# CHAPTER 3

# Mechanosensitive Ion Channels in *Caenorhabditis elegans*

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## I. OVERVIEW

*Caenorhabditis elegans* depends critically on mechanosensory perception to negotiate its natural habitat, the soil. The worm displays a rich repertoire of mechanosensitive behaviors, which can be easily examined in the laboratory. This, coupled with the availability of sophisticated genetic and molecular biology tools, renders *C. elegans* a particularly attractive model organism to study the transduction of mechanical stimuli to biological responses. Systematic genetic analysis has facilitated the dissection of the molecular mechanisms that underlie mechanosensation in the nematode. Studies of various worm mechanosensitive behaviors have converged to

*Current Topics in Membranes, Volume 59*
1063-5823/07 $35.00

DOI: 10.1016/S1063-5823(06)59003-9

identify highly specialized, plasma membrane ion channels that are required for the conversion of mechanical energy to cellular signals. Strikingly, similar mechanosensitive ion channels appear to function at the core of the mechanotransduction apparatus in higher organisms, including humans. Thus, the mechanisms responsible for the detection of mechanical stimuli are likely conserved across metazoans. The nematode offers a powerful platform for elucidating the fundamental principles that govern the function of metazoan mechanotransducers. In this chapter, we survey the current understanding of mechanotransduction in *C. elegans* and focus on the role of mechanosensitive ion channels in specific mechanosensory behavioral responses. Further, we aspire to highlight potential unifying themes, common to mechanosensory transduction in diverse species.

## II. INTRODUCTION

*C. elegans* is a small soil-dwelling nematode worm, with a simple body plan that is formed by just 959 somatic cells. *C. elegans* is primarily a hermaphroditic species but males, which can mate with hermaphodites are also found in natural populations at very low frequency. The transparent nature of both the egg and the cuticle of this nematode have facilitated exceptionally detailed developmental 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 are known (Sulston and Horvitz, 1977; Sulston *et al*., 1983). The anatomical characterization and understanding of neuronal connectivity in *C. elegans* are unparalleled in the metazoan world. Serial section electron microscopy has identified the pattern of synaptic connections made by each of the 302 neurons of the animal (including 5000 chemical synapses, 600 gap junctions, and 2000 neuromuscular junctions) so that the full ''wiring diagram'' of the animal is known (White *et al*., 1976, 1986). Although the overall number of neurons is small, 118 different neuronal classes, including many neuronal types present in mammals, can be distinguished. Other animal model systems contain many more neurons of each class (there are about 10,000 more neurons in *Drosophila* with approximately the same repertoire of neuronal types).

Thousands of mutations that disrupt development or various behaviors have been identified and positioned on a detailed genetic map (Brenner, 1974). Sequencing and high-quality annotation of the complete genome organized in six chromosomes (five autosomes and the sex chromosome X) have been accomplished (The *C. elegans* Sequencing Consortium, 1998); http://www.wormbase.org). Primary cell culture methodologies are available

for the analysis of specific groups of cells and neurons *ex vivo* (Christensen *et al.*, 2002). Electrophysiological study of nematode neurons and muscles has also become possible (Richmond and Jorgensen, 1999; O'Hagan *et al.*, 2005). Overall, the broad range of genetic and molecular tools available in *C. elegans* allows in-depth investigation of the cellular mechanisms underlying mechanotransduction.

## III. *C. ELEGANS* MECHANOSENSITIVE BEHAVIORS

Despite its anatomical simplicity, *C. elegans* displays an impressive repertoire of mechanosensitive behaviors (Table I). When touched gently on the posterior, an animal will move forward; when touched on the anterior body,

**TABLE I**
Main *C. elegans* Mechanosensitive Behaviors

| Mechanosensitive behavior | Stimulus | Mechanosensory neurons | References |
|---|---|---|---|
| Gentle body touch response | Light touch on the body | ALM, AVM, PLM, PVM | Chalfie *et al.*, 1985 |
| Harsh touch response | Prodding with a stiff object on the body | PVD, PVC, others? | Way and Chalfie, 1989; Chalfie and Wolinsky, 1990 |
| Head-on collision response | Nose tip collision with an obstacle | ASH, FLP, OLQ | Kaplan and Horvitz, 1993; Colbert *et al.*, 1997; Hart *et al.*, 1999 |
| Head withdrawal response | Light touch on nose side during foraging | OLQ, IL1 | Kaplan and Horvitz, 1993; Hart *et al.*, 1995 |
| Proprioception | Muscle contractions and relaxations | Ventral nerve cord motor-neurons, DVA | Wolinsky and Way, 1990; Francis and Waterston, 1991; Hresko *et al.*, 1994; Tavernarakis *et al.*, 1997; Li *et al.*, 2006 |
| Tap withdrawal reflex | Vibrations (taps) through the culture substrate | ALM, PVM, PLM, AVD | Wicks and Rankin, 1995 |
| Basal slowing response | Mechanical input from the culture substrate texture (i.e., the presence of a bacterial lawn) | CEP, ADE, PDE | Sawin *et al.*, 2000 |

it will move backward. This gentle body touch is sensed by the six touch receptor neurons (ALML/R, anterior lateral microtubule cell, left/right; AVM, anterior ventral microtubule cell; PLML/R, posterior lateral microtubule cell, left/right; PVM, posterior ventral microtubule cell; Chalfie, 1993, 1995; Chalfie and Sulston, 1981). The touch receptors are situated so that their processes run longitudinally along the body wall embedded in the hypodermis adjacent to the cuticle (Fig. 1). The position of the processes along the body axis correlates with the sensory field of the touch cell. Laser ablation of touch receptors, which have sensory receptor processes in the anterior half of the body, eliminates anterior touch sensitivity and laser ablation of the touch receptors, which have posterior dendritic processes, eliminates posterior touch sensitivity. In addition to mediating touch avoidance, the touch receptor neurons appear to control the spontaneous rate of locomotion since 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 environments or body stretch induced by locomotion itself.

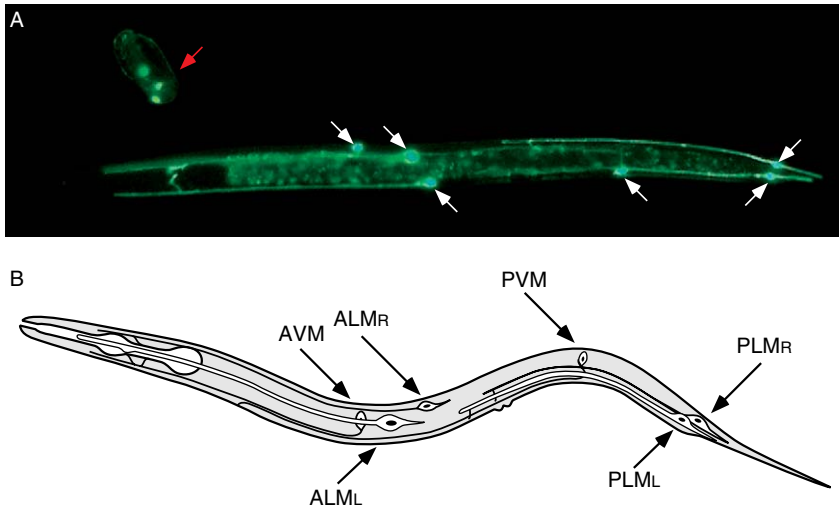


**FIGURE 1** The *C. elegans* touch receptor neurons. (A) Visualization of touch receptors. Worms are expressing the green fluorescent protein (GFP) under the control of the *mec-4* promoter, which is active only in the six touch receptor neurons. Arrows indicate touch receptor cell bodies. Some touch receptor axons are apparent. (B) 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.

Animals with defective touch receptor neurons can still respond to a harsh stimulus (push with a platinum wire; Way and Chalfie, 1989; Chalfie and Wolinsky, 1990). This is indicative of the presence of a separate neuronal circuit, which is responsible for harsh touch sensitivity. Worms also respond to mechanical stimuli applied at the tip of their head by initiating a backward movement. This behavior known as nose touch response is mediated by nose touch neurons (Kaplan and Horvitz, 1993; Colbert *et al*., 1997). The nose of *C. elegans* is highly sensitive to mechanical stimuli. This region of the body is innervated by many sensory neurons which mediate mechanosensitivity. Responses to touch in the nose can be classified into two categories, the head-on collision response and the foraging and head withdrawal response (Wicks and Rankin, 1995; Colbert *et al*., 1997; Bargmann and Kaplan, 1998; Hart *et al*., 1999).

Additional mechanosensitive behaviors include proprioception (the regulation of coordinated locomotion), the tap withdrawal reflex, and the basal slowing response (Chiba and Rankin, 1990; Liu and Sternberg, 1995; Tavernarakis *et al*., 1997; Wicks and Rankin, 1997; Sawin *et al*., 2000). In the laboratory, *C. elegans* moves through a bacterial lawn on a Petri plate with a readily observed sinusoidal motion. Proprioception facilitates the coordinated movement of body parts by synchronization of muscle contractions that produce the characteristic sinusoidal locomotory pattern of the nematode. Interactions between excitatory and inhibitory motorneurons produce a pattern of alternating dorsal and ventral contractions (Francis and Waterston, 1991; Hresko *et al*., 1994). Distinct classes of motorneurons control dorsal and ventral body muscles. To generate and sustain the sinusoidal pattern of movement, the contraction of the dorsal and ventral body muscles must be out of phase. For example, to turn the body dorsally, the dorsal muscles contract while the opposing ventral muscles relax. The adult motor system involves five major types of ventral nerve cord motorneurons defined by axon morphologies and patterns of synaptic connectivity.

The tap withdrawal reflex is a mechanosensitive behavior triggered by mechanical stimuli delivered as vibrations (taps) through the Petri dish and the agar medium on which the worms move. The response to taps consists of either accelerations or reversals (Wicks and Rankin, 1995). The basal slowing response occurs when moving worms encounter a bacterial lawn and is regulated by a circuit of dopaminergic mechanosensory neurons. Animals moving at high speed in the absence of food slow down when they enter a bacterial lawn. It is likely that mechanosensory input originating from textural differences in the substrate between areas with and without food drives this response. Indeed, the same response is observed if a lawn of sepharose beads is used instead of bacteria (Sawin *et al*., 2000).

**TABLE II**
Ion Channels Implicated in Mechanosensation, in *C. elegans*

| Ion channel | Sequence similarity | Expression pattern | Associated mechanosensitive behavior | References |
|---|---|---|---|---|
| MEC-4 | Epithelial $Na^+$ channel (degenerin) | Touch receptor neurons | Gentle body touch response | Chalfie and Au, 1989; Driscoll and Chalfie, 1991; Hamill *et al.*, 1992; O'Hagan *et al.*, 2005 |
| MEC-10 | Epithelial $Na^+$ channel (degenerin) | Touch receptor neurons | Gentle body touch response | Huang and Chalfie, 1994; O'Hagan *et al.*, 2005 |
| UNC-8 | Epithelial $Na^+$ channel (degenerin) | Motorneurons, interneurons, nose mechano-sensory neurons | Proprioception, coordinated locomotion | Park and Horvitz, 1986b; Shreffler *et al.*, 1995; Tavernarakis *et al.*, 1997 |
| DEL-1 | Epithelial $Na^+$ channel (degenerin) | Motorneurons | Proprioception, coordinated locomotion | Tavernarakis *et al.*, 1997 |
| UNC-105 | Epithelial $Na^+$ channel (degenerin) | Body wall muscles | Coordinated locomotion | Park and Horvitz, 1986a; Liu *et al.*, 1996; Garcia-Anoveros *et al.*, 1998 |
| OSM-9 | TRPV $Ca^{2+}$ channel | Nose mechano-sensory neu-rons, chemosen-sory neurons, osmosensory neurons | Nose touch response, nociception | Colbert *et al.*, 1997; Tobin *et al.*, 2002; Zhang *et al.*, 2004b |
| OCR-2 | TRPV $Ca^{2+}$ channel, OSM-9/ capsaicin receptor related protein | Amphid sensory neurons, phasmid neurons | Nose touch response | Tobin *et al.*, 2002 |
| TRP-4 | TRPN $Ca^{2+}$ channel | Dopaminergic mechanosensory neurons, interneurons | Proprioception, coordinated locomotion | Li *et al.*, 2006 |

This behavior allows animals to spend more time in food-rich areas and facilitates foraging.

*C. elegans* displays several additional behaviors that are based on sensory mechanotransduction and have been characterized to a lesser extend. For example, mechanotransduction appears to play a regulatory role in processes such as matting, egg laying, feeding, defecation, and maintenance of the pseudocoelomic body cavity pressure (Thomas, 1990; Avery, 1993; Bargmann and Kaplan, 1998; Syntichaki and Tavernarakis, 2004). These behaviors add to the large repertoire of mechanosensitive phenomena, amenable to genetic and molecular dissection in the nematode.

Extensive genetic studies have culminated in the identification and characterization of several genes, which encode components of specialized ion channels that mediate mechanosensitive behaviors in *C. elegans*. Similar channels with mechanosensitive properties have also been identified in diverse organisms including snails, flies, and vertebrates, and fall in two distinct classes: the degenerin (DEG)/epithelial $Na^+$ channel (ENaC) family and the transient receptor potential (TRP) family of ion channels (Table II). Below, we review the role of these mechanosensitive ion channels in specific *C. elegans* mechanosensory behavioral responses and discuss the molecular mechanisms that govern the function of nematode mechanotransducers.

## IV. *C. ELEGANS* DEG/ENaCs

The DEG/ENaC family of ion channels is a large group of proteins sharing a high degree of sequence and overall structure similarity. Members of the DEG/ENaC family have been identified in organisms ranging from nematodes, snails, flies, and many vertebrates including humans and are expressed in tissues as diverse as kidney, epithelia, muscles, and neurons (reviewed by Kellenberger and Schild, 2002). Specific *C. elegans* ion channels are referred to as degenerins because unusual, gain-of-function mutations in several family members induce swelling or cell death (Chalfie and Wolinsky, 1990). *C. elegans* degenerins exhibit ~25–30% sequence identity to subunits of the vertebrate amiloride sensitive, ENaCs, which are required for ion transport across epithelia (Hummler and Horisberger, 1999) and acid-sensing ion channels that may contribute to pain perception and mechanosensation (Waldmann and Lazdunski, 1998; Hummler and Horisberger, 1999; Kellenberger and Schild, 2002).

Despite their functional diversity they share a few common properties such as $Na^+$ selectivity and inhibition by amiloride, in addition to a highly

conserved overall structure. DEG/ENaC proteins range from about 550 to 950 amino acids in length and share several distinguishing blocks of sequence similarity. Subunit topology is invariable: all DEG/ENaC family members have two membrane-spanning domains (MSDs) with cysteine-rich domains (CRDs, the most conserved is designated CRD3) situated between these two transmembrane segments. DEG/ENaCs are situated in the membrane such that N- and C-termini project into the intracellular cytoplasm while most of the protein, including the CRDs, is extracellular (Fig. 2).

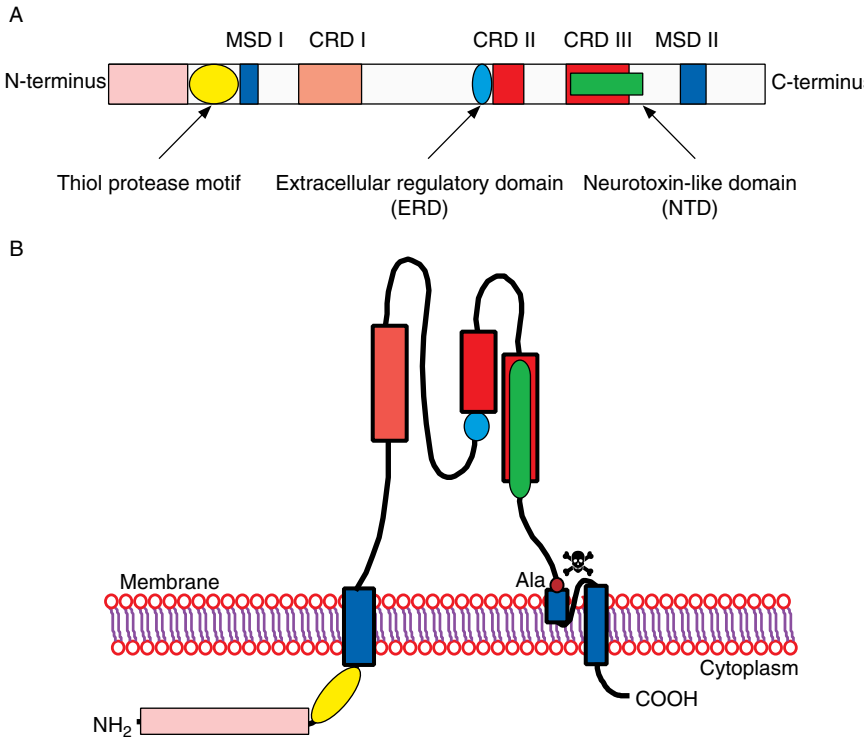


**FIGURE 2** Schematic representation of DEG/ENaC ion channel subunit structure and topology. (A) Functional/structural domains. Colored boxes indicate defined channel modules. These include the two membrane-spanning domains (MSDs; dark-blue shading) and the three cysteine-rich domains (CRDs; red shading; the first CRD is absent in mammalian channels and is depicted by light red shading). The small light-blue oval depicts the putative extracellular regulatory domain (ERD). The green box overlapping with CRDIII denotes the neurotoxin-related domain (NTD). The conserved intracellular region with similarity to thiol-protease histidine active sites is shown in yellow. Shown in pink is the N-terminal domain. (B) Transmembrane topology. Both termini are intracellular with the largest part of the protein situated outside the cell. The dot near MSD II represents the amino acid position (alanine 713 in MEC-4) affected in dominant, toxic degenerin mutants.

Highly conserved regions include the two MSDs (MSD I and II), a short amino acid stretch before the first MSD, the extracellular CRDs, an extracellular regulatory domain (ERD), and a neurotoxin-related domain (NTD) before predicted transmembrane domain II (Tavernarakis and Driscoll, 2000; Tavernarakis *et al*., 2001). The high degree of conservation of cysteine residues in these extracellular domains suggests that the tertiary structure of this region is critical to the function of most channel subunits and may mediate interactions with extracellular structures. The strong sequence and structure conservation across species suggests that DEG/ENaC family members shared a common ancestor relatively early in evolution (Fig. 3).

DEG/ENaC ion channels have been associated with mechanosensory responses in nematodes, flies, and mammals (Tavernarakis and Driscoll, 2001; Kellenberger and Schild, 2002; Syntichaki and Tavernarakis, 2004). At present, 30 genes encoding DEG/ENaC ion channels have been identified in the *C. elegans* genome. Genetic, molecular, and electrophysiological studies have implicated five nematode degenerins in mechanotransduction (DEL-1, MEC-4, MEC-10, UNC-8, and UNC-105; Table II; reviewed by Syntichaki and Tavernarakis, 2004). Below, we discuss the role of degenerins in *C. elegans* mechanosensory behaviors.

### *A. MEC-4 and MEC-10*

Genetic analysis revealed 18 genes, which, when mutated, disrupt specifically the gentle body touch sensation (Ernstrom and Chalfie, 2002). These genes are therefore thought to encode candidate mediators of touch sensitivity (these genes were named *mec* genes since when they are defective, animals are *mec*hanosensory abnormal; Chalfie and Au, 1989). Almost all of the *mec* genes have now been molecularly identified and most of them encode proteins postulated to make up a touch-transducing complex (Gu *et al*., 1996; Syntichaki and Tavernarakis, 2004). The core elements of this mechanosensory complex are the channel subunits MEC-4 and MEC-10, which can interact genetically and physically (Ernstrom and Chalfie, 2002; Goodman *et al*., 2002). Both these proteins are DEG/ENaC family members and interact to form the putative mechanotransducer in *C. elegans* touch receptor neurons, together with two other structural components, the stomatin-like protein MEC-2, and the paraoxonase-like protein MEC-6 (Chelur *et al*., 2002; Goodman *et al*., 2002).

Loss-of-function mutations in *mec-4* or *mec-10* do not affect the development and utlrastructure of the touch receptor neurons but render the animals touch insensitive (Chalfie and Au, 1989; Chalfie, 1995). The plasma membrane topology of these molecules has been elucidated by performing antibody and protease experiments (Lai *et al*., 1996). Evidence that MEC-4

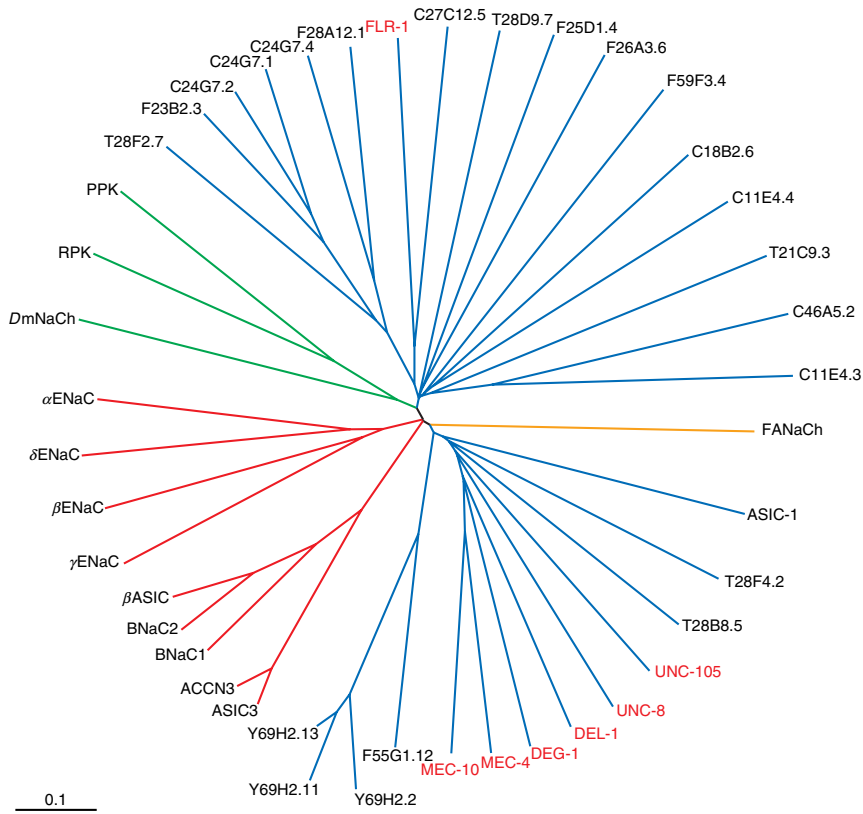


**FIGURE 3** Phylogenetic relations among DEG/ENaC proteins. The nematode degenerins are shown with blue lines. The current degenerin content of the complete nematode genome is included. The seven genetically characterized (DEG-1, DEL-1, FLR-1, MEC-4, MEC-10, UNC-8, and UNC-105) are shown in red. Representative DEG/ENaC proteins from a variety of organisms, ranging from snails to humans, are also included (mammalian: red lines; fly: green lines; snail: orange line). The scale bar denotes evolutionary distance equal to 0.1-nucleotide substitutions per site.

and MEC-10 coassemble into the same channel complex include that: (1) MEC-4 and MEC-10 subunits are coexpressed in the touch receptor neurons (Huang and Chalfie, 1994), (2) MEC-4 and MEC-10 proteins translated *in vitro* in the presence of microsomes can coimmunoprecipitate (Goodman *et al.*, 2002), and (3) genetic interactions between *mec-4* and *mec-10* have been observed (Gu *et al.*, 1996). MEC-4 exhibits a punctuate distribution along the axon of the touch receptor neurons which may represent the subcellular localization of the mechanotransducing complexes (Fig. 4).

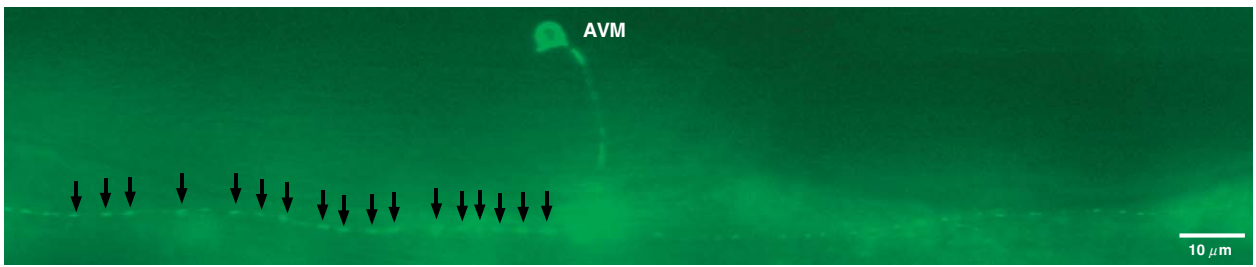


**FIGURE 4** Punctate localization of a putative mechanosensitive ion channel subunit. Image of an AVM touch receptor neuron expressing a GFP-tagged MEC-4 protein. Fluorescence is unevenly distributed along the process of the neuron in distinct puncta, which may represent the location of the mechanotransducing apparatus.

The MEC-4/MEC-10 mechanically gated ion channel is sensitive to the diuretic amiloride, which is a general inhibitor of mechanosensitive ion channels (Hamill *et al*., 1992; O'Hagan *et al*., 2005). It is proposed that at least two MEC-4 and MEC-10 subunits contribute to the channel formation (Huang and Chalfie, 1994). MEC-4 is required for touch neuron activity induced by light touch stimuli *in vivo*, as shown by measurements of physiological neural responses using a fluorescent calcium indicator reporter fusion (cameleon; Suzuki *et al*., 2003). Absence of MEC-4 does not alter the basic physiology of the touch neurons or their responses in harsh touch stimuli. Whole-cell patch clamp recordings from *C. elegans* touch receptor neurons, *in vivo*, provided experimental verification that the MEC-4/MEC-10 channel is actually mechanically gated. These studies show that the MEC-4/MEC-10 channel is directly activated by external forces, which results in the generation of mechanosensory currents carried by $Na^+$ and blocked by amiloride (O'Hagan *et al*., 2005).

Gain-of-function (dominant, d) mutations in *mec-4* induce necrotic cell death of the six touch receptor neurons (Syntichaki and Tavernarakis, 2003). Most such mutations encode substitutions of an alanine, adjacent to the second transmembrane domain, near the channel pore. Substitution of the small side chain alanine by a large side chain amino acid causes toxicity. Steric interference conferred by a bulky amino acid side chain causes the channel to close less effectively. Increased cation influx initiates neurodegeneration. That ion influx is critical for degeneration is supported by the fact that amino acid substitutions that disrupt the channel conducting pore can prevent neurodegeneration when present in *cis* to the A713 substitution. Other *C. elegans* degenerin family members (e.g., *deg-1* and *mec-10*) can be altered by analogous amino acid substitutions to induce neurodegeneration (Syntichaki and Tavernarakis, 2002). The mutant MEC-4(*d*) $Na^+$ channel conducts $Ca^{2+}$ both when heterologously expressed in *Xenopus* oocytes and *in vivo*. Thus, $Ca^{2+}$ influx via the MEC-4(*d*) channel directly contributes to the $Ca^{2+}$ increase in the cytoplasm and signals the initiation of necrosis (Bianchi *et al*., 2004). Necrosis induced by MEC-4(*d*) is similar in several respects to that associated with the excitotoxic cell death that occurs in higher organisms in response to injury, in stroke, and so on. Intragenic second-site mutations in *mec-4(d)* that encode amino acid substitutions near the pore domain disrupt the function of the hyperactive MEC-4(*d*) channel. Such mutations appear to influence the trafficking of the channel and suppress necrosis induced by *mec-4(d)* mutants in a temperature-dependent manner (Royal *et al*., 2005).

MEC-4 and MEC-10 together with MEC-2 and MEC-6 form the mechanosensitive channel complex that is thought to be linked to the extracellular mantle and to the cytoskeleton (Savage *et al*., 1989; Du *et al*., 1996).

These interactions are facilitated by other auxiliary molecules both extracellularly and intracellularly and may serve to convey mechanical forces to the channel. *mec-2* encodes a 481-amino acid protein and is expressed in the touch receptor neurons and in a few additional neurons in the nerve ring region (Huang *et al.*, 1995; Gu *et al.*, 1996; Du and Chalfie, 2001). The MEC-2 protein appears to be localized along the length of the touch receptor process as well as in the cell body (Huang *et al.*, 1995), and shares sequence similarity with human stomatin, a protein that has been implicated in regulating red blood cell plasma membrane conductance (Stewart, 1997). The mammalian stomatin physically interacts with G-protein-coupled receptors and colocalizes with glycophosphoinositol (GPI)-anchored proteins and lipid rafts (Snyers *et al.*, 1999; Tavernarakis *et al.*, 1999; Sedensky *et al.*, 2001). MEC-2 features a central region that encompasses an SPFH domain with a membrane-associated hydrophobic part (AA 114–141) and a cytoplasmic hydrophilic part that together exhibit 65% identity to stomatin (Huang *et al.*, 1995; Tavernarakis *et al.*, 1999). The SPFH domain is the common denominator of *s*tomatins, *p*rohibitins, *f*lotilins, and bacterial *Hfl*K/C proteins, all of which are membrane-associated regulators (Tavernarakis *et al.*, 1999). MEC-2 activates the MEC-4 channel in *Xenopus* oocytes and coimmunoprecipitates with the other members of the mechanosensitive complex (Goodman *et al.*, 2002). It is also required for neural responses to gentle mechanical stimuli *in vivo* (Suzuki *et al.*, 2003). MEC-2 interacts *in vitro* and colocalizes with MEC-4 through the SPFH domain. This interaction is necessary for channel activation (Zhang *et al.*, 2004a).

*mec-6* encodes a protein that is partially related to paraoxonases/acetylesterases and physically interacts with MEC-4 and MEC-10 (Chelur *et al.*, 2002). Although animals bearing recessive *mec-6* mutations are touch insensitive, the touch receptor neurons exhibit an apparent wild-type ultrastructure (Chalfie and Sulston, 1981). How MEC-6 contributes to channel function is not yet known.

In addition to MEC-4, MEC-10, MEC-2, and MEC-6, mechanotransduction in the touch receptor neurons also requires two groups of peripheral-associated proteins encoded by *mec* genes: the intracellular proteins MEC-7 and MEC-12 and the extracellular proteins MEC-1, MEC-5, and MEC-9 (reviewed by Syntichaki and Tavernarakis, 2004). The *mec-7* and *mec-12* genes encode a $\beta$- and an $\alpha$-tubulin, respectively, expressed at high levels in the touch receptor neurons (Savage *et al.*, 1989, 1994; Hamelin *et al.*, 1992; Fukushige *et al.*, 1999). These tubulins assemble to form 15-protofilament microtubules specific to touch receptor neurons. *mec-7* and *mec-12* mutations, which cause a touch-insensitive phenotype, disrupt tubulin subunit interactions, and protofilament assembly (Savage *et al.*, 1989, 1994; Gu *et al.*, 1996). The role of these microtubules in mechanosensation remains to be

determined. Perhaps these specialized structures are tethered to the mechanosensitive MEC-4/MEC-10 ion channel, providing an intracellular anchor required for channel gating.

*mec-1* encodes an extracellular matrix (ECM) protein with multiple epidermal growth factor (EGF) and Kunitz domains (Emtage *et al*., 2004). In *mec-1* mutants, touch cells lack the mantle and other specializations of the cuticle and have displaced processes (Chalfie and Sulston, 1981; Chalfie, 1993; Savage *et al*., 1994; Gu *et al*., 1996). MEC-1 colocalizes with MEC-5 and the mechanosensory complex in the touch neurons (Emtage *et al*., 2004). The *mec-5* gene encodes a collagen that is secreted by cells of the hypodermis (Du *et al*., 1996). These two ECM components are required for the correct localization of the degenerin channel (Emtage *et al*., 2004). The *mec-9* gene encodes two transcripts which direct the synthesis of proteins secreted by the touch receptor neurons (Chalfie and Sulston, 1981; Du *et al*., 1996). MEC-9L (encoded by one of the two *mec-9* transcripts) contains several domains related to the Kunitz type serine protease inhibitor domain, a $Ca^{2+}$-binding EGF repeat, a non-$Ca^{2+}$-binding EGF repeat, and a glutamic acid-rich domain (Du *et al*., 1996). How the extracellular MEC-1, MEC-5, and MEC-9 proteins influence the activity of the MEC-4/MEC-10 ion channel is not known. It is proposed that these proteins are components of the ECM and collectively serve to anchor the channel to extracellular structures and convey external mechanical forces to the core mechanotransducer complex (Fig. 5).

### *B. UNC-8 and DEL-1*

*C. elegans* shows a characteristic sinusoidal pattern of locomotion. Little is known about how the sinusoidal wave is propagated along the body axis. Adjacent muscle cells are electrically coupled via gap junctions, which could couple excitation of adjacent body muscles. Alternatively, ventral cord motorneurons could promote wave propagation since gap junctions connect adjacent motorneurons of a given class (White *et al*., 1976, 1986; Chalfie *et al*., 1985). A third possibility is that motorneurons could themselves act as stretch receptors so that contraction of body muscles could regulate adjacent motorneuron activities, thereby propagating the wave (Tavernarakis *et al*., 1997; Syntichaki and Tavernarakis, 2004). The adult neuronal circuit for locomotion comprises five major types of ventral nerve cord motorneurons (A motorneurons—12VA and 9 DA; B motorneurons—11VB and 7DB; D motorneurons—13 VD and 6 DD; AS motorneurons; and VC motorneurons; Francis and Waterston, 1991; Hresko *et al*., 1994). Mutations that affect the neuronal circuit for locomotion disrupt the sinusoidal pattern of

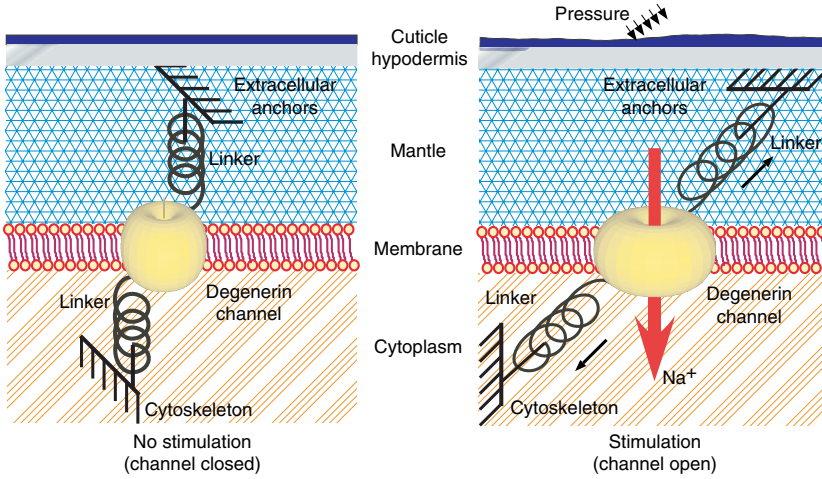


**FIGURE 5** A mechanotransducing complex in *C. elegans* touch receptor neurons. In the absence of mechanical stimulation, the channel is closed and therefore the sensory neuron is idle. Application of a mechanical force to the body of the animal results in distortion of a network of interacting molecules that opens the degenerin channel. $Na^+$ influx depolarizes the neuron, initiating the perceptory integration of the stimulus.

movement and generate locomotory defects, uncoordination, and paralysis (Park and Horvitz, 1986b; Tavernarakis and Driscoll, 1997).

Gain-of-function mutations in the *unc-8* gene (*unc-8(sd)*) induce transient neuronal swelling and severe uncoordination (Park and Horvitz, 1986a; Shreffler *et al*., 1995; Shreffler and Wolinsky, 1997). *unc-8* encodes a degenerin, which shares high sequence similarity to other DEG/ENaC family members as well as the same overall structure and topology (two transmembrane domains, three Cysteine-rich regions, and large extracellular region). It is expressed in several motorneuron classes and in some interneurons and nose touch sensory neurons (Tavernarakis *et al*., 1997). Interestingly, semidominant *unc-8* alleles alter an amino acid in the region hypothesized to be an extracellular channel-closing domain, defined in studies of *deg-1* and *mec-4* degenerins (Garcia-Anoveros *et al*., 1995; Tavernarakis *et al*., 1997). Another degenerin family member, *del-1* (for *de*generin-*l*ike) is coexpressed in a subset of neurons that express *unc-8* (the VA and VB motorneurons) and is likely to assemble into a channel complex with UNC-8 in these cells (Tavernarakis *et al*., 1997).

*unc-8* null mutants have a subtle locomotion defect (Tavernarakis *et al*., 1997). Wild-type animals move through an *E. coli* lawn with a characteristic

sinusoidal pattern. *unc-8* null mutants inscribe a path in an *E. coli* lawn that is markedly reduced in both wavelength and amplitude as compared to wild type (Fig. 6). This phenotype indicates that the UNC-8 degenerin channel functions to modulate the locomotory trajectory of the animal.

How does the UNC-8 motorneuron channel influence locomotion? One highly interesting morphological feature of some motorneurons (in particular, the VA and VB motorneurons that coexpress *unc-8* and *del-1*) is that their processes include extended regions that do not participate in neuromuscular junctions or neuronal synapses. These "undifferentiated" process regions have been hypothesized to be stretch-sensitive (discussed in White *et al*., 1976). Given the morphological features of certain motorneurons and the sequence similarity of UNC-8 and DEL-1 to candidate mechanically gated channels, we have proposed that these subunits coassemble into a stretch-sensitive channel that might be localized to the undifferentiated regions of the motorneuron process (Tavernarakis *et al*., 1997; reviewed by Syntichaki and Tavernarakis, 2004). When activated by the localized body stretch that occurs during locomotion, this motorneuron channel potentiates signaling at the neuromuscular junction, which is situated at a distance from

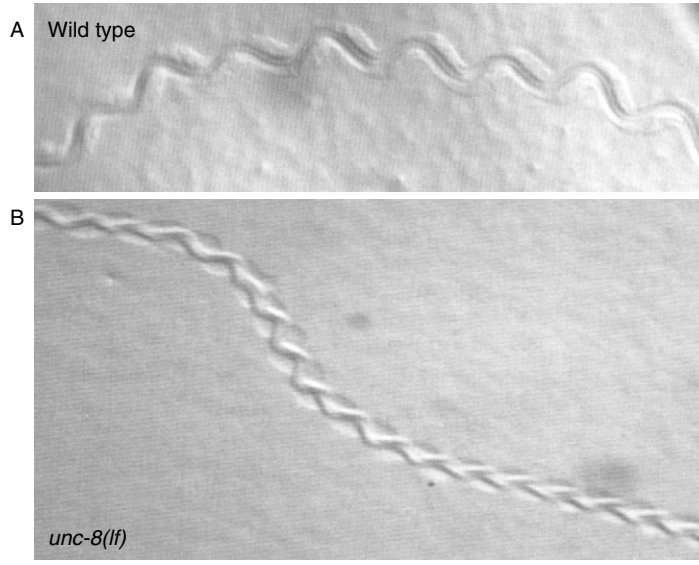


**FIGURE 6** Proprioception in the nematode. (A) Wild-type animals inscribe a sinusoidal track as they move on an agar plate evenly covered with an *E. coli* bacterial lawn. (B) The characteristic properties (amplitude and wavelength) of tracks inscribed by *unc-8(lf)* mutants are drastically reduced.

the site of the stretch stimulus. 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. This model bears similarity to the chain reflex mechanism of movement pattern generation. However, it does not exclude a central oscillator that would be responsible for the rhythmic locomotion. Instead, we suggest that the output of such an oscillator is further enhanced and modulated by stretch-sensitive motorneurons.

Genetic data indicates that UNC-8 interacts with UNC-1, a protein which is similar to MEC-2 and has an important role in determining volatile anesthetic sensitivity (Huang *et al*., 1995; Rajaram *et al*., 1998). UNC-1 is a close homologue of the mammalian stomatin protein (Rajaram *et al*., 1998). UNC-8 and UNC-1 colocalize along with another stomatin-like protein, UNC-24, in lipid rafts isolated from *C. elegans*. *unc-1* mutations eliminate UNC-8 from these structures (Sedensky *et al*., 2004). *unc-24* is expressed in a variety of motorneurons, interneurons, and sensory neurons, including the touch receptor neurons (Barnes *et al*., 1996; Zhang *et al*., 2002). Mutations in *unc-24* severely affect forward locomotion. Similarly to UNC-1, UNC-24 also affects anesthetic sensitivity and is required for the distribution of UNC-1 in the lipid rafts (Sedensky *et al*., 2004). These findings suggest that, in motorneurons, UNC-1 may play a role analogous to that of MEC-2 in touch receptor neurons; tethering the UNC-8/DEL-1 ion channels to intracellular structures.

One important corollary of the *unc-8* mutant studies is that the UNC-8 channel does not appear to be essential for motorneuron function; if this was 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. The model of UNC-8 and DEL-1 functions that is 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.

### C. UNC-105

The *unc-105* gene encodes a member of the DEG/ENaC family of ion channels and is mainly expressed in body wall muscles of *C. elegans*, where it is believed to mediate stretch sensitivity (Park and Horvitz, 1986a; Liu *et al*., 1996). UNC-105 contains ~150 amino acids at the C-terminus that are not represented in other degenerin proteins. Although loss-of-function

mutations in *unc-105* do not result in any readily observable phenotype, gain-of-function mutations cause muscle hyper contraction and result in severe paralysis of the animal (Park and Horvitz, 1986a). These mutations disrupt extracellular residues situated near the predicted transmembrane domain, where degeneration-causing mutations are found in MEC-4, MEC-10, and DEG-1. Therefore, these mutations in *unc-105* may result in constitutive channel activation producing the hypercontraction phenotype (Liu *et al.*, 1996). The muscle hyper contraction phenotype of dominant *unc-105* mutations can be suppressed by mutations near the C-terminus of *let-2*, a gene that encodes the α2 chain of type-IV collagen found in the basement membrane between muscle cells and the hypodermis (Liu *et al.*, 1996). The nature of the functional link, implied by the suppression effect, between UNC-105 and LET-2 collagen is unknown. A possible interpretation is that LET-2 normally carries gating tension to the UNC-105 channel when the muscle is stretched, thus providing regulatory feedback for muscle contraction (Liu *et al.*, 1996). Suppressor mutations in LET-2 may relieve conformational alterations to the UNC-105 channel induced by dominant mutations, allowing the channel to close. This putative connection between a collagen and a degenerin is reminiscent of a similar relationship between the MEC-5 collagen and MEC-4 in touch receptor neurons (Tavernarakis and Driscoll, 1997). Similarly, mechanosensory transduction in the auditory system requires the extracellular tip links that physically deliver mechanical energy to the mechanosensitive channels in the hair cell stereocillia of the inner ear (Section V.B; Pickles and Corey, 1992; Pickles, 1993).

Expression of the wild-type *unc-105* gene in two heterologous systems [*Xenopus* oocytes and human embryonic kidney (HEK) cells] resulted in no detectable currents, suggesting that the channel requires a mechanical stimulus for gating (Garcia-Anoveros *et al.*, 1998). By contrast, expression of two mutant forms of *unc-105*, carrying gain-of-function mutations predicted to cause constitutive activation, resulted in constitutive currents in both heterologous systems (Garcia-Anoveros *et al.*, 1998). These currents occurred without additional exogenous proteins, indicating that UNC-105 channels can assemble as homomultimers, at least in oocytes and HEK cells. Phylogenetic analysis suggests that UNC-105 is one of the most ancient degenerins, and thus may have not developed dependencies on other subunits (Corey and Garcia-Anoveros, 1996).

## V. *C. ELEGANS* TRP ION CHANNELS

TRP proteins are a family of cation-permeable channels that are present in diverse species ranging from yeast, flies, and worms to humans (Fig. 7). These channels bear structural similarities to the *Drosophila* TRP protein

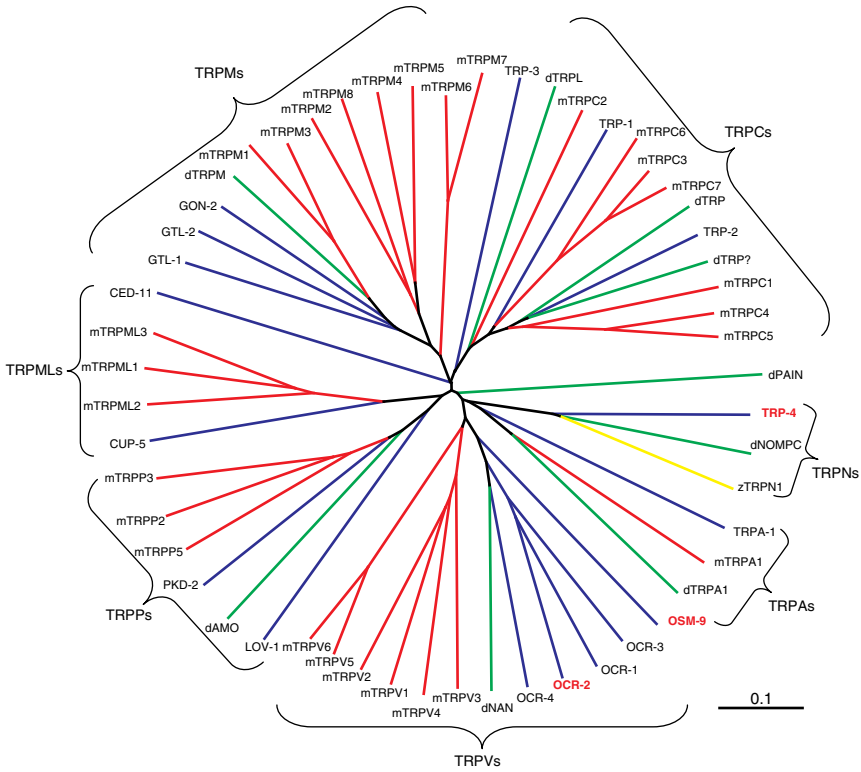


**FIGURE 7** Phylogenetic relations among TRP proteins. Nematode TRPs are indicated with blue lines together with mammalian TRP representatives (red lines), fly TRPs (green lines), and zebrafish TRPs (yellow line). The scale bar indicates relative evolutionary distance equal to 0.1 nucleotide substitution per site.

which is a light-activated $Ca^{2+}$ channel, expressed in photoreceptor cells (Montell and Rubin, 1989; Hardie and Minke, 1992; Montell, 2001). TRPs can form homo- or heteromultimeric channels composed of two or more TRP subunits and can associate with other macromolecular complexes to serve diverse cellular functions. Members of TRP family respond to several types of input such as mechanical and thermal stimuli, pH fluctuations, $Ca^{2+}$ and $Mg^{2+}$ ions, fatty acids, and chemicals that evoke thermal-like responses (Kahn-Kirby and Bargmann, 2006). Thus, TRP ion channels have been implicated in many physiological processes such as mechanosensation, thermosensation, osmosensation, phototransduction, responses to pheromones, ion absorption and homeostasis, lysosomal trafficking, and neurotransmitter release.

The TRP family of ion channels comprises seven subfamilies six of which include proteins that are conserved among worm, flies, and mammals (TRPC, classical/short TRP; TRPV, vallinoid TRP; TRPM, long/melastatin TRP; TRPM, mucolipin TRP; TRPP, polycystin TRP; TRPA; reviewed by Montell, 2005). The remaining subfamily, TRPN, contains members that are present in invertebrates and zebrafish (Walker *et al*., 2000; Sidi *et al*., 2003; Li *et al*., 2006), while a mammalian homologue has not been discovered yet. An additional distantly related subfamily, TRPY, named after the first member, the yeast vacuolar protein, Yvc1, includes proteins found only in fungi (Palmer *et al*., 2001; Bonilla and Cunningham, 2002; Denis and Cyert, 2002). All TRP members appear to form tetrameric assemblies and include six predicted transmembrane domains and a variable number of ankyrin motifs, which are suggested to mediate protein–protein interactions. Members of individual subfamilies may bear several other domains, such as coiled-coil motifs, protein kinase domains, transmembrane segments, and TRP domains (reviewed by Montell, 2005).

Sequence similarity searches of the *C. elegans* genome have identified 24 genes predicted to encode TRP proteins which are representatives of all seven TRP subfamilies. All the proteins contain the core regions of the TRP members, which include the six transmembrane domains, the gate domains, the pore loop, and the ankyrin repeats, distributed along the N-terminus. The C-terminus varies among different subfamilies and may contain coiled-coil motifs, lipid-binding domains, or other domains. Both the N- and C-termini are intracellular (reviewed by Kahn-Kirby and Bargmann, 2006). Three *C. elegans* TRP ion channels have been implicated in mechanotransduction (Fig. 8). OSM-9 and OCR-2 are members of the TRPV subfamily, and TRP-4 belongs to the TRPN group (Kahn-Kirby and Bargmann, 2006; Li *et al*., 2006).

Other members of the TRP ion channel family in *C. elegans* include GON-2 and GTL-1 which belong to the TRPM group and are localized in intestinal epithelial cells, where they control electrolyte homeostasis (Teramoto *et al*., 2005). GON-2 is also required for proper gonadal development (Sun and Lambie, 1997; West *et al*., 2001; Church and Lambie, 2003). *C. elegans* TRP-1, TRP-2, and TRP-3 are similar to TRPC ion channels. *trp-1* is expressed in motorneurons, sensory neurons, and interneurons, as well as in vulval and intestinal muscles (Colbert *et al*., 1997). TRP-3 is required for sperm-egg interactions during fertilization (Xu and Sternberg, 2003).

LOV-1 and PDK-2 are the nematode homologues of mammalian PDK-1 and PDK-2 TRPP ion channels, respectively (Corey, 2003). Mutations in the mammalian PDK-1 or PDK-2 result in autosomal dominant polycystic kidney disease (ADPKD). PDK-1 and PDK-2 form a $Ca^{2+}$-permeable ion

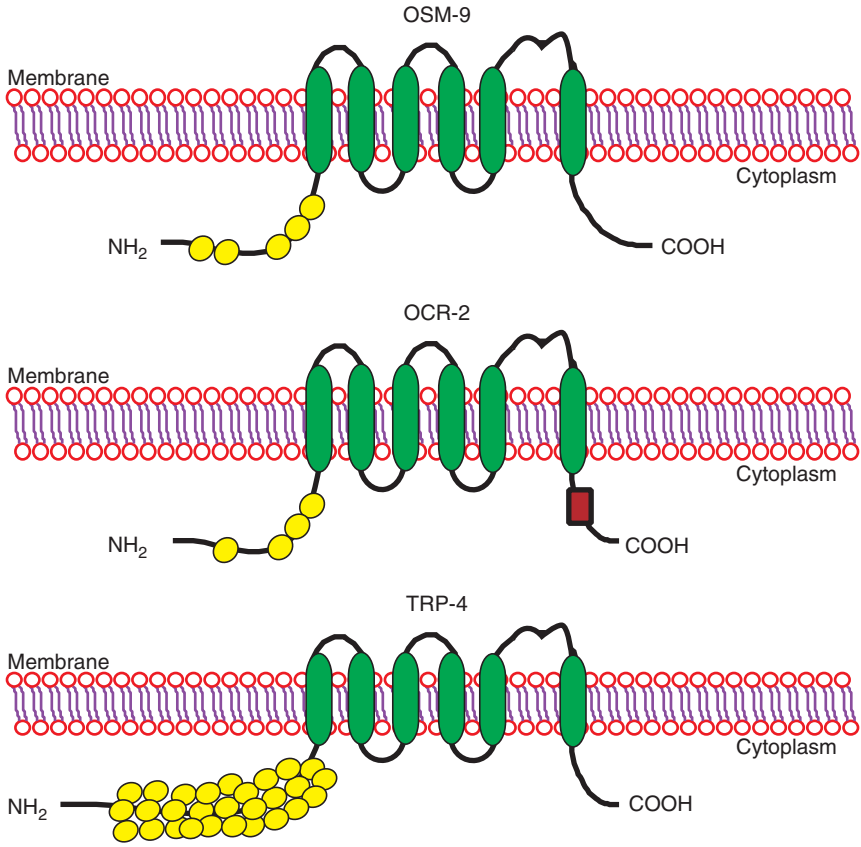


**FIGURE 8** Structure and topology of mechanosensitive TRP ion channel in *C. elegans*. Each protein contains six transmembrane domains, with the last two contributing to channel pore formation. The N-terminus is cytoplasmic and bears a variable number of ankyrin repeats (yellow circles). The C-terminus is also cytoplasmic and may contain several functional domains (see text) such as coiled coil domains (red box).

channel which is mechanically activated by fluid flow in certain epithelial cells (Nauli *et al.*, 2003). LOV-1 and PDK-2 act in nematode mating. *C. elegans* males deficient in either or both LOV-1 and PDK-2 are defective in attaching to hermaphrodites and locating the vulva (Barr and Sternberg, 1999; Barr *et al.*, 2001). Both proteins are localized in the cilia of sensory neurons in the male tail and to the CEM head neurons, consistent with a chemo- or mechanosensory function for these channels (Qin *et al.*, 2001).

The single TRPML ion channels in *C. elegans*, CUP-5, appears to be localized in lysosomes of many cell types. Mutations of *cup-5* result in defective endocytosis and degradation of proteins, and in the formation of large vacuoles (Fares and Greenwald, 2001; Hersh *et al*., 2002). *cup-5* null mutants cause maternal effect lethality, with an excess in lysosomes and high levels of apoptosis, which is rescued by the expression of mammalian TRPLM homologues (Treusch *et al*., 2004). Mammalian TRPML1, TRPML2, and TRPML3 also colocalize in the lysosomes and when mutated, they cause mucolipidosis IV, a disorder characterized by lysosomal dysfunction which leads to neurodegeneration (Qian and Noben-Trauth, 2005; Venkatachalam *et al*., 2006).

### *A. OSM-9 and OCR-2*

OSM-9 is the *C. elegans* homologue of the mammalian TRPV4 ion channel. The OSM-9 protein contains six predicted MSDs, three ankyrin motifs at the N-terminus, and a hydrophilic C-terminal domain. The *osm-9* gene is expressed in ciliated sensory neurons including QLQ, FLP, ADL, ADF, AWA, and ASH (Colbert *et al*., 1997). QLQ and ASH are polymodal nociceptive neurons which detect mechanical stimuli, osmotic pressure, and various odorants. These neurons have also been implicated in the response to light touch in the nose (Kaplan and Horvitz, 1993). The FLP neuron is a sensory neuron, also involved in nose touch responses. *osm-9* mutant animals fail to respond to nose touch stimuli while their response to gentle body touch, mediated by the six touch receptor neurons, is normal (Colbert *et al*., 1997). *mec-4* and *mec-10* are not required to sense nose touch and similarly *osm-9* is not required to sense body touch. These finding indicate that OSM-9 functions as mechanosensory channel in ciliated nose sensory neurons. Furthermore, *osm-9* mutants are also defective in olfactory responses mediated by the AWA and AWC neurons, and in osmotic avoidance responses mediated by the ASH neuron. The OSM-9 protein localizes to the sensory cilia of AWA and ASH, suggesting a direct role in sensory transduction (Colbert *et al*., 1997).

Four additional *osm-9*/capsaicin receptor-related TRPV genes are coexpressed with *osm-9* in specific subsets of cells (*ocr-1*, *ocr-2*, *ocr-3*, and *ocr-4*). These TRPV genes encode proteins which are 20-25% identical to OSM-9 and, similarly to OSM-9, contain six MSDs and three ankyrin repeats (Tobin *et al*., 2002). *ocr-1* is expressed in AWA and ADL chemosensory neurons, *ocr-2* is expressed in AWA, ADL, ASH, ADF, PHA, and PHB sensory neurons, *ocr-3* is expressed in the rectal gland cells and weakly in the glial socket cells, and finally *ocr-4* is expressed exclusively in the

mechanosensory QLQ neurons (Kahn-Kirby and Bargmann, 2006). All of these neurons, as well as the rectal gland cells coexpress *osm-9*. On the basis of the expression pattern of the *ocr* genes, OCR-2 and OCR-4 appear to be the strongest candidates for the formation of a TRPV mechanosensitive complex with OSM-9. Consistent with this notion, the nociceptive functions of the ASH neurons, including nose touch sensation, are severely compromised in *ocr-2* mutants (Tobin *et al.*, 2002). Both OSM-9 and OCR-2 are localized in the cilia of AWA and ASH cells and this localization is interdependent. In neurons that express *osm-9*, the absence of OCR-2 results in the translocation of OSM-9 from cilia to the cell body. In addition, ectopic expression of OCR-2 in the AWC drives OSM-9 to the cilia. These findings suggest a physical interaction between OSM-9 and OCR-2 that is required for normal nose touch sensation (Tobin *et al.*, 2002). Interestingly, in *Drosophila* the TRPV proteins NAN (Nanchung) and IAV (Inactive) interact to form a $Ca^{2+}$-permeable channel which senses mechanical vibrations and is required for auditory transduction (Kim *et al.*, 2003; Gong *et al.*, 2004). This is also indicative of the conserved function of TRPV proteins to mediate mechanosensitive behaviors. OSM-9 and OCR-2 also regulate the social feeding behavior in *C. elegans* (de Bono *et al.*, 2002). This behavior is characterized by a rapid movement toward the food source and the aggregation of animals during feeding (de Bono and Bargmann, 1998). Mutations in *osm-9* and *ocr-2* suppress this accumulation of animals in *C. elegans* strains, which are native social feeders.

Several genetic studies suggest that the function of the putative OSM-9/OCR-2 ion channel is regulated by G-protein signaling and specific polyunsaturated fatty acids (PUFAs), which act upstream of OSM-9/OCR-2 to modulate nocipteptive responses in ASH neurons, including the mechanosensory nose touch avoidance behavior (Roayaie *et al.*, 1998; Kahn-Kirby *et al.*, 2004). Rat TRPV4 expressed in the ASH neurons of nematode *osm-9* mutants rescues osmosensation and mechanosensation defects in these animals. However, this is not the case in *ocr-2* mutants (Liedtke *et al.*, 2003). Another mammalian TRPV homologue, the TRPV1 capsaicin receptor, is also capable of restoring the impaired avoidance behaviors of *osm-9* and *ocr-2* mutants (Tobin *et al.*, 2002). These results suggest that TRPV functions are at least partially conserved in metazoans.

### B. *TRP-4*

The *C. elegans* TRP-4 is a member of the TRPN subfamily of ion channels (Li *et al.*, 2006). This group also includes the zebrafish TRPN1 and the *Drosophila* NompC. TRPN1 is localized in the sensory hair cells of the inner

ear and is required for the response to vibrations and normal hearing (Sidi *et al*., 2003). NompC is a mechanosensory ion channel required for sensing bristle displacements (Walker *et al*., 2000). Similarly, TRP-4 appears to be involved in mechanosensory signaling in *C. elegans*. The *trp-4* gene is expressed in three sets of dopaminergic neurons (CEP, ADE, and PDE; Walker *et al*., 2000, p. 104), and in two interneurons (DVA and DVC; Li *et al*., 2006). Dopaminergic neurons in *C. elegans* mediate the basal slowing response, which is a tactile mechanosensory behavior (Sawin *et al*., 2000). Essentially, wild-type animals slow down when they encounter a bacterial lawn by sensing a mechanical attribute pertinent to the texture of the culture substrate and the bacterial lawn. This response is not specific to bacteria since animals respond similarly to sterile, artificial lawns made of sepharose beads (Sawin *et al*., 2000). Slowing originates from the decreased frequency of body bending and increases the amount of time animals spend in areas rich in food. *trp-4* mutant worms show fast and exaggerated body bending which is not modulated by the texture of the substrate. The frequency of body bending is regulated by dopaminergic neurons, while bending extend appears to be influenced by the DVA and DVC interneurons (Li *et al*., 2006). It is likely that TRP-4 functions in these neurons as a sensor of body bending, which provides the feedback necessary to sustain sinusoidal locomotion. Indeed, measurements $Ca^{2+}$ currents evoked by body bending suggest that the DVA interneuron is stretch sensitive and that the TRP-4 ion channel mediates stretch sensitivity in this neuron to facilitate proprioception (Li *et al*., 2006).

## VI. CONCLUDING REMARKS

Genetic analyses have been highly successful in identifying genes needed for mechanosensitive behaviors (Chalfie, 1997; Eberl *et al*., 1997; Nicolson *et al*., 1998; Gillespie and Walker, 2001; Hamill and Martinac, 2001). However, there are several limitations associated with genetic approaches aiming to dissect mechanotransduction mechanisms. 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 (Kernan *et al*., 1994; Eberl *et al*., 1997). 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 still remain to be discovered.

The detailed model for mechanotransduction in *C. elegans* touch receptor neurons accommodates genetic data and molecular properties of cloned genes. This model remains to be tested by determining subcellular channel localization, subunit associations and, most importantly, channel-gating properties. The proposed direct interactions between proteins that build the mechanotransducing complex have begun to be addressed experimentally (Chelur *et al*., 2002; Goodman *et al*., 2002; O'Hagan *et al*., 2005).

Despite the undeniably considerable progress that has been achieved during recent years in all fronts toward dissecting the process of sensory mechanotransduction at the molecular level, several thorny questions are still begging for answers. What is the gating mechanism of mechanosensitive ion channels? How is tension delivered to the mechanotransducing complex? What additional molecules play part in the biological response to mechanical stimuli? Are human sensory mechanotransducers similar in composition and function to nematode or *Drosophila* ones? It is important to emphasize that although specialized ion channels most likely comprise the core of every metazoan mechanotransducer, it is the other physically associated proteins that shape its properties. It is equally important to seek and identify these. Without them, our understanding of mechanical transduction will never be complete. Mechanical sensation at the molecular level in higher organisms is most likely a property of a complex structure involving many components and contacts and not of any single protein. Several tools could be employed toward this goal, such as yeast two hybrid screens and biochemical methods of copurification of channel complexes, together with anchoring proteins.

Electrophysiological studies of sensory mechanotransduction in *C. elegans* became possible, allowing direct recordings from nematode touch receptor neurons (O'Hagan *et al*., 2005). In a complementary approach, noninvasive monitoring and measurement technologies have been developed that allow the functional characterization of degenerin or other ion channels, while they are kept embedded in their natural surroundings (Bouevitch *et al*., 1993; Khatchatouriants *et al*., 2000; Suzuki *et al*., 2003). Direct, nondestructive recordings from touch receptor neurons coupled with the powerful genetics of *C. elegans* will hopefully allow the complete dissection of a metazoan mechanotransducing complex.

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