Molecules That Mediate Touch Transduction in the Nematode *Caenorhabditis elegans*

Monica Driscoll and Nektarios Tavernarakis
Department of Molecular Biology and Biochemistry, Rutgers University, Center for Advanced Biotechnology and Medicine, Piscataway, NJ 08855

ABSTRACT

Despite the widespread importance of mechanotransduction in biology, remarkably little is known about the nature of the molecules that mediate mechanical signaling. Mechanosensation in the nematode *Caenorhabditis elegans* is mediated by six mechanosensory neurons called touch receptor cells. Genetic analysis has resulted in the identification of over 400 mutations that disrupt the function of the touch receptors. Molecular characterization of the genes revealed has identified subunits of a candidate mechanosensory ion channel, tubulins expressed specifically in the touch receptors, and extracellular matrix proteins needed for mechanotransduction. *mec-4* and *mec-10* encode members of a *C. elegans* gene family related to the vertebrate epithelial Na^+ channel that are hypothesized to encode subunits of a mechanosensory channel. *mec-6* may encode another channel subunit. Inside the cell, α-tubulin MEC-12, ß-tubulin MEC-7 and a candidate linker protein MEC-2 may interact with the mechanotransducing channel to deliver gating tension. In the extracelluar matrix, collagen MEC-5 and MEC-9 and MEC-1 may interact with extracellular channel domains. A molecular model for mechanotransduction is discussed.

INTRODUCTION

Mechanotransduction, the conversion of a mechanical stimulus such as a minute stretch force into a cellular response, plays a central role in a broad range of biological processes (reviewed in French, 1992; Sackin, 1995). Cell volume regulation, fertilization, gravitaxis, involuntary movement and the senses of touch, balance and hearing all rely on mechanical transduction. Despite the widespread importance of mechanical signaling in biology, remarkably little is known about the nature of the molecules that mediate mechanotransduction. Elegant electrophysiological studies in several systems have established that mechanically-gated ion channels are the mediators of the response. However, mechanically responsive channels tend to be widely dispersed within a given tissue and their exceedingly low concentration has rendered biochemical purification of channels difficult. Biochemical purification of an *E. coli* mechanosensitve channel, MscL, has been accomplished (Sukharev et al., 1994), but to date no mechanically-gated ion channel has been definitively been cloned from a eukaryote.

An alternative approach toward identifying the molecules that are involved in mechanotransduction is to identify them genetically. This approach has been particularly fruitful in the simple nematode, *Caenorhabditis elegans* (reviewed in Driscoll and Kaplan, 1996; Herman, 1996). Genetic dissection of touch transduction in this worm has led to the identification of several molecules that are likely to assemble into a mechanotransducing complex. Here we discuss studies that led to the identification of these models and discuss a molecular model of touch transduction. This model resembles the one proposed for mechanotransduction in the vertebrate ear.

FEATURES OF THE *C. ELEGANS* MODEL SYSTEM

C. elegans is a small (1mm) free-living nematode that completes a life cycle in 2.5 days at 25°C. The most common sexual form is the hermaphrodite (XX), although males (X0) can be propagated. The simple body plan, the transparent egg and cuticle, and the nearly invariant developmental plan of this nematode have facilitated exceptionally detailed developmental and anatomical characterization of the animal. The complete sequence of cell divisions and the normal pattern of programmed cell deaths that occur as the fertilized egg develops into the 959-celled adult have been elaborated (Sulston and Horvitz, 1977; Sulston et al., 1983). In addition, the pattern of synaptic connections made by each of the 302 neurons of the animal has been described, so that the full "wiring diagram" of the animal is known (White et al., 1986). Researchers can take advantage of the fact that every cell is an identified cell and can perform laser microsurgery in which individual cells are killed by a laser microbeam (Bargmann and Avery, 1995).

C. elegans is well established as a powerful genetic system (Brenner, 1974). When a hermaphrodite parent is subjected to a mutagenizing agent, the F1 progeny self-fertilize to produce F2 animals that are homozygous for recessive mutations. In this way thousands of mutations that disrupt development or various behaviors have been identified and, after crossing with males and standard gene mapping, positioned on a detailed genetic map.

C. elegans molecular biology enables a considerable amount of information on *in vivo* activities of genes of interest to be determined rapidly. A physical map of the *C. elegans* genome, consisting of overlapping cosmid and YAC clones covering most of the six chromosomes, has been constructed to facilitate cloning of genes that have been positioned on the genetic map (Waterston and Sulston, 1995; Hodgkin et al., 1995). Impressive progress toward sequence analysis of the total *C. elegans* genome has been accomplished (Wilson et al., 1994). (Projected

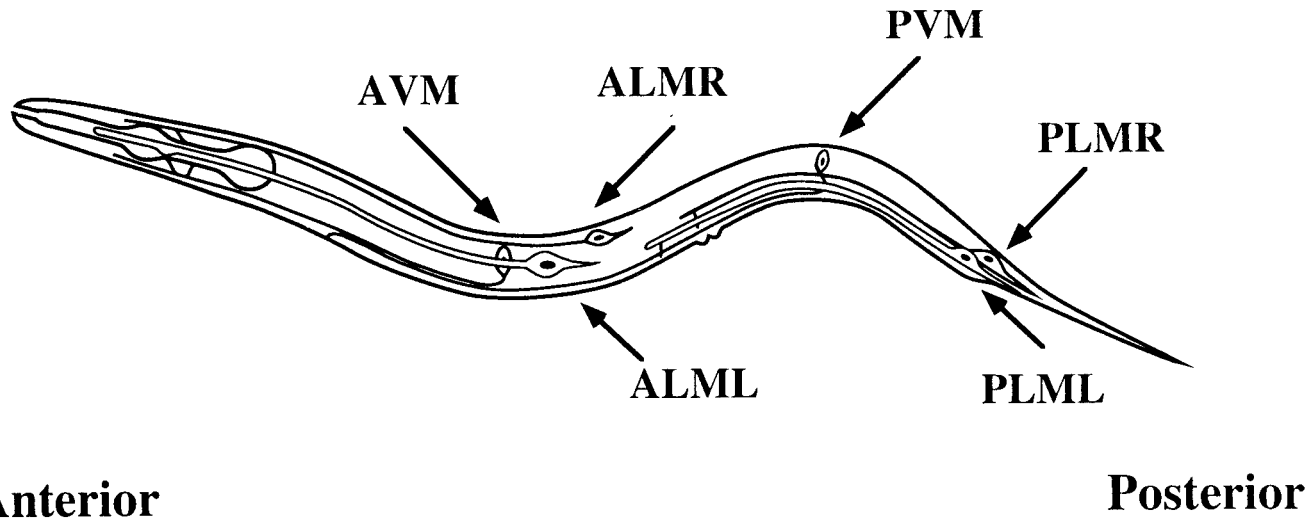

Figure 1. Arrangement of the Six Touch Receptor Neurons of C. Elegans.

completion date is 1998, data in regions believed to include 75% of all genes expected to be complete by mid 1996; R. Waterston, U. Washington, St. Louis, pers. comm.). In addition, ongoing efforts to obtain Expressed Sequence Tags for all *C. elegans* genes will soon provide a complete collection of the cDNAs of the nematode (Y. Kohara, National Institute of Genetics, Mishima, Japan). *C. elegans* is also particularly amenable to reverse genetics studies. DNA manipulated *in vitro* can be microinjected back into animals for functional assays (Fire, 1986; Mello et al., 1991). Vectors are available for identification of transformants, cell specific expression, and generation of fusions to marker genes such as *E. coli* ß-galactosidase (Fire et al., 1990) and the jellyfish green fluorescent protein (GFP) (Chalfie et al., 1994) so that individual cells can be visualized in stained or living animals.

CELLS THAT MEDIATE TOUCH-SENSITIVE BEHAVIOR IN *C. ELEGANS*

C. elegans normally moves along a petri plate coated with bacteria in a sinusoidal motion. If gently touched with an eyelash hair (typically attached to a toothpick) on the posterior, an animal will move forward; if touched on the anterior body, it will move backward. Both genetic and laser ablation studies have established that the mechanosensory component of this touch-sensitive behavior is mediated by six mechanosensory neurons, called the touch receptor neurons (Chalfie and Sulston, 1981; Chalfie et al., 1985; Chalfie and Au, 1989). The six touch receptors are depicted in Figure 1.

Touch receptor neurons are situated laterally along the body wall. Three touch receptor neurons are positioned in the posterior of the animal (these are named PLML, PLMR, and PVM). Three touch receptor neurons are situated anteriorly (ALML, ALMR and AVM). All send processes in an anterior direction. The positioning of touch cell processes is correlated with their sensory fields. For example, if all but the PLM cells are removed by laser ablation, the animal will remain touch sensitive in the posterior, but not in the anterior (Chalfie et al., 1985).

As noted above, the complete description of the pattern of all synapses made by *C. elegans* neurons has been described (White et al., 1986). The neuronal circuit for touch sensitivity requires the combined action of the mechanosensory touch receptor neurons, interneurons and the motorneurons that drive locomotion. The likely relay circuit for mechanotransduction has been worked out by examining the connectivity patterns and testing relationships using laser ablation (Chalfie et al., 1985). More detailed description of this circuit is beyond the scope of this article. It should also be noted that there is a separate touch sensory system in the *C. elegans* nose that we do not describe here (Kaplan and Horvitz, 1993).

ULTRASTRUCTURAL FEATURES OF THE TOUCH RECEPTOR NEURONS

Touch cell-specific microtubules.

The touch receptor cell processes are filled with a bundle of wide-diameter (15-protofilament) microtubules (Chalfie and Thomson, 1979; Chalfie and Thomson, 1982; see Figure 2). These 15-pf microtubules are unique to the touch receptor neurons (note that microtubules in most *C. elegans* cells contain 11 protofilaments; those in most organisms contain 13 protofilaments.) Individual microtubules do not span the complete length of the touch cell processes, which is about 400-500 μm. Instead, individual microtubules about 10-20 μm in length overlap within the microtubule bundles (Chalfie and Thomson, 1979). Interestingly, microtubule ends look structurally distinct in electron micrographs--the end proximal to the cell body appears darkened and is preferentially found on

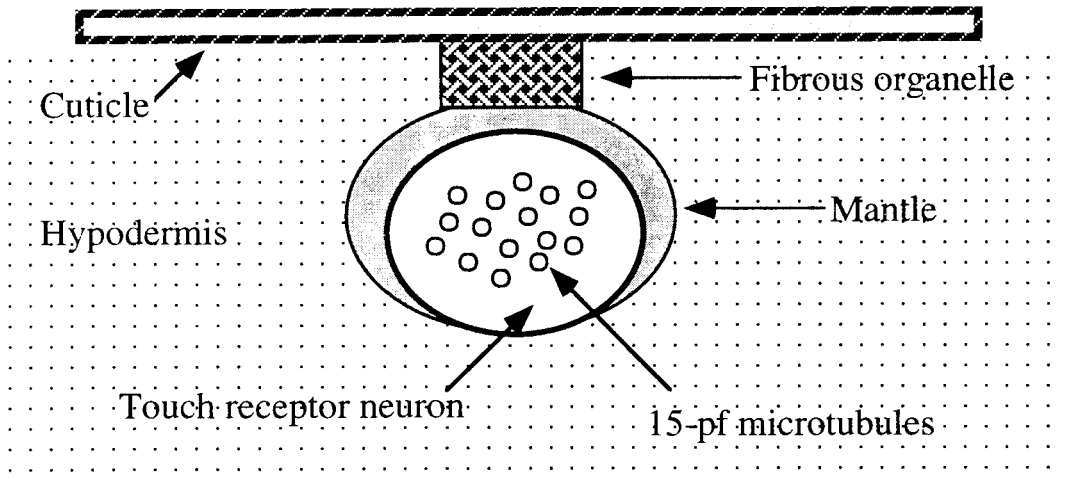

Figure 2. A Touch Receptor Neuron Process Filled with 15-pf Microtubules and Surrounded by the Mantle. *The cuticular specializations linking the the process to the cuticle are also shown (Fibrous organelle).*

Table I. Cloned mec Genes Encoding Proteins Involved in the Touch Response

Gene	Encoded Product
mec-2	Stomatin-like
mec-4	Degenerin
mec-5	Collagen
mec-7	ß-tubulin
mec-9	Protein with unknown function (Contains EGF and Kunitz repeats)
mec-10	Degenerin
mec-12	α-tubulin

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 interesting that the distal end is often juxtaposed to the plasma membrane and thus could potentially form a mechanical link between the microtubule network and mechanosensory receptors in the plasma membrane (Chalfie and Thomson, 1979; see discussion of mechanotransduction model below). The integrity of the 15-pf microtubules appears essential for touch receptor neuron function. If touch cell microtubules are disrupted by low concentrations of colchicine, touch sensitivity is lost (Chalfie and Thomson, 1982). Moreover, mutations that specifically disrupt the 15-pf microtubules support that these microtubules are more important for mechanotransduction than for process outgrowth.

The extracellular mantle.

Another distinguishing feature of the touch receptor neurons is that their processes are surrounded by a specialized extracellular matrix referred to as the mantle (Chalfie and Sulston, 1981; see Figure 2). Analysis of touch-insenstive mutants implicates the mantle in touch receptor function (see below). In addition to the mantle, darkly staining cuticular specializations are positioned periodicaly along the length of the touch receptor process, in close contact with the mantle. The cuticular specializations look similar to muscle attachment sites and thus may be sites at which the touch receptor process is fixed to the cuticle.

MUTATIONS DISRUPTING TOUCH SENSITIVITY

In order to identify genes that are essential for body touch sensitivity in *C. elegans*, M. Chalfie and colleagues mutagenized animals and screened their F2 progeny for insensitivity to gentle touch. In this screen, animals were tested to confirm that they could still move when strongly prodded. In this way, over 400 mutants that lack the ability to respond to gentle touch, but exhibit otherwise normal locomotion have been identified (Chalfie and Sulston, 1981; Chalfie and Au, 1985). These

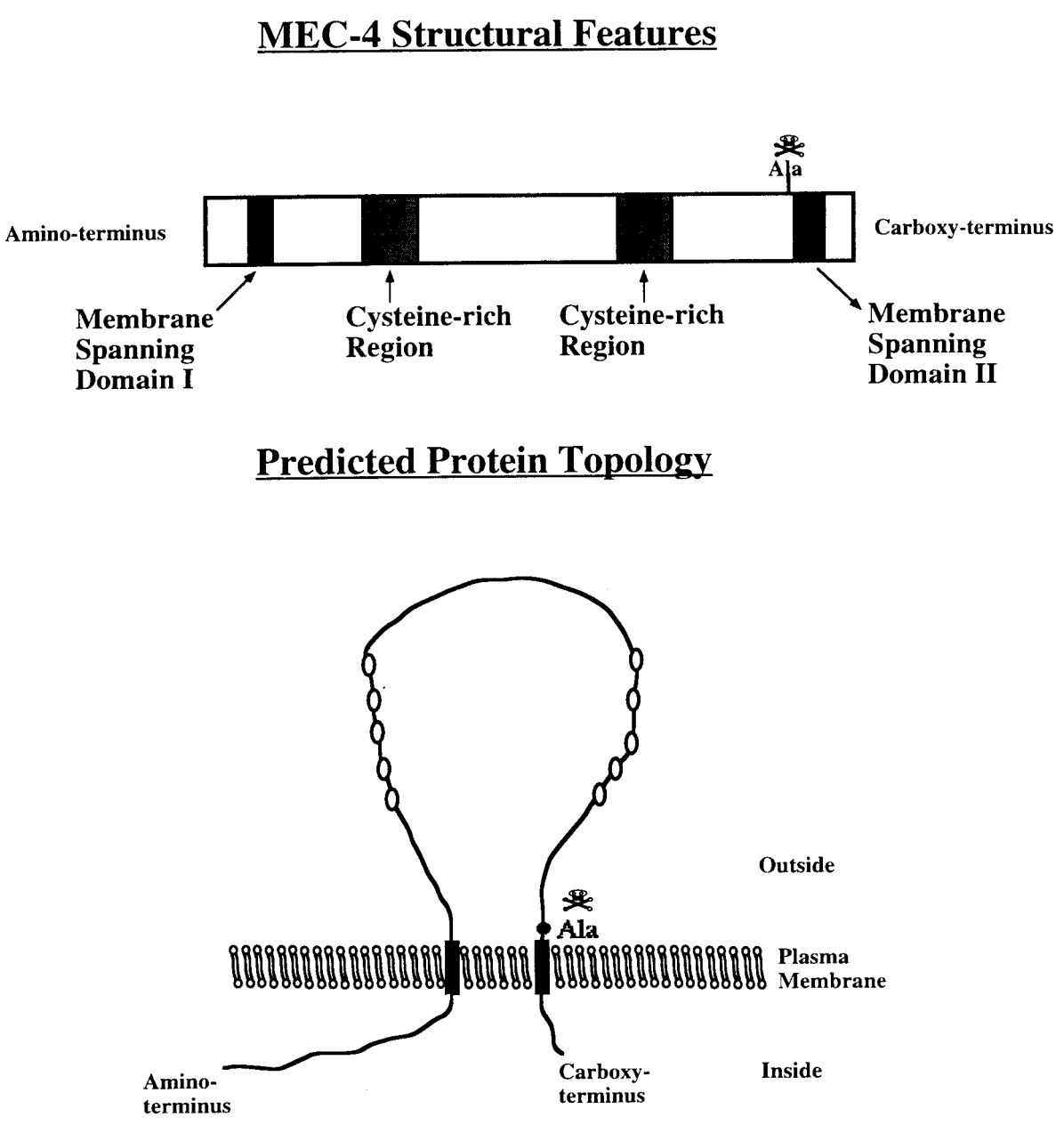

Figure 3. Structural Features of the MEC-4 Protein and the Predicted Topology with Respect to the Plasma Membrane. *The position of dominant mutations (ala713 substitutions) causing degeneration is indicated by skull and crossbones.*

mutations define 18 genes that play various roles in touch cell development and differentiation. Many of these genes have been named *mec* for mechanosensory defective. Genes that affect touch cell development include the homeobox transcription factors UNC-86 and MEC-3 and are discussed in more detail elsewhere (for example, Chalfie, 1993). Here we discuss those identified genes that have proven to encode molecules that might directly be involved in touch transduction (Table I).

mec-4 AND *mec-10*, GENES THAT ENCODE CANDIDATE MECHANOSENSITIVE ION CHANNEL SUBUNITS

Loss-of-function mutations in *mec-4* disrupt touch sensitivity but do not alter touch receptor ultrastructure (Chalfie and Sulston, 1981; Chalfie and Au, 1989). Unusual dominant gain-of-function *mec-4* alleles cause swelling and death of the touch receptor neurons. Molecular cloning of *mec-4* revealed that it encodes a protein of 768 amino acids that has two transmembrane domains (called MSDI and MSDII, Driscoll and Chalfie, 1991; Lai

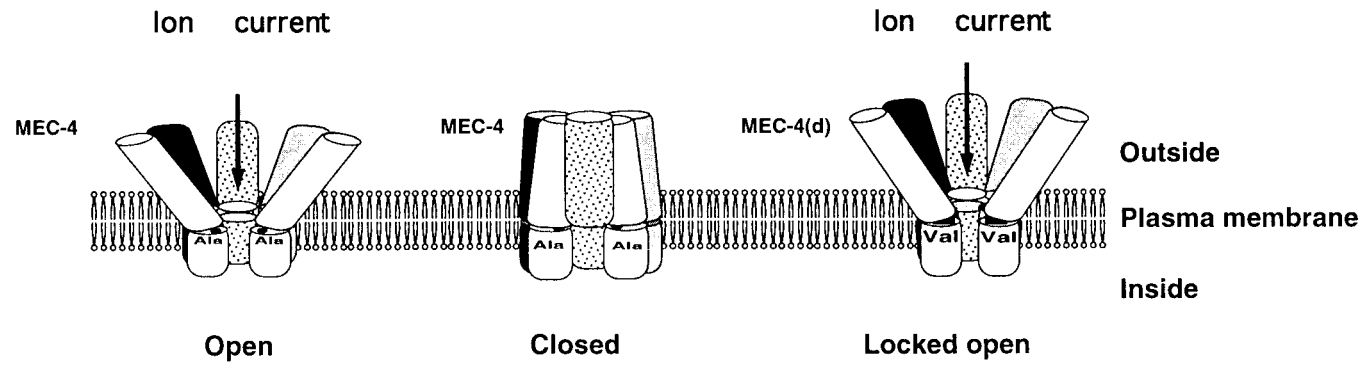

Figure 4. Schematic Representation of the Open and Closed States of the Channel. *When mutant MEC-4 carrying ala713 substitutions with larger side-chain amino-acids is assembled into a channel it may be locked into open conformation.*

et al., 1996; see Figure 3 for summary of protein features and transmembrane topology). Because dominant *mec-4* mutations could kill the touch receptor neurons, the MEC-4 protein was originally named a "degenerin". *mec-4* is expressed exclusively in the touch receptor neurons (Mitani et al., 1993). Interestingly, the *mec-4* protein is related to the mammalian ENaC genes, which encode epithelial amiloride-sensitive Na^+ channels that mediate ion transport across epithelia (Driscoll and Chalfie, 1991; Huang and Chalfie, 1993; Chalfie et al., 1994; Canessa *et al.*, 1993, 1994b; Lai et al., 1996). Thus, by analogy, *mec-4* is highly likely to encode an ion channel subunit. Although channel activity has not yet been directly demonstrated for either MEC-4 or MEC-10, certain nematode/rat chimeric proteins function in *C. elegans* and *Xenopus* oocytes, implying that the nematode and rat proteins are functionally similar (Hong and Driscoll, 1994, Waldmann et al., 1995).

Co-expression of three distinct but homologous subunits of the rat amiloride-sensitive Na^+ channel is required to *in vivo* reconstitute pharmacological properties of the channel in *Xenopus* oocytes (Canessa *et al.*, 1994b). This suggested that additional channel subunits might be expressed in the *C. elegans* touch receptor neurons. Indeed, the product of the *mec-10* gene, another gene whose activity is needed for touch receptor function is a degenerin family member that is expressed nearly exclusively in the touch receptor neurons (Huang and Chalfie, 1994). The *mec-6* gene may encode an additional subunit of the degenerin ion channels. *mec-6* mutations disrupt touch cell function and block *mec-4(d)*-induced degeneration (Chalfie and Wolinsky, 1990), genetic properties consistent with the hypothesis that *mec-6* encodes a third protein required for channel assembly or function. The *mec-6* gene has not been cloned yet.

A critical question to be addressed regards the function of the MEC-4/MEC-10 channel in the touch receptor neurons. Gene expression profiles, mutant phenotypes and sequence similarities support the working hypothesis that *mec-4* and *mec-10* could encode co-subunits of a mechanically-gated ion channel. *mec-4* and *mec-10* are needed only for the function of six mechanosensory neurons in *C. elegans* and they are expressed nearly exclusively in these sensory cells (Mitani et al., 1993; Huang and Chalfie, 1994). Their sequence similarity to the vertebrate amiloride-sensitive Na^+ channels is additionally intriguing since amiloride is a general channel blocker that affects a broad range of mechanosensitve ion channels (Hamill et al., 1992). For these reasons, it has been proposed that MEC-4 and MEC-10 function in a mechanically-gated ion channel--*i.e.*, one that opens in response to membrane stretch or to mechanical displacement of a channel domain. This hypothesis remains to be proven, however, and it should be noted that the MEC-4/MEC-10 channel could be simply required to maintain the appropriate ionic environment for another as yet unidentified mechanically gated ion channel.

MEC-4 STRUCTURE/ACTIVITY RELATIONSHIPS

Toxic alleles.

As mentioned above, dominant gain-of-function *mec-4* alleles induce swelling and death of the touch receptor neurons (Chalfie and Sulston, 1981; Chalfie and Au, 1989). DNA sequence analysis has revealed that *mec-4(d)* alleles encode protein variants with substitutions of large side-chain amino acids (Val or Thr) for a conserved Ala residue (AA713) situated adjacent to MSDII (Driscoll and Chalfie, 1991; Lai et al., 1996). Construction of site-directed mutations that introduce different amino acids at position 713 established that there is a correlation between the size of the amino acid side-chain and toxicity: large side-chain amino acids are toxic, whereas small side-chain amino acids (Ala, Ser, Cys) are not (Driscoll and Chalfie, 1991). This suggests 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, producing increased influx of ions that proves toxic (see Figure 4).

There is a second way in which *mec-4* can mutate

to induce neurodegeneration: *mec-4* alleles harboring a missense mutation (A404T) or a small deletion (Δ399-407) in the extracellular region induce degeneration in transgenic animals (García-Añoveros *et al.*, 1995), an observation consistent with the idea that these mutations disrupt a channel closing domain that is situated on the extracelluar side. Alternatively, death-inducing substitutions in the extracellular domain could change the MEC-4 three dimensional structure so as to favor the open channel conformation.

MSDII is a pore-lining domain.

All members of the degenerin/ENaC superfamily have two transmembrane domains. The more N-terminal of these (MSDI) is generally hydrophobic, whereas the more C-terminal of these (MSDII) is amphipathic. Amino acids on the polar face of amphipathic transmembrane MSDII are highly conserved and are essential for *mec-4* function (Hong and Driscoll, 1994). Consistent with the idea that these residues project into the channel lumen to influence ion conductance, amino acid substitutions in the candidate pore domain (predicted to disrupt ion influx) block or delay degeneration when the channel-opening A713V substitution is also present in MEC-4 (Hong and Driscoll, 1994). Electrophysiological characterization of rat and rat/nematode chimeras supports the hypothesis that MSDII constitutes a pore-lining domain and that highly conserved hydrophilic residues in MSDII face into the channel lumen to influence ion flow (Waldmann et al., 1995, Waldmann et al., 1996).

Other regions important for MEC-4 function.

Sequence analysis of recessive loss-of-function *mec-4* alleles has highlighted two other regions of MEC-4 that appear especially important in channel function (K. Hong and M. Driscoll, in preparation). Amino acid substitutions that disrupt MEC-4 function cluster within a conserved region that is situated on the intracellular side, close to MSDI. This region of the channel could interact with cytoskeletal proteins. A second hot-spot for channel-inactivating substitutions is situated near and within CRDII. This is a candidate region for interaction of the channel with the extracellular matrix.

EXTRACELLULAR COMPONENTS THAT MAY INTERACT WITH THE TOUCH RECEPTOR CHANNEL

Studies of mechanotransducing channels maintain that tension must be exerted on the channel for regulated opening and closing. Thus, it is expected that the touch receptor channel makes contacts inside and outside the touch receptor neuron that generate gating stress upon stimulation. Genetic analysis of touch receptor neurons has provided candidate molecules that could serve such a purpose.

MUTATIONS THAT DISRUPT THE SPECIALIZED MANTLE SURROUNDING THE TOUCH RECEPTOR NEURONS

The extracellular mantle that surrounds the touch receptor neurons shows ultrastructural alterations in two *mec* mutants, *mec-1* and *mec-5*. In *mec-1* mutants, touch cells 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 (Chalfie and Sulston, 1981). Interestingly, however, where portions of the touch processes are embedded within the hypodermis in *mec-1* mutants, mantle is present. It is not clear whether the mantle is essential for positioning the touch cell processes or, conversely, whether failure to correctly position the processes results in the lack of the mantle. *mec-1* is not yet cloned, but determining its molecular identity and expression pattern could help resolve this issue.

mec-5 mutations disrupt the extracellular matrix in a more subtle manner. The mantle in a wild-type animal can be stained with peanut lectin, whereas the mantle in *mec-5* mutants cannot. The exact significance of this mantle defect with regard to touch cell function is not immediately apparent. However, the cloning of *mec-5* revealed an interesting fact: *mec-5* encodes a special collagen that is expressed by the hypodermis (Du et al., 1996). Genetic experiments implicate *mec-5* in *mec-4* function (Gu et al., 1996). Thus, perhaps a specialized collagen could interact with the touch receptor channel, perhaps providing gating tension upon stimulation.

Another *mec* gene, *mec-9*, encodes a protein that appears to be secreted from the touch receptor neurons. Mutations in the *mec-9* gene do not alter the mantle in a way we can detect using electron microscopy (Chalfie and Sulston, 1981). The *mec-9* gene encodes two transcripts, the larger of which encodes a 834 amino acid protein that is expressed only in the touch receptors (Du et al., 1996). The predicted MEC-9 protein suggests that is is secreted and that it contains a glutamic acid-rich domain and several domains related to the Kunitz-type serine protease inhibitor domain, Ca^{+}-binding EGF repeats and non-Ca^{+}-binding EGF repeats. *mec-9* mutations are dominant enhancers of a *mec-5(ts)* allele, suggesting that these proteins might interact in the extracellular matrix outside the touch receptor neuron (Du et al., 1996).

Taken together, data support a working model where the products of the *mec-5*, *mec-9* and probably *mec-1* genes collaborate to create an extracellular environment that can interact with the channel to influence channel gating.

INTRACELLULAR COMPONENTS THAT MAY INTERACT WITH THE TOUCH RECEPTOR CHANNEL

As mentioned above, the touch receptor processes are characterized by bundles of 15-protofilament microtubules. Mutations in two genes, *mec-7* and *mec-12* disrupt the production of these microtubules (Chalfie and Sulston, 1981; Chalfie and Au, 1989). Interestingly, even in the absence of these microtubules, the touch receptor processes grow out, becoming filled with 11-pf microtubules (Chalfie and Thomson, 1982). Such touch receptors are non-functional, implicating the 15-pf microtubules in touch transduction.

mec-7 encodes a 440 amino acid ß-tubulin and *mec-12* encodes a 450 amino acid α-tubulin (Savage et al., 1989; M. Hamelin, M Chou, J. Culotti, pers. comm.). Both tubulin genes are expressed at high levels in touch neurons, although *mec-12* is expressed in additional neurons (Hamelin et al., 1992; Mitani et al., 1993; Savage et al., 1994; M. Hamelin, M. Chou and J. Culotti, pers. comm.). Taken together, studies of *mec-7* and *mec-12* mutants suggest that 15-protofilament microtubules are composed of MEC-7 and MEC-12 ß and α tubulins and are essential for mechanosensory transduction.

The 15-pf microtubules could contact the touch receptor channel directly to provide gating tension. However, there is some evidence suggesting a linker molecule, MEC-2, might serve to connect the microtubules to the touch receptor channel. The predicted 481-amino acid MEC-2 protein is expressed primiarily in the touch receptor neurons and includes some likely protein interaction domains (Huang et al., 1995). The carboxy-terminal domain of MEC-2 has a proline-rich region that is similar to SH3-binding domains. The central MEC-2 domain (AA 114-363) includes a membrane associated hydrophobic domain (AA114-141) and a cytoplasmic hydrophilic domain, that together exhibit 65% identity to the human red blood cell (RBC) protein stomatin. Stomatin is an integral membrane protein that associates with the cytoskeleton and affects ion balance via an unknown mechanism (Stewart *et al.*, 1993).

Genetic data suggest that MEC-2 interacts with the specialized microtubules. Normally, a MEC-2LacZ fusion protein is distributed along the touch receptor axon as well as in the cell body (Huang et al., 1995). The axonal distribution of a MEC-2LacZ fusion protein is mildly disrupted in a *mec-7* null or *mec-12* strong loss-of-function background. More dramatically, two specific *mec-12* missense alleles interfere with localization of MEC-2 fusion proteins, restricting the fusion proteins to the cell body (Huang et al., 1995). This can be interpreted to mean that MEC-2 and the MEC-12 α-tubulin could interact in the touch neurons.

Genetic evidence suggests that the MEC-2 protein functionally interacts with the touch receptor channel. For example, some recessive *mec-2* alleles act as dominant enhancers of a weak *mec-4(ts)* allele (Huang et al., 1995; Gu et al., 1996). Certain *mec-2* alleles partially suppress *mec-10(d)*-induced death (Huang and Chalfie, 1994). If the implied interactions are direct, a simple hypothesis is that MEC-2 may tether the 15 protofilament microtubules to the degenerin channel, an association that might enable mechanical deflection of microtubules to open the channel (Huang et al., 1995).

A MOLECULAR MODEL FOR TOUCH TRANSDUCTION IN *C. ELEGANS*

Taking the identities of molecules essential for touch transduction into account, a model for a mechanotransducing complex in the touch receptor neurons can be proposed (this model is discussed in more detail in Huang et al., 1995; Du et al., 1996, Gu et al., 1996)
The heteromeric ion channel, composed of MEC-4, MEC-10 and possibly MEC-6 subunits, plays a central role in the function of the mechanotransducing apparatus. Channel subunits assemble to form a transmembrane pore that is lined by hydrophilic residues in MSDII. Subunits are oriented such that their amino- and carboxy-termini project into the cytoplasm and their Cys-rich regions extend outside the cell. In order to experience gating tension, this channel must be tethered via extracellular channel domains to the specialized extracellular matrix and via intracellular domains to the microtubule cytoskeleton. On the extracellular side, channel subunits may interact with MEC-1, MEC-5 and/or MEC-9 in the touch receptor mantle. Inside the cell, channel subunits are likely to interact with the 15-protofilament microtubules, which may contact the channel at their distal ends via MEC-2, a linker protein that may interact both with the *mec-12* α-tubulin and with intracellular channel domains. To activate this mechanotransducing complex, a touch stimulus could deform the microtubule network, which could tug the channel open from the intracellular side. Alternatively, a touch stimulus could perturb the mantle connections and pull the channel open from the extracellular side. In either case, Na^+ influx would activate the touch receptor to signal to interneurons in the touch relay circuit, ultimately eliciting locomotion in the appropriate direction.

CONCLUDING REMARKS

It should be emphasized that the model for touch transduction in *C. elegans* constructed from genetic and molecular analysis is speculative and the predicted biochemical interactions await experimental verification. That the MEC-4/MEC-10 channel is functionally a mechanotransducing channel remains to be established. This may be a particularly difficult task since reconstitution of the tension-generating mechanism *in vitro* will be challenging. Perhaps pioneering work in recording from

tiny *C. elegans* neurons will provide the technological breakthrough required to perform electrophysiology directly on nematode neurons (Avery et al., 1995).

The model for touch transduction in *C. elegans* shares features with the model for mechanotransduction in the vertebrate inner ear (reviewed in Hudspeth, 1989; Pickles and Corey, 1992). The hair cell channels are situated towards the ends of specialized stereocilia near attachment sites of the tip-links (Assad et al., 1991; Hudspeth, 1982; Lumpkin and Hudspeth, 1995; Denk et al., 1996), which are thin filaments that connect adjacent stereocilia. When stereocilia are deflected, tension exerted via the tip-links appears to physically pull the channels into an open conformation. The molecular identity of the mechanotransducing ion channel that mediates hearing remains to be deduced. One exciting hypothesis is that various vertebrate mechanosensitive channels may bear structural and functional similarities to the *C. elegans* degenerin gene family. A mammalian superfamily member expressed in the nervous system has recently been identified (Price et al., 1996; Waldmann et al., 1996), but its involvement in mechanotransduction is as yet unexplored. Our hope is that by learning how the simple worm feels, we may gain significant insight into how we feel, hear and balance ourselves.

REFERENCES

Assad, J. A., G. M. Shephard and Corey D. P. 1991. Tip-link integrity and mechanical transduction in vertebrate hair cells. *Neuron* 7:985-994.

Avery, L., Raizen, D. and Lockery S. 1995. Electrophysiological methods. *Methods in Cell Biology* 48:251-269.

Bargmann, C. and Avery, L. 1995. Laser killing of cells in *Caenorhabditis elegans*. *Methods in Cell Biology* 48:225-250.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* 77:71-94.

Canessa, C. M., Horsiberger, J.D. and Rossier, B. C. 1993. Functional cloning of the epithelial sodium channel: relation with genes involved in neurodegeneration. *Nature* 361:467-470.

Canessa, C. M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.D. and Rossier, B. C. 1994a. Amilioride-sensitive epithelial Na^+ channel is made of three homologous subunits. *Nature* 367:463-467.

Canessa, C. M., Merillat, A. M. and Rossier, B. C. 1994b. Membrane topology of the epithelial sodium channel in intact cells. *American Journal of Physiology* 267:C1682-C1690.

Chalfie, M. 1993. Touch receptor development and function in *Caenorhabditis elegans*. *Journal of Neurobiology* 24:1433-1441.

Chalfie, M. and Au M. 1989. Genetic control of differentiation of the *Caenorhabditis elegans* touch receptor neurons. *Science* 243:1027-1033.

Chalfie, M. and Sulston, J. 1981. Developmental genetics of the mechanosensory neurons of *C. elegans*. *Developmental Biology* 82:358-370.

Chalfie, M. and Thomson J.N. 1979. Organization of neuronal microtubules in the nematode *C. elegans*. *Journal of Cellular Biology* 82:278-289.

Chalfie, M., and Thomson, J.N. 1982. Structural and functional diversity in the neuronal microtubules of *C. elegans*. *Journal of Cellular Biology* 93:15-23.

Chalfie, M. and Wolinsky, E. 1990. The identification and suppression of inherited neurodegeneration in *Caenorhabditis elegans*. *Nature* 345:410-416.

Chalfie, M., J.E. Sulston, J.G. White, E. Southgate, J.N. Thomson and Brenner S. 1985. The neural circuit for touch sensitivity in *C. elegans*. *Journal of Neuroscience* 5:956-964.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. and Prasher, D. C. 1994. Green fluorescent protein as a marker for gene expression. *Science* 263:802-805.

Denk, W., J. R. Holt, G. M. G. Shepherd and Corey D. P. 1996. Calcium imaging of single stereocilia in hair cells: localization of transduction channels at both ends of the tip links. *Neuron* 15:1311-1321.

Driscoll, M. and Chalfie, M. 1991. The mec-4 gene is a member of a family of *Caenorhabditis elegans* genes that can mutate to induce neuronal degeneration. *Nature* 349:588-593.

Driscoll, M. and Kaplan, J. 1996. Mechanotransduction, in The Nematode *C. elegans*, II. Cold Spring Harbor Press, Cold Spring Harbor, NY, in press.

Du, H., Gu, G., William, C. and Chalfie, M. 1996. Extracellular proteins needed for *C. elegans* mechanosensation. *Neuron* 16:183-194.

Fire, A. 1986. Integrative transformation of *Caenorhabditis elegans*. *EMBO Journal* 5:2673-2680.

Fire, A., Harrison, S. W., and Dixon, D. 1990. A modular set of lacZ fusion vectors for studying gene expression in *Caenorhabditis elegans*. *Gene* 93:189-198.

French, A. S. 1992. Mechanotransduction. *Annual Review of Physiology* 54:135-152.

García-Añoveros, J., Ma, C., and Chalfie, M. 1995. Regulation of *Caenorhabditis elegans* degenerin proteins by a putative extracellular domain. *Current Biology* 5:441-448.

Gu., G., Caldwell, G. A. and Chalfie, M. 1996. Genetic interactions affecting touch sensitivity in *Caenorhabdites elegans*. *Proceedings of the National Academy of Science USA*, in press.

Hamelin, M., I. M. Scott, J. C. Way and Culotti J. G. 1992. The mec-7 beta-tubulin gene of *Caenorhabditis elegans* is expressed primarily in the touch receptor neurons. *EMBO Journal* 11: 2885-2893.

Hamill, O. P., Lane, J. W. and McBride, D. W. 1992. Amiloride: a molecular probe for mechanosensitive channels. *Trends in Physiological Science* 13:373-376.

Herman, R. K. 1996. Touch sensation in *Caenorhabditis elegans*. *Bioessays*, in press.

Hodgkin, J., Plasterk, R. H. A. and Waterston, R. H. 1995. The nematode *Caenorhabditis elegans* its genome. *Science* 270:401-414.

Hong, K. and Driscoll, M. 1994. A transmembrane domain of the putative channel subunit of MEC-4 influences mechanotransduction and neurodegeneration in *C. elegans*. *Nature* 367:470-473.

Huang, M. and Chalfie, M. 1994. Gene interactions affecting mechanosensory transduction in *Caenorhabditis elegans*. *Nature* 367:467-470.

Huang, M., Gu, G., Ferguson, E. L., and Chalfie, M. 1995. A stomatin-like protein necessary for mechanosensation in *C. elegans*. *Nature* 378:292-295.

Hudspeth, A. J. 1982. Extracellular current flow and the site of transduction by vertebrate hair cells. *Journal of Neuroscience* 2:1-10.

Hudspeth, A. J. 1989. How the ear's works work. *Nature* 341:397-404.

Kaplan, J. M. and Horvitz H. R. 1993. A dual mechanosensory and chemosensory neuron in *C. elegans*. *Proceedings of the National Academy of Science USA* 90:2227-2231.

Lai, C.-C., Hong, K., Kinnell, M., Chalfie, M. and Driscoll, M. 1996. Sequence and transmembrane topopogy of MEC-4, an ion channel subunit required for mechanotransduction in *C. elegans*. *Journal of Cell Biology*, in press.

Lumpkin, E. A. and Hudspeth A. J. 1995. Detection of Ca^{2+} entry through mechanosensitive channels localizes the site of mechanoelectrical transduction in hair cells. *Proceedings of the National Academy of Science USA* 92:10297-10301.

Mello, C. C., Kramer, J. M., Stinchcomb, D. and Ambros, V. 1991. Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO Journal* 10: 3959-3970.

Mitani, S., Du, H., Hall, D., Driscoll, M. and Chalfie, M. 1993. Combinatorial control of touch receptor neuron expression in *Caenorhabditis elegans*. *Development* 119:773-783.

Pickles, J . O. and Corey, D. P. 1992. Mechanoelectrical transduction by hair cells. *Trends in Neuroscience* 15:254-259.

Price, M., Snyder, P. M. and Welsh, M. J. 1996. Cloning and expression of a novel human brain Na+ channel. *Journal of Biological Chemistry* 271:7879-7882.

Sackin, H. 1995. Mechanosensitive channels. *Annual Review of Physiology* 57:333-353.

Savage, C., M. Hamelin, J. G. Culotti, A. Coulson, D. G. Albertson and Chalfie M. 1989. mec-7 is a beta-tubulin gene required for the production of 15-protofilament microtubules in *Caenorhabditis elegans*. *Genes in Development* 3:870-881.

Savage, C., Y. Z. Xue, S. Mitani, D. Hall, R. Zakhary and Chalfie M. 1994. Mutations in the *Caenorhabditis elegans* ß-tubulin gene mec-7: effects on microtubule assembly and stability and on tubulin autoregulation. *Journal of Cell Science* 107:2165-2175.

Stewart, G.W., Argent A.C., and Dash B.C.J. 1993. Stomatin: a putative cation transport regulator in the red cell membrane. *Biochimica et Biophysica Acta* 1225:15-25.

Sukharev, S.I., Blount, P., Martinac, B., Blattner, F. R., and Kung, C. 1994. A large conductance mechanosensitive channel in E. coli encoded by mscL alone. *Nature* 368:265-268.

Sulston, J. E. and Horvitz, H. R. 1977. Post embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Developmental Biology* 56:110-156.

Sulston, J. E., Schierenberg, E., White, J. G. and Thomson, J. N. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Developmental Biology* 100:64-119.

Waldmann, R., Champigny, G. and Lazdunski, M. 1995. Functional degenerin-containing chimeras identify residues essential for amiloride-sensitive Na^+ channel function. *Journal of Biological Chemistry* 270:11735-11737.

Waldmann, R., Champigny, G., Voilley, N., Lauritzen, I. and Lazdunski, M. 1996. The mammalian degenerin MDEG, an amiloride-sensitive cation channel activated by mutations causing neurodegeneration in *Caenorhabditis elegans*. *Journal of Biological Chemistry* 271:10433-10436.

Waterston, R. and Sulston, J. 1995. The genome of *Caenorhabditis elegans*. *Proceedings of the National Academy of Science USA* 92:10836-10840.

White, J. G., Southgate, E., Thomson, J. N. and Brenner, S. 1986. The structure of the nervous system of the nematode *C. elegans*. *Philosophical Transactions of the Royal Society of London* 314B:1-340.

Wilson, R. et al. 1994. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature* 368:32-38.