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Mechanotransduction: Touch and Feel at the Molecular Level as Modeled in *Caenorhabditis elegans*

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Abstract The survival of an organism depends on its ability to respond to its environment through its senses. The sense of touch is one of the most vital; still, it is the least understood. In the process of touch sensation, a mechanical stimulus is converted into electrical signals. Groundbreaking electrophysiological experiments in organisms ranging from bacteria to mammals have suggested that this conversion may occur through the activation of ion channels that gate in response to mechanical stimuli. However, the molecular identity of these channels has remained elusive for a very long time. Breakthroughs in our understanding of the cellular and molecular mechanisms of touch sensation have come from the analysis of touch-insensitive mutants in model organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster.* This review will focus on the elegant genetic, molecular, imaging, and electrophysiological studies that demonstrate that a channel complex composed of two members of the DEG/ENaC gene family of channel subunits (named for the *C. elegans* degenerins and the related mammalian epithelial amiloride-sensitive Na channel), MEC-4 and MEC-10, and accessory subunits is gated by mechanical forces in touch-sensing neurons from *C. elegans*. I also report here electrophysiological and behavioral studies employing knockout mice that have recently shown that mammalian homologues of MEC-4, MEC-10, and accessory subunits are needed for normal mechanosensitivity in mouse, suggesting a conserved function for this channel family across species. The *C. elegans* genome encodes 28 DEG/ENaC channels: I discuss here the global role of DEG/ENaCs in mechanosensation, reporting findings on the role of other three nematode DEG/ENaCs (UNC-8, DEL-1, and UNC-105) in mechanosensitive and stretch-sensitive behaviors. Finally, this review will discuss findings in which members of another family of ion channels, the Transient Receptor Potential channels family, have been implicated in mechanosensitive behaviors in organisms ranging from *C. elegans* to mammals.

Keywords *Caenorhabditis elegans* · DEG/ENaC · Mechanotransduction

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Introduction

The sense of touch is the most mature sensory system in the first months of our life. Indeed, as infants, we experience the world primarily through touch. Moreover, touch remains profoundly important throughout our life, and we fully depend on it for most of our everyday activities including feeding, drinking, moving, protecting, and communicating. Despite its fundamental importance as one of the primary ways humans interact with the outside world, touch is the least understood of our senses, both at the cellular and molecular levels.

The sense of touch initiates when pressure on the nerve endings imbedded in sensory structures such as Pacinian and Meissner corpuscles, wrapped around our hair follicles or free in our skin, is translated into electrical signals. Electrophysiological studies have identified and characterized ion channels in peripheral neurons that are gated by mechanical forces and that may underline touch sensation [1–5]. However, for a long time, the genes encoding these channels eluded cloning efforts, precluding the execution of

experiments that could directly test this hypothesis. Reasons that contributed to the initial failure in identifying these genes are: (1) there are no known reagents that specifically bind mechanosensitive channels and that could aid in their purification, (2) there are only few mechanosensitive channels per neuron, and (3) reconstitution of these channels in expression systems for functional analysis has been difficult at best. The difficulty expressing candidate channels is likely due to their need to associate with intracellular and extracellular proteins to function upon application of mechanical forces.

Invertebrate genetics have enormously advanced our understanding of touch sensation. Indeed, the analysis of touch-insensitive mutants in model organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster* have allowed the cloning of genes that are directly implicated in touch sensation. Among them, there are genes that encode ion channel subunits that are thought to be at the core of mechanosensitive ion channel complexes. Evidence suggests that these channels may underlie the mechanosensitive currents that can be recorded in peripheral neurons. These genes belong to two distinct families: the *C. elegans* degenerins and the related mammalian epithelial amiloride-sensitive Na channel (DEG/ENaC) family of Na^+/Ca^{2+} channel subunits and the Transient Receptor Potential (TRP) family of Na^+/Ca^{2+} channel subunits.

C. elegans Experiences its Environment Primarily Through Mechanosensation

C. elegans is a soil nematode used in biology as a model organism to understand the genetics of development and neurobiology. The advantages of using *C. elegans* to study biological problems include: (1) its size (1 mm in length), (2) its rapid life cycle, short life-span (~2 weeks), and the large number of offspring (>200), (3) its hermaphroditic nature, (4) the transparency of its skin and eggshell, (5) the limited number of cells (only 6) and the knowledge of the complete developmental program of cell division and connectivity pattern of all 302 neurons, and (6) the facile isolation of mutants and molecular manipulability. *C. elegans* has no sense of sight. Thus, it relies on chemosensation and mechanosensation to interact with its environment. When tested in a laboratory, *C. elegans* responds to different types of mechanical stimulation placed virtually everywhere on its body (see below). Experiments in which a laser beam was used to kill selected neurons have identified the sensory neurons responsible for these mechanosensory behaviors [6–8].

Nose touch response. When *C. elegans* collides with an object on its path, it responds by reversing its direction. This response is known as the nose touch response and is mediated by mechanosensory neurons named ASH, FLP, and OLQ [7, 9, 10]. ASH neurons mediate also osmotic avoidance (nematodes respond to high-osmotic-strength solutions by avoiding them) and adverse response to noxious stimuli including heavy metals, octanol, and detergents. TRP channels OSM-9 and OCR-2 of the V subfamily have been implicated in all ASH-mediated behavioral responses and have been proposed to act in a heteromultimeric channel complex [11–13]. Indeed, *osm-9* and *ocr-2* knockout mutant *C. elegans* no longer avoids high-osmotic-strength solutions and octanol and no longer respond to touch to the nose by reversing direction [11, 12], and localization of GFP-tagged OCR-2 at the cilia of ASH sensory neurons depends on the presence of OSM-9 and vice versa, raising the possibility that these two subunits interact in vivo in a channel complex [12].

TRP channels are six-transmembrane domains proteins, with intracellular N and C termini that are thought to assemble in vivo to form tetramers. The first members of this family were cloned from *Drosophila* where they are required for phototransduction [14]. Based on their sequence homology, TRP channels can be divided into seven subfamilies: TRPC, TRPV, TRPM, TRPN, TRPA, TRPP, and TRML [15]. Cytosolic N and C termini vary in length and sequence between subfamilies and among members of the same subfamily. In many TRP-channel cytosolic domains, specialized structural features with hypothesized function have been recognized. For example, ankyrin repeats are in the N terminus of TRPV and C and L members (3 to 14 present). Ankyryn repeats are thought to function as protein–protein interaction domains.

The results implicating TRPV channels OSM-9 and OCR-2 in all ASH-mediated behaviors including nose touch, osmotic avoidance, and avoidance of noxious chemicals and detergents suggest that these channels respond to mechanical forces as well as other stimuli [12, 13]. Another possibility is that these channels act downstream of the primary sensory event or that they set a certain physiological state in ASH neurons such so that specialized sensory channels can act [12, 13]. Further studies are needed to determine if OSM-9 responds directly to mechanical forces and/or other noxious stimuli. Unfortunately, these studies have been so far hampered by the fact that OSM-9 cannot be functionally reconstituted in expression systems [12]. In vivo electrophysiological analysis of ASH neurons in situ should finally address this issue.

Head withdrawal reflex. When touched on the dorsal or ventral side of the nose, *C. elegans* responds by turning its head away from the stimulus. This response is mediated by OLQ and IL1 mechanosensory neurons and is known as the

head withdrawal response. OLQ and IL1 neurons also regulate spontaneous foraging movements, which are dorsal–ventral head movements occurring during normal foraging. Animals in which IL1 and OLQ neurons have been killed by a laser beam have exaggerated head bends and forage abnormally slowly [16]. TRPV channel OSM-9 is expressed in OLQ neurons (but not IL1 neurons); however, it is not known if it is needed for the head withdrawal reflex.

Gentle touch response. C. elegans responds to the gentle stroke of an eyelash hair dragged across their body by reversing direction [17, 18]. Laser ablation studies have identified five neurons that are required for the sensation of gentle touch. A sixth neuron (the posterior ventral microtubule cell [PVM] neuron) is not per se required for the gentle touch response, but it is structurally similar to the other five neurons (Fig. 1a) [6]. These six neurons, also known as touch neurons, were initially called microtubule cells because their processes are filled with 15-protofilament microtubules (Fig. 1b; other cells in *C. elegans* have 11-protofilament microtubules, and mammalian cells have 13-protofilament microtubules) [19]. Processes of touch neurons run longitudinally along the body of the worm in close proximity to the cuticle (the worm "skin") and are imbedded in the hypodermis. Around the process a specialized matrix called the mantle, clearly visible by electron microscopy in cross-sections, separates the neuron from the hypodermis (Fig. 1b). Of the six touch neurons, four are embryonically derived and two are added to the body plan during larval development. Two embryonically generated posterior lateral microtubule cell (PLM) neurons have their cell bodies situated in the tail, on right and left sides, and send neuronal processes to the anterior; two embryonically generated anterior lateral microtubule cell (ALM) neurons are situated in the anterior between the head and the vulva, on right and left sides, and send neuronal processes to the posterior. The anterior ventral microtubule cell (AVM) is added during the first larval stage. The PVM neuron arises in the body at the same time as AVM. PVM is ultrastructurally similar to the other touch cells because it also contains 15-protofilament microtubules. Moreover, its differentiation is controlled by the same genetic pathway that controls differentiation of PLMs, ALMs, and AVMs. However, PVM does not mediate a response to gentle touch on its own, and it was postulated to be a stretch receptor [18]. Touch neurons also mediate the "tap" response, which is elicited when the plate in which animals are reared is tapped on the side or dropped onto a surface. In the next section, I will discuss molecules expressed in touch neurons identified in a screen for gentle touch-insensitive nematodes that form the long sought mechanosensitive channel gated by gentle touch.

Harsh touch response. C. elegans in which gentle body touch neurons have been laser ablated no longer respond to the touch of an eyelash hair but still responds to probing with a metal wire. This response is known as the "harsh touch" response. Laser ablation studies have suggested that FLP, OLQ, and PVD neurons mediate the "harsh touch" response. However, the same laser ablation studies have indicated that other neurons may also participate [20]. Strong candidates are the gentle touch neurons. Support of this hypothesis is that fact that gentle body touch neurons respond to harsh touch by generating Ca^{2+} transients independent of the gentle touch receptor channel [21]. It

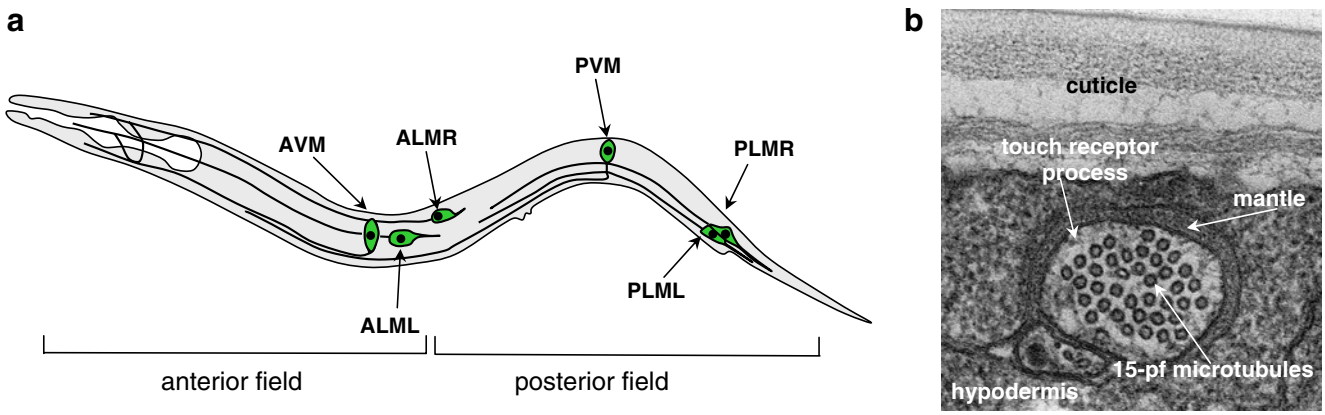

Fig. 1 *C. elegans* neurons that sense gentle body touch. **a** Schematic representation of *C. elegans* showing the position of the six neurons that sense the gentle stroke of an eyelash hair. There are two fields of touch sensitivity defined by the position of the touch neurons neuronal processes. The ALMs and AVM sense touch to the anterior field whereas PLMs sense touch to the posterior field. **b** Electron micrograph of a cross section of a touch receptor neuron process. The touch cell process is surrounded by the mantle and embedded in the hypodermis. It is also filled with 15-protofilament microtubules and is in very close proximity to the cuticle. This anatomical arrangement is thought to ensure the transmission of the mechanical forces applied on the cuticle to the touch neuron process. Dr. David Hall provided the electron micrograph

is currently not known what is the molecular determinant of these Ca^{2+} transients and whether they underlie harsh body touch sensation.

Mating. C. elegans males relay on mechanosensation to execute mating. During mating, the male tail containing the mating organs (the spicula) is used to search for the vulva. The male tail contains specialized sensory organs called rays. In each ray, a set of four sensory neurons detect tactile (and perhaps chemical) cues that are essential to locate the vulva. The spicula itself and other specialized organs in the tail (the hook and the postcloacae sensillia) also contain putative mechanosensory neurons and contribute to successful location of the vulva. A genetic screen for males that fail to locate the vulva identified TRP-like channels LOV-1 (Location of the Vulva Defective) and PKD-2 (Polycystic Kidney Disease) as candidate mechanoreceptor channels of ray sensory neurons [22, 23]. Mammalian homologues PKD1 and PKD2 are primarily expressed in the kidney and when mutant cause polycystic kidney disease. PKD1 and PKD2 are localized to the cilia of the kidney epithelial cells and are thought to help in clearing fluid from the tubules [24, 25]. Lack or malfunction of PKD1 or PKD2 is thought to lead to fluid accumulation in the tubules that ultimately leads to the formation of large fluid-filled cysts. It is currently not known how PKD1 and PKD2 help in clearing fluid, but this function may be linked to their putative mechanosensitivity.

Molecules that Mediate Gentle Body Touch

To identify molecules required for gentle body touch sensation, Chalfie et al. mutagenized *C. elegans* and screened progeny for rare mutants that no longer responded to gentle touch [18, 26]. Mutants selected fell into 16 groups identifying 16 genes that were named *mec* (mechanosensory abnormal) specifically required for the gentle touch response (Table 1). Indeed, one of the criteria used for the selection of the mutants was that they were otherwise normal: they reproduced and moved normally on a Petri dish. These criteria excluded genes that may be involved in gentle touch sensation but that are required for other locomotory activities. Mutations in redundant genes were also missed by this screen. Nonetheless, this pioneering work for the first time identified molecules needed for touch sensation.

DEG/ENaC Channel Subunits MEC-4 and MEC-10 at the Core of the C. elegans Touch Receptor. Among the 16 genes identified in the touch-insensitive screen, two encode channel subunits. *mec-4* and *mec-10* encode homologous proteins (48% identity) related to subunits of the amiloride-sensitive voltage-independent Na^+ channels in mammals (the ENaC channels) [27–32]. ENaC channels mediate Na^+ readsorption in renal, intestinal, and lung epithelia [29, 30]. *mec-4* and *mec-10* are expressed in touch neurons, and *mec-10* is additionally expressed in harsh touch receptors FLPs and PVDs. *mec-4* and *mec-10* loss-of-function mutants are touch insensitive, yet their touch receptor neurons develop normally and are ultrastructurally normal [18]. These data suggest that in touch neurons, *mec-4* and *mec-10* may form a channel responsive to mechanical forces.

MEC-4 at the molecular level. Because many more mutations were identified in the *mec-4* gene than in *mec-10*, MEC-4 structure–function is better understood (Fig. 2a). MEC-4 is 768 amino acids long and includes two transmembrane domains (MSDI and MSDII) oriented in the membrane such that MEC-4 N and C termini are projected intracellularly and a large central loop containing three cysteine-rich regions extends extraceullarly [32–34]. This topology is common to all the other members of the family including mammalian ENaC channels. Analysis of mutations that disrupt touch sensitivity and suppress hyperactivity of mutant MEC-4(d) (described below) in vivo lead to the identification of regions critical for MEC-4 function [27, 34, 35]. These regions include: (1) MSDII and a short pre-MSDII stretch of amino acids, both of which contribute to the channel pore: studies on mammalian ENaC channels have shown that this domain lines the pore of ENaCs as well [36–41]; (2) a highly conserved intracellular stretch adjacent to MSDI: in mammalian ENaCs, this domain influences ion permeation and selectivity [42]; (3) the cysteine-rich extracellular loops [43]: These domains may help tether MEC-4 to the specialized extracellular matrix of the touch neurons [44]; and (4) a residue in the C terminus (A745) critical for trafficking MEC-4 to or maintaining it at the plasma membrane [35]: in most neuronally expressed family members, this residue is an alanine or another nonpolar residue suggesting that it may have a conserved role in subunit trafficking [35]. In ENaC channels, stability at the surface is mediated by conserved PY motifs (consensus PPXY) also situated at the C terminus suggesting that the C terminus of DEG/ENaC channels may play a key role in subunit turnover. The PY motif interacts with Nedd-4 ubiquitin ligase, which mediates channel turnover [45, 46]. When the PY motif is missing or disrupted, it causes the channel to accumulate at the cell surface. This in turn causes enhanced Na^+ transport, constituting the basis of human Liddle's syndrome, a hypertensive disorder (reviewed in [47]).

A very interesting *mec-4* mutant was isolated in the original screen for touch-insensitive nematodes. *mec-4(d)*

Table 1 The *Caenorhabditis elegans mec* genes

Gene	Protein	Similarity	Function	Mutant phenotype
mec-1	MEC-1	EGF/Kunitz repeat protein	Mantle protein clusters the MEC channel in puncta	Touch insensitive. The mantle does not form and touch neurons are not attached to the cuticle
mec-2	MEC-2	Stomatin-like protein	Channel associated protein enhances channel function by acting on channel conductance or open probability	Touch insensitive
mec-3	MEC-3	LIM domain binding protein [20, 102]	Specification of touch neurons differentiation	Touch insensitive. Touch neurons small and lacking processes. ALM and PLM touch cells misplaced
mec-4	MEC-4	Amiloride-sensitive DEG/ENaC channel subunit	Mechanosensitive channel protein conducts Na^+ (Ca^{2+})	Touch insensitive
mec-5	MEC-5	Collagen	Extracellular matrix protein, participates with MEC-1 in clustering of the MEC channel	Touch insensitive. The mantle is not stained by peanut lectin
mec-6	MEC-6	Paraoxonase-like protein	Channel associated protein enhances channel function by acting on channel conductance or open probability	Touch insensitive
mec-7	MEC-7	β tubulin	15-protofilament microtubule component	Touch insensitive. Touch neurons lack 15 protofilaments microtubules
mec-8	MEC-8	RNA binding protein	RNA splicing [103, 104]	Touch insensitive. Disrupted fasciculation of amphid and phasmid channel cilia
mec-9	MEC-9	EGF/Kunitz repeat protein	Mantle protein required for MEC-1 localization in puncta around the touch cells processes	Touch insensitive.
mec-10	MEC-10	Amiloride-sensitive DEG/ENaC channel subunit	Mechanosensitive channel protein appears to act as accessory subunit of MEC-4	Touch insensitive
mec-12	MEC-12	α tubulin	15-protofilament microtubule component	Touch insensitive. Touch neurons lack 15 protofilaments microtubules
mec-14	MEC-14	Aldo-keto reductase	Controls MEC channel activity through an unknown mechanism	Partial touch insensitive. Temperature dependent
mec-15	MEC-15	F-box protein	Required for proper touch receptor neuron mechanosensation, morphology, and synapse development	Touch insensitive. Touch neurons lack 15 protofilaments microtubules
mec-16	MEC-16	Homeodomain protein	Required, during early larval development, for backward movement in response to anterior touch with a wire and during later larval stages, for response to gentle touch with a hair	Touch insensitive to metal wire in early development and to eye lash hair in later larval stages
mec-17	MEC-17	No distinct motif	Required for maintaining the differentiated state of the touch receptors component	Touch insensitive
mec-18	MEC-18	AMP binding protein	May negatively regulate the MEC channel	Partially touch insensitive

encodes a channel subunit mutated at position 713 just upstream of MSDII (A713V or T). *mec-4(d)* causes swelling and death of touch neurons (for this reason, *C. elegans* subunits were named "degenerins"—DEG [27, 48]). When reconstituted in *Xenopus* oocytes, the gene product of *mec-4(d)*, also named MEC-4(d), produces hyperactive channels [49, 50] (Fig. 2b). In addition to being hyperactive, MEC-4(d) is also Ca^{2+} permeable and may cause neurodegeneration by conducting toxic levels of Ca^{2+} into the cell [51]. It is currently not known if wild-type MEC-4 is Ca^{2+} permeable and, if so, whether Ca^{2+} permeability plays a key role in touch sensation.

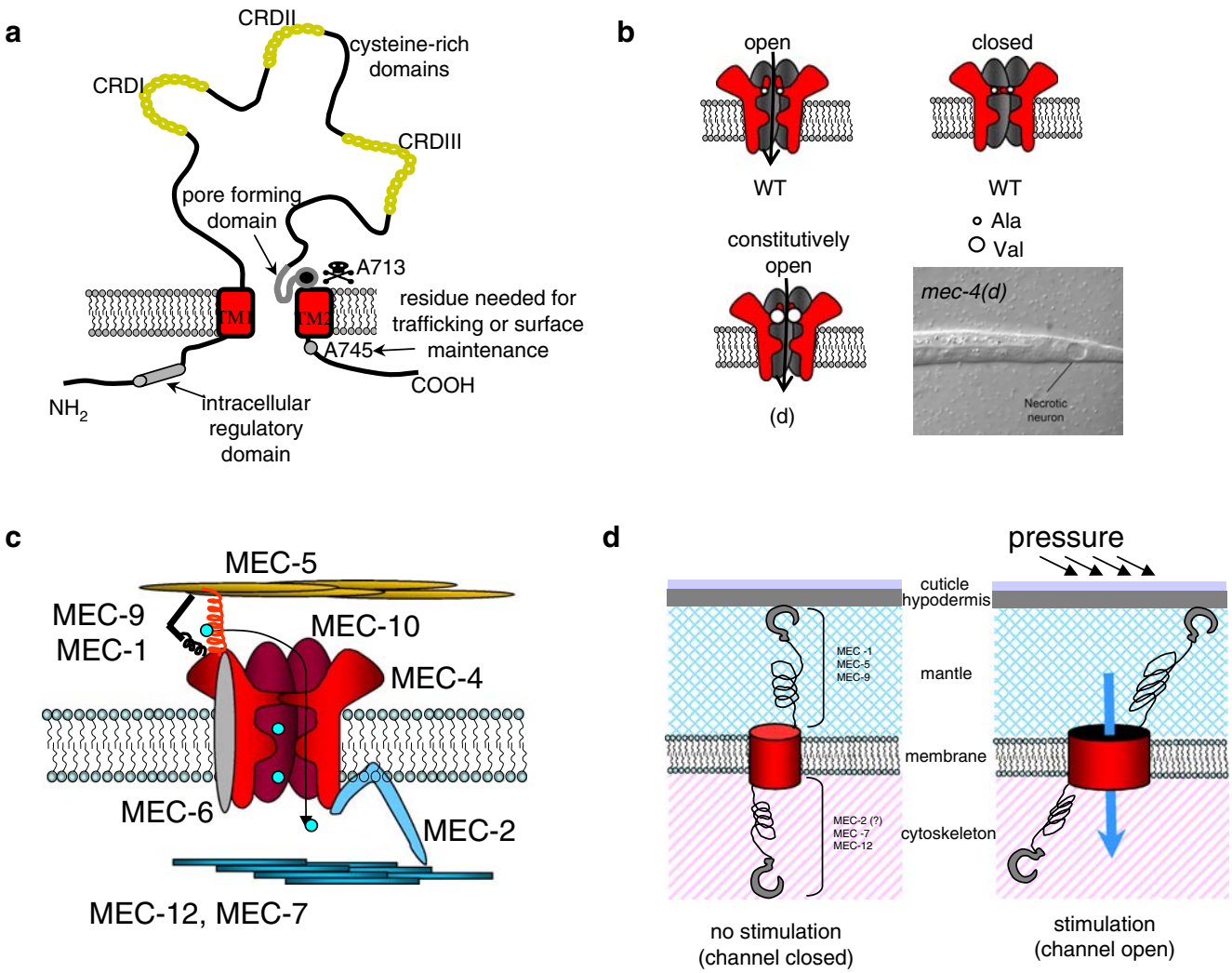

Fig. 2 MEC-4 structure/function and proposed mechanism of channel gating. **a** Structural features of a single MEC-4 subunit. The MEC-4 protein spans the membrane twice leaving the N and C termini in the cytosol. Ala713, which when replaced by a bulkier amino acid such as Valine or Threonine results in necrotic cell death, is indicated by the *skull* and *cross-bones icon*. MEC-4 also features three cysteine-rich domains (CRDI, II, III) that are thought to be involved in protein–protein interactions, perhaps anchoring MEC-4 to extracellular matrix proteins. Mammalian DEG/ENaC channels feature only one cysteine-rich domain, corresponding to MEC-4 CRDIII. Other important domains include: the intracellular regulatory domain, the pore forming domain, and residue A745, which is implicated in channel trafficking and/or maintenance at the cell surface [35]. **b** Model for *mec-4(d)*-induced toxicity. Wild-type MEC-4 channels open and close in response to mechanical forces. However, MEC-4(d) channels that encode substitutions for a conserved alanine adjacent to MSDII, are "locked" in an open conformation because of steric hindrance. This is postulated to result in excessive Na^+ and Ca^{2+} influx that triggers necrotic-like cell death. This necrosis manifests itself in the early stages as cell swelling (*lower right panel*) [27, 48, 51]. **c** Diagram of a touch-transducing complex in *C. elegans* touch receptor neurons. DEG/ENaC channel subunits MEC-4 and MEC-10 are at the core of the channel complex. They interact with accessory subunits MEC-2 and MEC-6 that regulate channel activity, selectivity, and perhaps gating [49, 50, 52, 56]. On the intracellular side of the membrane, α- and β-tubulins MEC-12 and MEC-7 form 15-protofilament microtubules that may interact directly or indirectly with the channel and help gate the channel when mechanical forces are applied [18, 100, 101]. On the extracellular side of the membrane, extracellular proteins MEC-1 (a EGF/Kunitz repeat protein), MEC-5 (collagen), and MEC-9 (another EGF/Kunitz repeat protein) are part of the mantle and are required to cluster the MEC channel in puncta structures that are needed for function [62]. MEC-1, MEC-5, and MEC-9 may be also needed to pull the channel open during mechanical stimulation. **d** Schematic representation of MEC channel gating. In the resting state (*left panel*), the molecular interactions between the various proteins of the complex are at rest, maintaining the channel in its closed conformation. When pressure is applied on the cuticle (the *C. elegans* skin), it causes a change in the molecular interactions of the complex leading to channel opening

MEC-10 at the molecular level. MEC-10 is a 730-amino acid-long protein 48% identical to MEC-4 over a stretch of 469 amino acids. MEC-10 topology is identical to MEC-4's, and several domains/amino acids that were found crucial to MEC-4 function are conserved in MEC-10, suggesting that they may be important for the function of MEC-10 as well. However, much less is known about MEC-10 structure/function and its physiological role within the channel complex. The main reasons for this are that much less mutations were isolated in *mec-10* than in *mec-4*

in the touch-insensitive genetic screen and that no deletion mutant has been available until recently [26, 31]. The *mec-10* mutations isolated in the original screen are all recessive and are localized within the intracellular N terminus (S105F, allele *e1515*) and in MSDII (G676R, allele *u20*; L679R, allele *u390*; G680E, allele *u332* and G684R, allele *e1715*) [31] known to be important for MEC-4 function [34]. Despite the low number of mutations isolated, behavioral, genetic, and functional data currently available suggest that MEC-10 is a regulatory subunit. Evidence that support a regulatory role of MEC-10 includes the following. (1) When *C. elegans* is transformed by *mec-10* that is mutated at the position corresponding to *mec-4 (d)* (*mec-10 (A673T)* or *mec-10(d)*), degeneration of the touch neurons is much weaker than in *mec-4(d)*. Only 36% of the larvae display degenerating PLMs, and ALMs are only rarely seen degenerating (>90% of the larvae display degeneration of both ALMs and PLMs in *mec-4(d)*) [31]. (2) *mec-10(d)*-induced neurodegeneration is strictly dependent on *mec-4* [31]. (3) While MEC-4(d) can form channels on its own, MEC-10 and MEC-10(d) are not functional when expressed alone [49]. (4) Introduction of the MEC-10 subunit to the MEC-4(d) channel in oocytes affects the K_d for amiloride and suppresses current amplitude [49–51]. Because only *mec-10* point mutations were isolated in the original screen [31], effects on touch sensitivity and on touch neurons physiology of a true *mec-10* null are at this time unknown. It is interesting to note that allele *u20* reduces the extent of *mec-10(d)*-induced neurodegeneration [31] and suppresses MEC channel activity in vivo [52], suggesting that mutation G676R exerts a strong dominant suppressive action on the channel. We are anxiously awaiting the behavioral and physiological analysis of the novel *mec-10* null mutant to learn more about the role of this subunit in channel function.

MEC-4 and MEC-10 at the functional level: Xenopus oocytes assays. When MEC-4 (or MEC-4(d)) and MEC-10 are coexpressed in *Xenopus* oocytes, they form Na^+-selective channels sensitive to the ENaC-blocking agent amiloride [49]. In *Xenopus* oocytes, MEC-4/MEC-10 channels are not gated by membrane stretch induced by hypotonic solutions [49]. This result suggests that hypotonic solutions do not induce sufficient membrane stretch because of the oocyte membrane invaginations [53]. Alternatively, sufficient membrane stretch is obtained when the oocyte is perfused with hypotonic solutions but other proteins or factors present in *C. elegans* touch neurons are needed for functional gating of the MEC channel. It is possible that intracellular and extracellular proteins normally present in vivo and absent in *Xenopus* oocytes are needed to exert gating tension on the channel during mechanical stimulation.

Stomatin-like protein MEC-2 enhances channel function. *mec-2* encodes a 481-amino acid protein expressed in the touch receptor neurons and in a few additional neurons in the head [54]. MEC-2 is a cytosolic protein, with one strong hydrophobic region that probably loops back in the membrane (Fig. 2c). MEC-2 shares a similarity with a membrane-associated protein found in red blood cells (RBC) called stomatin. Stomatin is missing in RBC from individuals affected by an inherited severe hemolysis called stomacytosis. Thus, stomatin was postulated to control ion permeation in RBC [55]. *mec-2* mutations were isolated in the genetic screen for touch-insensitive mutants suggesting that this protein is needed for proper channel function. More recent studies have revealed the nature of this requirement.

Coexpression of MEC-2 with MEC-4 and MEC-10 (both wild type and (d) variants) in *Xenopus* oocytes increases current amplitude up to 40 times without changing channel density at the cell surface [49]. This suggests that MEC-2 may increase current amplitude by changing channel conductance or open probability (or both). Single-channel experiments are needed to distinguish between these two possibilities. MEC-2 physically interacts with MEC-4 and changes its permeability to lithium, suggesting that portions of MEC-2 line the channel pore [49]. MEC-2 subcellular localization is worth noting because it plays a key role in touch sensitivity. MEC-2 is localized in puncta along the neuronal processes of touch neurons. The pattern of distribution of MEC-2 overlaps with MEC-4 [50, 56]. Zhang et al. [56] have shown that the punctated distribution of MEC-2 requires MEC-4, MEC-10, and MEC-6 and that this distribution is crucial for conferring touch sensitivity. *mec-2* mutations that drastically reduce the number of puncta are associated with severe touch insensitivity, whereas *mec-2* mutants in which the distribution of the channel is relatively undisturbed are only mildly touch insensitive. Almost half of the mutations that disrupt touch sensitivity are within the stomatin-like domain, which is critically needed for interaction with MEC-4, underscoring the vital role of this domain in MEC-2 function [56]. MEC-2 punctated distribution requires also interaction of the protein with cholesterol, suggesting that membrane microdomains play a key role in mechanotransduction [57]. This hypothesis is supported by the finding that MEC-2 homologue UNC-1 interacts with MEC-4 homologue UNC-8 in lipid rafts [58]. Wetzel et al. [59] have recently shown that the stomatin-like protein 3 (SLP3) is needed for normal touch perception in mouse, suggesting that stomatin-like proteins are essential component of mechanosensitive channels in mammals as well where they may associate with channel subunits homolog of MEC-4 (see below for more details).

Paraoxonase-like protein MEC-6 also enhances channel function. *mec-6* mutants are touch insensitive and suppress *mec-4(d)*-induced neurodegeneration suggesting the MEC-6 is needed for channel function [18, 60] (Fig. 2c). *mec-6* encodes a 377-amino acid transmembrane protein with a small intracellular N terminus and a large extracellular C terminus endowed with potential glycosylation sites. Two hundred and fifty amino acids at the C terminus of MEC-6 share similarity to vertebrate paraoxonase/arylesterases (25% identity and 45% similarity). Vertebrate serum paraoxonases are high-density lipoprotein-bound ester hydrolases that catalyze the hydrolysis of organic esters (including the pesticide metabolite paraoxon) and protect low-density lipoprotein from oxidation. As such, mammalian paraoxonases are thought to prevent atherosclerosis and coronary heart disease. MEC-6 is expressed in neurons, muscles, and the canal cell (the kidney of the worm) and may be needed for function of many other *C. elegans* DEG/ENaC channels. It is not known if mammalian paraoxonases play a role in the function of mammalian DEG/ENaC channels. When MEC-6 is coexpressed with MEC-4 (d) in *Xenopus* oocytes, they physically interact, and this results in an increase in current amplitude up to 24 folds [50]. Like MEC-2, MEC-6 does not change the channel density at the cell surface [50]. MEC-6 interacts also with MEC-10 and MEC-2, and the effects of MEC-2 and MEC-6 on current amplitude are synergistic [50]. This suggests that MEC-2 and MEC-6 enhance channel function acting through two distinct mechanisms.

Intracellular and extracellular proteins needed for touch transduction. In the original screen for touch-insensitive mutant nematodes, several mutations were isolated in genes that encode for extracellular and intracellular proteins [18]. These mutations do not affect the development of touch neurons, and thus, these proteins were proposed to be needed for trafficking, subcellular localization, or gating of the channel [18].

MEC-7 and MEC-12. Two of these proteins are the β- and α-tubulins MEC-7 and MEC-12 (Fig. 2c). Mutations in *mec-7* and *mec-12* disrupt the production of the 15-protofilament microtubules [19]. It is interesting to note that MEC-12 is acetylated (Lys40) in touch neurons but not in other neurons, suggesting this modification may be important for the assembly of 15-protofilament microtubules [61]. Acetylation of MEC-12 also occurs in cultured touch neurons indicating that this modification and possibly the assembly of 15-protofilament microtubules do not require the extracellular mantle [51]. A recent study from the Chalfie laboratory has shown that in *mec-7* mutants, the amiloride-sensitive current activated in touch neurons by mechanical forces and likely mediated by the MEC channel is still present (although reduced and less sensitive to pressure) [52]. These results indicate that MEC-7 may not be essential for channel gating.

MEC-1 and MEC-5. In *mec-1* mutants, the extracellular mantle is almost completely absent and touch neuron processes are not attached to the cuticle [18, 62]. However, this is not the reason why *mec-1* mutants are touch insensitive. Indeed, in *him-4* mutants, failure of touch neurons to attach to the cuticle is not associated with touch insensitivity [63]. *mec-1* encodes a 1,999-amino acid protein with an N-terminal signal sequence followed by a Kunitz-type serine protease domain, two Ca^{2+}-binding epidermal growth factor (EGF) domains, 14 additional Kunitz-type domains, and a C terminus of 160 amino acids. The Kunitz and EGF domains are likely protein–protein interaction domains. MEC-1 is secreted by the touch cells and coats the touch neurons processes [62]. *mec-5* encodes a collagen with unique Gly-X-Y repeats that is secreted by hypodermal cells that surround touch neurons [64]. MEC-1 and MEC-5 do not have obvious mammalian homologues; however, other extracellular proteins with Kunitz and EGF domains and other types of collagens, respectively, may serve a similar function in mammalian touch sensation. The pattern of distribution of MEC-1 and MEC-5 surrounding the processes of touch neurons is also punctuated and overlaps with MEC-4 localization, and it is needed for MEC-4 localization [62]. These data suggest that the mechanosensory channel localization at functionally relevant sites depends on the extracellular matrix. Both the MEC-1 and MEC-5 punctated patterns are completely absent in *mec-9* mutants, and it is substituted by a diffuse staining [62].

MEC-9. *mec-9* encodes a protein that is secreted by touch receptor neurons [64], and it is probably part of the mantle. However, *mec-9* mutations do not affect the structure of the mantle in any detectable way. MEC-9 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. These domains are postulated to function in protein–protein interactions and are also found in agrin, a protein that localizes acetylcholine receptors [65].

In summary, genetic and molecular data support a model in which a channel formed by DEG/ENaC subunits MEC-4 and MEC-10 and accessory subunits MEC-2 and MEC-6 is tethered to extracellular proteins that control channel subcellular localization. Presumably, based on the genetic data currently available, intracellular cytoskeletal proteins may also interact with the channel in a manner that may or may not be dependent on its interaction with extracellular

proteins. Thus, these molecular interactions may pull the channel open when mechanical forces are applied (Fig. 2c and d). Much of the future research will be dedicated to addressing if such molecular interactions exist and what is their functional role in the touch transduction event. One major step toward this direction was recently taken with the demonstration that the MEC channel is indeed gated by mechanical forces.

Other proteins needed for touch sensation. Another class of mec genes includes genes that are required for development of the touch neurons and for proper formation of synapses with interneurons (*mec-3*, *mec-8*, *mec-15*, *mec-16*, and *mec-17* [18, 20, 26, 66, 67]), as well as genes that encode for proteins that may have a regulatory action on the MEC channel (*mec-14* and *mec-18* [26, 67]; see Table 1).

Among these genes, *mec-14* and *mec-18* are particularly interesting because they may be part of the channel complex itself or they may act indirectly to regulate the channel activity. *mec-14* encodes a 453-amino acid-long protein with similarity to the beta-subunits of Shaker-type potassium channels, which are members of the aldo–keto reductase superfamily [68]. Although the similarity is weak, it is suggestive of possible regulation of the MEC channel activity by MEC-14. If MEC-14 regulates the activity of the MEC channel (which remains to be established), then this subunit is highly specific for the MEC-4/MEC-10 channel complex because it is expressed in touch neurons only, and therefore it cannot regulate the activity of any other DEG/ENaC channel, at least in wild-type animals [26, 67].

mec-18 encodes a 638-amino acid-long protein similar to firefly luciferase, plant protein 4-coumarate coA ligase, and yeast peroxisomal adenosine monophosphate-binding protein, involved in peroxisomal function [69]. Peroxisomes participate in the metabolism of fatty acids and rid the cell of toxic peroxides. Thus, one possibility is that MEC-18 influences touch sensitivity by controlling the fatty acids content of the plasma membrane. This would imply that DEG/ENaC channels are sensitive to membrane lipid content. One hint that this may be the case, at least for some DEG/ENaC channel subunits, is the fact that DEG/ENaC channel UNC-8 is localized in lipid rafts, and its presence in the rafts is dependent on stomatin-like protein UNC-1 [58].

The MEC Channel is Gated by Mechanical Forces

Although genetic studies have clearly identified proteins needed for normal touch sensation in *C. elegans*, they could not address if any of the MEC proteins is directly implicated in this process. Indeed, all the proteins identified in the screen could be simply required to maintain touch neurons in a certain physiological state so that these cells could function properly at baseline and/or when stimulated by mechanical forces.

Touch elicits a MEC-dependent rise of intracellular Ca^{2+} concentration in touch neurons. The first hint that the MEC channel is indeed a mechanosensor has come from studies employing a genetically encoded calcium sensor called "cameleon" [21]. In cameleon, the fluorophores cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) are linked by calmodulin and the myosin light-chain calmodulin-binding peptide M13 [70, 71]. When the concentration of intracellular Ca^{2+} is low, the two fluorophores are positioned far from each other resulting in low fluorescence resonance energy transfer (FRET). When Ca^{2+} rises, calmodulin binds Ca^{2+} and associates with M13, bringing the two fluorophores closer together and enabling FRET [70]. Measurements of ratiometric CFP/YFP signals thus give a report of changes in intracellular Ca^{2+} concentration. Because cameleon is genetically encoded, these measurements can be performed in vivo in transgenic nematodes that express the Ca^{2+} sensor in selected cell types.

Experiments employing cameleon expressed in touch neurons have revealed that the MEC channel is specifically required for responses to gentle touch and not for general function of touch neurons. First, a transient rise in intracellular Ca^{2+} concentration is observed in touch neurons of *C. elegans* mechanically stimulated by a probe that mimics the gentle stroke of an eyelash hair. Second, this Ca^{2+} rise is absent in *mec-4*, *mec-2*, and *mec-6* mutants, suggesting that the MEC channel is indeed needed for cellular responses to gentle touch (Fig. 3a, upper panels). Third, touch neurons from *mec-4* and *mec-2* mutants (*mec-6* was not tested) still respond to harsh touch with a distinct type of Ca^{2+} rise that is indistinguishable from the one observed in touch neurons from wild-type *C. elegans* (Fig. 3a, lower panels). Moreover, in vitro cultured touch neurons of *mec* mutants are endowed with background ionic currents identical to the ones found in wild-type touch neurons. They also respond normally by rising intracellular Ca^{2+} concentration when depolarized by high extracellular K^+ concentrations. In sum, the cellular response to gentle touch in touch neurons is a rise in intracellular Ca^{2+} concentration that depends on the activity of the MEC channel [21]. MEC-4 does not seem to be needed to maintain basal touch neurons physiology.

Other insights into touch neurons physiology revealed by cameleon measurements. Experiments employing cameleon revealed other aspects of the physiology touch neurons not

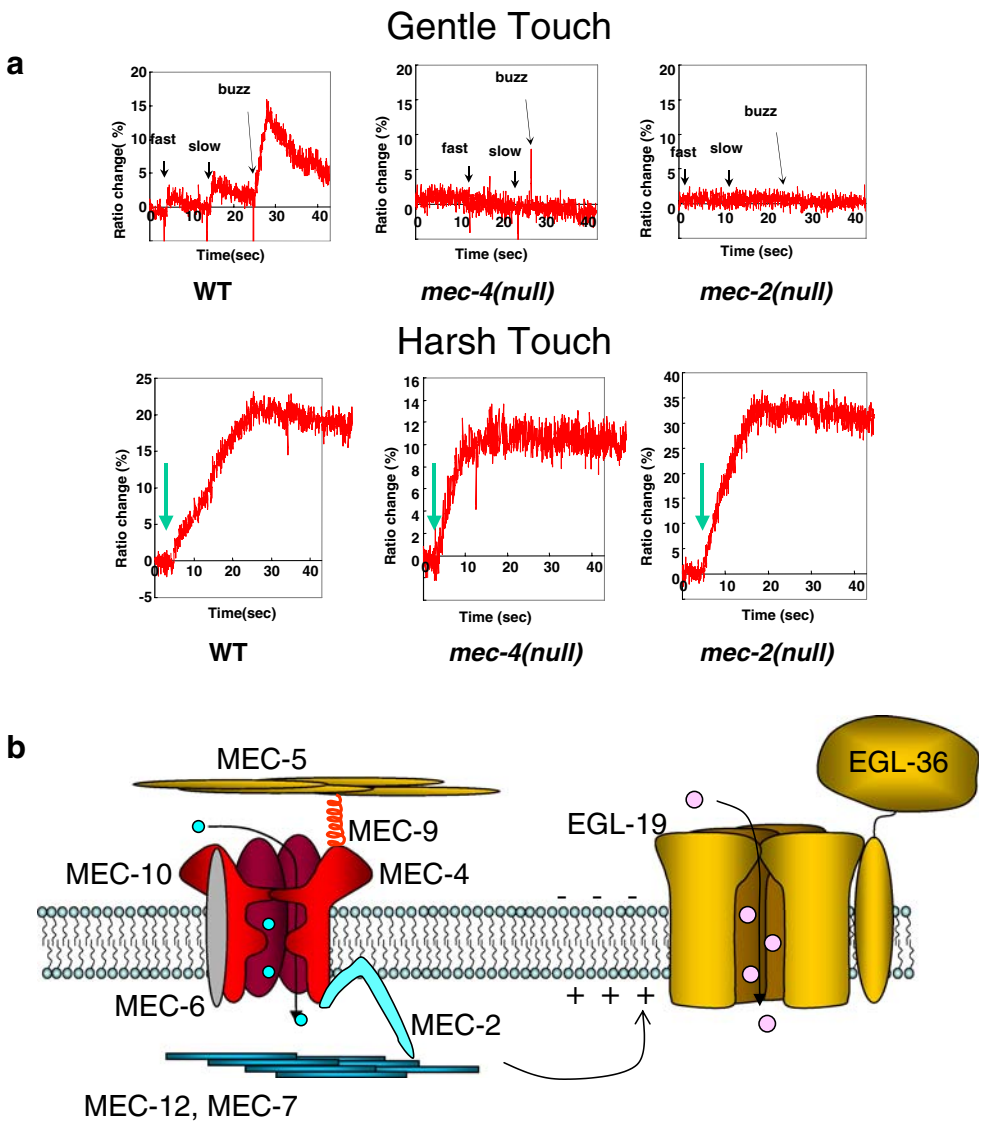

Fig. 3 Molecular model for body touch sensation. **a** Calcium imaging using the genetically encoded calcium sensor cameleon revealed that touch neurons undergo transient changes in intracellular calcium concentration during touch stimulation [21]. The *upper left panel* shows an example of one of these calcium transients evoked by a constantly moving probe (*buzz*). In contrast, fast and slow pokes cause only detectable calcium transients. *Middle* and *right upper panels* show lack of calcium transients in touch neurons from *mec-4* and *mec-2* null mutants. The *lower panels* show calcium transients recorded in all three strains when worms were stimulated by harsher touch. The molecular identity of the channel that produces these calcium transients is unknown at the moment. **b** Diagram of a touch-transducing complex in *C. elegans* touch receptor neurons and the possible mechanism of calcium transient activation. At rest, the channel is closed. Upon mechanical stimulation, a distortion in the network of interacting MEC molecules results in channel opening. Entry of Na^+ (and perhaps Ca^{2+}) through the channel depolarizes the neuron. Depolarization activates the voltage-gated L-type calcium channel EGL-19 (likely associated to accessory subunit UNC-36) that produces transient changes in intracellular calcium concentration

previously known. First, gentle touch neurons of *C. elegans* respond robustly to a moving probe (500-ms-long stimulation with a probe moving at a frequency of 20 Hz, "buzz" stimulation), only weakly to a "poke" (150 or 300 ms long) and not at all to constant pressure. These features indicate that touch neurons in *C. elegans* are motion sensors and may bare some resemblance to mammalian Pacinian corpuscles. Second, the rise of intracellular Ca^{2+} concentration is partially dependent on L-type voltage-gated Ca^{2+} channel EGL-19 and regulatory subunit UNC-36, suggesting that the MEC channel may depolarize the cell membrane and activate voltage-gated Ca^{2+} channels. Third, Ca^{2+} responses in touch neurons adapt with consecutive "buzz" stimulations. This may explain the behavioral adaptation to touch [72]. It is interesting to note that the small Ca^{2+} rises induced by "pokes" do not display adaptation, suggesting that the cellular signaling activated by a moving probe may involve the activation of additional mechanisms that require time to recover to a resting state [21]. Notably, both pokes and buzzes caused a deflection of

10–15 μm of the animal cuticle and were confirmed to generate thrashing activity in half-glued animals consistent with the animals responding to the stimuli. Lastly, as mentioned in the previous paragraph, touch neurons respond to harsh stimulation with a MEC-independent rise in intracellular Ca^{2+} concentration. Close analysis of this MEC-independent Ca^{2+} rise reveals that it is slower and longer lasting and seems to involve the activation of a distinct molecular machinery [21]. Because the only DEG/ENaC channels expressed in touch neurons are MEC-4 and MEC-10, it is highly likely that this sensory response is mediated by a different channel type.

Does MEC-4 conduct Ca^{2+}? The rise of intracellular Ca^{2+} concentration in touch neurons that follows gentle touch stimulation and that is strictly dependent on the MEC channel is partly dependent on the L-type Ca^{2+} channel EGL-19 (Fig. 3b, see above) [21]. However, in *egl-19* mutants, residual Ca^{2+} transients can be elicited by touch. This raises the possibility that the MEC channel itself could contribute to Ca^{2+} transients by directly conducting Ca^{2+} into the neuron. Toward this end, we have recently reported that the mutant MEC-4(d) channel complex is Ca^{2+} permeable [51]. We have argued that such Ca^{2+} permeability could play a key role in the neurodegenerative processes triggered by MEC-4(d) by inducing rise of intracellular Ca^{2+} concentration. A rise in intracellular Ca^{2+} concentration in turn would induce toxic release of Ca^{2+} from the endoplasmic reticulum resulting in cell death [48]. Importantly, hyperactivated mouse ASIC1a, a MEC-4(d) homologue, is also Ca^{2+} permeable and contributes to neuronal death in an ischemia model in mouse [73]. This indicates that MEC-4(d) Ca^{2+} permeability is not a nematode feature only. Our findings suggest that wild-type MEC-4 could also be Ca^{2+} permeable. A combination of electrophysiology, Ca^{2+} imaging, and behavioral tests should address if MEC-4 is Ca^{2+} permeable and if its Ca^{2+} permeability is important for touch sensation.

MEC current in touch neurons in situ is activated by mechanical stimulation. Experiments using cameleon have positioned the MEC channel very close to the actual mechanosensor. However, these experiments have not demonstrated if the MEC channel is gated by mechanical forces. Elegant electrophysiological experiments on in situ touch neurons finally demonstrated that the MEC channel is indeed activated by mechanical forces [52].

O'Hagan et al. [52] used an in vivo whole-cell patch clamp protocol on touch neurons that had been identified by their expression of green fluorescent protein (GFP) driven by the promoter of *mec-4*. They exposed the touch neuron cell body and stimulated the neuronal process with a glass probe that was pushed against the animal cuticle (Fig. 4a). As little as 100 nN of force activated an amiloride-blockable mechanosensitive Na^{+} current (Fig. 4b). This current was absent in *mec-4*, *mec-2*, and *mec-6* null mutants [52]. Furthermore, the mechanosensitive current displayed changed selectivity and amplitude in a mutant in which a *mec-4* residue thought to be located near the pore had been mutated. Importantly, other ionic currents of touch neurons were unaffected by *mec* mutations [52]. Taken together, these results strongly support the hypothesis that the MEC channel transduces mechanical signals.

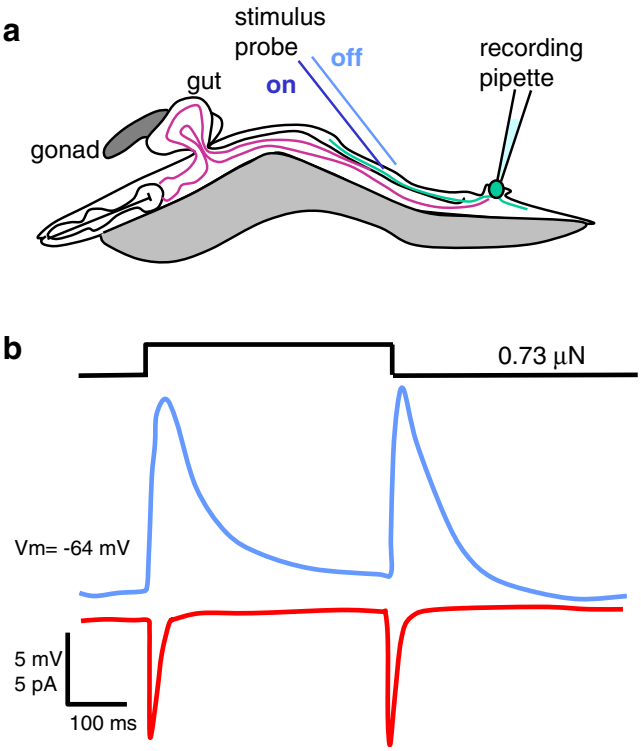

Fig. 4 The MEC channel transduces mechanical signals. **a** Schematic representation of the preparation used by O'Hagan et al. to record currents from PLM touch neurons in vivo in *C. elegans*. An animal was glued on a glass slide and submerged in physiological saline. Its cuticle was nicked to release some of the hydrostatic pressure and the PLM touch neuron was exposed to the external environment by opening the cuticle just above the neuron cell body. The PLM touch neuron was identified by expression of GFP under the control of *mec-4* promoter. After the PLM cell body was exposed, whole-cell patch clamp recordings were obtained. A glass probe (shown here in *blue*) was used to mechanically stimulate the animal body by pushing it against the animal cuticle (*ON*) from a resting position (*OFF*). **b** Examples of voltage (*blue*) and current (*red*) responses produced in a PLM touch neuron by application of pressure onto the cuticle just above the PLM neuronal process (0.73 μN of force was applied in this case). Note how the PLM neuron responds transiently when the force is applied or released but not during maintained application of the force. This was also seen in Ca^{2+} imaging using cameleon and suggests that *C. elegans* touch neurons are motion sensors. Adapted from O'Hagan et al. [52]

Features of the MEC current. The mechanoreceptor current (MRC) recorded in touch neurons in situ and carried by the MEC channel displays some very interesting properties. First, it is sensitive to pressure (force per unit area) rather than force per se. This is a feature that has been also observed in Pacinian corpuscles and may be due to the larger number of channels recruited by a larger probe. Second, it is activated both by application and release of pressure but not while constant pressure is applied (Fig. 4b). This suggests that the current is sensitive to a moving rather than immobile probe, as observed for the cameleon-detected Ca^{2+} transients [21].

The role of tubulin MEC-7 in channel activation. MEC-7 is a β-tubulin required for the formation of the 15-protofilament microtubules that are exclusively assembled in touch neurons. *mec-7* mutants are touch insensitive [19]. These data predict that the MRC should be absent in *mec-7* mutant *C. elegans*. When O'Hagan et al. [52] analyzed activation of the MRC in *mec-7(u142)* mutants, they found that the current was still present but its amplitude was 17 times smaller than in wild-type touch neurons. Moreover, the current was less sensitive to pressure, and higher forces were required to activate it. These results confirm that MEC-7 plays a role in channel activation but also suggest that it is not essential.

Comparison of cameleon and electrophysiological data. An interesting observation that emerges by comparing Ca^{2+} transients and MRC activated by touch is that while Ca^{2+} transients evoked by "buzz" stimulations display adaptation, MRC does not. However, as pointed out below, this may be due to the different stimuli used and may highlight an important feature of touch neurons. Suzuki et al. [21] tested adaptation using two different protocols. In the first one, they delivered 1.5-s stimuli using a continuously moving probe ("buzz") every minute. They observed significant reduction (~25%) of the response after only one stimulus. In the second protocol, they delivered a "buzz" stimulus every 10 s for 5 min and then tested the Ca^{2+} response. In this case, they observed 50% reduction in the Ca^{2+} transient. When measuring MRC, O'Hagan et al. delivered a "poke" stimulus every second for over a minute (64 s) and did not observe any change in the amplitude of the response [52]. The protocols used are different in one major way: adaptation of Ca^{2+} transients was observed using "buzz" stimuli, MRC adaptation was tested using a "poke" stimulus. It interesting to note that as mentioned above, Ca^{2+} transients evoked by "pokes" do not adapt either [52]. In fact, when animals are trained every 10 s for 5 min with "poke" stimulations, they do not display any reduction in the Ca^{2+} transients when they are tested after the training. These data suggest that "buzzes" activate a cellular machinery that adapts with consecutive stimuli. However, it should be noted that while Suzuki et al. [21] calibrated the stimuli by estimating the extent of deflection caused by the probe on the animal cuticle (10–15 μm), O'Hagan et al. [52] applied stimuli of known force (or pressure). This discrepancy raises the possibility that stimuli applied in the two set of experiments are not directly comparable.

Summary and Concluding Remarks on Gentle Body Touch Sensation in *C. elegans*

To conclude, genetic, molecular, and functional studies propose a model of gentle touch sensation in *C. elegans* in which a mechanosensitive channel is at the core of a molecular complex that translates the mechanical force into a cellular response (Fig. 2c). In this model, DEG/ENaC channel subunits MEC-4 and MEC-10 are at the core of the channel. Stomatin-like protein MEC-2 and paraoxonase-like protein MEC-6 associate with MEC-4 and MEC-10 and act as modulatory subunits that regulate channel permeability properties and probably gating. On the extracellular side of the membrane, matrix proteins MEC-5, MEC-1, and MEC-9 interact with the channel complex, perhaps through the large MEC-4 and MEC-10 extracellular Cys-rich domains, and help to localize the channel in functionally relevant sites along the neuronal process (the puncta). These same proteins may also aid in pulling the channel open when mechanical forces are applied. On the intracellular side of the membrane, microtubules composed of MEC-7 and MEC-12 may help in providing membrane tension or may interact directly with the channel thus favoring gating.

When a touch stimulus is applied to the animal cuticle, the deformation of the molecular interactions between all these components is thought to cause a change in the structure of the channel so that it opens (Fig. 2d). Sodium (and perhaps Ca^{2+} [51]) rushes into the cell causing membrane depolarization and consequent activation of the L-type voltage-gated Ca^{2+} channel EGL-19. Activation of EGL-19 leads to transmission of the electrical stimulus to gap junctions between the touch neuron and an interneuron. Consequently, the interneuron activates a motoneuron onto which it synapses. Activation of the motoneuron ultimately leads to contraction of the muscles and thus locomotion. Touch neurons also make chemical synapses with antagonistic interneurons that inhibit locomotion in the opposite direction. This reciprocal pattern of connectivity enables locomotion in the appropriate direction to be activated while locomotion in the inappropriate direction is inhibited.

Despite the fact that the studies described above have enormously advanced our understanding of touch sensation,

they have left key questions unanswered. For example, are all the MEC proteins required for channel gating and/or ion transport, or are some of them needed to maintain the normal physiology of touch neurons? Have all the proteins that are part of the channel complex been identified or have some been missed by the genetic screen because they are needed for other functions? How is the mechanical force converted into channel gating? Is force transduction a direct result of deformation of protein–protein interactions or the result of an indirect effect on membrane tension? Clearly, future research will be directed at addressing these points and will hopefully clarify the molecular mechanisms of touch sensation in mammals as well. The findings should also advance our understanding of other types of mechanosensitive mechanisms including proprioception, hearing and balance.

Other DEG/ENaC Channel Subunits in *C. elegans*, *Drosophila*, and Mouse Mediate Touch and Other Mechanosensory Behaviors

C. elegans DEG/ENaC channel and stretch-sensitive behaviors. The *C. elegans* genome encodes a total of 28 DEG/ENaCs. The mechanosensitivity of the channel complex formed by DEG/ENaC subunits MEC-4 and MEC-10 suggests that other DEG/ENaC channels may be mechanosensitive. Data on at least other two *C. elegans* family members suggest that mechanosensitivity is a common feature of members of this family. Data from one additional subunit called UNC-105 are controversial.

UNC-8 and DEL-1. unc-8 is expressed in interneurons, motoneurons, and some sensory neurons [74, 75]. Gain-of-function alleles that encode for hyperactive UNC-8 cause swelling and death of motoneurons, which in turn renders animals uncoordinated [76–78]. The hyperactivation-causing mutation is in the extracellular domain of UNC-8 (G387E) just after the first cysteine-rich domain, but its functional and cellular consequences are remarkably similar to the ones produced by MEC-4(d). *unc-8* loss-of-function mutants have a different and very interesting phenotype. They move quite normally on solid surfaces, but they inscribe shallower tracks on the bacterial lawn in which they travel. On agar surfaces, wild-type *C. elegans* moves through the bacterial lawn using an elegant sinusoidal motion. In *unc-8* null mutants, the sinusoidal wave is flattened. This phenotype and anatomical features of motoneurons have suggested that UNC-8 may function as a stretch receptor to mediate proprioception. Indeed, neuronal processes of motoneurons form neuromuscular junctions near the neuron cell body. The distal part of the neuronal process does not appear to have specialized anatomical structures resembling synapses. It was therefore suggested that this distal part may function as a stretch-sensitive structure to signal the extent and timing of body bends. Given the sequence similarity between UNC-8 and MEC-4, it has been suggested that UNC-8 channel could be localized in this part of the neuronal process and function as a stretch receptor in these neurons. *del-1* (for degenerin-like) is another DEG/ENaC channel subunit that is coexpressed with *unc-8* in a subset of neurons (the VA and VB motor neurons and the FLP nose touch neurons) and may assemble into a channel complex with UNC-8 in these cells [75]. Although, it is interesting to note that *del-1* deletion mutants do not share *unc-8* null locomotory phenotype suggesting that it may have a modulatory role within the channel complex, much like MEC-10 does (Thieringer et al., personal communication). How might UNC-8/DEL-1 channels contribute to proprioception and to setting body bends amplitude? It is possible that UNC-8/DEL-1 channels expressed on the stretch-sensitive portion of motoneuron processes sense stretch caused by the bending of the body during locomotion. The channels would then respond to the stretch by depolarizing the neuron, thus increasing the release of neurotransmitter at the neuromuscular junction. This ultimately would lead to stronger muscle contraction and thus deeper body bends through an autostimulatory mechanism. This model awaits experimental verification, which is now possible with the newly available Ca^{2+} imaging and physiological techniques.

UNC-105. unc-105 is expressed in body wall muscle cells, and semidominant *unc-105* alleles induce hypercontraction of body wall muscles [77]. One of these mutations encodes for an amino acid substitution in the extracellular domain of the channel protein just past MSDI (P134S) and causes hyperactivity of the channel as assayed in *Xenopus* oocytes [79]. Hyperactivity of the mutant channel is thought to cause excess ion influx with consequent hypercontraction [79]. It is interesting to note that specific alleles of *sup-20/let-2*, an essential type IV basement membrane collagen [80], suppress hypercontraction caused by *unc-105* semidominant mutations [77, 81]. These data suggest that *sup-20/let-2* collagen may function in muscle cells similarly to *mec-5* collagen in touch neurons. Thus, it was proposed that UNC-105 may be a stretch-sensitive channel gated by changes in molecular interactions with SUP-20/LET-2 that may occur during locomotion. Jospin et al. [82] have recently tested this hypothesis by searching for stretch-sensitive channels in body wall muscle cells. They applied mechanical forces to muscle cells in situ using the “filet” preparation in which, by microdissection, the gut is removed to expose body wall muscle [83]. They used two different types of mechanical stimulation: hypotonic solutions and membrane displacement induced by a glass rod. In neither case, they observed the activation of an amiloride-

sensitive current [82]. They were able to observe an amiloride-sensitive constitutive current in semidominant *unc-105* mutant muscle cells, although (allele *n506*, mutation P134S). This current was eliminated in *unc-105;sup-20/let-2* double mutants and restored in the double mutant treated with collagenase. These results indicate that *unc-105* is expressed in body wall muscle cells, but it does not seem to be sensitive to mechanical forces [82]. While it is possible that the mechanical stimulation used was not adequate for channel gating, these data suggest that UNC-105 may serve a different type of function in body wall muscle cells.

Drosophila DEG/ENaC channels. The *Drosophila* DEG/ENaC gene Pickpocket (*ppk1*) is expressed in the dendrites of the peripheral da/md abdominal sensory neurons that play roles in mechanosensory behaviors in insects [84]. *ppk1* mutants display hyperactive locomotion, suggesting that PPK1 may be required to control spontaneous locomotion. These data suggest a role for this gene in mechanical signaling [85].

Mouse DEG/ENaC channels in touch sensation and baroreception. Based on sequence similarity, the mammalian DEG/ENaC family of channels can be divided into two subgroups: (1) the ENaC subfamily that includes α-, β-, γ- and δ-subunits and (2) the ASIC subfamily. While ENaCs are involved primarily in Na^+ reabsorption in specialized kidney and lung epithelia [29, 30], members of the ASIC subfamily are neuronally expressed and play roles in touch and pain sensation, memory formation, and fear conditioning [86–89].

ASIC2. Introducing a mutation in mouse ASIC2 analogous to the mutation that causes hyperactivity of MEC-4 (MEC-4(d)) causes the channel to become hyperactive. Hyperactivated ASIC2, like MEC-4(d), causes swelling and death of human embryonic kidney cells (HEK) in which the mutated deoxyribonucleic acid has been introduced by transfection [90]. These results indicate a strong parallel between structure–function relationships in *C. elegans* MEC-4/MEC-10 and in mouse ASIC2. ASIC2 exists in two splice variants that vary at their C terminus: ASIC2a and ASIC2b. ASIC2a is expressed in medium- and large-diameter mechanosensory neurons of the dorsal root ganglia (DRG) and is localized to nerve termini that are known to function as cutaneous mechanosensors, including the Lanceolate endings of the hair shaft [86, 91]. To understand the role of ASIC2 in mechanosensation, the Welsh laboratory has generated an ASIC2 knockout mouse and analyzed the electrical responses to mechanical forces in a nerve/skin preparation [86]. They found that two types of low-threshold mechanosensory fibers (the rapidly adapting or RA and the slowly adapting or SA) responded abnormally to mechanical stimuli. While RA and SA fibers from wild-type mouse respond to larger displacement by sharply increasing their firing rate, RA and SA fibers from ASIC2 knockout mouse displayed a reduction in this response. The function of other nerve fibers known to mediate other types of sensations (pain, harsher touch) was unaffected in ASIC2 knockout mouse. These data strongly suggest that ASIC2 is specifically needed for the function of RA and SA mechanosensory fibers. Because RA and SA mechanosensors are believed to play a role in how mammals, including humans, sense gentle touch, these data indicate that ASIC2 may be a mammalian hortologue of MEC-4. It would be interesting to determine if this parallelism is maintained at the biophysical and cellular level by analyzing mechanical gating of ASIC2 and intracellular changes in Ca^{2+} concentration after ASIC2 activation.

ASIC3. Antibody staining on wild-type mouse reveals that ASIC3 is also localized to mechanosensory nerve terminals, and its expression pattern in part overlaps with ASIC2a [92]. Specifically, both channel subunits are expressed in RA mechanoreceptors. Studies on ASIC3 knockout mouse revealed that ASIC3 is also implicated in touch sensation [87]. The Welsh laboratory again used the nerve/skin preparation to study the electrical responses to touch in specific mechanosensory fibers of ASIC3 knockout mouse [87]. They found that the AM fibers (mechanociceptors) display a reduction in the capacity to increase the frequency of action potentials after large displacements. Because the AM fibers are thought to play a role in harsh touch sensation (like pinching), ASIC3 may be gated by harsh touch stimuli. It is interesting to note that in ASIC3 knockout mice, the response of RA fibers is doubled compared to the wild type. This result is intriguing because it suggests that ASIC3 may function as a negative regulator in RA fibers. Perhaps ASIC3 coassembles with ASIC2 in RA fibers and reduces its activity, similar to how MEC-10 reduces MEC-4 channel activity (as assayed in *Xenopus* oocytes [49]). The defects observed in the nerve/skin preparation are corroborated by mechanosensory behavioral defects in ASIC3 knockout mice. ASIC3 knockout mice are indeed less sensitive to mechanical stimulation after acid injection into the skeletal muscle (mechanical hyperalgesia).

γENaC. There is also evidence that members of the ENaC branch of the DEG/ENaC family are involved in mechanosensory signaling [93]. Based on the mechanosensitivity of *C. elegans* DEG/ENaC channels, Drummond et al. [93] hypothesized that arterial baroreceptor neurons that detect acute fluctuations in blood pressure could express DEG/ENaC channels. Using a combination of reverse transcriptase polymerase chain reaction and immunolabeling, they detected γENaC in baroreceptor neurons. They also showed that amiloride, a DEG/ENaC channel blocker,

inhibits the electrical activity of baroreceptor neurons that is stimulated by mechanical forces but has little or no effect on their basal activity. These data suggest that γENaC may be part of a mechanosensitive channel in baroreceptor neurons. However, it should be noted that the αENaC subunit that is needed in epithelia to produce functional channels was not detected in these cells. While it is possible that γENaC coassembles with another unidentified subunit to form mechanosensitive channels, amiloride is not a specific DEG/ENaC blocker. Still, the results are intriguing and may prompt further investigation if ASIC channels are present in these neurons and participate in their function.

A stomatin–domain protein is essential for touch sensation in mouse. As discussed above, in the touch neurons of *C. elegans* the stomatin-like protein MEC-2 is needed for channel function and may help to pull the channel open when mechanical forces are applied [49, 52, 54]. In mammals, stomatin was first identified in RBC. Furthermore, electrophysiological recordings from stomatin mutant mice revealed only a mild impairment in touch sensitivity [94]. However, Wetzel et al. [94] recently showed that another stomatin–domain protein named SLP3 (62% identical to stomatin [95]) is a key player in touch sensitivity in mice. They generated knockout mice for SLP3 and analyzed both electrophysiological and behavioral responses to touch. They used the nerve/skin preparation and looked at the mechanosensitivity of Aβ, Aδ, and C fibers (identified by analysis of their conduction velocity) thought to be implicated in touch sensation. They found that 30 and 40% of Aβ and Aδ fibers lacked mechanosensitivity in SLP3 knockout mice. They did not observe any change in mechanosensitivity of C fibers. Their experiments also show that there is no change in the ratio of the mechanoreceptor types (again identified by conduction velocity), indicating that the fibers are normally present in skin of SLP3 knockout mice but that they no longer respond to mechanical stimulation. Indeed, these fibers are lacking typical mechanosensitive currents. It is interesting to note that when they analyzed the effect of SLP3 on acid-activated current (both in HEK cells and DRG neurons), they found that SLP3 normally suppresses the current. This effect seems to be distinct from the lack of mechanosensitive currents in SLP3 knockout mice and suggests that SLP3 may regulate ASIC channels in addition to a mechanosensitive channel. To determine if the cellular impairments observed in SLP3 knockout mice are reflected in changes of touch sensitivity of these animals, Wetzel et al. developed a behavioral test employing grids placed on the bottom of a chamber (Fig. 5a). Wild-type mice displayed preference for the grid area, whereas SLP3 knockout mice did not, suggesting that touch sensitivity is impaired in these mice (Fig. 5b and c). To summarize, experiments on SLP3 knockout mice implicate SLP3 in touch sensation in mouse and suggest that this protein may function similarly to *C. elegans* MEC-2. The channel subunits that partner with SLP3 still await identification.

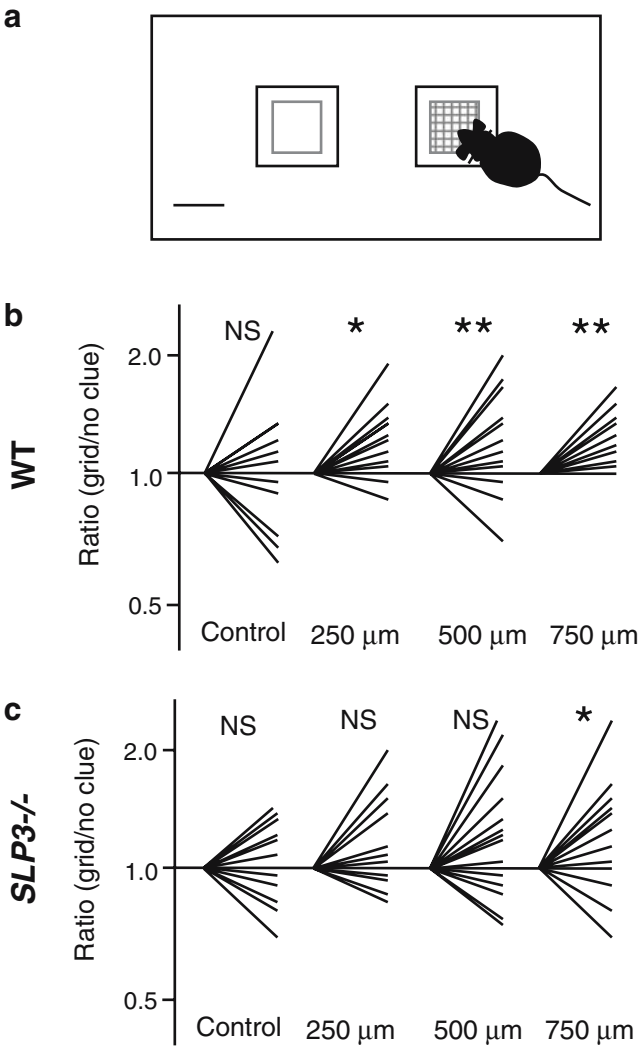

Fig. 5 Mammalian stomatin–domain protein SLP3 is essential for touch sensation in mouse. **a** Schematic drawing of the experimental arrangement used by Wetzel et al. to demonstrate that SLP3 is needed for normal touch sensitivity. A mouse was placed in a chamber containing a surface area with a grid pattern (*right square*) and one without grid (*left square*). *Scale bar*, 4 cm. **b** Wild-type mice display a marked preference for the surface with the grid pattern. The ratio of the distance traveled when the grid is present to when it is absent is shown as a *single line* for each animal (greater than 1.0 indicates more time spent on the grid; less than 1.0 indicates less time). It is interesting to note that the coarser the grid, the more it is preferred by the mice. **c** SLP3 knockout mice (*SLP3−/−*) display no preference for finer grids and only slight preference for a coarser grid (750 μm). These data, together with electrophysiological data reported by Wetzel et al., strongly suggest that SLP3 is needed for normal touch sensitivity in mouse. SLP3 is homologue to *C. elegans* MEC-2. These studies thus support the hypothesis that mammalian touch-sensitive channels are composed of the same types of subunits present in mechanosensitive channel MEC in *C. elegans*. Adapted from Wetzel et al. [94]. Control stands for surface without cue

TRP Channels and Mechanosensory Behaviors

In the first part of this review, when mechanosensory behavior in *C. elegans* was described, the TRP channels OSM-9/OCR-2 and LOV-1/PKD-2 were discussed regarding their putative function in nose touch response and mating. In this paper, I will briefly review other reports that implicate TRP channels in mechanosensory behaviors in *Drosophila* and zebrafish.

nompC (now known as *TRPN1*). Screens for touch-insensitive mutants in *Drosophila* have identified several genes that may play a role in mechanosensitivity. Among them, a gene named *nompC* (no mechano-receptor potential) encodes a TRP channel expressed in *Drosophila* mechano-sensory organs including bristles [96]. *nompC* mutants lack or have abnormal ionic currents in the bristle neurons when flies are stimulated by mechanical forces applied to the bristle. Zebrafish larvae treated with morpholino antisense oligonucleotides for the homologue of *nompC* are deaf, and sensory hair cells no longer respond to sound stimulation [97]. Taken together, these findings strongly implicate the *nompC* channels in mechanosensation in both invertebrates and vertebrates.

Nanchung. nan is a *Drosophila trp* gene that is homologous to the TRPV and TRPO (*osm-9*-like) subfamilies in mammals and *C. elegans*, respectively, and shows most similarity to *C. elegans* OCR-4 [98]. Kim et al. [98] showed that *nan* homozygote mutant flies do not respond to sound at all. This was observed even when the stimulus intensity was increased. These experiments suggest that TRP channel NAN plays a role in *Drosophila* hearing that is similar to the role played by *nompC* in zebrafish.

Painless. painless is another members of the TRP family in *Drosophila* that has been implicated in mechanosensory behavior. A screen for *Drosophila* mutants that do not respond to a probe heated above 38°C yielded *painless* [99]. *painless* is expressed in peripheral neurons that extend dendrites beneath the larval epidermis and is required for electrophysiological and behavioral responses to noxious heat and harsh mechanical stimulation. It is not needed, however, for responses to light touch [99].

Concluding Remarks

Simple model organisms, particularly *C. elegans* and *Drosophila*, have tremendously advanced our understanding of the cellular and molecular mechanisms of mechanotransduction. In particular, they have highlighted two ion channel families, the DEG/ENaC and the TRP channels, as directly implicated in this process. Recent studies employing knockout mice have shown that similar channels in mammals mediate mechanosensation and have suggested that channel composition may be remarkably similar to what seen in nematodes and flies. A looming question that remains is whether both these classes of channels function as mecanoreceptors or whether a member of one channel class is needed for a member of the other one to function properly (for example by setting the membrane potential or allowing secondary Ca^{2+} entry). If both channel types function as primary transducers of mechanical stimuli, other questions then arise: do the different channels respond at different thresholds (e.g., gentle vs harsh touch), or are they sensitive to different types of stimuli (e.g., touch vs sound waves)? To address these questions, further characterization of the properties of these mechanosensitive channels is needed as well as the identification of the cell types in which members of both families are expressed.

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