Analysis of Dominant Mutations Affecting Muscle Excitation in *Caenorhabditis elegans*

David J. Reiner, David Weinshenker and James H. Thomas

Department of Genetics, University of Washington, Seattle, Washington 98195

Manuscript received April 25, 1995
Accepted for publication July 24, 1995

ABSTRACT

We examined mutations that disrupt muscle activation in *Caenorhabditis elegans*. Fifteen of 17 of these genes were identified previously and we describe new mutations in three of them. We also describe mutations in two new genes, *exp-3* and *exp-4*. We assessed the degree of defect in pharyngeal, body-wall, egg-laying, and enteric muscle activation in animals mutant for each gene. Mutations in all 17 genes are semidominant and, in cases that could be tested, appear to be gain-of-function. Based on their phenotypes, the genes fall into three broad categories: mutations in 11 genes cause defective muscle activation, mutations in four genes cause hyperactivated muscle, and mutations in two genes cause defective activation in some muscle types and hyperactivation in others. In all testable cases, the mutations blocked response to pharmacological activators of egg laying, but did not block muscle activation by irradiation with a laser microbeam. The data suggest that these mutations affect muscle excitation, but not the capacity of the muscle fibers to contract. For most of the genes, apparent loss-of-function mutants have a grossly wild-type phenotype. These observations suggest that there is a large group of genes that function in muscle excitation that can be identified primarily by dominant mutations.

THE nervous system regulates behavior through a hierarchy of sensory input, information processing, and output. Output is achieved primarily through the coordinated activation of muscles by motor neurons. The stimulation of muscles by excitatory motor neurons causes depolarization of the muscle cell membrane (muscle excitation), which is coupled to mechanical contraction by release of calcium from intracellular stores in a process called excitation-contraction coupling (CATTERALL 1991). Much progress has been made in understanding the structure and regulation of the contractile components of the muscle cell (*e.g.,* EPSTEIN and FISCHMAN 1991), and significant progress has been made toward understanding the components of excitation and excitation-contraction coupling (CATTERALL 1991; LEVI *et al.* 1993; FOSTER 1994).

The nematode *Caenorhabditis elegans* is an excellent organism for the genetic analysis of muscle excitation and contraction. The organization of the animal is relatively simple (SULSTON and HORVITZ 1977; SULSTON *et al.* 1983; WHITE 1988), and the structure of the entire hermaphrodite nervous system has been described (WHITE *et al.* 1986). In particular, the genetic and biochemical analysis of muscle contraction and organization has been fruitful. More than 25 genes have been identified that play a role in muscle development and contraction (WATERSTON 1988). Genes have been identified that encode structural components of the myofilament, such as myosin heavy chain (MACLEOD *et al.* 1977; ARDIZZI and EPSTEIN 1987; WATERSTON 1989), paramyosin (WATERSTON *et al.* 1977) and actin (LANDEL *et al.* 1984; WATERSTON *et al.* 1984). Other genes involved in muscle attachment and development have been identified (BARSTEAD and WATERSTON 1989; VENOLIA and WATERSTON 1990). Additional genes have been identified that may be involved in excitation-contraction coupling, including *unc-22* (MOERMAN *et al.* 1988; BENIAN *et al.* 1989; BENIAN *et al.* 1993), which may regulate myosin activity directly (MOERMAN *et al.* 1988; HEIERHORST *et al.* 1994; HU *et al.* 1994; LEI *et al.* 1994).

The *C. elegans* hermaphrodite has four major muscle groups: the body-wall muscles used for locomotion, the pharyngeal muscles used for feeding, the vulval and uterine muscles used for egg laying, and the enteric muscles used for defecation. For each muscle group, a combination of electron microscopy, genetic analysis, and the selective killing of neurons has provided considerable information about the motor neurons and their apparent excitatory or inhibitory neurotransmitters. Body-wall muscle has been studied most extensively, including electrophysiological studies in the larger nematode *Ascaris* (*e.g.,* STRETTON *et al.* 1985). Several classes of motor neurons, including the VA, VB, DA, and DB neurons, are thought to excite body-wall muscles using acetylcholine as their primary transmitter (CHALFIE *et al.* 1985; WHITE *et al.* 1986; LEWIS *et al.* 1987). Two other classes of motor neurons, DD and VD, are inhibitory motor neurons on the body-wall muscle, using γ-aminobutyric acid (GABA) as their primary

Corresponding author: James H. Thomas, Department of Genetics, SK-50, University of Washington, Seattle, WA 98195.
E-mail: jht@genetics.washington.edu

transmitter (WHITE *et al.* 1986; MCINTIRE *et al.* 1993a,b). For the egg-laying muscles, a single class of putative motor neuron, HSN, has been described that probably uses serotonin as an excitatory transmitter (HORVITZ *et al.* 1982; HUANG *et al.* 1982; TRENT *et al.* 1983; WHITE *et al.* 1986). For the enteric muscles, two partially redundant motor neurons, AVL and DVB, use GABA as an excitatory transmitter (WHITE *et al.* 1986; MCINTIRE *et al.* 1993a,b). Finally, pharyngeal muscle activation appears to be myogenic, since no pharyngeal neurons are required for contraction (AVERY and HORVITZ 1989). Since the analysis of motor neurons and transmitter systems is incomplete for each of these muscle types, it is possible that additional motor neurons and transmitters are used.

In contrast, we currently understand little about how muscle cells respond to stimuli from motor neurons in *C. elegans*. Presumably these muscles have a variety of ionotropic and metabotropic receptors for neurotransmitters, as well as an array of ion channels and transporters that function in excitation, inhibition and recovery (HILLE 1992b). *C. elegans* muscles are presumed to have intracellular calcium stores in the form of the sarcoplasmic reticulum (WATERSTON 1988). *C. elegans* do not appear to have T-tubules similar to those observed in vertebrate skeletal muscle, presumably because the *C. elegans* muscle plasma membrane is directly apposed to the sarcoplasmic reticulum (WATERSTON 1988).

Large mutant screens have been performed to identify genes that function in a variety of behaviors, including (among others) the function of the four main muscle groups: locomotion (BRENNER 1974), egg laying (TRENT *et al.* 1983) feeding (AVERY 1993), and defecation (THOMAS 1990). However, these mutant screens did not distinguish genes required for muscle excitation from those required for muscle structure, neuronal function and neuronal development. We have begun to study how the various transmitter pathways converge or interact in the muscle cells by identifying mutations that generally disrupt muscle excitation and performing tests of the nature of their genetic and physiological defects. We have developed phenotypic criteria to select these genes from the many identified that affect behavior. Our analysis concentrated on the egg-laying and enteric muscles because their function is nonessential and their activity is easily measured (TRENT *et al.* 1983; THOMAS 1990).

MATERIALS AND METHODS

General methods: General maintenance of *C. elegans* and nomenclature are as previously described (BRENNER 1974; HORVITZ *et al.* 1979; WOOD 1988). The standard *N2* Bristol strain was used as wild type, and all animals were grown at 20°. We obtained mutant strains from the *Caenorhabditis* Genetics Center, from the MRC in England, from the HORVITZ lab at MIT, from the AVERY lab at the University of Texas Southwestern Medical Center, and from the GARRIGA lab at University of California, Berkeley. Observations and photographs using Nomarski optics were performed as described previously (SULSTON and HORVITZ 1977; STERNBERG and HORVITZ 1986).

Defecation assays: The frequency of enteric muscle contraction (EMC) was observed and recorded as described (THOMAS 1990; LIU and THOMAS 1994). Young adult hermaphrodites were staged by picking animals with two to eight eggs in the gonad. Several animals of the appropriate stage were picked to a separate plate and allowed to settle for at least 15 min before observation. To minimize selection bias in the assay procedure, animals were selected randomly from this plate for the beginning of assays and were observed for exactly 10 min. Activation of aBoc (anterior body-wall muscle contraction of defecation) was omitted from the scoring because observation of aBoc decreases the reliability of scoring EMCs. In all cases, aBoc was scored separately and was seen to be grossly normal.

In the course of this analysis, we discovered an age dependence of EMC frequency in the wild type and several mutants. Wild-type young adult animals, staged as described above, have 100% EMC (136 cycles in 10 animals), but 24 hr later (midadults) have only 79.3% EMC (140 cycles in 10 animals). This age-dependent difference in EMC frequency was also seen in mutants with an EMC defect mild enough to measure a change: for *egl-30(n715)*, *egl-30(ad805)*, *egl-30(n686)*, *unc-103(e1597)* and *unc-103(n500)* young adults, values were 59.0 (122), 85.1 (114), 78.1 (146), 75.8 (124) and 86.0 (114), respectively [%EMC (number of cycles)], but in midadults dropped to 39.8 (113), 53.6 (125), 61.8 (70), 47.1 (121) and 60.7 (117), respectively. The average decrease in EMC frequency between young adults and mid adults (~20%) was not substantially different for the wild type and these mutants.

We also found that mutant animals with weak EMC defects (particularly Mac heterozygotes) frequently had partial EMCs, in which the enteric muscles but not the intestinal muscles contract or vice versa. This phenomenon was observed for mutations in many of the Mac genes and was rarely observed in the wild type. These partial EMCs were counted as full EMCs in the data sets. In addition, we observed that *egl-23(n601sd)* and *egl-23(n2579sd)* mutants often leaked gut contents from the anus during motor program activation, perhaps due to very weak contraction of enteric muscles. No visible enteric muscle contraction accompanied these leaks, and they were not counted as EMCs.

Pharmacological assays: Pharmacological assays of egg-laying behavior were performed in microtiter wells in M9 buffer as described (Trent *et al.* 1983). Animals were staged by selecting individuals with a single full row of eggs in the gonad. The following concentrations were used: serotonin (10 mg/ml), levamisole (100 μg/ml), clomipramine (0.75 mg/ml) and imipramine (0.75 mg/ml). Because they dissolve very slowly, clomipramine and imipramine solutions were sonicated and mixed overnight at 37°. Serotonin and levamisole solutions were made freshly each day, and clomipramine and imipramine solutions were used only the day after mixing. These high drug concentrations are required for efficacy, probably due to cuticle impermeability (LEWIS *et al.* 1980).

Locomotion assays: Defects in locomotion were qualitatively analyzed using three methods. First, body length (either Long or Dpy; see RESULTS) was compared with the wild type and to other mutants. Second, locomotion of undisturbed mutant animals was compared with the wild type and to other mutants. Third, locomotion resulting from prodding mutant animals was compared with prodded wild type and other mutants. In all cases in which differences in degree of locomotion defect could be observed, they were observed using all three methods.

Evaluating contractile capability of muscles: Muscle ultra-

structure was examined using polarized light microscopy as described (SULSTON *et al.* 1980; WATERSTON *et al.* 1980). Wild-type, Mac mutant (Muscle activation; see RESULTS), and muscle structure mutant animals were examined in a blind experiment. Moderate detail was visible in the body-wall and pharyngeal musculature, but we were able to discern only gross perturbations of the egg-laying and enteric muscles. We used a laser microbeam to induce muscle contraction ectopically. The procedure used was as described for cell kills (AVERY and HORVITZ 1989), except that the laser beam was focused on the cell body adjacent to the nucleus, rather than on the nucleus itself, and no anesthetic was used. We speculate that damaging the cell body with the laser causes depolarization of the plasma membrane or disrupts internal Ca^{2+} stores. When the laser was directed at the muscle nucleus, it did not induce contraction. The laser beam used was strong enough to cause substantial pocks on the coverslip when focused there, and one to five shots were required to obtain full muscle contraction. Five young adult hermaphrodites of each genotype were assayed and the result was highly reproducible. We tested the anal depressor muscle and one of the body wall muscles posterior to the dorsal attachment of the anal depressor (C.appppd or C.pppppd). We did not observe reproducible response to laser stimulus of the egg-laying and pharyngeal muscles in the wild type. The muscle structure mutants used as controls were *unc-54(e190)*, *unc-60(e723)*, *unc-60(m35)* (a weak *unc-60* allele), *act-1,2,3(st15)*, and *act-2(ad468)*. *act-2(ad468)* affects only pharyngeal muscle (L. A. SCHRIEFER and R. H. WATERSTON, personal communication; AVERY 1993), while the other mutants affect body-wall, egg-laying, and enteric muscles (WATERSTON 1988).

Marker mutations used: *LGI, bli-3(e767), dpy-5(e61), unc-54(e190); LGII, sqt-2(sc3), dpy-10(e128), rol-6(e187), unc-52(e444); LGIII, dpy-1(e1), sma-3(e491), unc-32(e189), unc-71(e541); LGIV, dpy-9(e12), egl-4(n478), egl-18(n162), lin-1(e1275), unc-33(e204), unc-5(e53), daf-14(m77ts), mec-3(e1612), dpy-20(e1282ts), unc-31(n422), unc-31(e169), unc-22(s7), unc-26(e205), dpy-4(e1166); LGV, unc-34(e566), unc-60(m35), dpy-11(e224), dpy-21(e428), egl-12(n602sd), unc-51(e369), rol-9(sc148);* and *LGX, lon-2(e678), dpy-6(e14), unc-27(e155), aex-2(sa3), unc-115(e2225), egl-15(n484)*

Strains used for behavioral assays: DA468 *unc-31(e928); act-2(ad468) act-3(ad767)*, RW15 *act-1,2,3(st15)*, CB723 *unc-60(e723)*, DR35 *unc-60(m35)*, CB190 *unc-54(e190)*, DA758 *eat-12(ad695); him-5(e1490)*, DA674 *eat-11(ad541); unc-29-(e1072)*, JT5132 *+/eT1; exp-2(sa26)/eT1 let(n886)*, JT6428 *exp-3(n2372)*, JT6932 *exp-4(n2373)*, MT2056 *sup-10(n983)*, CB1500 *unc-93(e1500)*, MT200 *unc-93(n200)*, JT7229 *sup-9-(n1550)*, MT1092 *unc-43(n498)*, CB3533 *+/szT1; unc-110-(e1913)/szT1 lon-2(e678)*, JT6716 *egl-36(n728)*, MT6011 *egl-36(n2332)*, MT1520 *egl-30(n715)*, MT1434 *egl-30(n686)*, DA823 *egl-30(ad805)*, MT2509 *unc-103(n500)*, CB1597 *unc-103(e1597)*, JT7306 *egl-2(n693)*, JT5302 *egl-2(n693) sa304*, JT6713 *egl-2(n2656)*, JT5322 *egl-23(n601)*, JT6683 *egl-23-(n2579)*, JT180 *egl-23(n601 sa180)*, JT181 *egl-23(n601 sa181)*, JT6424 *egl-23(n601 sa179)*, MT2608 *egl-30(n715 n1189)*, MT180 *sup-9(n180)*, MT1684 *unc-105(n490 n785)*, MT1685 *unc-105(n490 n786)*, MT4535 *unc-93(e1500 n1912)*, MT2633 *unc-103(n500 n1211)*, MT2635 *unc-103(e1597 n1213)*, MT1927 *egl-2(n693 n904)*, MT1928 *egl-2(n693 n905)*, MT1929 *egl-2(n693 n906)*, MT1930 *egl-2(n693 n907)*, JT236 *egl-2(n693 sa236)*, JT6775 *unc-90(e1463 st215)*, CB4461 *unc-110(e1913 e2383)*, CB2842 *unc-58(e665 e2112)*, MT814 *unc-58(e665 n273)*, MT3365 *sup-10(n183)*, JT7524 *eat-12(ad695)*, MT6129 *eat-12(n2368)*, MT1084 *unc-105(n490)*, MT1098 *unc-105-(n506)*, MT2713 *unc-105(n1274)*, JT6759 *unc-90(e1463)*, CB665 *unc-58(e665)*, CB757 *unc-58(e757)*, CB778 *unc-58-(e778)*, CB1320 *unc-58(e1320)*, MT1089 *unc-58(n495)*, CB266 *unc-43(e266)*, CB755 *unc-43(e755)*, MT2598 *unc-43(n498 n1179)*, JT7369 *unc-43(e408)*, CB408 *unc-43(e408) sa306*, JT6052 *egl-2(n693) dpy-11(e224)*, JT6018 *egl-23(n601); dpy-11(e224)*, JT7148 *dpy-11(e224); sup-10(n983)*, JT6146 *exp-4(n2373); dpy-11(e224)*, JT6426 *dpy-11(e224) exp-3(n2372)*, JT7150 *unc-43(n498); dpy-11(e224)*, JT6019 *dpy-11(e224); egl-36(n728)*, JT7159 *egl-30(n715); dpy-11(e224)*, JT7149 *unc-103(n500); dpy-11(e224)*, JT7199 *unc-103(e1597); dpy-11-(e224)*, JT6020 *unc-93(e1500); dpy-11(e224)*, BE93 *dpy-2(e8)*, CB428 *dpy-21(e428)*.

Deficiency strains: JT5635 *+/eT1; sDf34 unc-46(e177)/eT1 let(s2165)*, BC1785 *dpy-18(e364)/eT1; unc-46(e177) sDf30/eT1*, MT1401 *+/szT1; dpy-7(e1324) nDf19/szT1 lon-2(e678)*, MT695 *nDf11/unc-79(e1068) dpy-17(e164)*, MT681 *nDf3/lin-31(n301) bli-2(e768)*, SP262 *mnDp1/+; mnDf1*, DR793 *dpy-13(e184) mDf7/nT1; +/nT1*, DR792 *sDf2/nT1; +/nT1*, CB3823 *eDf18/unc-24(e138) dpy-20(e1282)*, CB3824 *eDf19/unc-24(e138) dpy-20(e1282)*, SP802 *mnDf104 unc-4(e120)/mnC1 dpy-10(e128) unc-52(e444)*, JT6862 *nDf27 dpy-4(e1166)/nT1; +/nT1*, JT6883 *unc-22(s7) unc-31(e169) sDf23/nT1; +/nT1*.

Generating heterozygotes and homozygotes for assays: Most mutant heterozygotes were generated by crossing wild-type males to *mac; dpy-11(e224)* hermaphrodites and picking non-Dpy progeny (where *mac* is a muscle activation mutation). Because of potential confusion with the Mac-h Dpy phenotype, Mac-h heterozygotes were generated by crossing wild-type males to Mac-h homozygotes, and picking less severely Unc Dpy progeny. In all cases, these assayed animals segregated wild-type progeny, confirming their genotype. Most mutations were viable when homozygous, but *exp-2(sa26)* and *unc-110(e1913)* are recessive lethal. Presumed homozygotes for these mutations were obtained from synchronized broods of unbalanced heterozygotes, from which fast-growing progeny were removed. The remaining slow-growing progeny were picked to fresh plates and observed periodically for the duration of their life span.

Mapping: Five new Mac mutations, *n2332, n2372, n2373, n2579* and *n2656*, were isolated by E. JORGENSEN and H. R. HORVITZ in a screen for constipated mutants and were kindly provided to us. These new mutations were mapped to assign them to genes, and the map positions of many previously identified Mac mutations were further refined. These data are summarized in Table 1 and Figure 1. Assignment of new alleles to genes is described below. Linkage to chromosomes was established by two-factor mapping of Mac mutations with recessive markers on each chromosome (data not shown) as previously described (BRENNER 1974; WOOD 1988). After linkage was established, each Mac mutation was simultaneously two- and three-factor mapped with respect to recessive markers to refine its map position. An example of this strategy, used to map the *X*-linked *n2332* mutation, follows. Wild-type males were mated to *n2332* hermaphrodites and hemizygous male progeny were crossed to the double mutant *aex-2(sa3) unc-115(e2225)*. Non-Unc L4 progeny, of genotype *n2332/aex-2 unc-115*, were picked singly to plates. The total number of progeny was estimated, non-Unc non-Aex, Unc-non-Aex, and Aex-non-Unc recombinant progeny were picked singly to plates, and the position of each recombination event was inferred from progeny phenotypes. Non-Unc non-Aex recombinants could arise by a single recombination only if *n2332* mapped outside of the *aex-2 unc-115* interval. Five of 5 Aex-non-Unc recombinants segregated the Egl Exp phenotype caused by *n2332*, while 0 of 9 Unc-non-Aex recombinants segregated *n2332*. Seven non-Unc non-Aex recombinants were found among approximately 4,800 progeny, and all seven segregated Unc Aex progeny. These data indicated that *n2332* lies ~0.3 map units (7/2,400) to the right of *unc-115* (Table 1 and Figure 1). In cases where the phenotype of the Mac mutation interfered with picking recombinants,

TABLE 1

Map data for Mac genes and neighboring genes

Mutation	Relevant parental genotype	Phenotype picked	Recombinant genotype	Number
egl-2(n693sd)	*egl-2/unc-34 unc-60*	Unc-34 non Unc-60	*unc-34 egl-2/unc-34 unc-60*	12
		Unc-34 non Unc-60	*unc-34/unc-34 unc-60*	16
	egl-2/unc-34 unc-60	Wild type	*+/unc-34 unc-60*	0/1,400
egl-2(n2656sd)	*egl-2/unc-34 unc-60*	Wild type	*+/unc-34 unc-60*	0/1,000
egl-23(n601sd)	*egl-23/unc-26 dpy-4*	Unc non Dpy	*unc-26 egl-23/unc-26 dpy-4*	10
			unc-26/unc-26 dpy-4	11
		Dpy non Unc	*egl-23 dpy-4/unc-26 dpy-4*	5
			dpy-4/unc-26 dpy-4	6
	egl-23/unc-31 unc-22 sDf23	Unc-31 Unc-22 non Let	*unc-31 unc-22 egl-23/unc-31 unc-22 sDf23*	14
			unc-31 unc-22/unc-31 unc-22 sDf23	3
	egl-23/nDf27 dpy-4	Dpy non Let	*egl-23 dpy-4/nDf27 dpy-4*	10
			dpy-4/nDf27 dpy-4	5
egl-23(n2579sd)	*egl-23/unc-31 dpy-4*	Unc non Dpy	*unc-31 egl-23/unc-31 dpy-4*	19
			unc-31/unc-31 dpy-4	1
		Dpy non Unc	*dpy-4 egl-23/unc-31 dpy-4*	7
			dpy-4/unc-31 dpy-4	22
egl-36(n728sd)	*egl-36/aex-2 unc-115*	Aex non Unc	*aex-2 egl-36/aex-2 unc-115*	8
		Unc non Aex	*unc-115/aex-2 unc-115*	12
		Wild type	*+/aex-2 unc-115*	7/4,900
	egl-36/unc-115 egl-15	Unc non Egl	*unc-115/unc-115 egl-15*	9
		Wild type	*+/unc-115 egl-15*	0/5,600
egl-36(n2332sd)	*egl-36/aex-2 unc-115*	Aex non Unc	*aex-2 egl-36/aex-2 unc-115*	5
		Unc non Aex	*unc-115/aex-2 unc-115*	9
		Wild type	*+/aex-2 unc-115*	7/4,800
	egl-36/unc-115 egl-15	Unc non Egl	*unc-115/unc-115 egl-15*	1
		Wild type	*+/unc-115 egl-15*	0/2,600
exp-3(n2372sd)	*exp-3/dpy-21 unc-51*	Dpy non Unc	*dpy-21 exp-3/dpy-21 unc-51*	25
			dpy-21/dpy-21 unc-51	6
	exp-3/egl-12	Weak Egl	*+/egl-12*	3/6,000
egl-12(n602sd)	*egl-12/dpy-21 unc-51*	Dpy non Unc	*dpy-21 egl-12/dpy-21 unc-51*	6
			dpy-21/dpy-21 unc-51	16
exp-4(n2373sd)	*exp-4/dpy-9 lin-1*	Dpy non Lin	*dpy-9 exp-4/dpy-9 lin-1*	23
	exp-4/lin-1 unc-33	Unc non Lin	*exp-4 unc-33/lin-1 unc-33*	9
			unc-33/lin-1 unc-33	22
egl-4(n478)	*egl-4/dpy-9 lin-1*	Dpy non Lin	*dpy-9 egl-4/dpy-9 lin-1*	19
			dpy-9/dpy-9 lin-1	12
	egl-4/lin-1 unc-33	Unc non Lin	*egl-4 unc-33/lin-1 unc-33*	24
unc-43(e266)	*unc-43/daf-14 mec-3 dpy-20*	Daf non Dpy	*daf-14 unc-43/daf-14 mec-3 dpy-20*	4
			daf-14/daf-14 mec-3 dpy20	4
			daf-14 mec-3/daf-14 mec-3 dpy-20	27
unc-58(e665sd)	*unc-58/unc-27 aex-2*	Wild type	*+/unc-27 aex-2*	17/3,200
		Unc-27 non Aex	*unc-27/unc-27 aex-2*	10
	unc-58/aex-2 unc-115	Wild type	*+/aex-2 unc-115*	0/800
unc-110(e1913sd)	*unc-110/unc-27 aex-2*	Wild type	*+/unc-27 aex-2*	0/900
	unc-110/dpy-6 aex-2	Dpy Unc	*dpy-6 unc-110/dpy-6 aex-2*	4
		Unc Aex	*unc-110 aex-2/dpy-6 aex-2*	3
	unc-110/aex-2 unc-115	Wild type	*+/aex-2 unc-115*	8/720
			+/+	1/720[a]
		Aex non Unc	*aex-2/aex-2 unc-115*	9
unc-90(e1463sd)	*unc-90/dpy-6 egl-15*	Egl non Dpy	*egl-15/dpy-6 egl-15*	3
			unc-90 egl-15/dpy-6 egl-15	13
aex-2(sa3)	*aex-2/dpy-6 egl-15*	Dpy non Egl	*dpy-6 aex-2/dpy-6 egl-15*	10
			dpy-6/dpy-6 egl-15	3
		Egl non Dpy	*egl-15/dpy-6 egl-15*	6
			aex-2 egl-15/dpy-6 egl-15	8
	unc-27/dpy-6 aex-2	Dpy non Aex	*dpy-6 unc-27/dpy-6 aex-2*	3
			dpy-6/dpy-6 aex-2	9
		Aex non Dpy	*aex-2/dpy-6 aex-2*	4
			unc-27 aex-2/dpy-6 aex-2	9

TABLE 1

Continued

Mutation	Relevant parental genotype	Phenotype picked	Recombinant genotype	Number
aex-2(sa3)	*aex-2/unc-115 egl-15*	Unc non Egl	*unc-115/unc-115 egl-15*	3
		Egl non Unc	*aex-2 egl-15/unc-115 egl-15*	5
	unc-115/aex-2 egl-15	Aex non Egl	*aex-2 unc-115/aex-2 egl-15*	1
			aex-2/aex-2 egl-15	5
		Egl non Aex	*egl-15/aex-2 egl-15*	2
			unc-115 egl-15/aex-2 egl-15	7

Data for three-factor results are presented as the number of recombinants of the genotype listed found among the F_1 population screened. Data for two-factor results are presented as fractions, with the numerator representing the number of recombinants of the class found, and the denominator representing the total number of F_1 animals screened. The deficiency *nDf19* was tested and failed to complement *unc-27* and *egl-15,* but not *aex-2.* Since three-factor results clearly indicate that *aex-2* maps between *unc-27* and *egl-15,* this result is anomalous and may indicate a complex deficiency. The recessive mutation *unc-43(e266)* was deleted by *mDf7* but not by *eDf18, eDf19,* and *sDf2.*

[a] Anomalous recombinant. Presumably represents recombination events in both parents.

only the feasible recombinant classes were picked (*e.g.,* see the *egl-36(n2332)/ unc-115 egl-15* mapping cross in Table 1). A large number of Mac mutations map near the center of the *X* chromosome. To map these Mac mutations better, we also refined the genetic map locations of some of the recessive mutations in this region (*unc-27, aex-2* and *unc-115;* see Table 1 and Figure 1). Deficiency mapping was performed by testing complementation of recessive mutations by deficiencies (Table 1 legend).

Gene assignments: We mapped *n2332, n2372, n2373, n2579,* and *n2656* as described above. *n2332, n2579,* and *n2656* mapped to the same small intervals as previously identified Mac mutations (Table 1): *egl-36(n728)* and *n2332* mapped between *unc-115* and *egl-15; egl-23(n601)* and *n2579* mapped between *unc-26* and *dpy-4;* and *egl-2(n693)* and *n2656* mapped between *unc-34* and *unc-60.* Each of these mutations caused phenotypes that were very similar to the previous mutation in the nearby Mac gene. We also determined that both *egl-2(n693)* and *n2656* have the unusual characteristic that they are rescued for their egg-laying defect by imipramine and clomipramine, but not serotonin or levamisole (see Table 7). Based on these criteria, we have tentatively assigned *n2332, n2579* and *n2656* to *egl-36, egl-23,* and *egl-2,* respectively (see Figure 1). The two other new Mac mutations, *n2372* and *n2373,* each mapped to regions with no known Mac genes. Genes that were somewhat similar phenotypically were excluded on the basis of map data (Table 1) and distinct phenotypes (*egl-12, egl-4,* and *egl-18* mutations cause weaker Egl defects and no EMC defect, data not shown). On the basis of these observations, we have assigned *n2372* and *n2373* to the new genes *exp-3* and *exp-4,* respectively.

Other genetics: We found that strains carrying *egl-2(n693)* conferred a phenotype of repetition of the pBoc step of the defecation motor program (echo pBoc), in addition to its previously described egg-laying defect (TRENT *et al.* 1983) and its EMC defect (see RESULTS). In contrast, the putative new *egl-2* mutation *n2656* did not confer an echo pBoc phenotype. We readily isolated an *egl-2(n693)* recombinant without the echo pBoc, and this outcrossed *egl-2(n693)* strain was used for EMC assays in this study. We named the echo pBoc mutation *sa304.* The canonical recessive allele of *unc-43, e408,* was described to cause a progressive, moderate Egl phenotype (WOOD 1988). We found that no other *unc-43* loss-of-function mutations showed this phenotype, and we readily isolated an *e408* recombinant without the Egl defect. We named the Egl mutation *sa305.* The outcrossed *unc-43(e408)* conferred the same early-egg-laying phenotype as other *unc-43* loss-of-function mutations.

RESULTS

Mutations that cause defective egg-laying and enteric muscle contraction: The semidominant *exp-2(sa26)* mutation was isolated on the basis of a severe defect in the expulsion step of defecation (Exp), but also causes a strong egg-laying (Egl) defect (THOMAS 1990). Using polarized light microscopy, it was found that the defecation and egg-laying muscles were structurally normal in the *exp-2(sa26),* suggesting that this mutation disrupts activation of the muscles rather than their contractile fibers (THOMAS 1990). To analyze additional mutations that disrupt the activation of these muscle groups, we screened for defecation defects in all described Egl mutants that have grossly normal muscle structure. In addition to the *exp-2* mutation, we found that mutations in *egl-2, egl-23, egl-30, egl-36, unc-93, sup-9, sup-10, unc-103,* and *unc-43* conferred both Egl and Exp phenotypes (see Table 2 for references). In addition, five new mutations isolated in a screen for Exp defects were also found to disrupt egg-laying and were provided to us (E. JORGENSEN and H. R. HORVITZ, personal communication). Genetic analysis of these mutations (see MATERIALS AND METHODS) suggested that three of them, *n2656, n2579,* and *n2332,* are alleles of *egl-2, egl-23,* and *egl-36,* respectively (Table 1 and Figure 1). We found that the other two new mutations, *n2372* and *n2373,* were not allelic to previously identified Egl Exp mutations (see MATERIALS AND METHODS), and we assigned *n2372* to the new gene *exp-3* and *n2373* to the new gene *exp-4* (Table 1 and Figure 1). We will refer to the muscle activation phenotypes of this group of mutations as Mac-d (muscle activation-defective). In addition to mapping these new genes, we also refined the map position of seven other genes analyzed in this study (see MATERIALS AND METHODS, Table 1, and Figure 1).

Some Mac-d mutations also affect body-wall and pharyngeal muscles: In addition to their Egl and Exp defects, mutations in *unc-93, sup-9, sup-10, egl-30, unc-103* and *unc-43* cause strong defects in body-wall muscle

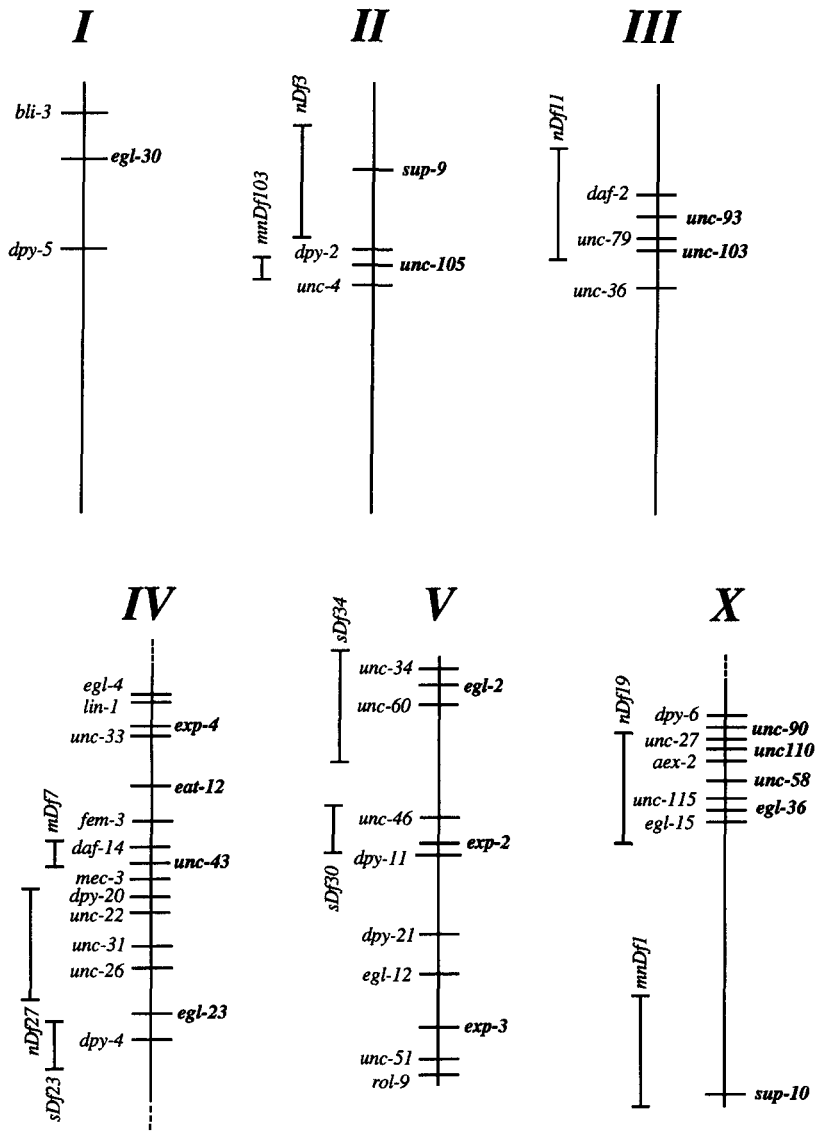

FIGURE 1.—Genetic map of the Mac genes. The six *C. elegans* chromosomes are represented as bold vertical lines labeled by roman numerals. Mac genes are labeled in bold type to the right of each chromosome. Genes used for mapping each Mac gene (Table 1) and for positional context are labeled to the left of each chromosome. Deficiencies used in these analyses are represented as vertical lines to the left of each chromosome. The entirety of some chromosomes is not shown, as indicated by dashed lines on the ends, and distances are not necessarily proportional.

contraction (Unc, for uncoordinated), as previously described (see Table 2). With the exception of *unc-43*, whose Unc phenotype is atypical (see below), the locomotion phenotype of these mutants is similar to that seen in mutants with defective muscle-structure (*e.g. unc-54, unc-60* and *unc-15*). Both Mac-d mutants and muscle-structure mutants display flaccid paralysis and are longer than the wild type, indicating that tonic muscle contraction contributes to animal length in the wild type. The Mac-d mutants, unlike severe muscle-structure mutants (WATERSTON *et al.* 1980), are capable of some locomotory movements in response to touch. An example of Mac-d response to touch is seen in *unc-93(e1500sd), sup-9(n1550), sup-10(n983),* and *unc-110(e1913),* which briefly recoil and then relax when touched (the "rubber-band" response; GREENWALD and HORVITZ 1980). When undisturbed, Mac-d animals move very little and adopt a straight body posture similar to that of the muscle-structure mutants. We show photographs of the wild type, *unc-54,* and *unc-93* to illustrate these body posture defects (Figure 2A–C). We also show a photograph of *egl-2,* an Egl Exp mutant that is not severely defective in locomotion (Figure 2G). Mutations in two of the Mac-d genes, *exp-2* and *sup-9,* also confer defects in pharyngeal muscle contraction. Based on these qualitative phenotypic observations, we show a synopsis of the muscle tissues affected by mutations in each of the Mac-d genes (Table 3).

In addition to their distinctive phenotypes, the Mac-d genes also share striking genetic characteristics. The Mac-d phenotypes of all eleven genes are caused by semidominant mutations (see below, Table 6). Recessive revertants of the dominant mutations in seven of these 11 Mac-d genes have been isolated (in our lab and by others, see Table 2). We and others have examined these loss-of-function mutants closely, and in each case their phenotype is grossly wild type (see Table 2 for references). While the null mutant phenotype is not known for most of the Mac-d genes (or the other Mac genes, see below), the frequency with which revertants of dominant Mac mutations are isolated suggests that these mutations represent strong loss-of-func-

TABLE 2

Summary of Mac mutations

Gene	Mutation	Phenotype	Publication or source
exp-2	*sa26*	Mac-d	THOMAS (1990)
exp-3	*n2372*	Mac-d	E. JORGENSEN and H. R. HORVITZ, personal communication
exp-4	*n2373*	Mac-d	E. JORGENSEN and H. R. HORVITZ, personal communication
egl-2	*n693*	Mac-d	TRENT *et al.* (1983)
	n2656	Mac-d	E. JORGENSEN and H. R. HORVITZ, personal communication
	n693 n904, n693 n905, n693 n906, n693 n907	wt	C. TRENT and H. R. HORVITZ, personal communication
	n693 sa236	wt	D. WEINSHENKER and J. THOMAS, unpublished data
egl-23	*n601*	Mac-d	TRENT *et al.* (1983)
	n2579	Mac-d	E. JORGENSEN and H. R. HORVITZ, personal communication
	n601 sa179, n601 sa180, n601 sa181	wt	D. REINER and J. THOMAS, unpublished data
egl-36	*n728*	Mac-d	TRENT *et al.* (1983)
	n2332	Mac-d	E. JORGENSEN and H. R. HORVITZ, personal communication
sup-9	*n1550*	Mac-d	LEVIN and HORVITZ (1993)
	n180	wt	GREENWALD and HORVITZ (1980)
sup-10	*n983*	Mac-d	GREENWALD and HORVITZ (1986)
	n183		
unc-93	*e1500, n200*	Mac-d	GREENWALD and HORVITZ (1980)
	e1500 n1912	wt	I. GREENWALD and H. R. HORVITZ, personal communication
egl-30	*n715, n686*	Mac-d	TRENT *et al.* (1983)
	ad805	Mac-d	L. AVERY, personal communication
	n715 n1189	wt	PARK and HORVITZ (1986a)
unc-103	*e1597*	Mac-d	J. HODGKIN, personal communication
	n500	Mac-d	PARK and HORVITZ (1986a)
	n500 n1211, e1597 n1213	wt	PARK and HORVITZ (1986a)
eat-12	*ad695*	Mac-h	AVERY (1993)
	n2368	Mac-h	R. LEE, M. HENGARTNER, L. AVERY, and H. R. HORVITZ, personal communication
unc-43	*n498*	Mac-m	PARK and HORVITZ (1986a)
	n498 n1179	Mac-m	PARK and HORVITZ (1986a)
	e266, e408, e755	Mac-m	BRENNER (1974)
unc-110	*e1913*	Mac-m	D. THIERRY-MIEG, personal communication
	e1913 e2383	wt	D. THIERRY-MIEG, personal communication
unc-58	*e665, e757, e778*	Mac-h	BRENNER (1974)
	e1320	Mac-h	BRENNER (1974)
	n495	Mac-h	PARK and HORVITZ (1986a)
	e665 e2112	wt	S. BRENNER, personal communication
	n495 n273	wt	I. GREENWALD and H. R. HORVITZ, personal communication
unc-90	*e1463*	Mac-h	R. WATERSTON, personal communication
	e1463 st215	wt	B. SCHRANK and R. WATERSTON, personal communication
unc-105	*n490, n506, n1274*	Mac-h	PARK and HORVITZ (1986a)
	n490 n785, n490 n786	wt	E. PARK and H. R. HORVITZ, personal communication

The source of these mutations and, in some cases, the initial descriptions of their phenotypes are listed. In all cases these phenotypes were confirmed by our observations.

tion or null alleles (see Table 2 for references). Their similar phenotypes and unusual genetic characteristics suggest that the Mac-d genes function in the same general process.

Mutations causing hyperactivated muscle: Some muscle activation mutations might be expected to cause phenotypes opposite of the Mac-d phenotypes. The phenotype of severely hypercontracted body-wall muscle has been described (EIDE and ANDERSON 1985). We call this phenotype hyperactivated muscle (Mac-h), which encompasses inappropriate activation and defective inactivation of muscles. Therefore, we screened for hyperactivated muscle (Mac-h) mutations. This term is intentionally vague, as we intend it to encompass mutations that generally cause inappropriate activation (or defective inactivation) of muscles. Mutations in four genes, *unc-58, unc-90, unc-105,* and *eat-12* cause a dumpy (Dpy) phenotype that is thought to be due to hyper-activation of body-wall muscle (PARK and HORVITZ 1986a; AVERY 1993; B. SCHRANK and R. WATERSTON, personal communication). We found that mutations in all four of these genes caused animals to retain many fewer eggs in their uterus and to lay eggs of an earlier stage than normal, suggesting that Mac-h mutations also cause hyperactivation of the egg-laying muscles. Severe mutations, such as the *unc-58* mutations

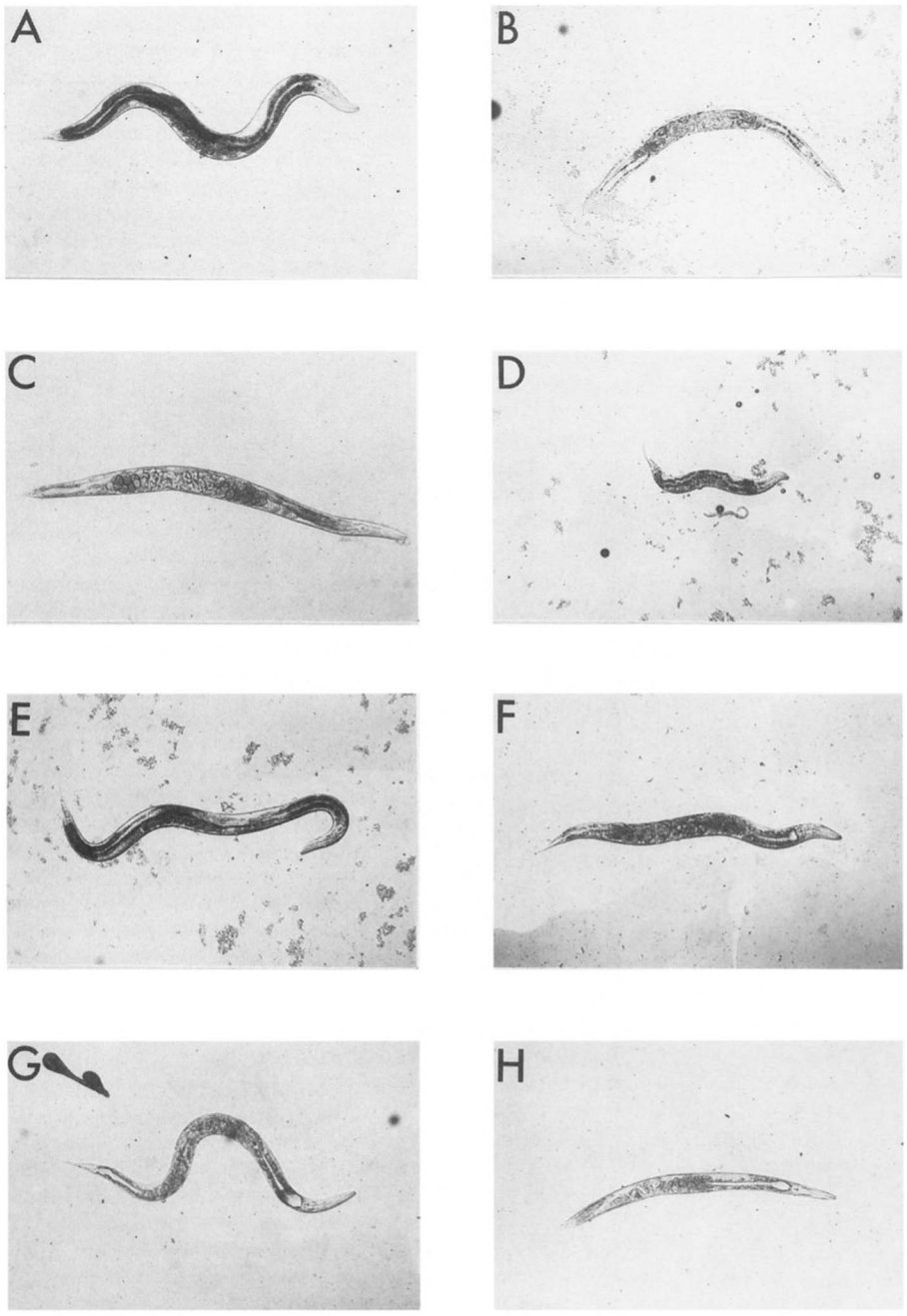

FIGURE 2.—Bright-field photomicrographs of live animals. (A) Wild type, (B) *unc-54(e190)*, (C) *unc-93(e1500)*, (D) *unc-105(n490)*, (E) *unc-110(e1913)/+*, (F) *unc-43(n498)*, (G) *egl-2(n693)*, and (H) *unc-54(e190); unc-105(n490)* double mutant.

e665, e495, e757, e1320, unc-90(e1463), unc-105(n490), and *eat-12(n2368),* dramatically perturb locomotion, perhaps because the muscles are nearly fully contracted in a phenomenon similar to tetanus (GORDON 1989). Less severe mutations, such as *eat-12(ad695), unc-58(e415),* and *unc-58(e778),* are less Dpy and cause only mildly perturbed locomotion, presumably because the muscles still retain capacity for further contraction. Mutations in *eat-12* also cause delayed relaxation of the pharyngeal terminal bulb muscles (AVERY 1993), suggesting that they cause hyperactivated pharyngeal muscle. We were unable to determine conclusively whether or not the Mac-h mutations cause hyperactivation of the enteric muscles, because of the very brief and quantal nature of enteric muscle contraction (EMC), in which a single contraction occurs approximately every 45 sec (THOMAS 1990). Though we could not measure the strength of these contractions, we determined that they do not occur at inappropriate times during the defecation motor program (data not shown). A photograph of *unc-105(n490),* which displays a severe Mac-h phenotype, is shown in Figure 2D. Based on these qualitative phenotypic observations, a synopsis of these Mac-h genes is shown (Table 4). As with the Mac-d genes, all four Mac-h genes are defined by semidominant mutations, and revertants of the dominant mutations in three of these genes have grossly wild-type phenotypes (see MATERIALS AND METHODS for references).

A Dpy phenotype is usually associated with cuticle defects (BRENNER 1974; LEVY *et al.* 1993) or with defects in dosage compensation (PLENEFISCH *et al.* 1989). We tested whether the Dpy and early-egg-laying phenotypes

TABLE 3

Summary of Mac-d genes and affected muscle groups

Gene	Pharyngeal muscle	Body-wall muscle	Egg-laying muscle	Enteric muscle
egl-2(sd)	+	+	d	d
egl-23(sd)	+	+	d	d
egl-36(sd)	+	+	d	d
exp-3(sd)	+	+	d	d
exp-4(sd)	+	+	d	d
exp-2(sd)[a]	d	+	d	d
unc-93(sd)	+	d	d	d
sup-9(sd)	d	d	d	d
sup-10(sd)	+	d	d	d
egl-30(sd)	+	d	d	d
unc-103(sd)	+	d	d	d

+, grossly normal function of the particular muscle group; d, muscle activation defective phenotype (Mac-d); sd, a semidominant mutation conferring the phenotypes listed.

[a] Phenotype of heterozygotes, since homozygotes are lethal.

of mutants for the four Mac-h genes were dependent on muscle contraction by building double mutants with mutations in *unc-54,* which encodes the major myosin isoform for the body-wall, egg-laying, and enteric muscles (EPSTEIN *et al.* 1974; WATERSTON 1988). The *unc-54* mutation was epistatic to mutations in all four Mac-h genes for both body-wall and egg-laying muscles. Specifically, all double mutant combinations were long, flaccid, paralyzed and egg-laying defective. Photographs of *unc-105(n490)* and the *unc-105(n490); unc-54* double mutant are shown (Figure 2, D and H). In each case, the *unc-54; mac-h* double mutant animals were slightly Dpy in comparison to *unc-54* single mutant animals, probably as a result of residual contraction of *unc-54* muscles. As controls, we constructed *unc-54* double mutants with *dpy-2(e8),* a mutation causing cuticle collagen defects (LEVY *et al.* 1993), and *dpy-21(e428),* a mutation causing dosage compensation defects (PLENEFISCH *et al.* 1989). In both cases, *unc-54* did not suppress the Dpy phenotypes (data not shown). Some of these observations have been previously reported (EIDE and ANDERSON 1985; PARK and HORVITZ 1986b). These observations demonstrate that the Mac-h defects depend on functional muscle, presumably because they inappropriately activate muscle contraction.

TABLE 4

Summary of Mac-h genes and affected muscle groups

Gene	Pharyngeal muscle	Body-wall muscle	Egg-laying muscle	Enteric muscle
unc-58(sd)	+	h	h	ND
unc-90(sd)	+	h	h	ND
unc-105(sd)	+	h	h	ND
eat-12(sd)	h	h	h	ND

+, grossly normal function of the particular muscle group; h, a hyperactivated muscle phenotype (Mac-h); ND, listed for all enteric muscle entries because we could not readily assay whether or not the Mac-h mutations caused enteric muscle hyperactivation; sd, a semidominant mutation conferring the phenotypes listed.

TABLE 5

Summary of Mac-m genes and affected muscle groups

Genotype	Pharyngeal muscle	Body-wall muscle	Egg-laying muscle	Enteric muscle
unc-43(sd)	+	h	d	d
unc-43(r)	+	d	h	ND
unc-110(sd)[a]	+	d	h	ND

+, grossly normal function of the particular muscle group; d, a muscle activation defective phenotype (Mac-d); h, a hyper-activated muscle phenotype (Mac-h); sd, a semidominant mutation conferring the phenotypes listed; r, recessive mutations. ND is listed for some enteric muscle entries because we could not readily assay whether or not the Mac-m mutations caused enteric muscle hyperactivation. Other symbols are as in Tables 3 and 4.

[a] Phenotype of heterozygotes, since homozygotes are lethal.

Mutations causing a mixture of defective and hyperactivated muscle: We found that mutations in two genes, *unc-43* and *unc-110,* caused phenotypes in locomotion, egg laying, or defecation that were reminiscent of Mac-d or Mac-h phenotypes. However, in contrast to the Mac-d and Mac-h genes, these mutations appeared to hyperactivate some muscle types and inactivate others (see below). We designate these phenotypes as Mac-m, for muscle activation-mixed. The *unc-43(n498)* mutation, described above as Egl and Exp (Mac-d), is also locomotion defective, and confers a moderate Dpy phenotype (Mac-h) that is suppressed by a mutation in *unc-54* (our data not shown). Putative loss-of-function mutations of *unc-43* have been identified both by their recessive phenotype (BRENNER 1974) and by reverting the dominant *unc-43(n498)* mutation (PARK and HORVITZ 1986a). The phenotype caused by these *unc-43* recessive mutations is a flaccid Unc somewhat reminiscent of Mac-d mutants, and an early-egg-laying phenotype similar to that of Mac-h mutants (the canonical recessive *unc-43* allele, *e408,* was mistakenly described as causing a mild egg-laying defective phenotype, see MATERIAL AND METHODS). The early-egg-laying phenotype of recessive *unc-43* mutations is also suppressed by mutations in *unc-54.* These loss-of-function phenotypes of *unc-43* are reciprocal to the semidominant mutant phenotypes of *unc-43(n498)* (Table 5).

The *unc-110(e1913)* mutation also causes a Mac-m phenotype but with a pattern of defects that is largely reciprocal to *unc-43(n498). unc-110(e1913)/+* animals are long and flaccidly paralyzed, with a body-wall phenotype very similar to the Mac-d mutation *unc-93(e1500),* but retain very few eggs, similar to the phenotype of Mac-h mutants for egg laying (Figure 2E). Like the Mac-h mutants, the early-egg-laying phenotype of *unc-110(e1913)* is suppressed by a mutation in *unc-54,*

TABLE 6

Semidominance of Mac mutations

Mutation	Percent EMC		Egg laying (Serotonin)		Heterozygote locomotion
	Homozygote	Heterozygote	Homozygote	Heterozygote	
Wild type	100 (143)	NA	7.9 (36)	NA	NA
egl-2(n693) (d)	0 (173)	24 (166)	0.1 (35)	2.0 (35)	NA[a]
egl-23(n601) (d)	3 (176)	24 (160)	0.0 (24)	0.2 (36)	NA[a]
egl-36(n728) (d)	30 (150)	43 (141)	0.0 (36)	1.6 (36)	NA
exp-3(n2372) (d)	16 (168)	42 (147)	0.0 (32)	0.0 (36)	NA
exp-4(n2373) (d)	17 (151)	32 (121)	0.2 (34)	1.1 (36)	NA
egl-30(n715) (d)	59 (122)	82 (146)	1.3 (35)	8.0 (36)	Unc
unc-103(e1597) (d)	47 (121)	58 (121)	0.0 (34)	0.1 (36)	Unc
unc-93(e1500) (d)	5 (144)	32 (133)	0.0 (36)	0.4 (35)	Unc
sup-10(n983) (d)	36 (142)	99 (115)	0.0 (36)	6.2 (34)	Unc[b]
unc-43(n498) (m)	9 (125)	99 (101)	0.2 (35)	5.5 (35)	Unc[b]
exp-2(sa26)[c] (d)	Lethal	2 (106)	Lethal	0.7 (36)	NA
sup-9(n1550)[c] (d)	Lethal	15 (145)	Lethal	0.1 (36)	Unc
unc-110(e1913)[c] (m)	Lethal	NA	Lethal	Egl-c[d]	Unc
unc-58(e665) (h)	NA	NA	Egl-c[d]	Egl-c[d]	Unc
unc-90(e1463) (h)	NA	NA	Egl-c[d]	Egl-c[d]	Unc
unc-105(n490) (h)	NA	NA	Egl-c[d]	Egl-c[d]	Unc
eat-12(ad695) (h)	NA	NA	Egl-c[d]	Egl-c[d]	Unc

The number in parentheses after the genotype represents the type of Mac mutation. d, muscle activation defective; h, hyperactivated muscle; m, muscle activation-mixed. Percent enteric muscle contraction per defecation cycle (%EMC) for homozygous and heterozygous canonical Mac-d mutations are shown. Ten animals were analyzed per genotype, and the number of defecation cycles observed is listed in parentheses. Additional semidominant alleles of each locus were assayed only as homozygotes, with the following results: *egl-2(n2656)* 0% (173), *egl-23(n2579)* 1% (173), *egl-36(n2332)* 33% (148), *egl-30(ad805)* 85% (114), *egl-30(n686)* 78% (146), *unc-103(n500)* 61% (117), and *unc-93(n200)* 50% (117). EMC frequency is normal for Mac-h mutations, but ND is listed because we could not readily assay hyperactivated enteric muscles. Egg-laying response of Mac-d homozygotes *vs.* heterozygotes to serotonin (10 mg/ml) is also shown. The number of animals tested for each genotype is listed in parentheses. For those Mac mutations affecting locomotion as homozygotes, the heterozygote locomotion phenotype is listed. Those Mac mutations that do not affect locomotion have NA listed.

[a] *egl-2* and *egl-23* confer modest recessive Unc phenotypes. These defects do not appear to be muscular in nature and may be due to these genes functioning in neurons that regulate locomotion.

[b] *sup-10(n983)* and *unc-43(n498)*, which cause weak locomotion defects as heterozygotes, were evaluated in blind experiments.

[c] Quantitative analysis of homozygotes of these genotypes are not possible due to lethality (*exp-2* and *unc-110*) or sub-viability (*sup-9*).

[d] Both homozygous and heterozygous Mac-h mutants laid early eggs even in the absence of serotonin (Egl-c; egg-laying constitutive), and were not easily distinguished from each other.

indicating that it requires functional egg-laying muscle (data not shown). A single revertant of *unc-110(e1913)* has been isolated and it has no obvious defects (D. THIERRY-MIEG, personal communication; and our observations). A qualitative synopsis of the tissues affected by the Mac-m mutations is shown in Table 5.

Quantitative analysis of dominance in Mac mutants: To assess phenotypes more quantitatively and to assess the degree of dominance of the Mac mutations, we measured the defects of homozygous and heterozygous Mac-d mutations for percent enteric muscle contraction (%EMC) and mean eggs laid per animal in response to serotonin (MATERIALS AND METHODS; Table 6). Heterozygotes were generated as described in MATERIALS AND METHODS. Where applicable, locomotion defects of homozygotes and heterozygotes were qualitatively assayed (MATERIALS AND METHODS; Table 6). Additional alleles at each locus (when available) were assayed as homozygotes for EMC and egg-laying response to serotonin (see legends to Tables 6 and 7 for data). Mac-h homozygotes and heterozygotes were also qualitatively compared for their muscle contraction abnormalities. In each case, the Mac-h heterozygote locomotion defect was clearly less severe than that of the homozygote. The early-egg-laying phenotype caused by Mac-h mutations was qualitatively similar in heterozygotes and homozygotes. These observations are summarized in Table 6. Homozygous and heterozygous phenotypes of Mac-m mutations were assayed according to their Mac-d or Mac-h character and these data are also summarized in Table 6. Although not all phenotypes of every mutation displayed dominance (*e.g., unc-43(n498)* EMC in Table 6), every mutation displayed clear semidominance for at least one phenotype (Table 6).

We were unable to assay behavior in homozygotes for three of the Mac genes because the Mac mutations conferred recessive lethality or sub-viability. *exp-2(sa26)* and *unc-110(e1913)* homozygotes arrest as early larvae, and *sup-9(n1550)* homozygotes are subviable and can barely be grown as a single mutant strain (THOMAS

1990; LEVIN and HORVITZ 1993; D. J. REINER, D. WEINSHENKER and J. H. THOMAS, unpublished observations). *sup-9(n1550)* homozygotes had very slow pharyngeal pumping, which may be responsible for their subviability. The extreme starved phenotype of *sup-9(n1550)* homozygotes made quantitative analysis of egg laying and EMC dubious, though we observed qualitatively that both behaviors were more severely defective in homozygotes than in heterozygotes, as was the locomotion phenotype. *exp-2(sa26)* homozygotes arrested as L1 larvae, but were viable for some days and moved about on the plate (THOMAS 1990). These *exp-2(sa26)* homozygote larvae did not have visible pharyngeal pumps, while *exp-2(sa26)/+* heterozygotes have weak, shallow pharyngeal pumps, demonstrating the semidominance of this mutation. We speculate that the L1 arrest of *exp-2(sa26)* homozygotes may be due to starvation caused by severely impaired pharyngeal muscle excitation. *unc-110(e1913)* homozygotes were extremely paralyzed, displayed infrequent pharyngeal pumping, and rarely reached adulthood to lay one or two eggs. In addition, their body morphology was variably distorted, with lumps in the head region. We were unable to discern whether the *unc-110(e1913)* lethality was likely to be due to this morphological defect, the pumping defect, or some other uncharacterized defect. Several recombinants in the *unc-27 aex-2* interval failed to separate *unc-110(e1913)* from the lethal phenotype (data not shown). However, the fact that *unc-110(e1913)* homozygotes are more severely Unc than heterozygotes suggests that this mutation also is semidominant.

One way that a mutation can cause dominance is by haploinsufficiency of the locus, in which the loss-of-function heterozygote does not have enough gene product to provide wild-type function (PARK and HORVITZ 1986a; HOMYK and EMERSON 1988; GREEN *et al.* 1990). Deficiency heterozygotes (*Df/+*) for some Mac genes (*exp-2, unc-93* and *unc-105*) have been analyzed previously (Table 2; see Figure 1). Here we have also analyzed deficiency heterozygotes for the following Mac genes: *egl-2, egl-36, sup-9, sup-10, unc-43, unc-58, unc-103* and *unc-110* (Figure 1). In each case the deficiency did not cause dominant muscle defects, indicating that the semidominant mutations in these Mac genes cannot be simple loss-of-function mutations. Though we were unable to analyze deficiencies that delete *egl-23, egl-30* and *unc-90,* wild-type revertants have been identified for each gene (see Table 2). There is no evidence bearing on the nature of the dominance for *eat-12, exp-3,* or *exp-4,* since neither revertants of the dominant mutations nor deficiencies of the loci have been identified. Based on these observations, we argue that most of the Mac mutations represent gain-of-function mutations. It has been suggested that such gain-of-function mutations produce mutant gene products that are toxic to the process in which the wild-type gene product functions (GREENWALD and HORVITZ 1980, 1986).

The Mac genes probably function in muscle: Based primarily on their mutant phenotypes, we hypothesized that the Mac genes act in muscle. In order to determine the nature of their muscle defects, we examined muscle ultrastructure using polarized light microscopy (WATERSTON *et al.* 1977). By this test, most of the Mac-d mutants had grossly normal muscle ultrastructure as scored in a blind experiment (see MATERIALS AND METHODS). However, as previously noted (GREENWALD and HORVITZ 1980), *unc-93(e1500)* causes slight disorganization of the body-wall muscle fibers, though no decrease in muscle birefringence. We observed a similar defect in *sup-9(n1550),* which is phenotypically similar to *unc-93(e1500)* and has been shown to interact genetically with *unc-93* (GREENWALD and HORVITZ 1980, 1982, 1986; LEVIN and HORVITZ 1993). We also noted slight disorganization of body-wall muscle in *unc-103(e1597)* and *unc-103(n500)* as previously described (PARK and HORVITZ 1986a). These slight abnormalities in muscle fiber organization are in sharp contrast to the severe disorganization observed in many muscle-structure mutants. We also examined the muscle birefringence of the Mac-h mutants. We found that the most severe Mac-h mutant, *unc-105(n490),* had modest disorganization of the muscle fibers in the body-wall muscles. *unc-90(e1463),* phenotypically the next most severe Mac-h mutation, displayed milder disorganization. The other Mac-h mutants appeared normal for muscle ultrastructure. We did not detect any defects in egg-laying and enteric muscles in any of the Mac mutants, but the small size of these muscles may have prevented visualizing minor defects. We conclude that most Mac mutations do not grossly affect the muscle contractile unit. In the few cases in which mild disorganization was observed, its severity was not in proportion to the severity of the muscle contraction defect (when compared with mutations in known muscle structure genes), suggesting that the disorganization is not a primary phenotype. We speculate that the modest muscle disorganization seen in the most severe Mac-h mutants is a side effect of their abnormal tonic muscle contraction.

We tested the hypothesis that Mac-d mutants have muscle that is capable of contraction by activating the muscles ectopically. We found that strong muscle contraction resulted when a laser microbeam was focused on the cell body of certain muscles in unanesthetized wild-type animals (see MATERIALS AND METHODS). Using this method, we consistently evoked contraction of the anal depressor muscle (an enteric muscle) and body-wall muscles, but not egg-laying and pharyngeal muscle. When the laser was used to activate the anal depressor and body-wall muscles in each Mac-d mutant, we consistently observed strong contractions similar to those seen in the wild-type (see MATERIALS AND METHODS for data). In a control experiment, we found that the muscles of *unc-54, unc-60* and *act-1,2,3* mutant animals responded poorly or not at all to laser excitation. These results, coupled with the muscle ultrastructure observations, suggest that the contractile apparatus of Mac muscles

TABLE 7

Pharmacology of egg laying in Mac-d mutants

Genotype	No drug	Serotonin	Levamisole	Clomipramine	Imipramine
Wild type	2.0 (48)	8.4 (311)	6.5 (66)	9.1 (60)	10.4 (59)
egl-2(n693)	0.0 (36)	0.1 (35)	0.4 (35)	9.2 (30)	3.9 (12)
egl-23(n601)	0.0 (12)	0.0 (36)	0.0 (36)	0.1 (36)	0.0 (12)
egl-36(n728)	0.0 (12)	0.0 (36)	0.0 (36)	0.1 (36)	0.0 (12)
exp-3(n2372)	0.0 (12)	0.0 (32)	0.0 (36)	0.0 (36)	0.0 (19)
exp-4(n2373)	0.0 (12)	0.2 (34)	0.2 (36)	0.0 (36)	0.0 (20)
unc-43(n498)	0.0 (12)	0.2 (35)	0.2 (35)	0.5 (36)	0.7 (12)
exp-2(sa26)/+	0.3 (12)	0.7 (36)	0.8 (36)	1.6 (36)	0.7 (12)
egl-30(n715)	0.4 (12)	1.3 (35)	0.5 (36)	0.0 (36)	0.0 (12)
sup-9(n1550)/+	0.0 (12)	0.1 (36)	0.4 (36)	0.0 (35)	0.5 (12)
unc-103(e1597)	0.0 (12)	0.1 (34)	0.2 (36)	0.0 (36)	0.0 (12)
unc-93(e1500)	0.1 (12)	0.0 (36)	0.1 (36)	0.0 (36)	0.0 (12)
sup-10(n983)	0.0 (12)	0.0 (36)	0.0 (36)	0.0 (36)	0.2 (12)

Egg laying was measured in no drug and in response to serotonin (10 mg/ml), levamisole (100 μg/ml), clomipramine (0.75 mg/ml), and imipramine (0.75 mg/ml). The numbers listed are mean eggs laid per animal in well assays (see MATERIALS AND METHODS) with number of animals assayed listed in parentheses. Additional alleles of each gene were assayed with no drug (data not shown) and for serotonin response, with the following results: *egl-2(n2656)* 0.0 (34), *egl-23(n2579)* 0.0 (48), *egl-36(n2332)* 0.6 (36), *egl-30(ad805)* 1.7 (36), *egl-30(n686)* 1.6 (35), *unc-103(n500)* 0.0 (35), and *unc-93(n200)* 0.0 (34).

is intact and functional. We conclude that the Mac mutants are defective either in aspects of muscle excitation, excitation-contraction coupling, or in presynaptic neuron function.

To test whether Mac mutations cause defects post- or presynaptically, we assayed the egg-laying response of mutant and wild-type animals to each described potent pharmacological activator of egg laying (see MATERIALS AND METHODS). Previous work suggests that serotonin functions as an excitatory neurotransmitter directly on egg-laying muscle (TRENT *et al.* 1983; DESAI *et al.* 1988; MENDEL *et al.* 1995; SÉGALAT *et al.* 1995). Mutants defective in the serotonergic HSN motor neurons have an Egl defect, but lay eggs well in response to exogenous serotonin. In contrast, mutants defective in the vulval muscles or vulval structure are resistant to exogenous serotonin (TRENT *et al.* 1983). The nicotinic acetylcholine agonist levamisole also activates egg laying (LEWIS *et al.* 1980), and the tricyclic antidepressants imipramine and clomipramine can induce egg laying both by blocking the reuptake of serotonin (TRENT *et al.* 1983) and in a serotonin-independent manner (D. WEINSHENKER, G. GARRIGA and J. THOMAS, unpublished data). We found that all mutations that confer a Mac-d phenotype for egg laying were strongly resistant to each of these pharmacological agents (Table 7), with the exception of *egl-2* (as previously noted by TRENT *et al.* 1983). The *egl-2* mutant is strongly resistant to levamisole and serotonin, like the other Mac-d mutants, but lays eggs well in response to imipramine and clomipramine (Table 7). These results suggest that the Egl phenotype of most Mac-d mutants is caused by postsynaptic rather than presynaptic defects. This suggestion is strongly supported by previous genetic analysis for *unc-93, sup-9,* and *sup-10* (see DISCUSSION). A more detailed analysis of *egl-2* pharmacology suggests that it also causes a post-synaptic defect, despite its response to tricyclic antidepressants (D. WEINSHENKER, G. GARRIGA and J. THOMAS, unpublished data). Since their muscles retain the capacity to contract but fail to contract in response to these agonists, we propose that the Mac genes function in excitation or excitation-contraction coupling. Supporting this model is the observation that egg-laying defects as severe as those seen in the Mac-d mutants are seen in only two other categories of mutants (our unpublished observations): those known to disrupt muscle structure or development strongly (WATERSTON 1988), and those in which the vulva is abnormal (FERGUSON *et al.* 1987). A limitation of our analysis is that we were unable to laser activate and measure pharmacological stimulation of the same muscle groups in Mac-d mutants and could not test Mac-h mutants pharmacologically. However, we hypothesize that our conclusions based on experiments on one muscle tissue in Mac mutants can be extended to other tissues.

DISCUSSION

Mac genes probably act in muscle: We think that most or all of the Mac mutations cause defects in muscle. Three lines of evidence support this hypothesis. First, the phenotypes caused by Mac-d mutations are characteristic of defects in muscle activation. Second, our pharmacological analysis of egg-laying behavior suggests that these mutations prevent synaptic activation of muscle contraction. Third, genetic mosaic analyses of two Mac genes, *unc-93* (HERMAN 1984) and *unc-105* (PARK and HORVITZ 1986b), indicate that these genes act in muscle. In addition, *unc-93* has been shown to encode a novel putative transmembrane protein (LEVIN and HORVITZ 1992), consistent with a role in muscle excitation, and *unc-105* has been shown to en-

code a protein with sequence similarity to a subunit of the amiloride-sensitive sodium channel (B. SCHRANK and R. WATERSTON, personal communication). Dominant activated mutations in *unc-105* cause a Mac-h phenotype, presumably due to inappropriate influx of Na^+ into the muscle. The phenotypic similarity of other Mac mutants to *unc-93* (Mac-d) and *unc-105* (Mac-h) supports the idea that they, too, act in muscle. Because the severity of phenotype conferred by most dominant Mac mutations is reduced by the presence of wild-type copies of the gene (our data, not shown; GREENWALD and HORVITZ 1980; PARK and HORVITZ 1986a), it is likely that the wild-type Mac gene product also functions in muscle.

An alternative explanation of the Mac mutations is that they affect the function of neurons that regulate the activation of muscles. In *C. elegans,* a number of examples have been described of abnormal neuronal function that cause either hyperactive (DESAI *et al.* 1988; MCINTIRE *et al.* 1993a,b; MENDEL *et al.* 1995; SÉGALAT *et al.* 1995) or defective (LEWIS *et al.* 1980; DESAI *et al.* 1988; THOMAS 1990; MCINTIRE *et al.* 1993a,b) muscle activation. However, in each of these cases only one muscle tissue was affected. This presumably results from the fact that each of these muscle tissues receives separate motor innervation using at least partially distinct neurotransmitters (CHALFIE *et al.* 1985; MCINTIRE *et al.* 1993a,b). All of the Mac mutations described here affect more than one muscle group, suggesting that they affect a process common to the different muscles.

We have focused on the common function of the Mac genes in muscle excitation. However, it is entirely plausible that Mac genes have additional functions in neurons. We note that several Mac mutations confer defects not described here, which we interpret as neuronal rather than muscular (our data, not shown). A precedent in *C. elegans* for a gene that controls both muscle activation and neuronal function is provided by the *goa-1* gene, which encodes a heterotrimeric G-protein α subunit (MENDEL *et al.* 1995; SÉGALAT *et al.* 1995).

Gene interactions within the Mac group: Because we propose that the Mac genes function in the muscle excitation process, one might expect to find interactions among these genes. However, with the exceptions noted below, when the majority of dominant Mac mutations were reverted, only tightly linked (presumably intragenic) revertants were found (see Table 2 for references). *unc-93, sup-9,* and *sup-10* have been shown to interact genetically, with dominant mutations in any one gene being suppressed by loss-of-function mutations in one of the others, as well as mutations in the genes *sup-11* and *sup-18* (GREENWALD and HORVITZ 1980, 1982 and 1986; LEVIN and HORVITZ 1993). Based on these and other genetic interactions, it has been proposed that these gene products function in a complex (GREENWALD and HORVITZ 1986; LEVIN and HORVITZ 1993). *unc-105(n490)* is suppressed by rare recessive mutations in *sup-20.* In contrast to genes in the *unc-93* group, *sup-20* has not been dominantly mutated to confer a Mac phenotype, and putative *sup-20* null mutations cause embryonic lethality (PARK and HORVITZ 1986b). Since extragenic suppressors of most of the Mac genes are not readily isolated, we hypothesize that these Mac gene products do not act in heteromultimeric complexes. Alternatively, interacting genes may be redundant or required for viability, as seen for *sup-11* (GREENWALD and HORVITZ 1982) and *sup-20* (PARK and HORVITZ 1986b).

The Mac process is perturbed mainly by semidominant mutations: A striking feature of many of the Mac genes is that only semidominant mutations cause strong muscle-defective phenotypes. What might explain this property? The regulation of excitation of neuronal and muscle cells can be very complex (HILLE 1992a,b), including components that are not required for excitation *per se,* since they make only modest contributions to the excitable state of the cell. Such components may be used for fine tuning the strength or frequency of contractions or to respond to varying environmental conditions. Loss-of-function mutations in such genes would not dramatically perturb muscle function. However, dominant mutations in such genes could perturb the function of the cell out of proportion to the contribution of the wild-type gene product. Alternatively, the Mac genes could be functionally redundant with other genes (THOMAS 1993). Again, dominant mutations in such a redundant gene could actively disrupt muscle excitation, in contrast to loss-of-function mutations, which would cause little or no defect.

On the basis of two findings, it has been proposed that loss-of-function mutations in many genes in *C. elegans* do not cause obvious visible phenotypes. First, in a study of dominant mutations in 10 genes, it was found that loss-of-function mutations in four of these genes conferred no obvious mutant phenotype (PARK and HORVITZ 1986a). Second, the number of putative genes identified by the *C. elegans* genome sequencing project is approximately five times larger than the number predicted by genetic estimates of the number of genes that mutate to produce obvious phenotypes (BRENNER 1974; CLARK *et al.* 1988; WATERSTON *et al.* 1992). Dominant mutations allow one to identify and study genes that would not be readily identified by loss-of-function mutations. In this context, we find it interesting that all of the genes that fit our phenotypic criteria for muscle excitation defects are defined by semidominant mutations.

We believe that many genes regulating muscle excitation in *C. elegans* are as yet unidentified. All but one of the Mac genes are defined by semidominant mutations, which appear to be quite rare, and for many of these genes we have only one such allele. Mathematical estimation of the number of genes involved in this process is not feasible, since the mutations were identified in many screens of different sizes, in different labs, and

using different criteria (see Table 2). However, the fact that only one dominant mutation has been identified for nine of the 17 Mac genes clearly indicates that the screens are far from saturation. It is also possible that mutations in some genes would be systematically missed due to dominant lethality, since complete elimination of embryonic muscle contractions (BARSTEAD and WATERSTON 1989; WATERSTON 1989; VENOLIA and WATERSTON 1990; LEVIN and HORVITZ 1993; WILLIAMS and WATERSTON 1994) or elimination of pharyngeal muscle contraction (AVERY and HORVITZ 1989) cause lethality. In particular, the observation that mutations in only three of the 17 Mac genes have an obvious effect on pharyngeal pumping may indicate that severe mutations of this class were missed due to lethality.

We could also be missing another class of Mac mutations entirely because the proper screens have not been done. Mac-d mutations have been isolated in screens for defects in each of the four muscle types. However, Mac-h mutations have been isolated only in screens for body-wall muscle and pharyngeal muscle defects. Given that six of the 12 Mac-d genes affected only egg-laying and enteric muscle contraction, it is plausible that more genes exist that affect only these tissues. Mac-h mutations in such genes would not have been isolated in previous screens (TRENT *et al.* 1983; THOMAS 1990), since these screens would probably not have detected hyper-activated muscle phenotypes.

A general model for Mac gene function: We hypothesize that many of the Mac genes function in muscle and encode either modulatory ion channels or regulatory proteins whose function is to modulate ion channel activity. As described earlier, the sequences of *unc-105* and *unc-93* are consistent with this hypothesis. In *C. elegans* and in a variety of other organisms, other inappropriately activated ion channels affecting muscle excitation have been described. For example, in humans dominant familial hyperkalemic periodic paralysis is caused by delayed inactivation of a skeletal muscle sodium channel (FONTAINE *et al.* 1990; CHAHINE *et al.* 1994; HOFFMAN *et al.* 1995), dominant hypokalemic periodic paralysis can be caused by mutations in a subunit of the muscle dihydropyridine receptor, a calcium channel (JURKATT-ROTT *et al.* 1994; PTÁCEK *et al.* 1994), and dominant mutations in the vertebrate ryanodine receptor, which mediates calcium release from the sarcoplasmic reticulum, can cause malignant hyperthermia by allowing excessive calcium influx into muscles (MACLENNAN *et al.* 1990; MACLENNAN and PHILLIPS 1992). It is also possible that some dominant Mac mutations perturb the response of the myofibril to Ca^{2+} or perturb the regulation of thin or thick filaments of the myofibril. It is also conceivable that indirect perturbations of cell excitability, such as the alteration of cell membrane composition described for mutants in the *easily shocked* gene in Drosophila (PAVLIDIS *et al.* 1994), could cause a Mac phenotype. It has been suggested that the overrepresentation of dominant mutations in inherited human muscle pathologies is due to lethality of loss-of-function mutations in the same genes (HOFFMAN *et al.* 1995). An alternative explanation, suggested by our work, is that loss-of-function of these genes does not cause a clinically significant defect.

It is interesting that dominant mutations in *unc-43* and *unc-110* each can cause what appear to be opposite muscle defects in different tissues, in contrast to the Mac-d and Mac-h mutations. Based on the properties of ion channels (HILLE 1992b), it seems unlikely that genes encoding ion channels could be mutated to such a phenotype. However, perturbation of a regulatory pathway with distinct ion channels as effectors in different muscle types could readily explain these findings. We speculate that *unc-43* and *unc-110* are good candidates for components of such ion channel regulatory pathways.

Many nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the National Institutes of Health National Center for Research Resources (NCRR). We thank ERIK JORGENSEN and H. ROBERT HORVITZ for providing five newly isolated Mac alleles, FRANS TAX and DENNIS LIU for helpful discussions, and LEON AVERY, KOUICHI IWASAKI, TAKAO INOUE and the reviewers for critical reading of the manuscript. We also thank the many members of the *C. elegans* community who analyzed some of the genes described in this study. This work was supported by U.S. Public Health Service Research Grant R01NS30187.

LITERATURE CITED

ARDIZZI, J. P., and H. F. EPSTEIN, 1987 Immunochemical localization of myosin heavy chain isoforms and paramyosin in developmentally and structurally diverse muscle cell types of the nematode *Caenorhabditis elegans*. J. Cell Biol. **105:** 2763–2770.

AVERY, L., 1993 The genetics of feeding in *Caenorhabditis elegans*. Genetics **133:** 897–917.

AVERY, L., and H. R. HORVITZ, 1989 Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of *C. elegans*. Neuron **3:** 473–485.

BARSTEAD, R. J., and R. H. WATERSTON, 1989 The basal component of the nematode dense-body is vinculin. J. Biol. Chem. **264:** 10177–10185.

BENIAN, G. M., J. E. KIFF, N. NECKELMANN, D. G. MOERMAN and R. H. WATERSTON, 1989 Sequence of an unusually large protein implicated in regulation of myosin activity in *C. elegans*. Nature **342:** 45–50.

BENIAN, G. M., S. W. L'HERNAULT, and M. E. MORRIS, 1993 Additional sequence complexity in the muscle gene, *unc-22*, and its encoded protein, twitchin, of *Caenorhabditis elegans*. Genetics **134:** 1097–1104.

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

CATTERALL, W. A., 1991 Excitation-contraction coupling in vertebrate skeletal muscle: a tale of two calcium channels. Cell **64:** 871–874.

CHAHINE, M., A. L. GEORGE, JR., M. ZHOU, S. JI, W. SUN *et al.*, 1994 Sodium channel mutations in paramyotonia congenita uncouple inactivation from activation. Neuron **12:** 281–294.

CHALFIE, M., and J. WHITE, 1988 The nervous system, pp. 337–392 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

CHALFIE, M., J. E. SULSTON, J. G. WHITE, E. SOUTHGATE, J. N. THOMSON *et al.*, 1985 The neural circuit for touch sensitivity in *Caenorhabditis elegans*. J. Neurosci. **5:** 956–964.

CLARK, D. V., T. M. ROGALSKI, L. M. DONATI and D. L. BAILLIE, 1988 The *unc-22* (IV) region of *Caenorhabditis elegans:* genetic analysis of lethal mutations. Genetics **119:** 345–353.

DESAI, C., G. GARRIGA, S. L. MCINTIRE and H. R. HORVITZ, 1988 A genetic pathway for the development of the *Caenorhabditis elegans* HSN motor neurons. Nature **336:** 638–646.

EIDE, D., and P. ANDERSON, 1985 The gene structures of spontaneous mutations affecting a *Caenorhabditis elegans* myosin heavy chain gene. Genetics **109:** 67–79.

EPSTEIN, H. F., and D. A. FISCHMAN, 1991 Molecular analysis of protein assembly in muscle development. Science **251:** 1039–1044.

EPSTEIN, H. F., R. H. WATERSTON and S. BRENNER, 1974 A mutant affecting the heavy chain of myosin in *Caenorhabditis elegans*. J. Mol. Biol. **90:** 291–300.

FERGUSON, E. L., P. W. STERNBERG and H. R. HORVITZ, 1987 A genetic pathway for the specification of the vulval cell lineages of *Caenorhabditis elegans*. Nature **326:** 259–267.

FONTAINE, B., T. S. KHURANA, E. P. HOFFMAN, G. A. BRUNS, J. L. HAINES *et al.*, 1990 Hyperkalemic periodic paralysis and the adult muscle sodium channel alpha-subunit gene. Science **250:** 1000–1002.

FOSTER, P. S., 1994 The role of phosphoinositide metabolism in Ca^{2+} signaling of skeletal muscle cells. Int. J. Biochem. **26:** 449–468.

GORDON, A. M., 1989 Contraction of skeletal muscle, pp. 197–213 in *Textbook of Physiology: Excitable Cells and Neurophysiology*, Ed. 21, edited by H. D. PATTON, A. F. FUCHS, B. HILLE, A. M. SCHER and R. STEINER. W. B. Saunders Co., Philadelphia.

GREEN, L. L., N. WOLF, K. L. MCDONALD and M. T. FULLER, 1990 Two types of genetic interaction implicate the whirligig gene of *Drosophila melanogaster* in microtubule organization in the flagellar axoneme. Genetics **126:** 961–973.

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

GREENWALD, I. S., and H. R. HORVITZ, 1982 Dominant suppressors of a muscle mutant define an essential gene of *Caenorhabditis elegans*. Genetics **101:** 211–225.

GREENWALD, I. S., and H. R. HORVITZ, 1986 A visible allele of the muscle gene *sup-10X* of *C. elegans*. Genetics **113:** 63–72.

HEIERHORST, J., W. C. PROBST, F. S. VILIM, A. BUKU and K. R. WEISS, 1994 Autophosphorylation of mulluscan twitchin and interaction of its kinase domain with calcium/calmodulin. J. Biol. Chem. **269:** 21086–21093.

HERMAN, R. K., 1984 Analysis of genetic mosaics of the nematode *Caenorhabditis elegans*. Genetics **108:** 165–180.

HILLE, B., 1992a G protein-coupled mechanisms and nervous signaling. Neuron **9:** 187–195.

HILLE, B., 1992b *Ionic Channels of Excitable Membranes*. Sinauer Associates Inc., Sunderland, MA.

HOFFMAN, E. P., F. LEHMANN-HORN and R. RUDEL, 1995 Overexcited or inactive: ion channels in muscle disease. Cell **80:** 681–686.

HOMYK, T., JR., and C. P. EMERSON, JR., 1988 Functional interactions between unlinked muscle genes within haploinsufficient regions of the Drosophila genome. Genetics **119:** 105–121.

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

HORVITZ, H. R., M. CHALFIE, C. TRENT, J. SULSTON and P. EVANS, 1982 Serotonin and octopamine in the nematode *Caenorhabditis elegans*. Science **216:** 1012–1014.

HU, S. H., M. W. PARKER, J. Y. LEI, M. C. WILCE, G. M. BENIAN *et al.*, 1994 Insights into autoregulation from the crystal structure of twitchin kinase. Nature **369:** 581–584.

HUANG, S. P., T. A. TATTAR, R. A. ROHDE and B. M. ZUCKERMAN, 1982 *Caenorhabditis elegans:* effects of 5-hydroxytryptophan and dopamine on behavior and development. Exp. Parasitol. **54:** 72–79.

JURKAT-ROTT, K., F. LEHMANN-HORN, A. ELBAZ, R. HEINE, T. GREGG *et al.*, 1994 A calcium channel mutation causing hypokalemic periodic paralysis. Hum. Mol. Genet. **3:** 1415–1419.

LANDEL, C. P., M. KRAUSE, R. H. WATERSTON and D. HIRSH, 1984 DNA rearrangements of the actin gene cluster in *Caenorhabditis elegans* accompany reversion of three muscle mutants. J. Mol. Biol. **180:** 497–513.

LEI, J., X. TANG, T. C. CHAMBERS, J. POHL and G. M. BENIAN, 1994 Protein kinase domain of twitchin has protein kinase activity and an autoinhibitory region. J. Biol. Chem. **269:** 21078–21085.

LEVI, A. J., P. BROOKSBY and J. C. HANCOX, 1993 One hump or two? The triggering of calcium release from the sarcoplasmic reticulum and the voltage dependence of contraction in mammalian cardiac muscle. Cardiovasc. Res. **27:** 1743–1757.

LEVIN, J. Z., and H. R. HORVITZ, 1992 The *Caenorhabditis elegans unc-93* gene encodes a putative transmembrane protein that regulates muscle contraction. J. Cell Biol. **117:** 143–155.

LEVIN, J. Z., and H. R. HORVITZ, 1993 Three new classes of mutations in the *Caenorhabditis elegans* muscle gene *sup-9*. Genetics **135:** 53–70.

LEVY, A. D., J. YANG and J. M. KRAMER, 1993 Molecular and genetic analyses of the *Caenorhabditis elegans dpy-2* and *dpy-10* collagen genes: a variety of molecular alterations affect organismal morphology. Mol. Biol. Cell **4:** 803–817.

LEWIS, J. A., C. H. WU, H. BERG and J. H. LEVINE, 1980 The genetics of levamisole resistance in the nematode *Caenorhabditis elegans*. Genetics **95:** 905–928.

LEWIS, J. A., J. S. ELMER, J. SKIMMING, S. MCLAFFERTY, J. FLEMING *et al.*, 1987 Cholinergic receptor mutants of the nematode *Caenorhabditis elegans*. J. Neurosci. **7:** 3059–3071.

LIU, D. W., and J. H. THOMAS, 1994 Regulation of a periodic motor program in *C. elegans*. J. Neurosci. **14:** 1953–1962.

MACLENNAN, D. H., and M. S. PHILLIPS, 1992 Malignant hyperthermia. Science **256:** 789–794.

MACLENNAN, D. H., C. DUFF, F. ZORZATO, J. FUJII, M. PHILLIPS *et al.*, 1990 Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. Nature **343:** 559–561.

MACLEOD, A. R., R. H. WATERSTON and R. M. FISHPOOL, 1977 Identification of the structural gene for a myosin heavy-chain in *Caenorhabditis elegans*. J. Mol. Biol. **114:** 133–140.

MCINTIRE, S. L., E. JORGENSEN and H. R. HORVITZ, 1993a Genes required for GABA function in *Caenorhabditis elegans*. Nature **364:** 334–337.

MCINTIRE, S. L., E. JORGENSEN, J. KAPLAN and H. R. HORVITZ, 1993b The GABAergic nervous system of *Caenorhabditis elegans*. Nature **364:** 337–341.

MENDEL, J. E., H. C. KORSWAGEN, K. S. LIU, Y. M. HADJU-CRONIN, M. I. SIMON *et al.*, 1995 Participation of the protein G_o in multiple aspects of behavior in *C. elegans*. Science **267:** 1652–1655.

MOERMAN, D. G., G. M. BENIAN, R. J. BARSTEAD, L. A. SCHRIEFER and R. H. WATERSTON, 1988 Identification and intracellular localization of the *unc-22* gene product of *Caenorhabditis elegans*. Genes Dev. **2:** 93–105.

PARK, E. C., and H. R. HORVITZ, 1986a Mutations with dominant effects on the behavior and morphology of the nematode *Caenorhabditis elegans*. Genetics **113:** 821–852.

PARK, E. C., and H. R. HORVITZ, 1986b *C. elegans unc-105* mutations affect muscle and are suppressed by other mutations that affect muscle. Genetics **113:** 853–867.

PAVLIDIS, P., M. RAMASWAMI and M. A. TANOUYE, 1994 The Drosophila *easily shocked* gene: a mutation in a phospholipid synthetic pathway causes seizure, neuronal failure, and paralysis. Cell **79:** 23–33.

PLENEFISCH, J. D., L. DELONG and B. J. MEYER, 1989 Genes that implement the hermaphrodite mode of dosage compensation in *Caenorhabditis elegans*. Genetics **121:** 57–76.

PTÁCEK, L., R. TAWIL, R. GRIGGS, A. ENGEL, R. LAYZER *et al.*, 1994 Dihydropyridine receptor mutations cause hypokalemic periodic paralysis. Cell **77:** 863–868.

SÉGALAT, L., D. A. ELKES and J. M. KAPLAN, 1995 Modulation of serotonin-controlled behaviors by G_o in *Caenorhabditis elegans*. Science **267:** 1648–1651.

STERNBERG, P. W., and H. R. HORVITZ, 1986 Pattern formation during vulval development in *C. elegans*. Cell **44:** 761–772.

STRETTON, A. O. W., R. E. DAVIS, J. D. ANGSTADT, J. E. DONMOYER and C. D. JOHNSON, 1985 Neural control of behaviour in *Ascaris*. Trends Neurosci. **8:** 294–300.

SULSTON, J. E., and H. R. HORVITZ, 1977 Post-embryonic cell lineage of the nematode, *Caenorhabditis elegans*. Dev. Biol. **56:** 110–156.

SULSTON, J. E., D. G. ALBERTSON and J. N. THOMSON, 1980 The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev. Biol. **78:** 542–576.

SULSTON, J. E., E. SCHIERENBERG, J. G. WHITE and J. N. THOMSON, 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. **100:** 64–119.

THOMAS, J. H., 1990 Genetic analysis of defecation in *Caenorhabditis elegans*. Genetics **124:** 855–872.

THOMAS, J. H., 1993 Thinking about genetic redundancy. Trends Genet. **9:** 395–399.

TRENT, C., N. TSUNG and H. R. HORVITZ, 1983 Egg-laying defective

mutants of the nematode *Caenorhabditis elegans*. Genetics **104:** 619–647.

VENOLIA, L., and R. WATERSTON, 1990 The *unc-45* gene of *Caenorhabditis elegans* is an essential muscle-affecting gene with maternal expression. Genetics **126:** 345–353.

WATERSTON, R. H., 1988 Muscle, pp. 281–336 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

WATERSTON, R. H., 1989 The minor myosin heavy chain, *mhcA*, of *Caenorhabditis elegans* is necessary for the initiation of thick filament assembly. EMBO J. **8:** 3429–3436.

WATERSTON, R. H., R. M. FISHPOOL and S. BRENNER, 1977 Mutants affecting paramyosin in *Caenorhabditis elegans*. J. Mol. Biol. **117:** 679–697.

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

WATERSTON, R. H., D. HIRSCH and T. R. LANE, 1984 Dominant mutations affecting muscle structure in *Caenorhabditis elegans* that map near the actin gene cluster. J. Mol. Biol. **180:** 473–496.

WATERSTON, R., C. MARTIN, M. CRAXTON, C. HUYNH, A. COULSON *et al.*, 1992 A survey of expressed genes in *Caenorhabditis elegans*. Nat. Genet. **1:** 114–123.

WHITE, J., 1988 The anatomy, pp. 81–122 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

WHITE, J. G., E. SOUTHGATE, J. N. THOMSON and S. BRENNER, 1986 The structure of the nervous system of the nematode *Caenorhabditis elegans*. Phil. Trans. R. Soc. Lond. B Biol. Sci. **314:** 1–340.

WILLIAMS, B. D., and R. H. WATERSTON, 1994 Genes critical for muscle development and function in *Caenorhabditis elegans* identified through lethal mutations. J. Cell Biol. **124:** 475–490.

WOOD, W. B., 1988 *The Nematode Caenorhabditis elegans*. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.

Communicating editor: I. GREENWALD