II including *sup-9* and *lin-31* (GREENWALD and HORVITZ 1980). If *n1550* maps to the same region as *sup-9*, the phenotype of *sup-9(n1550)/nDf3* worms should probably be similar to the strong-severe rubber-band phenotype (see RESULTS for the classification of rubber-band phenotypes) of *sup-9(n1550)/sup-9(0)* worms. We crossed wild-type males with single *nDf3/lin-31 bli-2* hermaphrodites, so that half the F_1 cross progeny males were expected to have the genotype *nDf3/+*. We crossed 10 of these F_1 males individually with *sup-9(n1550)/+* hermaphrodites. Seven of these F_1 males mated successfully, based on the production of F_2 male progeny. For six of the seven matings, we picked eight F_2 hermaphrodites with a strong-severe rubber-band phenotype. We picked F_2 hermaphrodites younger than the oldest males to increase our odds of picking cross-progeny. Two of the six F_1 males carried the *lin-31 bli-2* chromosome, because some of their F_2 hermaphrodites segregated Lin Bli nonrubber-band F_3 progeny. The other four F_1 males probably carried the *nDf3* chromosome, because none of their F_2 hermaphrodites segregated Lin Bli progeny. These F_2 progeny either segregated some wild-type progeny or all rubber-band progeny. The latter class was too sick and produced too few progeny to maintain as a viable strain. These worms are likely to have been of the genotype *sup-9(n1550)/nDf3*.

Two-factor mapping of sup-9(dsp) mutations: To show that the dominant-suppressor mutation (dsp) mutations *n1435* and *n242* map very close to *sup-9*, we constructed the following strains and looked for recombinants between *sup-9(0)* alleles, *n180* or *n1553*, and the putative *sup-9(dsp)* mutations. We crossed *sup-9(n1435); sup-10(n983)* males with *sup-9(n1553) dpy-10; sup-10(n983)* hermaphrodites to generate *sup-9(n1435) +/sup-9(n1553) dpy-10; sup-10(n983)* F_1 hermaphrodites. From these hermaphrodites, we screened the F_2 progeny for recombinants of a *sup-9(+)/sup-9(n1553); sup-10(n983)* genotype, which would have a rubber-band phenotype. We found no rubber-band worms among 3,177 F_2 progeny (recombination frequency $P < 0.063\%$), indicating tight linkage between *n1435* and *sup-9(n1553)*. Similarly for *n242*, we crossed *sup-9(n242); unc-93(e1500)* males with *sup-9(n180) dpy-10; unc-93(e1500)* hermaphrodites to generate *sup-9(n242) +/sup-9(n180) dpy-10; unc-93(e1500)* F_1 hermaphrodites. We found no rubber-band worms among 5,842 F_2 progeny (recombination frequency $P < 0.034\%$), indicating tight linkage between *n242* and *sup-9(n180)*.

Strain constructions: In general, double mutant combinations were constructed using balancer mutations in *trans* when necessary. Some constructions were checked by complementation tests or, as described above, by PCR analysis. If a mutation caused no visibly abnormal phenotype in a given strain, we checked that the mutation was present by crossing the mutation into a genetic background in which the mutation causes a phenotype that can be scored. For example, *sup-9(n242)* causes no visibly abnormal phenotype alone, but by crossing *sup-9(n242)* males with *sup-10(n983)* hermaphrodites, we generated nearly wild-type male cross progeny and thereby demonstrated the presence of a dominant suppressor in the *sup-9(n242)* strain. The heterozygous strains with phenotypes we scored in this work (listed in Table 3) were constructed so that cross progeny could be distinguished from self progeny. To identify cross progeny, we sometimes used hermaphrodite parents with an additional recessive marker mutation, such as a dumpy mutation, so that the cross progeny did not have the marker phenotype. In other crosses, cross progeny were shown to be present by the presence of male progeny. Because hermaphrodites do not usually produce self-progeny males, male progeny are generally cross progeny; hermaphrodite progeny of the same age as male progeny are often cross progeny as well. Table 3 lists the full genotypes of the heterozygous strains used in this study, the male and hermaphrodite parents, and the methods used to identify cross progeny.

Construction of sup-11(d); sup-9(n1550) double mutants: We tested the interaction of the rubber-band *sup-9(n1550)* mutation with all 10 existing *sup-11(d)* mutations, nine isolated as dominant suppressors of *unc-93(e1500)* (GREENWALD and HORVITZ 1982) and one isolated by us as a recessive suppressor of *sup-9(n1550); sup-18(n1014)*. The *sup-11(n628)* mutation is lost and could not be tested. The inviability of *sup-9(n1550)* homozygotes and the extreme sickness, low brood size and locomotory defects associated with the *sup-11(d)* scrawny phenotype complicated these constructions and led us to use two different approaches. In the first approach, we picked scrawny weak-moderate rubber-band progeny from worms heterozygous for the *sup-11(d)* mutation and the *sup-9(n1550)* mutation. The scrawny rubber-band worms that segregated no nonrubber-band worms had the genotype *sup-11(d); sup-9(n1550)*. (A worm of genotype *sup-9(n1550)/+* would have segregated one quarter nonrub-

TABLE 3

Crosses used to generate heterozygotes

	Heterozygote	Male parent	Hermaphrodite parent	Identification of cross progeny
A	*sup-9(n1550) lin-31/sup-9(n180) +*	*sup-9(n180)*	*sup-9(n1550) lin-31/+ lin-31*	non-Lin progeny; new phenotype[a]
	sup-9(n1550)/nDf3	*nDf3/+*	*sup-9(n1550)/+*	Rubber-band prog. seg. only rubber-band prog.[b]
	unc-93(e1500)/unc-93(e1500 n234); him-5/+	*unc-93(e1500 n234); him-5*	*unc-93(e1500)*	progeny that seg. non-Rubber-band
	sup-10(n983)/sup-10(n183)	*sup-10(n183)*	*sup-10(n983)*	progeny that seg. non-Rubber-band
	unc-93(e1500)/unc-93(e1500 n234); him-5/+; sup-10(n983)/sup-10(n183)	*unc-93(e1500 n234); him-5; sup-10(n983)*	*unc-93(e1500); sup-10(n183)*	progeny that seg. Rubber-band
	sup-9(n1550)/sup-9(n180); unc-93(e1500) +/unc-93(e1500 n1415) dpy-17	*sup-9(n180); unc-93(e1500)*	*sup-9(n1550); unc-93(e1500 n1415) dpy-17*	non-Dpy progeny; new phenotype
	sup-9(n1550)/sup-9(n180); unc-93(n200) + /unc-93(e1500 n1415) dpy-17	*sup-9(n180); unc-93(n200)*	*sup-9(n1550); unc-93(e1500 n1415) dpy-17*	non-Dpy progeny; new phenotype
	sup-9(n1550)/sup-9(n180); + sup-10(n983)/lin-15 sup-10(e2127)	*sup-9(n180); sup-10(n983)*	*sup-9(n1550); lin-15 sup-10(e2127)*	non-Lin progeny; new phenotype
B	*sup-9(n1550 n2359)/sup-9(n180); unc-93(e1500)*	*sup-9(n180); unc-93(e1500)*	*sup-9(n1550 n2359); unc-93(e1500)*	male progeny
	sup-9(n1550 n2359)/+; unc-93(e1500)	*unc-93(e1500); sup-10(n183)*	*sup-9(n1550 n2359); unc-93(e1500)*	male progeny
	sup-9(n1550 n2360)/sup-9(n180); unc-93(e1500)	*sup-9(n180); unc-93(e1500)*	*sup-9(n1550 n2360); unc-93(e1500)*	male progeny
	sup-9(n1550 n2360)/+; unc-93(e1500)	*unc-93(e1500); sup-10(n183)*	*sup-9(n1550 n2360); unc-93(e1500)*	male progeny
	+ sup-9(n180) +/lin-42(n1089) + lin-31(n301); unc-93(e1500)	*sup-9(n180); unc-93(e1500)*	*lin-42(n1089) lin-31(n301); unc-93(e1500)*	male progeny
	sup-9(n1550 n2359)/sup-9(n1550)	*sup-9(n1550 n2359); sup-10(n983)*	*sup-9(n1550)/+*	male progeny[c]
	sup-9(n1550 n2360)/sup-9(n1550)	*sup-9(n1550 n2360); sup-10(n983)*	*sup-9(n1550)/+*	male progeny[c]
C	*sup-9(n1435)/sup-9(n242)*	*sup-9(n1435)*	*sup-9(n242)*	males and hermaphrodites of same age[d]
	sup-9(n1435) +/sup-9(n1553) dpy-10; sup-10(n983)	*sup-9(n1435); sup-10(n983)*	*sup-9(n1553) dpy-10; sup-10(n983)*	non-Dpy progeny
	sup-9(n1435)/+; dpy-17/+; sup-10(n983)	*sup-9(n1435); sup-10(n983)*	*dpy17; sup-10(n983)*	non-Dpy progeny
	sup-9(n242) +/sup-9(n1553) dpy-10; sup-10(n983)	*sup-9(n242); sup-10(n983)*	*sup-9(n1553) dpy-10; sup-10(n983)*	non-Dpy progeny
	sup-9(n242)/+; dpy-17/+; sup-10(n983)	*sup-9(n242); sup-10(n983)*	*sup-9(n1435); dpy-17; sup-10(n983)*	non-Dpy progeny
	sup-9(n242)/sup-9(n1435); dpy-17/+; sup-10(n983)	*sup-9(n242); sup-10(n983)*	*sup-9(n1435); dpy-17; sup-10(n983)*	non-Dpy progeny
	sup-9(n1435)/sup-9(n180); unc-93(e1500)	*sup-9(n180); unc-93(e1500)*	*sup-9(n1435); unc-93(e1500)*	males and hermaphrodites of same age[d]
	sup-9(n1435)/+; unc-93(e1500)	*unc-93(e1500); sup-10(n183)*	*sup-9(n1435); unc-93(e1500)*	male progeny
	sup-9(n242)/sup-9(n180); unc-93(e1500); dpy-11/+	*sup-9(n180); unc-93(e1500)*	*sup-9(n242); unc-93(e1500); dpy-11*	non-Dpy progeny
	sup-9(n242)/+; unc-93(e1500); dpy-11/+	*unc-93(e1500); sup-10(n183)*	*sup-9(n242); unc-93(e1500); dpy-11*	male progeny
	sup-9(n242)/sup-9(n1435); unc-93(e1500)	*sup-9(n242); unc-93(e1500)*	*sup-9(n1435); unc-93(e1500)*	males and hermaphrodites of same age; new phenotype[d,e]
	sup-9(n242) +/sup-9(n1550) lin-31	*sup-9(n242)*	*sup-9(n1550) lin-31/+ lin-31*	non-Lin progeny[f]
	sup-9(n1435) +/sup-9(n1550) lin-31	*sup-9(n1435)*	*sup-9(n1550) lin-31/+ lin-31*	non-Lin progeny[f]
D	*unc-93(e1500 n234)/+; him-5/+; sup-10(n983)*	*unc-93(e1500 n234); him-5*	*sup-10(n983)*	male progeny
	sup-9(n180)/+; sup-10(n983)	*sup-9(n180)*	*sup-10(n983)*	male progeny
	sup-18(n1010)/+; sup-10(n983)	*sup-18(n1010)*	*sup-10(n983)*	male progeny
	sup-9(n180)/+; unc-93(e1500) dpy-17/++	*sup-9(n180)*	*unc-93(e1500) dpy-17*	non-Dpy progeny
	sup-93(e1500) dpy-17/++; sup-10(n183)/+	*sup-10(n183)*	*unc-93(e1500) dpy-17*	non-Dpy progeny
	unc-93(e1500) dpy-17 +/++ sup-18(n1010)	*sup-18(n1010)*	*unc-93(e1500) dpy-17*	non-Dpy progeny
	sup-9(n180)/+; unc-93(e1500)	*sup-9(n180); unc-93(e1500)*	*unc-93(e1500) dpy-17*	non-Dpy progeny
	unc-93(e1500); sup-10(n183)/+	*unc-93(e1500); sup-10(n183)*	*unc-93(e1500) dpy-17*	non-Dpy progeny

(A) Heterozygotes mentioned in the section entitled "The *sup-9(n1550)* rubber-band mutation."
(B) Heterozygotes mentioned in the section entitled "Partial loss-of-function *sup-9*-alleles."
(C) Heterozygotes mentioned in the section entitled "*sup-9* dominant-suppressor alleles."
(D) Heterozygotes mentioned in the section entitled "Semidominant suppression of *unc-93(e1500)* and *sup-10(n983)*."
[a] Half the cross progeny contain one copy of *sup-9(n1550)* and have a strong-severe rubber-band phenotype.
[b] See MATERIALS AND METHODS for details.
[c] Half the male cross progeny contain one copy of *sup-9(n1550)* and have a rubber-band phenotype.
[d] Males are cross progeny, and most hermaphrodites of the same age are likely to be cross progeny as well.
[e] Cross progeny have a wild-type phenotype, whereas self progeny have a moderate rubber-band phenotype.
[f] Half the cross progeny contain one copy of *sup-9(n1550)* and have a strong rubber-band phenotype.

ber-band progeny.) In the second approach, we picked the strongest rubber-band (strong-severe and severe) nonscrawny progeny from worms heterozygous for the *sup-11(d)* mutation and the *sup-9(n1550)* mutation. The rubber-band worms that segregated only rubber-band progeny were homozygous for *sup-9(n1550)*, and those that segregated scrawny weak-moderate rubber-band worms were heterozygous for *sup-11(d)*. For all the *sup-11(d)* alleles except *n402*, the *sup-11(d); sup-9(n1550)* worms had a similar weak-moderate rubber-band phenotype in addition to the scrawny phenotype. The construction of *sup-11(n402); sup-9(n1550)* was not completed, but we did find scrawny weak-moderate rubber-band worms that segregated both nonrubber-band and rubber-band progeny, which is consistent with a genotype of *sup-11(n402); sup-9(n1550)/+*. The failure of *sup-11(n402)* to completely suppress the rubber-band phenotype of *sup-9(n1550)* is identical to what we have observed for the other nine *sup-11(d)* mutations.

Attempted construction of sup-9(n1550 n2288); unc-93(e1500) animals: We attempted to construct *sup-9(0); unc-93(e1500)* strains for all the *sup-9* mutations generated from mutageneses of *sup-9(n1550); sup-18(n1014)* worms. These constructions yielded phenotypically wild-type strains, *i.e.*, full suppression of the rubber-band phenotype of *unc-93(e1500)*, for all of the *sup-9(0)* revertants but not for the *sup-9(p)* revertants. In the case of the putative *sup-9(p)* allele, *sup-9(n1550 n2288)*, we were unable to construct a phenotypically wild-type strain of genotype *sup-9(n1550 n2288); unc-93(e1500)*. We attempted to construct worms of genotype *sup-9(n1550 n2288); unc-93(e1500)* in the following way. We crossed *lin-42 lin-31/+ +* males with *sup-9(n1550 n2288); sup-18(n1014)* hermaphrodites, so that half of the male progeny were of genotype *+ sup-9(n1550 n2288) +/ lin-42 + lin-31; sup-18(n1014)/+*. We crossed the male cross progeny with *lin-42 lin-31; unc-93(e1500)* hermaphrodites and picked eight non-Lin-42 non-Lin-31 nonrubber-band F_1 hermaphrodites. Each of these F_1 hermaphrodites had a two-thirds probability of being heterozygous for *sup-9(n1550 n2288)*. From the F_2 self progeny of each F_1 hermaphrodite, we picked eight rubber-band non-Lin-31 non-Lin-42 hermaphrodites. If an F_1 hermaphrodite were heterozygous for *sup-9(n1550 n2288)* and if *sup-9(n1550 n2288)* were a typical *sup-9(0)* revertant, there would be a two-thirds probability that each rubber-band non-Lin-31 non-Lin-42 F_2 progeny picked would be heterozygous for *sup-9(n1550 n2288)* and would generate phenotypically wild-type progeny. In the F_3 progeny, we found wild-type progeny from only one of the eight F_1-derived groups of F_2 progeny, and these wild-type progeny did not seem to be of genotype of *sup-9(0); unc-93(e1500)*. These wild-type hermaphrodites were crossed with *unc-93(e1500); sup-10(n183)* males and the resulting cross-progeny were not as rubber-band as would be expected for worms of genotype *sup-9(0)/ +; unc-93(e1500); sup-10(n183)/+*. (The presence of *sup-18(n1014)/+* in these worms would not explain the suppression either.) From this experiment in which *sup-9(n1550 n2288)* did not act as would have been expected if it were a *sup-9(0)* allele, it seems most likely that *sup-9(n1550 n2288); unc-93(e1500)* worms did not have a wild-type phenotype, and we tentatively assign *n2288* as a *sup-9(p)* mutation (see below).

RESULTS

Classification of rubber-band phenotypes: In this paper, we use the following classification system to describe the severity of the rubber-band phenotype, which includes defects in movement, body shape and egg laying (GREENWALD and HORVITZ 1980, 1986). We define four broad classes of rubber-band phenotypes: "weak" corresponds to the phenotype of *unc-93(n200)* animals, "moderate" corresponds to the phenotype of *sup-10(n983)* animals, "strong" corresponds to the phenotype of *unc-93(e1500)* animals and "severe" corresponds to the phenotype of *sup-9(n1550)* (see below) animals (Table 4). *unc-93(n200)* animals move backward and forward fairly well, but in an uneven manner rather than with a wild-type sinusoidal motion; adult hermaphrodites have an egg-laying defect that causes them to become bloated with eggs, although most of the eggs are laid (GREENWALD and HORVITZ 1980). *sup-10(n983)* animals move forward poorly but do move backward well enough to disperse randomly over a bacterial lawn in a Petri plate; adult hermaphrodites have a pronounced egg-laying defect, although they lay some eggs before forming "bags of worms" (GREENWALD and HORVITZ 1986). *unc-93(e1500)* animals move poorly and do not disperse over a bacterial lawn on a Petri plate; adult hermaphrodites do not lay any eggs (GREENWALD and HORVITZ 1980). *sup-9(n1550)* animals usually do not move more than a body-length during their life, never lay eggs, and cannot be maintained as a strain because they grow very slowly, and produce few, if any, progeny. If a phenotype falls between two classes (*e.g.*, weak and moderate), the phenotype is designated as mixed (weak-moderate). These qualitative assessments of the strength of different rubber-band phenotypes allowed us to compare the relative effects of suppressors and enhancers of a given rubber-band mutation.

TABLE 4

Classification of rubber-band phenotypes

Genotype	Phenotype
sup-9(n1550)	Severe rubber-band
unc-93(e1500)	Strong rubber-band
sup-10(n983)	Moderate rubber-band
unc-93(n200)	Weak rubber-band

These distinct rubber-band phenotypes are described in the text.

The *sup-9(n1550)* rubber-band mutation: *Identification and characterization of sup-9(n1550):* A new rubber-band mutation, *n1550*, was isolated by M. HERMAN as a heterozygote in an unrelated screen in which the F_2 self progeny of single F_1 worms were examined (unpublished data). Worms of genotype *n1550/+* have a rubber-band phenotype that is almost as strong as that of *unc-93(e1500)* homozygotes. Worms of genotype *n1550/n1550* cannot be maintained as a strain and have a severe rubber-band phenotype that includes extreme sluggishness, very slow growth, and the production of few, if any, progeny.

We suspected that *n1550* mutation could be an allele of *sup-9* based on the following genetic experi-

ments. First, the map distance between *n1550* and *lin-31* was estimated by two-factor crosses to be about 8–10 map units (see MATERIALS AND METHODS). The distance between *sup-9* and *lin-31* was previously measured as 8.2 map units (FERGUSON and HORVITZ 1985). Second, *sup-9* null mutations *in trans* to *n1550* enhanced the rubber-band phenotype of the *n1550* mutation. In the case of the *unc-93(e1500)* and *sup-10(n983)* rubber-band mutations, the phenotype of "rubber-band/null" worms is more severe than that of "rubber-band/+" worms (GREENWALD and HORVITZ 1980, 1986). Thus, if *n1550* were a *sup-9* allele, *n1550/sup-9(0)* worms might have a more pronounced rubber-band phenotype than that of *n1550/+* worms. Consistent with this hypothesis, worms of genotype *n1550/sup-9(n180)* are extremely sick, move very little, and fail to lay eggs—a strong-severe rubber-band phenotype, which is stronger than that of *n1550/+* animals. We tested the interaction of *n1550* with the large deficiency *nDf3*, which deletes *sup-9* (GREENWALD and HORVITZ 1980), and showed that *nDf3* produced a similar phenotype in *trans* to *n1550* and that *n1550/nDf3* worms could not be maintained as a strain (see MATERIALS AND METHODS). We suspect that this slightly more extreme phenotype might be a consequence of additional sickness associated with *nDf3*, a common feature of deficiency heterozygotes in *C. elegans* (MENEELY and HERMAN 1979; SIGURDSON, SPANIER and HERMAN 1984). These mapping experiments suggest that *n1550* might be an allele of *sup-9*.

To test the hypothesis that *n1550* is an allele of *sup-9*, we generated *cis*-dominant mutations that eliminated the dominant phenotype of *n1550* and showed these new mutations to be *sup-9(0)* alleles (Figure 1). The rationale for this experiment is the assumption that if *n1550* were a *sup-9* allele, a *sup-9(0)* mutation in *cis* would abolish the dominant rubber-band phenotype of *n1550*. If *n1550* were not a *sup-9* allele, a *sup-9(0)* mutation in *cis* would enhance the dominant rubber-band phenotype of *n1550*, as does a *sup-9(0)* mutation in *trans* to *n1550* (Figure 1A and see above). We crossed EMS-mutagenized *n1550; unc-93(0) dpy-17* hermaphrodites with *lin-42/+* males and screened for non-Rubber-band non-Dpy F_1 progeny (Figure 1). The *unc-93(0)* mutation is a complete recessive suppressor of *n1550* (see below). Self progeny from this cross were Dpy non-Rubber-band, and most of the cross progeny were Rubber-band non-Dpy. We isolated three non-Dpy non-Rubber-band revertants from 8,868 haploid genomes screened. Each of these revertants contained a *sup-9(0)* mutation based upon its failure to complement previously identified *sup-9(0)* mutations for suppression of the rubber-band phenotype caused by *unc-93(e1500)* or *sup-10(n983)* (see MATERIALS AND METHODS). Whereas *sup-9(n1550)/sup-9(0)* worms had a rubber-band phenotype, these three revertants with a *sup-9(0)* mutation in *cis* to *n1550* had a wild-type phenotype, indicating that *n1550* is an allele of *sup-9*. This mutagenesis strategy allowed the recovery of *sup-9* mutations that inactivated *n1550* in *cis* without any bias about the phenotype of the homozygotes. Given that deficiencies that delete *sup-9* are viable as heterozygotes (GREENWALD and HORVITZ 1980), this screen had the potential to identify *sup-9* null mutations. Since all three newly identified *sup-9* alleles result in a wild-type phenotype when homozygous, these experiments strongly support the hypothesis (GREENWALD and HORVITZ 1980) that the null phenotype of *sup-9* is wild-type.

Suppression of sup-9(n1550): To test whether mutations that suppress the previously identified rubber-band mutations *unc-93(n200)*, *unc-93(e1500)* and *sup-10(n983)* can also suppress the *n1550* rubber-band mutation, we constructed double mutant combinations with *n1550* (Table 5). Worms of genotypes *sup-9(n1550); unc-93(0) dpy-17* and *sup-9(n1550); lin-15 sup-10(0)* were completely suppressed for all aspects of the rubber-band phenotype. (The *dpy-17* and *lin-15* mutations were used as markers for strain constructions and are not otherwise relevant.) While constructing these strains, we noticed that the suppressors were weakly semidominant, such that *n1550/+; sup/+* worms had a strong rubber-band phenotype that was slightly weaker than that of *n1550/+* worms.

sup-18(0) mutations are recessive complete suppressors of *sup-10(n983)* and recessive partial suppressors of *unc-93(e1500)* (GREENWALD and HORVITZ 1986). Worms of genotype *sup-9(n1550); sup-18(n1014)* had a strong-severe rubber-band phenotype (Table 5). This incomplete suppression of *sup-9(n1550)* by *sup-18(0)* mutations is similar to that of *unc-93(e1500)* by *sup-18(0)* mutations. Given these results, we investigated the interaction of *sup-18(0)* mutations with the weak rubber-band mutation *unc-93(n200)*. Both *unc-93(n200) sup-18(n1010)* worms and *unc-93(n200) sup-18(n1014)* worms displayed a subtle rubber-band phenotype that was less severe than that of *unc-93(n200)* worms, indicating partial suppression by *sup-18*. These results reveal that partial suppression by *sup-18(0)* of *sup-9(n1550)* was not simply a consequence of an inability of *sup-18(0)* to fully suppress a severe rubber-band allele, because the weak rubber-band allele *unc-93(n200)* was also only partially suppressed. Worms of genotype *sup-9(n1550)/+; sup-18(n1014)/+* had a slightly less pronounced rubber-band phenotype than that of worms of genotype *sup-9(n1550)/+*. Thus, *sup-18(0)* mutations are weak semidominant suppressors of *sup-9(n1550)*.

sup-11(d) mutations are dominant suppressors of *unc-93(e1500)* and *unc-93(n200)* and recessive sup-

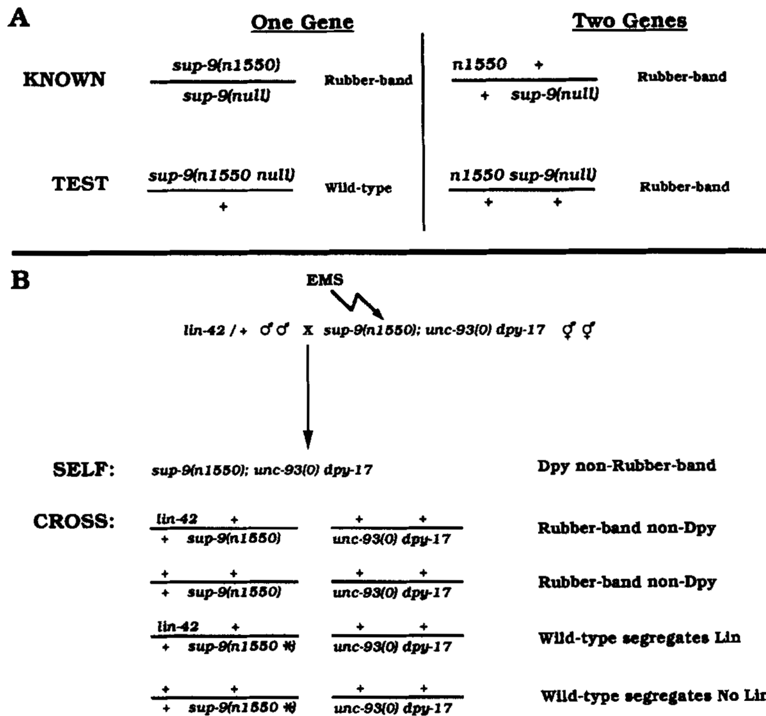

FIGURE 1.—*n1550* is an allele of *sup-9*. (A) A genetic *cis-trans* test. The *n1550* mutation causes a dominant rubber-band phenotype. We have shown that a *sup-9* null mutation in *trans* to *n1550* does not eliminate the *n1550* dominant rubber-band phenotype (Table 6B). If *n1550* is an allele of *sup-9*, a *sup-9* null mutation in *cis* to *n1550* will eliminate the *n1550* dominant rubber-band phenotype (left panel). If *n1550* is not an allele of *sup-9*, a *sup-9* null mutation in *cis* will not eliminate the *n1550* dominant rubber-band phenotype (right panel). We induced a *sup-9* null mutation in *cis* to *n1550* using the mutagenesis scheme shown in (B). (B) We generated *sup-9* null mutations in *cis* to *n1550* and showed that they eliminated the dominant rubber-band phenotype caused by *n1550*. The *lin-42* and *dpy-17* mutations are recessive; the *n1550* mutation is dominant; the *unc-93(0)* mutation is *unc-93(e1500 n1415)* and acts as an essentially recessive suppressor of *n1550*. We screened the F_1 cross progeny as shown for phenotypically wild-type revertants. The asterisk in the genotype of the *sup-9* intragenic revertant indicates a new *sup-9* mutation that eliminates *n1550* activity. Theoretically, half of the revertants will segregate Lin progeny and will be of genotype *lin-42 +/+ sup-9(n1550 *); + +/unc-93(0) dpy-17*. The other half will not segregate Lin progeny and will be of genotype *+ +/+ sup-9(n1550 *); + +/unc-93(0) dpy-17*. For the latter class of revertants, the new *sup-9* mutation could not be recovered because in the absence of the *lin-42* balancer mutation, there is no way to follow the new *sup-9* mutation. We isolated three *sup-9* null mutations from 8,868 haploid genomes screened. See MATERIALS AND METHODS for details.

TABLE 5

Suppression of the *sup-9(n1550)* rubber-band phenotype

Genotype	Phenotype
sup-9(n1550)	Severe rubber-band
sup-9(n1550)/+	Strong rubber-band
sup-9(n1550); unc-93(0)	Wild-type
sup-9(n1550); sup-10(0)	Wild-type
sup-9(n1550); sup-18(0)	Strong-severe rubber-band
sup-11(d); sup-9(n1550)	Weak-moderate rubber-band, scrawny
sup-11(d)/+; sup-9(n1550)	Strong-severe rubber-band

unc-93(e1500 n1415) is the *unc-93(0)* mutation; *sup-10(e2127)* is the *sup-10(0)* mutation. Complete genotypes are listed in the text. Designations indicating the strength of the rubber-band phenotypes are explained in the text and in Table 4. The rubber-band phenotype of *sup-11(d)/+; sup-9(n1550)* worms is stronger than that of *sup-9(n1550); sup-18(0)* worms.

pressors of *sup-10(n983)* (GREENWALD and HORVITZ 1982, 1986). We tested nine *sup-11(d)* mutations, including *sup-11(n710)*, which is a weaker suppressor of *unc-93(e1500)* than the other *sup-11(d)* mutations (GREENWALD and HORVITZ 1982), for their abilities to suppress *sup-9(n1550)* (see MATERIALS AND METHODS). In general, *sup-11(d); sup-9(n1550)* worms have a weak-moderate rubber-band phenotype in addition to the scrawny phenotype associated with the *sup-11(d)* mutation. Worms of genotype *sup-11(d)/+; sup-9(n1550)* had a strong-severe rubber-band phenotype and were viable as a strain. We could not detect any differences in the extent of suppression by the nine *sup-11(d)* mutations, but minor differences were difficult to assess because the *sup-11(d)* mutations cause a slight uncoordinated (nonrubber-band) phenotype that presumably is part of the overall sickness of the scrawny phenotype. Thus, *sup-11(d)* mutations are semidominant, partial suppressors of *sup-9(n1550)* (Table 5). For four *sup-11(d)* alleles (*n403*, *n405*, *n711* and *n2298*), we constructed *sup-11(d); sup-10(n983)* double mutants and showed that these worms had a subtle rubber-band phenotype that was weaker than a weak rubber-band phenotype. Therefore, *sup-11(d)* mutations are partial suppressors of both *n1550* and *n983* and full suppressors of *e1500* and *n200*.

Interactions of sup-9(n1550) with other rubber-band mutations: We determined the interactions of *sup-9(n1550)* with the three previously identified rubber-band mutations. GREENWALD and HORVITZ (1986) showed that *unc-93(e1500); sup-10(n983)* worms have a phenotype that is less severe than either single mutant and that *unc-93(n200); sup-10(n983)* worms have a phenotype that is more severe than either single mutant. Because *sup-9(n1550)* homozygotes have a severe rubber-band phenotype and cannot be maintained as a strain, we employed an alternative strategy using heterozygotes to examine the interactions of *sup-9(n1550)* with the other rubber-band mutations. Our approach is based upon the observation for each of the four rubber-band mutations that the genotypes, in order of increasing severity of phenotype, can be

TABLE 6

Interactions between rubber-band mutations

	Genotype[a]			Rubber-band phenotype		
	unc-93	*sup-10*		Strength[b]	Average brood size[c]	
A	*e1500*			Strong	36 ± 1.8 (*n* = 12)	
	e1500/0			Moderate-strong	45 ± 2.4 (*n* = 11)	
		n983		Moderate	70 ± 7.5 (*n* = 12)	
		n983/0		Weak-moderate	125 ± 12.8 (*n* = 10)	
	e1500/0	*n983/0*		Very weak	273 ± 12.2 (*n* = 4)	
					% viable[d]	
	unc-93	*sup-10*	*sup-9*	Strength[b]	Expt. 1	Expt. 2
B			*n1550/0*	Strong-severe	90 (*n* = 163)	107 (*n* = 60)
	e1500/0		*n1550/0*	Severe	15 (*n* = 461)	9 (*n* = 307)
	n200/0		*n1550/0*	Severe		7 (*n* = 396)
		n983/0	*n1550/0*	Severe	12 (*n* = 78)	

[a] The crosses used to generate the hetrozygotes are listed in table 3; only relevant genotypes are shown here. *sup-9(0)* = *sup-9(n180)*. *sup-10(0)* = *sup-10(n183)* (A) or *sup-10(e2127)* (B). *unc-93(0)* = *unc-93(e1500 n234)* (A) or *unc-93(e1500 n1415)* (B).
[b] Designations indicating the strength of the rubber-band phenotype are explained in the text and in Table 4.
[c] Brood size is one measure of the strength of the rubber-band phenotype. Average brood size, the standard error of the mean, and the number of broods counted (*n*) are listed for worms of these genotypes. Wild-type hermaphrodites have an average brood size of about 330 (HODGKIN, HORVITZ and BRENNER 1979).
[d] The viability of *n1550/0* worms is derived from the number of rubber-band male progeny divided by the number of wild-type male progeny observed when *sup-9(0)* males were crossed with *sup-9(n1550) lin-31/+ lin-31* hermaphrodites. For the last three genotypes in part B, progeny were considered viable if they were L2 larvae or older on day 6 of the cross. The number of progeny examined for each genotype is listed in parentheses. Expt. 1 and Expt. 2 refer to two similar experiments done at different times.

ranked as rubber-band/rubber-band > rubber-band/null > rubber-band/+ (GREENWALD and HORVITZ 1980, 1986; also see above). We constructed worms with genotypes including two different rubber-band mutations such that for each locus the rubber-band mutation was in *trans* to a null mutation, *i.e.*, rubber-band1/null; rubber-band2/null. We found that the phenotype of *unc-93(e1500)/unc-93(e1500 n234); sup-10(n983)/sup-10(n183)* worms was much less severe than that of either *unc-93(e1500)/unc-93(e1500 n234)* alone or *sup-10(n983)/sup-10(n183)* alone (Table 6A); thus, the mutual suppression of *unc-93(e1500)* and *sup-10(n983)* reported by GREENWALD and HORVITZ (1986) was also observed in this experiment. Worms of genotypes *sup-9(n1550)/sup-9(n180); unc-93(e1500)/unc-93(e1500 n1415)* or *sup-9(n1550)/sup-9(n180); unc-93(n200)/unc-93(e1500 n1415)* or *sup-9(n1550)/sup-9(n180); sup-10(n983)/sup-10(n183)* had more severe phenotypes than do *sup-9(n1550)/sup-9(n180)* worms (Table 6B). Thus, the *sup-9(n1550)* rubber-band phenotype was enhanced by rubber-band mutations in *unc-93* or *sup-10*. All of these double rubber-band mutants had a severe rubber-band phenotype that varied in expressivity between dead three-fold embryos and extremely sick and uncoordinated adults (data not shown; also see Table 6). (Normal three-fold embryos are late-stage embryos that are undergoing morphogenesis after completing cell proliferation; SULSTON *et al.* 1983; WOOD 1988.) Thus, the interaction of *sup-9(n1550)* with the other three rubber-band mutations was similar to that seen between *unc-93(n200)* and *sup-10(n983)*, consistent with there being independent or additive effects of the two rubber-band mutations.

The arrest stage of these double rubber-band mutant embryos coincides with the time of muscle differentiation and the inception of muscle contraction. To assay muscle function in these mutant embryos, we identified 10 *sup-9(n1550)/sup-9(n180); unc-93(n200)/unc-93(e1500 n1415)* embryos (see Table 3) and examined them using Nomarski differential interference contrast optics. Wild-type embryos stop moving at the end of elongation and resume movement by the time pharyngeal pumping starts (SULSTON *et al.* 1983; WOOD 1988). These mutant embryos developed normally through most of embryogenesis, including the elongation stage, which involves movements controlled by the body-wall muscle cells (SULSTON *et al.* 1983). Late in embryogenesis, the mutant embryos had weak and infrequent pharyngeal pumping and were almost totally paralyzed. This severe rubber-band phenotype differs from the Pat (*P*aralyzed and *A*rrested at *T*wo-fold) phenotype, in which muscle-defective embryos are paralyzed, fail to elongate and arrest at the two-fold stage (WATERSTON 1989; BARSTEAD and WATERSTON 1991). Thus, this severe rubber-band phenotype does not completely disrupt the functioning of body-wall muscle.

Reversion of sup-9(n1550): To determine if genes in addition to *sup-9*, *sup-10*, *sup-11*, *sup-18* and *unc-93* can mutate to suppress the rubber-band phenotype of *sup-9(n1550)*, we screened for suppressors of *n1550*.

We mutagenized worms of genotype *sup-9(n1550); sup-18(n1014)*, which are rubber-band and viable (see above). The *sup-18(n1014)* mutation was included because *sup-9(n1550)* homozygotes cannot be maintained as a strain. We isolated 15 independent nonrubber-band revertants from the F_2 progeny of EMS-mutagenized *sup-9(n1550); sup-18(n1014)* worms. Reversion events occurred at a frequency of about 1×10^{-3} per haploid genome, which is similar to the frequency previously seen for reversion of *unc-93(e1500)* worms and *sup-10(n983)* worms (GREENWALD and HORVITZ 1980, 1986). Using complementation tests, we assigned all 15 revertants to known genes (see MATERIALS AND METHODS). We found seven *sup-9(0)* mutations, three *sup-10(0)* mutations, three *unc-93(0)* mutations, one *sup-11(d)* mutation and one additional putative *sup-9* allele, *n2288* (see below) (Table 2). The *sup-11(d)* mutation *n2298* had the properties of previously isolated *sup-11(d)* mutations (GREENWALD and HORVITZ 1982, 1986), including a recessive scrawny phenotype and the same pattern of suppression of the *sup-9(n1550)*, *sup-10(n983)*, and *unc-93(e1500)* mutations (data not shown). *sup-11(n2298)* was isolated as a revertant with completely wild-type movement as a consequence of the combined suppressor activities of *sup-11(n2298)* and *sup-18(n1014)*, two partial suppressors of *sup-9(n1550)*.

To identify other types of mutations that could interact with *sup-9(n1550)*, we performed a similar mutagenesis experiment to find dominant suppressors of *sup-9(n1550)*. We screened the F_1 progeny of EMS-mutagenized *sup-9(n1550); sup-18(n1014)* worms for nonrubber-band revertants (see MATERIALS AND METHODS). From a minimum of about 3.3×10^6 haploid genome equivalents, we isolated 25 wild-type revertants. All of these revertants contained recessive, rather than dominant, suppressors of *sup-9(n1550)*. Based on the results of complementation tests, there were two *sup-10(0)* mutations, four *unc-93(0)* mutations, 16 *sup-9(0)* mutations and three other putative *sup-9* mutations (*n2359*, *n2360* and *n2361*; see below).

Partial loss-of-function *sup-9* alleles: In our screen for wild-type F_1 revertants of EMS-mutagenized *sup-9(n1550); sup-18(n1014)* worms (see above), we found three mutations, *n2359*, *n2360* and *n2361*, that behaved differently from null mutations in *unc-93*, *sup-9* and *sup-10* (see MATERIALS AND METHODS). These mutations proved to be partial loss-of-function *sup-9* alleles that acted anomalously when combined with the altered-function rubber-band alleles. These *sup-9* alleles contain second-site mutations that seem to have reduced the altered function of *n1550*, but have not eliminated all *sup-9* function. These alleles did not consistently suppress or enhance the rubber-band phenotype. In some cases, one allele enhanced the phenotype of a rubber-band mutation, whereas the other allele suppressed the *same* rubber-band mutation. As discussed below, these data suggest that the Sup-9, Sup-10 and Unc-93 proteins physically interact in a protein complex and do not support a model in which each of these proteins independently contributes to the rubber-band phenotype. The interactions of *sup-9(n1550 n2359)* and *sup-9(n1550 n2360)* with the different rubber-band alleles are summarized in Table 7. It is possible that *n2360* and *n2361* are nonindependent isolates of the same mutation (see MATERIALS AND METHODS); in all cases tested, *sup-9(n1550 n2360)* and *sup-9(n1550 n2361)* behaved identically, and we report here only the results for *sup-9(n1550 n2360)*.

TABLE 7

Summary of *sup-9(p)* interactions with rubber-band alleles

Rubber-band allele	*sup-9(n1550 n2359)*	*sup-9(n1550 n2360)*
sup-10(n983)	Partial suppressor	Partial suppressor
unc-93(e1500)	Enhancer	Enhancer
unc-93(n200)	Enhancer	No effect
sup-9(n1550)	Enhancer	Suppressor

These designations are explained in the text and in Table 8.

To test whether *sup-9(n1550 n2359)* and *sup-9(n1550 n2360)* are *sup-9(0)* alleles, we constructed multiple mutants containing either *n1550 n2359* or *n1550 n2360* and *sup-10(n983)*. These strains displayed a subtle rubber-band phenotype weaker than the weak rubber-band phenotype, indicating an incomplete suppression of the rubber-band phenotype of *sup-10(n983)* (Table 8A). We showed that the mutations in these revertants failed to complement a *sup-9(0)* mutation for suppression of *sup-10(n983)*. Therefore, these strains are *sup-9* intragenic revertants. These intragenic alleles alone caused a wild-type phenotype (Table 8A). From these experiments, we hypothesize that these revertants contain partial loss-of-function or "*sup-9(p)*" mutations, because although these mutations appeared to inactivate in *cis* the *sup-9* function required by *sup-9(n1550)* to cause its rubber-band phenotype, they inactivated only partially the *sup-9* function required by *sup-10(n983)* to cause its rubber-band phenotype.

Although these *sup-9(p)* alleles partially suppressed the effects of the *sup-10(n983)* rubber-band mutation, they enhanced the effects of the *unc-93(e1500)* rubber-band mutation (Table 8A). *sup-9(n1550 n2359); unc-93(e1500)* worms had a strong rubber-band phenotype that was slightly stronger than that of *unc-93(e1500)* worms based on uncoordinated movement. *sup-9(n1550 n2360); unc-93(e1500)* worms had a strong-severe rubber-band phenotype that includes being more uncoordinated, having a lower brood size, and being generally sicker than *unc-93(e1500)* worms. Because *sup-9(n1550)* enhanced the *unc-93(e1500)* rubber-band phenotype (see above), it is possible that these *sup-9(p)* alleles have residual *n1550* activity that

TABLE 8

***sup-9(p)* alleles can enhance or suppress rubber-band phenotypes**

A *sup-9* allele	Rubber-band mutation			
	Alone	*sup-10(n983)*	*unc-93(e1500)*	*unc-93(n200)*
sup-9(+)	Wild-type	Moderate rubber-band	Strong rubber-band	Weak rubber-band
sup-9(0)	Wild-type	Wild-type	Wild-type	Wild-type
sup-9(n1550 n2359)	Wild-type	Very weak rubber-band	Strong rubber-band[a]	Moderate rubber-band
sup-9(n1550 n2360)	Wild-type	Very weak rubber-band	Strong-severe rubber-band	Weak rubber-band

B Genotype	Phenotype[b]
sup-9(n1550 n2359)/sup-9(0); unc-93(e1500)	Weak-moderate rubber-band
sup-9(n1550 n2360)/sup-9(0); unc-93(e1500)	Strong-severe rubber-band
sup-9(n1550 n2359)/+; unc-93(e1500)	Strong rubber-band
sup-9(n1550 n2360)/+; unc-93(e1500)	Strong rubber-band[c]
sup-9(0)/+; unc-93(e1500)	Strong rubber-band

For *p* = *n1550 n2359*: *sup-9(p); e1500* > *e1500* = *sup-9(0)/+; e1500* = *sup-9(p)/+; e1500* > *sup-9(p)/sup-9(0); e1500*

For *p* = *n1550 n2360*: *sup-9(p); e1500* = *sup-9(p)/sup-9(0); e1500* > *sup-9(p)/+; e1500* > *sup-9(0)/+; e1500* = *e1500*

C Genotype	Phenotype[d]
sup-9(n1550 n2359)/sup-9(n1550)	Strong-severe rubber-band
sup-9(n1550 n2360)/sup-9(n1550)	Moderate-strong rubber-band
sup-9(0)/sup-9(n1550)	Strong-severe rubber-band
sup-9(+)/sup-9(n1550)	Strong rubber-band

Crosses to generate the heterozygotes are listed in Table 3. *sup-9(0)* = *sup-9(n180)*. *sup-9(n1550)* is a rubber-band allele. Designations indicating the strength of the rubber-band phenotype are explained in the text and in Table 4.

[a] The rubber-band phenotype of *sup-9(n1550 n2359); unc-93(e1500)* worms is slightly stronger than that of *unc-93(e1500)* worms.

[b] These phenotypes were scored relative to each other based on uncoordinated movement and in some cases also brood size.

[c] The rubber-band phenotype of *sup-9(n1550 n2360)/+; unc-93(e1500)* worms is slightly stronger than that of *unc-93(e1500)* worms.

[d] These phenotypes were scored relative to each other based on uncoordinated movement.

causes this enhancement of the *unc-93(e1500)* rubber-band phenotype (see DISCUSSION).

We showed that the *sup-9(p)* allele *n1550 n2360* is a dominant enhancer of *unc-93(e1500)* by constructing strains carrying *n1550 n2360* in *trans* to *sup-9(+)* or *sup-9(0)* alleles in combination with *unc-93(e1500)*. By observing the phenotypes of these animals, we found the genotypes, in order of decreasing strength of the rubber-band phenotype, were *p/p; unc-93(e1500)* = *p/0; unc-93(e1500)* > *p/+; unc-93(e1500)* > *+/+; unc-93(e1500)* (*p* is *n1550 n2360*; see Table 8B). The phenotypes of *sup-9(n1550 n2360); unc-93(e1500)* and *sup-9(n1550 n2360)/sup-9(0); unc-93(e1500)* animals were indistinguishable based upon their movements (Table 8B) and brood sizes (data not shown). Because *sup-9(n1550 n2360)/+; unc-93(e1500)* worms had a stronger rubber-band phenotype than did *unc-93(e1500)* worms, *sup-9(n1550 n2360)* is a dominant enhancer of *unc-93(e1500)*.

Similar experiments with the *sup-9(p)* allele *n1550 n2359* indicate that *n1550 n2359* has lost some of the *sup-9* function required for the rubber-band phenotype of *unc-93(e1500)*. We observed that *sup-9(n1550 n2359)/sup-9(0); unc-93(e1500)* worms had a weaker rubber-band phenotype than *unc-93(e1500)* worms and *sup-9(0)/+; unc-93(e1500)* worms (Table 8B). Thus, unlike *n1550 n2360*, *n1550 n2359* was not dominant to a *sup-9(0)* mutation, and in this case *n1550 n2359* acted as a suppressor of *unc-93(e1500)*. This result suggests that *n1550 n2359* has a reduction in the *sup-9* function required for the rubber-band phenotype of *unc-93(e1500)* and that the enhancement seen in *sup-9(n1550 n2359); unc-93(e1500)* worms might be a result of residual *n1550* activity. We were unable to distinguish the strong rubber-band phenotype of *sup-9(n1550 n2359)/+; unc-93(e1500)* worms from that of *unc-93(e1500)* worms (Table 8B). The difference between the phenotypes of *unc-93(e1500)* and *sup-9(n1550 n2359); unc-93(e1500)* worms is small, and a weak dominant enhancement of *unc-93(e1500)* by *sup-9(n1550 n2359)* might not have been detectable.

By constructing these two *sup-9(p)* alleles with the rubber-band mutation *unc-93(n200)*, we showed that these *sup-9(p)* alleles show allele-specificity in their interaction with the two *unc-93* rubber-band mutations. Worms of genotype *sup-9(n1550 n2359); unc-93(n200)* had a moderate rubber-band phenotype that was more severe than the weak rubber-band phenotype of *unc-93(n200)* worms (Table 8A). We could not detect any effect on the *unc-93(n200)* rubber-band phenotype by *sup-9(n1550 n2360)* (Table 8A). Because *unc-93(n200)* resulted in a weak rubber-band phenotype, a small enhancement or suppression might be too subtle to have been detectable. Although the *sup-9(p)* alleles interacted similarly in some ways with the two *unc-93* rubber-band mutations, the *sup-9(n1550 n2359)* mutation is a stronger enhancer of

unc-93(n200) and *sup-9(n1550 n2360)* is a stronger enhancer of *unc-93(e1500)*.

We also studied interactions between *sup-9* alleles in worms of genotype *sup-9(p)/sup-9(n1550)*. *sup-9(n1550 n2359)/sup-9(n1550)* worms had a strong-severe rubber-band phenotype stronger than the strong rubber-band phenotype of *sup-9(n1550)/+* worms and similar to that of *sup-9(n180)/sup-9(n1550)* worms (Table 8C). By contrast, *sup-9(n1550 n2360)/sup-9(n1550)* worms had a moderate-strong rubber-band phenotype that was slightly weaker than the strong rubber-band phenotype of *sup-9(n1550)/+* worms (Table 8C). These results suggest that *sup-9(n1550 n2360)*, but not *sup-9(n1550 n2359)*, could be antagonizing the rubber-band phenotype of *sup-9(n1550)* in *trans*.

The *sup-9(p)* class of mutations is relatively rare, since seven of eight *sup-9* mutations isolated in the F_2 reversion screen of *sup-9(n1550)* and two of two *sup-9* mutations isolated in the F_1 reversion screen of *sup-9(n1550)/+* are typical *sup-9(0)* alleles, which suppress rather than enhance *unc-93(e1500)*. In addition, all nine *sup-9* mutations isolated as suppressors of *sup-10(n983)* by GREENWALD and HORVITZ (1986) suppress *unc-93(e1500)*. In summary, unlike *sup-9(0)* mutations, the *sup-9(p)* mutations exhibited gene-specific and allele-specific interactions with rubber-band mutations in the *sup-9*, *sup-10* and *unc-93* genes.

The *n1550 n2288* revertant, which we identified in our F_2 reversion screen of *sup-9(n1550); sup-18(n1014)* worms (see above), might also contain a *sup-9(p)* allele. *n1550 n2288* failed to complement a *sup-9(0)* mutation for suppression of *sup-10(n983)* (see MATERIALS AND METHODS). In addition, like *sup-9(p)* mutations, *sup-9(n1550 n2288)* probably did not fully suppress the rubber-band phenotype of *unc-93(e1500)* (see MATERIALS AND METHODS). We have not mapped the *n2288* mutation.

***sup-9* dominant-suppressor alleles:** We have identified two dominant-suppressor (dsp) alleles of *sup-9*, both of which were isolated as suppressors of rubber-band mutants. We obtained one of these alleles, *n1435*, while seeking dominant suppressors of the rubber-band phenotype of *sup-10(n983)* animals. We mutagenized *sup-10(n983)* hermaphrodites with EMS and isolated one dominant suppressor, *n1435*, from about 10^6 F_1 progeny. Animals of genotype *n1435; sup-10(n983)* had a wild-type phenotype, and animals of genotype *n1435/+; sup-10(n983)* had a weak rubber-band phenotype (Table 9). *n1435; sup-10(+)* worms had a wild-type phenotype (Table 9). No other abnormalities were observed to be associated with *n1435* either in combination with *sup-10(n983)* or alone. By two-factor mapping, we showed that *n1435* is linked to *dpy-10 II* (recombination frequency of about 13%; data not shown) and *lin-31 II* (recombination frequency of about 6.6%; data not shown). We used further two-factor mapping to show that *n1435* maps within 0.07 map units of *sup-9* (see MATERIALS AND METHODS). These data suggest that *n1435* might be an allele of *sup-9*. We constructed *n1435; unc-93(e1500)* worms and observed that they had slightly better movement than did *unc-93(e1500)* worms and laid some eggs, whereas *unc-93(e1500)* worms never lay eggs (GREENWALD and HORVITZ 1980) (Table 9). We could not distinguish the phenotype of *n1435/+; unc-93(e1500)* worms from that of *unc-93(e1500)* worms (Table 9). Thus, *n1435* is a semidominant suppressor of *sup-10(n983)* and a weak recessive suppressor of *unc-93(e1500)*.

The other dominant-suppressor allele of *sup-9*, *n242*, was isolated after mutagenesis with gamma irradiation some years ago (GREENWALD and HORVITZ 1980; and unpublished data) as a suppressor of the rubber-band phenotype of *unc-93(e1500)* animals (noted in Table 3 of GREENWALD and HORVITZ 1980). Further analysis showed that *n242* is a dominant suppressor of both *unc-93(e1500)* and *sup-10(n983)* rubber-band mutations. Specifically, we found that *n242/+; unc-93(e1500)* worms had a weak rubber-band phenotype, and *n242/+; sup-10(n983)* worms had a phenotype between a weak rubber-band phenotype and a wild-type phenotype (Table 9). Two-factor mapping placed *n242* within 0.04 map units of *sup-9* (see MA-

TABLE 9

***sup-9(n1435)* and *sup-9(n242)* are dominant suppressors of the rubber-band phenotype**

Genotype[a]		
sup-9 allele(s)	Rubber-band mutation	Phenotype[b]
n1435/+	*sup-10(n983)*	Weak rubber-band
n1435/0	*sup-10(n983)*	Wild-type
n1435	*sup-10(n983)*	Wild-type
n242/+	*sup-10(n983)*	Very weak rubber-band
n242/0	*sup-10(n983)*	Wild-type
n242	*sup-10(n983)*	Wild-type
n1435/n242	*sup-10(n983)*	Wild-type
n1435/+	*unc-93(e1500)*	Strong rubber-band
n1435/0	*unc-93(e1500)*	Moderate rubber-band
n1435	*unc-93(e1500)*	Moderate rubber-band
n242/+	*unc-93(e1500)*	Weak rubber-band
n242/0	*unc-93(e1500)*	Wild-type
n242	*unc-93(e1500)*	Wild-type
n1435/n242	*unc-93(e1500)*	Wild-type
n1435	None	Wild-type
n242	None	Wild-type
n1435/n242	None	Wild-type

[a] Crosses that generated the heterozygotes are listed in Table 3; only relevant genotypes are shown here. The *sup-9(0)* alleles were *n180* or *n1553*.

[b] Designations indicating the strength of the rubber-band phenotype are explained in the text. *sup-10(n983)* worms have a moderate rubber-band phenotype that is weaker than the strong rubber-band phenotype of *unc-93(e1500)* worms (GREENWALD and HORVITZ 1986).

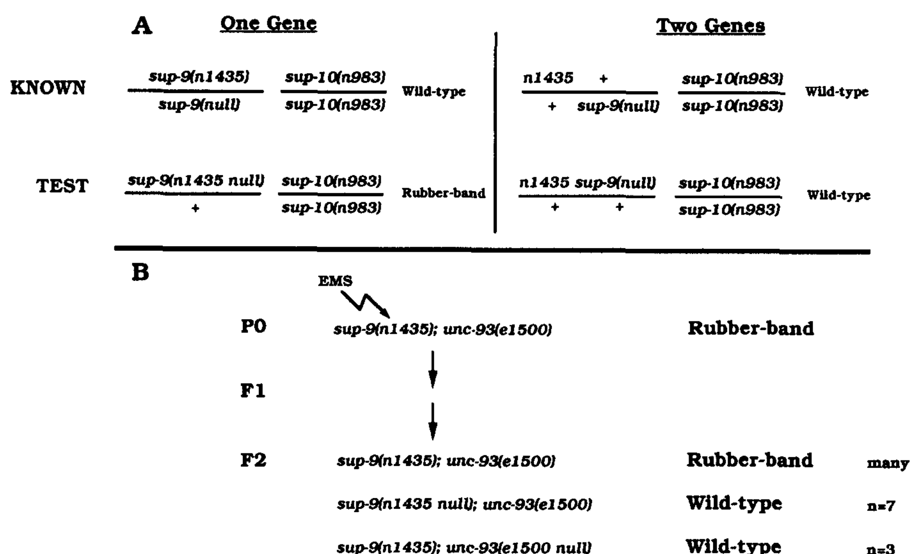

FIGURE 2.—*n1435* is an allele of *sup-9*. (A) A genetic *cis-trans* test. The *n1435* mutation is a dominant suppressor of the rubber-band phenotype of *sup-10(n983)* animals. We have shown that a *sup-9* null mutation in *trans* to *n1435* does not eliminate the *n1435* dominant suppressor activity (Table 9). If *n1435* is an allele of *sup-9*, a *sup-9* null mutation in *cis* to *n1435* will eliminate the *n1435* dominant suppressor activity (left panel). If *n1435* is not an allele of *sup-9*, a *sup-9* null mutation in *cis* will not eliminate the *n1435* dominant suppressor activity (right panel). We induced a *sup-9* null mutation in *cis* to *n1435* using the mutagenesis scheme shown in (B). (B) The *n1435* mutation has only a very weak suppressor effect on the rubber-band phenotype of *unc-93(e1500)* animals. We mutagenized *sup-9(n1435); unc-93(e1500)* hermaphrodites with EMS and screened the F_2 progeny for phenotypically wild-type revertants. We generated *sup-9* null mutations in *cis* to *n1435* and demonstrated that these *sup-9* null mutations eliminate the *n1435* dominant suppressor activity, confirming the one gene hypothesis. "n," number of revertants isolated of genotype indicated. See MATERIALS AND METHODS for details.

TERIALS AND METHODS). As is the case for *n1435*, *n242* caused no visibly abnormal phenotype alone.

We demonstrated that *n1435* is an allele of the *sup-9* gene by the following experiment. We generated *cis*-dominant mutations that eliminated the dominant suppressor activity associated with *n1435* and showed that these new mutations are loss-of-function alleles of the *sup-9* gene. The rationale for this experiment is the assumption that if *n1435* were a *sup-9* allele, a *sup-9(0)* mutation in *cis* would abolish the dominant suppressor activity of *n1435* (Figure 2A). We used an indirect strategy to isolate such *sup-9(0)* mutations (Figure 2B). We picked wild-type revertants from among the F_2 progeny of EMS-mutagenized worms of genotype *n1435; unc-93(e1500)*. Based on complementation testing of the mutations generated in this screen, we isolated three *unc-93(0)* mutations, no *sup-10(0)* mutations and seven *sup-9(0)* mutations (Figure 2B). We showed that all three of the new *sup-9(0)* mutations tested (*n2276*, *n2279* and *n2281*) lacked the dominant suppressor activity associated with *n1435* (see MATERIALS AND METHODS). Thus, *n1435* is an allele of *sup-9*. The overall frequency of suppressor mutations obtained from the reversion of the *n1435; unc-93(e1500)* phenotype was about 8.5×10^{-4}, which is similar to the frequency of 1×10^{-3} obtained by GREENWALD and HORVITZ (1980) for the reversion of the *unc-93(e1500)* phenotype. These earlier *e1500* EMS reversion experiments generated nine *unc-93(0)* mutations, four *sup-10(0)* mutations, nine *sup-9(0)* mutations and one *sup-11(d)* mutation (GREENWALD and HORVITZ 1980). To determine whether *sup-10(0)* mutants could have been recovered in our screen, we tested *sup-10(n183)*, the canonical *sup-10(0)* mutation, for its ability to suppress the rubber-band phenotype of *unc-93(e1500)* in a strain with the *sup-9(n1435)* mutation present. We constructed the triple mutant *sup-9(n1435); unc-93(e1500); sup-10(n183)* and observed complete suppression of the rubber-band phenotype of *unc-93(e1500)* by *sup-10(n183)*. Thus, our failure to recover *sup-10(0)* mutations in this screen is not likely to be significant. We believe that *n242* is also likely to be an allele of *sup-9* based on genetic mapping experiments (see above) and the similar genetic properties of *n242* and *n1435*.

The mutations *n242* and *n1435* define a new class of *sup-9* alleles that act as dominant suppressors of the *sup-10(n983)* rubber-band mutation. We compared the suppression by these dominant-suppressor mutations in *trans* to *sup-9(0)* and *sup-9(+)* alleles. Our data are summarized in Table 9. In general, the genotypes, in order of decreasing suppression of the rubber-band phenotype, can be ranked as dsp/dsp ≥ dsp/null ≥ dsp/+ ≥ +/+. (This ranking uses the term "≥" because the phenotypes are sometimes indistinguishable for two genotypes, *e.g.*, *sup-9(n1435); sup-10(n983)* and *sup-9(n1435)/sup-9(0); sup-10(n983)* worms are both phenotypically wild-type.) Since the dsp/null heteroallelic combinations were better suppressors than were the dsp/+ combinations, these results are incon-

sistent with a model in which *n242* and *n1435* have increased *sup-9* activity.

The *n1435* mutation does not totally eliminate the *sup-9* activity required for the *unc-93(e1500)* rubber-band mutant phenotype, since *sup-9(n1435); unc-93(e1500)* worms displayed a rubber-band phenotype (see above). The *sup-9(n1435)* mutation might alter a domain of the Sup-9 protein that is required more by the *sup-10(n983)* altered protein to cause a rubber-band phenotype than by the *unc-93(e1500)* altered protein. Alternatively, the *sup-9(n1435)* mutation might suppress the rubber-band phenotype of *sup-10(n983)* better than that of *unc-93(e1500)* because *sup-10(n983)* is a weaker rubber-band mutation than *unc-93(e1500)*.

The *sup-9(n242)* mutation is a stronger suppressor than the *sup-9(n1435)* mutation of the rubber-band phenotype caused by *sup-10(n983)* and *unc-93(e1500)*. Worms of genotype *sup-9(n242)/+; sup-10(n983)* had a weaker rubber-band phenotype, *i.e.*, were more suppressed than worms of genotype *sup-9(n1435)/+; sup-10(n983)* (Table 9). The suppression of the *unc-93(e1500)* rubber-band phenotype by *sup-9(n242)* was also stronger than that of the *sup-9(n1435)* mutation in genotypes heterozygous, hemizygous or homozygous for a *sup-9(dsp)* mutation (Table 9). In addition, worms of genotypes *sup-9(n242)/sup-9(n1435)*, and *sup-9(n242)/sup-9(n1435); sup-10(n983)*, and *sup-9(n242)/sup-9(n1435); unc-93(e1500)* had a wild-type phenotype (Table 9). In *sup-9(n242)/sup-9(n1435); unc-93(e1500)* animals, the phenotype caused by the *n242* mutation is dominant to the phenotype caused by the *n1435* mutation, because the worms resemble *sup-9(n242); unc-93(e1500)* animals (wild-type) and not *sup-9(n1435); unc-93(e1500)* animals (moderate rubber-band). This result is consistent with the hypothesis that *n242* is a stronger *sup-9(dsp)* allele than is *n1435*.

To determine how the dominant-suppressor *sup-9* mutations and the *sup-9(n1550)* rubber-band mutation interact, we constructed strains heterozygous for these two types of mutations. Both *sup-9(n242)/sup-9(n1550)* and *sup-9(n1435)/sup-9(n1550)* worms had a strong rubber-band phenotype similar to that of *sup-9(n1550)/+* worms. These results indicate that *sup-9(dsp)* mutations neither suppress nor enhance the phenotype caused by *sup-9(n1550)*. Worms of genotype *sup-9(n1550)/sup-9(n180)* had a stronger phenotype than did *sup-9(n1550)/+* worms, so that the *sup-9(dsp)* mutations behaved differently from *sup-9(0)* in *trans* to *sup-9(n1550)*. This experiment suggests that the *sup-9(dsp)* mutations may have some *sup-9(+)* function.

Semidominant suppression of *unc-93(e1500)* and *sup-10(n983)*: Because *sup-10(0)*, *sup-18(0)* and *unc-93(0)* mutations are weakly semidominant suppressors of *sup-9(n1550)* (see above), we wondered whether the extragenic suppressors of other rubber-band mutations also were semidominant in their action. We tested for semidominant suppression of the *sup-10(n983)* rubber-band mutation by *sup-9(n180)*, *unc-93(e1500 n234)* and *sup-18(n1010)*. In all three cases, we found that worms of genotype *suppressor/+; sup-10(n983)* had a slightly weaker moderate rubber-band than *sup-10(n983)* worms, based upon the uncoordinated movement of these worms. We also tested for semidominant suppression of *unc-93(e1500)*. Worms of genotype *unc-93(e1500)/+* displayed a weak rubber-band phenotype (GREENWALD and HORVITZ 1980). We found that *unc-93(e1500)/+* worms had a more pronounced rubber-band phenotype than that of *sup-9(n180)/+; unc-93(e1500)/+* worms and *unc-93(e1500)/+; sup-10(n183)/+* worms. By contrast, worms of genotype *unc-93(e1500) +/+ sup-18(n1010)* were phenotypically indistinguishable from worms of genotype *unc-93(e1500)/+*. We could not detect any difference between the phenotypes of animals of genotype *unc-93(e1500)* and that of animals of genotype *sup-9(n180)/+; unc-93(e1500)* or *unc-93(e1500); sup-10(n183)/+*. Thus, we observed weak semidominant suppression of *unc-93(e1500)* and *sup-10(n983)* in some but not all circumstances. These results suggest that there is a sensitivity of the rubber-band phenotype to the dosage of each of these gene products.

DISCUSSION

Our results support the hypothesis of GREENWALD and HORVITZ (1980, 1986) that the Sup-9, Sup-10, and Unc-93 products interact as a protein complex to function in the regulation or coordination of muscle contraction. First, we have identified a rubber-band allele of *sup-9* and shown that this mutation is suppressed by loss-of-function mutations in any of the other genes. When combined with previous observations (GREENWALD and HORVITZ 1980, 1986), this finding establishes that a rubber-band mutation in *sup-9*, *sup-10* or *unc-93* is suppressed by a loss-of-function mutation in any of the same three genes. Thus, each of these genes seems to be needed for the function of each of the others, suggesting that they do not act sequentially but rather act together. Second, null alleles of *sup-9*, *sup-10* and *unc-93* act as weak semidominant suppressors of the *sup-9(n1550)*, *sup-10(n983)* and *unc-93(e1500)* rubber-band mutations. This finding indicates that the relative stoichiometry of these gene products is important, consistent with the hypothesis that these genes interact by encoding components of a protein complex. Third, the characteristics of the *sup-9* dominant suppressor (dsp) mutations also are consistent with there being protein-protein interactions between the products of *sup-9* and *unc-93* and between the products of *sup-9* and *sup-10*, as we discuss below. Fourth, the gene- and

allele-specific interactions of the *sup-9* partial loss-of-function (p) alleles with the rubber-band mutations support a model in which the *sup-9*, *sup-10* and *unc-93* gene products physically contact each other in a protein complex, as we explain below.

The Sup-18 protein might also interact with the Sup-9, Sup-10 and Unc-93 proteins. Null mutations in *sup-18* partially suppress the effects of the *sup-9(n1550)*, *unc-93(e1500)* and *unc-93(n200)* rubber-band mutations and completely suppress the effects of the *sup-10(n983)* rubber-band mutation. The basis for this differential suppression is not likely to be simply the strength of the rubber-band mutations, because *sup-10(n983)* is intermediate in strength. Perhaps *sup-18* encodes a peripheral component of the proposed protein complex, such that the absence of the *sup-18* gene product does not necessarily completely inactivate the functioning of this complex; the extent of this inactivation would depend on the requirement of each rubber-band mutant gene product for the *sup-18* gene product. Alternatively, *sup-18* could be a positive regulator of *unc-93*, *sup-9* and *sup-10* or a negative regulator of components of a functionally parallel pathway. These possibilities were previously considered by GREENWALD and HORVITZ (1980, 1986).

How do the *sup-9(dsp)* mutations cause dominant suppression of the Unc-93 or Sup-10 rubber-band phenotype? Three mechanisms seem appropriate to consider. First, the Sup-9(dsp) protein could antagonize the action of the Sup-9(+) protein, thus eliminating *sup-9* function. Since *sup-9(0)* alleles suppress the rubber-band phenotype, such dominant-negative or antimorphic action would lead to a wild-type phenotype in animals of genotype *sup-9(dsp)/sup-9(+); rubber-band*. In this case, the Sup-9(dsp) protein either could inactivate the Sup-9(+) protein directly as a result of interactions between these two different Sup-9 proteins or could inactivate the Sup-9(+) protein indirectly by titrating a factor that interacted with and was needed for Sup-9(+) function. Second, the Sup-9(dsp) protein could directly compensate for the abnormality in the protein product of the *unc-93* or *sup-10* rubber-band allele, possibly by protein-protein interactions that restored a functionally normal complex of the Unc-93, Sup-10 and Sup-9 proteins. Third, the Sup-9(dsp) protein could indirectly compensate for the abnormality in the protein product of the *unc-93* or *sup-10* rubber-band allele, for example by having a novel "anti-rubber-band" activity that antagonizes the effects of a protein complex containing the product of a rubber-band mutation. The differences among these three models can be exemplified by considering our hypothesis (LEVIN and HORVITZ 1992) that the rubber-band phenotype is caused by an inappropriately open ion channel: the first model predicts that the ion conductance of this channel would be eliminated, the second model predicts that the ion conductance of this channel would be restored, and the third model predicts that there would be a compensatory change in another ion channel or pump.

Two of the intragenic *sup-9(n1550)* revertants, *sup-9(n1550 n2359)* and *sup-9(n1550 n2360)*, define a new class of *sup-9* alleles. The simplest, most obvious interpretations for intragenic suppressors of *sup-9(n1550)* would be either a restoration of wild-type *sup-9* function or an elimination of *sup-9* function, but neither of these explanations fits the complex patterns of suppression observed. Instead, we term these mutations "*sup-9(p)*" alleles for their partial loss of function, which we propose is a loss of certain aspects of *sup-9* function rather than a simple reduction in the overall level of *sup-9* function. Both *sup-9(p)* alleles display a gene-specific interaction with different rubber-band mutations, based on the observations summarized in Table 7. These *sup-9(p)* alleles are partial suppressors of *sup-10(n983)*, suggesting that they cause a partial loss of the *sup-9* function needed for the *sup-10(n983)* rubber-band phenotype. However, the *sup-9(p)* alleles are not suppressors, and in some cases are enhancers, of the *unc-93* rubber-band mutations. These data suggest that the *sup-9(p)* alleles do not disrupt the *sup-9* function needed for the *unc-93* rubber-band phenotypes. Furthermore, in *trans* to *sup-9(n1550)*, *sup-9(n1550 n2359)* behaves like a *sup-9* null mutation, but *sup-9(n1550 n2360)* weakly antagonizes the *sup-9(n1550)* mutant phenotype. These *sup-9(p)* mutations also display allele specificity (Table 8). *sup-9(n1550 n2359)* is a weak enhancer of *unc-93(e1500)* and a strong enhancer of *unc-93(n200)*, whereas *sup-9(n1550 n2360)* is a strong enhancer of *unc-93(e1500)* and apparently not an enhancer of *unc-93(n200)*. The allele-specificity and gene-specificity of the interaction of these *sup-9(p)* alleles with the rubber-band mutations argues against a model involving bypass or informational suppression of the rubber-band mutations and in favor of a protein-protein interaction among the Sup-9, Sup-10 and Unc-93 proteins. In the context of such a model, the gene-specificity of these interactions further reveals that the Sup-9 protein interacts differently with the Sup-10 and Unc-93 proteins.

Why do the *sup-9(n1550 n2359)* and *sup-9(n1550 n2360)* alleles enhance some Unc-93 rubber-band phenotypes? Since the rubber-band mutation *sup-9(n1550)* also enhances the Unc-93 rubber-band phenotype, it seems plausible that cryptic *sup-9(n1550)* activity remaining in these *sup-9* revertants is responsible. This cryptic activity could be manifested because the portion of the Sup-9 protein required for expression of the Unc-93 rubber-band phenotype remains functional. Why are there allele-specific differences in

the interactions of these *sup-9(p)* alleles with *unc-93(e1500)* and *unc-93(n200)*? Given that there was no detectable difference in the interaction of *sup-9(n1550)* with *unc-93(e1500)* and *unc-93(n200)* (Table 6B), a simple reduction in the level of function of cryptic *sup-9(n1550)* activity should affect their interaction with the two *unc-93* rubber-band mutations very similarly. The interactions of *sup-9(n1550 n2359)* and *sup-9(n1550 n2360)* with *unc-93(e1500)* and *unc-93(n200)* are not consistent with a higher level of cryptic *n1550* activity for either one of the *sup-9(p)* alleles (Table 8B and see RESULTS). Thus, it seems more likely that the allele-specific defects in the Sup-9(p) proteins caused by *sup-9(n1550 n2359)* and *sup-9(n1550 n2360)* are the result of a functional difference between the two Sup-9(p) proteins in their interactions with the other proteins in the proposed protein complex. Alternatively, these *sup-9(p)* mutations could have a novel activity, unrelated to *sup-9(n1550)*, that differentially enhances the rubber-band phenotype of *unc-93(e1500)* and *unc-93(n200)*. Given the genetic interactions seen for these *sup-9(p)* mutations and also, perhaps, the interactions of *sup-9(1435dsp)* with *unc-93(e1500)* and *sup-10(n983)*, we postulate that the Sup-9 protein probably has independently mutable functions with respect to its interactions with other proteins in the proposed protein complex. Furthermore, these *sup-9* mutations may identify regions of the Sup-9 protein involved in the allele- and gene-specific interactions with other proteins in this complex.

Certain double rubber-band mutants containing *sup-9(n1550)* and *unc-93(e1500)*, *unc-93(n200)* or *sup-10(n983)* display embryonic lethality (Table 6B), with the mutant embryos showing normal body-wall muscle movements during the elongation stage of embryogenesis but almost no movement later in embryogenesis. This phenotype differs from that of *myo-3* and *deb-1* null mutants, which probably lack all body-wall muscle function, have no body-wall muscle movements and fail to elongate (WATERSTON 1989; BARSTEAD and WATERSTON 1991). Why do the body-wall muscle cells in these double rubber-band mutant embryos function during elongation but not later during embryogenesis or after hatching? One possibility is that the Unc-93, Sup-9 and Sup-10 proteins do not function until after elongation. The mechanism of body-wall muscle movement during elongation is thought to differ from that of later body-wall muscle movement, because motor neurons have not formed neuromuscular junctions with the body-wall muscle cells at the start of elongation in most, if not all, regions of the body (DURBIN 1987). In addition, *unc-104* null mutants, which form very few synapses, and *cha-1* null mutants, which lack choline acetyltransferase and hence the cholinergic inputs that drive body-wall muscle contractions, have normal body-wall muscle movements during elongation (HALL and HEDGECOCK 1991; RAND and RUSSELL 1984; ROGALSKI and RIDDLE 1988; RAND 1989). Thus, it is possible that the control of body-wall muscle contractions during elongation is myogenic rather than neurogenic. Therefore, it appears that the myogenic aspects of muscle contraction may be normal in these mutants, but that the neurogenic aspects may be disrupted. Based upon the putative membrane localization of the Unc-93 protein and the disruption of the regulation of muscle contraction by the rubber-band mutations, we suggested previously that the Unc-93, Sup-9 and Sup-10 proteins might act in excitation-contraction coupling (LEVIN and HORVITZ 1992). Taken together, these observations leads us to a model in which the proposed protein complex functions in a neuronal-input-specific step in excitation-contraction coupling. For example, a complex consisting at least in part of the Unc-93, Sup-9 and Sup-10 proteins might constitute or regulate an ion channel localized to the muscle cell plasma membrane and gated by neuronal activity.

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