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INVITED REVIEW

Touch sensitivity in *Caenorhabditis elegans*

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Abstract The nematode *Caenorhabditis elegans* was the first organism for which touch insensitive mutants were obtained. The study of the genes defective in these mutants has led to the identification of components of a mechanosensory complex needed for specific cells to sense gentle touch to the body. Multiple approaches using genetics, cell biology, biochemistry, and electrophysiology have characterized a channel complex, containing two DEG/ENaC pore-forming subunits and several other proteins, that transduces the touch response. Other mechanical responses, sensed by other cells using a variety of other components, are less well understood in *C. elegans*. Many of these other senses may use TRP channels, although DEG/ENaC channels have also been implicated.

Keywords Mechanosensitivity · Sensory neurons · Mechanosensitive channel · Epithelial Na^+ channel · TRP channel

Introduction

The detection of mechanical forces is a basic sense present in virtually all organisms. Bacteria sense osmotic pressure, plants sense and respond to gravity, and humans have a variety of mechanosensory behaviors, including touch and hearing. In fact, Kung [46] has argued that the sensing of mechanical force may be the most ancient of the senses. Despite their ubiquity and importance, these senses are more poorly understood at the molecular level than other senses like smell and vision.

Understanding mechanosensation in higher eukaryotes has proven especially difficult. Molecules needed for transducing mechanical force are thought to be present in low quantities; this rarity prevents isolation and identification of the molecules via biochemical means. Therefore, genetic approaches have been used to identify candidate molecules required for touch sensation. The first organism in which this approach was used was the nematode *Caenorhabditis elegans*.

C. elegans lends itself to easy genetic analysis. The animal has two sexes: self-fertilizing hermaphrodites, which allow easy maintenance of severely defective strains, and males, which allow cross-fertilization. In addition, much is known about *C. elegans* biology. Its entire genome has been sequenced [35], its entire cellular development from zygote to adult has been described [80, 81], and all 302 neurons of the hermaphrodite nervous system have been identified and their anatomical connections mapped by electron microscopy [91]. Because the worm is transparent, these neurons can be ablated to test their roles. Of particular importance for this review, *C. elegans* has several mechanosensory behaviors, including responses to gentle touch, harsh touch, and nose touch, food-mediated slowing, osmotic avoidance, and male mating behaviors [56].

In this review, we primarily discuss *C. elegans* gentle touch mechanosensation. Gentle touch sensitivity is usually tested by stroking the worm with an eyebrow hair attached to a toothpick. Touches to the anterior half of the worm cause the animals to reverse and move backward, while

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stimulating the tail causes the animal to accelerate forward [12]. More intense stimuli, such as prodding with a platinum wire, constitute harsh touch and are mediated by different sensory neurons than gentle touch mechanosensation [12, 90]. The neurons involved in responding to gentle touch also respond to taps to the culture plate upon which the worms are grown. Plate tap produces a nondirectional mechanical stimulus on the worm and evokes a tap withdrawal reflex; young larvae move forward half the time, whereas adults almost always move backward [18]. Because tapping appears to activate both anterior and posterior responses simultaneously [59], the change in tap withdrawal as animals mature demonstrates a change in the reflex circuitry.

Touch receptor neurons

Six touch receptor neurons (Fig. 1a) sense gentle touch and plate tap [12, 93]. Two bilaterally symmetric pairs of cells are located anterior of the vulva (ALML and ALMR) and in the tail (PLML and PLMR). These cells arise embryonically and have laterally-directed processes. A third pair of cells (AVM and PVM) originate from bilaterally symmetric precursors, but lie in different positions (in the mid anterior and posterior of the worm, respectively) because of the migrations of their precursors [80]. These cells arise after hatching and have processes that are located in the ventral cord.

The behavior of laser-ablated animals indicates that touch to the anterior half of the animal is sensed primarily by the ALM neurons in all stages and also by the AVM neuron in mature animals. Touch to the posterior half of the animal is sensed by the PLM neurons [13, 93]. The PVM neuron alone does not produce a response to touch or tap, but synapses formed by the neuron suggests that it may have a subtle role in anterior touch sensation [13].

As with most *C. elegans* neurons the touch receptor neurons have a very simple morphology [14]. Each of the six cells has a single anteriorly directed process that extends from the touch receptor cell bodies. All the cells, except for PVM, branch near the anterior end of the process. In addition, the PLM cells have single posteriorly directed processes (the ALM cells sometimes have a short posteriorly directed process, but when present, it is much shorter). The processes appear to have a dual purpose. First, they are likely to be the sensory endings of the neurons because touch is sensed over their entire lengths. The position of the touch receptor processes next to the cuticle [12] presumably puts it in an optimal position to detect external mechanical signals. Second, the processes make synaptic connections

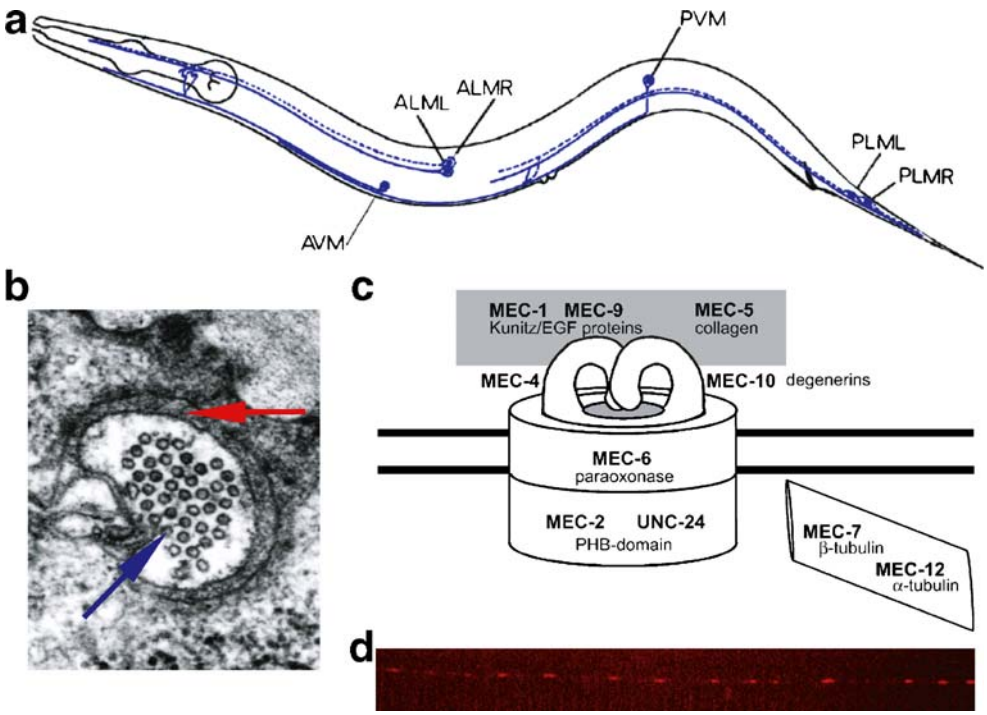

Fig. 1 Touch receptor neurons needed to sense gentle touch stimuli. **a** Diagram of touch receptor neurons (*blue*) in *C. elegans*; adapted with permission from Chalfie and Sulston [12]. **b** Electron micrograph of a cross-section of a process of a touch receptor neuron. These cells contain specialized large-diameter microtubules (*blue arrow*) and are enveloped by an extracellular mantle (ECM) (*red arrow*); reproduced with permission from Chalfie and Sulston [12]. **c** Proteins required for mechanosensation in the touch receptor neuron include components of a DEG/ENaC channel, tubulins that comprise the large-diameter microtubules, and molecules in the ECM; adapted with permission from O'Hagan and Chalfie [56]. **d** Components of the mechanosensory channel complex localize to discrete puncta along the touch neuron process, as visualized with MEC-4::GFP (*false colored*). Reproduced with permission from Emtage et al. [25]

with interneurons, either directly or through their anterior branch [12].

All six neurons have common physical features that differentiate them from other neurons, including prominent extracellular matrix (called the mantle) that periodically enlarges along the process and large-diameter (15-protofilament) microtubules that fill it (Fig. 1b) [12, 14]. These cells are the only ones with these specialized microtubules.

Proteins required for generation and differentiation

Screens for mutants that are insensitive to gentle touch have identified genes necessary for both touch receptor neuron development and function. Most of the genes identified in these screens were designated as mechanosensory abnormal or *mec* [10, 12, 78]. Genes that are needed for the generation and differentiation of these cells are described in this section, whereas those needed for touch neuron function are described in the next section.

Several transcription factors are necessary for the production of the touch receptor neurons. Two genes, *lin-32* and *vab-15*, are needed for the differentiation and division of the neuroblasts that give rise to the touch neurons. *lin-32* encodes a basic helix–loop–helix protein; *vab-15* encodes a *msh*-type homeobox gene [22, 100]. Mutations in either gene prevent the production of AVM, PVM, and the PLM neurons and affect the proper migration of the ALM neurons. *lin-32* and *vab-15* are partially redundant in the ALM cells because the double mutants lack all six touch receptor neurons [10, 12, 22]. LIN-32 (the protein product of the *lin-32* gene) appears to act early in development to regulate neuroblast cell fate [10, 100]. Because *vab-15* is genetically redundant with *lin-32* in ALM development, VAB-15 may act similarly [22].

unc-86, which encodes a POU-type homeodomain protein, affects later development in the lineages that give rise to the touch neurons [27]. *unc-86* is expressed in the touch receptor neurons and their precursors, among other cells [26]; mutations in *unc-86* affect the precursors so that the correct lineages are not followed and none of the six touch receptor neurons are made [11, 12].

The touch neurons require *unc-86* not only for their generation but also their differentiation [26]. UNC-86 activates expression of *mec-3*, another gene required for touch cell fate [12, 94]. In *mec-3* mutants, cells that normally become touch receptor neurons are present but do not undergo proper differentiation and lack the characteristic touch neuron features: they no longer have the 15-protofilament microtubules or mantle, and the ALM cells develop a prominent posterior process [12]. The *mec-3* gene encodes a LIM-type homeodomain protein that is expressed in the six touch neurons as well as the FLP and PVD neurons [89, 90]. UNC-86 and MEC-3 transcription factors bind cooperatively as heterodimers, increasing their DNA-binding specificity and stability [95]. UNC-86::MEC-3 heterodimers then bind promoters of *mec-3* and other genes, maintaining *mec-3* expression and activating transcription of downstream genes necessary for touch receptor neuron function [24, 94]. The requirement for *mec-3* for touch neuron differentiation has been used to identify additional *mec-3*-dependent genes using DNA microarrays [99]. The roles of most of these new genes in touch sensitivity have not been tested.

The differentiation of touch receptor neurons is controlled not only transcriptionally, but also posttranscriptionally. Specifically, the *mec-8* gene appears to be needed for the correct splicing of the touch function gene *mec-2* (Calixto et al., unpublished data). *mec-8* encodes a widely-expressed protein with two RNA recognition motifs and is known to be needed for correct mRNA processing in other tissues [53, 96]. The number of genes requiring *mec-8*-processing in the touch receptor neurons is not known.

Proteins required for mechanosensation

Differentiated touch receptor neurons express several genes required to sense gentle touch and tap. The products encoded by these genes include subunits of a mechanoreceptor channel complex, components of the extracellular matrix, tubulins that form the 15-protofilament microtubules, and additional proteins of unknown function (Fig. 1c).

The mechanoreceptor channel complex contains at least five proteins that are needed to transduce the touch stimulus. The main pore-forming subunits of this complex are encoded by the *mec-4* and *mec-10* genes. MEC-4 and MEC-10 are degenerins, a class of ion channels in *C. elegans* named after the neuronal degeneration phenotype caused by gain-of-function alleles [16, 21, 38]. Degenerins are part of the degenerin/epithelial sodium channel (DEG/ENaC) protein superfamily found in invertebrates and vertebrates. DEG/ENaC proteins form ion channels that conduct sodium and are blocked by the diuretic amiloride [2, 33, 44]. Whereas *C. elegans* degenerins function in mechanosensation and, perhaps, proprioception [84], DEG/ENaC channels in vertebrates have also been implicated in nociception, sodium homeostasis, regulation of the composition and volume of lung fluids, and memory and learning [44, 45].

DEG/ENaC proteins contain cytoplasmic NH_2-terminal and COOH-terminal ends, two transmembrane domains, and an extracellular loop containing two cysteine-rich

domains (CRDs). The extracellular loops in degenerins (as represented by six of the 23 *C. elegans* DEG/ENaC proteins) have an additional CRD as well as an extracellular regulatory domain (ERD) located N-terminal to the other CRDs (Fig. 2) [44]. The second transmembrane domain (TMII) and the sequence preceding it are thought to contribute to the pore of the channel; mutations in these regions cause constitutive channel activity, blockage of the constitutive activity, or changes in ion selectivity [36, 37, 57]. The mutations that cause constitutive channel activity were first identified as changes in MEC-4, MEC-10, and other *C. elegans* degenerins that cause neurodegeneration [16, 21, 38]. Substitutions of large-side chain residues for an alanine in the region preceding TMII cause hyperactivation and toxic influx of ions [21, 36, 75]. Additional degeneration-causing and gain-of-function mutations have been found affecting the degenerin ERD, suggesting that this region of the extracellular loop gates channel activity [30, 84]. The most C-terminal CRD, which is highly conserved, exhibits similarity to venom neurotoxin, suggesting a possible role in modulating DEG/ENaC channel gating [83]. *mec-4* mutations in this region disrupt channel function and may affect appropriate gating [37]. Finally, parts of the cytoplasmic COOH-terminal domain of MEC-4 may be required for channel trafficking or maintenance of the channel in the cell surface [67].

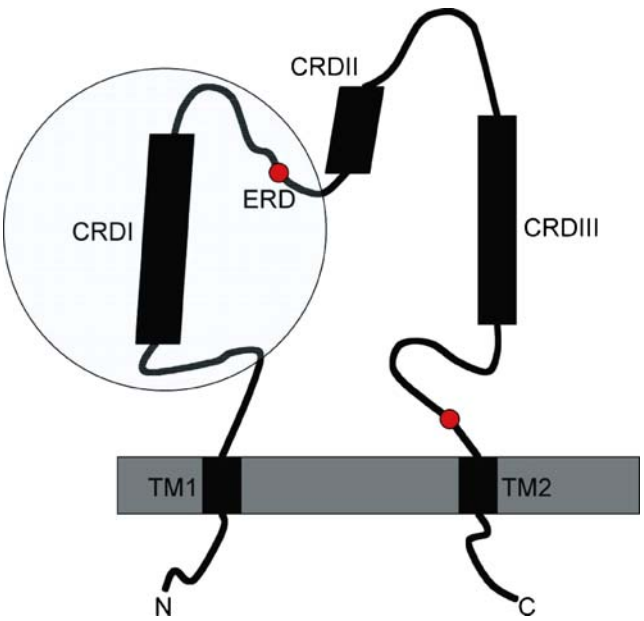

Fig. 2 Conserved domains in DEG/ENaC channels. DEG/ENaC channels have cytoplasmic N- and C-terminal ends, two transmembrane (TM) domains, and two cysteine-rich domains (CRD). Degenerins such as MEC-4 have an additional CRD domain as well as an extracellular regulatory domain (ERD), shown in the *shaded circle*. Mutations in the ERD and in the pore forming residues preceding TM2 (*red*) can cause neurodegeneration. Figure redrawn with permission from Kellenberger and Schild [44]

Substantial evidence supports the idea that thw *mec-4*- and *mec-10*-encoded degenerins transduce gentle touch. Mutations in either gene produce touch insensitivity [12], and gain-of-function mutations as described above lead to touch receptor neuron degeneration [16, 21, 30, 38]. Both genes are coexpressed in the six touch neurons [38]. Along the touch neuron process, MEC-4 localizes to discrete puncta where mechanosensory proteins are believed to exist as a complex (Fig. 1d) [17]. When expressed in heterologous systems, MEC-4 and MEC-10 coimmunoprecipitate with each other and with other putative mechanosensory channel subunits [17, 31]. In frog oocytes, expression of the mutant form of MEC-4 that produces degeneration, MEC-4d, can induce an amiloride-sensitive sodium current. MEC-10d alone cannot generate a current in the oocytes, but when coexpressed with MEC-4d, it becomes a part of the channel and modifies the current's properties [31]. These data are consistent with the fact that in vivo, *mec-4 (d)* produces strong degeneration independent of *mec-10*, while *mec-10(d)* degeneration is less frequent and requires *mec-4*, and suggest that MEC-4 and MEC-10 have functional differences [38]. Finally, in vivo electrophysiology recordings of touch receptor neurons demonstrate that MEC-4 and MEC-10 transduce gentle touch stimuli ([57], see below).

In addition to the MEC-4 and MEC-10 pore-forming subunits, the mechanoreceptor channel complex includes proteins encoded by *mec-6*, *mec-2*, and *unc-24*. MEC-6, a transmembrane protein that is similar to mammalian paraoxonases, is widely expressed, including in the touch receptor neurons. In the touch neurons, MEC-6 colocalizes with MEC-4 puncta along the neuronal process. MEC-6 coimmunoprecipitates with the degenerins and other channel subunits [17], and mutations in *mec-6* produce touch insensitivity [12], eliminate MEC-4 puncta [17] and suppress *mec-4(d)*- and *mec-10(d)*-induced degeneration [16, 38]. Additionally, MEC-6 increases MEC-4d currents expressed in frog oocytes [17]. These results suggest that MEC-6 is a subunit of the mechanosensory complex required for proper channel localization, formation, and function. Because it is widely expressed in other tissues and required for degeneration caused by gain-of-function mutations in other degenerins, MEC-6 may play similar roles in other DEG/ENaC channels [16, 30, 50, 73].

mec-2 and *unc-24* encode PHB (prohibitin homology)-domain membrane proteins [3, 39]. The PHB domain is a 150 amino acid domain found in a large number of proteins including stomatin, flotillin, prohibitin, and podocin in vertebrates. Stomatin may regulate ion permeability because loss of stomatin in red blood cells of individuals with hereditary stomatocytosis leads to cell lysis [76]. Stomatin, podocin, MEC-2, UNC-24, and several other PHB-domain proteins have a hydrophobic

region N-terminally adjacent to the PHB domain [3, 8, 39, 77]. This domain in MEC-2, stomatin, and podocin attaches the proteins to the inner leaflet of the plasma membrane [40].

MEC-2 and UNC-24 are expressed in the touch receptor neurons, while UNC-24 is also expressed in other neurons and is required for proper movement and coordination [3, 39]. Both proteins colocalize with MEC-4 puncta and coimmunoprecipitate with MEC-4 and other mechanoreceptor channel subunits [17, 31, 98]. While both MEC-2 and UNC-24 appear to be part of the touch receptor complex, the effects of their loss on mechanosensation differ. *mec-2* null mutants are completely touch insensitive [12], whereas *unc-24* mutants display only subtle defects in touch sensitivity [98]. This difference is also reflected in frog oocytes, where MEC-2 increases MEC-4d currents 40-fold [31], while UNC-24 has no affect on MEC-4d current alone and decreases it by 30% in the presence of MEC-2 [98].

Proper function of both MEC-2 and UNC-24 require the conserved PHB domain. In MEC-2, the domain is required for homooligomerization, localization to puncta, and amplification of MEC-4d currents in frog oocytes [98]. Recently, MEC-2 has also been shown to bind cholesterol; this binding requires the PHB domain and five amino acids in the adjacent hydrophobic region [40]. In HEK 293T cells, expression of MEC-2 can bind and recruit cholesterol to rat αENaC (a DEG/ENaC protein whose TM2 domain can substitute for that of MEC-4 in vivo [36]), and MEC-2 probably plays a similar role in *C. elegans* touch receptor neurons. While cholesterol is not required for MEC-2 multimerization or association with DEG/ENaC proteins, gentle touch sensitivity in worms is dependent on sterols [40]. The PHB domain of UNC-24 has not been tested for the ability to bind cholesterol, but it is necessary for the association of UNC-24 with other channel subunits [98]. UNC-24 also contains a nonspecific lipid-transfer domain [3, 72]. Such a domain could be needed for the insertion of cholesterol into the membrane. The association of these proteins with themselves, the channel pore subunits, and cholesterol might regulate channel activity by organizing the lipid environment around the mechanoreceptor complex.

In addition to encoding genes that form the mechanosensory channel complex at the membrane, touch sensitivity genes also encode components of the extracellular matrix (ECM, mantle). Three such genes are *mec-1*, *mec-5*, and *mec-9*. *mec-1* and *mec-9* encode proteins with EGF- and Kunitz-like domains that are expressed and secreted by the touch neurons [23, 25], while *mec-5* encodes a novel collagen generated by surrounding cells [23]. Both MEC-1 and MEC-5 proteins colocalize with the MEC-4 puncta along the touch neuron process, suggesting that they are concentrated around the mechanoreceptor channel complex [25].

The punctate distribution of the mechanoreceptor channel complexes requires *mec-1*, *mec-5*, and *mec-9*. Mutations in any one of these genes abolish proper localization of both mechanosensory channel subunits (MEC-4 and MEC-2) and ECM proteins MEC-1 and MEC-5 to their characteristic puncta along the process [25, 97], but loss of the channel subunits does not affect the localization of MEC-1 and MEC-5 puncta [25]. Because the puncta appear to align with annuli, the periodic ridges of the cuticle created by the hypodermis, the hypodermis probably contributes to the distribution of the puncta [25].

MEC-1 appears to have several functions in the touch receptor neurons. Complete loss of *mec-1* produces touch insensitive animals whose touch processes lack much of the ECM and are no longer attached to the body wall [12, 25]. The attachment and ECM defects can be separated by mutations from touch insensitivity; partial loss-of-function mutations of *mec-1* result in completely touch-insensitive animals that have normal appearing ECM, puncta, and attachment. Moreover, mutations in the hemicentin *him-4* gene also prevent touch cell process attachment but have little effect on touch sensitivity [87].

The touch receptor neurons are distinguished from other cells in *C. elegans* by having 15-protofilament rather than 11-protofilament microtubules [15]. *mec-7* and *mec-12* encode the β-tubulin and α-tubulin subunits of these large-diameter microtubules, respectively [29, 69]. Mutations in these genes and drugs that depolymerize the specialized microtubules cause animals to become touch insensitive [12, 15]. The cell processes are still present in the mutants because 11-protofilament microtubules have replaced the 15-protofilament microtubules and allow process outgrowth [15]. The 15-protofilament microtubules are arranged in bundles and fill the axon process, and their distal ends appear to associate with the plasma membrane [14].

The large-diameter microtubules appear to have multiple roles in the touch receptor neurons, so their specific role in mechanosensation is unclear. For example, mutations in these genes can affect the distribution but not the production of MEC-2 and MEC-4 puncta, restricting them to the cell body and proximal parts of the axon and suggesting a role of microtubules in transport of the channel complexes in the process [25, 39]. We have recently found, however, conditions in which the microtubules can be disrupted in adults (Bounoutas and Chalfie, unpublished data). These animals have a normal distribution of puncta but are touch insensitive. Thus, the microtubules may have a direct role in mechanosensation.

Additional proteins required for touch sensitivity have been identified but not fully characterized. Mutagenesis screens for touch insensitivity identified alleles for *mec-14*,

mec-15, *mec-17*, and *mec-18*, and all but *mec-15* have been cloned. *mec-14* encodes an oxido-reductase-like protein expressed in the six touch receptor neurons (Chalfie et al., unpublished data). Because MEC-14 is somewhat similar to the β-subunit of the Drosophila *shaker* K^+ channel, an intriguing speculation is that it regulates the mechanoreceptor channel complex. *mec-17* encodes a novel protein with no readily identifiable motifs [98]. Finally, *mec-18* encodes a protein similar to plant firefly luciferase and plant CoA ligase (Gu and MC, unpublished data).

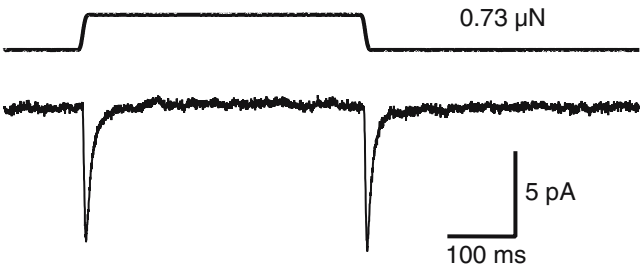

Fig. 3 Electrophysiological recording of a mechanoreceptor current (MRC). Both onset and offset of force (*top trace*) to the touch receptor neuron generates a current response (*bottom trace*). Figure reproduced and modified with permission from O'Hagan et al. [57]

Mechanoreceptor current and calcium influx

A better understanding of touch receptor function has come from in vivo electrophysiology recordings and from calcium imaging of the neurons. Using techniques developed for patch-clamp recording from *C. elegans* neurons [32, 52], PLM neurons were recorded from while mechanical stimuli were applied to the posterior body wall [57]. The onset of stimuli produced a mechanoreceptor current (MRC) that peaked rapidly and then decayed during continued force; removal of force produced an additional MRC of equal amplitude (Fig. 3). Forces as small as 100 nN generated MRCs, and increasing the amount of force increased the amplitude of the current (responses saturated for forces exceeding 1–2 μN) [57]. Although worms habituate to repeated gentle touch and plate tap [12, 60], the size of the MRCs did not decrease with repeated stimuli, suggesting that habituation occurs subsequent to the generation of the MRC. Finally, the MRCs were carried primarily by Na^+ and were reversibly blocked by amiloride, as expected if they require the MEC-4/MEC-10 DEG/ENaC channel [57].

Electrophysiology recordings of touch receptor neurons allow direct examinations of the effects of mutations on mechanosensory transduction. Null mutations in *mec-2*, *mec-4*, or *mec-6* eliminate MRCs, suggesting that each of these subunits is needed for channel function [57]. These mutations do not affect other currents in the touch receptor neurons, showing specificity for affecting MRCs [57, 82]. More significantly, point mutations in *mec-4* and *mec-10* that modify the selectivity of the degenerin channel alter the properties of the MRC. This alteration and the rapid onset of the effect (<0.5 ms) argue that the MEC-4 channel complex is transducing touch in these cells [57]. In contrast to the loss of MRCs when channel subunits are mutated, MRCs are reduced (~17-fold) but not abolished when the *mec-7* β-tubulin is absent [57]. These data suggest that the microtubules may not be essential for transduction.

Depolarization by the MRC leads to a subsequent calcium influx into the touch receptor neurons. Calcium imaging experiments of ALM neurons in vivo show that this influx is dependent on *mec-4* and *mec-2* as well as *egl-19*, which encodes an α-subunit of an L-type voltage-gated calcium channel [82]. Imaging of cultured touch receptor neurons also suggests that UNC-36, an α_2/δ subunit of L-type channels, may function as an accessory subunit to enhance calcium influx and voltage sensitivity [28]. The role of this calcium influx in response to the MRC is unclear. *egl-19* reduction-of-function mutants and *unc-36* null mutants are not fully touch sensitive; in addition, they have subtle defects in touch neuron migration and axon guidance that preclude attributing the cause of touch insensitivity to reduced calcium influx [28]. *C. elegans* lacks voltage-gated sodium channels, so voltage-gated calcium channels may contribute to the propagation of action potentials down the touch neuron process. The influx of extracellular calcium may also act on downstream secondary messengers in the neuron, triggering neurotransmitter release to targets in the touch reflex circuit that inhibit the opposite touch response and regulate habituation.

Models for mechanosensation

The initial model for mechanosensation in the touch receptor neurons was a dual-tether model (Fig. 4a) [39]. In this model, the mechanoreceptor channel complex is tethered intracellularly by the large-diameter microtubules and extracellularly by the proteins in the ECM. Application of force to the membrane would displace the microtubules, producing tension on the channel attached to the ECM. Movement of the channel between these two attachment points would then force the channel open.

Although the microtubules associate with the plasma membrane [14] and can affect transport of channel subunits throughout the axon [25, 39], recent evidence casts doubt on their role as intracellular tethers. Null mutations in *mec-7* and *mec-12* do not eliminate MRCs [57], suggesting that the large-diameter microtubules are not essential for the gating of the channel. Furthermore, stretching of the channel because of the displacement of the two tethering points seems unlikely to produce equal responses when force is both applied and removed, although this is what is seen in vivo [57].

We currently favor a model, derived from suggestions made by Kung [46], in which the channel complex is only tethered extracellularly. In this model, movement of the channel relative to the membrane, for example by pushing the channel into the cell when the animal is touched, would change the forces in the bilayer on the channel. These changes in force would cause the channel to open in analogy with the MscL mechanosensory channel in bacteria (Fig. 4b). The rapid adaption seen when animals are touched could result from the redistribution of forces in the bilayer. Elastic changes would again displace the channel complex when the force is removed leading to a second MRC that would quickly adapt. The role of the microtubules in this model is unclear. One possibility is that the microtubules organize the cell cortex so that movement of the bilayer is restricted. Similarly, the ECM outside the puncta might also constrain the movement of the membrane. These constraints could slow adaptation.

Touch reflex circuitry

Sensation of mechanical stimuli by the touch receptor neuron triggers changes in locomotion and other behaviors. Movement away from the touch stimulus is controlled by a three-neuron reflex circuit. *C. elegans* locomotion results from alternating contraction of dorsal and ventral wall body muscles. This muscle contraction is activated by ventral cord motor neurons (A motor neurons for backward movement and B motor neurons for forward movement), which in turn are regulated by four pairs of interneurons that extend throughout the ventral cord (AVA and AVD for backward movement; AVB and PVC for forward movement) [13]. The touch neurons form gap junctions with these interneurons to activate movement away from the touch stimulus (anterior touch neurons to AVD and posterior to PVC; secondary connections involve the other interneurons) [13]. To prevent simultaneous activation of both forward and reverse responses, anterior and posterior touch neurons also form putative inhibitory glutamatergic synapses with the interneurons activated by the other touch neurons (anterior touch neurons to PVC and posterior with AVD) [13, 47].

The relative strength of the motor circuit appears to change during development as seen by the response to plate-tap, which produces a nondirectional stimulus that presumably activates both the anterior and posterior touch response [93]. In early larva, *C. elegans* responds to plate tap by either moving forward or reversing at equal frequencies, suggesting the head and tail-touch circuits display equal strength and sensitivity. However, older animals almost always reverse, pointing to a developmental preeminence of the anterior touch circuit that corresponds to the rise of the postembryonic touch receptor neurons AVM and PVM [18].

In addition to locomotion, touch sensed by the touch receptor neurons modulates feeding behaviors, egg laying,

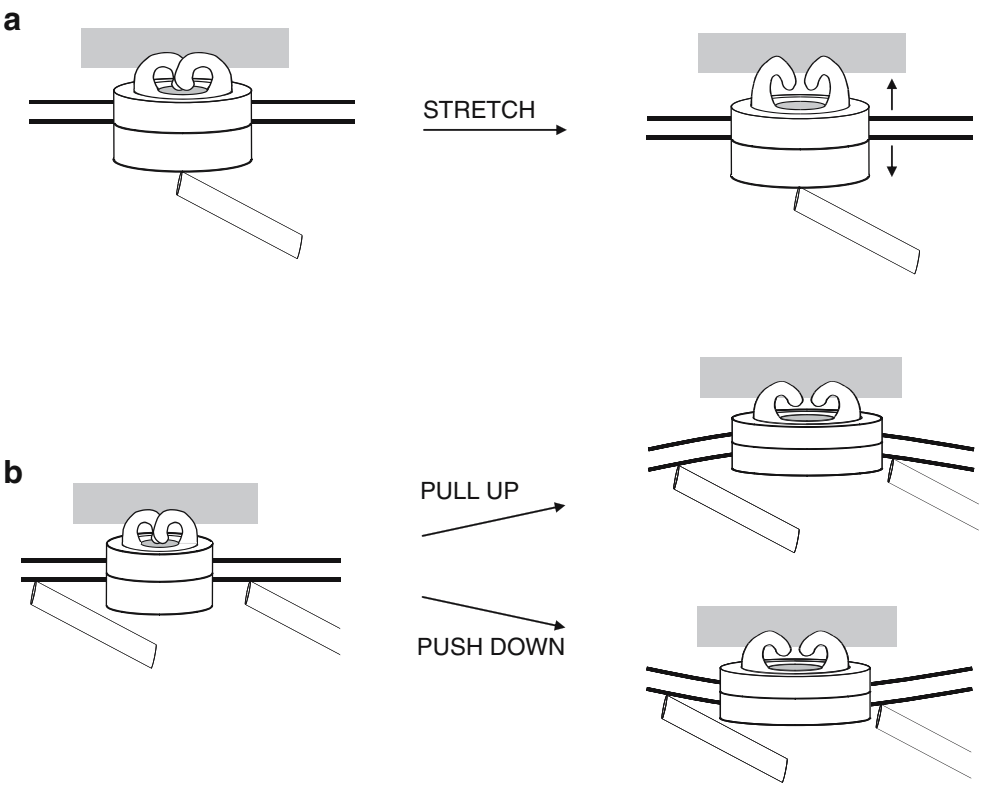

Fig. 4 Models of mechanotransduction in the touch receptor neurons. **a** Duel-tether model, in which the channel complex is attached to both the ECM and the large-diameter microtubules. In this model, separating the two tethering points forces the channel open. **b** A single-tether model of mechanosensation. In this model, displacement of the surrounding lipid bilayer around the channel produces forces that change the conformation state of the channel

and defecation. *C. elegans* slows upon encountering bacterial food, a texture-sensing behavior requiring the dopaminergic CEP, ADE, and PDE neurons [68]. The touch neurons synapse onto these neurons [13] and may override their signaling in preference of moving away from the touch stimulus [56]. Stimulating touch receptor neurons also suppresses pharyngeal pumping and head oscillations in foraging behavior. The mechanism for suppressing pharyngeal pumping is unknown, though the touch neurons form connections with PVR neurons that synapse onto neurons in the pharynx [13]. Suppression of head oscillations results from anterior touch stimulation of the AVD and AVA interneurons, which trigger RIM neurons to release tyramine that inhibits motor neurons and contraction of muscles radially surrounding the base of the head [1]. In both cases, the feeding behavior of the worm is suspended as touch avoidance becomes paramount. Touch stimulation also suppresses egg-laying behavior. Egg laying requires HSN neurons, which receive chemical synapses from PLM neurons that may inhibit their function [13]. Finally, touch stimulation also resets the defecation cycle of the animal [49], though no intermediate neural connections have been identified.

Habituation

The response to gentle touch decreases as *C. elegans* is repeatedly touched [12, 60]. This phenomenon is known as habituation. Habituation to gentle touch has been studied primarily by Catharine Rankin and her colleagues using the tap-withdrawal response [65]. Plate tap causes mature animals to reverse, and the frequency and distance of reversals diminish progressively with repeated taps. By measuring the distance of the reversals, habituation of the tap withdrawal response can be quantified [60]. This habituation depends on the frequency of the stimuli; animals habituate faster and more completely if taps are presented at a short interstimulus interval (ISI), such as every 2 s, than if taps are presented at a longer intervals, such as 1 min [61]. Worms trained at shorter ISIs spontaneously recover from habituation quicker than worms trained at longer ISIs. These results suggest that the mechanisms of habituation for tap withdrawal response differ for short and long ISIs [61, 65]. Training *C. elegans* with blocks of taps at longer ISIs produces long-term memory (LTM) for habituation that can be retained in optimal conditions for about 24 h. This memory cannot be generated with training blocks of short ISIs and can be disrupted by heat-shocking the animals in between blocks of stimuli [6, 65]. *C. elegans* are also susceptible to context conditioning, as plate tap training at long ISIs can be associated with other environmental cues to increase retention of habituation [58].

The mechanisms behind habituation to tapping are not fully understood. In vivo recordings from touch receptor neurons show that repeated stimuli do not decrease MRC amplitude, suggesting that habituation results from signals further downstream in the touch neurons themselves or postsynaptic to the neurons [57]. Because the chemical synapses between the touch neurons and interneurons involved in gentle touch mechanosensation appear to be glutamatergic in nature, habituation studies have focused on genes involved in glutamate transport and reception. *eat-4*, which encodes a protein with homology to rat brain-specific sodium-dependent inorganic phosphate cotransporter (BNPI), is expressed in touch receptor neurons [47]. In rat hippocampal neurons, BNPI transports glutamate into synaptic vesicles [7], and *eat-4* presumably plays a similar role in the presynapses of touch receptor neurons. *eat-4* mutants display faster than normal habituation of responses to plate-tap at all ISIs, slower spontaneous recovery, and an inability to produce LTM [62, 64]. Meanwhile, *glr-1* encodes a non-NMDA glutamate receptor expressed in all four interneurons (AVA, AVB, AVD, and PVC) that synapse with the touch receptor neurons, and mutations in *glr-1* also block LTM of habituation [63]. It is interesting to note that tap training of *C. elegans* modifies the density of postsynapse GLR-1 of the interneurons [63, 66]. These results suggest that glutamatergic transmission from the touch neuron to interneuron plays an important role in habituation.

Dopamine, which had previously not been implicated in gentle touch mechanosensation behavior, also affects habituation of the tap withdrawal reflex. ALM and PLM neurons express the dopaminergic receptor gene *dop-1*, and mutations in the gene cause the worm to habituate faster than normal in response to plate tap. This effect of *dop-1* on habituation is specific to the touch receptor neurons, as a wild-type copy of the gene expressed under a touch neuron-specific promoter rescues the habituation defect in *dop-1* mutants [68]. The touch receptor neurons synapse onto dopamine producing neurons (CEP, ADE, and PDE) [13], and synaptic input from the touch neurons could control their release of dopamine. Touch receptor neurons are presynaptic to the dopaminergic neurons, but dopamine may act as a neurohumoral agent or even be secreted extrasynaptically. The resulting feedback loop between touch neurons and dopaminergic neurons may modulate habituation of gentle touch mechanosensation [68].

Other mechanosensory behaviors

Although the response to gentle touch is the best understood mechanosensory behavior in *C. elegans*, other mechanosensory behaviors have also been examined. These

behaviors include withdrawal from nose touch [20] and harsh touch to the body [12], texture-mediated slowing in the presence of food [70], mechanical detection of hermaphrodites by males during mating [51], and responses to body stretch (proprioception) [48, 84]. Some of these behaviors are thought to involve DEG/ENaC channels, whereas others appear to require transient receptor potential (TRP) channels.

Nose touch, which is sensed by three types of ciliated neurons in the head, the ASH, FLP, and OLQ cells [43], may utilize both types of channel proteins. Two TRPV proteins, OSM-9 and OCR-2, are required for nose touch; they are coexpressed and possibly interact in the ASH neurons [19, 85]. OSM-9 is also expressed in the FLP and OLQ neurons. In OLQ neurons, OSM-9 may form a heteromeric channel with another TRPV protein, OCR-4 [85]. Several DEG/ENaC proteins are expressed in these cells as well (UNC-8 in ASH and FLP neurons; DEL-1 and MEC-10 in FLP neurons) [38, 84], but their role, if any, in nose touch is not known.

Sensation of harsh body touch requires the PVD neurons [90]. These two nonciliated neurons, which lie between the midbody and the tail, extend long anterior and posterior processes that extend almost the entire length of the worm [92]. As the animal matures, these processes develop elaborate dentritic branches that cover most of the surface of the animal, giving them the appearance of multidendritic cells in other organisms [86]. The molecular machinery involved in sensing harsh touch is unknown. The degenerin MEC-10 is expressed in PVD neurons [38], but *mec-10* mutations do not abolish sensitivity to harsh touch to the body [90]. One TRPV protein, OSM-9, is expressed in PVD cells, but the effect of its loss on the harsh touch response has not been reported.

C. elegans slows when it encounters a bacterial lawn or a patch of Sephadex beads the same size as bacteria, and this slowing is thought to be a response to surface texture. The ability to sense these textural differences requires the eight dopaminergic ADE, CEP, and PDE neurons [70]. Four CEP neurons are found in the head and extend ciliated sensory processes to the tip of the nose; pairs of ADE and PDE neurons are positioned in the head and posterior to the midbody, respectively, and send ciliated processes along the body's lateral midlines [78, 91]. These neurons express the TRPN channel TRP-4 [48]. The *trp-4* gene is an ortholog of the fruitfly and zebrafish *nompC* genes [74, 88]. Because *trp-4* mutants do not slow on entering a bacterial lawn [48], TRP-4 may have a role in transducing textural stimuli.

Male mating behavior requires a series of movements of the male tail, including contact with the hermaphrodite, sensing vulva location, insertion of spicules into the vulva, and sperm release [51]. This behavior is carried out by 87 neurons exclusive to the male; 42 of these neurons are ciliated neurons in the tail and many of these may be mechanoreceptors [79]. Two TRPP proteins, the polycystin-like proteins LOV-1 and PKD-2, are expressed in a subset of these neurons and required for male mating response and vulva location [4, 5, 54]. The human polycystins, PC-1 and PC-2, the respective homologs of *C. elegans* LOV-1 and PKD-2, are defective in inherited polycystic kidney disease [41]; they localize to primary cilium of kidney epithelial cells and sense fluid sheer stress, presumably as a heteromeric channel complex [55]. LOV-1 and PKD-2 colocalize at the cilia of certain male-specific neurons in the tail [4] and may interact similarly to their human homologues to transduce mechanical stimuli during male mating.

C. elegans moves by bending its body in a sinusoidal wave. The shape of the sine wave is affected by several mutations, which have been hypothesized as interfering with proprioception, the ability to sense relative body positioning and stretch. One such gene is *trp-4* [48]. As previously mentioned, TRP-4 is expressed in the dopaminergic neurons required for food-mediated slowing, but it is also expressed in the DVA interneuron [88]. The DVA cell body is located in the head, but it sends a process that extends for the entire length of the ventral cord [91]. *trp-4* mutants bend their bodies deeper and more frequently than wild-type animals, and these defects can be rescued by expressing TRP-4 exclusively in the DVA neuron [48]. In addition, expression of the calcium sensor G-CaMP shows that body bends trigger a significant Ca^{2+} spike in the DVA, strongly suggesting that the DVA neuron senses body-stretch [48].

Another potential candidate for sensing proprioreception is the UNC-8 degenerin. UNC-8 is expressed in several neurons including the VA and VB motor neurons, which have regions of long, undifferentiated processes that may act as stretch receptors [84, 91]. While no imaging or recordings from VA and VB motor neurons in response to bending have been done, the neurons also express another degenerin DEL-1, which may interact with UNC-8 to form a heterologous mechanosensitive channel [84]. Unlike *trp-4* mutants that display deeper body bending, *unc-8* mutants demonstrate more shallow body bends compared to wild-type worms [84]. Thus, if UNC-8-expressing motor neurons do function in proprioreception, they may act opposite to the TRP-4/DVA system to promote instead of inhibit *C. elegans* body bending [71].

Future directions

Although *C. elegans* has proven to be a very useful model for the study of mechanically driven senses, much work remains. Much of the essential transduction machinery has

been identified for the response to gentle touch, but several problems exist. First, the nature of the mechanosensory complex is not completely understood. Although we know that five proteins form the channel complex, we do not know their stoichiometry or structure, and we do not know what other proteins are required. Screens for touch-insensitive mutants have identified many genes whose products are needed for touch sensitivity, but even though these screens are saturated, they cannot give a complete picture of the genes needed for touch sensitivity. Microarray analysis of RNAs expressed in the touch receptor neurons have provided many more candidate genes [99], but their importance in transduction has not been determined. Second, we do not know how that channel complex interacts with the ECM, nor how the ECM is structured. Third, we do not know how the association of cholesterol (or other sterols) with the channel complex affects its activity. Fourth, the role of the 15-protofilament microtubules in mechanosensation remains to be elucidated. Fifth, and perhaps most important, we do not know exactly how touch is transduced by the channel complex.

For the other mechanically stimulated senses in *C. elegans*, the main area of future research will be the determination of the molecular bases of transduction. Although the sensory neurons and putative channels responsible for many of these behaviors have been identified, the means by which they sense mechanical stimuli has yet to be ascertained. Genetic approaches, using both classical procedures [9] and improved RNA interference methods [42], as well as DNA microarray methods (e.g., [99]) should provide additional candidates for components of the transduction machinery in these cells. Wild-type and mutant strains can then be analyzed by calcium imaging, as has been done with the ASH neurons needed for nose touch [34] and the DVA neuron needed for proprioception [48], and direct electrophysiological recording to obtain a description of the mechanosensory currents in these sensory neurons.

The study of gentle touch in *C. elegans* has identified DEG/ENaC channels as transduction channels for mechanosensation. This work also indicates that the sensing of mechanical force requires a rather complex molecular apparatus at the cell membrane. Future work will determine whether the lessons learned in *C. elegans* are applicable to mechanosensation in other organisms.

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