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MOLECULAR MODELING OF MECHANOTRANSDUCTION IN THE NEMATODE *CAENORHABDITIS ELEGANS*

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ABSTRACT

Genetic and molecular studies of touch avoidance in the nematode *Caenorhabditis elegans* have resulted in a molecular model for a mechanotransducing complex. *mec-4* and *mec-10* encode proteins hypothesized to be subunits of a mechanically gated ion channel that are related to subunits of the vertebrate amiloride-sensitive epithelial Na^+ channel. Products of *mec-5*, a novel collagen, and *mec-9*, a protein that includes multiple Kunitz-type protease inhibitor repeats and EGF repeats, may interact with the channel in the extracellular matrix. Inside the cell, specialized 15-protofilament microtubules composed of *mec-12* α-tubulin and *mec-7* β-tubulin may be linked to the mechanosensitive channel by stomatin-homologous MEC-2. MEC-4 and MEC-10 are members of a large family of *C. elegans* proteins, the degenerins. Two other degenerins, UNC-8 and DEL-1, are candidate components of a stretch-sensitive channel in motor neurons. Implications for advancing understanding of mechanotransduction in other systems are discussed.

INTRODUCTION

Despite the fundamental importance of mechanical signaling in a myriad of biological processes ranging from cell volume regulation to sensory transduction in touch and hearing (reviewed in 27, 66), remarkably little is known about the nature of the molecules that mediate mechanotransduction. Although elegant

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electrophysiological studies have shown that mechanically gated ion channels play central roles in the conversion of mechanical forces into cellular responses, aspects of channel biology have rendered the genes encoding channel components elusive: Reagents that could facilitate protein isolation by specifically associating with channel subunits at high affinity are not available, and even in specialized mechanotransducing structures such as the vertebrate cochlea, mechanically gated channels are distributed at low concentrations. To date, biochemical purification and cloning of only one mechanosensitive channel, the *Escherichia coli* MscL, has been heroically accomplished (75).

An alternative approach toward isolating molecules involved in mechanotransduction is to isolate mutants defective in mechanosensitive behaviors and then to clone the identified genes by standard methods. Such a strategy has resulted in the isolation and characterization of several genes required for mechanosensitive behaviors in the nematode *C. elegans* (reviewed in 20, 22, 36). Here we review genetic and molecular studies in *C. elegans* that have culminated in a molecular model of body touch transduction and the identification of candidate mechanically gated ion channels.

The C. elegans Experimental System

C. elegans, a small (1 mm) free-living nematode normally found in soil, is easily reared on an *E. coli* diet in the laboratory. The life cycle of this animal, which can be completed in just 2.5 days at 25°C, includes a period of embryonic development within an eggshell, four larval stages (L1–L4), and adulthood, which is distinguished by sexual maturity. The most common sexual form is the hermaphrodite (XX), although males (X0) can be easily propagated for use in genetic studies. *C. elegans* is fairly transparent such that the nucleus of every cell can be visualized using Normarski differential interference contrast microscopy. This feature enabled the complete sequence of cell divisions that occur as the fertilized egg develops into the 959-celled adult to be recorded (77, 78). Of these cells, 302 are neurons, and the pattern of synaptic connections made by each neuron has been determined using serial section electron microscopy (88). Knowledge of the complete wiring diagram of the animal has enabled neural circuits for specific mechanosensory behaviors to be predicted. Such predictions have been tested by laser ablation experiments in which individual cells are killed by a laser microbeam (15, 44).

A key advantage to investigating biological processes in *C. elegans* is its powerful genetic system (6). Thousands of mutations affecting development or behavior have been identified and assigned to complementation groups that are positioned on a detailed genetic map. Once genes are mapped, it is increasingly straightforward to clone them. A collection of overlapping cosmid and YAC clones that cover most of the six chromosomes (the physical map of the *C.*

elegans genome) is correlated with the genetic map (37, 84) so that the position of a mapped candidate gene can usually be restricted to a collection of cosmids spanning only a few hundred kilobases of DNA. Verification of gene identity is frequently accomplished by constructing transgenic lines and testing for complementation of identified mutations (26, 55). Sequence analysis of the *C. elegans* genome is underway (76, 91) with a projected completion date of 1998.

Taken together, the abilities to identify and experimentally verify individual neurons specialized for mechanosensation, to isolate mutations specifically affecting the function of these cells, and to clone the identified genes rapidly has led to significant insight into molecular mechanisms of mechanotransduction in *C. elegans*.

Mechanosensitive Behaviors in C. elegans

Mechanical stimuli regulate many *C. elegans* behaviors including locomotion, foraging, egg laying, feeding (pharyngeal pumping), and defecation. The mechanosensitive response best characterized at the cellular, genetic, and molecular levels is the movement away from a light touch delivered to the body with an eyelash hair (14). Another behavioral paradigm elegantly utilized to study mechanosensory control of locomotion is the response to nose touch—the reversal of direction as a consequence of head-on collision or a light touch on the side of the nose (35, 44, 53; reviewed in 22). Other touch-mediated locomotory responses such as a reaction to harsh touch (a strong prod with a metal wire best assayed in the absence of gentle touch mechanosensory neurons; 86) or to tap (a diffuse stimulus as delivered by a tap on the plate on which worms are reared; 19, 60, 89, 90), have been less extensively studied at the genetic level. Here we review the molecular genetics of body touch, with an emphasis on a recently identified family of ion channel subunits postulated to function in mechanically gated ion channels.

GENTLE BODY TOUCH

The Mechanosensory Touch Receptor Neurons

In the laboratory, *C. elegans* moves through a bacterial lawn on a petri plate with a readily observed sinusoidal motion. When gently touched with an eyelash hair (typically attached to a toothpick) on the posterior, an animal will move forward; when touched on the anterior body, it will move backward. This gentle body touch is sensed by the touch receptor neurons ALML/R (anterior lateral microtubule cell left, right), AVM (anterior ventral microtubule cell), and PLML/R (posterior lateral microtubule cell left, right) (14, 15; see Figure 1). The touch receptors are situated so that their processes, embedded in the hypodermis adjacent to the cuticle, run longitudinally along the body wall.

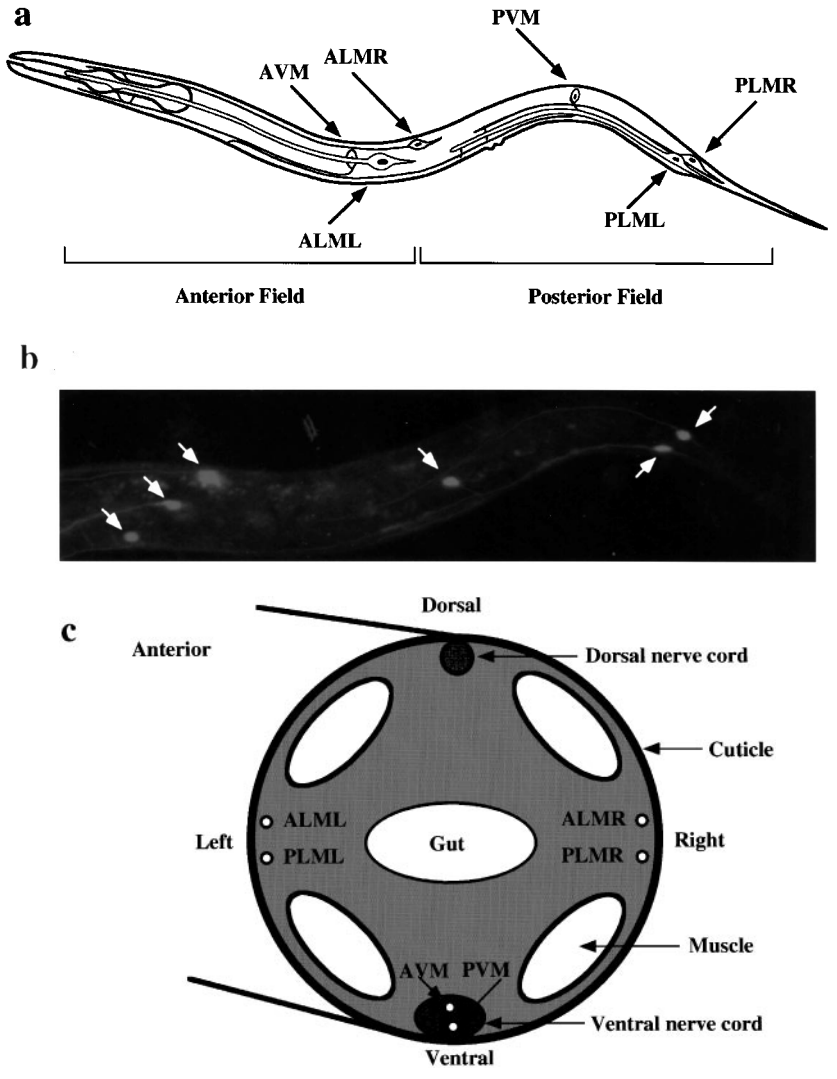

Figure 1 The *C. elegans* touch receptor neurons. (*a*) Schematic diagram showing the position of the six touch receptor neurons in the body of the adult nematode. Note the two fields of touch sensitivity defined by the arrangement of these neurons along the body axis. The ALMs and AVM mediate the response to touch over the anterior field, whereas PLMs mediate the response to touch over the posterior field. (*b*) Visualization of touch receptors in living worms expressing the green fluorescent protein under the control of the *mec-4* promoter, which is active only in the six touch receptor neurons. Arrows indicate touch receptor cell bodies. Some touch receptor axons are apparent. (*c*) Schematic representation of a nematode in theoretical cross section. Position of the touch cell processes are depicted. Other landmarks of *C. elegans* anatomy are also shown.

The position of the processes along the body axis correlates with the sensory field of the touch cell. Laser ablation of AVM and the ALMs, which have sensory receptor processes in the anterior half of the body, eliminates anterior touch sensitivity, and laser ablation of the PLMs, which have posterior dendritic processes, eliminates posterior touch sensitivity (15). In addition to mediating touch avoidance, the touch receptor neurons appear to control the spontaneous rate of locomotion because animals that lack functional touch cells are lethargic. The mechanical stimuli that drive spontaneous locomotion are unknown but could include encounters with objects in their environment or body stretch induced by locomotion itself.

One additional neuron, PVM (posterior ventral microtubule cell) is also categorized as a touch cell because it is ultrastructurally similar to the others and its differentiation is controlled by the same genetic pathway (see below). Laser ablation studies have shown, however, that the PVM neuron does not play a critical role in gentle touch-modulated locomotion because it cannot mediate touch avoidance by itself (15).

Distinguishing Ultrastructual Features of the Touch Receptor Neurons

TOUCH CELL-SPECIFIC MICROTUBULES The touch receptor cell processes are distinguished by the presence of a bundle of wide-diameter [15-protofilament (pf)] microtubules (16, 17; see Figure 2). These 15-pf microtubules are only found in the six touch receptor neurons (microtubules in most *C. elegans* cells contain 11 protofilaments; those in most organisms contain 13 protofilaments). Individual microtubules do not span the full length (about 400—500 mm) of the touch cell processes. Rather, individual microtubules 10–20 mm in length overlap within the microtubule bundle to fill the process (16). Interestingly, microtubule ends appear structurally distinct in electron micrographs—the end proximal to the cell body is darkened and is preferentially found on the inside of a microtubule bundle, whereas the distal end is diffusely stained and is always situated outside of the microtubule bundle. It is particularly intriguing that the distal end of the microtubule is often situated adjacent to the plasma membrane; such an arrangement suggests a mechanical link could be formed between the microtubule network and mechanosensitive channels in the plasma membrane (16; see discussion of mechanotransduction model below). The integrity of the 15-pf microtubules is required for touch receptor neuron function. If touch cell microtubules are disrupted by low concentrations of colchicine or by mutation, touch sensitivity is lost (14, 17).

THE EXTRACELLULAR MANTLE Touch receptor processes are surrounded by a specialized extracellular matrix referred to as the mantle (14; see Figure 2).

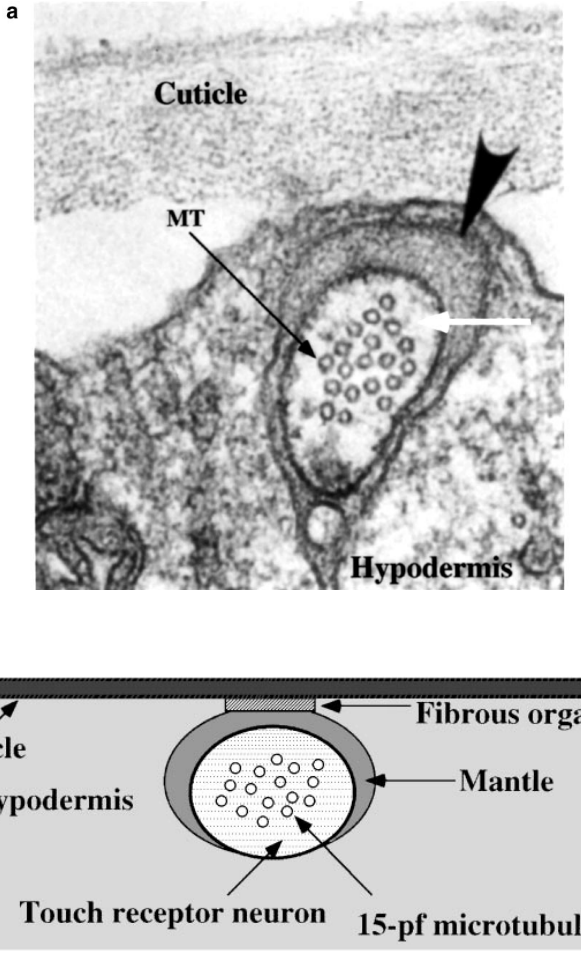

Figure 2 Ultrastructural features of the touch receptor neurons. (*a*) Electron micrograph of a touch receptor neuron process. The touch cell process (*white arrow*) is filled with 15-pf microtubules (MT, *thin black arrow*). The process is embedded in the hypodermis and surrounded by the mantle (*large black arrowhead*). (*b*) Schematic representation of a touch receptor neuron cross section. A darkly staining region labeled fibrous organelle is depicted here as a bar-shaded rectangle connecting the mantle and the cuticle. Such specializations appear periodically along the length of the touch receptor process and may serve to attach the process to the cuticle. This figure is adapted from Reference 36.

The mantle can be stained with peanut lectin and is most prominent on the side of the touch receptor neuron closest to the cuticle. Darkly staining cuticular specializations are positioned periodically along the length of the touch receptor process in close contact with the mantle. These cuticular specializations look similar to muscle attachment sites and thus may be sites at which the touch receptor process, via the mantle, is fixed to the cuticle. The integrity of the mantle appears important for touch receptor function (see below).

Genetic and Molecular Analysis of Body Touch

To identify genes needed for function of the touch receptor neurons, Chalfie and colleagues mutagenized animals and screened their F2 progeny for the failure to respond to gentle touch. Mutants were retested by prodding with a wire (harsh touch) to confirm that although they were touch-insensitive, they were still capable of locomotion. Using this approach, more than 400 mutants that (more or less) specifically lack the ability to respond to gentle touch were identified (11, 14). Many of these were designated as *mec* mutants because the phenotype they confer is mechanosensory abnormal (Mec). The identified mutations define 16 genes that contribute in specific ways to touch cell development and function (summarized in Table 1).

Transcription Factors Needed for Touch Cell Development

UNC-86 AND MEC-3 Touch cell development requires the combinatorial action of genes needed for appropriate execution of touch cell lineages, specification of touch cell fate, restricted cellular expression of structural genes, cell migration, and process outgrowth (10, 22). Two genes, *unc-86* and *mec-3*, are particularly important for differentiation of the touch receptor neurons.

UNC-86 is a POU homeodomain transcription factor needed for the proper differentiation of neuroblasts that generate all six touch receptor neurons (13, 25). A key function of UNC-86 in touch receptor development is to bind to DNA to stimulate transcription of *mec-3*, which encodes a LIM homeodomain protein (28) expressed in the six touch receptors (and in two other neurons that may be stretch sensitive, FLP and PVD; 44, 85–87, 92). *mec-3* is needed for the specification of touch cell fate. In the absence of *mec-3*, the lineages that generate the touch cells are executed normally and cells produced differentiate as neurons. However, the neurons generated fail to express touch cell–specific features such as the 15-pf microtubules and the mantle (14, 85). The MEC-3 homeodomain protein forms a heterodimer with UNC-86 and binds to well-defined sites in the *mec-3* promoter and in other touch cell–specific promoters (48, 93), thereby functioning as a transcriptional activator of touch cell–specific structural genes, including the *mec-7* β-tubulin and the *mec-4* channel subunit (33, 56, 87, 92). Once *mec-3* is expressed in the touch cells, it activates its own

Table 1 The touch cell genes of *C. elegans*

Gene[a]	Encoded product	Likely function	References
unc-86	POU homeodomain transcription factor	Execution of cell lineage	24, 25, 48, 92, 93
mec-3	LIM homeodomain transcription factor	Specification of touch cell differentiation	48, 85, 86, 87, 92, 93
mec-4	Channel subunit	Mechanosensitive channel	12, 21, 38, 39, 46
mec-10	Channel subunit	Mechanosensitive channel	12, 40
mec-2	Stomatin-like protein	Channel-cytoskeleton linker	41
mec-7	β-tubulin	15-pf microtubule formation	33, 67, 68
mec-12	α-tubulin	15-pf microtubule formation	32, 30
mec-5	Collagen	Extracellular matrix	23, 30
mec-9	EGF and Kunitz repeats	Extracellular interactions	23, 30
mec-8	Likely RNA-binding protein	Splicing	51, 52
mec-14	Aldo-keto reductase	Channel activity	11, 14, 54
mec-1[b]	?	Mantle	11, 14
mec-6[b]	?	Channel activity	11, 14
mec-17[b]	?	Maintenance of transcription	11, 14
mec-15[c]	?	?	11, 14
mec-18[c]	?	?	11, 14

[a]Genes identified by mutations in *C. elegans* that are required for normal response to gentle body touch mediated by the six touch receptor neurons.
[b]Even though the molecular identity of these genes has yet to be established, genetic analysis suggests a function for their encoded products.
[c]Mutations in these genes disrupt or attenuate touch response, but the mode of action of the encoded proteins has not been determined.

transcription (86). Later in development, *mec-17* also contributes to maintenance of *mec-3* expression. The mechanism of action of *mec-17* is not known because the gene has not been characterized molecularly. *unc-86* continues to be expressed in differentiated touch receptors and is continuously needed for their function (24).

Structural Genes Required for Touch Receptor Function

MEC-4 AND *MEC-10* CHANNEL PROTEINS Animals bearing loss-of-function mutations in *mec-4* or *mec-10* are touch insensitive despite the fact that in these mutant backgrounds, the touch receptor neurons develop normally and exhibit no apparent defects in ultrastructure (14). *mec-4* and *mec-10* encode homologous proteins related to subunits of the multimeric amiloride-sensitive Na^+ channel, which mediates Na^+ readsorption in vertebrate kidney, intestine, and lung epithelia (the ENaC channel; 7, 8, 12, 21, 40, 46). By analogy, MEC-4 and MEC-10 are likely ion channel subunits. Indeed, although channel activity has not yet been directly demonstrated for either MEC-4 or MEC-10, certain chimeric nematode/rat proteins function in *C. elegans* and in *Xenopus*

oocytes, implying that the nematode and rat proteins are functionally similar (38, 82).

What is the role of the MEC-4/MEC-10 ion channel in the touch receptor neurons? Intriguingly, *mec-4* is expressed only in the six touch receptor neurons (56) and *mec-10* is expressed in the six touch receptor neurons and in two other neuron pairs that may mediate stretch-sensitive responses (FLPL/R and PVDL/R; 40). Because the MEC-4 and MEC-10 subunits are expressed exclusively in mechanosensitive neurons and are essential for the function of these neurons, it has been proposed that MEC-4 and MEC-10 coassemble into a mechanically gated ion channel that plays a central role in touch transduction. The relationship of these channel subunits to subunits of an amiloride-sensitive channel is also intriguing because amiloride is a general inhibitor of mechanosensitive ion channels (34). Experimental verification that the MEC-4/MEC-10 channel is mechanically gated remains a challenge for the future.

MEC-4 primary sequence and transmembrane topology MEC-4 and MEC-10 are members of a superfamily of homologous proteins that includes additional members from *C. elegans* and vertebrates. Here we briefly describe features of MEC-4 (21, 46), most of which are also generally conserved in other *C. elegans* family members. MEC-4 is 768 amino acids in length and includes two membrane-spanning domains (MSDI, MSDII; Figure 3). Both predicted hydrophobic domains are slightly longer than required for a single transmembrane pass, and it has been proposed that MSDI and MSDII in other superfamily members may include residues that loop back into the membrane forming a pore, similar to H5 domains of several characterized channel types (29, 43, 61). MEC-4 also includes three cysteine-rich domains (CRDI, CRDII, CRDIII) and one region similar to venom neurotoxins (NTD) (N Tavernarakis & M Driscoll, submitted) situated between the two transmembrane domains.

Mechanically gated channels are thought to be tethered to proteins inside and outside the cell to provide gating tension (e.g. work on the hair cell channel reviewed in 42, 59). In MEC-4 the N- and C-termini are cytoplasmic and are thus candidate domains for interaction with the cytoskeleton; the central region of MEC-4 is extracellular and thus the CRD and NTD domains situated outside the cell are candidate regions for interaction with the extracellular matrix (46). Other superfamily members have the same transmembrane topology (8, 29, 61, 72).

MEC-4 structure/activity relationships—toxic alleles Dominant gain-of-function *mec-4* alleles induce swelling and death of the touch receptor neurons (11, 14). These *mec-4*(*d*) alleles encode substitutions of large side chain amino acids (Val or Thr) for a conserved Ala residue (aa 713) situated adjacent to MSDII

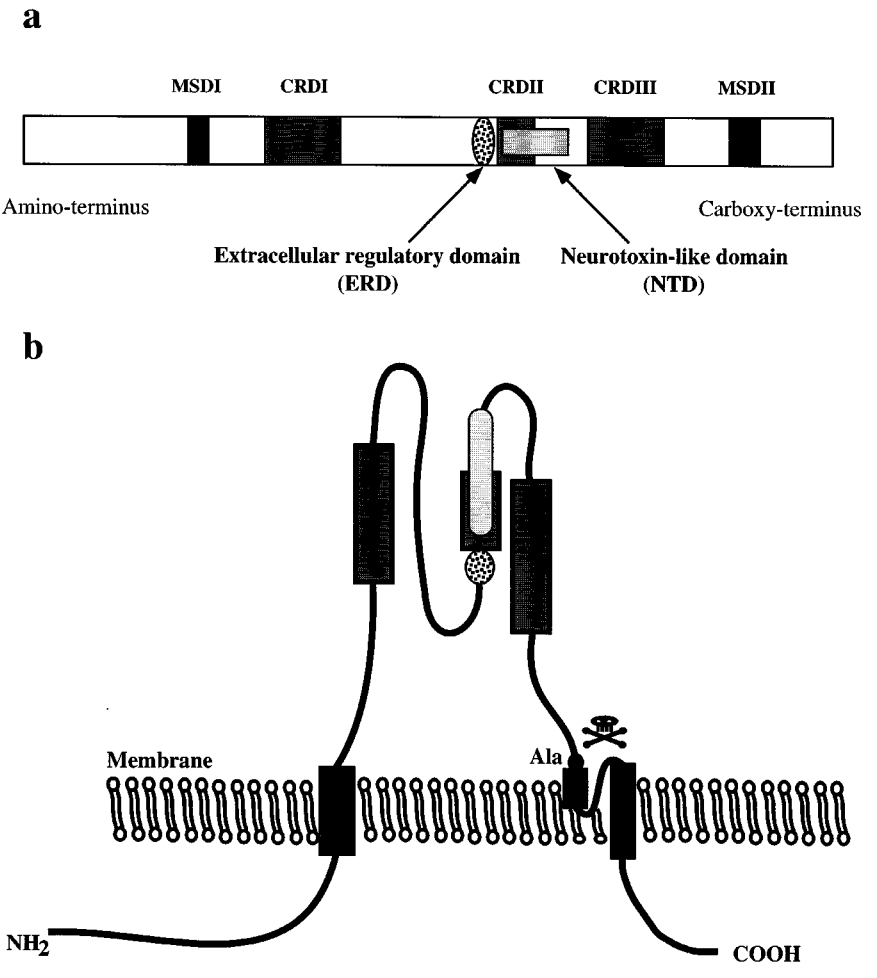

Figure 3 Features of the MEC-4 protein. (*a*) Structural features of MEC-4. The two membrane-spanning domains (MSDI, II; *black boxes*) are shown together with the three cysteine-rich domains (CRDI, II, III; *gray boxes*). The putative extracellular regulatory domain (ERD) is depicted by the oval. The neurotoxin-related domain (NTD) is represented by the lightly shaded rectangle overlapping with CRDII. (*b*) Transmembrane topology of MEC-4. Both the amino- and carboxy-termini are intracellular; the central part of the protein, which includes the CRDs, ERD, and NTD, is extracellular. The second membrane-spanning domain is longer than required for a single transmembrane pass and may loop back to form a pore-lining structure. Ala713, which when replaced by a bulkier amino acid results in degenerative cell death, is indicated by the skull and cross-bones icon.

(see Figure 3; 21, 46). There is a correlation between the size of the amino acid side chain at position 713 and toxicity: Tests of *mec-4* mutant alleles engineered to include all possible amino acid substitutions established that a large side chain amino acid at this site is toxic to touch neurons, whereas a small side chain amino acid (Ala, Ser, Cys) is not lethal (21). These results suggest that steric hindrance plays a critical role in the degeneration mechanism. A working model for the initiation of cell death is that the presence of a bulky side chain at this site prevents the channel from closing effectively, resulting in an increased influx of ions that is toxic (Figure 4). Interestingly, small amino acid side chains are present at the position corresponding to MEC-4 (Ala713) in all superfamily members. Other *C. elegans* family members (e.g. *deg-1* and *mec-10*) can be altered by analogous amino acid substitutions to induce neurodegeneration, and thus the *C. elegans* branch of the gene family has been named the degenerin family. Strikingly, a mutant variant of neuronally expressed mammalian family member MDEG, engineered to encode Val or Phe at the corresponding position, induces swelling and death when introduced into *Xenopus* oocytes and hamster embryonic kidney cells (83).

An alternative way in which *mec-4* can be engineered to induce neurodegeneration has been determined based on studies of an unusual toxic recessive allele of degenerin *deg-1* (29). *mec-4* alleles harboring a missense mutation (A404T) or a small deletion (Δ399–407) in the extracellular region induce degeneration in transgenic animals. Again, increased channel activity is thought to initiate degeneration. Thus these toxic alterations in degenerin proteins may disrupt an extracellular domain that functions in channel closing (the extracellular regulatory domain, ERD). Alternatively, death-inducing substitutions in the ERD could modify the MEC-4 tertiary structure to favor the open channel conformation. The ERD is highly conserved among *C. elegans* family members.

MSDII is a predicted pore-lining domain The more C-terminal MEC-4 transmembrane domain MSDII is amphipathic. Amino acids on the hydrophilic face are highly conserved and essential for *mec-4* function (38). These observations underlie the hypothesis that MSDII forms part of the channel pore with polar residues projecting into the lumen to influence ion conductance. Consistent with this suggestion, amino acid substitutions in the candidate pore domain (designed to disrupt ion influx) block or delay neurodegeneration when present in *cis* to the channel-opening A713V substitution (38). Electrophysiological characterization of rat ENaC mutants and rat/nematode chimeras supports the hypothesis that specific polar residues in MSDII influence channel conductivity (82).

Other regions important for MEC-4 function In the saturation genetic screen for touch-insensitive mutants, more than 50 *mec-4* loss-of-function alleles were

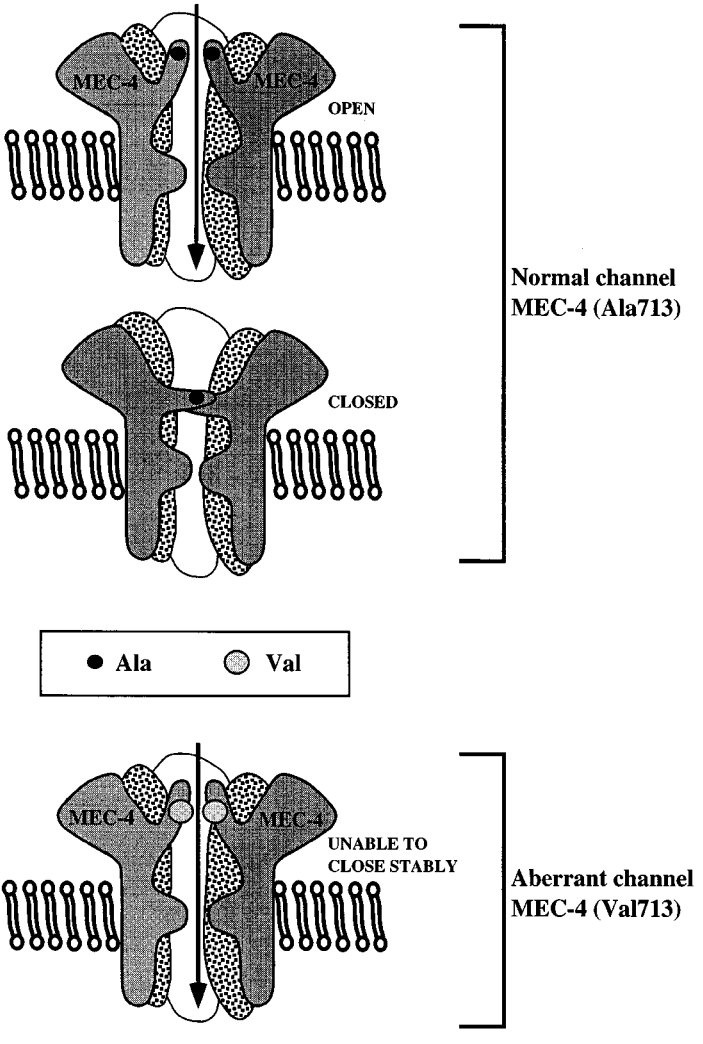

Figure 4 Model for *mec-4*(*d*)-induced toxicity. Gain-of-function mutations in *mec-4* encode substitutions for a conserved alanine adjacent to MSDII and result in neuronal degeneration. Amino acids with bulkier side chains at this position are thought to lock the channel in an open conformation by causing steric hindrance, resulting in Na^+ influx that triggers necrotic-like cell death.

isolated (11, 14). DNA sequence analysis of these alleles revealed that in addition to MSDII, there are three regions where single amino acid substitutions that inactivate MEC-4 are clustered (39). Two of the sites are in the extracellular domain, situated either near or within the extracellular CRDIII. The role of these regions in channel function remains to be determined but is speculated to be to facilitate protein interactions. One short stretch in the intracellular N-terminal domain (aa 87–95) may define a site of interaction of the MEC-4 subunit with an intracellular protein.

Subunit Composition of the Candidate Mechanotransducing Channel

The subunit compositions and stoichiometries for DEG/ENaC channels have not been determined. Biochemical analyses suggest that the mammalian ENaC channel has as many as six different subunits (1, 4). Electrophysiological assays of the rat ENaC channel reconstituted in oocytes established that at least three homologous subunits (α-, β-, and γ-rENaC) must be coexpressed to assemble an active channel with the pharmacological properties similar to the in vivo channel (9).

The touch receptor channel also appears to be multimeric. Evidence that MEC-4 and MEC-10 coassemble into the same channel complex include (*a*) MEC-4 and MEC-10 subunits are coexpressed in the touch receptor neurons, (*b*) MEC-4 and MEC-10 proteins translated in vitro in the presence of microsomes can coimmunoprecipitate (C Lai & M Driscoll, unpublished observations), and (*c*) genetic interactions between *mec-4* and *mec-10* have been observed (30, 40). For example, *mec-10* can be engineered to encode a death-inducing amino acid substitution *mec-10*(A673V) (40). However, if *mec-10*(A673V) is introduced into a *mec-4* loss-of-function background, neurodegeneration does not occur. This result is consistent with the hypothesis that MEC-10 cannot form a functional channel in the absence of MEC-4.

Genetic experiments also suggest that MEC-4 subunits interact with each other. The toxic protein MEC-4(A713V) can kill cells even if it is coexpressed with wild-type MEC-4(+) [as occurs in a *trans* heterozygote of genotype *mec-4*(*d*)/*mec-4*(+)]. However, if toxic MEC-4(A713V) is coexpressed with a specific *mec-4* allele that encodes a single amino acid substitution in MSDII, e.g. *mec-4*(*d*)/*mec-4*(E732K), neurodegeneration is partially suppressed (38; Figure 5). Because one MEC-4 subunit can interfere with the activity of another, it is likely that there may be more than one MEC-4 subunit in the channel complex. Interestingly, genetic experiments also suggest interactions between MEC-10 subunits (40). Thus a model for the minimum touch receptor channel is that the multimeric complex includes at least two MEC-4 and two MEC-10 subunits.

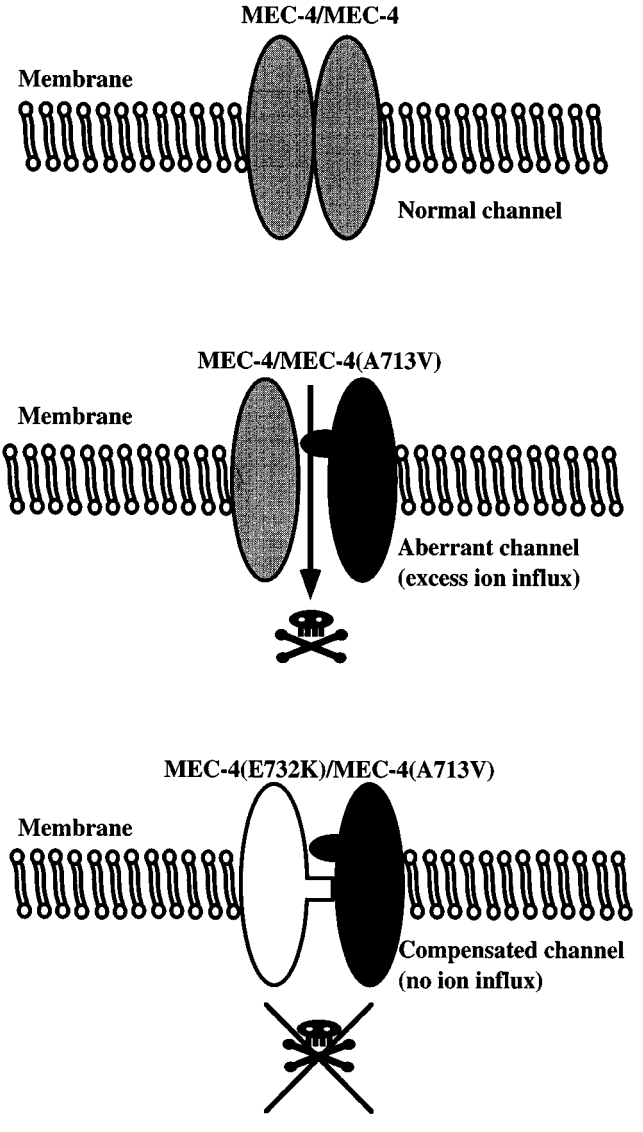

Figure 5 Multiple MEC-4 subunits are likely to participate in the formation of a touch receptor neuron channel. Allele-specific compensatory mutations in *mec-4* can neutralize the deleterious effects of the toxic gain-of-function mutation. Such an effect would be difficult to explain if only a single MEC-4 subunit were present in each channel complex and thus implies there is more than one MEC-4 molecule in each channel.

mec-6 AND CHANNEL ACTIVITY Recessive *mec-6* mutations disrupt touch sensitivity but do not cause detectable changes in touch cell ultrastructure (14). *mec-6* alleles have the interesting property that they completely block *mec-4*(*d*)- and *mec-10*(A673V)-induced touch cell degeneration, i.e. in *mec-6*; *mec-4*(*d*), and *mec-6*; *mec-10*(A673V) double mutant strains, cell death is suppressed. How exactly *mec-6* acts to influence MEC-4/MEC-10 channel activity is unknown in part because the gene remains to be cloned. It appears that *mec-6* mutations do not affect *mec-4* transcription, although they do cause a *mec-4/lacZ* reporter fusion product to be rapidly degraded (N Tavernarakis et al, unpublished observations). Working hypotheses concerning the function of *mec-6* thus focus on the possibilities that *mec-6* could encode either another subunit needed for channel function/assembly or a protein that mediates localization or a posttranslational modification essential for MEC-4 and MEC-10 activity. It should be noted that *mec-6* action is not exclusive to the MEC-4/MEC-10 touch receptor channel. *mec-6* mutations also suppress deleterious consequences of vacuole-inducing mutations in other *C. elegans* degenerins, including *deg-1* (18), *unc-8* (80), and *unc-105* (N Tavernarakis & M Driscoll, unpublished).

Intracellular Proteins Needed for Touch Transduction

As described above and shown in Figure 1, the touch receptor processes are filled with bundled 15-pf microtubules. Mutations in two genes, *mec-7* and *mec-12*, disrupt the production of these microtubules (11, 14). Interestingly, even in the absence of the 15-pf microtubules, the touch receptor processes grow out seemingly normally and become filled with 11-pf microtubules (17). Such touch receptors do not function, however, suggesting that the extensively cross-linked 15-pf microtubules contribute a specific role in touch transduction.

MEC-7 *mec-7* encodes a β-tubulin (68) expressed at high levels in the touch receptor neurons (33, 69). MEC-7 is highly conserved; apart from the C-terminal domain that is characteristically highly variable, only seven amino acids differ from other β-tubulins. It is not known whether any of the unique residues are instructive for the formation of 15-pf microtubules, although it is interesting that one of the amino acid differences affects a strictly conserved Cys residue (MEC-7 Cys293) that has been implicated in protofilament assembly by analysis of *Drosophila* mutants (64). *mec-7* mutations isolated in the screen for touch-insensitive mutants range in severity from recessive to strongly dominant, and most of the amino acid changes that disrupt MEC-7 function are known (67, 68). Domains affected by mutations include sites for GTP binding and hydrolysis, sites for heterodimerization with α-tubulin, and sites for higher-order microtubule assembly.

MEC-12 *mec-12* encodes an α-tubulin expressed at high levels in the touch receptor neurons but also expressed in several other neurons that do not assemble 15-pf microtubules (32). Thus the presence of the MEC-12 tubulin is not sufficient to nucleate assembly of the touch cell–specific microtubules. As is the case for *mec-7*, many *mec-12* mutations are semidominant or dominant and thus may disrupt subunit interactions or protofilament assembly. MEC-12 is the only *C. elegans* tubulin that is acetylated.

Taken together, studies of *mec-7* and *mec-12* strongly support that unique α- and β-tubulins assemble to form the 15-pf microtubules required for touch receptor function. Whether these specialized microtubules play a direct role in the function of the mechanotransducing complex remains to be determined.

MEC-2, A CANDIDATE LINKER PROTEIN How might the touch cell microtubule network influence the activity of a mechanically gated ion channel? Given that the specialized microtubules in the touch cells appear to associate with the plasma membrane at their distal ends, a simple hypothesis is that the 15-pf microtubules might contact the touch receptor channel directly to provide gating tension. There is some genetic evidence, however, that implicates another molecule, MEC-2, as a link between the microtubules and the touch receptor channel.

mec-2, required for the function of the touch receptors, encodes a predicted 481-amino acid protein expressed in the touch receptor neurons (and in a few additional neurons in the head) that appears to be localized all along the length of the touch receptor process, as well as in the cell body (41). The *mec-2* primary sequence features three candidate protein interaction domains (Figure 6*a*): (*a*) the carboxy-terminal domain includes a proline-rich region similar to SH3-binding domains; (*b*) the central MEC-2 domain (aa 114–363) includes a membrane-associated hydrophobic domain (aa 114–141) and a cytoplasmic hydrophilic domain that together exhibit 65% identity to the human red blood cell protein stomatin [stomatin is an integral membrane protein that associates with the cytoskeleton and affects ion balance via an unknown mechanism (74)]; and (*c*) part of the N-terminal domain (situated in part between aa 42–118) is needed for the localization of a MEC-2/LacZ fusion protein to the touch receptor process. Some of MEC-2 interactions appear to be with other MEC-2 proteins: Many of the 54 mutant *mec-2* alleles have dominant effects and exhibit a complex pattern of interallelic complementation (14). However, genetic data also suggest that MEC-2 interacts with the specialized touch cell microtubules (30, 41). Normally, a MEC-2/LacZ fusion protein is distributed along the touch receptor axon (41). The axonal distribution of a MEC-2/LacZ fusion protein is mildly disrupted in a *mec-7* null or *mec-12* strong loss-of-function background, implying that the 15-pf microtubules are not essential for the localization of

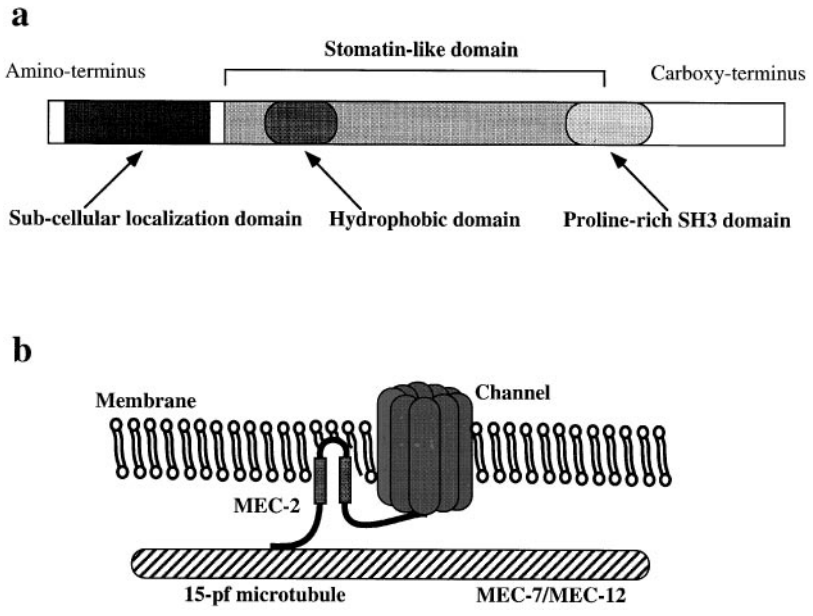

Figure 6 The MEC-2 protein and proposed interactions in the touch receptor neurons. (*a*) Structural features of the candidate linker protein MEC-2. The central region of the protein is similar to stomatin and contains a hydrophobic region capable of inserting into the membrane. A proline-rich SH3-binding domain partially overlaps with the stomatin-like region at the carboxy-terminus and may mediate interactions with channel subunits. Sequences in the N-terminus are needed for MEC-2 localization to neuronal processes. (*b*) Monotopic arrangement of MEC-2 with respect to the plasma membrane and predicted interacting proteins. Both N- and C-termini are intracellular with the amino-terminus hypothesized to interact with microtubules and the carboxy-terminus hypothesized to interact with the channel. This arrangement is reminiscent of a lever structure with the membrane attachment serving as a fulcrum to relay a mechanically induced microtubule deflection to the channel, pulling it open.

MEC-2 to the neuronal process. However, two specific *mec-12* missense alleles interfere dramatically with localization of MEC-2 fusion proteins, restricting the fusion proteins to the cell body (41). One of the *mec-12* alleles encodes a single amino acid substitution close to a MAP-binding region in other α-tubulins; the other affects a residue in the C-terminal domain. Taken together, analyses of the MEC-2/lacZ fusion protein suggest that residues in the MEC-2 N-terminus and the MEC-12 α-tubulin C-terminus could interact (Figure 6*b*).

There is also some evidence that the MEC-2 protein functionally interacts with the touch receptor channel. Certain *mec-2* alleles partially suppress *mec-10*(A673V)-induced death (40). In addition, some recessive *mec-2* alleles

act as dominant enhancers of a weak *mec-4*(*ts*) allele (30, 41). In other words, when a temperature-sensitive *mec-4* mutant is reared at the maximum temperature at which the touch receptors still function, adding a single mutant copy of the *mec-2* gene to the strain background can push the touch receptor neuron over the threshold into a nonfunctional state. Although such genetic studies do not in any way prove a direct interaction, they are consistent with the simple hypothesis that MEC-2 may tether the 15-pf microtubules to the degenerin channel (Figure 6*b*; see below; 41). Clearly, it is imperative that the implied interactions be tested biochemically.

Proteins that Affect or Act in the Extracellular Matrix: MEC-1, MEC-5, and MEC-9

MEC-1 In *mec-1* mutants, touch cells generally lack the mantle and associated periodic specializations of the overlying cuticle; the ALM processes are somewhat displaced and run along body wall musculature rather than within the hypodermis (14). However, where portions of the touch processes are embedded within the hypodermis in *mec-1* mutants, the mantle is present. Whether the mantle acts to position the touch cell processes or, alternatively, whether incorrect positioning of the process leads to the failure to produce the mantle remains to be determined. Cloning of *mec-1* and analysis of its expression pattern should aid in distinguishing between these possibilities.

MEC-5 *mec-5* mutations disrupt the extracellular matrix in a subtle manner; the mantle in a wild-type animal can be stained with peanut lectin, whereas the mantle in *mec-5* mutants cannot (14; E Hedgecock & M Chalfie, unpublished data). *mec-5* encodes a novel collagen type that is secreted by hypodermal cells (23). The central portion of the MEC-5 protein is made up of Pro-rich Gly-X-Y repeats. *mec-5* mutations (many of which are temperature sensitive) cluster toward the carboxy-terminus of the protein and affect these repeats. What role the unique sequences in the amino- and carboxy-termini contribute to MEC-5 function is not clear because no *mec-5* mutations map to these regions. Genetic interactions suggest that *mec-5* influences MEC-4/MEC-10 channel function (e.g. *mec-4* and *mec-10* mutations can enhance the *mec-5*(*ts*) mutant phenotype; 30). Thus a specialized collagen could interact with the touch receptor channel, perhaps acting to provide gating tension. The potential importance of collagen:degenerin interactions is underscored by studies of another degenerin family member, *unc-105*, that is expressed in muscle (50). Semidominant gain-of-function mutations in *unc-105* cause severe muscle hypercontraction (58). Specific alleles of *let-2/sup-20*, which encodes a type-IV basement membrane collagen, suppress the *unc-105*(*sd*) phenotype (50, 58). Thus although direct

interactions of collagens and degenerin channels remain to be proven, such associations may emerge as a common theme in the function of this channel class.

MEC-9 *mec-9* mutations do not alter mantle ultrastructure in a detectable manner (14) despite the fact that *mec-9* encodes a protein that appears to be secreted from the touch receptor neurons (23). The *mec-9* gene actually encodes two transcripts, the larger of which encodes a 834 amino acid protein (MEC-9L) expressed only by the touch receptors. The predicted MEC-9L protein contains several domains related to the Kunitz-type serine protease inhibitor domain, the Ca^{2+}-binding EGF repeat, the non-Ca^{2+}-binding EGF repeat, and a glutamic acid–rich domain. Single amino acid substitutions that disrupt MEC-9 function affect the two Ca^{2+}-binding EGF repeats, the sixth EGF repeat, and the third Kunitz-type domain, thus implicating these regions as important in MEC-9 function. How MEC-9 is needed for touch cell activity is not clear, but it is interesting that MEC-9 appears specialized for protein interactions and that agrin, a protein that acts to localize acetylcholine receptors, has a domain structure similarly specialized (agrin features multiple EGF and Kazal-type serine protease inhibitor repeats; 65). *mec-9* mutations are dominant enhancers of a *mec-5*(*ts*) allele, suggesting that these proteins might interact in the unique mantle matrix outside the touch receptor neuron (23, 30).

Molecular Biology of Other mec Genes

Two other genes needed for body touch sensitivity, *mec-14* and *mec-8*, have been cloned. *mec-14* mutations do not perturb touch receptor ultrastructure but can partially suppress *mec-10*(A673V)-induced death, suggesting that MEC-14 could influence channel function (40). *mec-14* encodes a member of the aldo-keto reductase superfamily (M Chalfie, personal communication). Interestingly, the β-subunit of the Shaker type K^+ channels, which modifies channel properties (62), is also a member of this superfamily (54).

mec-8 is an example of a gene that affects touch cell function because it is needed for expression of at least one other *mec* gene. *mec-8* alleles disrupt touch sensitivity (14), but they also affect other sensory structures, attachment of body wall muscle to the hypodermis and cuticle, and embryonic and larval development (51). Molecular analysis has shown that the *mec-8* protein, which includes two RNA-binding motifs, is required for splicing of several messages, including that of the *mec-2* protein (41, 52).

The molecular identities of *mec-1, mec-6, mec-15, mec-17,* and *mec-18* remain to be determined to elaborate a more detailed understanding of touch transduction in *C. elegans*.

A Model for Mechanotransduction in the Touch Receptor Neurons

The molecular features of cloned touch cell structural genes and genetic data suggesting interactions between them constitute the basis of a model of the touch receptor mechanotransducing complex (Figure 7*a*; see 23, 30, 41, 46 for discussion). The central component of this model is the candidate mechanosensitive ion channel that includes multiple MEC-4 and MEC-10 subunits. These subunits assemble to form a channel pore that is lined by hydrophilic residues in MSDII. Subunits adopt a topology in which the Cys-rich and NTD domains extend into the specialized extracellular matrix outside the touch cell and the amino- and carboxy-termini project into the cytoplasm. Although it is not known what type of protein *mec-6* encodes, available data are consistent with the hypothesis that it could encode another channel subunit.

Regulated gating is expected to depend on mechanical forces exerted on the channel. Tension is hypothesized to be delivered by tethering the extracellular channel domains to the specialized extracellular matrix and anchoring intracellular domains to the microtubule cytoskeleton. Outside the cell, channel subunits may contact MEC-5 and/or MEC-9 (MEC-5 and MEC-9 may associate with one another; 23) in the touch receptor mantle. Inside the cell, channel subunits may interact with MEC-2, which is proposed to link the channel to the distal ends of the 15-pf microtubules through an association with the MEC-12 α-tubulin. A touch stimulus could deform the microtubule network or could perturb the mantle connections to deliver the gating stimulus. In either scenario, Na^+ influx would activate the touch receptor to signal the appropriate locomotory response (see below).

Interestingly, the model proposed for mechanotransduction in the touch receptor neurons shares features of the proposed gating mechanism of mechanosensory channels that respond to auditory stimuli in the hair cells of the vertebrate inner ear (Figure 7*b*; 42, 59). Stereocilia situated on the hair cell apical surface are connected at their distal ends to neighboring stereocilia by filaments called tip links. Directional deflection of the stereocilia relative to each other introduces tension on the tip links, which is proposed to open the mechanosensitive hair cell channels directly.

Neuronal Circuitry for Body Touch Avoidance

Upon conversion of a mechanical stimulus into an electrochemical response, the touch receptor neurons activate a simple reflex circuit. Although the molecules involved in signaling to interneurons are not determined, the likely neuronal circuit has been identified (Figure 8; 15). The touch cells provide direct input to the interneurons that control locomotion. Two interneurons, AVD and PVC,

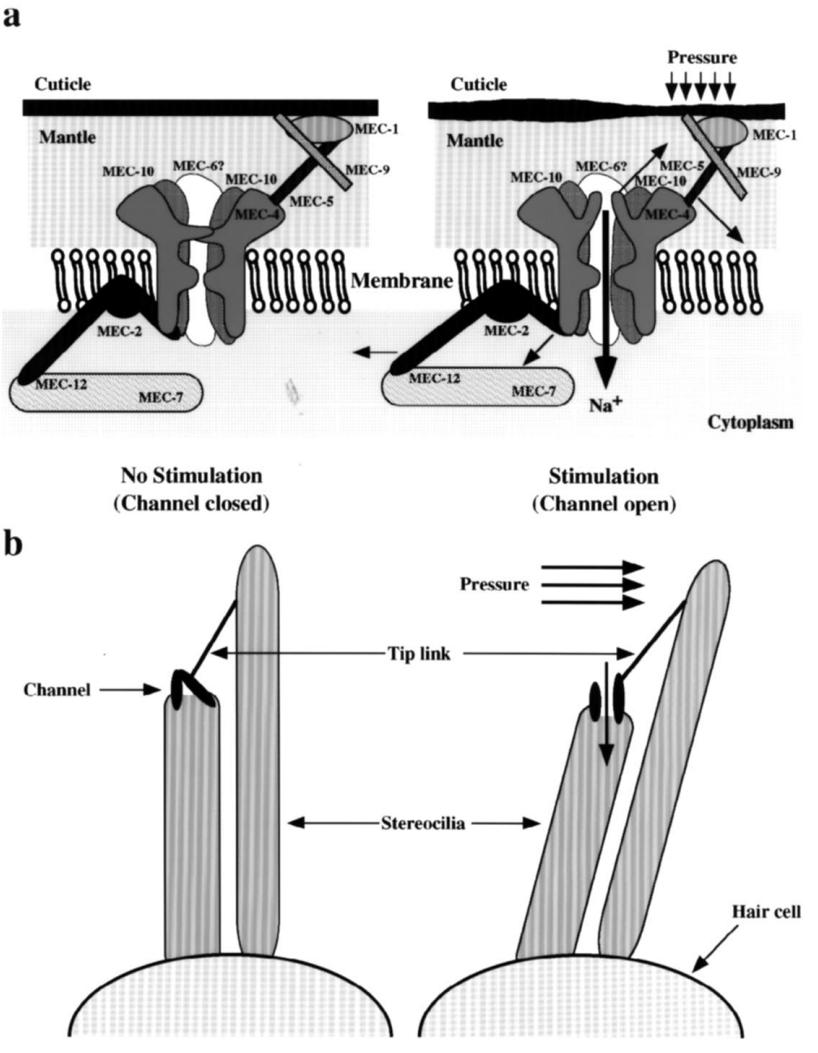

Figure 7 Models of mechanotransduction. (*a*) A touch-transducing complex in *C. elegans* touch receptor neurons (see text). In the absence of mechanical stimulation, the channel is closed and therefore the sensory neuron is idle. Application of a mechanical force to the body of the animal results in distortion of a network of interacting molecules that opens the channel. Na^+ influx depolarizes the neuron initiating the perceptory integration of the stimulus. (*b*) Model for mechanical gating of the channels in vertebrate hair cells. Mechanosensory channels situated at the stereocilla tips may be pulled open by the tip link when stereocilia are deflected. Note that although the channel is shown at only one end of the tip link, evidence suggests that channels can be situated at both ends (20a, 50a).

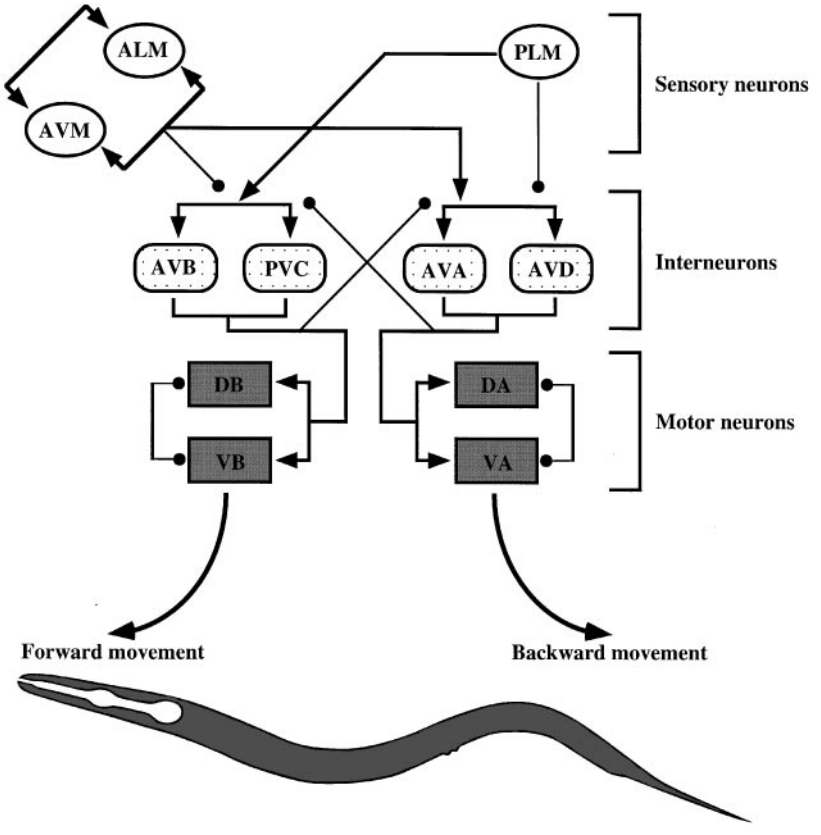

Figure 8 Neuronal circuitry for locomotion in response to gentle body touch. Interconnections between sensory neurons (*white ovals*), interneurons (*stippled*), and motor neurons (*gray rectangles*) are shown. Stimulatory connections are represented by arrowheads and inhibitory connections are represented by dark circles.

have been experimentally demonstrated to participate in touch circuits. The AVD interneuron promotes backward locomotion and is required for anterior touch sensitivy in larvae; when AVD is removed by laser microsurgery, larvae cannot back up (15). Likewise, the PVC interneuron promotes forward locomotion and is required for posterior touch sensitivity; animals that lack this neuron cannot move forward. (Two other interneurons that the touch cells contact, AVA and AVB, have a bit more complex involvement in locomotion). The anterior touch cells ALM and AVM form gap junctions with the backward interneuron AVD and provide synaptic input to the forward interneurons AVB

and PVC. Conversely, PLMR forms gap junctions with the forward interneuron PVC and provides synaptic input to the backward interneurons AVA and AVD. In other words, the touch cells form gap junctions with agonist interneurons and apparent chemical synapses with the antagonist interneurons. This reciprocal pattern of connectivities enables locomotion in the appropriate direction to be stimulated at the same time that locomotion in the inappropriate direction is inhibited.

The connectivity of the touch receptors suggests roles in the regulation of multiple behaviors (88). Among the neurons the touch cells synapse onto are several putative sensory neurons, interneurons that connect the somatic nervous system with the pharyngeal nervous system, and motor neurons that control egg laying. Because body touch has been shown to regulate pharyngeal pumping (15), egg laying (B Sawin, personal communication), and defecation (81), at least some of the connections are likely to be functional. As noted above, the PVM touch receptor does not appear to play a role in the touch-mediated locomotory response (15). Possibly, PVM mediates mechanosensory control of some of these other behaviors.

C. ELEGANS DEGENERINS AND STRETCH-SENSITIVE CHANNELS

The *C. elegans* degenerin gene family includes at least 15 members [the characterized *mec-4* (21, 46), *mec-10* (40), *deg-1* (18, 29), *unc-105* (50), *unc-8* and *del-1* (79) genes, as well as 9 additional members predicted by the *C. elegans* genome sequencing project]. The expression patterns of all these family members have been assayed (31, 40, 56, 79; N Tavernarakis & M Driscoll, unpublished data). In general, degenerin subunits are expressed in different combinations in different cell types, including neurons, muscle, and hypodermis (Table 2). Whether these degenerins actually function in mechanically gated ion channels remains to be determined. However, it has been suggested that at least three degenerins, *unc-105, unc-8,* and *del-1,* influence stretch-sensitive behaviors.

unc-105

Semidominant *unc-105* alleles induce hypercontraction of body wall muscles (57) and encode amino acid substitutions in the extracellular domain (50). These substitutions are presumed to cause muscle hypercontraction because muscle cells are depolarized by inappropriate ion influx. Specific alleles of *sup-20/let-2*, an essential type-IV basement membrane collagen (71), suppress *unc-105*(*sd*) hypercontraction (50, 58). It has been proposed that *unc-105* may function in a stretch-responsive channel in body wall muscle that is gated via

Table 2 Characterized *C. elegans* degenerins[a]

Protein	Cellular expression pattern	Postulated function	Reference
MEC-4	Touch receptors	Touch-sensitive channel	12, 21, 38, 39, 46
MEC-10	Touch receptors Other sensory neurons	Touch-sensitive channel	12, 40
DEG-1	Interneurons Sensory neurons Muscle Hypodermis	?	18, 29
UNC-105	Muscle	Stretch-sensitive channel	50
UNC-8	Motor neurons Interneurons Sensory neurons	Stretch-sensitive channel	70, 79
DEL-1[b]	Motor neurons Sensory neurons	Stretch-sensitive channel	79

[a]Except for DEL-1, all degenerins have been identified genetically.
[b]DEL for degenerin-like. This gene was identified by homology searches for new degenerins in the *C. elegans* genomic sequences database compiled by the *C. elegans* Genome Sequencing Consortium.

attachment to collagen in the extracellular matrix (50). Interestingly, *unc-105* null mutations have no apparent phenotype (58), suggesting that another degenerin coexpressed with *unc-105* could redundantly supply the same function in muscle. Another possibility is that, as is the case for *unc-8* null mutations (see below), the *unc-105* null phenotype may be a subtle behavioral defect that is difficult to detect.

unc-8

Unusual semidominant gain-of-function *unc-8* alleles induce transient neuronal swelling (70) and severe uncoordination (6, 57). *unc-8* encodes a degenerin expressed in several motor neuron classes and in some interneurons and nose touch sensory neurons (80). Interestingly, semidominant *unc-8* alleles alter an amino acid in the region hypothesized to be an extracellular channel closing domain (80) defined in studies of *deg-1* and *mec-4* (29, 80). The genetics of *unc-8* are also similar to those of *mec-4* and *mec-10*: (*a*) Specific *unc-8* alleles can suppress or enhance *unc-8*(*sd*) mutations in *trans*, suggesting that UNC-8::UNC-8 interactions occur, and (*b*) *mec-6* mutations suppress *unc-8*(*sd*)-induced phenotypes (70), suggesting that MEC-6 is needed for UNC-8 channel function. Another degenerin family member, *del-1* (for degenerin-like) is coexpressed in a subset of neurons that express *unc-8* (the VA and VB motor neurons and the FLP nose touch neurons) and may assemble into a channel complex with UNC-8 in these cells (80).

What function does the UNC-8 degenerin channel serve in motor neurons? *unc-8* null mutants have a subtle locomotion defect (80). Wild-type *C. elegans*

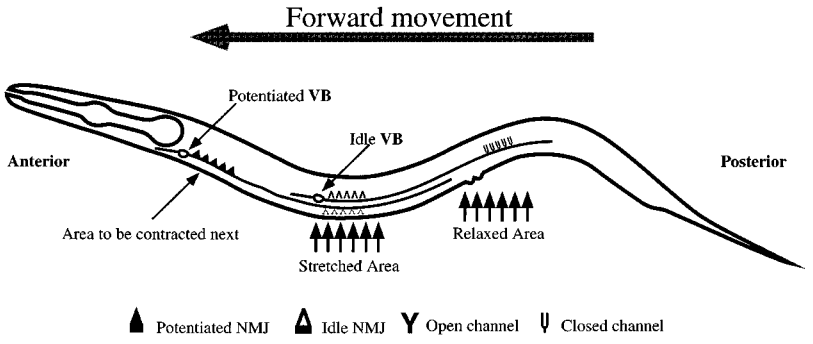

Figure 9 Model for modulation of locomotion by stretch-responsive channels in motor neurons. Two VB motor neurons in the ventral nerve cord are shown with stretch-sensitive channels situated in their undifferentiated processes. The anterior VB is potentiated by opening of ion channels during the process of stretch. This motor neuron will signal to the anterior muscles to then become fully contracted. At the same time another motor neuron in the middle of the body remains idle because its process does not receive a stretch stimulus. Sequential activation of motor neurons, which are distributed along the ventral nerve cord and signal non-overlapping groups of muscles, amplifies and propagates the sinusoidal body wave (neuromuscular junction, NMJ).

moves through an *E. coli* lawn with a characteristic sinusoidal pattern (this occurs by localized alternating contraction and relaxation of body wall muscles; see 22). *unc-8* null mutants inscribe a path in an *E. coli* lawn that is markedly reduced in both wavelength and amplitude as compared with wild-type worms. This phenotype suggests that the UNC-8 degenerin channel functions to modulate the locomotory trajectory of the animal.

How does the UNC-8 motor neuron channel influence locomotion? One highly interesting morphological feature of motor neurons (in particular, the VA and VB motor neurons that coexpress *unc-8* and *del-1*) is that their processes include extended regions that do not participate in neuromuscular junctions or neuronal synapses (Figure 9). These undifferentiated process regions have been hypothesized to be stretch sensitive (originally by RL Russell & L Byerly and discussed in Reference 88). Given the morphological features of certain motor neurons and the homology of UNC-8 and DEL-1 to candidate mechanically gated channels, it has been proposed that these subunits coassemble into a stretch-sensitive channel that might be localized to the undifferentiated regions of the motor neuron process. When activated by the localized body stretch that occurs during locomotion, this motor neuron channel can potentiate signaling at the neuromuscular junction, which is situated at a distance from the site of stretch stimulus. The stretch signal enhances motor neuron excitation of muscle, increasing the strength and duration of the pending muscle contraction

and directing a full-size body turn. In the absence of the stretch activation, the body wave and locomotion still occur, but with significantly reduced amplitude because the potentiating stretch signal is not transmitted. Because *unc-8* is expressed in several motor neuron classes and there are no known mutations in *del-1*, it is not clear which (if any) are the critical motor neurons that mediate this stretch-sensitive behavior.

One important corollary of the *unc-8* mutant studies is that the UNC-8 channel does not appear to be essential for motor neuron function; if this were the case, animals lacking the *unc-8* gene would be severely paralyzed. This observation strengthens the argument that degenerin channels function directly in mechanotransduction rather than merely serving to maintain the osmotic environment so that other channels can function. Still, it cannot be ruled out that another yet-to-be-discovered degenerin family member that performs the same cellular function as *unc-8* is coexpressed with *unc-8* in motor neurons. As is true for MEC-4 and MEC-10, the model of UNC-8 and DEL-1 function based on mutant phenotypes, cell morphologies, and molecular properties of degenerins remains to be tested by determining subcellular channel localization, subunit associations and, most importantly, channel gating properties.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Limitations of Genetic Approaches

Genetic analyses in *C. elegans* have been highly successful in identifying genes needed for mechanosensitive behaviors. Still, limitations of the genetic approach to dissection of mechanotransduction mechanisms should be mentioned. Genes that encode products needed for the activities of mechanotransducing complexes in multiple cell types or that perform multiple cellular functions might have evaded genetic detection because mutations in such genes would be expected to be severely uncoordinated or even lethal. [Indeed, many mutations that affect mechanosensation in *Drosophila* render animals severely uncoordinated and nearly inviable (45)]. Moreover, genes whose functions are redundantly encoded cannot be readily identified in genetic screens. Thus additional cellular proteins essential for the mechanotransducing complex in the well-studied *C. elegans* body touch receptor neurons may remain to be identified. Alternative experimental approaches for isolating such genes, for example library screens for interacting proteins, should fill in gaps that genetic studies leave.

Verification of the Model for the Touch-Transducing Complex

The detailed model for touch transduction in the *C. elegans* body touch receptor neurons accommodates genetic data and molecular properties of cloned *mec*

genes. However, apart from unpublished findings that MEC-4 and MEC-10 coimmunoprecipitate in vitro, no direct interactions between proteins proposed to be present in the mechanotransducing complex have been demonstrated. Given that genes for candidate interacting genes are in hand, it should be possible to test hypothesized associations biochemically. More challenging and most critical, the hypothesis that a degenerin-containing channel is mechanically gated must be addressed. This may be particularly difficult because at present it is not straightforward to record directly from tiny *C. elegans* neurons (2). Expression of the MEC-4/MEC-10 or (UNC-8/DEL-1) channel in heterologous systems such as *Xenopus* oocytes will be complicated by the presence of the many endogenous mechanically gated ion channels (47) and by the likely possibility that not only the multimeric channel, but essential interacting proteins, will have to be assembled to gate the channel.

A Global Role of Degenerins in Mechanotransduction?

A key question that remains regards how broadly DEG/ENaC superfamily members will prove to be involved in mechanotransduction. It should be noted that the *E. coli* mechanosensitive ion channel MscL is not homologous at the level of primary sequence to the *C. elegans* degenerins. Nonetheless, both subunit types share general structural features including similar transmembrane topologies (5, 29, 46) and participation in a multimeric complex. It has also been proposed that MscL and DEG/ENaC superfamily members could share a structurally related channel pore structure (43). Themes in the structures of mechanosensitive channels will be better elaborated as more are characterized at the molecular level.

Will any mammalian DEG/ENaC family members be mechanically gated? At present, although *C. elegans* degenerins share many sequence motifs and are approximately 20–30% identical to their cloned vertebrate counterparts, clear differences distinguish *C. elegans* and characterized mammalian family members. CRDI and the 22-amino acid region in the ecto-domain implicated in channel closing (29) are unique to the *C. elegans* proteins. In addition, the C-termini of *C. elegans* degenerins lack the Pro-rich regions important to the localization and turnover of the vertebrate proteins (63, 69, 73). Such distinctions may reflect true differences in functional specialization. Gating of at least one distant member of the DEG/ENaC superfamily, *Helix* FaNaC, is regulated by FMRF-amide (49), and thus it is clear that DEG/ENaC family members will not be exclusively mechanically gated.

On the other hand, analyses of the mammalian αENaC channel reconstituted in lipid bilayers suggest that its gating can be influenced by hydrostatic tension (3). This result should be considered tentative; see discussion in Reference 34a. One recently identified mammalian family member, MDEG, is expressed exclusively in the nervous system and behaves analogously to *C. elegans*

degenerins in that it can be similarly altered to create a toxic form that induces cell degeneration (83). Given the strong implication of multiple *C. elegans* degenerin family members in stretch-sensitive behaviors and the large size of the *C. elegans* gene family, it is likely that mammalian members more closely related to *C. elegans* degenerins will be identified in the future. Such genes will be plausible candidate genes for the elusive mammalian stretch-sensitive channels.

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