Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 6577–6582, June 1996
Neurobiology

Genetic interactions affecting touch sensitivity in *Caenorhabditis elegans*

(mechanotransduction/enhancer mutations/suppressor mutations)

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Communicated by H. Robert Horvitz, Massachusetts Institute of Technology, Cambridge, MA, February 29, 1996 (received for review January 17, 1996)

ABSTRACT **At least 13 genes (*mec-1*, *mec-2*, *mec-4–10*, *mec-12*, *mec-14*, *mec-15*, and *mec-18*) are needed for the response to gentle touch by 6 touch receptor neurons in the nematode *Caenorhabditis elegans*. Several, otherwise recessive alleles of some of these genes act as dominant enhancer mutations of temperature-sensitive alleles of *mec-4*, *mec-5*, *mec-6*, *mec-12*, and *mec-15*. Screens for additional dominant enhancers of *mec-4* and *mec-5* yielded mutations in previously known genes. In addition, some *mec-7* alleles showed allele-specific, dominant suppression of the *mec-15* touch-insensitive (Mec) phenotype. The dominant enhancement and suppression exhibited by these mutations suggest that the products of several touch genes interact. These results are consistent with a model, supported by the known sequences of these genes, that almost all of the touch function genes contribute to the mechanosensory apparatus.**

The response to gentle touch in the nematode *Caenorhabditis elegans* is mediated by a set of six mechanosensory receptor neurons (1, 2). Saturation mutageneses for touch-insensitive animals have led to the identification of 13 genes (called *mec* for *mec*hanosensory abnormal) that are needed for the function of these touch receptors (1, 3). Mutant animals are touch insensitive (the Mec phenotype) but have differentiated touch receptor neurons [other genes affect the differentiation of these cells (4–6)]. Many of these touch function genes have been cloned and characterized [one of the genes, *mec-8*, encodes a putative RNA splicing regulator (7) that will not be discussed further, since it does not appear to contribute directly to the touch apparatus].

Two of these genes, *mec-4* (8, 9) and *mec-10* (10) encode similar membrane proteins, called degenerins, that, because of their similarity to the subunits of the vertebrate epithelial Na^+ channel (11, 12), are likely to be channel components. Both genes are expressed in the touch receptor neurons and can be mutated to cause the degeneration of these cells, presumably by making a hyperactive channel (1, 10, 13). An extracellular domain present in these *C. elegans* proteins appears to regulate channel function because mutations within it also cause the degeneration phenotype (14). Preliminary sequence analysis suggests that the *mec-6* gene may also encode a degenerin (C. Ma and M.C., unpublished data).

Two distinguishing features of the touch cells are the associated extracellular matrix, called the mantle, and the bundle of large (15-protofilament) microtubules that fills most of the axonal cytoplasm (1, 15). Proteins that contribute to the mantle include the *mec-5*-encoded collagen, which is produced by the surrounding epidermal cells, the secreted protein product of the *mec-9* gene produced by the touch cells (16), and probably the *mec-1* gene product. The *mec-1* gene has not been cloned, but it is needed for mantle production (1).

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The touch cell-specific microtubules are formed from the *mec-12* α-tubulin (M. Hamelin, M. Chou, and J. Culotti, personal communication) and the *mec-7* β-tubulin (17). In electron micrographs, these microtubules appear to be cross-linked to each other and have their distal ends (the ends furthest from the cell body) near the plasma membrane (18). Most of the distal ends appear to have associated material that could link the microtubules with the plasma membrane.

One candidate linker protein is the product of the *mec-2* gene because the proper localization of MEC-2LacZ fusion proteins in touch cell axons requires *mec-7* and *mec-12* (19). *mec-2* encodes a putative integral membrane protein whose central portion shares extensive similarity with stomatin, a membrane protein of human red blood cells that is thought to regulate ion conductance (20, 21). We have hypothesized that MEC-2 similarly regulates degenerin channel activity (19). Another protein that may regulate the channel is the product of the *mec-14* gene (N. Hom, S. Gangadharan, Y. Tu, M. Huang, L. Chen, and M.C., unpublished data), which shares sequence similarity to β-subunits of *Shaker*-type K^+ channels (22) and aldo-keto reductases (23, 24).

To investigate the function of the touch gene products further, we have identified several genetic interactions among these genes. Genetic interactions, such as interallelic complementation, suppression, and enhancement, can reveal important relationships among gene products (25–33). For example, Simon *et al.* (32) identified several genes whose products were needed for the signaling cascade initiated by the *Drosophila sevenless* gene product by searching for dominant enhancers of a temperature-sensitive (ts) *sevenless* allele.

Some genetic interactions have already been noted among the *mec* genes. Interallelic complementation and enhancement occurs with *mec-2* (1) and *mec-10* (10), respectively, suggesting that both gene products are components of multimeric complexes. In addition, degeneration-causing mutations of *mec-4* are suppressed by *mec-6* mutations (34), while a degeneration-causing mutation in *mec-10* is suppressed by mutations in *mec-2*, *mec-4*, *mec-6*, *mec-12*, *mec-14*, and *mec-15* and enhanced by mutations in *mec-18* (ref. 10; the latter two genes have not been cloned). In this paper, we describe several dominant enhancing effects revealed by a protocol similar to that of Simon *et al.* (32). Our results show that extensive interactions exist among these touch genes. These data suggest a model in which many of the *mec* gene products form a multiprotein complex needed for mechanosensory transduction.

MATERIALS AND METHODS

Strain Maintenance and Construction. Wild type (N2) and mutant strains were grown and cultured as described by Brenner (35). The following mutations were used. LG I, *dpy-5(e61)*, *mec-6(e1342, u3, u247)*; LG II, *dpy-2(e8)*, *mec-15(u75)*, *mnDf29* (36); LG III: *mec-12(e1605, u50, u63, u67,*

Abbreviation: ts, temperature sensitive.

u1607), *dpy-17*(*e164*), *mec-14*(*u55*, *u61*, *u310*), *dpy-11*(*e224*), *nDf16* (37); LG V: *mec-1*(*e1066*), *sDf20* (38); *mec-9*(*e1494*, *u151*, *u164*, *u338*), *nDf31* (39); LG X: *mec-18*(*u182*, *u452*), *lon-2*(*e678*), *mec-2*(*e75*, *e1514*, *e1804*, *u26*, *u227*), *mec-7*(*e1506*, *u428*, *u429*, *u443*), *mec-10*(*e1515*, *e1715*), *stDf5* (R. Francis and R. H. Waterston, personal communication), *dpy-6*(*e14*), *unc-7*(*e5*), *lin-15*(*n765*) (40), *mec-5*(*e1790*, *u213*, *u444*), *mec-4*(*u45*, *u25*, *u29*), *yDf1* (41), *sup-10*(*n983*).

The *mec* mutations were described by Chalfie and Sulston (1) and Chalfie and Au (3). The *unc* and *dpy* mutations were described by Brenner (35).

Multiple mutant combinations were constructed by using standard *C. elegans* genetic protocols (35). In some cases marker mutations were included to identify the mutation-containing chromosome. To construct recombinants between other X-linked *mec* mutations and *mec-4*(*u45*) or *mec-5*(*u213*), we plated individual Mec nonLon progeny from *mec*+ +/+ *lon-2 mec-4* (or *mec-5*) heterozygotes and picked nonLon progeny from Lon-containing plates. These animals were tested for homozygosity for both the additional X-linked *mec* mutation and the *mec-4* or *mec-5* mutation by complementation tests. To construct the *mec-5 mec-4* double mutants, we examined the progeny of *lin-15*+ *mec-4*+/+ *mec-5*+ *sup-10* animals for the loss of both *lin-15* and *sup-10* markers, and verified the genotype by complementation tests. Double *mec* mutant strains of *mec-6*(*u247*), *mec-12*(*u67*), and *mec-15*(*u75*) with other *mec* mutations contained *dpy-5*, *dpy-17*, or *dpy-2*, respectively.

Enhancement and Suppression Assays. Dominant enhancement assays of ts *mec* mutations were carried out at temperatures at which virtually all (>99%) of the animals homozygous for the mutation were touch sensitive. The optimum temperature was determined by growing strains at various temperatures for at least three generations and testing their touch sensitivity as described by Chalfie and Sulston (1). In all cases males and hermaphrodites responded the same to various temperatures.

To test enhancement of *mec-4*(*u45*) and *mec-5*(*u213*) by previously identified autosomal mutations, we crossed homozygous *mec* males to *mec-4* or *mec-5* hermaphrodites at 21°C, and the resulting males were examined for touch sensitivity. When other X-linked *mec* mutations were tested with the *mec-4* and *mec-5* mutations, males from the double mutants were mated with *lon-2*-marked *mec-4* or *mec-5* hermaphrodites. To test enhancement of *mec-6*(*u247*), *mec-12*(*u67*), or *mec-15*(*u75*), we mated *dpy*-marked hermaphrodites with *mec-6*, *mec-12*, or *mec-15* males and tested the resulting non-Dpy hermaphrodites for touch sensitivity. In these experiments animals were always compared in parallel to animals of the same sex that lacked the heterozygous *mec* mutation.

The amount of enhancement was usually determined by testing three batches of animals (90–300 animals were examined totally for each strain) and calculating the number of animals that were touch insensitive (Mec). The results of the repeated tests were analyzed using the one-tailed Fisher exact probability test (42). Enhancement was assumed if $P \leq 0.01$.

Dominant suppression was tested similarly except that animals were mated and tested at higher temperatures [23°C for *mec-4*(*u45*) and *mec-5*(*u213*); 23°C and 25°C *mec-15*(*u75*)].

To test for interallelic complementation between *mec-7* and *mec-12* mutations, *dpy-11*(*e224*); *mec-7* hermaphrodites were mated with *mec-12* males and the resulting non-Dpy hermaphrodites were scored for touch sensitivity. Dominant suppression by *mec-7* mutations of semidominant *mec-12* alleles was tested similarly. Recessive suppression was also tested in some *mec-12; mec-7* hermaphrodites generated by standard genetic procedures. All these tests were performed at 25°C.

Enhancer Screens. We identified new dominant enhancer mutations of *mec-4*(*u45*) and *mec-5*(*u213*) by mutating *mec-4*(*u45*) and *mec-5*(*u213*) L4 larvae or young adults with ethyl methanesulfonate as described by Brenner (35). Two or three worms were plated together and grown at 20°C. F_1 progeny were counted and tested for touch sensitivity. Strains with putative enhancer mutations were outcrossed with wild-type worms and the mutations were tested for dominance or complementation with existing *mec* mutations. Eleven X-linked recessive *mec* mutations were identified in the *mec-4* screen and 10 were found in the *mec-5* screen. These mutations failed to complement *mec-4* and *mec-5* mutations, respectively, at 15°C (the permissive temperature for the ts allele). To verify that these mutations were *mec-4* or *mec-5* alleles, we mapped them relative to *unc-7*, *lin-15*, and *sup-10*. All these mutations mapped between *lin-15* and *sup-10*, the location of *mec-4* and *mec-5*.

Sequencing. *mec-2* alleles *e75*, *u26*, and *u733* were sequenced as in Huang *et al.* (18); *mec-5* alleles *u728*, *u732*, and *u735* were sequenced as in Du *et al.* (15).

RESULTS

Recessive, ts alleles are available for five of the touch function genes: *mec-4*, *mec-5*, *mec-6*, *mec-12*, and *mec-15* [refs. 1 and 3; dominant ts alleles are also known for *mec-7* and *mec-12* (refs. 15 and 17; and M.C., unpublished data) but they have not been used in this study]. To use these alleles to identify dominant enhancing effects, we first determined the maximum temperatures at which >99% of the animals were touch sensitive [i.e., were wild type (Fig. 1)]. All of the mutants we used, except *mec-15*(*u75*), showed very sharp phenotypic changes as the temperature was raised (males and hermaphrodites gave the same results). This property is useful because it allows the percentage of touch insensitive (Mec) animals to serve as a sensitive indicator of enhancement. We examined enhancement at the following temperatures: 21°C for *mec-4*(*u45*), *mec-5*(*u213*), and *mec-12*(*u67*), 15°C for *mec-6*(*u247*), and 18°C for *mec-15*(*u75*). Several otherwise recessive mutations were found to enhance the touch insensitive phenotypes of these mutations in a dominant fashion. The results for each gene are presented in Table 1 and described in the following sections.

***mec-4*(*u45*).** The strongest enhancement of *mec-4*(*u45*) occurred with mutant alleles of the two tubulin genes *mec-7* and *mec-12*. Some *mec-2* and *mec-10* alleles also produced significant enhancement. In a screen for new dominant enhancers of *mec-4*(*u45*), we identified 18 enhancing mutations among 65,000 F_1 progeny of ethyl methanesulfonate-mutagenized animals. Eleven of these mutations were *mec-4* alleles (pre-

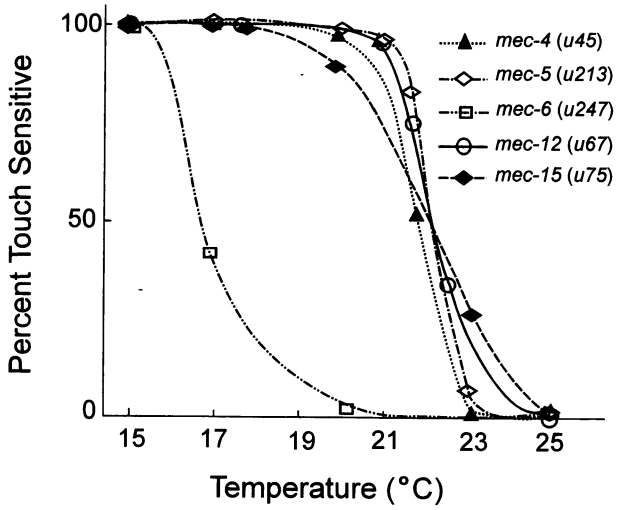

FIG. 1. The effect of temperature on the touch sensitivity of different *mec* mutants.

Table 1. Dominant enhancement of ts Mec phenotypes

Tester Mutations			ts alleles				
Gene	Allele	Defect†	*mec-4(u45)*	*mec-5(u213)*	*mec-6(u247)*	*mec-12(u67)*	*mec-15(u75)*
Wild Type			− (1)	− (0.4)	− (1)	− (0.4)	− (0)
mec-1,	*e1066*		− (1)	− (2)	− (0)	− (2)	− (0)
Unknown	*sDF20*	Deletion	− (0)	− (3)	− (4)	+ (4)	− (1)
mec-2,	*e75*	A204V	+ (57)	+ (19)	+ (45)	− (0)	− (5)§
stomatin-like	*e1084*	R319*	+ (5)	+ (39)			
protein	*e1514*	E299K	+ (22)	− (5)	+ (27)	+ (21)	− (2)§
	u26	R385H	+ (19)	+ (12)	+ (9)	+ (39)	− (2)§
	u227	A234V	+ (45)		+ (4)	+ (16)	− (1)§
	u733‡	Q453*	− (4)			+ (21)	
	u750‡	T246I	+ (76)			+ (7)	
mec-4,	*u25*	T91I			+ (14)	− (3)	− (0)
degenerin	*u29*	E461K			+ (48)	− (0)	− (0)
	u765‡			+ (11)			
	yDf1	Deletion			+ (41)	+ (8)	− (0)
mec-5,	*e1790*	G249E	+ (5)		− (0)	− (2)	− (2)
collagen	*u444*	Deletion	− (3)		− (0)	+ (10)	− (0)
	u728‡	G354R	+ (6)				
	u732‡	G190R	+ (6)				
	u735‡	G190R	+ (5)				
mec-6,	*e1342*		− (0)	+ (77)		+ (100)	− (1)
possible degenerin	*u3*		− (0)	+ (56)		− (2)	− (0)
mec-7, β-tubulin	*e1506*	M1−	− (2)	+ (7)		+ (87)	S¶
	u428	G369E	+ (71)	+ (4)	− (0)	+ (93)	+ (88)
	u429	G141E	+ (71)		− (2)	+ (55)	S¶
	u443	Deletion	− (1)	+ (10)	− (0)		S¶
mec-9,	*e1494*	G315E	− (1)	+ (49)		− (0)	− (0)
putative secreted protein	*u151*	R631G	− (3)	+ (40)	− (1)	− (1)	− (0)
	u164		− (4)	+ (31)	− (0)	− (0)	− (1)
	u338	Q123*	− (1)	+ (60)			− (1)
	nDf31	Deletion	− (2)	+ (57)	− (1)	− (4)	− (0)
mec-10,	*e1515*	S105F	+ (31)	+ (43)	+ (36)	− (0)	− (3)§
degenerin	*e1715*	G684R	+ (33)	+ (12)	− (3)	− (4)	− (4)§
	u731‡		+ (11)				
	stDf5	Deletion	+ (73)	+ (71)	+ (35)	+ (11)	+ (6)§
mec-12,	*e1605*			+ (7)	− (0)		− (0)
α-tubulin	*e1607*		+ (83)	+ (54)	− (0)		− (4)
	u50			+ (45)			− (0)
	u63	E415K	+ (83)	+ (72)	+ (7)		− (1)
	u734‡		+ (74)				
	u765‡			+ (43)			
	u766‡			+ (49)			
mec-14,	*u55*	Splice site	− (0)	+ (30)	− (2)	− (0)	− (0)
putative	*u61*	G127D	− (0)	− (6)	− (0)	− (4)	− (0)
channel	*u310*	G134E		− (4)	+ (5)		
modulator	*nDf16*	Deletion	− (3)	+ (4)	+ (6)		− (2)
mec-15,	*u53*		− (1)	− (0)	− (1)	+ (15)	
unknown	*u75*		+ (5)	− (3)	− (0)	+ (12)	
	u267		− (1)	− (0)	− (1)	+ (8)	
	mnDf29	Deletion	+ (7)	+ (21)	− (2)	− (0)	
mec-18,	*u182*		− (2)	− (1)	− (0)	− (0)	− (2)
unknown	*u452*		− (0)	− (0)	− (0)	− (0)	− (2)

Strains were homozygous for the ts allele and heterozygous for the tester allele. Positive enhancement (+) was scored if $P \leq 0.01$ by the one-tailed Fisher exact probability test. The numbers in parentheses represent the percentages of Mec animals.

†We determined the sites of some *mec-2* and *mec-5* mutations. Other mutations were sequenced in the following: *mec-2*, ref. 19; *mec-4(u25)*, M. Driscoll, personal communication, *mec-4(u29)*, ref. 43; *mec-5*, ref. 16; *mec-7*, ref. 44; *mec-9*, ref. 16; *mec-10*, ref. 10; *mec-12*, ref. 19; *mec-14*, L. Chen and M.C., unpublished data. In *mec-7*(e1506) the initial Met codon is mutated. We have designated this mutation as M1−. Deficiencies (Df) contain the deletion of the indicated genes and are described in *Materials and Methods*. The asterisk stands for the stop codon.

‡These alleles were isolated in screens for new enhancer mutations.

§These strains, while not touch insensitive, responded much less to the touch stimulus (i.e., animals moved less and more slowly). These results suggest a weak enhancement of the *mec-15(u75)* phenotype.

¶These three alleles dominantly suppressed (S) the *mec-15* Mec phenotype at 25°C (98% of *u75*; *e1506*/+, 86% of *u75*; *u429*/+, and 96% of *u75*; *u443*/+ animals are nonMec; 75–150 animals were examined for each strain).

sumably null or strong alleles). Alleles of *mec-2*, *mec-5*, *mec-10*, *mec-12* were also found (Table 1). We also tested for suppression by representative alleles from different *mec* genes by using *mec-4(u45)*, but did not identify any suppressor mutations.

The enhancement by *mec-2* mutations was allele specific: *e75*, *u227*, and *u750* were strong enhancers, whereas *e1084*, *u26*, and *u733* were relatively weak enhancers. The three stronger enhancers altered the N-terminal half of the stomatin-

like region of the MEC-2 protein (19), whereas the three weaker enhancers either mutate the C-terminal portion of the stomatin-like region (*e1084*) or the more C-terminal domain that is unique to the MEC-2 sequence (e.g., *u733* truncates the final 28 amino acids of MEC-2). Since stomatin is thought to regulate red blood cell permeability (20, 21) and previous gene interaction studies suggested that MEC-2 may interact with the degenerin MEC-10 (10), the stomatin-like region may interact with the degenerin channel.

Allele-specific enhancement was also observed with *mec-7* mutations. Specifically, missense mutations, *u428* and *u429*, strongly enhanced the *mec-4(u45)* mutation, but null alleles, *e1506* and *u443*, did not. Although *u428* and *u429* produce the same phenotype as null alleles, they result in immunodetectable MEC-7 (44). The altered MEC-7 protein in these mutants may interact with a component or components of the putative touch apparatus in a dominant negative manner. Although we cannot rule out direct interactions with MEC-4, this dominant negative effect of missense *mec-7* mutations on *mec-4* could be achieved indirectly through interactions with MEC-12 or MEC-2, for example.

Mutations in the *mec-10* degenerin gene and deletion of the *mec-10* region also enhanced the *mec-4(u45)* phenotype. This interaction is consistent with our hypothesis that MEC-4 and MEC-10 may be part of the degenerin channel (10, 45).

Alleles of two other genes, *mec-5* and *mec-15*, also showed weak but statistically significant enhancement. Although only one of two previously identified *mec-5* alleles enhanced *mec-4(u45)*, three *mec-5* alleles were obtained in our screen for enhancers of *u45*. All three *mec-5* mutations alter Gly residues (two cause identical changes) in the C-terminal part of the Gly-x-y repeat region of the MEC-5 collagen, a region that is required for *mec-5* function (16). Although the enhancement was slight (5–6%), all three *mec-5* mutations produced it. In addition, more alleles were found in *mec-5* by this screen than for any other gene except *mec-4*. We believe that this number is particularly significant because ≈60% of *mec-5* alleles isolated at 25°C are ts (3), and virtually all of these ts alleles produce a wild-type phenotype at 15°C (16). [Because *mec-5* and *mec-4* map so close to each other, we have not separated the new *mec-5* mutations from *mec-4(u45)*.]

mec-5(u213). The Mec phenotype of *mec-5(u213)* was enhanced by mutations in more genes than any other ts mutation we tested. Since MEC-5 is a collagen that is abundantly produced by the hypodermal cells of the worm (16), limiting its amount to a threshold level may be important in revealing its interaction with most other *mec* genes. Previously known mutations in *mec-2*, *mec-6*, *mec-9*, *mec-10*, and *mec-12* strongly enhanced the Mec phenotype of *mec-5(u213)*, while mutations in *mec-7*, *mec-14*, and *mec-15* either weakly or variably enhanced it (Table 1; *mec-4* mutations were not tested). In addition, a search for additional dominant enhancer mutations among 65,000 F_1 progeny of ethyl methanesulfonate-mutagenized *mec-5(u213)* animals led to the identification of ten *mec-5* mutations, two *mec-12* alleles, and a single *mec-4* mutation. In addition, we did not identify any suppressors among known *mec* mutations or in a screen of 35,000 F_1 progeny from mutagenized *u213* animals conducted at 23°C.

Strong enhancement of *mec-5(u213)* occurred with various *mec-9* alleles. This enhancement is also seen with other ts alleles of *mec-5* (16). Since both genes encode putative extracellular proteins, we believe these proteins may interact and form extracellular attachment points for the touch apparatus (16). The strong enhancement by mutations in the degenerin genes *mec-10* and *mec-6* supports this hypothesis.

mec-6(u247). Mutations in *mec-2*, *mec-4*, and *mec-10* strongly enhanced the Mec phenotype of *mec-6(u247)*. Since preliminary data suggests that *mec-6* encodes another degenerin homologue (C. Ma and M.C., unpublished data), we expected an enhancement pattern similar to that of *mec-4(u45)*. The enhancement patterns were similar except that mutations in the tubulin genes, *mec-7* and *mec-12* did not enhance *mec-6(u247)*. Since enhancement of *mec-4(u45)* was generally stronger than of *mec-6(u247)*, the former mutation may be a more sensitive reporter.

Although *mec-4* mutations enhanced the *mec-6(u247)* Mec phenotype, the reverse enhancement was not seen. This result may reflect a greater relative abundance of MEC-6 over MEC-4. In these experiments, we combined a threshold amount of one component (the product of the ts allele) and a haploid amount of the wild-type product of another gene (with, at most, a haploid amount of an altered product from the gene). Under these conditions, the reduction to threshold of the most abundant product should be the more sensitive reporter of interactions between the products. Alternatively, the *mec-6* alleles used for *mec-4(u45)* may not be null, but may provide partial gene activity. In any event, *mec-10* mutations enhance both *mec-4(u45)* and *mec-6(u247)*. These data are consistent with the previous observations that *mec-4* and *mec-6* mutations suppress a degeneration-causing mutation in *mec-10* (10), and support the suggestion that MEC-4, MEC-6, and MEC-10 contribute to the degenerin channel. Consistent with this hypothesis, Canessa *et al.* (12) have shown that three subunits similar to degenerins are required for the mammalian epithelial Na^+ channel.

mec-12(u67). Mutations in *mec-2*, *mec-5*, *mec-7*, and *mec-15* enhanced the Mec phenotype of *mec-12(u67)*. The *mec-12* gene encodes an α-tubulin (M. Hamelin, M. Chou, and J. Culotti, personal communication); we expected and found that the strongest enhancement of *mec-12(u67)* occurred with mutations in the β-tubulin gene *mec-7*.

One *mec-6* allele, *e1342,* also strongly enhanced the *u67* Mec phenotype, while another, *u3,* did not. The *e1342* allele may encode a product that interferes with the organization of the touch apparatus when the *mec-12(u67)* defect is present. *mec-2* mutations also enhanced *mec-12(u67)* in an allele-specific manner. *mec-2(e75)* strongly enhanced *mec-6(u247)* but not *mec-12(u67)*. In contrast, *mec-2(u26)* strongly enhanced *mec-12* but only weakly enhanced the *mec-6* ts mutation [it also weakly enhanced *mec-4(u45)*]. These *mec-2* mutations alter different regions of the *mec-2* product (19). The *e75* mutation changes Ala-204 to Val in the stomatin-like domain of MEC-2, while the *u26* mutation changes Arg-385 to His in the MEC-2 unique C-terminus. These data support the hypothesis that the stomatin-like region of MEC-2 interacts with the degenerins, and the nonstomatin-like sequence interacts with cytoskeletal elements.

Since otherwise recessive alleles of α and β tubulins in *Drosophila* (46) and yeast (47) fail to complement each other in double heterozygotes, we tested the phenotype of double heterozygotes of *mec-7* and *mec-12* mutants. We found that several *mec-12/+*; *mec-7/+* strains with the *mec-7* mutations *e1506*, *u9*, *u50*, *u80*, *u88*, *u142*, *u170*, *u173*, *u178*, *u278*, *u305*, *u382*, and *u388* produced a slightly touch-insensitive phenotype with *mec-12* mutations *u63*, *u67*, *u76*, and *u172*. In addition, dominant and recessive suppression were also observed among different *mec-7* and *mec-12* alleles. We found that in the *mec-12/+* and *mec-7/+* strains, *mec-7* alleles *u88*, *u222*, and *u275* suppressed the semidominant *mec-12* mutation *u174*, *mec-7(e1505)* suppressed semidominant *mec-12(u159)*, and *mec-7(e1506, u388)* suppressed semidominant *mec-12(u94)*. In *mec-12*; *mec-7* homozygous animals, *mec-7u305*; *mec-7(u10)*, *mec-12(u204)*; *mec-7(u234)*, *mec-12(u241)*; *mec-7(u222)*, and *mec-12(u279)*; *mec-7(u10)* were weakly sensitive, thus exhibiting slight suppression.

mec-15(u75). Strong enhancement of the *mec-15* Mec phenotype was only seen with one mutation, the *mec-7* allele *u428*. Weaker enhancement was seen with mutations in *mec-2* and *mec-10* (see Table 1 legend). An unexpected result was that three other *mec-7* alleles (two null alleles and one missense

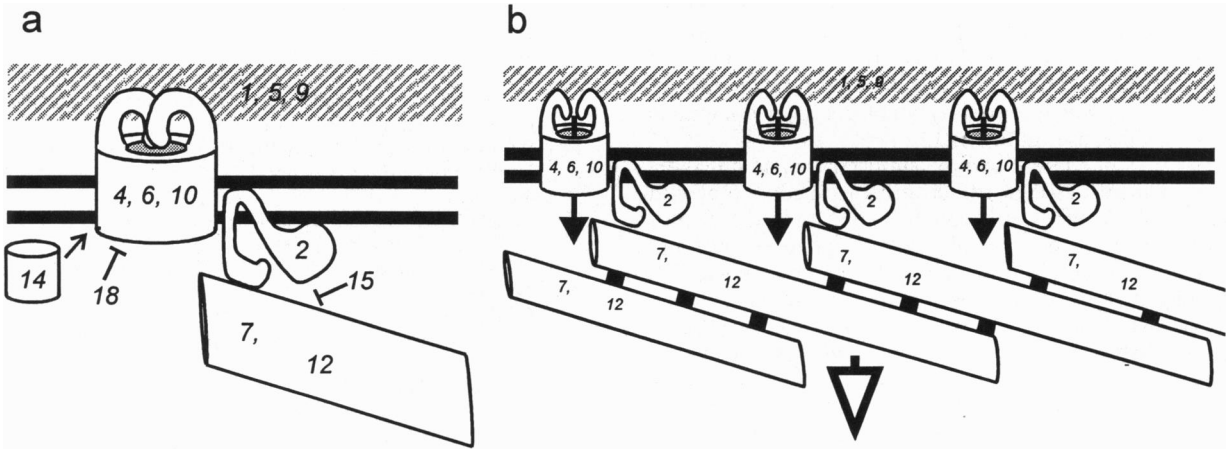

FIG. 2. A model for mechanosensory transduction in the *C. elegans* touch receptor neurons. (*a*) The mechanosensory apparatus. Gene products are indicated only by the number of the *mec* gene. Arrows symbolize the activation of the channel, whereas T-bars symbolize the suppression of the channel activity. Since the *mec-1*, *mec-15*, and *mec-18* genes have not been cloned, their positions are suggested only from genetic data and mutant phenotypes. See text for details. (*b*) Activation of channels by the displacement of the bundle of 15-protofilament microtubules (comprised of the *mec-12* α-tubulin and *mec-7* β-tubulin). Because the channels are attached to both the microtubules (through MEC-2) and the extracellular matrix (through MEC-5 and MEC-9), movement of the microtubules (open arrowhead) could lead to channel opening and subsequent ion flow (black arrows). The coupled opening of several channels could result from the cross-linking of the microtubules in the bundle.

mutation) dominantly suppressed the *mec-15*(*u75*) Mec phenotype at both 23°C and 25°C. Although we tested representative alleles of other *mec* genes, we did not identify any other suppressors of *mec-15*(*u75*). The suppression by *mec-7* loss-of-function mutations suggest that *mec-15*(*u75*) produces an abnormal product whose effects require a sufficient amount of *mec-7* β-tubulin. One possibility is that *mec-15* encodes a microtubule-associated protein that normally down-regulates the interaction of the microtubules with the degenerin channel. This suggestion that the *u75* mutation and other *mec-15* alleles may be acting as gain-of-function alleles is supported by the finding that a deletion of the *mec-15* gene gave a different pattern of enhancement with respect to the action of *mec-5*(*u213*) and *mec-12*(*u67*) (see Table 1).

DISCUSSION

By using ts *mec* alleles to sensitize our touch assay, we have shown that several genetic interactions exist among the touch genes. In screens for new dominant enhancers of *mec-4*(*u45*) and *mec-5*(*u213*), we found mutations only in previously identified *mec* genes. We have suggested that screens for *mec* mutations were probably saturated for genes that could be mutated to touch insensitivity (3). These new results suggest that few, if any, additional dosage-limited genes exist that affect touch sensitivity. Dominant enhancement could result from the disruption of protein interactions, the reduction of activity in the same biochemical pathway, or the weakening of a second, partially redundant pathway (48). Given that most of the cloned *mec* genes appear to encode structural proteins, we

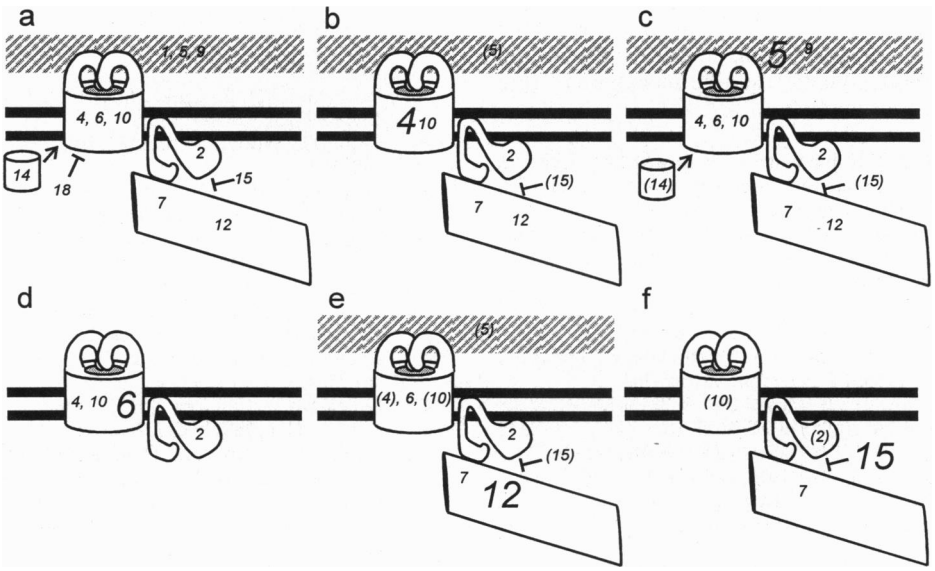

FIG. 3. A pictorial representation of the *mec* gene interactions as deduced from the dominant enhancement data. (*a*) Data are the same as in Fig. 2. The other panels (*b–f*) Data show only the components for which genetic interactions were detected between an individual ts mutation of one *mec* gene (as indicated by a large, bold number) and other mutations in other *mec* genes. The presence of the parenthesis around specific components indicates that marginal or variable enhancement with the highlighted gene was found. In addition to these data, the studies of Huang and Chalfie (10) suggest that the *mec-2*, *mec-4–6*, *mec-10*, *mec-12*, and *mec-14* genes are needed to activate *mec-10*-induced degeneration, while wild-type *mec-18* appears to inhibit this degeneration.

believe that the enhancement and suppression described here are mostly due to disruption of protein-protein interactions. Enhancement may result from a reduction in the number of effective touch receptor complexes (null alleles) or a reduction in the effectiveness of the existing touch receptor complexes (for non-null missense mutations).

The data presented here and the suppression and enhancement studies on *mec-10* (ref. 10; see above) are consistent with a model in which the touch cell proteins form a receptor complex that transduces mechanosensory signals (Figs. 2 and 3). Specifically, in this model, channels formed by the MEC-4, MEC-6, and MEC-10 degenerin proteins are attached externally to the extracellular matrix formed by MEC-5, MEC-9, and possibly MEC-1. These attachment points might involve the extracellular regulatory domain that we have previously identified in the degenerins (14). Intracellularly, the channels are attached to the array of large-diameter microtubules (formed by the MEC-12 α-tubulin and the MEC-7 β-tubulin) via interactions with MEC-2, the stomatin-like protein. Mechanical stimuli activate the channel by causing it to be stretched between its extracellular and intracellular attachment points. The length of the microtubules ($\approx$20 μm; ref. 18) would make them very sensitive levers that could detect the touch stimulus. The apparent cross-bridging of the microtubules seen in electron micrographs (18) would permit the coupled activation of several channels.

We envision that the products of the *mec-14*, *mec-15*, and *mec-18* genes modulate directly or indirectly the activity of the mechanosensory complex. Since *mec-14* encodes a protein with some similarity to the β-subunits of *Shaker*-type potassium channels (N. Hom, S. Gangadharan, Y. Tu, M. Huang, L. Chen, and M.C., unpublished data), it may modulate the degenerin channel directly. *mec-15* may affect microtubule function or its coupling with the channel. Since *mec-18* mutations enhanced a *mec-10*-induced degeneration (10), the wild-type *mec-18* gene appears to negatively affect degenerin channel activity. The nature of this interaction is not known.

This model is analogous to the model for the gating of the hair cells in the vertebrate auditory and vestibular systems (49). In hair cells, the transduction channel is hypothesized to associate with components of the extracellular matrix, such as the tip-link (50). Intracellularly, the channel is thought to associate with an actin cytoskeleton (49). The physical manipulation of the apparatus opens the channel through the displacement of a channel gating domain. Mechanosensation though direct physical manipulation may be a common feature of this type of signal transduction.

We thank D. Kelly and D. Rabinowitz for help with the statistical analysis and M. Treinin for the helpful discussions. The deletion (Df) strains were kindly provided by the Caenorhabditis Genetics Center. This research was supported by National Institutes of Health Grants GM30997 and GM34775 to M.C. and National Research Service Postdoctoral Fellowship NS09968 to G.A.C.

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