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REVIEW

Mechanotransduction in the Nematode *Caenorhabditis elegans*

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ABSTRACT

Mechanotransduction is the conversion of a mechanical stimulus into a biological response and constitutes the basis for a plethora of fundamental biological processes such as the senses of touch, balance and hearing and contributes critically to development and homeostasis in all organisms. Recent, genetic and electrophysiological studies have shown that specialized macromolecular complexes, encompassing mechanically gated ion channels, play a central role in the transformation of mechanical forces into a cellular signal, which takes place in mechanosensory organs of diverse organisms. These complexes are highly efficient sensors, closely entangled with their surrounding environment. Such association appears essential for proper channel gating, and provides proximity of the mechanosensory apparatus to the source of triggering mechanical energy. Genetic and molecular evidence collected in model organisms such as the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and the mouse highlight distinct classes of mechanically gated ion channels and interacting molecules, which are likely parts of the mechanotransducing apparatus. In this article, we review the progress towards deciphering mechanotransduction in *C. elegans*. The exceptional amenability of this simple worm to genetic and molecular manipulations has facilitated the dissection of a metazoan mechanotransducer complex to unprecedented detail.

CONTENTS

1. INTRODUCTION

Perception of incident mechanical stimuli is critically important for interfacing with the physical world. Ubiquitous mechanical stimuli permeate the environment of every living cell and every organism. The process by which cells convert mechanical energy into electrical or chemical signals is called *mechanotransduction* and appears to be a universal property of all living organisms ranging from bacteria to humans [8, 44, 52, 118]. The capacity to respond and adjust to mechanical inputs plays a pivotal role in numerous fundamental physiological phenomena such as the perception of sound and gravity, which underlie our senses of hearing and balance [46, 56, 78]. Touch sensation and proprioception (the coordinated movement of our body parts) are additional manifestations of responsiveness to mechanical stimulation [47, 135, 137, 150]. Somewhat less appreciated but by far not less important is the critical role of mechanotransduction in the stretch-activated reflexes of vascular epithelia and smooth muscle and in the regulation of systemic fluid homeostasis and blood pressure [47, 86, 133, 138, 150]. Mechanotransduction is also critical for the prevention of polyspermy during fertilization, cell volume and shape regulation, cell locomotion, and tissue development and morphogenesis [75, 81, 113]. In plants, mechanotransduction is the basis of gravitaxis and turgor control [90, 104]. In protists (*Paramecium*, *Stentor*) mechanotransduction underlies gravikinesis (the swimming against the gravity vector in order to avoid sedimentation [7, 50, 66, 94].

All living organisms have developed highly specialized structures that are receptive to mechanical forces originating either from the surrounding environment or from within the organism itself. Among the most elaborate

and greatly efficient, such structures are the mechanotransducers that are responsible for sensory awareness, for example, those facilitating touch, balance proprioception and hearing [46, 52, 56, 133]. The mechanisms underlying the capability of living cells to receive and act in response to mechanical inputs are among the most ancient, implemented during evolution. Proteins with mechanosensitive properties are ubiquitously present in eubacteria, archaea and eukarya, and are postulated to have been an essential part of the physiology of the Last Universal Ancestor [46, 80, 82, 84, 95]. The first mechanosensitive processes may have evolved as backup mechanisms for cell protection, e.g. to reduce intracellular pressure and membrane tension during osmotic swelling. Subsequent organismal diversification and specialization resulted in variable requirements for mechanotransduction in different organisms [99]. Hence, evolutionary pressure has shaped a large repertoire of mechanotransducers, optimized for a great assortment of tasks that range from maintenance of intracellular osmotic balance and pressure to our impressive ability of hearing and discriminating sounds, and reading Braille code with our fingertips [52, 60].

In this article, we introduce the nematode *Caenorhabditis elegans* as a platform for investigating the molecular mechanisms of mechano-transduction and survey our current understanding of mechanosensitive behaviours in this simple metazoan.

2. *C. ELEGANS:* A SIMPLE NEMATODE WORM

C .elegans is a small (1 mm), hermaphroditic soil nematode. It completes a reproductive life cycle in 2.5 days at 25^0C. Under normal conditions it develops from fertilized embryo through 4 larvae stages to become egg-laying adult and lives for 2 weeks thereafter [9]. Under adverse conditions such as high temperature, food deprivation and overcrowding, larvae enter to an alternative stage called “dauer” and can survive for months [53].

The simple body plan, the transparency of the egg and the cuticle of the nematode facilitated the detailed developmental exploitation and the anatomical characterization of the animal [145, 152; www.wormatlas.org]. The complete sequence of cell divisions during the development of the fertilized embryos into the 959-celled adult has been elucidated [130]. In addition, the structure and connectivity of the whole nervous system of the animal has been determined by means of electron microscopy sectioning. This has allowed the complete reconstruction of synaptic connection patterns made by each of the 302 neurons of the animal and the full “wiring diagram” of the *C. elegans* nervous system has been elucidated. Neurons have been categorized in regard to anatomical and biochemical properties into 118 different classes [151, 152]. Moreover, the function of individual neurons

has been defined through laser beam microsurgery experiments and neuronal circuits responsible for different behaviours have been characterized.

C. elegans is a powerful genetic system. Random mutagenesis and precise genetic mapping can be achieved through a dense single nucleotide polymorphism map [83, 155]. Cloning of mutagenized genes is a simple process due to the availability of a whole genome physical map consisting of overlapping cosmid and YAC clones which cover most of the chromosomes [16, 28]. The *C. elegans* genome is organized in six chromosomes and it is fully sequenced and annotated [139; www.wormbase.org]. On the whole, the broad range of genetic and molecular approaches that can be utilized in *C. elegans* greatly facilitates thorough and multifaceted investigation of fundamental problems in biology such as mechanical signalling.

3. *C. ELEGANS* MECHANOSENSITIVE BEHAVIOURS

Many behaviours displayed by *C. elegans* are direct manifestations of mechanosensitivity, making it exceptionally attractive for investigating mechanotransduction [4, 5, 12, 17, 18, 34, 67, 77, 91, 111, 131, 135, 154, 157]. The best characterized such behaviour is the response to a gentle mechanical stimulus delivered transversely along the body of the animal, typically by means of an eyelash hair attached onto a toothpick (the 'gentle body touch response'; [17, 18, 67, 135]). We discuss studies elucidating the molecular mechanisms of this touch response in the following section. Other mechanosensory responses are the generation and maintenance of the characteristic coordinated sinusoidal pattern of locomotion (analogous to proprioception; [135, 137]; see below), and the nose touch response, which can be further categorized into the head-on collision response and the head withdrawal response [33, 77].

When animals collide with an obstacle in a nose-on fashion during the course of normal locomotion they respond by reversing their direction of movement [4, 26]. Three classes of mechanosensory neurons, ASH, FLP, and OLQ, mediate this avoidance response [4, 67, 77, 154]. Each of these sensory neurons accounts for a part of the normal response, which is quantitative with normal animals responding about 90% of the time. Laser ablation and genetic studies have demonstrated that each sensory neuron contributes to the overall responsiveness as follows: ASH, 45%; FLP, 29%; and OLQ, 5%. The remaining 10% of the responses are mediated by the ALM and AVM neurons, which sense anterior body touch [33, 77]. It is unclear what distinguishes the function of the three nose touch neurons. One attractive possibility is that these cells differ in their sensitivities and that the intensities of nose touch stimuli vary according to the violence of the collision. If this were the case, it would be expected that the most sensitive

neuron (ASH) would account for the majority of responses while less sensitive neurons (FLP and OLQ) would account for the remainder. In addition to their mechanosensory properties, the ASH neurons are part of a chemosensory organ, the amphid sensilla, with their sensory endings exposed to the external environment [103, 145]. The ASH neurons serve chemosensory and osmosensory functions, mediating avoidance of osmotic repellents [63, 64]. Several classes of chemosensory neurons respond to multiple chemical stimuli in *C. elegans*. However, ASH is unique among them in responding to such divergent stimuli. In this respect, ASH neurons are similar to vertebrate neurons that sense painful stimuli, which are called nociceptors. For their multi-sensory capabilities, the ASH neurons have been categorized as polymodal sensory neurons [33, 77].

An additional mechanosensory response in the worm is that to harsh mechanical stimuli [22, 33, 49]. Although animals lacking functional touch cells are insensitive to touch with an eyelash, they remain sensitive to prodding with a platinum wire (typically responding by undergoing backward movement; [33, 134, 135]). This result suggests that a separate mechanosensory circuit mediates sensitivity to harsh touch stimuli. The PVD neurons are thought to be harsh touch sensory neurons for several reasons. First, similarly to the touch receptor neurons and motorneurons, the PVD neurons have long undifferentiated processes that run along the lateral body wall, which could be mechanosensory [154]. Second, the PVD neurons express genes involved in touch cell differentiation (e.g. *mec-3*), implying that they may also be mechanosensory [38, 147, 148]. Third, killing the PVD neurons in animals that lack touch cell function eliminates harsh touch sensitivity [18, 33]. The locomotion interneurons AVA and PVC are direct synaptic targets of PVD. Mutants lacking the GLR-1 glutamate receptors, which is expressed by the locomotory interneurons are insensitive to harsh touch, which suggests that these synapses are functional and that glutamate is the PVD transmitter [93]. The involvement of PVC in relaying harsh touch stimuli is exemplified by the phenotype of specific mutations in the *deg-1* degenerin gene. Animals carrying dominant, gain-of-function mutations in *deg-1* are touch abnormal [22, 49, 124], and they do not respond to gentle touch in the tail or prodding with a wire.

Another mechanosensitive behaviour in *C. elegans* is the tap withdrawal reflex, where animals retreat in response to a tap on the culture plate [24, 112, 153]. Worms respond to a diffuse mechanical stimulus (a tap to the side of the dish that they are resting on) by either accelerating forward movement or by initiating backward movement [24, 112]. Given that the stimulus is not spatially coherent and that the animal's response is variable, it was proposed that the tap response reflects the simultaneous activation of the anterior and posterior touch cells. The behavioural outcome is likely determined by the

integration of these two antagonistic circuits.

Mechanotransduction appears to also play a regulatory role in processes such as matting, egg laying, feeding, defecation, and maintenance of the pseudocoelomic body cavity pressure [3, 37, 87, 89, 140, 157]. These behaviours add to the large repertoire of mechanosensitive phenomena, amenable to genetic and molecular dissection in the nematode [4, 131, 134].

4. THE GENTLE BODY TOUCH RESPONSE

The laboratory assay for the gentle body touch response involves a mild stroke of the animal with an eyelash hair attached to a toothpick, transversely to the anterior-posterior body axis [17, 18, 131]. When no response is observed, animals are prodded with a thin platinum wire to confirm that they are touch insensitive rather than paralyzed (gentle-touch insensitive animals typically still respond to a strong stimulus-the harsh touch response; [18, 22, 33, 157]). Depending on the part of the body touched, animals will either accelerate or initiate forward movement (when stimulated at the posterior or the tail), or reverse and move backwards (when stimulated at the anterior part of the body). Hermaphrodite, male, juvenile (except L1), and dauer animals respond identically to touch. The response is adaptive: repetitive stimulation leads to short periods of insensitivity [91, 112, 154].

4.1. The touch receptor neurons

The touch reflex of the mature animal involves six touch receptor neurons, 5 pairs of interneurons and 69 motorneurons [12, 18]. The six touch receptor neurons were originally designated as the microtubule cells because of distinctive bundles of 15-protofilament (pf; tubulin dimmer filaments) microtubules that fill their processes (ALML/R: anterior lateral microtubule cell, left/right; AVM: anterior ventral microtubule cell; PLML/R; posterior lateral microtubule cell, left/right; and PVM: posterior ventral microtubule cell; [12, 14, 18-20]). All six cells are dispensable for the viability of the organism. Apart from insensitivity to gentle body touch, laser ablation of all six neurons does not result in any additional adverse effects [10, 18, 135]. Two fields, anterior and posterior, of touch sensitivity are defined by the arrangement of the six touch receptor neurons along the body axis ([18, 135]; Fig. 1). All the touch receptor neuron cell bodies have anteriorly directed processes.

Laser microsurgery established that PLML and PLMR are required for response to a touch to the tail. If either is present, tail touch sensitivity is observed. When both are ablated, animals are completely insensitive to gentle touch stimuli administered to the posterior [18, 21, 79]. Either ALML

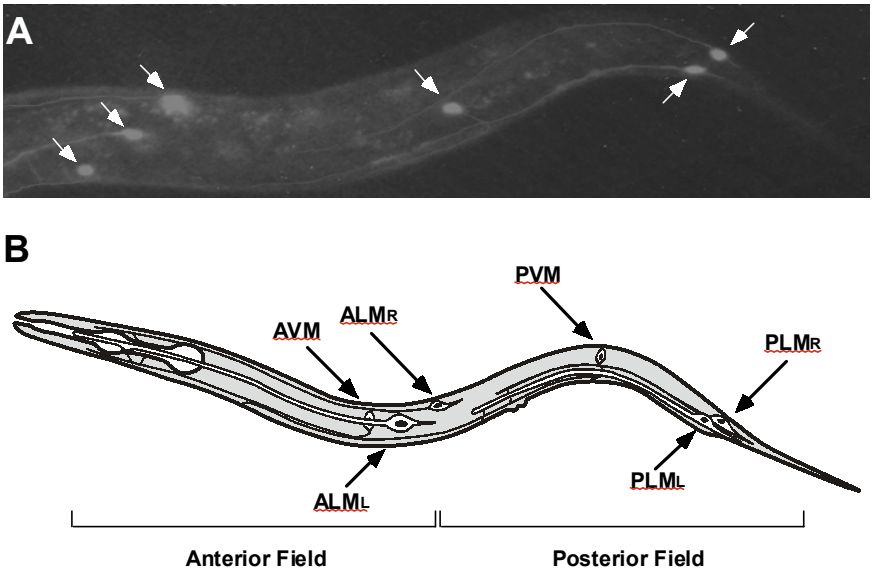

Figure 1: The two fields of touch sensitivity are defined by the arrangement of touch receptor neurons along the body axis. The ALMs and AVM mediate the response to touch over the anterior field whereas PLMs mediate the response to touch over the posterior field. PVM does not mediate touch response by itself [10, 11, 18, 135].

or ALMR can mediate a response to a mechanical stimulus delivered to the anterior part of the body. AVM, which is added into the touch circuitry postembryonically, can mediate a weak response to some touches but not all, by itself. In animals in which both ALM cells are killed, partial touch sensitivity returns 35-40 hours after hatching, which is attributable to AVM being added. PVM cannot mediate a touch response by itself. Other cells or neurons cannot differentiate and take the place of missing touch receptor neurons [12, 13, 18].

Bundles of darkly staining large diameter mictotubules distinguish the touch receptor neurons [19, 151]. Cross bridges between microtubules of a bundle are observed in micrographs obtained by electron microscopy, and may increase the structural integrity of the bundle. These microtubules are unique to the nematode touch receptor neurons and contain 15-protofilament microtubules, a unique feature of these six cells [20, 135]. In most eukaryotic cells, α- and β-tubulin co-assemble into 13-protofilament microtubules, whereas the vast majority of microtubules in *C. elegans* cells have 11-protofilament [20, 151]. In normal touch receptors, 11-protofilament

microtubules typical of most other cells in this nematode are occasionally observed. If the 15-protofilament microtubules are eliminated by mutation, the number of 11-protofilament microtubules in the touch cell processes increases [12, 14, 19, 45, 121].

Microtubules may provide a rigid intracellular 'point of reference', against which a touch stimulus could exert mechanical force to the mechanotransducing apparatus [33, 135]. Microtubules also appear to play a role in process outgrowth since processes are lacking in cells that have been treated with colchicine and benomyl (benomyl interferes with the 11-protofilament microtubules that take over in the absence of 15-protofilament microtubules; [32]). Continuity of the microtubules does not appear necessary for axonal outgrowth [14, 125]. Examination of serial section electron micrographs revealed that the 15-protofilament microtubules do not span the entire length of the touch receptor process. The processes are 400-500 microns long whereas the microtubules are 10-20 microns long [145, 151]. The average microtubule length varies with cell type, with lateral cell processes containing more microtubules than the ventral cell processes [19, 151]. Such short microtubules may facilitate sliding relative to each other, which would be required to accommodate changes in cell length that is likely to accompany the sinusoidal motion of the animal. Microtubules have a distinct polarity: the distal end is found on the outside of the microtubule bundle and the proximal end is preferentially found within the bundle [19, 151]. The distal end is distinguished by its diffuse ending, which is a diffuse patch of stained material with a diameter up to twice that of the microtubules. Proximal ends often have a filled appearance. Intriguingly, the diffusely stained structure of the distal end often appears to associate with the plasma membrane [19, 151].

4.2. The *C. elegans* mechanosensory apparatus

To identify molecules dedicated to touch transduction, Martin Chalfie and colleagues mounted a forward genetics approach to isolate gentle body touch-insensitive nematode mutants [13, 14, 17, 37, 55, 71, 135]. Briefly, populations of wild type, touch sensitive animals were mutagenized and touch insensitive individuals were sought among their descendants by stroking with an eyelash hair and prodding with a platinum wire [11]. During the course of this very tedious screening process, over 417 mutations in 17 different genes, randomly distributed in all six chromosomes of *C. elegans* were isolated [33, 135]. By design, the screen yields mutations in genes that are fairly specific for normal gentle body touch perception. For example, gene mutations with pleotropic effects that result in lethality or uncoordinated and paralyzed phenotypes would have been missed. In addition to being touch insensitive *mec* mutants tend to be lethargic when

grown normally in the presence of amble food [33]. Reduced spontaneous movement is probably due to their inability to sense micro vibrations in their environment, interaction with external objects or stretch produced by the locomotory movements themselves. However, when starved or during mating they move as well as wild type. The 17 genes isolated are designated as the *mec* genes for their '*mec*hanosensory abnormal' phenotype. Corroborating the high specificity of the screen, while most of the alleles generated cause complete touch insensitivity, only few other abnormalities accompany the mutants [33]. Depending on their role and point of action, *mec* genes can be loosely classified into three main categories. First, the regulatory/specification genes which control the expression of the touch receptor neuron specific genes or modify the activity of the mechanotransducer complex; second, the *mec* genes encoding core structural components of the mechanosensitive ion channel; and third the genes encoding peripheral, associated proteins.

Four *mec* genes can be classified in the category of core structural components of the putative mechanosensory ion channel in touch receptor neurons, *mec-2*, *mec-4*, *mec-6* and *mec-10*. MEC-4 and MEC-10 form the core ion channel, while MEC-2 and MEC-6 physically interact with the channel subunits to shape and modulate their gating properties. Animals bearing loss-of-function mutations in *mec-4* or *mec-10* are touch-insensitive despite the fact that in these mutant backgrounds the touch receptor neurons develop normally and exhibit no apparent defects in ultra structure [31, 71, 135]. *mec-4* and *mec-10* encode homologous proteins related to subunits of the multimeric amiloride sensitive Na^+ channel which mediates Na^+ re-absorption in vertebrate kidney, intestine and lung epithelia (the ENaC channel; [78, 114]). In addition to being involved in mechanotransduction, MEC-4, MEC-10 and several other related nematode proteins have a second, unusual property. Specific amino acid substitutions result in aberrant channels that induce the swelling and subsequent necrotic death of the cells in which they are expressed [30, 31, 57, 62]. This pathological property is the reason that this family of proteins was originally called degenerins [15, 22, 132, 133]. *C. elegans* degenerins, together with their mammalian relatives, the ENaCs, comprise the large DEG/ENaC family of ion channels (Fig. 2). Channel activity has recently been directly demonstrated for MEC-4 and MEC-10 [54]. In addition, chimeric nematode/rat proteins function in *C. elegans* and in *Xenopus* oocytes, implying that the nematode and rat proteins are functionally similar [15, 78]. *mec-4* is expressed only in the six touch receptor neurons and *mec-10* is expressed in the six touch receptor neurons and in two other neuron pairs that may mediate stretch-sensitive responses (FLPs and PVDs; [31, 34, 71, 135]). Interestingly, a MEC-4::GFP fusion localizes in distinct puncta along the processes of the

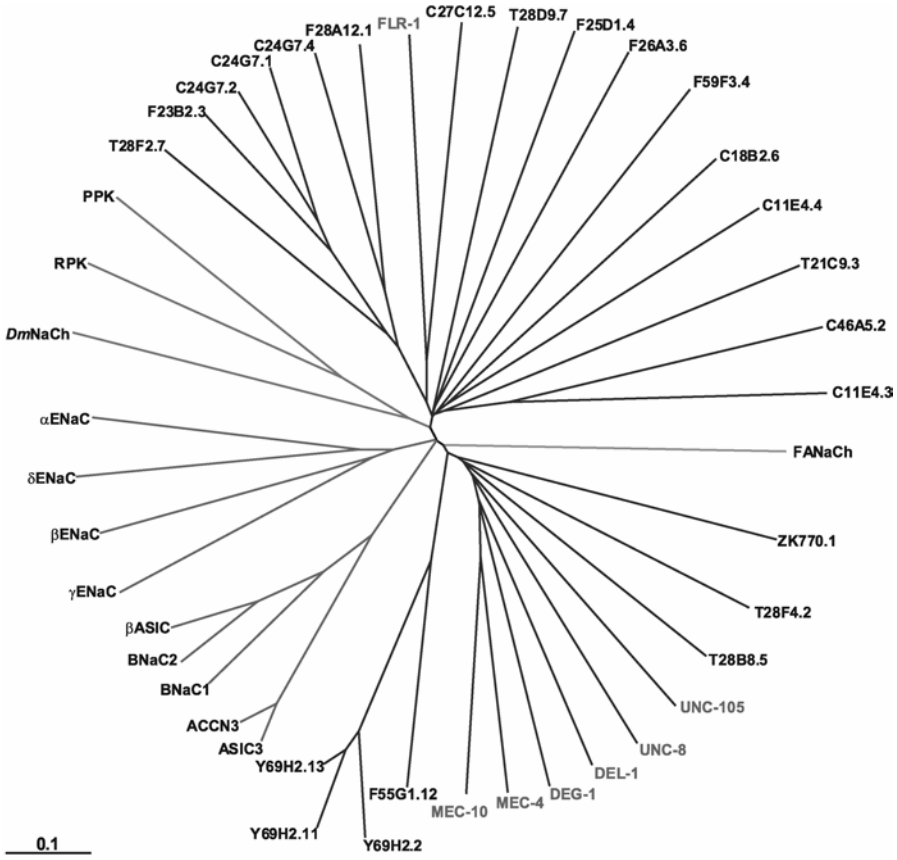

Figure 2: Phylogenetic relationships among DEG/ENaC proteins. Nematode degenerins are shown with blue lines. The current degenerin content of the complete nematode genome is included. The seven genetically characterized (DEG-1, DEL-1, FLR-1, MEC-4, MEC-10, UNC-8 and UNC-105) are shown in red. Representative 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 relative evolutionary distance equal to 0.1 nucleotide substitutions per site [119]. (See color insert # 1).

touch receptor neurons (Fig. 3). Such punctuate localization may reflect the distribution of mechanotransducing complexes on the axon of the touch

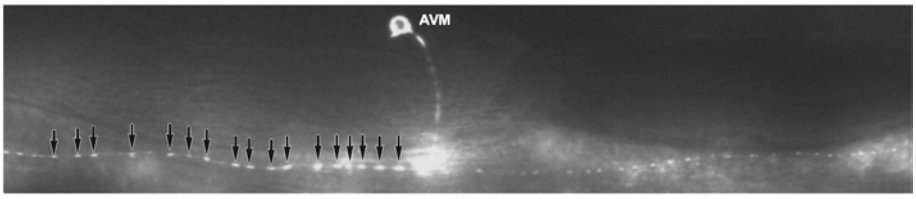

Figure 3: Punctuate localization of a putative mechanosensitive ion channel subunit. Image of an AVM touch receptor neuron expressing a GFP-tagged MEC-4 protein. Fluorescence is unevenly distributed along the process of the neuron in distinct puncta, which may represent the location of the mechanotransducing apparatus. (See color insert # 2).

receptor neuron. Since the MEC-4 and MEC-10 subunits are expressed exclusively in mechanosensitive neurons and are essential for the function of these neurons, it has been proposed that MEC-4 and MEC-10 co-assemble into a mechanically-gated ion channel that plays a central role in touch transduction (the relationship of these channel subunits to subunits of an amiloride-sensitive channel is also intriguing because amiloride is a general inhibitor of mechanosensitive ion channels; [1, 59, 61, 68, 85, 113, 141]). *In vivo* whole-cell patch clamp recording of touch evoked currents in *C. elegans* touch neurons showed that depolarization occurs rapidly and is produced only during positive or negative changes of the applied force. Sustained forces result in adaptation of either the touch receptor neuron or the mechanosensory apparatus itself. The membrane current produced is analogous to the applied force [100]. Mechanosensitive currents are carried by Na^+ ions and are reversibly blocked by amiloride, indicating that the MEC-4/MEC-10 degenerin ion channel complex is the core putative mechanoreceptor. Absence of MEC-4, in the *mec-4(u253)* loss-of-function mutant abolished mechanosensory currents. Loss of MEC-10, also eliminates touch evoked membrane currents [100].

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 [37, 55, 72]. MEC-2 features three candidate protein-protein interaction domains (Fig. 4). First, part of the amino-terminal domain (situated in part between AA 42-118) is needed for the proper localization of a *mec-2/lacZ* fusion protein to the touch receptor process. Second, the carboxy-terminal domain includes a proline-rich region that is similar to SH3-binding domains. Third, the central region (AA 114-363) encompasses an SPFH domain with a membrane-associated hydrophobic part (AA 114-141) and a cytoplasmic hydrophilic part that together exhibit 65% identity to the human

red blood cell protein stomatin [72, 136]. The SPFH domain is the common denominator of stomatins, prohibitins, flotilins and bacterial *Hfl*K/C proteins, all of which are membrane associated regulators (Fig. 5; [136]).

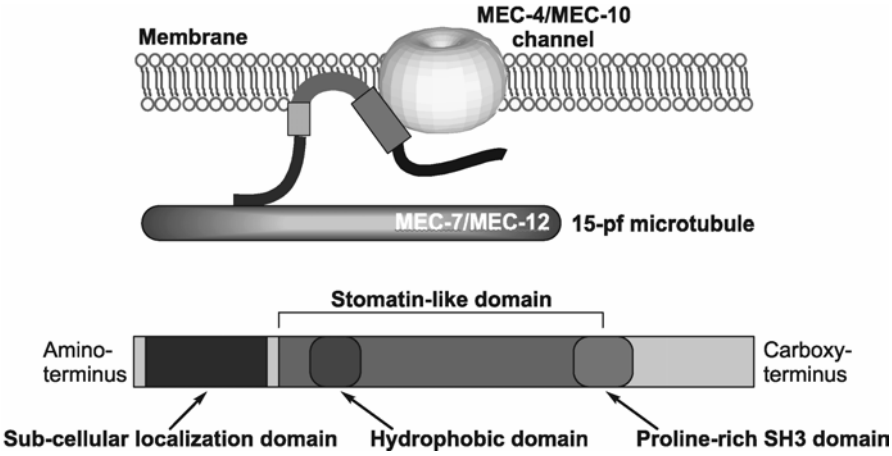

Figure 4: Schematic representation and topology of the MEC-2 protein. Conserved domains as well as hydrophobic regions are highlighted. Putative interactions with the degenerin channel and the cytoskeleton are indicated [54]. (See color insert # 3).

Stomatin, also known as band 7.2b protein, is a membrane-associated protein originally identified as a component of human red cells [29, 123, 127-129, 159]. In humans, stomatin is missing from erythrocyte membranes in autosomal dominant hemolytic disease overhydrated hereditary stomatocytosis, despite an apparent normal stomatin gene. Many of the 54 mutant *mec-2* alleles have dominant effects and exhibit a complex pattern of inter-allelic complementation [17, 55], indicating that MEC-2 protein molecules form higher order complexes. However, there is also genetic data suggesting that MEC-2 interacts with the specialized touch cell microtubules encoded by *mec-7* and *mec-12* (β-tubulin and α-tubulin respectively; [55, 72]). Normally, a *mec-2/lacZ* fusion protein is distributed along the touch receptor axon [72]. The axonal distribution of a MEC-2::lacZ fusion protein is mildly disrupted in a *mec-7* null or *mec-12* strong loss-of-function background, implying that the 15-protofilament microtubules are not

essential for the localization of MEC-2 to the neuronal process. However, two specific *mec-12* missense alleles interfere dramatically with localization of MEC-2 fusion proteins, restricting the fusion proteins to the cell body [72]. MEC-2 colocalizes with MEC-4 in the six touch receptor neurons and is distributed along neuronal processes in punctuate pattern [158]. This is consistent with the coimmunoprecipitation of the two proteins in Xenopus oocytes [54]. The stomatin-like domain of MEC-2 interacts specifically with

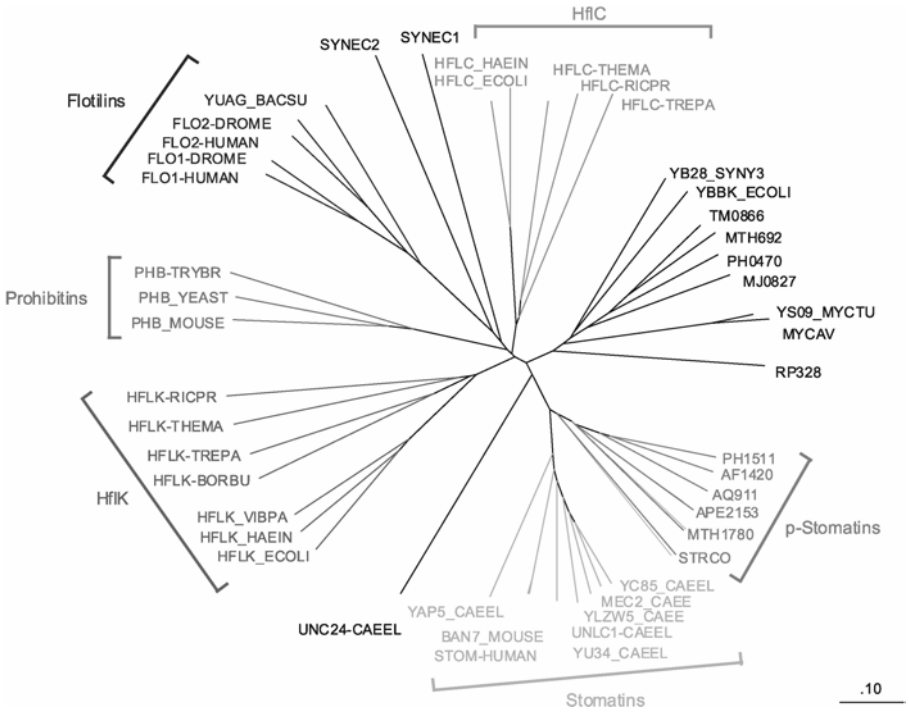

Figure 5: Phylogenetic relations among SPFH domain proteins. A dendrogram showing distance relationships among most of the stomatin protein super-family members (the complete ClustalW generated alignment on which the dendrogram was based is available at http://www.imbb.forth.gr/worms/worms/alignment.gif). The dendrogram was constucted with the neighbor-joining method (120) based on pairwise distance estimates of the expected number of amino acid replacements per site (0.10 in the scale bar), and visualized by TreeTool (http,//geta.life.uiuc.edu). Protein sub-families are denoted in different colors [136]. (See color insert # 4).

the N-terminus cytoplasmic region of MEC-4 [158]. Punctuate expression of MEC-2 is disrupted in the *mec-4(u253), mec-6(u450)* and *mec-10(u20)* loss-of-function mutants indicating that the MEC-2 subcellular localization depends on the other partners of the mechanosensory complex [158].These genetic studies, which do not by themselves prove a direct interaction, have recently been complemented by elegant heterologous expression experiments in *Xenopus* oocytes that support physical interaction between MEC-2 and the channel subunits MEC-4 and MEC-10 [54]. Reconstitution of channel activity in *Xenopus* oocytes revealed that MEC-2 regulates the activity of the MEC-4/MEC-10 channel, providing the first direct support for the hypothesis that stomatin-like proteins interact with and regulate ion channels [54, 129]. This interaction appears to dramatically potentiate the conductivity of the channel in oocytes. Co-expression of MEC-2 with the hyperactive MEC-4(d) and MEC-10(d) derivatives in *Xenopus* oocytes resulted in about 40-fold increase in the amplitude of amiloride-sensitive ionic currents, and this amplification allowed currents to be detected even with wild-type MEC-4 and MEC-10 proteins [54]. Visualization of tagged MEC-4(d) and MEC-10(d) in live oocytes demonstrated that MEC-2 does not increase the number of MEC-4(d)/MEC-10(d) channels that reach the plasma membrane, and probably acts by regulating their activity. Taken together these results are consistent with the simple hypothesis that MEC-2 tethers the 15-protofilament microtubules to the degenerin channel and largely determines its physiological properties. In *mec-2(u37)* loss-of-function mutants the touch-evoked currents are abolished confirming that MEC-2 is one of the major components needed for the proper function of the MEC-4/MEC-10 ion channel [100]. Another *C. elegans* stomatin-like protein expressed in the touch neurons is UNC-24. It shows a punctuate expression and colocalizes with MEC-4 and MEC-2. UNC-24 appears to interact through its stomatin-like domain with MEC-2 and MEC-4 [158].

Recessive *mec-6* mutations disrupt touch sensitivity but do not cause detectable changes in touch cell ultrastructure [17, 135]. *mec-6* alleles have the interesting property that they completely block *mec-4(d)* and *mec-4*(A673V)-induced touch cell degeneration, *i.e*. in *mec-6; mec-4(d)* and *mec-6; mec-10*(A673V) double mutant strains, cell death is suppressed [62, 71, 135]. MEC-6 encodes a protein with limited similarity to Paraoxonases/ Arylesterases that physically interacts with MEC-4 and MEC-10 [23]. How exactly MEC-6 acts to influence MEC-4/MEC-10 channel activity is unknown. Nevertheless, it appears that *mec-6* mutations do not affect *mec-4* transcription, although they do cause full-length MEC-4::LacZ or MEC-4::GFP reporter fusion chimeras to be rapidly degraded (N T. unpublished observations; [23]). Thus, working hypotheses concerning the function of MEC-6 focus on two possibilities. First, MEC-6 is another subunit needed

for channel function or assembly, or second, it mediates localization or post-translational modification essential for MEC-4 and MEC-10 activity/stability. It should be noted that MEC-6 function is not exclusively related to the MEC-4/MEC-10 touch receptor channel. *mec-6* mutations also suppress the deleterious consequences of neurodegeneration-inducing mutations in other *C. elegans* degenerins including *deg-1*, *unc-8* and partly *unc-105* ([22, 88, 124, 137]; N. T. unpublished observations). *mec-6* loss-of-function mutations affect localization of the MEC-4 channel and disrupt touch evoked membrane currents [23].

Two subgroups of *mec* genes encoding peripheral components required for mechanotransduction in the touch receptor neurons can be defined, those encoding intracellular (*mec-7*, *mec-12*) and those encoding extracellular (*mec-1*, *mec-5*, *mec-9*) proteins [33, 34, 135]. As described previously, the touch receptor processes are filled with bundled 15-protofilament microtubules. Mutations in two genes, *mec-7* and *mec-12*, disrupt the formation of these microtubules [13, 17, 45, 58, 121, 122]. Such touch receptors do not function, suggesting that the extensively cross-linked 15-protofilament microtubules play a specific role in touch transduction. *mec-7* encodes a β-tubulin expressed at high levels in the touch receptor neurons [58, 121, 122]. MEC-7 is highly conserved--apart from the carboxy-terminal domain that is characteristically highly variable; only 7 amino acids differ from other β-tubulins. *mec-12* encodes an α-tubulin expressed at high levels in the touch receptor neurons but also expressed in several other neurons that do not assemble 15-protofilament microtubules [45]. Thus, the presence of the MEC-12 tubulin is not sufficient to nucleate assembly of the touch-cell specific microtubules. As is the case for *mec-7*, many *mec-12* mutations are semi-dominant or dominant and are likely to disrupt subunit interactions or protofilament assembly [55]. The totality of the studies on *mec-7* and *mec-12* strongly support that unique α- and β-tubulins assemble to form the 15-protofilament microtubules required for touch receptor function.

In *mec-1* mutants, touch cells generally lack the mantle and associated periodic specializations of the overlying cuticle [17, 55, 122]. *mec-1* is expressed in touch receptor neurons, other lateral neurons and intestinal muscles. It encodes a likely secreted protein with multiple Kunitz-type serine protease inhibitor and EGF domains. The Kunitz and EGF domains are likely to be protein interaction domains. The C terminus of MEC-1 is needed for touch sensitivity, while the N terminus mediates the attachment of the touch neuron processes to the hypodermis [41]. MEC-1 is localized along the touch receptor processes in a punctuate manner and colocalizes with MEC-5 and the MEC-4/MEC-10 mechanosensory channel complex [41]. *mec-5* mutations disrupt the extracellular matrix in a subtle manner. The mantle in a wild-type animal can be stained with peanut lectin, whereas the

mantle in *mec-5* mutants cannot [17, 38, 55]. *mec-5* encodes a novel collagen type that is secreted by hypodermal cells [38]. The central portion of the *mec-5* protein is made up of Pro-rich Gly-X-Y repeats. *mec-5* mutations, many of which are temperature-sensitive, cluster toward the carboxy terminus of the protein and affect these repeats. Genetic interactions suggest that *mec-5* influences MEC-4/MEC-10 channel function (for example, *mec-4* and *mec-10* mutations can enhance the *mec-5(ts)* mutant phenotype; [55]). Thus, a specialized collagen could interact with the touch receptor channel, perhaps acting to provide gating tension.

mec-9 mutations do not alter mantle ultrastructure in a detectable manner, despite the fact that *mec-9* encodes a protein that appears to be secreted from the touch receptor neurons [17, 38]. The *mec-9* gene generates two transcripts, the larger of which encodes an 834 amino acid protein (MEC-9L) that is expressed only by the touch receptors (38). Akin to MEC-1, the predicted MEC-9L protein contains several domains related to the Kunitz-type serine protease inhibitor domain, the Ca^{2+}-binding EGF repeat, the non-Ca^{2+}-binding EGF repeat and a glutamic acid-rich domain [38]. Single amino acid substitutions that disrupt MEC-9 function affect the two Ca^{2+}-binding EGF repeats, the sixth EGF repeat and the third Kunitz-type domain, thus implicating these regions as important in MEC-9 function [38]. How MEC-9 is needed for touch cell activity is not clear, but it is interesting that MEC-9 appears specialized for protein interactions and that agrin, a protein that acts to localize acetylcholine receptors, has a domain structure that appears similarly specialized (agrin features multiple EGF and Kazal-type serine protease inhibitor repeats; [115-117]). *mec-9* mutations are dominant enhancers of a *mec-5(ts)* allele, suggesting that these proteins might interact in the unique mantle extracellular matrix outside the touch receptor neuron [38, 55].

5. PROPRIOCEPTION

C. elegans locomotion results from alternate contraction and relaxation of dorsal and ventral body wall muscles, which generates a canonical sinusoidal pattern of movement [151, 157]. The arrangement of the body wall muscles and their synaptic inputs restricts locomotion to dorsal and ventral turns of the body. The body wall muscles are organized into two dorsal and two ventral rows. Each row consists of 23 or 24 diploid mononucleate muscle cells arranged in an interleaved pattern [43, 97, 146, 156]. 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.

Interactions between excitatory and inhibitory motorneurons produce a pattern of alternating dorsal and ventral contractions ([43, 70, 135]; Fig. 6). 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 since gap junctions connect adjacent motorneurons of a given class [18, 151, 152]. 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 [134, 135, 137].

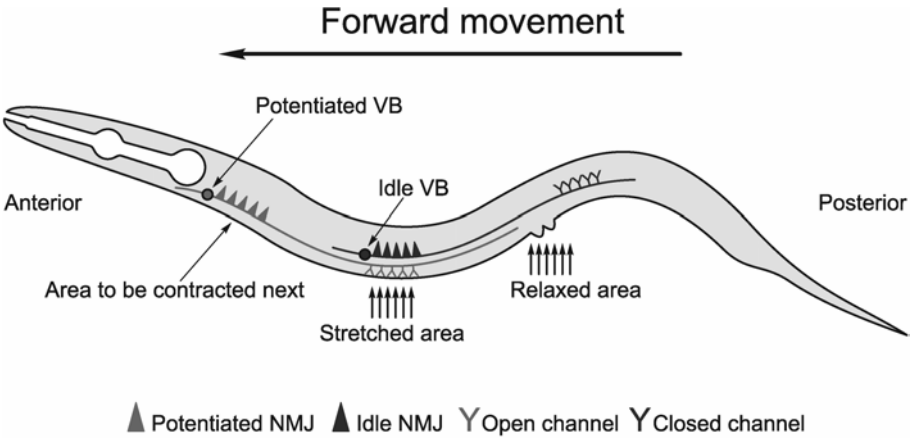

Figure 6: A model for UNC-8 involvement in stretch-regulated control of locomotion. Schematic diagram of potentiated and inactive VB class motor neurons. Neuro-muscular junctions (signified by triangles) are made near the cell body [135, 151]. Mechanically-activated channels postulated to include UNC-8 (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 which potentiate the motor neuron signal that excites a specific muscle field. Sequential activation of motor neurons that are distributed along the ventral nerve cord and signal non-overlapping groups of muscles, amplifies and propagates the sinusoidal body wave (NMJ: neuromuscular junction). (See color insert # 5).

Numerous mutations disrupt normal sinusoidal locomotion in *C. elegans*, resulting in animals with movement defects ranging from total paralysis, to severe uncoordination, to subtle and almost imperceptible irregularities in movement [135, 137]. Unusual, semi-dominant (sd), gain-of-function mutations in the gene *unc-8* induce transient neuronal swelling of embryonically derived motorneurons as well as some neurons in the head and tail ganglia, and severe uncoordination [102, 124, 137]. Swelling is absent at hatching and peaks in severity late in L1 and L2. *unc-8* encodes a degenerin expressed in several motor neuron classes and in some interneurons and nose touch sensory neurons. 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. 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 [124, 137]. Another degenerin family member, *del-1* (degenerin-like) 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 [137]. The UNC-8 and DEL-1 proteins include all domains characteristic of degenerin family members and are likely to adopt similar transmembrane topologies (amino and carboxy termini situated inside the cell and a large extracellular domain that includes three cysteine-rich regions). Neither degenerin has any primary sequence features that are markedly different from other *C. elegans* family members although one somewhat atypical feature of UNC-8 is that it has a relatively long C-terminal domain that shares some primary sequence homology with the extended C-terminus of another degenerin implicated in locomotion, UNC-105 [88, 101].

The exact function of the UNC-8 degenerin channel in motorneurons was elucidated through genetic approaches. *unc-8* null mutants have a subtle locomotion defect; they inscribe a path in an *E. coli* lawn that is markedly reduced in both wavelength and amplitude as compared to wild type [137]. 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? As mentioned earlier, 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 be stretch-sensitive [151]. Given the morphological features of certain motor neurons and the sequence similarity of UNC-8 and DEL-1 to the candidate mechanically-gated channels MEC-4 and MEC-10, 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 [137]. 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 stretch stimulus. 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 [135, 137]. 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 phenotypes, cell morphologies and molecular properties of degenerins remains to be tested by determining sub cellular channel localization, subunit associations and, most importantly, channel gating properties.

6. MECHANOTRANSDUCTION IN OTHER ORGANISMS: SIMILARITIES AND DIFFERENCES

Investigations on the genetics of sensory mechanotransduction, which were initiated in *C. elegans* and are now also being carried out in *Drosophila* and in mammals, have converged to reveal a limited set of underlying mechanisms [40, 52, 65, 78]. For example, 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 [42, 52, 60, 73, 74, 76, 105, 106, 135, 149]. 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 each other introduces tension on the tip links, which is proposed to open the mechanosensitive hair cell channels directly. This remarkable convergence of independent studies in distant species, strongly suggests that different mechanotransducers in different systems have evolved to strictly adhere to the same set of principles. Members of two major ion channel families, the DEG/ENaC and the unrelated in amino acid sequence TRP (Transient Receptor Potential) group, have emerged as the common denominators within a metazoan mechanosensory apparatus [1, 39, 96, 98, 133, 150]. For example, NompC, a TRP cation channel is required for normal mechanosensitive currents in fly hair bristles [144]. Another member of the TRP protein family, the TRPA1 channel has been identified as a candidate mechanotransducing channel in the mouse [27]. In situ hybridization revealed that the TRPA1 channel is expressed in the cochlea organ of Corti, which contains the auditory hair cells. Additional colocalization experiments link TRPA1 to mechanosensation: TRPA1 is expressed together with two accessory proteins of the mechanosensory apparatus, myosin 1c and cadherin 23, at the tips of stereocilia throughout the kinocilium and in the pericuticular zone. Consistently, TRPA1 is necessary for either normal function or development of embryonic lateral line hair cells in zebrafish. Whole-cell patch clamp recording of inner hair cells in mice show that the transduction current produced is significantly reduced in the absence of TRPA1, indicating that this channel is a component of the mechanosensitive transduction channel of vertebrate hair cells [27].

In all cases examined, genetic, molecular and physiological data portray a similar architecture for mechanotransducing complexes. This architecture implements variations of the tethered-ion channel concept. It is striking that regardless of the identity of the core ion channel (DEG/ENaC or TRP) both intracellular and extracellular tethers appear to be required to render the core channel mechanosensitive [6, 25, 92]. The mechanosensory function of the complex dictates its highly specialized structure. The nematode model of mechanotransduction in touch receptor neurons best illustrates this point, with a unique cytoskeletal network intracellularly and a dedicated extracellular mantle being essential for mechanosensory transduction. Furthermore, the requirement for anchoring of mechanosensitive ion channels is signified by the presence of exceptionally long ankyrin repeats in the NompC mechanosensory channel of *Drosophila*. The conjecture that mechanotransduction dictates an explicit structure has predictive powers; an arrangement of ion channel proteins and associated components that is aligned with the specifications of the tethered-ion–channel model is likely to have mechanotransducing properties.

Despite enormous progress on the illumination of vertebrate mechanosensory cell biology achieved in recent years, there is still a striking gap between the biophysical information that has accumulated and our understanding of the molecular aspects of mechanosensation. Sophisticated experiments in mice and humans revealed many genes involved in the development and function of the mammalian cochlea and have cumulated in the formulation of the gating-spring model for hair cell mechanotransduction [51, 52]. However, many pieces of the mechanotransducing apparatus puzzle are still missing. Work in lower vertebrates such as birds, amphibians and fish has also contributed significantly in complementing and extending the studies with mammals. In these animals mechanosensory structures are often much easier to access, follow and monitor providing large potential for investigating the molecular basis of auditory transduction [2, 126].

An increasing amount of evidence suggests that some mammalian DEG/ENaC proteins may play a role in mechanosensation similarly to their nematode counterparts. In mammals, there are strong indications that ENaC subunits may be components of the baroreceptor mechanotransducer, one of the most potent regulators of arterial pressure and neurohumoral control of the circulation [35, 36]. 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; [48, 108, 142, 143]) has emerged as promising candidate for a mechanosensitive channel. In BNC1 null mice touch receptor neurons of the skin produce fewer action potentials than in wild type animals over a comparable range of stimuli.

7. CONCLUSIONS AND PERSPECTIVES

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. 7; [11, 33, 135]). The architecture of this mechanotransducer complies with the general principle of the tethered mechanosensitive ion channel. 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 [134, 135]. These subunits assemble to form a channel pore that is lined by the hydrophilic residues of membrane-spanning domain II [69]. The mechanosensory apparatus encompassing MEC-4 and MEC-10 subunits appears to be localized at the long processes of touch receptor neurons. Subunits adopt a topology in which the cysteine-rich and neuroto-

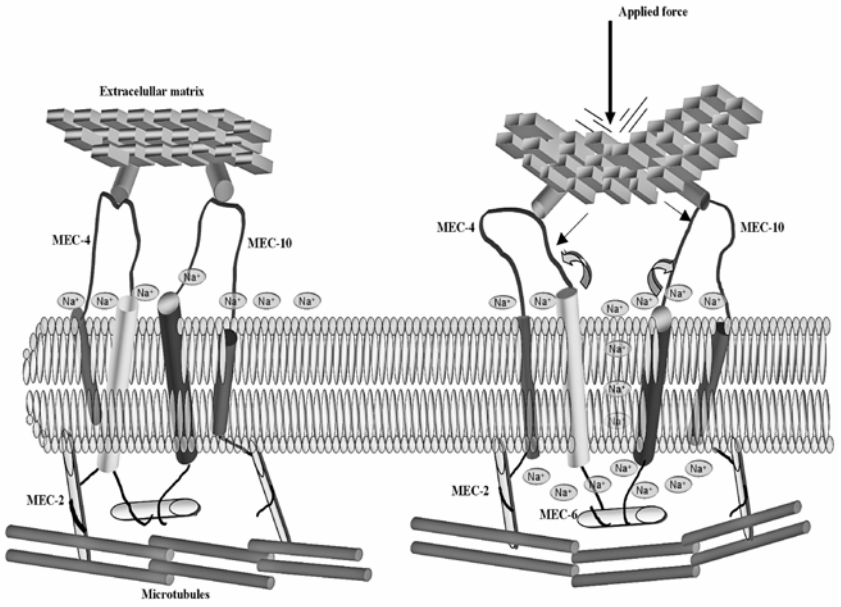

Figure 7: A mechanotransducing complex in *C. elegans* touch receptor neurons. In the absence of mechanical stimulation the channel is closed and therefore the sensory neuron is idle. Application of a mechanical force to the body of the animal results in distortion of a network of interacting molecules that opens the degenerin channel. Na^+ influx depolarizes the neuron initiating the preceptory integration of the stimulus [47]. (See color insert # 6).

xin-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; [34, 38, 49, 55, 135]). 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; [54, 55, 72, 109, 110]). A touch stimulus either could deform the microtubule network, or could perturb the mantle connections to deliver the gating stimulus. In both scenarios, Na^+ influx would activate the touch receptor to signal the appropriate locomotory response.

The detailed model for mechanotransduction in *C. elegans* neurons accommodates genetic data and molecular properties of cloned genes. This model also based on mutant phenotypes, cell morphologies heterologous degenerin expression approaches and structural features of degenerins remains to be tested by determining sub-cellular channel localization, subunit associations and, most importantly, channel gating properties. It should be emphasized that the proposed direct interactions between proteins that build the mechanotransducing complex remain largely hypothetical and only recently have they begun to be addressed experimentally [23, 54].

An additional major 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 [107]. 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 [144]. 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 [144]. Therefore, NompC homologues in other organisms, including humans, emerge 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.

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