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The Role of DEG/ENaC Ion Channels in Sensory Mechanotransduction

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Abstract: All living organisms have the capacity to sense and respond to mechanical stimuli permeating their environment. Mechanosensory signaling constitutes the basis for the senses of touch and hearing and contributes fundamentally to development and homeostasis. Intense genetic, molecular, and elecrophysiological studies in organisms ranging from nematodes to mammals have highlighted members of the DEG/ENaC family of ion channels as strong candidates for the elusive metazoan mechanotransducer. These channels have also been implicated in several important processes including pain sensation, gametogenesis, sodium re-absorption, blood pressure regulation, and learning and memory. In this chapter, we review the evidence linking DEG/ENaC ion channels to mechanotransduction and discuss the emerging conceptual framework for a metazoan mechanosensory apparatus.

1.1. Introduction

Highly specialized macromolecular structures allow organisms to sense mechanical forces originating either from the surrounding environment or from within the organism itself. Such structures function as mechanotransducers, converting mechanical energy to biological signals. At the single-cell level, mechanical signaling underlies cell volume control and specialized responses such as the prevention of polyspermy in fertilization. At the level of the whole organism, mechanotransduction underlies processes as diverse as stretch-activated reflexes in vascular epithelium and smooth muscle, gravitaxis and turgor control in plants, tissue development and morphogenesis, and the senses of touch, hearing, and balance.

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Elegant electrophysiological studies in several systems have established that mechanically-gated ion channels are the mediators of the response. For years, however, these channels have eluded intense cloning efforts. Why are these channels so particularly resistant to our exploitation? These channels are rare. In skin pads, mechanoreceptors are spread out so there are only 17,000 in the finger and palm skin pad (Koltzenburg et al., 1997). This is an extremely low concentration. In the specialized hair cells of our ears, only a few hundred mechanically gated channels may exist. To make our prospects of directly encountering them even more slim, mechanosensory channels are embedded and intertwined with materials that attach them to the surrounding environment—contacts probably critical to function that are hard or even impossible to reconstitute or mimic in a heterologous system such as *Xenopus* oocytes, for example. Finally, there are no known biochemical reagents that interact with the mechanically gated channels with high specificity and high affinity, thwarting efforts for biochemical purification. Biochemical purification and structural analysis of an *E. coli* mechanosensitve channel, *Msc*L, has been accomplished (Blount and Moe, 1999; Sukharev et al., 1997), but until recently, eukaryotic mechanosensitive ion channels have eluded cloning efforts, and thus little is understood of their structures and functions.

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* (Syntichaki and Tavernarakis, 2004). 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. These genetic studies revealed several genes that encode subunits of candidate mechanically gated ion channels involved in mediating touch transduction, proprioception, and coordinated locomotion (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994; Liu et al., 1996; Tavernarakis et al., 1997). These channel subunits belong to a large family of related proteins in *C. elegans* 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 approximately 25–30% sequence identity to subunits of the vertebrate amiloride-sensitive epithelial Na^+ channels (ENaC), which are required for ion transport across epithelia, and acid-sensing ion channels that may contribute to pain perception and mechanosensation (ASICs, BNC) (Hummler and Horisberger, 1999; Kellenberger and Schild, 2002; Price et al., 2000; Waldmann and Lazdunski, 1998). Together, the *C. elegans* and vertebrate proteins define the DEG/ENaC (degenerin/epithelial sodium channel) family of ion channels (Kellenberger and Schild, 2002). Additional members of this large group of proteins are the snail FMRF-amide gated channel FaNaC (Lingueglia et al., 1995), the *Drosophila* ripped pocket and pickpocket (RPK and PPK) (Adams et al., 1998; Darboux et al., 1998) and *C. elegans flr-1* (Take-Uchi et al., 1998).

To summarize, members of the DEG/ENaC family have now been identified in organisms ranging from nematodes, snails, flies, and many vertebrates including humans, and are expressed in tissues as diverse as kidney and lung epithelia, muscle, and neurons. Intense genetic, molecular, and elecrophysiological studies have

TABLE 1.1. DEG/ENaC proteins implicated in mechanotransduction

Protein	Expression pattern	Postulated function	Organism	Reference
DEL-1	Motorneurons Sensory neurons	Stretch sensitivity Proprioception	*Caenorhabditis elegans*	Tavernarakis et al., 1997
DEG-1	Interneurons Sensory neurons Muscle Hypodermis	Harsh touch sensitivity?	*Caenorhabditis elegans*	Chalfie and Wolinsky, 1990
MEC-4	Touch receptor neurons	Touch sensitivity	*Caenorhabditis elegans*	Driscoll and Chalfie, 1991
MEC-10	Touch receptor neurons Other sensory neurons	Touch sensitivity	*Caenorhabditis elegans*	Huang and Chalfie, 1994
UNC-8	Motorneurons Interneurons Sensory neurons	Stretch sensitivity Proprioception	*Caenorhabditis elegans*	Tavernarakis et al., 1997
UNC-105	Muscle	Stretch sensitivity	*Caenorhabditis elegans*	Liu et al., 1996
PPK	Sensory dendrites of peripheral neurons	Touch sensitivity Proprioception	*Drosophila melanogaster*	Adams et al., 1998
DmNaCh	Multiple dendritic sensory neurons	Stretch sensitivity	*Drosophila melanogaster*	Darboux et al., 1998
BNC1	Lanceolate nerve endings that surround the hair follicle	Touch sensitivity	*Mus musculus*	Price et al., 2000
γENaC	Baroreceptor nerve terminals innervating the aortic arch and carotid sinus	Pressure sensitivity	*Rattus norvegicus*	Drummond et al., 1998
ASIC3/ DRASIC	Dorsal root ganglia neurons; large-diameter mechanoreceptors; small-diameter peptidergic nociceptors	Mechanosensation; acid-evoked nociception	*Mus musculus*	Price et al., 2001

implicated these channels in mechanotransduction in nematodes, flies, and mammals (Kellenberger and Schild, 2002; Tavernarakis and Driscoll, 2001a). Therefore, these proteins are strong candidates for a metazoan mechanosensitive ion channel (Table 1.1).

Here, we review the studies that led to the identification of nematode degenerins and discuss their role in mediating mechanosensitive behaviors in the worm. Furthermore, we correlate the mechanotransducer model that has emerged from investigations in *C. elegans* with recent findings in mammals, also implicating members of the DEG/ENaC family of ion channels in mechanotransduction. The

totality of the evidence in such diverse species suggests that structurally related ion channels shape the core of a metazoan mechanotransducer.

1.2. Mechanosensory Signaling in *C. elegans*

C. elegans is a small (1 mm) soil-dwelling hermaphroditic nematode that completes a life cycle in 2.5 days at 25°C. Animals progress from a fertilized embryo through four larval stages to become egg-laying adults, and live for about 2 weeks. The simple body plan and 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 both 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). Overall, the broad range of genetic and molecular techniques applicable in the *C. elegans* model system allow a unique line of investigation into fundamental problems in biology such as mechanical signaling.

In the laboratory, *C. elegans* moves through a bacterial lawn on a petri plate with a readily observed sinusoidal motion. 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 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. A motorneurons (12 VA and 9 DA), B motorneurons (11 VB and 7DB), D motorneurons (13 VD, 6 DD), AS motorneurons and VC motorneurons command body wall muscles arranged in four quadrants along the body axis (Francis and Waterston, 1991; Hresko et al., 1994; Walthall, 1995). Relatively 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 because gap junctions connect adjacent motorneurons of a given class (Chalfie et al., 1985; White et al.,

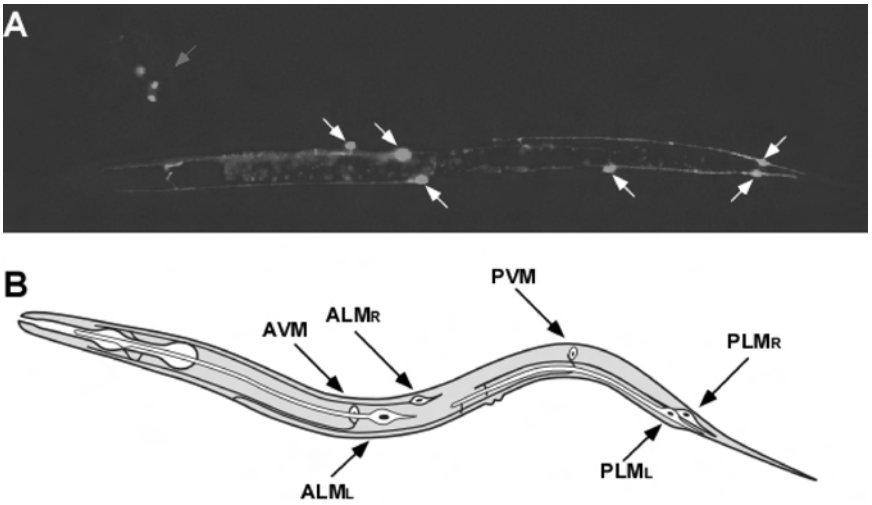

FIGURE 1.1. The *C. elegans* touch receptor neurons. (A) Visualization of touch receptors. Worms are expressing the green fluorescent protein (GFP) under the control of the *mec-4* promoter, which is active only in the six touch receptor neurons. Arrows indicate touch receptor cell bodies. Some touch 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. (See Color Plate 1 in Color Section)

1976; White et al., 1986). 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 (Syntichaki and Tavernarakis, 2004; Tavernarakis et al., 1997).

When gently touched with an eyelash hair (typically attached to a toothpick) on the posterior, an animal will move forward; when touched on the anterior body, it will move backward. This gentle body touch is sensed by the six touch receptor neurons ALML/R (anterior lateral microtubule cell left, right), AVM (anterior ventral microtubule cell), and PLML/R (posterior lateral microtubule cell left, right; Fig. 1.1).

PVM (posterior ventral microtubule) is a neuron that is morphologically similar to the touch receptor neurons and expresses genes specific for touch receptor neurons but has been shown to be incapable of mediating a normal touch response by itself (Chalfie, 1993; Chalfie, 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. The position of the processes along the body axis correlates with the sensory field of the touch cell. Laser ablation of AVM and the ALMs, which have sensory receptor processes in the anterior half of the body, eliminates anterior touch sensitivity and laser ablation of the PLMs,

which have posterior dendritic processes, eliminates posterior touch sensitivity. In addition to mediating touch avoidance, the touch receptor neurons appear to control the spontaneous rate of locomotion because animals that lack functional touch cells are lethargic. The mechanical stimuli that drive spontaneous locomotion are unknown, but could include encounters with objects in their environment or body stretch induced by locomotion itself. Touch receptor neurons have two distinguishing features. First, they are surrounded by a specialized extracellular matrix called the mantle which appears to attach the cell to the cuticle. Second, they are filled with unusual 15-protofilament microtubules (Chalfie and Thomson, 1982). Genetic studies suggest that both features are critical for the function of these neurons as receptors of body touch (reviewed in Syntichaki and Tavernarakis, 2004).

C. elegans displays several additional behaviors that are based on sensory mechanotransduction which have been characterized to a lesser extent. The nose of *C. elegans* is highly sensitive to mechanical stimuli. This region of the body is innervated by many sensory neurons that 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 (Bargmann and Kaplan, 1998; Colbert et al., 1997; Hart et al., 1999; Wicks and Rankin, 1995). Other mechanosensitive behaviors include the response to harsh mechanical stimuli, and the tap withdrawal reflex, where animals retreat in response to a tap on the culture plate (Mah and Rankin, 1992; Rankin, 2002). Furthermore, mechanotransduction appears to also play a regulatory role in processes such as mating, egg laying, feeding, defecation, and maintenance of the pseudocoelomic body cavity pressure (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.

1.2.1. Degenerins and Mechanotransduction in C. elegans

With the sequencing of the *C. elegans* genome now complete, it is possible to survey the entire gene family within this organism. Presently, 30 genes encoding members of the DEG/ENaC family have been identified in the *C. elegans* genome, seven of which have been genetically and molecularly characterized (*deg-1*, *del-1*, *flr-1*, *mec-4*, *mec-10*, *unc-8* and *unc-105*; Table 1.2).

While DEG/ENaC proteins are involved in many diverse biological functions in different organisms, they share a highly conserved overall structure (Benos and Stanton, 1999; Kellenberger and Schild, 2002; Syntichaki and Tavernarakis, 2004). This strong conservation across species suggests that DEG/ENaC family members shared a common ancestor relatively early in evolution (Fig. 1.2).

The basic subunit structure may have been adapted to fit a range of biological needs by the addition or modification of functional domains. This conjecture can be tested by identifying and isolating such structural modules within DEG/ENaC ion channels.

DEG/ENaC proteins range from about 550 to 950 amino acids in length and share several distinguishing blocks of sequence similarity (Fig. 1.3). Subunit topology

TABLE 1.2. The current list of *C. elegans* DEG/ENaC family members and their chromosomal distribution. Genes have been listed alphabetically with the seven genetically characterized ones on top. Phenotypes are those of loss-of-function alleles. All 23 uncharacterized putative degenerin genes encode proteins with the sequence signature of amiloride-sensitive channels. However, some lack certain domains of typical DEG/ENaC ion channels (ND: Not Determined)

Gene name	ORF	Chromosome	Behavior/Phenotype	Reference
deg-1	C47C12.6	X	Touch abnormality	Chalfie and Wolinsky, 1990
del-1	E02H4.1	X	Locomotory defects	Tavernarakis et al., 1997
mec-4	T01C8.7	X	Touch insensitivity	Driscoll and Chalfie, 1991
mec-10	F16F9.5	X	Touch insensitivity	Huang and Chalfie, 1994
flr-1	F02D10.5	X	Fluoride resistance	Katsura et al., 1994
unc-8	R13A1.4	IV	Locomotory defects	Tavernarakis et al., 1997
unc-105	C41C4.5	II	Muscle function defects?	Liu et al., 1996
	C11E4.3	V		
	C11E4.4	X		
	C18B2.6	X		
	C24G7.1	I		
	C24G7.2	I		
	C24G7.4	I		
	C27C12.5	X		
	C46A5.2	X		
	F23B2.3	IV		
	F25D1.4	V		
	F26A3.6	I	ND	The *C. elegans* Sequencing Consortium, 1998
	F28A12.1	V		
	F55G1.12	IV		
	F59F3.4	IV		
	T21C9.3	V		
	T28B8.5	I		
	T28D9.7	II		
	T28F2.7	I		
	T28F4.2	I		
	Y69H2.2	V		
	Y69H2.11	V		
	Y69H2.13	V		
	ZK770.1	I		

is invariable: all DEG/ENaC family members have two membrane-spanning domains with cysteine-rich domains (CRDs, the most conserved is designated CRD3) situated between these two transmembrane segments (Tavernarakis and Driscoll, 2000; Tavernarakis and Driscoll, 2001a). DEG/ENaCs are situated in the membrane such that amino- and carboxy-termini project into the intracellular cytoplasm while most of the protein, including the CRDs, is extracellular (Fig. 1.3) (Garcia-Anoveros and Corey, 1997; Syntichaki and Tavernarakis, 2004). Highly

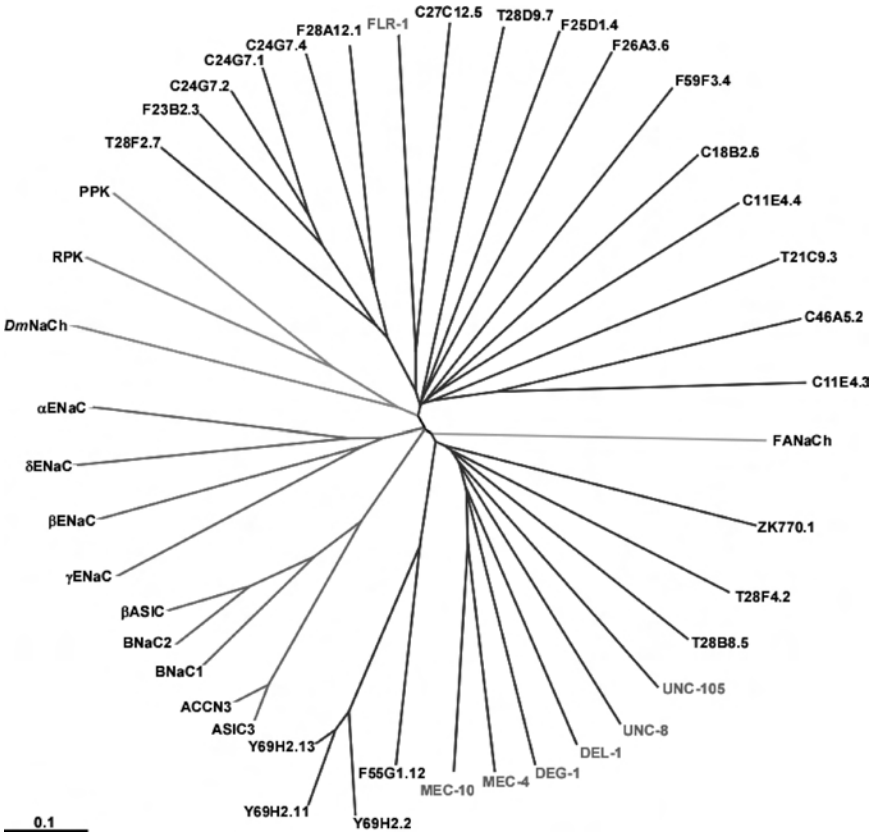

FIGURE 1.2. Phylogenetic relations among DEG/ENaC proteins in nematode degenerins are shown with blue lines. The current degenerin content of the complete nematode genome is included. The seven genetically characterized (DEG-1, DEL-1, FLR-1, MEC-4, MEC-10, UNC-8 and UNC-105) are shown in red. Representative 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. (See Color Plate 2 in Color Section)

conserved regions include the two membrane-spanning domains (MSD I and II), a short amino acid stretch before the first membrane-spanning domain, extracellular cysteine-rich domains (CRDs), an extracellular regulatory domain and a neurotoxin-related domain (NTD) before predicted transmembrane domain II (Tavernarakis and Driscoll, 2000). 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. Interestingly, the NTD is also distantly related to domains in several other proteins including the *Drosophila crumbs* protein, required for epithelial organization (Tepass et al., 1990), *agrin*, a basal lamina

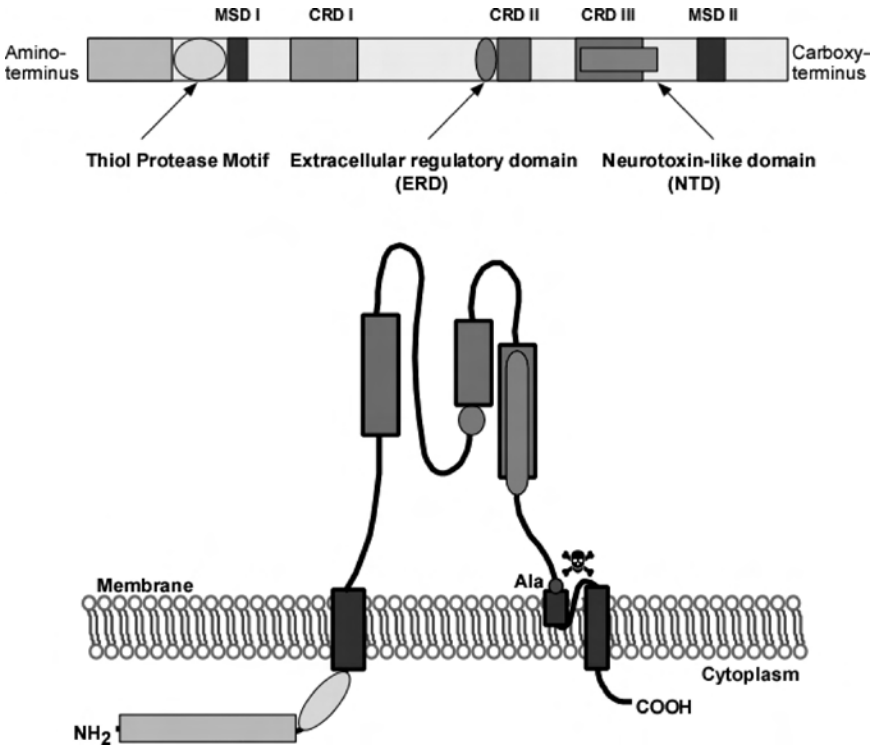

FIGURE 1.3. Schematic representation of DEG/ENaC ion channel subunit structure and topology. (A) Functional/structural domains. Colored boxes indicate defined channel modules. These include the two membrane-spanning domains (MSDs; dark-blue shading), and the three cysteine-rich domains (CRDs; red shading; 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 amino-terminal domain modeled based on protease pro-domains (see Fig. 1.7). (B) Transmembrane topology. Both termini are intracellular with the largest part of the protein situated outside the cell. The dot near MSDII represents the amino-acid position (Alanine 713 in MEC-4) affected in dominant, toxic degenerin mutants. (See Color Plate 3 in Color Section)

protein that mediates aggregation of acetylcholine channels (Rupp et al., 1992), and the selectins that participate in cell adhesion (such as ELAM-1) (Bevilacqua et al., 1989). The presence of related domains in proteins such as *crumbs* and *agrin* implies that such domains might act as interaction modules that mediate analogous interactions needed for tissue organization or protein clustering. We hypothesize that the appearance of neurotoxin-related domains in a specific class of ion channels may be the result of convergent evolution, driven by the requirement for high affinity interaction modules in these proteins.

Amino and carboxy termini are intracellular and a single large domain is positioned outside the cell (Fig. 1.3; Kellenberger and Schild, 2002; Tavernarakis and Driscoll, 2001b). The more amino-terminal of the two membrane-spanning domains (MSDI) is generally hydrophobic, whereas the more carboxy-terminal of these (MSDII) is amphipathic (Hong and Driscoll, 1994; Hong et al., 2000). In general, MSDI is not distinguished by any striking sequence feature except for the strict conservation of a tryptophan residue (corresponding to position W111 in MEC-4), and the strong conservation of a Gln/Asn residue (corresponding to position N125 in MEC-4). MSDII is more distinctive, exhibiting strong conservation of hydrophilic residues (consensus GLWxGxSxxTxxE) that has been implicated in pore function (Hong and Driscoll, 1994). The short highly conserved region before the minimal transmembrane domain is thought to loop back into the membrane to contribute to the channel pore (Benos and Stanton, 1999; Garty and Palmer, 1997; Kellenberger et al., 1999). The extended MSDII homology region (loop + transmembrane part) can be considered a defining characteristic of DEG/ENaC family members.

Below we discuss two nematode mechanosensitive behaviors that involve degenerins: the gentle body-touch response and locomotion. Furthermore, we highlight similarities in the structure and function of these proteins.

1.2.1.1. The Gentle Touch Response

Approximately 15 genes have been identified by genetic analysis, which, when mutated, specifically disrupt gentle body touch sensation. These genes are therefore thought to encode candidate mediators of touch sensitivity (these genes were named *mec* genes because when they are defective, animals are *mech*anosensory 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; Tavernarakis and Driscoll, 1997). 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.

MEC-4, MEC-10 and several related nematode degenerins have a second, unusual property: specific amino acid substitutions in these proteins result in aberrant channels that induce the swelling and subsequent necrotic death of the cells in which they are expressed (Syntichaki and Tavernarakis, 2003). This pathological property is the reason that proteins of this subfamily were originally called degenerins (Chalfie and Wolinsky, 1990). For example, unusual gain-of-function (dominant; *d*) mutations in the *mec-4* gene induce degeneration of the six touch receptor neurons required for the sensation of gentle touch to the body. In contrast, most *mec-4* mutations are recessive loss-of-function mutations that disrupt body touch sensitivity without affecting touch receptor ultrastructure or viability (reviewed in Syntichaki and Tavernarakis, 2004).

Evidence that MEC-4 and MEC-10 co-assemble into the same channel complex include the following: (1) MEC-4 and MEC-10 subunits are co-expressed 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 co-immunoprecipitate (Goodman et al., 2002); and (3) genetic interactions between *mec-4* and *mec-10* have been observed (Gu et al., 1996). For example, *mec-10* can be engineered to encode a death-inducing amino acid substitution *mec-10* (A673V) (Huang and Chalfie, 1994). However, if *mec-10* (A673V) is introduced into a *mec-4* loss-of-function background, neurodegeneration does not occur. This result is consistent with the hypothesis that MEC-10 cannot form a functional channel in the absence of MEC-4. Genetic experiments also suggest that MEC-4 subunits interact with each other. The toxic protein MEC-4 (A713V) encoded by the *mec-4(d)* allele can kill cells even if it is co-expressed with wild-type MEC-4(+) (as occurs in a *trans* heterozygote of genotype *mec-4(d)/mec-4(+)*). However, if toxic MEC-4 (A713V) is co-expressed with a specific *mec-4* allele that encodes a single amino acid substitution in MSDII (e.g., *mec-4(d)/mec-4* (E732K)), neurodegeneration is partially suppressed (Gu et al., 1996). Because one MEC-4 subunit can interfere with the activity of another, it can be inferred that there may be more than one MEC-4 subunit in the channel complex.

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, Ala713Val substitution is also present in MEC-4 (Hong and Driscoll, 1994; Kellenberger et al., 1999; Kellenberger and Schild, 2002). 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 (Schild and Kellenberger, 2001; Schild et al., 1997).

mec-4(d) alleles encode substitutions for a conserved alanine that is positioned extracellularly, adjacent to pore-lining membrane-spanning domain (Fig. 1.3; alanine 713 for MEC-4; Driscoll and Chalfie, 1991). The size of the amino acid sidechain at this position is correlated with toxicity. Substitution of a small sidechain amino acid does not induce degeneration, whereas replacement of the Ala with a large sidechain amino acid is toxic. This suggests that steric hindrance plays a role in the degeneration mechanism and supports the following working model for *mec-4(d)*-induced degeneration: MEC-4 channels, like other channels, can assume alternative open and closed conformations. In adopting the closed conformation, the sidechain of the amino acid at MEC-4 position 713 is proposed to come into close proximity to another part of the channel. Steric interference conferred by a bulky amino acid sidechain prevents such an approach, causing 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* family members (e.g., *deg-1* and *mec-10*) can be altered by analogous amino acid substitutions to

induce neurodegeneration (Chalfie and Wolinsky, 1990; Huang and Chalfie, 1994). In addition, large sidechain substitutions at the analogous position in some neuronally expressed mammalian superfamily members do markedly increase channel conductance (Garcia-Anoveros et al., 1998; Waldmann et al., 1995).

Interestingly, the cell death that occurs appears to involve more than the burst of a cell in response to osmotic imbalance (Syntichaki and Tavernarakis, 2002). Rather, it appears that the necrotic cell death induced by these channels may activate a death program that 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. Electron microscopy studies of degenerating nematode neurons that express the toxic *mec-4(d)* allele have revealed a series of distinct events that take place during degeneration, involving extensive membrane endocytosis and degradation of cellular components (Hall et al., 1997). Thus, the toxic degenerin mutations provide the means with which to examine the molecular genetics of injury-induced cell death in a highly manipulable experimental organism.

1.2.1.2. Sinusoidal Locomotion

Unusual, *s*emi-*d*ominant gain-of-function mutations in another degenerin gene, *unc-8*, (*unc-8(sd)*) induce transient neuronal swelling and severe lack of coordination (Park and Horvitz, 1986; Shreffler et al., 1995; Shreffler and Wolinsky, 1997). *unc-8* encodes a degenerin expressed in several motor neuron classes and in some interneurons and nose touch sensory neurons (Tavernarakis et al., 1997). Interestingly, semi-dominant *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). The genetics of *unc-8* are further similar to those of *mec-4* and *mec-10*; specific *unc-8* alleles can suppress or enhance *unc-8(sd)* mutations *in trans*, suggesting that UNC-8::UNC-8 interactions occur. Another degenerin family member, *del-1*(for *deg*enerin-*l*ike) is co-expressed in a subset of neurons that express *unc-8* (the VA and VB motor neurons) and is likely to assemble into a channel complex with UNC-8 in these cells (Tavernarakis et al., 1997).

What function does the UNC-8 degenerin channel serve in motorneurons? *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. 1.4).

This phenotype indicates that the UNC-8 degenerin channel functions to modulate the locomotory trajectory of the animal.

How does the UNC-8 motor neuron channel influence locomotion? One highly interesting morphological feature of some motorneurons (in particular, the VA and VB motorneurons that co-express *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

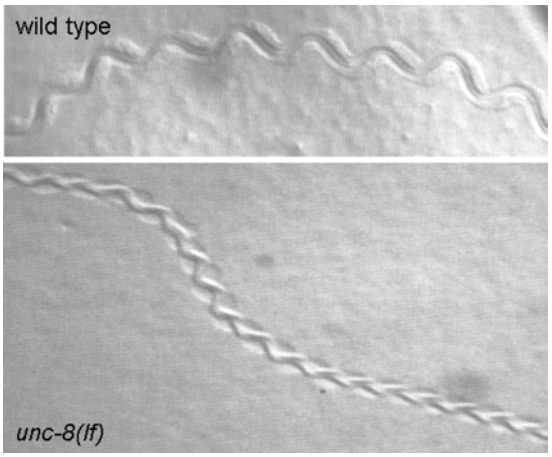

FIGURE 1.4. Proprioception in the nematode. (A) Wild-type animals inscribe a sinusoidal track as they move on an agar plate evenly covered with an *E. coli* bacterial lawn. (B) The characteristic properties (amplitude and wavelength) of tracks inscribed by *unc-8(lf)* mutants are drastically reduced. (See Color Plate 4 in Color Section)

be stretch-sensitive (discussed in White et al., 1976). Given the morphological features of certain motor neurons and the sequence similarity of UNC-8 and DEL-1 to candidate mechanically gated channels, we have proposed that these subunits co-assemble into a stretch-sensitive channel that might be localized to the undifferentiated regions of the motor neuron process (Tavernarakis et al., 1997; reviewed in Syntichaki and Tavernarakis, 2004). When activated by the localized body stretch that occurs during locomotion, this motor neuron channel potentiates signaling at the neuromuscular junction, which is situated at a distance from the site of the stretch stimulus (Fig. 1.5).

The stretch signal enhances motorneuron excitation of muscle, increasing the strength and duration of the pending muscle contraction and directing a full size body turn. In the absence of the stretch activation, the body wave and locomotion still occur, but with significantly reduced amplitude because the potentiating stretch signal is not transmitted. 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.

One important corollary of the *unc-8* mutant studies is that the UNC-8 channel does not appear to be essential for motor neuron function; if this were the case, animals lacking the *unc-8* gene would be severely paralyzed. This observation strengthens the argument that degenerin channels function directly in mechanotransduction rather than merely serving to maintain the osmotic environment so that other channels can function. As is true for the MEC-4 and MEC-10 touch receptor channel, the model of UNC-8 and DEL-1 function that is based on mutant

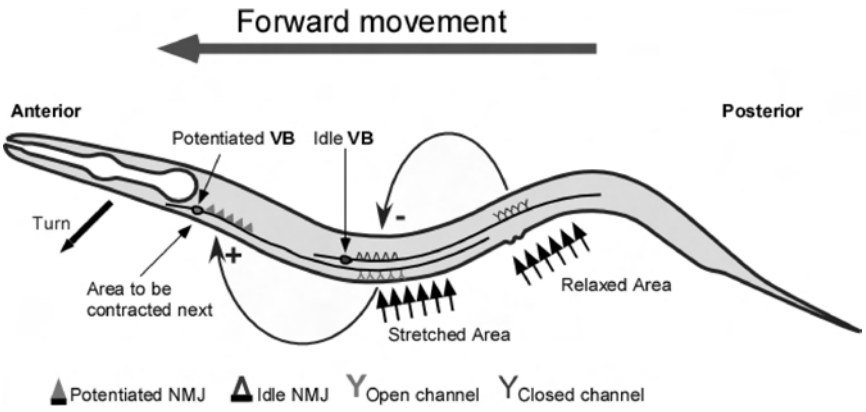

FIGURE 1.5. A model for UNC-8 involvement in stretch-regulated control of locomotion. Schematic diagram of potentiated and inactive VB class motor neurons. Neuro-muscular junctions (signified by triangles) are made near the cell body. Mechanically-activated channels postulated to include UNC-8 (and, possibly in VB motor neurons, DEL-1) subunits (signified by Y figures) are hypothesized to be concentrated at the synapse-free, undifferentiated ends of the VB neuron. Mechanically gated channels could potentiate local excitation of muscle. Body stretch is postulated to activate mechanically gated channels that potentiate the motor neuron signal that excites a specific muscle field. A strong muscle contraction results in a sustained body turn. In *unc-8(lf)* mutants, VB motor neurons lack the stretch-sensitive component that potentiates their signaling and consequently elicit a muscle contraction that is shortened in intensity or duration so that the body turns less deeply. Note that although we depict VB as an example of one motor neuron class that affects locomotion, other motor neuron classes must also be involved in the modification of locomotion in response to body stretch. Sequential activation of motor neurons that are distributed along the ventral nerve cord and signal nonoverlapping groups of muscles, amplifies and propagates the sinusoidal body wave (NMJ: neuromuscular junction). (See Color Plate 6 in Color Section)

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.

1.2.2. A Model for the Nematode Mechanotransducer

The features of cloned touch cell and motorneuron structural genes together with genetic, molecular and electrophysiological data that suggest interactions between them constitute the basis of a model for the nematode mechanotransducing complex (Fig. 1.6).

The central component of the mechanotransduction apparatus is the putative mechanosensitive ion channel that includes multiple MEC-4 and MEC-10 subunits in the case of touch receptor neurons, and UNC-8 and DEL-1 subunits in the case of motorneurons (reviewed in Goodman and Schwarz, 2003; Syntichaki and Tavernarakis, 2004). These subunits assemble to form a channel pore that is lined

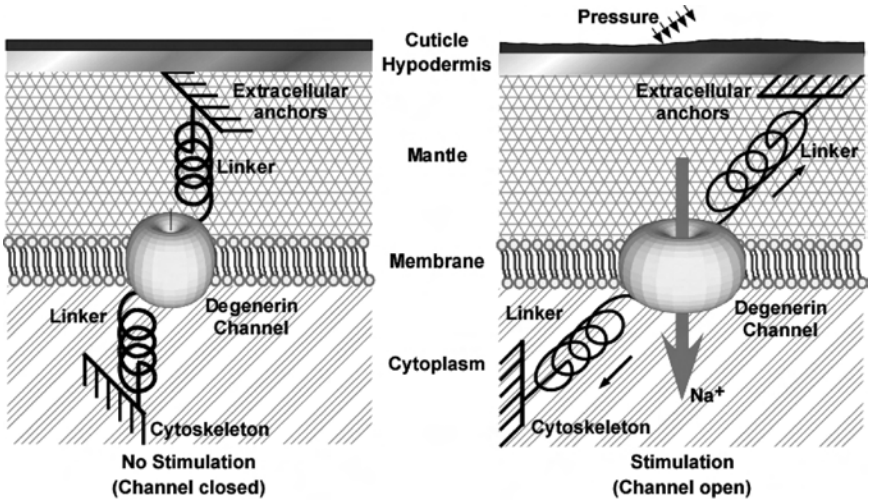

FIGURE 1.6. A mechanotransducing complex in *C. elegans* touch receptor neurons. In the absence of mechanical stimulation the channel is closed and therefore the sensory neuron is idle. Application of a mechanical force to the body of the animal results in distortion of a network of interacting molecules that opens the degenerin channel. Na^+ influx depolarizes the neuron initiating the perceptory integration of the stimulus. (See Color Plate 7 in Color Section)

by the hydrophilic residues of membrane-spanning domain II. Subunits adopt a topology in which the cysteine-rich and neurotoxin-related domains extend into the specialized extracellular matrix outside the touch cell and the amino- and carboxy-termini project into the cytoplasm. Regulated gating depends on mechanical forces exerted on the channel. Tension is delivered by tethering the extracellular channel domains to the specialized extracellular matrix and anchoring intracellular domains to the microtubule cytoskeleton. Outside the cell, channel subunits may contact extracellular matrix components (such as *mec-1*, *mec-5* and/or *mec-9* in the case of the touch receptor mantle; Du et al., 1996; Ernstrom and Chalfie, 2002; Garcia-Anoveros et al., 1995). Inside the cell, channel subunits may interact with the cytoskeleton either directly or via protein links (such as MEC-2 in the touch receptor neurons or UNC-1 in motorneurons; Goodman et al., 2002; Rajaram et al., 1999).

Sequence analysis of recessive loss-of-function *mec-4* alleles has highlighted two regions of MEC-4, which appear especially important in channel gating. Amino acid substitutions that disrupt MEC-4 function cluster within a conserved region that is situated on the intracellular side, close to MSDI (Hong et al., 2000). This region of the channel could interact with cytoskeletal proteins (Fig. 1.7).

Interestingly, the effects of semi-dominant alleles of *unc-8* can be completely blocked by mutations in this conserved region, highlighting its functional importance (Shreffler et al., 1995; Shreffler and Wolinsky, 1997; Tavernarakis et al., 1997). This suppression is observed both when such mutations reside *in cis*, on the same protein molecule as the semi-dominant mutations or *in trans*, on different

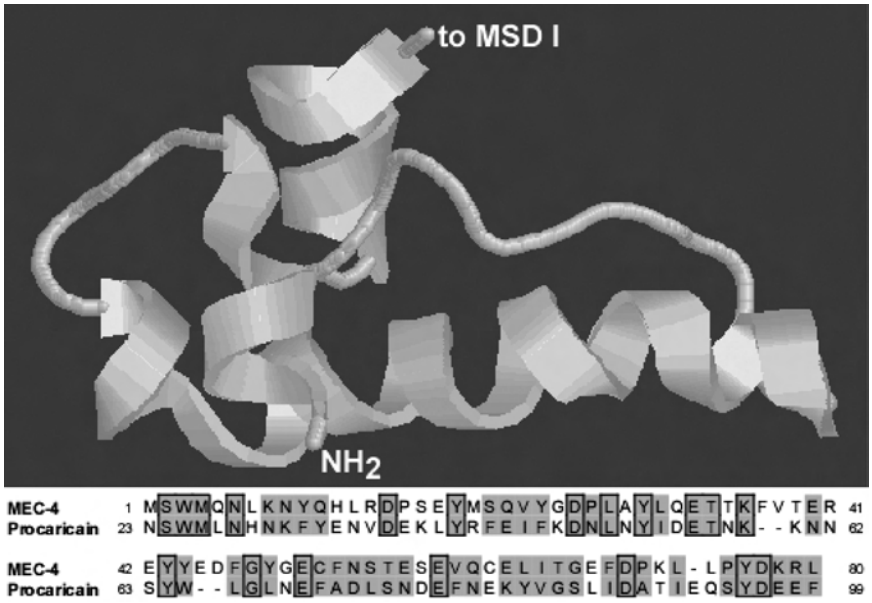

FIGURE 1.7. A three-dimensional model of the extreme, intracellular amino-terminus of MEC-4. The domain has been modeled by homology to the protease procaricain (the relevant alignment is shown at the bottom). The resulting structure appears to have the capacity for protein-protein interactions with a potential hydrophobic surface (Tavernarakis et al., 2001). (See Color Plate 8 in Color Section)

co-expressed genes, as observed in heterozygote animals carrying a semi-dominant allele on one chromosome and a mutation in the conserved intracellular amino terminal region on the other (Shreffler et al., 1995; Tavernarakis et al., 2001). Such a pattern of genetic suppression suggests that UNC-8 proteins interact to form a dimeric or multimeric complex where more than one molecules associate to form a channel. The conserved intracellular amino terminal region could play a role in facilitating such interactions. A second hot-spot for channel-inactivating substitutions is situated near and within NTD or within CRDII (Tavernarakis and Driscoll, 2000). This is a candidate region for interaction of the channel with the extracellular matrix.

The mechanosensory apparatus encompassing MEC-4 and MEC-10 subunits appears to be localized at the long processes of touch receptor neurons (Fig. 1.8). A touch stimulus either could deform the microtubule network, or could perturb the mantle connections to deliver the gating stimulus (Fig. 1.6). In both scenarios, Na^+ influx would activate the touch receptor to signal the appropriate locomotory response. This is an attractive hypothesis, but confirmation has been stonewalled by the technical challenge of stimulating and recording directly from the *C. elegans* touch neurons, which are tiny (soma on the order of 1 μm) and embedded in the hypodermis. Furthermore, reconstitution of the mechanotransducing complex in a

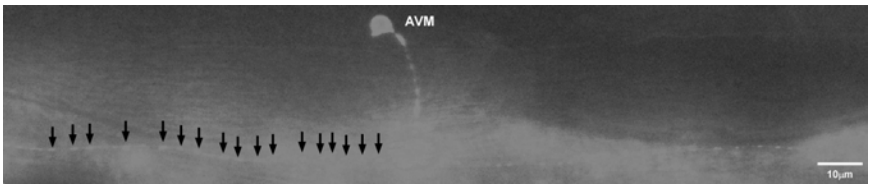

FIGURE 1.8. Punctate localization of a putative mechanosensitive ion channel subunit. Image of an AVM touch receptor neuron expressing a GFP-tagged MEC-4 protein. Fluorescence is unevenly distributed along the process of the neuron in distinct puncta, which may represent the location of the mechanotransducing apparatus. (See Color Plate 5 in Color Section)

heterologous system is likely to require both channel expression and regeneration of gating contacts, which would be no small feat. Nonetheless, ongoing efforts to surmount technical difficulties in direct recording from nematode sensory neurons may soon provide decisive information.

Because it has not yet been possible to directly demonstrate mechanical gating of the MEC-4/MEC-10 touch receptor channel or the UNC-8 channels using electrophysiological approaches, two models for the biological activities of degenerin channels have been considered (Syntichaki and Tavernarakis, 2004). In the simplest model, the degenerin channel mediates mechanotransduction directly. The alternative model is that the degenerin channel acts indirectly to maintain a required osmotic balance within a neuron so that a mechanosensitive channel, yet to be identified, can function. In the case of the touch receptor channel, the absence of either MEC-4 or MEC-10 renders the mechanosensory neuron nonfunctional, making it impossible to distinguish between the two alternative hypotheses. The situation with the UNC-8 channel is different. It is clear from the phenotype of *unc-8* null mutants that the majority of neurons that express *unc-8* must remain functional in the absence of *unc-8* activity (Tavernarakis et al., 1997). Our understanding of neuronal circuitry and characterized behavioral mutants argues that if these neurons were not functional, *unc-8* null mutants would exhibit severely defective locomotion. Given that *unc-8* null mutants move in a manner only marginally different from wild-type animals, the case that the UNC-8 channel maintains an osmotic milieu required for the function of other neuronal channels is weakened. One caveat to this discussion is that we cannot rule out the possibility that a functionally redundant and as yet unidentified degenerin family member might be co-expressed with *unc-8* and could nearly compensate for its absence.

The model proposed for mechanotransduction in the touch receptor neurons and motorneurons of *C. elegans* shares the same underlying principle and features of the proposed gating mechanism of mechanosensory ion channels in *Drosophila* sensory bristles, and the channels that respond to auditory stimuli in the hair cells of the vertebrate inner ear (Gillespie and Walker, 2001; Hamill and Martinac, 2001; Hudspeth, 1989; Pickles and Corey, 1992) Hair cells have bundles of a few hundred stereocilia on their apical surface, which mediate sensory transduction. Stereocilia are connected at their distal ends to neighboring stereocilia by filaments

called tip links. The integrity of the tip links is essential for channel opening and the mechanosensitive channels appear to be situated at the ends of the stereocilia, near the connecting tip links. Directional deflection of the stereocilia relative to one another introduces tension on the tip links, which is proposed to open the mechanosensitive hair cell channels directly.

1.3. DEG/ENaC Mechanosensitive Channels in *Drosophila*

Two members of the DEG/ENaC family of ion channels have been implicated in mechanotransduction in *Drosophila*, pickpocket (PPK) and DmNaCh. PPK was found in the sensory dendrites of a subset of peripheral neurons in late-stage embryos and early larvae. In insects, such multiple dendritic neurons play key roles in touch sensation and proprioception and their morphology resembles human mechanosensory free nerve endings. These results suggest that PPK may be a channel subunit involved in mechanosensation (Adams et al., 1998). DmNaCh is expressed in the dendritic arbor subtype of multiple dendritic (md) sensory neurons in the *Drosophila* peripheral nervous system. DmNaCh mRNA was first detected during late embryogenesis. While the origin and specification of md neurons are well documented, their roles are still poorly understood. They could function as stretch or touch receptors, raising the possibility that DmNaC could also be involved in mechanotransduction (Darboux et al., 1998).

1.4. DEG/ENaC Mechanosensitive Channels in Mammals

An increasing amount of evidence suggests that some mammalian DEG/ENaC proteins may play a role in mechanosensation similarly to their nematode counterparts. Mammalian members of the DEG/ENaC family fall into two classes. The first class includes α, β, and γ, ENaC, which form a multimeric epithelial channel that performs critical functions in Na^+ reabsorption in the kidney and in fluid clearance in neonatal lung (Kellenberger and Schild, 2002). A second ENaC subfamily includes proteins more prevalently found in neurons (Alvarez de la Rosa et al., 2003; Wemmie et al., 2003). Channels in this group are known to be gated by protons and have thus been classified as the ASIC family (acid-sensing ion channel) (Deval et al., 2004; Waldmann et al., 1999). The acid-sensitive gating properties of certain neuronally expressed homomeric or heteromeric channels fuel speculation that these ASIC channels might respond to the local acidosis that occurs in injured or inflamed tissue and thus play critical roles in nociception (Alvarez de la Rosa et al., 2002; Reeh and Steen, 1996).

ENaC proteins are expressed in epithelial cells of the colon, in the apical membranes of specific epithelial structures such as the renal cortical collecting ducts (CCDs) and in airways cells. ENaC subunits are also detected in epithelial and nonepithelial structures in the rat cochlea. (Kellenberger and Schild, 2002). ENaC proteins in endothelial cells are likely to be involved in responses to mechanical

stimuli. Because of their location, endothelial cells are subjected to various types of mechanical forces such as osmotic pressure, hydrostatic pressure, fluid shear stress, mechanical stress by blood flow, air breathing and air vibration on the hair cells of the inner ear. Because ENaC channels are sensitive to membrane stretch and changes to the cell volume it is possible that ENaC conductance is regulated by these biomechanical forces.

There are strong indications that ENaC subunits may be components of the baroreceptor mechanotransducer, one of the most potent regulators of anterial pressure and neurohumoral control of the circulation (Drummond et al., 1998; Drummond et al., 2001). Baroreceptors innervate the aortic arch and carotid sinuses and are activated by pressure-induced vessel-wall stretch. Furthermore, it has been shown that β- and γENaC, but not αENaC, are located in tactile sensory receptors in the hairless skin of the rat paw, suggesting that these subunits may be components of a mechanosensory receptor for touch (Drummond et al., 2000). ENaC immunoreactivity was also detected in mechanosensory lanceolate nerve endings of the rat mystacial pad in the vibrassae (whisker).

Members of the ASIC (acid-sensing ion channel) subgroup of the DEG/ENaC family have been implicated in mechanotransduction in mammals. BNC1 (brain Na^+ channel; also known as MDEG, BNaC1, ASIC2) (Garcia-Anoveros et al., 1997; Price et al., 1996; Waldmann et al., 1996; Waldmann and Lazdunski, 1998) has emerged as promising candidate for a mechanosensitive channel; it is the ASIC member most similar in amino acid sequence to nematode MEC-10 and can be can be genetically altered analogously to MEC-4 and MEC-10 to generate hyperactive, toxic channels (Champigny et al., 1998; Waldmann et al., 1996). There exist two splice variants of BNC1 (a and b; also known as MDEG1 and MDEG2; Lingueglia et al., 1997; Price et al., 1996; Waldmann et al., 1999) that share a common carboxy-terminal half.

In rodent hairy skin, several specialized nerve termini function as mechanoreceptors, including rapidly adapting (RA), slowly adapting (SA), and D-hair receptors (Koltzenburg et al., 1997). Antisera raised against BNC1 identifies large numbers of central nervous system neurons, but also reveals that BNC1 specifically localizes to the palisades of lanceolate nerve terminals—fine parallel processes projected in the hair follicle and surrounding the hair shaft, a likely site for sensation of hair movement (Price et al., 2000). These nerve terminals house one type of rapidly adapting mechanoreceptor. Interestingly, in these studies BNC1 immunoreactivity is not prevalent in other nerve termini intimately associated with the hair follicle and implicated in mechanotransduction, such as the pilo-Ruffini endings that also circle the hair shaft terminal, or other mechanoreceptors or nociceptors (Price et al., 2000). The specific subcellular localization is striking in that many large- and small-diameter dorsal root ganglion neurons express messages homologous to BNC1, yet the protein is localized to only a few mechanosensory termini (Price et al., 2000). Broad transcript expression in large and small diameter neurons, but rare localization of the protein in nerve termini, has been observed for ENaCs in the dorsal root ganglion and in baroceptor neurons (Chen et al., 1998; Drummond et al., 2000; Garcia-Anoveros et al., 2001; Waldmann

and Lazdunski, 1998). Such specificity indicates that mechanisms for localized or selective positioning of DEG/ENaC channels are operative in peripheral neurons. Alternatively, channel proteins may not sufficiently concentrated to be easily detected by immunological methods, a characteristic of typical mechanoreceptor channels.

Does BNC1 play a role in mechanosensation or nociception? Either (or both) is plausible because BNC1 is detectable in both large-diameter neurons (mostly mechanosensitive neurons) and small-diameter neurons (mostly nociceptors) of the dorsal root ganglion (Price et al., 2000; Waldmann and Lazdunski, 1998). Generation of a BNC1 mouse knockout enabled testing of these possibilities. Both splice variants of BNC1 were eliminated in this mouse (Price et al., 2000). At a gross level, the BNC1 null mice appear generally normal in development, size, fertility, and behavior.

To address a potential function in mechanotransduction, detailed characterization of skin sensory neurons was performed on a skin-nerve preparation in which nerve terminals are tested for response to an applied displacement force (Price et al., 2000). This hairy skin preparation houses all five specialized mechanoreceptor types, classified based on their electrophysiological properties: rapidly adapting (RA) low-threshold mechanoreceptors, slowly adapting (SA) low-threshold mechanoreceptors, D-hair mechanoreceptors, A-fiber mechano-nociceptors, and polymodal C-fiber mechano-nociceptors (Koltzenburg et al., 1997). In BNC1 $-/-$ animals, neither the stimulus-response curves nor the median force required to activate D-hair mechanoreceptors, A-fiber mechano-nociceptors and C-fiber mechano-nociceptors is altered, compared to BNC1$+/+$ controls (Price et al., 2000).

Likewise, all efforts to test for changes in acid-induced responses and nociception in dorsal root ganglion neurons and poly-modal C fibers failed to indicate an essential role for BNC1 in modulating H^+-gated currents in these cells. In contrast, a striking change in the function of RA and SA low-threshold mechanoreceptors was observed in the BNC1 null mutant. Although the minimal force detectable for activation of these mechanoreceptors remains the same, the stimulus-response curve for RA, and to a lesser extent SA, BCN1–/– neurons is significantly different (Price et al., 2000).

In wild-type nerve terminals, increasing the force exerted on the fiber elicits increasing numbers of action potentials. Mutant neurons still respond to displacement, but produce fewer action potentials over a comparable range of stimuli. Interestingly, the effects on the action potential do not appear to result from developmental defects in the neurons involved. There are no apparent differences in the proportion of RA and SA fibers in skin preparations of wild type and mutant mice. In addition, the number and morphology of lanceolate fibers (one, but not the only, type of RA receptor) is similar in BNC1$+/+$ and –/– animals (Price et al., 2000). Similarly, in nematodes the touch receptor neurons can develop normally in the absence of MEC-4 channels. Also important is that the defects in action potential firing in the BNC1 mutant appear to affect something other than the capacity to generate an action potential. Injection currents required to elicit action

potentials in cultured low-threshold mechanoreceptor neurons from BNC1+/+ and BNC1–/– mice are similar. Because the basic capacity to convert a depolarizing inward current to an action potential appears to be normal in the BNC1–/– sensory neurons, it appears that the problem in BNC1–/– neurons is the actual generation of a mechanically induced depolarizing potential, consistent with the hypothesis that BNC1 participates directly in a mechanosensitive channel.

The consequences of the BNC1 channel deficiency, although somewhat modest at first glance, may be of profound biological importance because in humans the dynamic sensitivity of RA and SA receptors is thought to be critical for perception and discrimination of touch sensation (Jarvilehto et al., 1976; Jarvilehto et al., 1981). Why might the response be modified rather than eliminated in mechanosensitive neurons of the BNC1 knockout? One plausible reason is that DEG/ENaC channels are most often hetero-multimeric and BNC1 might act more as a modulatory subunit than as the core of a mechanotransducing complex, much as β and γENaC are less critical than αENaC function in kidney epithelia. Alternatively, different DEG/ENaC channels (or other channels) may perform redundant functions in the same neurons. Consistent with this possibility, ENaC subunits have been immunologically detected in neurons expressing BNC1, suggesting ENaC subunits could be components of mechanotransducing channels in neurons as well.

In addition to BNC1, ASIC3 (also known as DRASIC) is a good candidate for transduction of mechanical stimuli because it is expressed in mechanosensors and nociceptors (Price et al., 2001). ASIC3 was detected in DRG neurons, both small- and large-diameter cells, in rapidly adapting (RA) mechanoreceptors (fibers of Meissner corpuscles, guard hair follicles), in slowly adapting mechanoreceptors (Merker cells) and in AM mechano-nociceptors. RA mechanoreceptors respond to light touch and AM nociceptors respond to noxious stimuli (pain sensation). Loss of ASIC3 in mice enhanced mechanosensitivity in RA mechanoreceptors and reduced mechanosensitivity in AM nociceptors. ASIC3$^{-/-}$ mice displayed decreased responses in acidic and noxious heat stimulus. Thus, it is suggested that ASIC3 participates in mechanosensation in response to mechanical stimuli and changes in pH (Price et al., 2001).

1.5. Outlook

Genetic analyses have been highly successful in identifying genes needed for mechanosensitive behaviors (Chalfie, 1997; Eberl et al., 1997; Gillespie and Walker, 2001; Hamill and Martinac, 2001; Nicolson et al., 1998). Still, limitations of the genetic approach to dissection of mechanotransduction mechanisms should be mentioned. Genes that encode products needed for the activities of mechanotransducing complexes in multiple cell types or that perform multiple cellular functions might have evaded genetic detection because mutations in such genes would be expected to be severely uncoordinated or even lethal. Indeed, many mutations that affect mechanosensation in *Drosophila* render animals severely uncoordinated and nearly inviable (Eberl et al., 1997; Kernan et al., 1994). 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 identified.

The detailed model for mechanotransduction in *C. elegans* 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 recently begun to be addressed experimentally (Chelur et al., 2002; Goodman et al., 2002).

More challenging and most critical, the hypothesis that a degenerin-containing channel is mechanically gated must be addressed. This may be particularly difficult because at present it is not straightforward to record directly from tiny *C. elegans* neurons. Expression of the MEC-4/MEC-10 or (UNC-8/DEL-1) channel in heterologous systems such as *Xenopus* oocytes is complicated by the presence of the many endogenous mechanically gated ion channels (Hamill et al., 1992; McBride and Hamill, 1992; Zhang et al., 2000), and by the likely possibility that not only the multimeric channel, but essential interacting proteins will have to be assembled to gate the channel (Gu et al., 1996; Syntichaki and Tavernarakis, 2004).

A question that remains to be addressed is whether the mammalian counterparts of the *C. elegans* degenerins play specialized roles in mechanical signaling in humans. A significant step toward addressing this question has been accomplished with the demonstration that BNC1 is involved in mechanosensory signaling in the skin as we have described above. Even though, the candidacy of BNC1 for being in the core of a mechanotransducing complex was greatly boosted by these results, a demanding critic would argue that albeit very strong, it still remains just a candidacy. The potential role of BNC1 as part of the core mechanotransducing channel can still only be inferred from these experiments and is not directly proven. It is still possible that BNC1 forms or participates in an auxiliary channel that facilitates the function of the actual mechanotransducing channel. A BNC1 knockout does not completely eliminate the responses to mechanical stimuli (Price et al., 2000). The incomplete nature of the BNC1 deficiency effects indicates that even if BNC1 is indeed part of the core mechanosensory channel, it most likely is not the only critical one. Alternatively, there might be more than one, different mechanotransducing complexes within one neuron, with different properties and composition. The above arguments however, are by no means confined to BNC1. On the same basis, MEC-4/MEC-10 and UNC-8/DEL-1 in *C. elegans* as well as PPK in *Drosophila* might not be parts of the real mechanotransducer but only auxiliary ion channels.

The recent identification of another strong candidate mechanosensory channel, the *Drosophila* NompC, adds to the list of candidate mechanosensitive ion channels (Walker et al., 2000). NompC is unrelated in amino acid sequence to DEG/ENaC channels and is required for normal mechanosensitive currents in fly hair bristles (Walker et al., 2000). Evidence implicating NompC in mechanotransduction is especially convincing given the supporting electrophysiological analysis that is

feasible in this system, and the availability of mutants with altered properties and intermediate effects. Therefore, NompC homologues in other organisms, including humans, emerge as putative mechanosensitive ion channels. Even in this case, however, there are caveats; the absence of NompC does not completely eliminate mechanosensitive currents in *Drosophila* hair bristles. Furthermore, the identities and properties of force-generating tethers of NompC in mechanotransducing complexes will need to be determined. Another issue that needs to be addressed is the potential interplay between DEG/ENaC and NompC channels in mechanosensory cells before a clear understanding of mechanotransduction can be achieved.

Despite the considerable progress that has been achieved on all fronts in recent years toward dissecting the process of sensory mechanotransduction at the molecular level, several thorny questions still beg for answers. What is the gating mechanism of mechanosensitive ion channels? How is tension delivered to the mechanotransducing complex? What additional molecules play a part in the biological response to mechanical stimuli? Are human sensory mechanotransducers similar in composition and function to nematode or *Drosophila* ones? Are DEG/ENaC truly the core mechanosensitive channels or are they merely auxiliary channels/components?

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 wonderful properties. It is equally important to seek and identify these. Without them, our understanding of mechanical transduction will never be complete even if the identity of the core ion channel is revealed. Let us keep in mind that 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 towards this goal, such as yeast two-hybrid screens and biochemical methods of co-purification of channel complexes together with anchoring proteins. The advent of the human genome sequence will provide the full set of testable DEG/ENaC candidates for mechanotransduction in humans. Some of these may be more closely related to nematode proteins specialized for mechanotransduction than currently identified family members, and may be the long-sought human mechanosensors. In addition, fine mutations that do not dramatically incapacitate a candidate channel, might be engineered back into mice to then examine how these correlate to the characteristics of mechanically induced currents.

Characterization of expression patterns of all ASIC and ENaC family members in these animals and genetic knockouts of other candidate mechanotransducer channels will be required to address the question of functional redundancy, work that can be easily pursued in the post-genome era. Obviously such studies should also reveal whether other DEG/ENaC family members are needed for the function of other mechanoreceptors or nociceptors in mouse skin. A question that remains to be resolved is how broadly DEG/ENaC family members will prove to be involved in mechanotransduction. Analyses of the mammalian ENaC channel in lipid bilayers suggests that its gating can be influenced by membrane stretch (Awayda et al., 1995;

Ismailov et al., 1997), although interpretation of these studies requires attention to experimental caveats (Rossier, 1998).

A tremendous boost to sensory mechanotransduction studies will be provided when the necessary technology that allows direct recordings from nematode neurons is achieved Although, electrophysiological recording from some *C. elegans* neurons and muscles are possible, the touch receptor neurons and other sensory neurons are beyond the realm of feasibility given the current state of the art (Avery et al., 1995; Goodman et al., 1998). The capacity to perform electrophysiological studies on degenerin or other ion channels while they are kept embedded in their natural surroundings is the currently missing tool. Perhaps, the development of new, noninvasive monitoring and measurement technologies will be required in the case of the tiny *C. elegans* neurons (Bouevitch et al., 1993; Khatchatouriants et al., 2000). 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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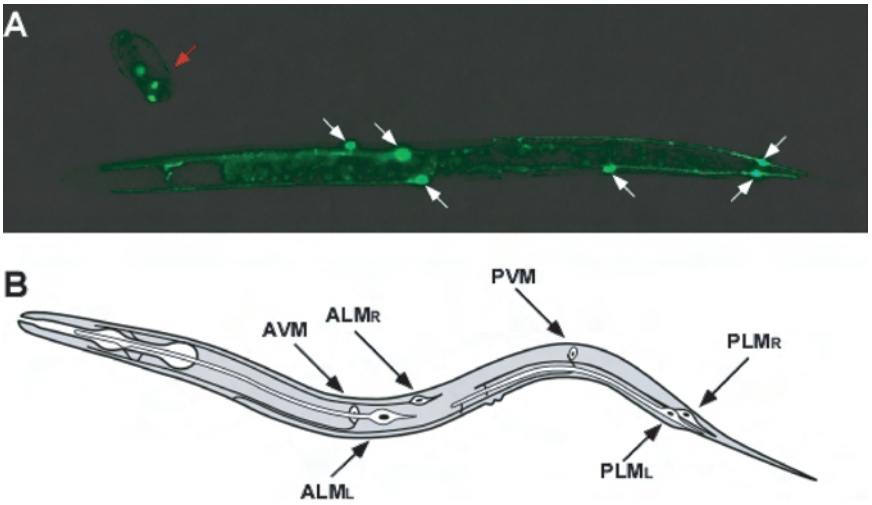

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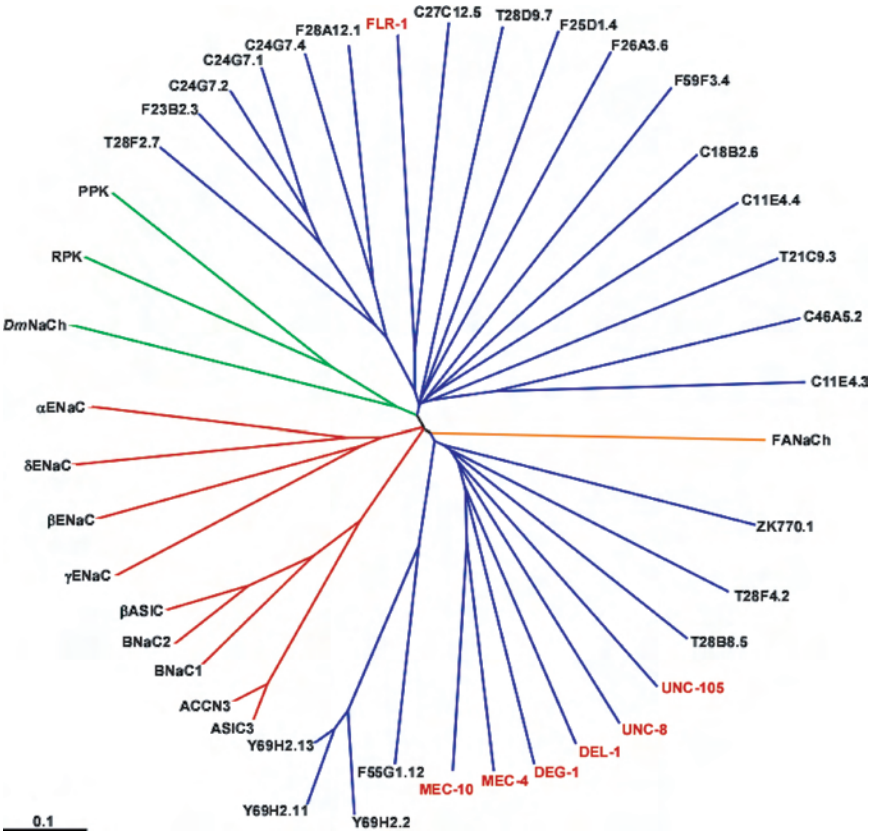

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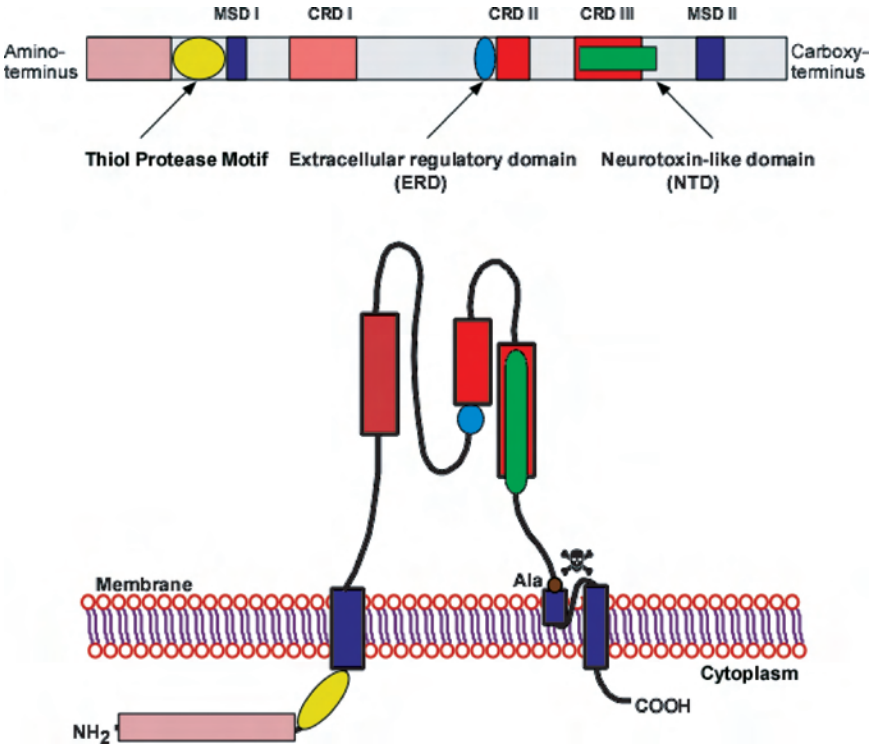

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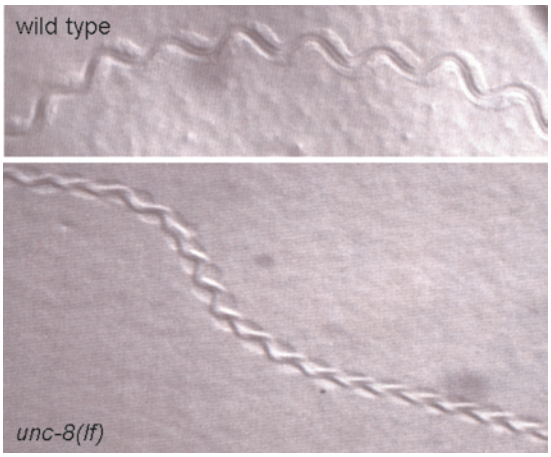

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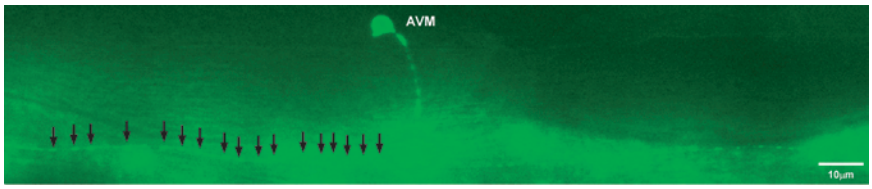

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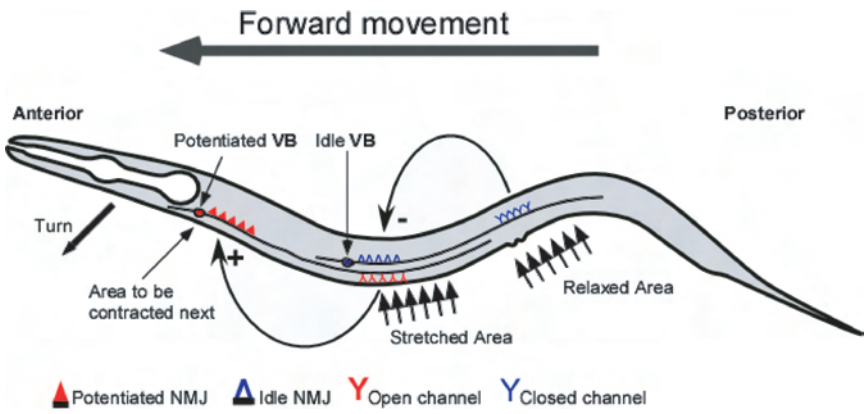

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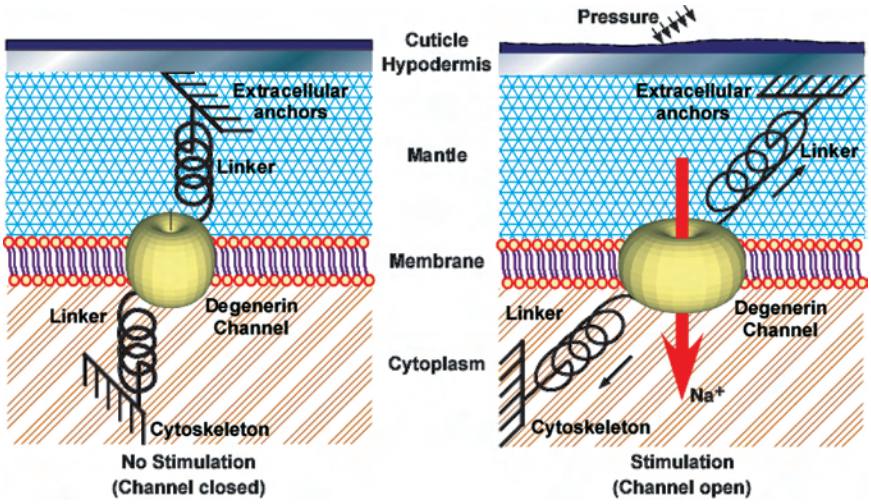

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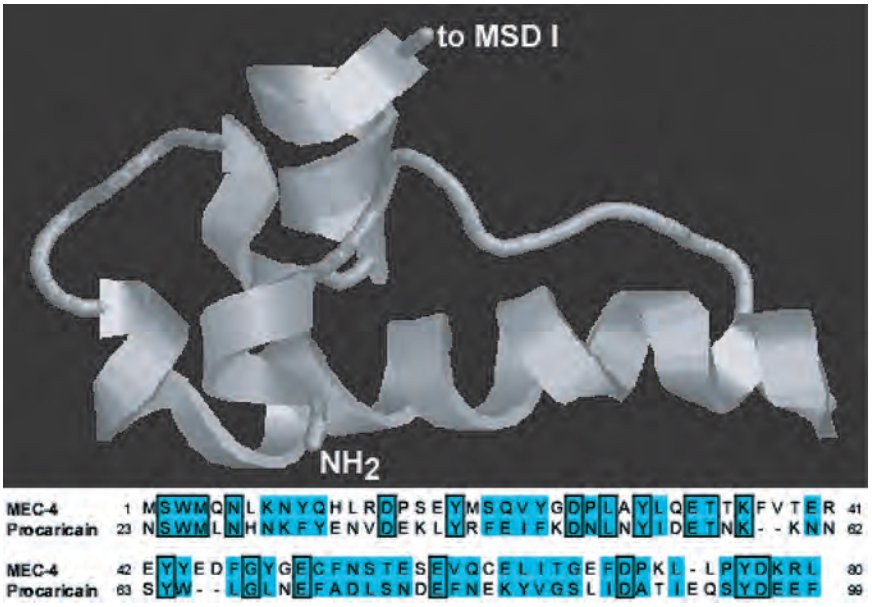

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