The *mec-8* Gene of *Caenorhabditis elegans* Affects Muscle and Sensory Neuron Function and Interacts With Three Other Genes: *unc-52*, *smu-1* and *smu-2*

Erik A. Lundquist and Robert K. Herman

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108

Manuscript received March 14, 1994
Accepted for publication May 23, 1994

ABSTRACT

Mutations in the *Caenorhabditis elegans* gene *mec-8* were previously shown to cause defects in mechanosensation and in the structure and dye filling of certain chemosensory neurons. Using noncomplementation screens, we have identified eight new *mec-8* alleles and a deficiency that uncovers the locus. Strong *mec-8* mutants exhibit an incompletely penetrant cold-sensitive embryonic and larval arrest, which we have correlated with defects in the attachment of body muscle to the hypodermis and cuticle. Mutations in *mec-8* strongly enhance the mutant phenotype of *unc-52(viable)* mutations; double mutants exhibit an unconditional arrest and paralysis at the twofold stage of embryonic elongation, a phenotype characteristic of lethal alleles of *unc-52*, a gene previously shown to encode a homolog of the core protein of heparan sulfate proteogylcan, found in basement membrane, and to be involved in the anchorage of myofilament lattice to the muscle cell membrane. We have identified and characterized four extragenic recessive suppressors of a *mec-8; unc-52(viable)* synthetic lethality. The suppressors, which define the genes *smu-1* and *smu-2*, can weakly suppress all *mec-8* mutant phenes. They also suppress the muscular dystrophy conferred by an *unc-52(viable)* mutation.

MUTANT analysis has proved to be very useful in the study of the development and function of both the nervous system and the musculature of the nematode *Caenorhabditis elegans*. Many genes identified by mutations that affect animal movement, mechanosensation, egg-laying, chemosensation, thermosensation, feeding or defecation have been shown to be important in nervous system development (for reviews, see CHALFIE and WHITE 1988; BARGMANN 1993), and among the class of mutants affected in movement, many have been shown to have defects in muscle development, structure or function (WATERSTON 1988; WILLIAMS and WATERSTON 1994).

Mutations in the *mec-8* gene were originally identified in a screen for mechanosensory-defective (Mec) mutants (CHALFIE and SULSTON 1981; CHALFIE and AU 1989). When a fine hair is drawn gently across the body of a wild-type worm in the region of the head or tail, the animal moves quickly backward or forward, respectively. Mec mutants do not respond to this gentle touch, although they do recoil to a prod with a wire and move with apparently wild-type coordination. Laser ablation studies have shown that the wild-type response to light touch is mediated by a set of six sensory neurons, the touch cells, which have long, anteriorly directed, longitudinal processes (CHALFIE and SULSTON 1981). The processes of the touch cells are embedded in the animal's hypodermis and lie near the cuticle; associated with each process is extracellular material called the mantle, which appears to be connected to the cuticle by fibrous bodies (CHALFIE and SULSTON 1981; FRANCIS and WATERSTON 1991). It is presumed that *mec-8* mutants are defective in touch cell function, although no morphological defects in the touch cells of *mec-8* animals have been discerned by electron microscopy (CHALFIE and SULSTON 1981; CHALFIE and AU 1989). The results of temperature-shift experiments with *mec-8(u218* ts), a temperature-sensitive allele, suggest that only a small amount of *mec-8* product is required for touch cell function; the Mec phenotype can be rescued if the mutants are grown at permissive temperature either during embryogenesis or for a time during larval development (CHALFIE and AU 1989).

Mutations in *mec-8* also cause defects in chemosensory neurons (PERKINS *et al.* 1986). Neurons required for various chemosensory behaviors have been identified by laser ablation studies (BARGMANN *et al.* 1990, 1993; BARGMANN and HORVITZ 1991a,b). All of the implicated neurons belong to a pair of bilaterally symmetric sensilla in the head called amphids. Each wild-type amphid consists of the ciliated dendrites of 12 neurons plus two non-neuronal support cells, a sheath cell and a socket cell (WARD *et al.* 1975; WARE *et al.* 1975; WHITE *et al.* 1986; PERKINS *et al.* 1986). PERKINS *et al.* (1986) showed by serial section electron microscopy that mutation in *mec-8* disrupts the fasciculation of the amphid cilia. A convenient method for scoring chemosensory defects in *mec-8* mutants (as well as many other chemosensory mutants) has been described (PERKINS *et al.* (1986). When living wild-type animals are bathed in a solution of fluorescein isothiocyanate (FITC), the cell bodies and processes of six neurons of each amphid,

which are exposed to the external environment via a pore, fill with dye and can be readily visualized by fluorescence microscopy (HEDGECOCK *et al.* 1985). Two neurons belonging to each of a bilaterally symmetric pair of sensilla in the tail, called phasmids (SULSTON *et al.* 1980; WHITE *et al.* 1986; HALL and RUSSELL 1991), are also exposed to the outside and fill with dye. PERKINS *et al.* (1986) reported that each of two different *mec-8* mutations invariably block FITC filling of both phasmid neurons and all but two amphid neurons, a phenotype we refer to as Dyf, for abnormal dye filling.

In this report we further characterize *mec-8* mutants. We have identified eight new *mec-8* alleles and a deficiency of the locus by noncomplementation screens, and we have analyzed the Mec and Dyf phenes caused by all alleles to try to determine the *mec-8* null phenotype. We have also discovered that strong *mec-8* mutations cause a cold-sensitive embryonic and larval lethality at low penetrance and cold-sensitive disruptions in the structure of body wall muscle. *C. elegans* body wall muscle cells are located in four longitudinal strips that run the length of the animal, subventrally and subdorsally. To generate animal movement, the contractile force of the body wall muscles must be transmitted to the external cuticle (WATERSTON 1988). Interposed between the body muscle cells and the cuticle are a basement membrane and a thin layer of hypodermis, which is responsible for secreting the cuticle. The muscle cells are attached to the adjoining basement membrane, which in turn is anchored to the cuticle via filament-associated structures called fibrous organelles that bridge the thin hypodermal layer and thus complete the mechanical coupling of muscle to cuticle (FRANCIS and WATERSTON 1991; HRESKO *et al.* 1994). We have found that *mec-8* animals grown at 16° frequently exhibit defects in attachment of the myofilament lattice to the hypodermis and cuticle. In sum, *mec-8* mutations cause defects in the functions of at least three distinct types of cells: mechanosensory and chemosensory neurons and body muscle.

One of the virtues of *C. elegans* genetics is its suitability for identifying interacting genes through the analysis of the phenotypes of double mutants. We report here two types of genetic interaction involving *mec-8*. The first is a synthetic enhancement of an *unc-52* mutant phenotype by *mec-8* mutation. Products of the *unc-52* gene are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan (ROGALSKI *et al.* 1993); and an epitope encoded by *unc-52* sequence (ROGALSKI *et al.* 1993) is concentrated in those regions of the basement membrane that contribute to the muscle-hypodermal attachments (FRANCIS and WATERSTON 1991; HRESKO *et al.* 1994). Different classes of *unc-52* mutation have been characterized. Viable *unc-52* alleles cause a gradual disruption of the myofilament lattice posterior to the pharynx beginning at the third or fourth larval stage; the disruption of muscle is correlated with a progressive paralysis (MACKENZIE *et al.* 1978; WATERSTON 1988; GILCHRIST and MOERMAN 1992). Other *unc-52* alleles cause a paralysis and arrest at the twofold stage of embryonic elongation (ROGALSKI *et al.* 1993; WILLIAMS and WATERSTON 1994), which is a phenotype characteristic of mutations in other muscle-affecting genes (WATERSTON 1989; VENOLIA and WATERSTON 1990; BARSTEAD and WATERSTON 1991; WILLIAMS and WATERSTON 1994). We have found that when viable *unc-52* mutations are combined with any of several *mec-8* mutations, the phenotype of the double mutant is very similar to that of an embryonic lethal *unc-52* mutant.

Suppression of a mutant phenotype by mutation in another gene provides a second method for identifying interactions between genes. We have identified extragenic suppressors of *mec-8; unc-52* synthetic lethality that define two loci, *smu-1* and *smu-2*. We show that mutations in these two loci are capable of suppressing three distinct *mec-8* phenes as well as the paralysis conferred by certain viable *unc-52* alleles.

MATERIALS AND METHODS

General genetic methods: Nematodes were cultured as described by BRENNER (1974). The wild-type strain was N2. We have followed standard *C. elegans* genetic nomenclature (HORVITZ *et al.* 1979). Unless otherwise noted, all genetic experiments were done at 20°. Genes, mutations and chromosome rearrangements used in this work were the following (for references, see HODGKIN *et al.* 1988): LG (linkage group) I: *dpy-5(e61)*, *unc-40(e271)*, *mec-6(e1342)*, *unc-13(e51)*, *unc-55(e402)*, *unc-29(e193)*, *mec-8(e398*, *u303*, *u74*, *u391*, *u218* ts, *u456*, *u314)* (CHALFIE and AU 1989), *mec-8(rh170)* (E. HEDGECOCK, personal communication), *dpy-24(s71)*, *unc-75(e950)*, *unc-101(m1)*, *unc-59(e261)*, *unc-95(su33)*, *unc-54(e190)*, *nDf23*, *nDf24*, *nDf25*, *nDp4*. LGII: *sqt-2(sc3)*, *mup-1(e2346)*, *unc-85(e1414)*, *dpy-10(e128)*, *unc-4(e120)*, *sqt-1(sc13)*, *unc-52(e669*, *e669su250*, *e444*, *e998*, *e1421*, *su54*, *su200)* and *mnC1*. LGIII: *dpy-18(e364)*. LGIV: *dpy-13(mn278)*, *emb-9(hc70)*, *bli-6(sc16)*, *mec-3(e1338)*, *him-8(e1491)*. LGV: *unc-60(e667)*, *mec-1(e1066)*, *unc-23(e324)*, *mec-9(e1494)*, *him-5(e1467)*. LGX: *unc-1(e538)*, *lon-2(e678)*, *mec-2(e75)*, *unc-97(su110)*, *unc-6(ev400)*, *mec-7(e1343)*, *mec-10(e1515)*, *him-4(e1267)*, *unc-3(e151)*, *daf-6(e1377)*, *unc-7(bx5)*, *mec-5(e1340)*, *let-2(b246)*, *osm-1(p808)*, *mec-4(e1497)*.

Genetic mapping of *mec-8*: We have refined the map position of *mec-8*, which earlier was mapped to the right of *dpy-5 I* (CHALFIE and SULSTON 1981). For mapping purposes, *mec-8* mutants were scored either with respect to their defect in mechanosensation (Mec), by gently stroking the animals with an eyebrow hair (CHALFIE and SULSTON 1981), or with respect to their defect in dye filling (Dyf; see below). The results of three-factor crosses, which are given in Table 1, put *mec-8* between *unc-29* and *dpy-24* and closer to *unc-29* (see Figure 1). The following two-factor data yield an *unc-29-dpy-24* map distance of 1.2 map units: 8 of 250 Unc self progeny of *unc-29/dpy-24* hermaphrodites segregated Dpy (Unc) progeny, and 6 of 328 Dpy self progeny from the same parents segregated Unc (Dpy) progeny. Among 210 Unc self progeny of *unc-29/mec-8(e398)* hermaphrodites, one segregated Mec self progeny,

TABLE 1

Three-factor map data

Genotype of parent	Phenotype of selected recombinants	Number	Genotype of recombinant chromosome
dpy-5 unc-13 mec-8(e398)/+++	Dpy non-Unc	10/10	*dpy-5 unc-13(+) mec-8(+)*
	Mec non-Dpy	9/17	*dpy-5(+) unc-13 mec-8*
		8/17	*dpy-5(+) unc-13(+) mec-8*
dpy-5 unc-29 +/+ + mec-8(e398)	Dpy non-Unc	19/19	*dpy-5 unc-29(+) mec-8*
	Unc non-Dpy	20/20	*dpy-5(+) unc-29 mec-8(+)*
unc-29 + dpy-24/+ mec-8(e398)+	Unc non-Dpy	9/12	*unc-29 mec-8(+) dpy-24(+)*
		3/12	*unc-29 mec-8 dpy-24(+)*
	Dpy non-Unc	14/15	*unc-29(+) mec-8 dpy-24*
		1/15	*unc-29(+) mec-8(+) dpy-24*
unc-29 mec-8(e398) dpy-24/+++	Unc non-Dpy	13/16	*unc-29 mec-8 dpy-24(+)*
		3/16	*unc-29 mec-8(+) dpy-24(+)*
	Dpy non-Unc	10/10	*unc-29(+) mec-8(+) dpy-24*

corresponding to an *unc-29-mec-8* distance of about 0.2 map unit. Among 150 Mec self progeny of *mec-8(e398)/dpy-24* hermaphrodites, two segregated Dpy self progeny, corresponding to a *mec-8-dpy-24* distance of about 0.7 map unit.

Deficiency mapping was performed by mating males heterozygous for the chromosome of interest to hermaphrodites heterozygous for the deficiency. F_1 cross progeny were examined for the presence of mutant animals. The mutation being tested was linked to *dpy-5* or *unc-75*. For example, *dpy-5 mec-8(e398)/++* males were mated to *mnDf111/unc-13 lin-11* hermaphrodites, and F_1 progeny were scored for Mec and Dyf. The deficiency *mnDf111* uncovers *mec-8(e398)* (and all other *mec-8* alleles tested; see RESULTS), *unc-55* and *unc-29* but not *unc-13, dpy-5, dpy-24* or *lin-11*. None of the three deficiencies *nDf23, nDf24* or *nDf25* uncovers *mec-8(e398)* or *dpy-24*. For each of the latter three deficiencies, we identified complementing and viable *mec-8/Df* animals by showing that they segregated extremely few self progeny carrying the *mec-8(+)*-containing chromosome (the chromosome not tagged with *dpy-5* or *unc-75* or with the markers balancing the deficiency stock), presumably because the *mec-8(+)*-bearing chromosome also carried a homozygous inviable deficiency.

A stock of genotype *dpy-5 unc-13 mec-8(e398); nDp4* was constructed by mating *dpy-5 unc-13 mec-8(e398)/+++* males with *unc-13; nDp4* hermaphrodites and picking non-Unc-13 progeny, some of which gave rise to many Dpy non-Unc-13 animals. Twenty of the latter animals were picked; they all segregated Dpy Unc Mec self progeny, as expected for a *dpy-5 unc-13 mec-8(e398); nDp4* parental genotype if *nDp4* carries *unc-13(+)* but not *dpy-5(+)*. The Dpy non-Unc parents were all non-Mec, which indicates that *nDp4* carries *mec-8(+)*.

Identification of new *mec-8* alleles: Ethyl methanesulfonate (EMS) treatment was performed according to BRENNER (1974) and SULSTON and HODGKIN (1988). Trimethylpsoralen (TMP) treatment was based on the methods of YANDELL *et al.* (1994): a mixture of N2 males and hermaphrodites was washed with M9 buffer (SULSTON and HODGKIN 1988) and resuspended in 30 μg/ml TMP in M9 buffer. After 15 min in the dark, the animals were transferred to an unseeded NGM plate (SULSTON and HODGKIN 1988) and exposed to longwave ultraviolet radiation, using a Blak-Ray lamp, model UVL-21, for a dose of 700 $\mu W/cm^2$ (measured with a Superdose dose meter) for 45 sec. A drop of OP50 bacteria (BRENNER 1974) was added to the plate, and the worms were allowed to recover for 5 hr in the dark.

After exposure to either EMS or TMP, N2 males were mated with *unc-29 mec-8(e398) dpy-24 I; unc-7(bx5) X* L4 hermaphrodites. The mating parents (2–5 of each on 35-mm diameter plates) were transferred to fresh plates daily. Non-Unc-7 F_1 hermaphrodite progeny were scored for touch sensitivity by gently stroking each worm with an eyebrow hair, and all Mec animals were picked. Only one mutant per plate was saved, to ensure independence of mutants. The deficiency *mnDf111* was isolated as an allele of *mec-8* in a separate F_1 noncomplementation screen: EMS-mutagenized N2 males were mated with *dpy-5 unc-13 mec-8(e398) I; unc-9 X* hermaphrodites, and F_1 progeny were scored for Mec. The X-linked markers *unc-7* and *unc-9* were included in these crosses to prevent male cross-progeny from mating with hermaphrodite cross-progeny; Unc-7 and Unc-9 males do not mate (HODGKIN 1983). Three *mec-8* mutations (*mn450, mn455* and *mn459*) were identified among 9,645 F_1 animals scored in the first EMS screen, a frequency of 3×10^{-4}. The deficiency *mnDf111* was the only *mec-8* mutation recovered among 3,553 F_1 animals screened. Molecular analysis has shown that the *mec-8* region is entirely missing in *mnDf111* (R. ELLIS, personal communication; E. LUNDQUIST, J. SHAW and R. HERMAN, unpublished experiments). Four TMP-induced *mec-8* mutations (*mn462, mn463, mn464* and *mn465*) were identified among 18,712 F_1 animals scored, a frequency of 2×10^{-4}. Each mutation recovered in the EMS and TMP screens was outcrossed five times to N2.

To obtain potential transposon-tagged alleles, hermaphrodites of the mutator strain RW7097 (MORI *et al.* 1988) were mated with N2 males; the heterozygous male cross-progeny were mated to *unc-29 mec-8(e398) dpy-24 I; unc-7 X* hermaphrodites, as in the EMS and TMP mutagenesis experiments, and the hermaphrodite cross progeny were screened for rare Mec animals. Five *mec-8* mutations were identified among 3,316 F_1 hermaphrodites scored. Because of their high incidence and because each of the five mutations resulted in a similar temperature-sensitive Mec and Dyf phenotype (see RESULTS), we suppose that the mutations were not independent, and only one, *mn472* ts, was analyzed further, after outcrossing ten times to N2.

Microscopy: Living animals were mounted for Nomarski differential interference contrast microscopy or epifluorescence microscopy on a 5% agar pad (sometimes containing 5 mM sodium azide as an anesthetic) in a drop of M9 buffer under a coverslip. Animals viewed by polarized light optics were mounted on a slide (without agar) in a drop of M9 plus 5 mM sodium azide. A rotary compensator was used to enhance contrast of birefringent muscles. Samples were photographed with Ilford XP2 400 film.

Dye filling of amphid and phasmid neurons: Two fluorescent dyes were used: FITC (Sigma) and 3,3′-dioctadecyl-

oxacarbocyanine perchlorate (DiO; Molecular Probes). FITC was used for scoring *mec-8* in crosses. DiO, which gives better resolution and less fading than FITC, was used to quantitate defects in dye filling in various *mec-8* mutants. FITC filling was performed according to HEDGECOCK *et al.* (1985). For DiO filling (HERMAN and HEDGECOCK 1990), animals were bathed in a solution of 20 μg/ml DiO in M9 buffer for 2 hr (a dilution of a DiO stock solution containing 2 mg/ml DiO in dimethylformamide). Excess dye was removed by allowing the animals to crawl on a clean NGM plate for a few minutes before putting them on a slide for microscopy. Most strains were scored in at least two independent experiments (most were scored three times). Little variation in filling was seen among experiments done on different days or with different stock solutions of DiO.

Scoring developmental arrest of *mec-8* mutants: L4 hermaphrodites were picked individually to NGM plates and transferred daily. Self progeny were scored as follows: embryonic arrest (any unhatched embryos remaining 1 day after hermaphrodite removal at 20° or 25°, 2 days after hermaphrodite removal at 16°) ; L1 and L2 arrest (any L1 or L2 larvae remaining 2 days after hermaphrodite removal, 3 days after hermaphrodite removal at 16°); all other animals. Scored animals were removed from the plates so they would not interfere with subsequent counts.

Fixation and antibody staining: A drop of 1 mg/ml polylysine was placed on a clean slide and allowed to dry. Animals were washed from NGM plates with M9 buffer or water and placed on the polylysine spot. A coverslip was placed over the sample; liquid was drawn from underneath the coverslip using the edge of a paper towel until the animals appeared to flatten slightly; and the slide was placed in liquid nitrogen for at least 5 min. Immediately after removal from liquid nitrogen, the coverslip was flipped off with a razor blade, and the slide was put in methanol at −20° for 10 min, acetone at −20° for 5–10 min and then allowed to dry.

A drop of undiluted goat serum was placed on the sample and incubated at 37° for 1 hr, room temperature for 2–3 hr, or overnight at 4° in a humidified chamber. Excess goat serum was removed, and a drop (20–70 μl) of primary antibody diluted in phosphate-buffered saline (PBS) (SULSTON and HODGKIN 1988) was added to the sample. Samples were incubated in a humidified chamber for 1 hr at 37°, 2–3 hr at room temperature or overnight at 4°. Each slide was washed three times in PBS, 20 min per wash. A drop of fluorescein-conjugated secondary antibody, diluted 1:100 to 1:150 in PBS, was added to the sample. After incubation in a humidified chamber at 37° for 1 hr or at room temperature for 2–3 hr, the sample was washed twice in PBS, 20 min per wash, and once in PBS with 4 mM ascorbic acid. The sample was mounted in a drop of PBS with 4 mM ascorbic acid, and the coverslip was sealed with fingernail polish.

The following antibodies were used, each at a final dilution of 1:500: DM 5.8 (MILLER *et al.* 1983), which recognizes MHC-B, the major body wall muscle myosin heavy chain; MH2 (FRANCIS and WATERSTON 1991), which recognizes UNC-52 (ROGALSKI *et al.* 1993) in the basement membrane adjacent to muscle strips ; and MH27 (WATERSTON 1988), which recognizes an antigen present in the junctions of hypodermal cells and therefore outlines hypodermal cells.

Construction and analysis of *mec-8; unc-52* double mutants: Several *mec-8; unc-52(e444)* strains were constructed and maintained using the chromosome balancer *mnC1 dpy-10 unc-52(e444) II*. Males of genotype *mec-8/+; unc-4/+* were mated to homozygous *mnC1 dpy-10 unc-52* hermaphrodites. F_1 cross-progeny that segregated both Mec and Unc-4 progeny were selected, and Mec non-Unc-4 F_2 hermaphrodites, genotype *mec-8; mnC1 dpy-10 unc-52(e444)/unc-4*, were picked. These animals failed to segregate viable Dpy Unc-52 self-progeny; eggs laid by them were collected and mounted on agar in S medium under a coverslip (SULSTON and HODGKIN 1988) and sealed around the edges with Vaseline. The developing zygotes were then incubated at 20° (or other indicated temperature) and monitored periodically by Nomarski microscopy. In all cases, close to one quarter of the embryos were paralyzed and arrested at the twofold stage of embryogenesis, as described in RESULTS. Essentially no embryos from *mnC1 dpy-10 unc-52/unc-4* parents or various *mec-8* mutants showed twofold embryonic arrest under comparable conditions. In other experiments, the *mec-8* mutation was balanced by a *dpy-5 unc-29* chromosome, which also does not lead to embryonic arrest at the twofold stage. For example, *mec-8(e398)/dpy-5 unc-29; unc-52(e669)* animals were generated from *mec-8/ dpy-5 unc-29; unc-52/+* parents, which segregated both Unc-52 and Mec self progeny; the homozygous *unc-52* animals exhibited the progressive paralysis conferred by *unc-52*, were non-Mec and non-Dyf, and segregated Dpy Unc-29 self progeny but no Mec Dyf self progeny. Eggs laid by these animals were collected and scored, as already described, and in every case about one-quarter of the embryos were paralyzed and arrested at the twofold stage of elongation. Finally, in a few instances, neither the *mec-8* nor the *unc-52* mutation was balanced. For example, *mec-8(e398)/+; unc-52(e444)/+* animals were generated and recognized by the fact that they segregated both Unc-52 and Mec Dyf self progeny. No Unc-52 Mec Dyf self progeny were produced, however. A large number of Mec Dyf progeny (two-thirds of which were expected to be heterozygous for *unc-52*) were picked and allowed to lay eggs for a few hours. The eggs were collected and inspected periodically by Nomarski microscopy; about one-sixth showed the characteristic arrest at the twofold stage.

Identification of genetic suppressors of *mec-8(u218* ts*); unc-52(e669su250* ts*)* synthetic lethality: The double mutant *mec-8(u218* ts*) I; unc-52(e669su250* ts*)* II is viable at 16° and inviable at 25°. Hermaphrodites of this genotype grown at 16° were mutagenized with EMS and cultured on plates at 16° for two generations. The plates were then shifted to 25°, and viable and coordinated F_3 progeny were identified and picked. No more than one suppressed line from each plate was saved. Four independent mutants were analyzed. Each of the four mutations suppressed, at 25°, the paralysis conferred by *unc-52(e669su250* ts*)*, both the Mec and Dyf phenes conferred by *mec-8(u218* ts*)* and the synthetic lethality conferred by the *unc-52* and *mec-8* mutations together (see RESULTS); all four lines bred true for these characteristics.

All mapping and complementation experiments involving the suppressors were done at 25°. The suppressor stocks were crossed to N2 males, and F_1 cross progeny were picked and allowed to self. Among the F_2 progeny in all four cases were Mec and Unc-52 animals that were indistinguishable from the original *mec-8(u218* ts*)* and *unc-52(e669su250* ts*)* strains, which indicates that all four of the suppressor mutations are extragenic to both *mec-8* and *unc-52*. Many of Unc-52 progeny of the triply heterozygous strains segregated about one-quarter non-Unc-52 (suppressed) animals, which indicates that the suppressors are recessive for suppression of the paralysis conferred by *unc-52(e669su250* ts*)*. Analogous results indicated that the suppressors are also recessive for suppression of the Mec phene conferred by *mec-8(u218* ts*)*. The suppressor mutations were assigned allele names *mn415*, *mn416*, *mn417* and *mn433*, and the loci defined by the suppressor mutations are called *smu* (suppressor of *mec-8* and *unc-52*).

Complementation tests between suppressor mutations: The characteristic of suppressing the paralysis conferred by *unc-52(e669su250* ts*)* was used for complementation testing

between pairs of suppressors. In generating new strains, the presence of the suppressor was ascertained by first identifying Unc-52 animals, which must be homozygous for *unc-52(e669su250* ts), and then identifying non-Unc-52 (homozygous suppressor) self progeny. Complementation tests were performed by crossing *unc-52(e669su250* ts); *smu(a); him-5* males to *dpy-5; unc-52(e669su250* ts); *smu(b)* hermaphrodites (for these tests, we made no special effort to follow the fate of the original *mec-8(u218* ts) mutation; the construction of suppressor stocks in which *mec-8(u218* ts) was clearly absent is described below). Non-Dpy hermaphrodite progeny were then scored for suppression of the paralysis conferred by *unc-52(e669su250* ts). The F_2 progeny were also checked for suppression; in all cases of noncomplementation, no nonsuppressed segregants were found in the F_2 generation, suggesting that noncomplementing alleles were closely linked. The suppressor mutations fell into two complementation groups: *mn415*, *mn417* and *mn433* were assigned to *smu-1*, and *mn416* was assigned to *smu-2*.

Mapping *smu-1 I*: For each of the *smu-1* alleles, N2 males were mated to *mec-8(u218* ts) *smu-1; unc-52(e669su250* ts) hermaphrodites. From the triply heterozygous F_1 hermaphrodites, many non-Unc-52 progeny hermaphrodites were picked individually, and the proportion of animals that segregated about three-quarters Mec self progeny was determined. The ratios obtained–5/100 for *mn415*, 1/20 for *mn417* and 2/87 for *mn333*, for an average of 0.04–indicate that *smu-1* is situated on LGI, about 9 map units from *mec-8*. The location of *smu-1* on LGI (Figure 1) was determined more precisely by two- and three-factor crosses. *dpy-5 unc-75*/+ males were used to generate *dpy-5* + *unc-75* +/+ *mec-8(u218* ts) + *smu-1(mn415); unc-52(e669su250* ts)/+ hermaphrodites. The progeny of these animals were screened for Dpy non-Unc-75 non-Unc-52 and Unc-75 non-Dpy non-Unc-52 recombinants. Only recombinants that segregated Unc-52 animals among their self progeny were saved. Ten Unc-52 progeny from each selected recombinant were picked and scored for the segregation of non-Unc-52 (suppressed) progeny. Among the Dpy non-Unc recombinants, 13/13 harbored *smu-1(mn415)*; only 1/10 Unc-75 non-Dpy recombinants harbored *smu-1(mn415)*. These data suggest that *smu-1* is either to the right of *unc-75* (in which case one of the recombinant chromosomes was generated by two crossovers) or very close and to the left. A similar cross involving a *dpy-5 unc-59* chromosome gave the following results: 8/10 Dpy non-Unc-59 recombinants and 1/7 Unc-59 non-Dpy recombinants carried *smu-1(mn415)*, which positions *smu-1* nearer but to the left of *unc-59*. A similar cross involving *dpy-5 unc-101* gave the following results: 9/9 Dpy non-Unc-101 recombinants and 0/8 Unc-101 non-Dpy recombinants carried *smu-1(mn415)*, which indicates that *smu-1* is near or to the right of *unc-101*. That it is to the right was determined by the following cross: *dpy-5 mec-8(u218* ts)/++ males were mated to *mec-8(u218* ts) *unc-101 smu-1(mn415)* hermaphrodites. F_1 Mec animals were picked, and non-Unc-101 non-Mec recombinants (recombinant between *unc-101* and *smu-1*) were selected. Four of five such recombinants segregated Dpy self progeny. The one recombinant chromosome without *dpy-5* could have been involved in two crossovers. Finally, two-factor crosses were used to measure the *unc-101-smu-1* map distance: *mec-8(u218* ts) *unc-101*/++ males were mated with *mec-8(u218* ts) *smu-1; unc-52(e669su250* ts) hermaphrodites, and Mec progeny (*mec-8 unc-101*+/*mec-8* + *smu-1; unc-52*/+) were picked. Among the self progeny of these animals, Unc-101 hermaphrodites were picked; their self progeny were then scored for the presence of non-Mec (suppressed) segregants. For *mn415*, 3/189 Unc-101 parents segregated recombinants. The data for *mn417* and *mn433* were 2/55 and 1/56, respectively. Taken together, these results confirm that the three *smu-1* alleles map close together and that *smu-1* maps about 0.7 map unit–correcting for growth at 25° (Rose and Baillie 1979)–to the right of *unc-101*. We have shown that *smu-1(mn415)* and *unc-101* complement: *mec-8(u218* ts) *smu-1(mn415) I; unc-52(e669su250* ts) *II; him-5 V* males were crossed to *dpy-5 unc-101 I; unc-52(e669su250* ts) *II* hermaphrodites; the non-Dpy progeny were all Unc-52 (not suppressed). Furthermore, *unc-101/smu-1* animals (see above) were non-Unc-101.

Mapping *smu-2 II*: The progeny ratios from hermaphrodites heterozygous for *smu-2(mn416), mec-8(u218* ts) and *unc-52(e669su250* ts) suggested that *smu-2* is loosely linked to *unc-52 II* (Figure 1). This surmise was confirmed by subsequent mapping experiments. For example, *smu-2* was shown to be very near or to the left of *dpy-10 II* by the following three-factor cross: *mec-8(u218* ts); *smu-2 unc-52(e669su250* ts); *him-5* males were mated to *mec-8(u218* ts); *dpy-10 unc-4* hermaphrodites, and Mec hermaphrodite progeny were picked. Dpy non-Unc-4 and Unc-4 non-Dpy F_2 recombinant progeny were picked, and their self progeny were screened for animals showing suppression of Mec: 10/10 Unc-4 non-Dpy recombinants and 0/8 Dpy non-Unc recombinants carried *smu-2*. The following three-factor cross placed *smu-2* near or to the left of *unc-85*: 0/6 Unc-85 non-Dpy recombinants and 4/4 Dpy non-Unc-85 recombinants among the self progeny of + *unc-85 dpy-10* +/*smu-2* ++ *unc-52(e669su250* ts) hermaphrodites carried *smu-2*, as ascertained by segregants showing suppression of paralysis in *unc-52(e669su250* ts) homozygotes. The following cross placed *smu-2* between *sqt-2* and *unc-85*: 6/8 Sqt non-Unc-85 recombinant and 0/2 Unc-85 non-Sqt recombinants among the self progeny of *sqt-2* + *unc-85* +/+ *smu-2* + *unc-52(e669su250* ts) hermaphrodites carried *smu-2*. This position is also supported by the following results: 0/29 Sup non-Sqt recombinants and 5/5 Sqt non-Sup recombinants among the self progeny of *sqt-2 smu-2* + *unc-52(e669su250* ts)/++ *unc-85 unc-52(e669su250* ts) hermaphrodites carried *unc-85*. Finally, the following two-factor cross gives a *smu-2-unc-85* map distance of 3.8 map units, corrected to about 2.5 map units because of the growth at 25° (Rose and Baillie 1979): 9/120 non-Unc-52 (suppressed) segregants from *smu-2* + *unc-52(e669su250* ts)/+ *unc-85 unc-52(e669su250* ts) hermaphrodites carried *unc-85*.

We have shown that *smu-2* and the closely linked gene *mup-1* (Goh and Bogaert 1991) complement, a cross between *smu-2 unc-4 unc-52(e669su250* ts)/+++ males and *mup-1(e2436) dpy-10 unc-52(e669su250* ts) hermaphrodites, yielded equal proportions of Unc-52 (not suppressed) and wild-type cross progeny.

Isolation of *smu-1* alleles away from *mec-8(u218* ts) and *unc-52(e669su250* ts): Three strains, each carrying a different *smu-1* allele alone, were constructed by the following procedure. *unc-52(e669su250* ts)/+ males were mated to *dpy-5 smu-1; unc-52(e669su250* ts) hermaphrodites (derived from three-factor mapping crosses) and Unc-52 progeny were picked. Non-Dpy non-Unc-52 (suppressed) recombinants were picked among the progeny and allowed to self. Several non-Dpy hermaphrodite progeny were picked, and a line that segregated no Dpy progeny was kept. The *smu-1; unc-52(e669su250* ts) stock so generated was crossed to *unc-52(e669su250* ts)/ + males five times, always retaining the *unc-52* allele in homozygous form to score for the presence of *smu-1*. Next, *unc-101*/+ males were mated to *smu-1; unc-52(e669su250* ts) hermaphrodites, and progeny were picked. Non-Unc-101 progeny were picked from broods that contained Unc-101 animals. Non-Unc-101 progeny were then

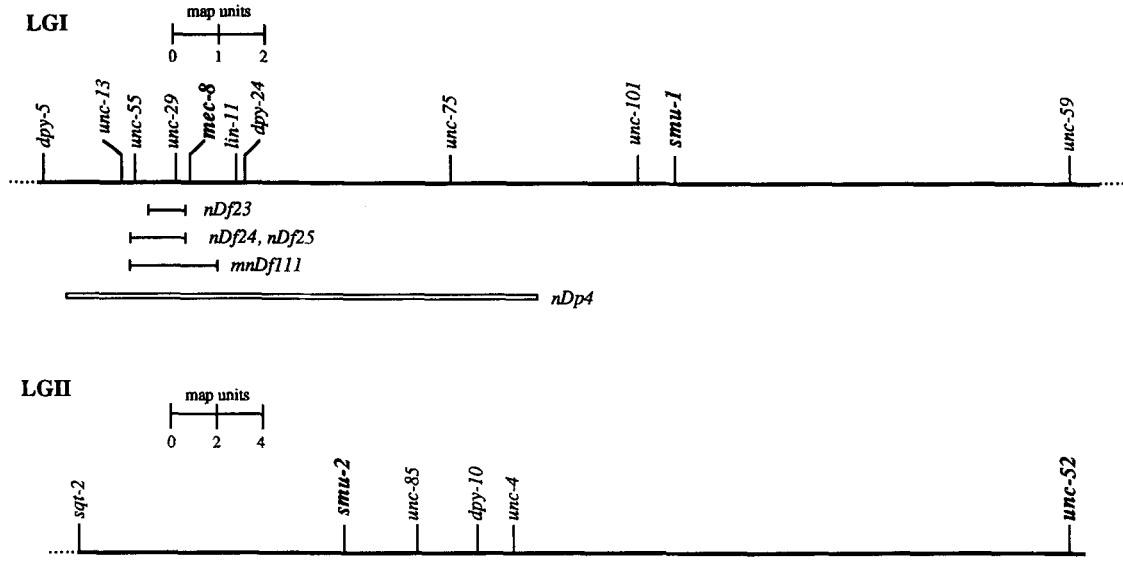

FIGURE 1.—Genetic map showing positions of *mec-8 I, unc-52 II, smu-1 I, smu-2 II* and other loci and chromosome rearrangements used in mapping.

picked from broods that segregated Unc-101 but no Unc-52 self progeny. A line that segregated no Unc-101 animals was assumed to be homozygous for *smu-1*. The presence of *smu-1* was confirmed by crossing in *unc-52(e669su250* ts) and identifying non-Unc-52 (suppressed) descendants of Unc-52 segregants. The absence of *mec-8(u218* ts) was confirmed by crossing to *mec-8(u218* ts)/+ males and finding no Mec cross progeny.

Isolation of *smu-2* away from *mec-8(u218* ts) and *unc-52(e669su250* ts): Beginning with the *mec-8(u218* ts) *I; smu-2(mn416) unc-52(e669su250* ts) *II* strain, *mec-8(u218* ts) was crossed out by segregation against *dpy-5*, to derive the stock *dpy-5; smu-2 unc-52(e669su250* ts). Next, *unc-52(e669su250* ts)/+ males were mated to *dpy-5; smu-2 unc-52(e669su250* ts) hermaphrodites, and non-Dpy Unc-52 progeny were picked. Non-Dpy non-Unc-52 (suppressed) self progeny were picked, and the resulting *smu-2 unc-52(e669su250* ts) homozygous line was then outcrossed to *unc-52(e669su250* ts)/+ males five times. Finally, *unc-85*/+ males were crossed to *smu-2 unc-52(e669su250* ts) hermaphrodites, and hermaphrodite progeny were picked. Many non-Unc-52 non-Unc-85 self progeny hermaphrodites were picked from a brood that contained Unc-85 animals and scored for segregation of Unc-52 animals. From broods containing Unc-85 animals but no Unc-52 animals, several non-Unc-85 hermaphrodites were picked. Those segregating no Unc-85 animals were assumed to be *smu-2* homozygotes. The presence of *smu-2* was confirmed by crossing in *unc-52(e669su250* ts) and identifying non-Unc-52 (suppressed) descendants of Unc-52 segregants. The absence of *mec-8(u218* ts) was confirmed by crossing to *mec-8(u218* ts)/+ males and finding no Mec cross progeny.

Construction of *mec-8 smu-1* double mutants: Each of the three *smu-1* alleles was combined with various *mec-8* alleles by the following procedure. *mec-8 unc-101*/+ males were mated with *smu-1* homozygotes. Many hermaphrodite progeny were picked, and Mec non-Unc-101 recombinants were picked from the progeny of those F_1 hermaphrodites that gave rise to Mec Unc-101 self progeny. The F_3 progeny were scored for the presence of Unc-101 animals, and several non-Unc-101 F_3 animals were picked. Those that segregated no Unc-101 progeny were assumed to be *mec-8 smu-1* homozygotes. There is a small chance (1–2%) that a *smu-1(+)* allele is present in these stocks, owing to recombination between *unc-101* and *smu-1*. Suppression of the *mec-8* mutation provides evidence that the strain is in fact homozygous for *smu-1*. In a few cases where suppression was not observed, a second, independent stock was constructed. Patterns of suppression of a given *mec-8* allele by different suppressors were generally consistent (see RESULTS).

Construction of *mec-8; smu-2* double mutants: *mec-8*/+; *unc-85*/+ males were mated to *smu-2* hermaphrodites and many progeny were picked. From the progeny of those F_1 hermaphrodites that segregated Mec and Unc-85 self progeny, several Mec non-Unc-85 animals were picked. Those that segregated no Unc-85 self progeny were assumed to be the genotype *mec-8; smu-2*. Because of the possibility of recombination between *smu-2* and *unc-85* (which are about 4 map units apart; see Figure 2), we cannot be positive that a given stock displaying no suppression of *mec-8* is homozygous for *smu-2*. However, only *mec-8(u74)* showed no apparent suppression by *smu-2*, and in this case, none of the *smu-1* alleles showed significant suppression either (see RESULTS).

Construction of *smu-1; unc-52* and *smu-2 unc-52* double mutants: *smu-1* and *smu-2* alleles were tested for suppression of the paralysis conferred by several *unc-52* alleles. *unc-52*/+ males were mated to *smu-1* or *smu-2* hermaphrodites, and F_1 progeny were picked. From F_2 broods containing Unc-52 animals, many Unc-52 animals were picked, three per plate. Their progeny were then scored with respect to (Unc-52) paralysis. Non-Unc-52 animals were presumed to be *smu-1; unc-52* or *smu-2 unc-52*.

RESULTS

***mec-8* map position and identification of new alleles:** We have used two- and three-factor genetic crosses (data given in MATERIALS AND METHODS) to refine the genetic map position of *mec-8* on linkage group I. Both touch insensitivity and dye-filling defects conferred by *mec-8* mutations were used in the mapping. The resulting genetic map in the neighborhood of *mec-8* is given in Figure 1.

Eight *mec-8* mutations were identified previously by other workers: *e398* (CHALFIE and SULSTON 1981), *u74*, *u218* ts, *u303*, *u314*, *u391* and *u456* (CHALFIE and AU 1989) were identified in screens for mechanosensory-defective mutants, and *rh170* was identified in a screen

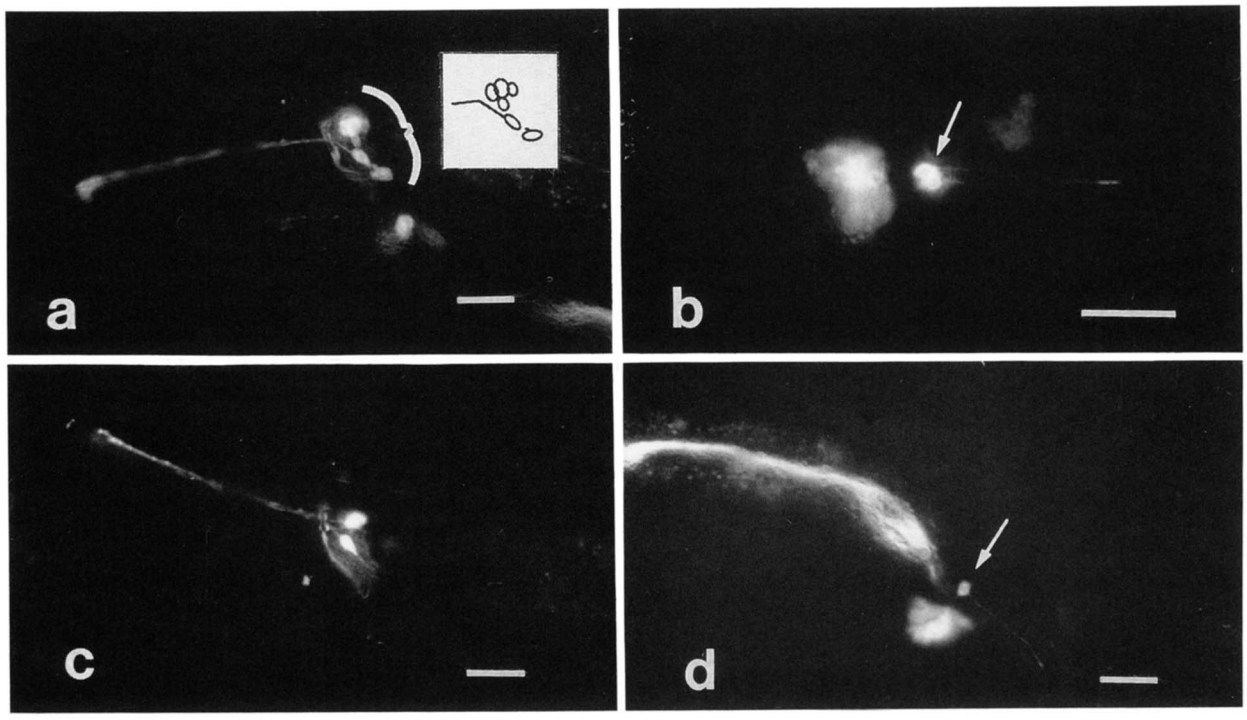

FIGURE 2.—Neuron filling of wild-type and *mec-8* animals with the fluorescent dye DiO. Anterior is to the left, and dorsal is up. Scale bar in each panel represents 20 μm. (a) Six neuron cell bodies (marked by a bracket and depicted in the inset), all belonging to one amphid of a wild-type animal, have filled with dye and are visualized by fluorescence microscopy. The processes of the neurons also show staining. The fluorescence below the bracketed amphid neurons is due to two phasmid neurons of a separate but nearby worm. (b) Two neuron cell bodies (arrow) of a single phasmid in a wild-type animal are shown; the broad staining to the left of the phasmid cell bodies is due to dye in the intestine. (c) A *mec-8(u456)* mutant is defective in dye filling; only two neuron cell bodies of an amphid have filled with dye. (d) Only one phasmid neuron cell body (arrow) of a *mec-8(u456)* animal has filled with dye. (The intestine also contains dye, and the animal has expelled some dye, seen below the phasmid cell body, by defecation.)

for mutants defective in dye filling (E. HEDGECOCK, personal communication). The two alleles *u456* and *u391* were γ-ray-induced (M. CHALFIE, personal communication); the others were identified following EMS mutagenesis. To identify new and possibly null alleles of *mec-8*, we conducted F_1 noncomplementation screens and identified eight new independent *mec-8* mutations. Males that were either mutagenized or derived from a mutator stock were mated to homozygous *unc-29 mec-8(e398) dpy-24 I; unc-7 X* hermaphrodites, and the heterozygous wild-type hermaphrodite cross-progeny were scored for their ability to respond to light touch. Touch-insensitive animals were picked; and their self progeny were checked for touch insensitivity and possible homozygosity for the new mutation. This screen is capable of identifying complete loss-of-function (null) mutations because the recessive lethal deficiency *mnDf111* was isolated in a similar noncomplementation screen against *mec-8(e398)* (see MATERIALS AND METHODS) and uncovers the *mec-8* locus, *i.e.*, *mec-8(e398)/mnDf111* animals are touch insensitive, viable and fertile. All eight new alleles, like the previous eight, are homozygous viable and fertile at 20°.

Three *mec-8* alleles (*mn450*, *mn455* and *mn459*) were recovered among 9,645 F_1 heterozygotes scored following EMS mutagenesis. This mutation frequency, 3×10^{-4}, is approximately the frequency expected for the generation of loss-of-function mutations in an average *C. elegans* gene (BRENNER 1974). Trimethylpsoralen mutagenesis yielded four *mec-8* mutations (*mn462*, *mn463*, *mn464* and *mn465*) out of 18,712 F_1 heterozygotes scored, a mutation frequency of 2×10^{-4}.

The eighth new *mec-8* allele was recovered from a mutator strain, RW7097, which exhibits enhanced germline transposition of the transposable element Tc1 (MORI *et al.* 1988). In this case, male progeny from a cross between N2 males and RW7097 hermaphrodites were employed in the same non-complementation screen described above. Five Mec mutants were recovered among 3,316 F_1 heterozygotes scored, but all five mutations when homozygous conferred a similar temperature-sensitive phenotype. We suppose that the five mutants all carried the same mutation, which was already present in our RW7097 stock; we therefore restricted our focus to one mutation in this set, *mn472* ts. This mutation was outcrossed to N2 10 times. The other seven new mutations were outcrossed five times.

As will be shown in detail below, all of the eight new *mec-8* alleles confer a stronger defect in dye filling than does *mec-8(e398)*, with the exception of *mn472* ts, which confers a weaker dye-filling defect; these results indicate that the new alleles are not

TABLE 2

Dye-filling defects and mechanosensory defects of *mec-8* mutants

Genotype (temperature)[a]	Proportion of phasmid neurons filled (*n*)[b]	Touch sensitivity	Genotype (temperature)[a]	Proportion of phasmid neurons filled (*n*)[b]	Touch sensitivity
N2 (wild-type)	0.99 (240)	++	*mec-8(rh170)*	0.01 (180)	−
mec-8(e398)	0.11 (176)	−	*mec-8(rh170)/mnDf111*	0.01 (116)	−
mec-8(e398)/mnDf111	0.02 (404)	−	*mec-8(u74)*	0.02 (136)[c]	−
mec-8(mn450)	0.03 (324)	−	*mec-8(u74)/mnDf111*	0.04 (80)[c]	−
mec-8(mn450)/mnDf111	0.02 (260)	−	*mec-8(u74)/mec-8(mn472* ts) (25°)	0.06 (268)	−
mec-8(mn455)	0.02 (328)	−	*mec-8(u74)/mec-8(mn472* ts) (16°)	0.20 (280)	−
mec-8(mn455)/mnDf111	0.03 (260)	−	*mec-8(u74)/mec-8(u218* ts) (25°)	0.05 (192)[c]	−
mec-8(mn459)	0.04 (336)	−	*mec-8(u74)/mec-8(u218* ts) (16°)	0.39 (208)	−
mec-8(mn459)/mnDf111	0.02 (248)	−	*mec-8(u218* ts) (25°)	0.18 (368)	−
mec-8(mn462)	0.04 (380)	−	*mec-8(u218* ts)*/mnDf111* (25°)	0.08 (336)	−
mec-8(mn462)/mnDf111	0.03 (288)	−	*mec-8(u218* ts) (16°)	0.94 (120)[c]	++
mec-8(mn463)	0.03 (488)	−	*mec-8(u218* ts)*/mnDf111* (16°)	0.36 (240)[c]	−
mec-8(mn463)/mnDf111	0.02 (288)	−	*mec-8(u303)*	0.05 (228)	−
mec-8(mn463)/mec-8(mn472 ts) (25°)	0.06 (164)	−	*mec-8(u303)/mnDf111*	0.01 (200)	−
mec-8(mn463)/mec-8(mn472 ts) (16°)	0.18 (300)	−	*mec-8(u314)*	<0.01 (172)	−
mec-8(mn463)/mec-8(u218 ts) (25°)	0.07 (216)[c]	−	*mec-8(u314)/mnDf111*	0.02 (200)	−
mec-8(mn463)/mec-8(u218 ts) (16°)	0.34 (128)[c]	−	*mec-8(u391)*	0.02 (184)	−
mec-8(mn464)	0.02 (404)	−	*mec-8(u391)/mnDf111*	0.03 (228)	−
mec-8(mn464)/mnDf111	0.04 (288)	−	*mec-8(u391)/mec-8(mn472* ts) (25°)	0.05 (264)	−
mec-8(mn465)	0.02 (400)	−	*mec-8(u391)/mec-8(mn472* ts) (16°)	0.18 (276)	−
mec-8(mn465)/mnDf111	0.03 (288)	−	*mec-8(u391)/mec-8(u218* ts) (25°)	0.05 (212)[c]	−
mec-8(mn472 ts) (25°)	0.10 (396)	−	*mec-8(u391)/mec-8(u218* ts) (16°)	0.44 (180)[c]	−
mec-8(mn472 ts)*/mnDf111* (25°)	0.05 (208)	−	*mec-8(u456)*	<0.01 (284)	−
mec-8(mn472 ts) (16°)	0.57 (132)[c]	+	*mec-8(u456)/mnDf111*	0.02 (256)	−
mec-8(mn472 ts)*/mnDf111* (16°)	0.23 (208)[c]	−			

++ = touch sensitive; + = partially touch sensitive; − = touch insensitive.

[a] Animals were scored at 20° unless otherwise noted.

[b] *n* is the total number of phasmid neurons scored; each animal has four phasmid neurons.

[c] Scored in one experiment; all other strains were scored in at least two independent experiments.

reisolates of *e398*, the allele used in the noncomplementation screens.

Mec and Dyf phenes of *mec-8* mutants: All 16 *mec-8* mutations result in an insensitivity to light touch, the Mec phene (CHALFIE and AU 1989; Table 2). Indeed, all but *rh170* were originally identified on the basis of this phenotype. CHALFIE and AU (1989) showed earlier that *u218* ts causes a temperature-sensitive touch response; we have confirmed this result and shown that *mn472* ts is also temperature-sensitive: *mn472* ts animals are weakly touch sensitive at 16° and touch insensitive at 25° (Table 2). None of the other 14 alleles confers a temperature-sensitive touch response. For all 16 mutations, mutant animals respond to a prod with a wire and generally move normally. All 16 alleles are recessive to *mec-8(+)* with respect to the touch response.

As noted in the introduction, when living wild-type animals are bathed in a solution of a fluorescent dye, either FITC or DiO, 16 chemosensory neurons, six in each of a pair of amphids in the head and two in each of pair of phasmids in the tail, fill with dye and can be readily visualized by fluorescence microscopy (HEDGECOCK *et al.* 1985). PERKINS *et al.* (1986) reported that when either *mec-8(e398)* or *mec-8(u74)* animals were exposed to FITC, usually only two neurons per amphid and no phasmid neurons filled with dye, the Dyf phene. Figure 2 shows fluorescence micrographs of wild-type and *mec-8(u456)* amphids and phasmids after exposure to DiO. When neurons fill with dye, cell bodies as well as processes are stained. Only two neurons of the *mec-8(u456)* amphid illustrated in Figure 2c filled with dye, and one neuron of the phasmid in Figure 2d filled. All 16 *mec-8* mutations caused dye-filling defects and were recessive to *mec-8(+)* with respect to this phene. Both amphids and phasmids were affected, but for ease of scoring, we have counted the proportion of phasmid neurons that filled with dye for each homozygous *mec-8* allele, each *mec-8* allele over a deficiency, and various *trans*-heterozygous combinations of *mec-8* alleles (Table 2). When phasmid dye filling was rare, animals exhibiting staining usually had only one phasmid neuron filled.

All but three of the *mec-8* alleles show about 1–4% dye filling of phasmid neurons with DiO. The two alleles that were temperature-sensitive with respect to the touch response were also temperature sensitive with respect to dye filling, showing 10% (*mn472* ts) and 18% (*u218* ts) dye filling at 25° and considerably more dye filling at 16° (Table 2). The allele *e398* was also weaker than the others, showing 11% dye filling at 20°. The Dyf phenes caused by *u218* ts, *mn472* ts or *e398* over *mnDf111*, a deficiency that does not complement *mec-8*, are more severe than those caused by the homozygous alleles, suggesting that *u218* ts, *mn472* ts and *e398* are incomplete

TABLE 3

Cold-sensitive embryonic arrest and early larval arrest of *mec-8* mutants

Genotype	°C	*n*	Proportion of embryonic or early larval arrest	Genotype	°C	*n*	Proportion of embryonic or early larval arrest
N2 (wild-type)	16	731	<0.01	*mec-8(mn472* ts)	16	1106	0.01
N2 (wild-type)	25	659	<0.01	*mec-8(rh170)*	16	385	0.16
mec-8(e398)	16	683	0.06	*mec-8(rh170)*	25	561	0.02
mec-8(e398)	25	363	0.02	*mec-8(u74)*	16	597	0.31
mec-8(mn450)	16	1828	0.26	*mec-8(u74)*	25	369	0.14
mec-8(mn450)	25	327	0.02	*mec-8(u218* ts)	16	391	0.00
mec-8(mn455)	16	750	0.17	*mec-8(u218* ts)	25	302	0.02
mec-8(mn455)	25	370	0.03	*mec-8(u303)*	16	459	0.03
mec-8(mn459)	16	752	0.14	*mec-8(u303)*	25	165	0.02
mec-8(mn459)	25	414	0.02	*mec-8(u314)*	16	410	0.27
mec-8(mn462)	16	641	0.23	*mec-8(u314)*	25	328	0.01
mec-8(mn462)	25	506	0.01	*mec-8(u391)*	16	601	0.29
mec-8(mn463)	16	501	0.28	*mec-8(u391)*	25	370	0.02
mec-8(mn463)	25	580	0.03	*mec-8(u456)*	16	425	0.28
mec-8(mn464)	16	588	0.23	*mec-8(u456)*	25	666	0.05
mec-8(mn464)	25	625	0.01				
mec-8(mn465)	16	516	0.30				
mec-8(mn465)	25	622	<0.01				

loss-of-function (hypomorphic) alleles of *mec-8*. The Dyf phenes caused by all other *mec-8* alleles over *mnDf111* are similar to those caused by the homozygous alleles. Three of the strong alleles (*u74*, *mn463* and *u391*) behaved as did *mnDf111* when placed in *trans* to *u218* ts and *mn472* ts (Table 2); for example, *mec-8(u74)/mec-8(u218* ts) and *mnDf111/mec-8(u218* ts) at 25° gave 5% and 8% phasmid filling, respectively. These results are consistent with the hypothesis that *u74*, *mn463* and *u391* are null.

Cold-sensitive embryonic and early larval lethality of *mec-8* mutants: We have discovered that many *mec-8* mutants grown at 16° display variable embryonic or early larval arrest. Table 3 lists the results of self-progeny counts for *mec-8* mutants grown at 16° and 25°. The arrest phenotype is clearly cold-sensitive; *mec-8* animals grown at 16° show more lethality than those grown at 20° or 25°. The two alleles that conferred a temperature-sensitive touch response and a temperature-sensitive dye-filling defect, *u218* ts and *mn472* ts, did not display the cold-sensitive arrest. The *e398* allele, which was not among the strongest alleles with respect to its Dyf phene, also showed little arrest at 16°. *u303* is a weak allele by the criterion of cold-sensitive arrest, and *mn455*, *mn459* and *rh170* seem also to be somewhat weaker than the other alleles (Table 3). This leaves nine alleles that gave 23–31% embryonic or larval arrest when grown at 16°; these include all of the strong alleles as defined by other criteria. We suggest that the variable cold-sensitive embryonic or early larval arrest is a null phene. Six of the seven weaker *mec-8* alleles were EMS-induced; the seventh, *mn472* ts, was derived from the mutator strain RW7097. Among the nine strong alleles, four were induced by trimethylpsoralen and two were γ-ray-induced.

Arrested *mec-8* embryos and larvae are deformed and display muscle defects: We have used light microscopy to inspect arrested *mec-8* embryos and larva reared at 16°. Arrested embryos and larvae homozygous for six strong alleles–*mn450*, *mn463*, *u74*, *u314*, *u391* and *u456*–and two weaker ones–*e398* and *rh170*–were inspected by both Nomarski differential interference microscopy and polarized light microscopy. Arrested embryos frequently showed incomplete elongation; some of these embryos subsequently hatched, but others did not. Wild-type embryos elongate to approximately 3.5 times the length of the egg before hatching (PRIESS and HIRSH 1986). Figure 3 shows Nomarski micrographs of wild-type 2- and 3.5-fold embryos (Figure 3, a and b) and defective *mec-8(mn463)* embryos (Figure 3, c and d) reared at 16°. The *mec-8(mn463)* embryos pictured have arrested at about the twofold stage of elongation but show pharyngeal development characteristic of later embryonic development. This is the earliest stage of *mec-8* arrest we saw; other embryos arrested at later stages of elongation and many of these hatched. All of the arrested embryos, even those that failed to elongate beyond the twofold stage, exhibited some movement. In addition to showing elongation defects, virtually all arrested *mec-8* embryos displayed hypodermal constrictions and bulges; the tips of the noses, particularly, were often pinched and bent (Figure 3, c and d). Arrested *mec-8* L1 and L2 larvae reared at 16° also displayed hypodermal kinks and bulges. Indeed, for the strong alleles, about 5–10% of *mec-8* adults reared at 16° exhibited morphological deformities–bulges and kinks–in the dissecting microscope, and such animals were frequently uncoordinated in their movement as well.

We used polarized light microscopy to inspect the birefringent body muscle cells (WATERSTON 1988) of the

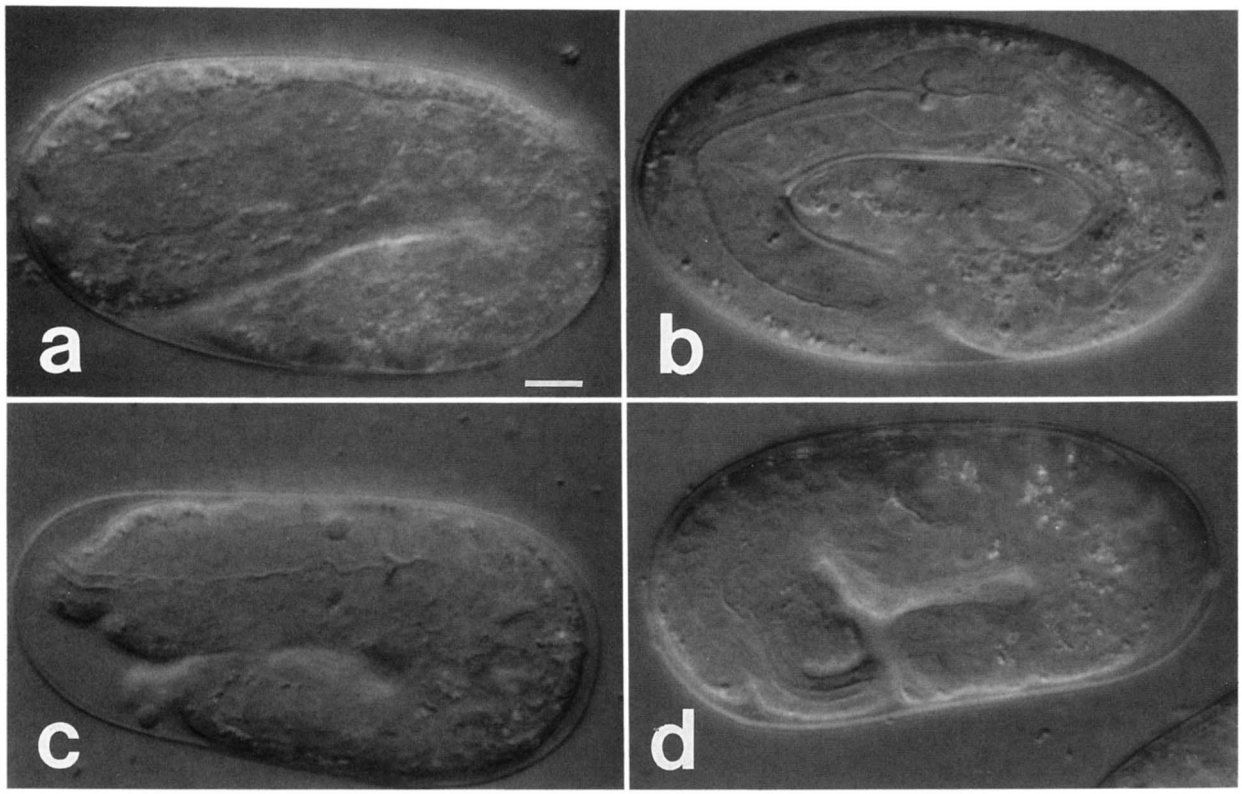

FIGURE 3.—Wild-type and *mec-8* embryos, as observed by Nomarski differential interference microscopy. All embryos were reared at 16°. (a) Wild-type embryo at nearly twofold stage of elongation (approximately 450 min after fertilization). The pharynx has not yet undergone extensive morphogenesis to form distinct bulbs and a grinder. (b) Wild-type embryo at about 3.5-fold stage of elongation (approximately 750 min after fertilization, just prior to hatching). The pharynx has undergone morphogenesis and is pumping. The nose of the animal is below the plane of focus. (c) A *mec-8(mn463)* embryo arrested at nearly the twofold stage of elongation. The pharynx is well developed but the hypodermis shows constrictions and bulges. (d) A *mec-8(mn463)* embryo arrested at about a 2.5-fold stage of elongation. This animal also shows severe hypodermal constrictions and bulges. The scale bar, shown in (a), represents 5 μm; all panels are at the same magnification.

same set of eight *mec-8* mutants arrested as embryos or young larvae. Body wall muscle in *C. elegans* is arranged in four longitudinal strips that run the length of the animal (WATERSTON 1988). All arrested embryos and larvae displayed disruptions in the patterns of birefringence (data not shown). A bulge or kink in the hypodermis invariably corresponded to an area of defective muscle birefringence; usually in such regions, a muscle strip appeared to be detached from the hypodermis, as has been observed for mutants affected in *myo-3* (WATERSTON 1989), *unc-45* (VENOLIA and WATERSTON 1990) and *mup-1* (GOH and BOGAERT 1991). We also inspected the two ventral strips of body muscle in adult *mec-8* animals reared at 16°. Animals chosen at random were anesthetized and rolled onto their dorsal sides so that the complete lengths of the ventral strips could be inspected for possible local disruptions. For each genotype, 20–30 animals were scored. For each of the alleles *e398*, *rh170*, *u74*, *u314*, *u391* and *u456*, over half of the animals displayed at least one localized disruption in birefringence. For the alleles *u218* and *u303*, which caused little cold-sensitive embryonic arrest (Table 3), 3/22 and 5/30 animals showed at least one disruption in birefringence, respectively. None of 20 N2 animals showed any disruption. The same set of *mec-8* mutants reared at 25° exhibited very few or no disruptions as adults.

We have also used antibody staining to visualize the body muscle defects in *mec-8* mutants. We stained wild-type and *mec-8(mn463)* mutants grown at 16° with DM 5.8 (MILLER *et al.* 1983), a mouse monoclonal antibody that recognizes MHC B, the major muscle myosin heavy chain, and the results are illustrated in Figure 4. The muscle strips in *mec-8(mn463)* arrested embryos were often disorganized; the strips frequently appeared locally unattached to the hypodermis and cuticle as if they had peeled away from the body wall and collapsed inward. Many muscle cells gave a pattern of staining characteristic of an organized myofilament lattice, but other muscle cells showed only a general cytoplasmic staining, with nuclei outlined, as if an organized lattice structure had not formed. About 40% of *mec-8(mn463)* embryos and larvae reared at 16° and stained with anti-MHC B showed some abnormality in muscle structure. Qualitatively similar results were obtained for *mec-8(u74)* and *mec-8(u456)* animals.

Distribution of *unc-52* protein in *mec-8* mutants: The *C. elegans unc-52* gene encodes a homolog of perlecan,

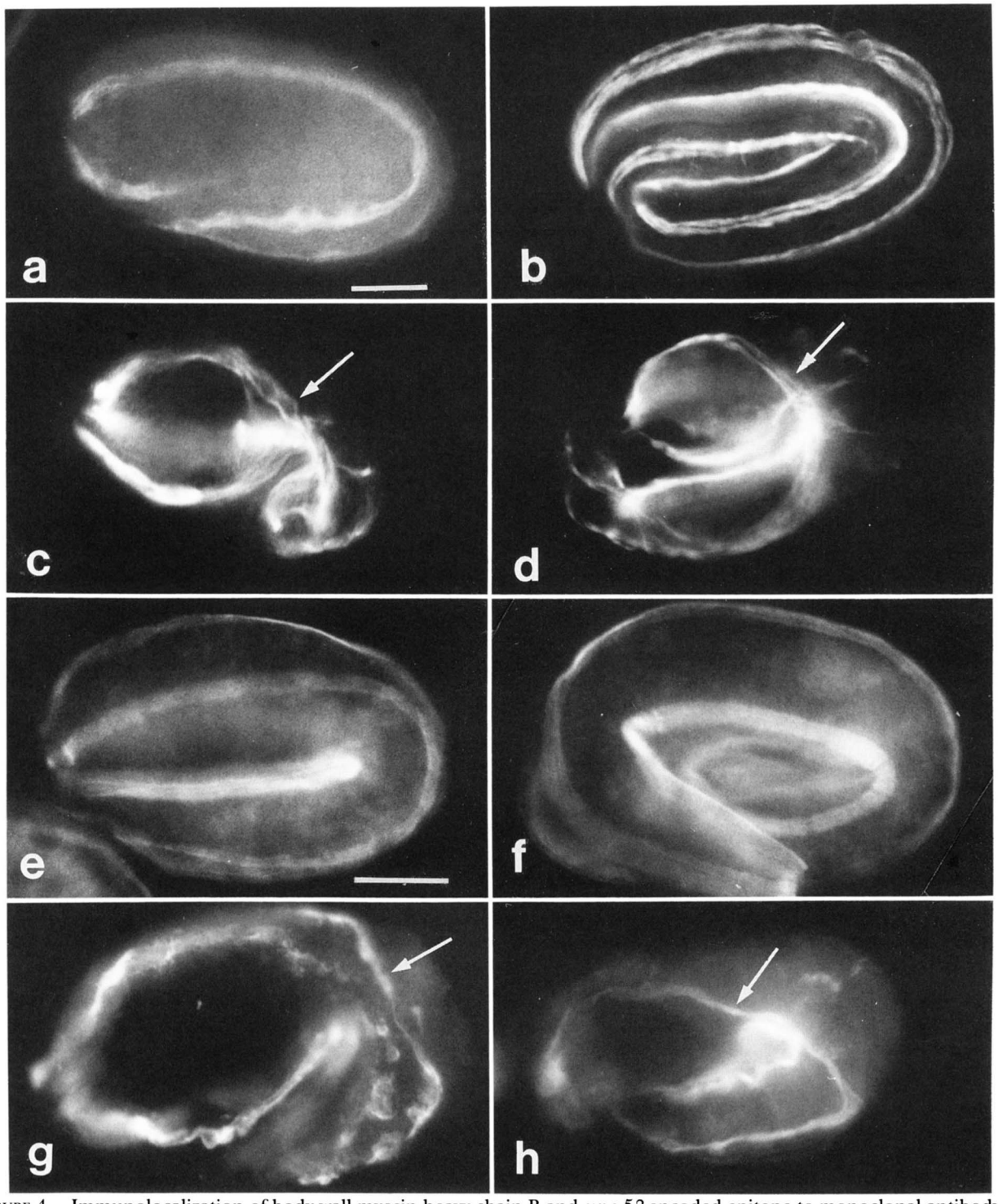

FIGURE 4.—Immunolocalization of body wall myosin heavy chain B and *unc-52*-encoded epitope to monoclonal antibody MH2 in wild-type and *mec-8* animals. All embryos were reared at 16°. The scale bar in (a) represents 10 μm for (a–d); the scale bar in (e) represents 10 μm for (e–h). Embryos in (a–d) were stained with DM 5.8 (MILLER *et al.* 1983), an antibody against myosin heavy chain B, the major myosin heavy chain of the body wall muscles; embryos in (e–h) were stained with MH2 (FRANCIS and WATERSTON 1991); the antigen recognized by MH2 has been localized to the basement membrane of contractile tissues (FRANCIS and WATERSTON 1991) and has been shown to be encoded by *unc-52* sequence (ROGALSKI *et al.* 1993). (a) A wild-type embryo at nearly the twofold stage of elongation. One of the four longitudinal muscle strips can be seen throughout its length. The myofilament lattices of some cells have not yet organized, as is indicated by the rounded appearance and outlined nuclei of some muscle cells in the tail. The anterior portion of a second muscle strip is in the plane of focus. (b) An N2 embryo at about the threefold stage of elongation. Two longitudinal muscle strips can be seen. (c and d) Two *mec-8(mn463)* embryos displaying muscle defects. Strips of body wall muscle are displaced, as if they are unattached and have collapsed away from the body wall (arrows). (e) Wild-type embryo at twofold stage of elongation, stained with MH2. (f) Wild-type embryo at 3.5-fold stage of elongation. (g and h) Two *mec-8(mn463)* arrested embryos. Strips of MH2 staining are displaced from the body wall (arrows), much like defective muscle strips. The embryo in (g) shows some MH2 staining that has not organized into strips, but remains diffuse and unorganized.

TABLE 4

Synthetic lethal combinations of *mec-8* and *unc-52*

	mec-8 allele					
unc-52 allele	*u218* ts	*u456*	*e398*	*mn455*	*mn463*	*mn472* ts
e444[a]	† (16°), † (25°)	†	†	†	†	
e669	† (25°)	†	†			
e669su250 ts	V (16°), † (25°)		† (25°)			† (16°)
e998			†			
e1421			†			
su54			†			

Unless otherwise noted, the growth temperature was 20°. Where no symbols are given, the double mutant was not made. † = synthetic lethal combination, showing paralyzed arrest at two-fold stage of embryonic elongation, V = viable.

[a] All *unc-52(e444)*-bearing animals carried the *mnC1 dpy-10 unc-52* chromosome (see MATERIALS AND METHODS).

the core protein of the mammalian basement membrane heparan sulfate proteoglycan (ROGALSKI *et al.* 1993). A monoclonal antibody, MH2, identified and described by FRANCIS and WATERSTON (1991), recognizes an antigen localized to basement membrane immediately adjacent to the body muscle cells; the positions in the basement membrane directly adjacent to the dense bodies and M-lines of the muscle show greatly intensified staining with MH2. ROGALSKI *et al.* (1993) have shown that a recessive lethal *unc-52* mutation abolishes MH2 staining; these workers also mapped the epitope recognized by MH2 to a region of *unc-52*-encoded sequence. (There are alternative *unc-52* transcripts, which presumably correspond to alternate protein products, and MH2 probably does not recognize them all.) We have stained wild-type and *mec-8* embryos reared at 16° with MH2, and the results are illustrated in Figure 4, e–h. The wild-type embryos show staining that corresponds to the body muscle strips, but the arrested *mec-8* animals show strips of staining that are disorganized and that appear to be locally unattached to the hypodermis, patterns that are very similar to those already described for staining muscle myosin of arrested *mec-8* animals. Furthermore, in some areas the staining was diffuse and not organized into strips. Defects visualized by MH2 staining were seen as early as the 1.5-fold stage of embryonic elongation. As was the case with staining myosin, the defects seen with MH2 staining were cold-sensitive and variable from animal to animal.

Synthetic lethality of *mec-8; unc-52(viable)* double mutants: The *unc-52* gene was first defined by mutations that lead to a progressive dystrophy of body musculature (BRENNER 1974). Mutant animals develop normally as larvae, but a gradual paralysis posterior to the pharynx begins late in larval development. The progression of the paralysis is correlated with a gradual disorganization of muscle structure (MACKENZIE *et al.* 1978; WATERSTON *et al.* 1980), the cause of which has been ascribed to a disruption of weak anchorages of the myofilament lattice (WATERSTON 1988). The lattice of the muscle cells is normally attached at many points to the muscle cell membrane and to the adjacent basement membrane; the basement membrane in turn is anchored to an adjacent and very thin hypodermis and also tethered through the hypodermis to external cuticle (FRANCIS and WATERSTON 1985, 1991; HRESKO *et al.* 1994). Animals homozygous for viable *unc-52* alleles are fertile, although they do show some abnormalities in gonadal structure (GILCHRIST and MOERMAN 1992). More recently, a zygotic lethal class of *unc-52* mutation has been discovered; embryos homozygous for lethal *unc-52* mutations arrest at the twofold stage of elongation and remain virtually paralyzed, a phenotype referred to as Pat, for *p*aralyzed and *a*rrested at the *t*wofold stage of elongation (WILLIAMS and WATERSTON 1994; GILCHRIST and MOERMAN 1992; ROGALSKI *et al.* 1993). The Pat phenotype is characteristic of muscle-defective mutants, such as *myo-3* (WATERSTON 1989), *unc-45* (VENOLIA and WATERSTON 1990), *deb-1* (BARSTEAD and WATERSTON 1991) and many other loci (WILLIAMS and WATERSTON 1994).

We have discovered that *mec-8* mutations strongly enhance the mutant phenotype of otherwise viable and fertile *unc-52* alleles such that the double mutant animals mimic the Pat phenotype conferred by lethal *unc-52* alleles. The synthetic lethality of *mec-8; unc-52(viable)* double mutants is not specific to particular pairs of alleles, and it is not cold sensitive. We tested six alleles of each gene in various combinations (Table 4), and all resulted in a 100% penetrant Pat phenotype. An example of these experiments is illustrated in Figure 5. In this case, animals of genotype *mec-8(mn463) I; mnC1 dpy-10 unc-52(e444)/unc-4 II*, which are touch insensitive and dye-filling defective, were generated; they segregated Mec Unc-4 and Mec non-Unc self progeny but no (Mec) Dpy Unc-52 self progeny. In place of the latter, one quarter of the self progeny were Pat embryos (Figure 5), which were not produced by *mec-8(mn463)* or *mnC1 dpy-10 unc-52/unc-4* parents. Some, but not all, Pat embryos hatched. The Pat embryos typically had hypodermal constrictions and bumps, particularly near the nose, which were similar to those seen in the arrested *mec-8* embryos reared at 16° and to those observed with lethal *unc-52* mutants (WILLIAMS and WATERSTON 1994; B. WILLIAMS, personal communication).

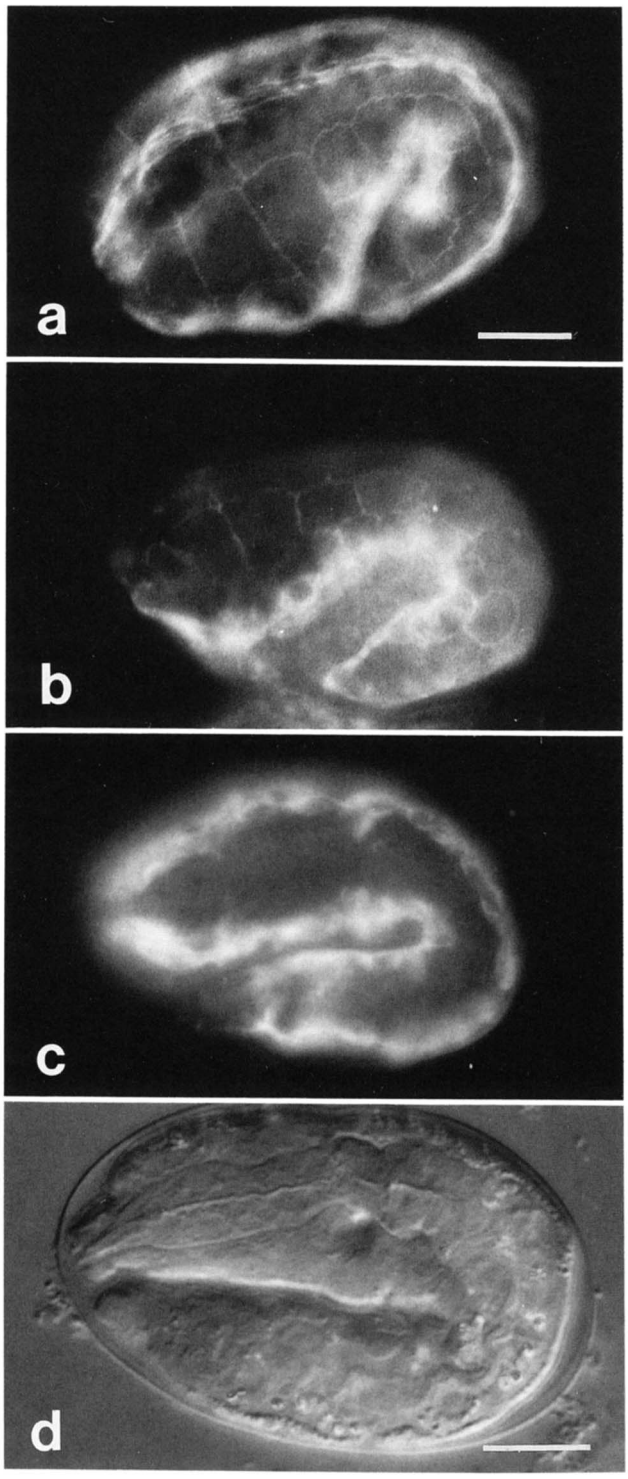

FIGURE 5.—Micrographs showing embryonic self progeny of *mec-8(mn463) I; mnC1 dpy-10 unc-52(e444)/unc-4 II* hermaphrodites. Anterior is to the upper left. The scale bar in (a) represents 10 μm for panels (a–c); the scale bar in (d) represents 10 μm for panel (d). All embryos were reared at 20°. (a) A 1.5-fold embryo double-stained with DM 5.8 (anti-MHC-B) and MH27, which recognizes junctions of hypodermal cells. This embryo displays wild-type organization of developing myofilament lattices into longitudinal strips, so we presume that it is not homozygous for *unc-52*. (b) This 1.7-fold embryo, which is double-stained as in (a), shows about the same hypodermal staining as in (a) but shows no organization of the myofilament lattice; the muscle cells maintain a rounded appearance with staining outlining the nuclei. We presume that the genotype of this animal is *mec-8; mnC1 dpy-10 unc-52*. (c) This 1.75-fold embryo is stained with anti-MHC B only. It shows the phenotype characteristic of *mec-8; unc-52* double mutants. No myofilament lattice organization is apparent; muscle cells are rounded, and muscle cell nuclei are outlined by MHC B staining. (d) Nomarski micrograph showing the terminal phenotype of a *mec-8(mn463); mnC1 dpy-10 unc-52(e444)*. The embryo is paralyzed and arrested at the twofold stage of elongation (Pat phenotype); it also shows a well developed pharynx and hypodermal constrictions and lumps.

TABLE 5

***mec-8(e398)* or *unc-52(e669)* in double mutant combinations that are viable and fertile**

Fertile with *mec-8(e398)*		Fertile with *unc-52(e669)*	
bli-6	*sqt-1*	*bli-6*	*mec-6*
dpy-5	*unc-1*	*daf-6*	*mec-7*
dpy-10	*unc-4*	*dpy-5*	*mec-9*
dpy-13	*unc-6*	*dpy-10*	*osm-1*
dpy-18	*unc-7*	*dpy-24*	*unc-1*
dpy-24	*unc-13*	*him-4*	*unc-3*
emb-9[a]	*unc-23*	*lon-2*	*unc-4*
him-4	*unc-29*	*mec-1*	*unc-23*
him-5	*unc-40*	*mec-2*	*unc-29*
him-8	*unc-54*	*mec-3*	*unc-55*
let-2[b]	*unc-60*	*mec-4*	*unc-97*
lon-2	*unc-75*	*mec-5*	
mec-1	*unc-95*		
mec-2	*unc-97*		

[a] *emb-9(hc70)* is a temperature-sensitive embryonic lethal mutation (WOOD *et al.* 1980). *mec-8(e398); emb-9(hc70)* doubles show temperature-sensitive embryonic arrest like *emb-9(hc70)* alone.

[b] *let-2(b246)* is a temperature-sensitive embryonic lethal mutation (HIRSH and VANDERSLICE 1976). *mec-8(e398); let-2(b246)* double mutants show temperature-sensitive embryonic arrest like *let-2(b246)* alone.

When temperature-sensitive alleles for both genes were combined, the resulting *mec-8(u218* ts*); unc-52(e669su250* ts*)* strain survived at 16° but was inviable at 25°. The somewhat stronger temperature-sensitive *mec-8* allele *mn472* ts was inviable in combination with *unc-52(e669su250)* even at 16°. *mec-8(u218* ts*)* in combination with *unc-52(e444)* even at 16° resulted in a Pat phenotype. No semidominant interactions between *mec-8* and *unc-52* mutations were detected. For example, at 20° *mec-8(e398); unc-52(e444)/+* animals were Mec and fully viable and fertile, and *mec-8(e398)/+; unc-52(e444)* animals were Unc-52, viable and fertile (and neither Mec nor Dyf).

The *mec-8; unc-52(viable)* interaction seems to be gene-specific. Table 5 lists other double mutants that were constructed involving either *mec-8(e398)* or *unc-52(e669)*. Some of the other mutations that were tested are in genes that encode components of the basement membrane, such as *unc-6(ev400)*, which encodes a laminin B2-like molecule (ISHII *et al.* 1992), and *emb-9* and *let-2*, which encode basement membrane collagen (GUO *et al.* 1991; SIBLEY *et al.* 1993) or in genes that have

been implicated in attachment of muscle cells to the hypodermis and cuticle, such as *unc-23* (WATERSTON 1988), *unc-95* and *unc-97* (ZENGEL and EPSTEIN 1980; WATERSTON 1988) or in genes that affect the response to light touch (CHALFIE and AU 1989). None of the mutations tested in combination with *mec-8(e398)* or *unc-52(e669)* resulted in a Pat phenotype or showed any other discernable interaction.

Mutations in *smu-1* and *smu-2* suppress *mec-8* alleles, *unc-52* alleles and *mec-8; unc-52* synthetic lethality: We have identified extragenic suppressors of the *mec-8; unc-52* synthetic lethality by making use of the conditional lethality of the *mec-8(u218* ts*); unc-52(e669su250* ts*)* strain. Animals grown at 16° were treated with EMS and allowed to propagate for at least two generations at 16° before shifting to 25°. Four independent suppressed mutants were identified as survivors at 25°. In the triple mutants at 25°, each of the suppressor mutations suppresses, in addition to the synthetic lethality of the *mec-8; unc-52* combination, the paralysis conferred by *unc-52(e669su250* ts*)* and the Mec and Dyf phenes conferred by *mec-8(u218* ts*)*. All four suppressors are recessive to their wild-type alleles for suppression of the paralysis conferred by *unc-52(e669su250* ts*)*, as well as for suppression of the Mec phene of *mec-8(u218* ts*)*. By complementation tests for suppression of *unc-52(e669su250* ts*)* paralysis, we determined that one suppressor locus, *smu-1*, has three alleles (*mn415*, *mn417* and *mn433*) and another, *smu-2*, has one allele (*mn416*), where *smu* stands for suppressor of *mec-8* and *unc-52*. We mapped the three *smu-1* alleles to a position about 1 map unit to the right of *unc-101 I*, and we mapped *smu-2* about 2.5 map units left of *unc-85 II* (Figure 1).

Stocks containing each of the homozygous suppressor mutations alone were constructed. The *smu-1* mutants have no highly-penetrant visible phenotype. However, they do show a slight tendency to coil their bodies, and they are slower growing and have slightly smaller brood sizes than N2 (data not shown). *smu-2(mn416)* mutants also show no highly penetrant visible phenotype but, like the *smu-1* mutants, have a tendency to coil their bodies.

Double mutants involving each of the *smu-1* and *smu-2* suppressors with 11 different *mec-8* alleles were constructed and scored for suppression of the Mec and Dyf phenes of *mec-8*. Table 6 gives the results. The data for the *mec-8* alleles alone are repeated from Table 2. The two temperature-sensitive alleles of *mec-8*, *u218* ts and *mn472* ts, were well suppressed for both Dyf and Mec phenes at 25°. None of the other *mec-8* alleles was suppressed for mechanosensation, but many were suppressed by at least one suppressor for Dyf, many by all four suppressors. Apart from the two temperature-sensitive alleles, the two *mec-8* alleles showing the greatest suppression for all four suppressors were *mec-8(u303)* and *mec-8(mn455)*, neither of which, on the basis of its cold-sensitive embryonic arrest phene, is likely to be null. Conversely, the Dyf phene of *mec-8(u74)*, which is a candidate for being null, was not suppressed by any of the suppressors. The cold-sensitive lethality phene of two strong *mec-8* alleles, *u74* and *mn463*, was scored in a *smu-1* and a *smu-2* background (Table 7). Both *mec-8* alleles were suppressed by *smu-1* and *smu-2* mutations. Overall, the data suggest that *smu-1* and *smu-2* mutations are fairly general suppressors of the *mec-8* mutations. The weaker *mec-8* alleles are suppressed better than the strong alleles for the Mec and Dyf phenes, but the strong alleles are suppressed for the cold-sensitive lethality.

Finally, each of the suppressors was checked for its ability to suppress the paralysis conferred by each of seven viable *unc-52* alleles: *e444*, *e669*, *e669su250* ts, *e998*, *e1421*, *su54*, and *su200*. As expected, all four suppressor mutations suppressed *unc-52(e669su250* ts*)*. The *e669su250* ts mutation confers a temperature-sensitive paralysis, but even at 16° mutant adults move slowly. The suppression by all four suppressors was strong at 25° but was also significant at 16° Adult *unc-52(e669su250* ts*)* animals reared at 25° display disrupted myofilament lattice structure posterior to the pharynx when viewed by polarized light microscopy. We observed that double mutants carrying *unc-52(e669su250* ts*)* and any one of the four suppressor mutations showed partial restoration of myofilament lattice structure when grown at 25° (data not shown). None of the other *unc-52* mutations was suppressed by any of the *smu-1* alleles, and only one other *unc-52* mutation, *e669*, was suppressed by *smu-2*, albeit weakly. The temperature-sensitive allele *e669su250* ts, was derived as a partial revertant of *e669* (MACKENZIE *et al.* 1978; GILCHRIST and MOERMAN 1992).

GILCHRIST and MOERMAN (1992) identified suppressors of *unc-52(viable)* mutations that define the *sup-38 IV* gene. Because their screen was for dominant suppressors, it is not surprising that they did not recover *smu-1* or *smu-2* alleles. The fact that we did not identify *sup-38* mutations may be the consequence of our focus on mutations that suppress both the Mec phene of *mec-8(u218* ts*)* and the paralysis of *unc-52(e669su250* ts*)*.

DISCUSSION

We have shown that mutations in the *mec-8* gene of *C. elegans* cause, in addition to the previously reported mechanosensory defects and defects in the amphid and phasmid chemosensory neurons, an incompletely penetrant, cold-sensitive embryonic and larval arrest. The strongest *mec-8* alleles cause about 30% of the animals reared at 16° to arrest development as embryos or larvae. The arrested embryos often fail to complete the elongation phase of embryonic morphogenesis and exhibit morphological abnormalities, including kinks and

TABLE 6

Suppression of dye-filling defects and mechanosensory defects of *mec-8* mutants by *smu-1* I and *smu-2* II

Genotype (temperature)[a]	Proportion of phasmid neurons filled (*n*)[b]	Touch sensitivity
N2 (wild-type)	0.99 (240)	++
mec-8(mn455)	0.02 (328)	−
mec-8(mn455) smu-1(mn415)	0.30 (192)[c]	−
mec-8(mn455) smu-1(mn417)	0.30 (152)[c]	−
mec-8(mn455) smu-1(mn433)	0.31 (176)[c]	−
mec-8(mn455); smu-2(mn416)	0.29 (196)[c]	−
mec-8(mn459)	0.04 (336)	−
mec-8(mn459) smu-1(mn415)	0.06 (176)[c]	−
mec-8(mn459) smu-1(mn417)	0.02 (176)[c]	−
mec-8(mn459) smu-1(mn433)	0.15 (198)[c]	−
mec-8(mn459); smu-2 (mn416)	0.13 (200)[c]	−
mec-8(e398)	0.11 (176)	−
mec-8(e398) smu-1(mn415)	0.08 (312)	−
mec-8(e398) smu-1(mn417)	0.09 (212)[c]	−
mec-8(e398) smu-1(mn433)	0.15 (416)	−
mec-8(e398); smu-2(mn416)	0.19 (180)[c]	−
mec-8(mn463)	0.03 (408)	−
mec-8(mn463) smu-1(mn415)	0.04 (108)[c]	−
mec-8(mn463) smu-1(mn417)	0.12 (352)	−
mec-8(mn463) smu-1(mn433)	0.23 (296)	−
mec-8(mn463); smu-2(mn416)	0.18 (184)	−
mec-8(mn472 ts*)* (25°)	0.10 (396)	−
mec-8(mn472 ts*) smu-1(mn415)* (25°)	0.55 (524)	+
mec-8(mn472 ts*) smu-1(mn417)* (25°)	0.52 (132)[c]	+
mec-8(mn472 ts*) smu-1(mn433)* (25°)	0.54 (180)[c]	+
mec-8(mn472 ts*); smu-2(mn416)* (25°)	0.69 (144)[c]	+
mec-8(rh170)	0.01 (180)	−
mec-8(rh170) smu-1(mn415)	0.22 (212)[c]	−
mec-8(rh170) smu-1(mn417)	0.06 (228)[c]	−
mec-8(rh170) smu-1(mn433)	0.12 (180)[c]	−
mec-8(rh170); smu-2(mn416)	0.08 (124)[c]	−
mec-8(u74)	0.02 (136)[c]	−
mec-8(u74) smu-1(mn415)	0.02 (372)	−
mec-8(u74) smu-1(mn417)	0.04 (332)	−
mec-8(u74) smu-1(mn433)	0.02 (196)[c]	−
mec-8(u74); smu-2(mn416)	0.02 (204)[c]	−
mec-8(u218 ts*)* (25°)	0.18 (368)	−
mec-8(u218 ts*) smu-1(mn415)* (25°)	0.79 (448)	++
mec-8(u218 ts*) smu-1(mn417)* (25°)	0.80 (448)	++
mec-8(u218 ts*) smu-1(mn433)* (25°)	0.73 (400)	++
mec-8(u218 ts*); smu-2(mn416)* (25°)	0.90 (432)	++
mec-8(u303)	0.05 (228)	−
mec-8(u303) smu-1(mn415)	0.20 (152)[c]	−
mec-8(u303) smu-1(mn417)	0.21 (308)	−
mec-8(u303) smu-1(mn433)	0.21 (336)	−
mec-8(u303); smu-2(mn416)	0.43 (180)[c]	−
mec-8(u391)	0.02 (184)	−
mec-8(u391) smu-1(mn415)	0.08 (220)[c]	−
mec-8(u391) smu-1(mn417)	0.04 (168)[c]	−
mec-8(u391) smu-1(mn433)	0.06 (204)[c]	−
mec-8(u391); smu-2(mn416)	0.20 (172)[c]	−
mec-8(u456)	<0.01 (284)	−
mec-8(u456) smu-1(mn415)	0.02 (180)[c]	−
mec-8(u456) smu-1(mn417)	0.02 (124)[c]	−
mec-8(u456) smu-1(mn433)	0.10 (312)	−
mec-8(u456); smu-2(mn416)	0.14 (196)[c]	−

++ = touch-sensitive; + = partially touch-sensitive; − = touch-insensitive.
[a] Animals were scored at 20° unless otherwise noted.
[b] *n* is the number of phasmid neurons scored; each animal has four phasmid neurons.
[c] Scored in one experiment; other strains were scored in at least two separate experiments.

TABLE 7

Suppression of the cold-sensitive (16°) arrest of *mec-8* mutants by alleles of *smu-1* and *smu-2*

Genotype	*n*	Proportion embryonic or early larval arrest
N2 (wild type)	731	<0.01
smu-1(mn415)	639	0.01
smu-1(mn417)	719	0.02
smu-1(mn433)	708	<0.01
smu-2(mn416)	498	0.02
mec-8(mn463)	501	0.28
mec-8(mn463) smu-1(mn415)	574	0.05
mec-8(mn463) smu-1(mn417)	1197	0.06
mec-8(mn463) smu-1(mn433)	619	0.01
mec-8(mn463); smu-2(mn416)	416	0.04
mec-8(u74)	597	0.31
mec-8(u74) smu-1(mn415)	340	0.11
mec-8(u74) smu-1(mn417)	1030	0.09
mec-8(u74) smu-1(mn433)	649	0.18
mec-8(u74); smu-2(mn416)	642	0.08

bulges in the hypodermis. Some, but not all, animals exhibiting defects in embryonic elongation hatch; other arrested larvae are normally elongated. Upon inspection of body wall muscle cells in arrested *mec-8* embryos and larvae by both polarized light microscopy and antibody staining of body wall myosin, we found disruptions in body wall muscle organization that suggested muscle attachment defects; strips of body wall muscle were frequently displaced as if they had been pulled away from the body wall. Furthermore, an *unc-52*-encoded epitope (ROGALSKI *et al.* 1993), which in wild-type animals is localized to regions of basement membrane involved in attachments to body wall muscle (FRANCIS and WATERSTON 1991), showed disruptions in arrested animals that were very similar to those seen by myosin staining. We suggest that the cold-sensitive lethality and morphological abnormalities of *mec-8* mutants may all be a direct consequence of defects in muscle cell attachment. Embryonic elongation appears to be driven by the squeezing of the outermost layer of cells, the hypodermis (PRIESS and HIRSH 1986), but elongation beyond the twofold stage appears to depend, perhaps in addition to hypodermal squeezing, on functional body wall musculature; embryos lacking muscle function have a common (Pat) phenotype of paralysis

and arrest at the twofold stage of elongation (WATERSTON 1989; VENOLIA and WATERSTON 1990, BARSTEAD and WATERSTON 1991; WILLIAMS and WATERSTON 1994; HRESKO *et al.* 1994), which is the earliest arrest we see in *mec-8* animals. Unlike the Pat embryos, however, all of the *mec-8* arrested embryos exhibited some movement.

We characterized all 16 *mec-8* alleles with respect to the Mec, Dyf and lethal arrest phenes, and the effects of different alleles on the different phenes were quite consistent. Two alleles, *u218* ts and *mn472* ts, exhibited a weak Mec phene; both also showed the weakest Dyf phene, were temperature-sensitive for both Mec and Dyf and exhibited no cold-sensitive lethality. A third allele, *e398*, was incompletely Dyf and showed very little cold-sensitive lethality. Supporting the idea that *u218* ts, *mn472* ts and *e398* are all hypomorphs were the results showing that animals carrying these mutations opposite a deficiency were more strongly Dyf. It is worth noting that *e398* is an amber mutation (CHALFIE and AU 1989), so apparently the truncated *mec-8* protein produced by the *e398* mutant has residual function. At least four more alleles–*u303*, *mn455*, *mn459* and *rh170*–appear to be non-null, since they gave only 17% or less embryonic or larval lethality at 16°. The other nine alleles were fully Mec and Dyf and gave 23–31% embryonic or early larval arrest at 16°. We also showed that three of these nine were equivalent to a deficiency when placed opposite weak alleles for the Dyf assay. We could not readily gauge the effect on cold-sensitive lethality of putting our strongest alleles opposite a deficiency. Such animals are generally viable, however, because we easily generated them at 16°, 20° and 25°. We suggest that the *mec-8* null phenotype is Mec, Dyf, and incompletely penetrant, cold-sensitive lethal. Consistent with this view is the fact that five of the strong alleles arose in noncomplementation screens that also identified a deficiency of the locus. Finally, the frequency at which we recovered EMS-induced alleles was approximately that expected for the inactivation of an average *C. elegans* gene (BRENNER 1974).

It might be considered surprising that a null mutation can result in an incompletely penetrant and cold-sensitive lethality. There are clear examples in yeast, however, in which complete inactivation of a gene results in lethality only at low temperature. One example is the *SAC1* gene, which was first defined by mutations that suppress a temperature-sensitive mutation in an actin gene (NOVICK *et al.* 1989). Additional examples include the *CIN1*, *CIN2* and *CIN4* genes; mutations in each of these lead to defects in microtubule-mediated processes (HOYT *et al.* 1990). It may be relevant that all of these genes are involved in cytoskeletal functions. If we assume, as already suggested, that the cold-sensitive lethality of *mec-8* mutants is a consequence of defects in muscle attachment, we are led to suggest that the *mec-8(+)* product is not absolutely essential but contributes to the reinforcement of muscle attachments, particularly at low temperature, and that the complete absence of *mec-8(+)* leads to weak muscle attachment at low temperature, either because attachments are not secured in the first place or because they are made but are fragile. In either case, those animals in which the defects are particularly severe are arrested, whereas other animals, although frequently exhibiting minor attachment defects, survive and reproduce.

The relationship between the muscle attachment defects and the Mec and Dyf phenes of *mec-8* mutants is unclear. One possible common theme could be cell attachments or adhesion, perhaps involving extracellular matrix. The fasciculation or bundling defects of the process endings of the chemosensory neurons, which underlie the Dyf defects, may be the consequence of defects in cell-cell attachments, perhaps mediated by the extracellular matrix in which the process endings are embedded (PERKINS *et al.* 1986). The touch cell processes are also embedded in an extracellular matrix, the mantle, which plays a role in anchoring the touch cells to the external cuticle (CHALFIE and SULSTON 1981).

We have discovered a synthetic lethal interaction between mutations in *mec-8* and viable alleles of *unc-52*. The interaction is not allele specific for either gene, except that the *unc-52* mutations must be partial loss-of-function because null alleles of *unc-52* confer a phenotype (ROGALSKI *et al.* 1993; WILLIAMS and WATERSTON 1994) that is similar if not identical to that of the *mec-8; unc-52(viable)* double mutant, *i.e.*, *mec-8* mutations enhance the phenotype conferred by *unc-52* viable alleles to that of *unc-52* null. The synthetic interaction appears to be gene specific: no interactions were found involving either *mec-8* or *unc-52* with any of a variety of mutations in other genes. It seems relevant that the cold-sensitive muscle defects observed in the strong *mec-8* mutants appear to be similar to the muscle defects conferred by mutation in *unc-52*, except that in comparison to *unc-52(lethal)* mutants, the defects are much weaker, exhibiting incomplete penetrance and variable expressivity, and in contrast to *unc-52(viable)* mutants, there is no evidence that the *mec-8* cold-sensitive defects worsen during postembryonic development. The Mec and Dyf phenes of *mec-8* seem completely independent of *unc-52*, however. All of the seven viable *unc-52* mutants we have studied are wild-type with respect to both of these phenes. We therefore suggest that the *mec-8 unc-52* interaction involves defects in muscle attachment specifically.

One possible interpretation of a synthetic enhancement is that the two loci involved encode partially redundant functions (GUARENTE 1993; THOMAS 1993). An example of this phenomenon in *C. elegans* is provided by the *ace-1*, *ace-2* and *ace-3* genes, each of which encodes an acetylcholinesterase; the single mutants have no obvious phenotype, but the *ace-1 ace-2* double mutant is severely uncoordinated (CULOTTI *et al.* 1981) and

the *ace-1 ace-2 ace-3* triple mutant arrests development shortly after hatching (JOHNSON *et al.* 1988). Another example is provided by the *glp-1* and *lin-12* genes, which encode very similar molecules and confer a synthetic lethal phenotype (LAMBIE and KIMBLE 1991). FERGUSON and HORVITZ (1989) have characterized redundant genetic pathways that direct *C. elegans* vulva development; a mutant phenotype is revealed only when each pathway has been mutated. We do not believe that the *mec-8; unc-52* synthetic lethality reveals a redundancy of function between *mec-8* and *unc-52* or redundant pathways, however, because the double mutant phenotype is the same as that of the *unc-52* null. Instead, we suggest that *mec-8(+)* and *unc-52(+)* contribute nonredundantly to the process of muscle attachment. This makes their interaction analogous to one seen between *fem-1* and *fem-2*, which are involved in the *C. elegans* sex determination pathway; a double mutant consisting of partial loss-of-function alleles of each gene is synthetically enhanced to resemble the null phenotype for each single gene (KIMBLE *et al.* 1984). We suggest that the absence of *mec-8* function renders the residual *unc-52* activity provided by *unc-52(viable)* insufficient for establishing or maintaining the muscle cell attachments necessary for successful embryogenesis. (This suggests that *mec-8(+)* functions in muscle attachment at higher temperatures as well as low, which is not surprising because it functions to make animals non-Mec and non-Dyf at all temperatures.) One possibility in line with this view would be that *mec-8* encodes a component of the muscle attachment machinery, which would both strengthen muscle attachment in *unc-52(+)* animals at low temperature and be required for muscle attachment in *unc-52(viable)* embryos. A second possibility would be that *mec-8* product controls the formation of a component of the muscle attachment machinery, perhaps even *unc-52* protein.

Preliminary molecular analysis of *mec-8* supports the idea that *mec-8* protein controls the expression of other genes (E. LUNDQUIST, J. SHAW and R. HERMAN, unpublished results). The gene encodes two 80-amino acid RNA recognition domains found in many RNA-binding proteins, including the 70-kD protein component of the U1 small nuclear ribonucleoprotein (snRNP) complex (QUERY *et al.* 1989) and various RNA splicing factors (KENAN *et al.* 1991), which suggests that the *mec-8* protein may regulate patterns of alternative RNA splicing. *unc-52* has been shown to undergo extensive alternative splicing (ROGALSKI *et al.* 1993; D. MOERMAN, personal communication), so it is possible that it is a target of *mec-8* control. According to this picture, in *unc-52(+)* animals *mec-8(+)* directs the RNA splicing necessary for the synthesis of a species of *unc-52* protein that stabilizes muscle cell anchorage at low temperature. In *unc-52(viable)* mutants, a species of *unc-52* protein that contributes to muscle cell attachment would be missing or defective, and as a consequence, adult muscle cell attachments would be fragile and embryonic attachments would become dependent on *unc-52* products that require *mec-8(+)* splicing function. We emphasize that this picture is speculative. An alternative possibility is that *mec-8(+)* regulates the splicing of a transcript for a protein that interacts with *unc-52* protein. In either case, we suggest that *mec-8* may control the splicing of transcripts of one or more additional genes that are responsible for the Mec and Dyf phenes.

We have identified and characterized four suppressors of the *mec-8 unc-52* synthetic lethality conferred by weak temperature-sensitive alleles of each gene. The four suppressors were assigned to two genes, *smu-1 I*, and *smu-2 II*. The suppressor mutations by themselves seem to be mildly deleterious but confer no easily scored phenotype that we have been able to discern. We checked all four suppressors for their ability to suppress the Mec and Dyf phenes of 11 *mec-8* alleles. Only the two weakest *mec-8* alleles showed suppression of Mec– by all four suppressors. All but one of the 11 *mec-8* alleles were suppressed at least weakly for Dyf by at least one *smu-1* allele and the *smu-2* mutation. None was completely suppressed, *i.e.*, the difference between the Mec and Dyf responses may simply be a consequence of our ability to measure a weak suppression of Dyf. The general rule from these results seems to be that the weakest alleles are most readily suppressed. By this criterion, *u74* may be the best candidate for a null, since it was the only one for which we were unable to detect suppression of the Dyf phenotype. We also looked at suppression of the cold-sensitive lethality of two strong *mec-8* alleles, including *u74*, and both were suppressed by *smu-1* and *smu-2* mutations. Because the weak *mec-8* alleles did not exhibit any cold-sensitive lethality, it seems that only a little *mec-8* activity is needed to suppress this mutant phene.

The action of *smu-1* and *smu-2* suppressors on *mec-8* mutations seems to be allele-nonspecific. The suppressors seem able to compensate for a small amount of missing *mec-8* function for each of the three mutant phenes. The recessive nature of the mutations suggests that they may be loss-of-function. We think it very unlikely that the suppressors are informational because informational suppressors should be allele-specific. The suppressor genes do not map near any of the known amber suppressors (KONDO *et al.* 1990) or *smg* suppressors (HODGKIN *et al.* 1989), nor do they exhibit any of the morphological features of *smg* mutants. We also think it unlikely that the suppressors encode proteins that interact directly with altered *mec-8* protein to compensate for the *mec-8* defects. Here too we would expect such suppression to be allele-specific (and perhaps also dominant). We suggest that the suppressors result in a small amount of bypass function. A bypass function would explain how a *mec-8* null mutation could be suppressed; we

propose that *u74* is null, and it is suppressed with respect to the cold-sensitive lethality. We would explain the weak suppression of Mec and Dyf phenes by supposing that the amount of bypass function provided is small. What is the nature of the bypass function? If we are correct that *mec-8* acts by controlling alternative splicing of target genes, then the bypass could be generated by loss-of-function mutations in genes involved in the general splicing machinery such that the normal splicing restrictions are loosened and splices that in wild-type animals are *mec-8*-dependent are now permitted, albeit at low levels, in the absence of *mec-8* protein.

We also tested the ability of the suppressors to suppress the paralysis conferred by seven *unc-52* mutations in a *mec-8(+)* genetic background. All four suppressors suppressed the paralysis conferred by *unc-52(e669su250* ts). The only other allele suppressed was *unc-52(e669)*, which was suppressed weakly by *smu-2*. If, as we have just supposed, the suppressor mutations result in loosened restrictions on alternative splicing, then novel products generated in the *smu mec-8(+) unc-52* background could possibly lead to the strengthening of muscle attachments in adult animals. This kind of suppression might be allele-specific and in any case would be expected to be weak.

We thank MARTY CHALFIE for generously providing *mec-8* strains and encouragement, DON MOERMAN for helpful discussion, BOB WATERSTON and MICHELLE COUTU HRESKO for strains and antibody, ED HEDGECOCK for *mec-8(rh170)*, LOIS EDGAR for her TMP mutagenesis protocol and RON ELLIS for communicating unpublished results. We also owe special thanks to JOCELYN SHAW for stimulating discussion and guidance, to CLAIRE KARI for isolating *smu-1* and *smu-2* alleles and to all members of the St. Paul worm laboratories for helpful discussion. Some strains in this work were provided by the Caenorhabditis Genetics Center. E.A.L. was supported in part by a University of Minnesota Graduate School Dissertation Fellowship. This work was supported by U. S. Public Health Service grant GM22387.

LITERATURE CITED

BARGMANN, C. I., 1993 Genetic and cellular analysis of behavior in *C. elegans*. Annu. Rev. Neurosci. **16:** 47–71.

BARGMANN, C. I., and H. R. HORVITZ, 1991a Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in C. elegans. Neuron **7:** 729–742.

BARGMANN, C. I., and H. R. HORVITZ, 1991b Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. Science **251:** 1243–1246.

BARGMANN, C. I., J. H. THOMAS and H. R. HORVITZ, 1990 Chemosensory cell function in the behavior and development of *Caenorhabditis elegans*. Cold Spring Harb. Symp. Quant. Biol. **55:** 529–538.

BARGMANN, C. I., E. HARTWIEG and H. R. HORVITZ, 1993 Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell **74:** 515–527.

BARSTEAD, R. J., and R. H. WATERSTON, 1991 Vinculin is essential for muscle function in the nematode. J. Cell Biol. **114:** 715–724.

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

CHALFIE, M., and M. AU, 1989 Genetic control of differentiation of the *Caenorhabditis elegans* touch receptor neurons. Science **243:** 1027–1033.

CHALFIE, M., and J. SULSTON, 1981 Developmental genetics of the mechanosensory neurons of *Caenorhabiditis elegans*. Dev. Biol. **82:** 358–370.

CHALFIE, M., and J. WHITE, 1988 The nervous system, pp. 337–391 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

CULOTTI, J. G., G. VON EHRENSTEIN, M. R. CULOTTI and R. L. RUSSELL, 1981 A second class of acetylcholinesterase-deficient mutants of the nematode *Caenorhabditis elegans*. Genetics **97:** 281–305.

FERGUSON, E. L., and H. R. HORVITZ, 1989 The multivulva phenotype of certain *Caenorhabditis elegans* mutants results from defects in two functionally redundant pathways. Genetics **123:** 109–121.

FRANCIS, G. R., and R. H. WATERSTON, 1985 Muscle organization in *Caenorhabditis elegans*: localization of proteins implicated in thin filament attachment and I-band organization. J. Cell Biol. **101:** 1532–1549.

FRANCIS, R., and R. H. WATERSTON, 1991 Muscle cell attachment in *Caenorhabditis elegans*. J. Cell Biol. **114:** 465–479.

GILCHRIST, E. J., and D. G. MOERMAN, 1992 Mutations in the *sup-38* gene of *Caenorhabditis elegans* suppress muscle-attachment defects in *unc-52* mutants. Genetics **132:** 431–442.

GOH, P. Y., and T. BOGAERT, 1991 Positioning and maintenance of embryonic body wall muscle attachments in *C. elegans* requires the *mup-1* gene. Development **111:** 667–681.

GUARENTE, L., 1993 Synthetic enhancement in gene interaction: a genetic tool come of age. Trends Genet. **9:** 362–366.

GUO, X. D., J. J. JOHNSON and J. M. KRAMER, 1991 Embryonic lethality caused by mutations in basement membrane collagen of *C. elegans*. Nature **349:** 707–709.

HALL, D. H., and R. L. RUSSELL, 1991 The posterior nervous system of the nematode *Caenorhabditis elegans*: serial reconstruction of identified neurons and complete pattern of synaptic interactions. J. Neurosci. **11:** 1–22.

HEDGECOCK, E. M., J. G. CULOTTI, J. N. THOMSON and L. A. PERKINS, 1985 Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. Dev. Biol. **111:** 158–170.

HERMAN, R. K., and E. M. HEDGECOCK, 1990 Limitation of the size of the vulval primordium of *Caenorhabditis elegans* by *lin-15* expression in surrounding hypodermis. Nature **348:** 169–171.

HIRSH, D., and R. VANDERSLICE, 1976 Temperature-sensitive developmental mutants of *Caenorhabditis elegans*. Dev. Biol. **49:** 220–235.

HODGKIN, J., 1983 Male phenotypes and mating efficiency in *Caenorhabditis elegans*. Genetics **103:** 43–64.

HODGKIN, J., M. EDGLEY, D. L. RIDDLE and D. G. ALBERTSON, 1988 Genetics, pp. 491–584 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

HODGKIN, J., A. PAPP, R. PULAK, V. AMBROS and P. ANDERSON, 1989 A new kind of informational suppression in the nematode *Caenorhabditis elegans*. Genetics **123:** 301–313.

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.

HOYT, M. A., T. STEARNS and D. BOTSTEIN, 1990 Chromosome instability mutants of *Saccharomyces cerevisiae* that are defective in microtubule-mediated processes. Mol Cell. Biol. **10:** 223–234.

HRESKO, M. C., B. D. WILLIAMS and R. H. WATERSTON, 1994 Assembly of body wall muscle and muscle cell attachment structures in *Caenorhabditis elegans*. J. Cell Biol. **124:** 491–506.

ISHII, N., W. G. WADSWORTH, B. D. STERN, J. G. CULOTTI and E. M. HEDGECOCK, 1992 UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in C. elegans. Neuron **9:** 873–881.

JOHNSON, C. D., J. B. RAND, R. K. HERMAN, B. D. STERN and R. L. RUSSELL, 1988 The acetylcholinesterase genes of *C. elegans*: identification of a third gene (*ace-3*) and mosaic mapping of a synthetic lethal phenotype. Neuron **1:** 165–173.

KENAN, D. J., C. C. QUERY and J. D. KEENE, 1991 RNA recognition: towards identifying determinants of specificity. Trends Biochem. Sci. **16:** 214–220.

KIMBLE, J., L. EDGAR and D. HIRSH, 1984 Specification of male development in *Caenorhabditis elegans*: the *fem* genes. Dev. Biol. **105:** 234–239.

KONDO, K., B. MAKOVEC, R. H. WATERSTON and J. HODGKIN, 1990 Genetic and molecular analysis of eight tRNA (Trp) amber suppressors in *Caenorhabditis elegans*. J. Mol. Biol. **215:** 7–19.

LAMBIE, E. J., and J. KIMBLE, 1991 Two homologous regulatory genes, *lin-12* and *glp-1*, have overlapping functions. Development **112:** 231–240.

MACKENZIE, J. M., JR., R. L. GARCEA, J. M. ZENGEL and H. F. EPSTEIN, 1978 Muscle development in Caenorhabditis elegans: mutants exhibiting retarded sarcomere construction. Cell **15:** 751–762.

MILLER, D. M., III, I. ORTIZ, G. C. BERLINER and H. F. EPSTEIN, 1983 Differential localization of two myosins within nematode thick filaments. Cell **34:** 477–490.

MORI, I., D. G. MOERMAN and R. H. WATERSTON, 1988 Analysis of a mutator activity necessary for germline transposition and excision of Tc1 transposable elements in *Caenorhabditis elegans*. Genetics **120:** 397–407.

NOVICK, P., B. C. OSMOND and D. BOTSTEIN, 1989 Suppressors of yeast actin mutations. Genetics **121:** 659–674.

PERKINS, L. A., E. M. HEDGECOCK, J. N. THOMSON and J. G. CULOTTI, 1986 Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. **117:** 456–487.

PRIESS, J. R., and D. I. HIRSH, 1986 *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. Dev. Biol. **117:** 156–173.

QUERY, C. C., R. C. BENTLEY and J. D. KEENE, 1989 A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70K U1 snRNP protein. Cell **57:** 89–101.

ROGALSKI, T. M., B. D. WILLIAMS, G. P. MULLEN and D. G. MOERMAN, 1993 Products of the *unc-52* gene in *Caenorhabditis elegans* are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan. Genes & Dev. **7:** 1471–1484.

ROSE, A. M., and D. L. BAILLIE, 1979 The effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*. Genetics **92:** 409–418.

SIBLEY, M. H., J. J. JOHNSON, C. C. MELLO and J. M. KRAMER, 1993 Genetic identification, sequence, and alternative splicing of the *Caenorhabdtitis elegans* alpha2(IV) collagen gene. J. Cell Biol. **123:** 255–264.

SULSTON, J., and J. HODGKIN, 1988 Methods, pp. 587–606 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

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.

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

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

WARD, S., N. THOMSON, J. G. WHITE and S. BRENNER, 1975 Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. J. Comp. Neurol. **160:** 313–337.

WARE, R. W., D. CLARK, K. CROSSLAND and R. L. RUSSELL, 1975 The nerve ring of the nematode *Caenorhabditis elegans*: sensory input and motor output. J. Comp. Neurol. **162:** 71–110.

WATERSTON, R. H., 1988 Muscle, pp. 281–335 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

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

WHITE, J. G., E. SOUTHGATE, J. N. THOMSON and S BRENNER, 1986 The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B **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., T. HECHT, S. CARR, R. VANDERSLICE, N. WOLF and D. HIRSH, 1980 Parental effects and phenotypic characterization of mutations that affect early development in *Caenorhabditis elegans*. Dev. Biol. **74:** 446–469.

YANDELL, M. D., L. G. EDGAR and W. B. WOOD, 1994 Trimethylpsoralen induces small deletion mutations in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci USA **91:** 1381–1385.

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

Communicating editor: I. GREENWALD