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How we feel: Ion channel partnerships that detect mechanical inputs and give rise to touch and pain perception

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Summary

Every moment of every day, our skin and its embedded sensory neurons are bombarded with mechanical cues that we experience as pleasant or painful. Knowing the difference between innocuous and noxious mechanical stimuli is critical for survival and relies on the function of mechanoreceptor neurons that vary in their size, shape, and sensitivity. Their function is poorly understood at the molecular level. This review emphasizes the importance of integrating analysis at the molecular and cellular levels and focuses on the discovery of ion channel proteins co-expressed in the mechanoreceptors of worms, flies, and mice.

Introduction

All sensory neurons are alike. Each detects a physical stimulus and produces an electrical signal that gives rise to behavioral responses, conscious perceptions, or both. Many operate near the physical limits of detection over a dynamic range of several orders of magnitude (Bialek, 1987; Block, 1992). These properties suggest that they are endowed with a detector, an amplifier, and mechanisms for gain-control. One of the most striking and well-understood examples is the ability of photoreceptors to detect single photons while retaining sensitivity to light intensities that vary by nine orders of magnitude (Rieke and Rudd, 2009).

Each somatosensory neuron is distinct. The somatosensory system is a collection of neurons innervating the skin, muscle, joints, tendons and internal organs to establish and maintains sensitivity across a range of stimulus intensities and frequencies. This collection includes nociceptors that require stimulation above a high threshold for activation. Nociceptors are responsible for informing the brain about damage to peripheral tissues, an essential function that enables the nervous system to execute and coordinate appropriate protective behaviors. They are often polymodal neurons responding to multiple types of stimulation including extreme temperatures, intense force, acid, and noxious chemicals. Other somatosensory neurons respond to less intense stimulation and detect either temperature changes or mechanical stimulation, but not both. These cells provide information about warmth, cooling or the shape and texture of objects.

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Theater of sensation

The skin is our largest sensory surface, extending nearly two square meters in an average human. Mechanoreceptor neurons are principal actors in this theater. They are responsible not only for detecting mechanical cues, but also for encoding and transmitting all relevant information to the central nervous system. Their performance is shaped by ion channels that include, but are not limited to, sensory transduction channels. Agents that activate or inhibit mechanoreceptor neurons can exert their influence by acting on channels other than transduction channels. For example, naked mole rats are insensitive to the persistent skin acidification that is a feature of their environment. These animals have acid-gated ion channels (ASICs) with a similar sensitivity to protons (H^+) as those found in mice (Smith et al., 2011). However the voltage-gated Na^+ channels expressed in their C-fiber nociceptors are hypersensitive to inhibition by protons and this inhibition counterbalances the excitation due by ASIC activation, rendering animals insensitive to acidification. Thus, the difference in nociceptor sensitivity stems from variation in voltage-gated Na^+ sodium channels that are essential for action potential generation rather than any variation in sensory transduction.

Though mechanoreceptor neurons were first studied more than 75 years ago (Adrian, 1926; Adrian and Zotterman, 1926a, b), the events that link sensory stimulation to neuronal activation are only beginning to be understood. Today, the protein partners responsible for detecting mechanical stimuli have been identified only for a few mechanoreceptor neurons in the nematode *Caenorhabditis elegans*. Genetic screens for animals defective in touch sensation have revealed critical roles for genes encoding TRP channels and DEG/ENaCs in behavioral responses to mechanical inputs. The key insights derived from genetic approaches have been reviewed elsewhere (Arnadottir and Chalfie, 2010; Ernstrom and Chalfie, 2002). We review data demonstrating that TRP channels and DEG/ENaC channels are widely distributed in the sensory neurons of vertebrates and invertebrates and examine the idea that these channels have conserved, but distinct functions. We rely on investigations of somatosensory mechanoreceptors in nematodes, flies, and mice, but recognize that on-going investigations in humans and other animals have the potential to deepen and expand understanding of how mechanoreceptors function.

Sensing with ion channels

For several decades, attention has focused on the idea that mechano-electrical transduction (MeT) channels are formed by proteins enriched in mechanoreceptor neurons and required for their function. Figure 1 summarizes current knowledge about proteins proposed to form MeT channels in animals. The *mec-4* DEG/ENaC and *osm-9* TRP channel genes were the first candidates identified from classical genetic screens. The DEG/ENaC genes are conserved in animals, but absent from plants, bacteria, and fungi (Goodman and Schwarz, 2003; Hunter et al., 2012) and encode proteins with two transmembrane domains and a large extracellular domain. As revealed in high-resolution crystal structures (Gonzales et al., 2009; Jasti et al., 2007), three DEG/ENaC proteins assemble to form an ion channel. Both homomeric and heteromeric channels has been observed (Akopian et al., 2000; Deval et al., 2004; Donier et al., 2008; Gründer et al., 2000; Hesselager et al., 2004; Lingueglia et al., 1997). The TRP channel genes comprise a large superfamily conserved in eukaryotes and encode proteins predicted to have six transmembrane domains. Four TRP channel proteins assemble into homomeric or heteromeric ion channels (Venkatachalam and Montell, 2007). Recently, two additional classes of membrane proteins (Piezo and TMC) have been linked to mechanotransduction (Coste et al., 2010; Coste et al., 2012; Kawashima et al., 2011; Kim et al., 2012).

Both TRPs and DEG/ENaCs are broadly expressed in somatosensory neurons. Several mechanoreceptor neurons are known to co-express multiple TRPs and multiple DEG/ENaC channels. Figure 2 aggregates evidence that these channels are co-expressed in mechanoreceptor neurons from the growing, but essentially independent literatures on TRP and DEG/ENaC channels expression and function. Excluding reviews, PubMed lists 1687 entries for DEG/ENaCs, 2341 for TRP channels, and only 15 entries for both ion channel families.[1] Here we focus on two invertebrates, *Caenorhabditis elegans* nematodes and *Drosophila melanogaster* fruitflies, and one mammal, the laboratory mouse. Despite the fact that members of these gene families are coexpressed, the cellular and behavioral function of individual TRP and DEG/ENaC channels is often explored subunit by subunit. But, genetic redundancy within each ion channel family and the potential for functional redundancy between the two families limits insight derived from this approach. Additional complications include alteration of channel function by their association with heteromeric channel complexes and through alternative splicing of ion channel genes.

Stagecraft for sensory biology

A fundamental block to progress in understanding how mechanoreceptor neurons function is that studying stimulus-initiated behavior, action potential generation or intracellular calcium dynamics does not allow researchers to separate the initial step of mechanotransduction from amplification, gain control and transmission. Behavior, spikes, and calcium dynamics depend on the action of all of the ion channels expressed by sensory neurons. These channels are interconnected in feedback loops regulated by a common control variable—membrane potential. The voltage-clamp uncouples such feedback loops by holding membrane potential constant and allows researchers to examine transduction independently of amplification, gain control, and spike generation. Within this heuristic, deleting molecules needed for the formation or function of MeT channels should eliminate mechanoreceptor currents, but leave other ionic currents and mechanisms for amplification, gain control and spike generation intact. Conversely, deleting molecules essential for post-transduction signal should leave mechanoreceptor currents intact, but produce defects in other ionic currents or in amplification, gain control, and spike generation. Marrying *in vivo* voltage clamp with genetic dissection of identified mechanoreceptor neurons in *C. elegans* has revealed that the pore-forming subunits of MeT channels are DEG/ENaCs in two classes of mechanoreceptors (Geffeney et al., 2011; O'Hagan et al., 2005) and a TRP channel in a third class of mechanoreceptors (Kang et al., 2010).

Neuron by neuron

C. elegans nematodes are microscopic animals with a compact nervous system consisting of only 302 neurons, about 30 of which are classified as mechanoreceptor neurons (Goodman, 2006). Because the mechanoreceptor neurons can be identified in living animals, and because of their small size, it is possible to record mechanoreceptor currents (MRCs) and mechanoreceptor potentials (MRPs) *in vivo*. MRCs have been recorded from the body touch receptor neurons known collectively as the TRNs, the cephalic CEP neurons and two classes of nociceptors, the ciliated ASH neurons and the multi-dendritic PVD neurons. In all four of these mechanoreceptors, stimulation activates inward currents (Figure 3) and evokes transient increases in intracellular calcium. Strikingly, MRCs are activated in response to both the application and withdrawal of stimulation. Such response dynamics were first described 50 years ago in recordings from Pacinian corpuscles in mammals (Alvarez-Buylla

[1]Search conducted on 15 April 2012 using search terms: "TRP channels", "ENaC OR ASIC OR degenerin", and the union of both terms.

and Ramirez de Arellano, 1953; Gray and Sato, 1953) and are emerging as a conserved property of somatosensory mechanoreceptor neurons.

The TRNs (ALM, PLM, AVM, and PVM) express several DEG/ENaC channel proteins, but no TRP channel subunits have been reported (Figure 2A). External mechanical loads open sodium-dependent, amiloride-sensitive mechanotransduction (MeT) channels. MEC-4 is essential, while MEC-10 is dispensable for the generation of MeT currents (Arnadottir et al., 2011; O'Hagan et al., 2005). Both proteins are pore-forming subunits of the native MeT channel since missense mutations of a conserved glycine in the second transmembrane domain alter the permeability of the MeT current (O'Hagan et al., 2005). These protein partners were the first to be linked to native MeT currents in any animal.

CEP expresses at least one DEG/ENaC and one TRP channel protein, TRP-4 (Figure 2A). Two lines of evidence support the idea that the TRP-4 protein is an essential pore-forming subunit of MeT channels in CEP: 1) loss of TRP-4 eliminates MRCs in CEP and 2) mutations in the putative pore domain of the channel alter the reversal potential of MRCs (Kang et al., 2010). These latter data are strong indicators that TRP-4 is a pore-forming subunit of the MeT channel in CEP.

The ASH neurons function as nociceptors in the animal because they require more intense forces for activation than PLM and larger displacements for activation than CEP (Geffeney et al., 2011). These cells express multiple DEG/ENaC and TRP channel proteins (Figure 2A), but the major mechanoreceptor current is carried by a MeT channel formed by the DEG/ENaC channel protein, DEG-1. A minor current remains in *deg-1* null mutants and is carried by a biophysically-distinct channel (Geffeney et al., 2011). Though it is possible that DEG-1 and the channel responsible for the minor current function in series with DEG-1 amplifying the minor current, the data support a model where the channels function in parallel because loss of DEG-1 does not alter the rise rate of MRCs in ASH. The TRPV proteins OSM-9 and OCR-2 are essential for ASH-mediated behaviors (Colbert et al., 1997; Tobin et al., 2002), but loss of these channel subunits has no effect on either the major or minor current in ASH (Geffeney et al., 2011). In ASH, TRPV channels likely regulate cell activity downstream of mechanotransduction, as suggested by their importance for calcium signaling in ASH following mechanical stimulation (Hilliard et al., 2005).

From analysis of ASH, we learn that DEG/ENaC channels can act in parallel with a second MeT channel and that TRPV channels are important for post-transduction signaling. This complex pathway for mechanoreceptor neuron signaling may be shared with other nociceptors responsible for detecting noxious and potentially damaging sensory stimuli. An additional, conserved function of nociceptors is their sensitization in response to injury and their regulation by biogenic amines (Walters and Moroz, 2009). Such sensitization is also apparent in *C. elegans* and reflected in the finding that ASH-dependent behaviors are regulated by various biogenic amines, including serotonin (Chao et al., 2004). Collectively, these observations raise the possibility that biogenic amines might regulate the sensitivity of nociceptors to mechanical cues and that such regulation may affect MeT channels, post-transduction signaling or both.

The multidendritic PVD neuron is a polymodal neuron activated by mechanical and thermal stimuli and is proposed to function as a nociceptor. Like ASH, PVD expresses multiple TRP and DEG/ENaC channel subunits (Figure 2A). As in ASH and the touch receptor neurons, mechanoreceptor currents in PVD are amiloride-sensitive and sodium-dependent (Li et al., 2011b). These biophysical properties suggest that MRCs are carried primarily by a DEG/ENaC channel. Consistent with this idea, Chatzigeorgiou et al. (2010) demonstrated that both DEGT-1 and MEC-10 are required for mechanically-evoked calcium transients in

PVD. But, as reported for the touch receptor neurons (Arnadottir et al., 2011), loss of *mec-10* had no effect on mechanoreceptor currents (Li et al., 2011b). Thus, *mec-10* may function redundantly with the three other DEG/ENaC channels expressed in PVD: *degt-1*, *del-1*, *asic-1*. Additional studies are needed to clarify this issue and to determine the function of all of these ion channel proteins in PVD. One approach developed recently exploits optogenetics to identify PVD-expressed genes needed for post-transduction signaling (Husson et al., 2012). Using this strategy, Husson et al. (2012) show that PVD-selective knockdown of *asic-1* alters light-evoked behavioral responses. (Light responses were unaffected in animals by *mec-10*, *del-1*, and *degt-1* knockdown.)

PVD appears to express seven TRP channels, including TRPA-1 and OSM-9 (Figure 2A). Neither TRPA-1 nor OSM-9 are required for calcium transients induced by noxious mechanical stimuli. However, TRPA-1 is needed for responses to noxious cold (Chatzigeorgiou et al., 2010). Less is known about the function of the other TRP channels, but an optogenetics-based approach reveals that GTL-1 is required for normal light-evoked behaviors and strongly suggests that this TRPM channel plays an essential role in post-transduction signal amplication (Husson et al., 2012). Collectively, these studies paint a picture of PVD function in which a DEG/ENaC channel acts as a force sensor, TRPA-1 detects thermal stimuli, and ASIC-1 and GTL-1 contribute to post-transduction signaling.

The FLP neurons are multidendritic neurons and are activated by noxious mechanical and thermal stimuli (Chatzigeorgiou and Schafer, 2011; Chatzigeorgiou et al., 2010). The FLP neurons innervate the body surface anterior to PVD and co-express *osm-9* and three DEG/ENaC genes: *mec-10*, *unc-8*, and *del-1* (Figure 2A). Apart from the observation that mechanical stimuli activate calcium transients in FLP in a MEC-10-dependent manner (Chatzigeorgiou and Schafer, 2011), little is known about the function of MEC-10, UNC-8, and DEL-1 in FLP. The contribution of TRP channel genes to FLP function is complex, as FLP mechanosensitivity depends on OSM-9 and TRPA-1-dependent signaling in the OLQ mechanoreceptors.

In a cell that expresses multiple TRP channel subunits and no DEG/ENaC subunits, each TRP protein appears to have a distinct cellular function. The OLQ neuron expresses three TRP channel subunits and two of these have been examined for their role in initiating behavioral responses and calcium transients in response to mechanical stimulation. Loss of TRPA-1 decreases two behaviors influenced by OLQ, the cessation of foraging for food and reversal of forward movement induced by mechanical stimulation (Kindt et al., 2007). Surprisingly, loss of TRPA1 had a very subtle effect on calcium transients in OLQ. The first response to mechanical stimulus was not affected, but the magnitude of the second response was reduced (Kindt et al., 2007). A role for the TRPV channel subunit OSM-9 is evident from the finding that *osm-9* mutant OLQ neurons lack mechanically-evoked calcium transients (Chatzigeorgiou et al., 2010). Because MRCs have yet to be measured in this mechanoreceptor neuron, it is not known whether loss of TRPA-1 or OSM-9 affect MRCs or the events that follow their activation.

These examples in *C. elegans* nematodes establish the rule that mechanoreceptor neurons commonly express multiple DEG/ENaC and TRP channel proteins and that these channels operate together to enable proper sensory function. The ability to directly measure MRCs *in vivo* has revealed that both DEG/ENaC and TRP channels can form MeT channels. Evidence from the ASH and PVD nociceptors suggest that some TRP channels are essential for post-transduction events essential for sensory signaling. These case studies provide evidence for the idea that TRP channels can be crucial elements in both sensory transduction and in post-transduction signaling. They also illustrate the powerful insights available when

detailed physiological analysis of identified mechanoreceptor neurons is merged with genetic dissection.

It is rare for deletion of a single DEG/ENaC gene to induce strong behavioral defects in *C. elegans*. Indeed, there is only one such DEG/ENaC gene known so far: *mec-4*. By contrast, deleting the DEG/ENaC genes *mec-10*, *deg-1*, *unc-8*, and *unc-105* fails to produce clear behavioral phenotypes, although gain-of-function alleles significantly disrupt several behaviors. Though only a subset of the DEG/ENaC genes have been studied in this way, these findings suggest there is considerable redundancy in *C. elegans* mechanosensation. The case of *mec-4* and *mec-10* illustrate this idea clearly: both genes are co-expressed in the TRNs and encode pore-forming subunits of the MeT channel required for gentle touch sensation (O'Hagan et al., 2005). Whereas deleting *mec-4* eliminates mechanoreceptor currents and behavioral responses to touch, deleting *mec-10* produces a mild defect in touch sensation and has little effect on mechanoreceptor currents (Arnadottir et al., 2011).

Case studies on the fly

The peripheral nervous system of *Drosophila* larvae has three main types of neurons (Bodmer et al., 1987; Bodmer and JAN, 1987; Ghysen et al., 1986). External sensory and chordotonal neurons have a single sensory dendrite and innervate specific mechanosensory organs. In contrast, multidendritic neurons have a variable number of fine dendritic processes that lie beneath the epidermis and do not innervate a specific structure. Different subclasses of these neurons provide information about touch and body position as well as function as nociceptors (Hughes and Thomas, 2007; Song et al., 2007; Zhong et al., 2010). In the adult, external sensory and chordotonal neurons innervate more elaborate structures formed by the cuticle including bristles and antennae. These cells continue to be responsible for proprioception and touch sensation. Multidendritic neurons persist into adulthood after extensive arbor rearrangements (Shimono et al., 2009).

Polymodal nociceptor neurons in *Drosophila* larvae called Class IV multidendritic or md neurons innervate the body surface and express both TRP and DEG/ENaC channel subunits (Figure 2B). These neurons initiate aversive, nocifensive responses to heat, mechanical loads and UV light (Hwang et al., 2012; Tracey et al., 2003; Xiang et al., 2010; Zhong et al., 2012; Zhong et al., 2010). The md neurons express three TRPA genes: *painless*, *pyrexia* and *dTRPA1* (Figure 2B). None are expressed exclusively in md neurons, suggesting that these genes have additional functions. Painless is present in the larval cardiac tube (Sénatore et al., 2010) and in adult sensilla, including gustatory bristles in the proboscis, the leg and the wing margin (Al-Anzi et al., 2006); Pyrexia is expressed in neurons that innervate sensory bristles and antennae (Lee et al., 2005); and dTRPA1 is expressed both in chemoreceptor neurons and in central neurons required for temperature-sensing in adult flies (Hamada et al., 2008; Kim et al., 2010).

The contribution of Pyrexia to the mechanosensitivity of md neurons has not been studied, but genetic deletion of Painless and dTRPA1 increase the threshold for aversive responses to heat and force (Tracey et al., 2003; Zhong et al., 2012). In contrast, loss of either a DEG/ENaC channel subunit, Pickpocket, or DmPiezo reduces the response to intense mechanical stimuli, but has no effect on the response to noxious heat (Kim et al., 2012; Zhong et al., 2010). Decreasing the expression of both Pickpocket and DmPiezo renders larvae insensitive to noxious mechanical stimuli, but has little effect on responses to noxious heat. Additionally, cultured md neurons from *DmPiezo* knockout mutants lack mechanically activated currents that are present in cell isolated from wild-type animals (Kim et al., 2012). These findings suggest that Pickpocket and DmPiezo could function in parallel as MeT channels in md neurons.

Recent studies reveal that the *painless* and *dTRPA1* genes encode multiple isoforms (Hwang et al., 2012; Zhong et al., 2012). The longest isoform of Painless, Painless^{p103}, has eight ankyrin repeats in the amino-terminal domain and the shortest, Painless^{p60}, has none. Both isoforms are expressed in md neurons, but only the shortest isoform rescues mechanonociception (Hwang et al., 2012). In contrast, dTRPA1 isoforms differ in regions of the protein that flank the ankyrin repeats (Zhong et al., 2012) and two isoforms of the gene are expressed in md neurons. One isoform, dTrpA1-C, restores normal thermal nociception but not mechanonociception. It is not clear whether dTRPA1-D or an as-yet-unidentified isoform is important for mechanonociception in md neurons. Thus, an unexpectedly complex model for md neuron-mediated mechanonociception is emerging—Pickpocket and DmPiezo detect mechanical loads in parallel, while Painless^{p60} and perhaps a dTRPA1 isoform are required for post-transduction signaling, including amplification.

The Johnston's organ (JO) of adult *Drosophila* antennae is a near-field sound receptor and like other animal ears, the JO relies on mechanical amplification and frequency-selective tuning to optimize sound sensitivity (Göpfert et al., 2006; Göpfert et al., 2005; Robert and Göpfert, 2002; Tsujiuchi et al., 2007). Sound is not the only mechanical stimulus detected by the JO, however. This array of hundreds of mechanoreceptor neurons also responds to displacements induced by wind and gravity (Kamikouchi et al., 2009; Sun et al., 2009; Yorozu et al., 2009). Mechanoreceptor cells in the JO project their axons into the antennal nerve and express five TRP channels (Figure 2B): NOMPC, Nan, Iav, Painless, and Pyrexia. Genetic dissection of hearing and gravitaxis reveals that some channels (Painless, Pyrexia) are needed to sense gravity, others for hearing (NOMPC), and that the TRPV proteins Nan and Iav are expressed broadly and needed for both hearing and gravity sensing.

In sound-sensitive chordotonal neurons, the exact function of each TRP channel is matter of continuing investigation. One model (Göpfert et al., 2006) is that NOMPC is essential for detecting sound-induced mechanical stimuli and Nan and Iav work together to both refine mechanical amplification and ensure the proper transmission of stimulus-evoked action potentials in the antennal nerve. In this schema, NOMPC functions like its *C. elegans* homolog, TRP-4, and forms the pore of a sensory MeT channel. Techniques for measuring mechanoreceptor currents in the JO are needed to directly test this model, but functional specialization of NOMPC and Nan/Iav is supported by the fact that they occupy distinct compartments in the sensory cilium of JO mechanoreceptors (Lee et al., 2010; Liang et al., 2011).

Several TRP proteins may be co-expressed in the chordotonal organs of the adult leg that provide information about joint position (Gong et al., 2004; Kim et al., 2003; Liang et al., 2011). These include, NOMPC, Iav and Waterwitch (Wtrw), which appear to be co-expressed in the campaniform sensilla that detect cuticle deformation in the wings and halteres (Gong et al., 2004; Kim et al., 2003; Liang et al., 2011). The coexpression of these proteins in other mechanoreceptor neurons suggests that an understanding of how these cells enable mechanosensitivity may depend on cellular context and the entire ensemble of ion channels expressed in each mechanoreceptor. Finally, NOMPC is famous for its expression in the mechanoreceptors that innervate large bristle sensilla on the fly's body (Walker et al., 2000). *NompC* mutants lack transient, but retain sustained trans-epithelial mechanoreceptor currents (Walker et al., 2000). Thus, in bristle mechanoreceptors, there appears to be another mechanotransduction channel. Bristle receptors express many other TRP channel subunits as well as DEG/ENaC channel subunits (Figure 2B). These data raise the possibility that another channel may function in parallel to the NOMPC channel in the bristle receptor neuron.

The company of flies and nematodes

There is evidence that DEG/ENaC and TRP channels function as MeT channel subunits in distinct mechanoreceptor neurons in both *C. elegans* and *Drosophila*. One evolving paradigm is that mechanonociceptors (md neurons in *Drosophila* and ASH neurons in *C. elegans*)rely on DEG/ENaC proteins to detect noxious mechanical stimuli and on TRP channels for essential post-mechanotransduction signaling. Another is the notion that TRP channels can play multiple roles within a single mechanoreceptor neuron, exemplified by the finding that a trio of TRP channels is critical for mechanotransduction and post-transduction signal essential for hearing in *Drosophila*. A third paradigm is the presence of multiple MeT channels as found in *Drosophila* bristles, md neurons and *C. elegans* ASH nociceptors, suggesting that functional redundancy may be a shared feature of mechanoreceptors.

The mouse and its skin

As in nematodes and flies, mechanoreceptor neurons in mice co-express DEG/ENaC and TRP channel proteins and are among the principal actors that give rise to somatic sensations. Except for nociceptors associated with painful perceptions, the performance of mechanoreceptors in mammals depends on affiliation with specialized sensory organs in the skin presumed to be the locus of mechanotransduction. Figure 2C summarizes current research showing that DEG/ENaC and TRP channel proteins localize to the skin in mice, a property that suggests these proteins could form MeT channels in mammals. Most investigations of mechantransduction in mammals have relied on behavioral studies, analysis of dorsal root ganglia (DRG) or trigeminal (TG) neurons in culture, and extracellular, single-unit recordings *in vivo* and *ex vivo*. The recent demonstration of *in vivo*, whole-cell patch-clamp recordings from DRG neurons (Ma et al., 2010) is an exciting new tool that is just beginning to be applied.

The nerve endings emanating form TG and DRG neurons are diverse and are classified according to the expression of signaling peptides and receptors, their functional properties or their morphology and anatomy (Delmas et al., 2011; Lewin and Moshourab, 2004). For most somatosensory neurons, however, anatomical and functional properties are only loosely connected (Boulais and Misery, 2008). For instance, fibers that share multiple electrophysiological features such as Aβ-like conduction velocities as well as rapidly-adapting, low-threshold responses to mechanical stimuli innervate multiple peripheral end organs (Brown and Iggo, 1967; Vallbo et al., 1995). An example of the converse situation in which fibers with distinct electrophysical properties innervate a common peripheral end organ has recently come to light. In particular, Li et al. (2011a) show that Aβ, Aδ, and C fibers all form lanceolate endings that surround hair follicles. This discovery relied on developing a suite of genetic markers that were exploited in two ways. First, they were used to determine the peripheral endings associated with marked sensory sub-types. Second, they were used to link electrophysiological properties derived from *in vivo* intracellular recordings to the marker suite and hence to mechanoreceptor subtype. Thus, knowledge of conduction velocity and force sensitivity is not sufficient to infer the identity of the peripheral organ being stimulated. Nevertheless, many investigations of the contribution of ion channel proteins to somatosensation rely on functional classifications (see Figure 4).

Genetic deletion of single DEG/ENaC or TRP channel proteins in mice alters sensitivity to mechanical stimulation, but leaves both functions largely intact. While these studies cast doubt on the idea that DEG/ENaC or TRP channel proteins are essential for mechanotransduction in mammals, they also suggest that the mammalian somatosensory system is robust to genetic deletion. Such robustness could reflect molecular redundancy within or between ion channel gene families. Additionally, robustness could be conferred by

functional degeneracy among mechanoreceptor neurons. The potential for degeneracy arises from the fact that each and every skin dermatome contains a mixture of peripheral sensory structures and is innervated by multiple classes of somatosensory neurons that fasciculate into a common nerve. For example, low-threshold, rapidly adapting Aβ fibers are thought to innervate both Pacinian and Meissner corpuscles in the skin (Brown and Iggo, 1967; Burgess et al., 1968; Vallbo et al., 1995). In addition to having distinct morphologies, each of these endings also expresses different DEG/ENaC and TRP channel proteins (Calavia et al., 2010; García-Añoveros et al., 2001; Kwan et al., 2009; Price et al., 2001; Suzuki et al., 2003B). In this scenario, loss of a single ion channel protein is expected to have only a minor effect on the entire class of such fibers.

The acid-sensing ion channels or ASICs are a vertebrate sub-division of the conserved DEG/ENaC superfamily. Most, if not all of the ASIC proteins are expressed in cell bodies in the trigeminal and dorsal root ganglia (reviewed in Deval et al., 2010) and localize to the peripheral endings in the skin (Figure 2C). For instance, ASIC1 is expressed in nerves innervating Pacinian corpuscles in human skin (Calavia et al., 2010; Montaño et al., 2009). However, genetic deletion of ASIC1 has no effect on the threshold or firing frequency of fibers innervating mouse skin (Page et al., 2004), but alters visceral sensory function (Page et al., 2004; Page et al., 2005). ASIC2 and ASIC3 are expressed in the majority of mechanoreceptor endings in mouse skin (Figure 2C; García-Añoveros et al., 2001; Price et al., 2000; Price et al., 2001), but deleting ASIC2 or ASIC3 has only subtle effects on the activity of mechanoreceptor fibers (Price et al., 2000; Price et al., 2001). Moreover, neither ASIC2 nor ASIC3 is essential for MeT currents studied in cultured DRG neurons (Drew et al., 2004). Collectively, these investigations indicate that no single ASIC subunit is essential for the function of mechanoreceptor neurons in mice and suggest that the function of such neurons is robust to genetic deletion of channel proteins.

In principle, genetic deletion of a shared auxiliary subunit could reveal more severe deficits in behavioral and cellular responses to touch because such proteins might affect the function of multiple channel-forming subunits. The impact of genetic deletion of SLP3 illustrates the power of this idea (Wetzel et al., 2007). SLP3 is a stomatin-like protein that binds to both ASIC2 and ASIC3 and alters the activity of ASIC channels in heterologous cells. It is orthologous to the *C. elegans* protein MEC-2, which is required for MeT currents *in vivo* and enhances MEC-4-dependent currents in heterologous cells (Goodman et al., 2002; Huang and Chalfie, 1994; O'Hagan et al., 2005). Genetic deletion of SLP3 decreases the proportion of mechanically sensitive Ab and Ad fibers that innervate the skin and the proportion of dissociated DRG neurons with mechanosensitive currents (Wetzel et al., 2007). Additionally, loss of SLP3 disrupts texture sensing. These data suggest that many, but not all mechanoreceptors depend on SLP3 and its DEG/ENaC binding partners to detect mechanical stimuli.

Mirroring the effects of single ASIC gene deletions are those of TRP channel gene deletions: loss of a single channel gene has only subtle effects on somatosensory nerve fiber function and no single TRP channel gene deletion leads to a loss of mechanosensitivity in an individual fiber class (Kwan et al., 2006; Kwan et al., 2009; Liedtke and Friedman, 2003; Suzuki et al., 2003a). But, loss of TRP channel proteins has clear effects on the response of nociceptors to inflammation. Here we provide three examples. First, noxious chemical agents such as mustard oil potentiate behavioral responses to mechanical stimuli, an effect which is muted in TRPA1 knockout mice (Bautista et al., 2006). Loss of TRPA1 also disrupts sensitization produced by injection of bradykinin, a peptide released by damaged tissue (Kwan et al., 2009). Second, genetic deletion of TRPV4 has subtle effects on behavioral and neural responses to mechanical cues (Chen et al., 2007; Suzuki et al., 2003a), but produces significant deficits in inflammation-induced sensitization. Compounds induced

by inflammation (prostaglandin E2 and serotonin) decrease the threshold for mechanical activation of C fiber nociceptors in wild type, but not in TRPV4−/− (Alessandri-Haber et al., 2005; Chen et al., 2007). In addition, the spontaneous activity of C fibers is increased following inflammation, but there is no change in the rate of spontaneous activity in C fibers isolated from TRPV4−/− mice. Finally, TRPC1-deficient mice have reduced firing rates in Aβ-fibers and reduced probability of withdrawal from light touch (Garrison et al., 2012). A reduction of TRPC1 has a much more dramatic effect on the ability of the animals to respond to the inflammation mediators, prostaglandin E2 and serotonin (Alessandri-Haber et al., 2009). Thus, loss of TRP channels has subtle effects on baseline responses and significant effects on the ability of animals to respond to inflammation.

Conclusions and Perspectives

Over the past decades, a great deal of attention has focused on discovering the protein partners that form MeT channels in somatic mechanoreceptors. Two classes of ion channel proteins are leading candidates: DEG/ENaC and TRP channel proteins. Two others have recently joined their ranks: Piezo and TMC. Here, we surveyed the literature to establish that most, if not all mechanoreceptor neurons in worms, flies, and mice express multiple DEG/ENaC and TRP channel proteins. Piezo is expressed in a subset of somatosensory neurons in mice, but its representation relative to other channels is not known. Little is known about expression of TMC proteins in mechanoreceptor neurons. But, the landscape of ion channel co-expression in mechanoreceptor neurons is only beginning to be mapped. Future work aimed at refining such maps for mammalian mechanoreceptor neurons will be critical for deeper understanding. Also, each of these potential MeT channel subunits operates within a large company of other ion channel actors that increase the complexity, flexibility, and robustness of somatosensory neuron function.

Both DEG/ENaC and TRP channel proteins can function as essential, pore-forming subunits of MeT channels in three classes of mechanoreceptor neurons in worms: the touch receptor neurons, the CEP texture sensors, and the ASH nociceptors. Unexpectedly, some mechanoreceptor neurons rely on a single class of channels to detect mechanical cues (TRNs and CEP), while others (ASH) use at least two, genetically- and biophysically-distinct channels. Although such functional redundancy has been established only in invertebrates so far, it could explain some of the notable failures of genetic deletion of putative MeT channel subunits to disrupt touch and pain sensation in mice. From worms we also learn that some, but not all pore-forming MeT channel subunits are essential for mechanosensation. This situation is likely to exist in other mechanoreceptor neurons, including those responsible for touch and pain sensation in mammals. These findings strongly recommend adopting a cautionary stance in interpreting the modest effects of genetic deletion of a single MeT channel subunit.

Epilogue

Somatic sensation of gentle and noxious mechanical cues gives rise to our sense of touch and acute pain and also provides crucial information that regulates body movements and essential functions like blood pressure. The robustness that makes full loss of somatosensory function extremely rare complicates traditional genetic dissection of somatic sensation in mammals. Progress towards identifying the composition of MeT channels in mammalian mechanoreceptor neurons would be enhanced by refining current methods for categorizing somatosensory neurons and their fibers to better reflect their true functional organization. Perhaps, following the example of Li, et al. (2011a) and mapping the channel proteins co-expressed in peripheral endings in the skin will provide a reliable method for linking morphological subtypes to specific neuronal functions. When robust categorization of DRG and TG neurons can be combined with sub-type selective gene markers, the curtain could

rise on a new scene in which selected classes of sensory neurons can be identified and targeted for *in vivo* whole-cell patch recording in transgenic mice, as they are in worms.

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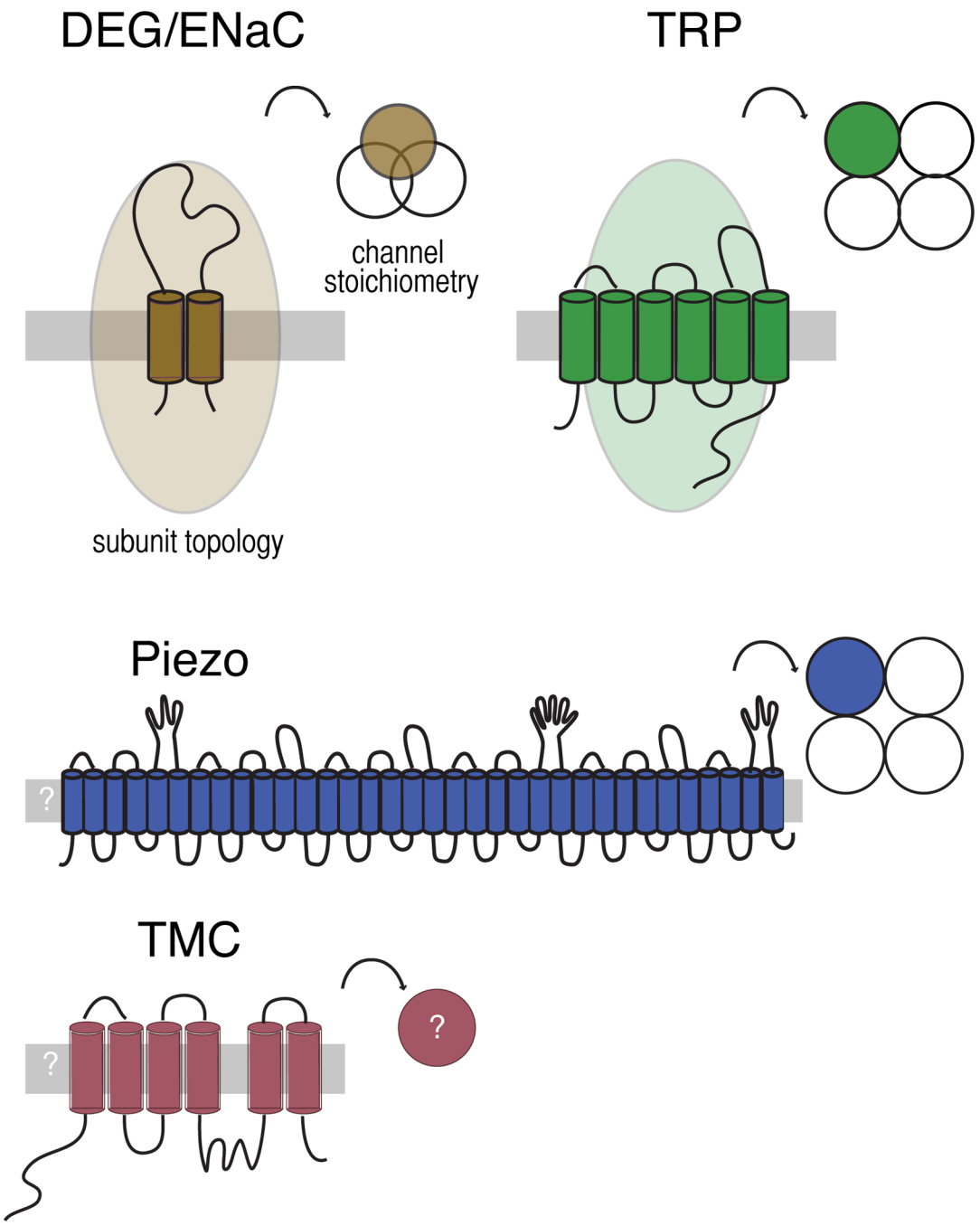

Figure 1. Topology and stoichiometry of proteins proposed to form MeT channels in animals

The TRP channel genes are conserved in eukaryotes and encode proteins predicted to have six transmembrane domains and assemble into tetrameric ion channels. Many TRPs have ankyrin repeats in their intracellular amino terminal; some have more than ten such repeats (Venkatachalam and Montell, 2007). The DEG/ENaC genes are absent from plants, yeast and other microbes, but conserved in animals (Goodman and Schwarz, 2003). They encode proteins with two transmembrane domains and a large extracellular domain. Three DEG/ENaC proteins assemble to form an ion channel. Both TRP channels and DEG/ENaC proteins can form homomeric and heteromeric channels, increasing the potential for channel diversity. Recently, two additional classes of membrane proteins (Piezo and TMC) have

been linked to mechanotransduction in mammals (Coste et al, 2010; Kawashima et al, 2011) and *Drosophila* fruit flies (Kim et al., 2012). Piezo is sufficient to produce stretch activated channels in heterologous cells (Coste et al, 2010; Coste et al., 2012; Bae et al., 2011) and purified Piezo forms a channel in lipid bilayers (Coste et al., 2012). TMC1 and TMC2 are required for mechanotransduction by sound- and vibration-sensing hair cells in mice (Kawashima et al, 2011). Both Piezo and TMC have homologs in invertebrates; the *C. elegans* TMC homolog is expressed in the multidendritic PVD nociceptors. The predicted topology and stoichiometry of Piezo and TMC await further experimental confirmation.

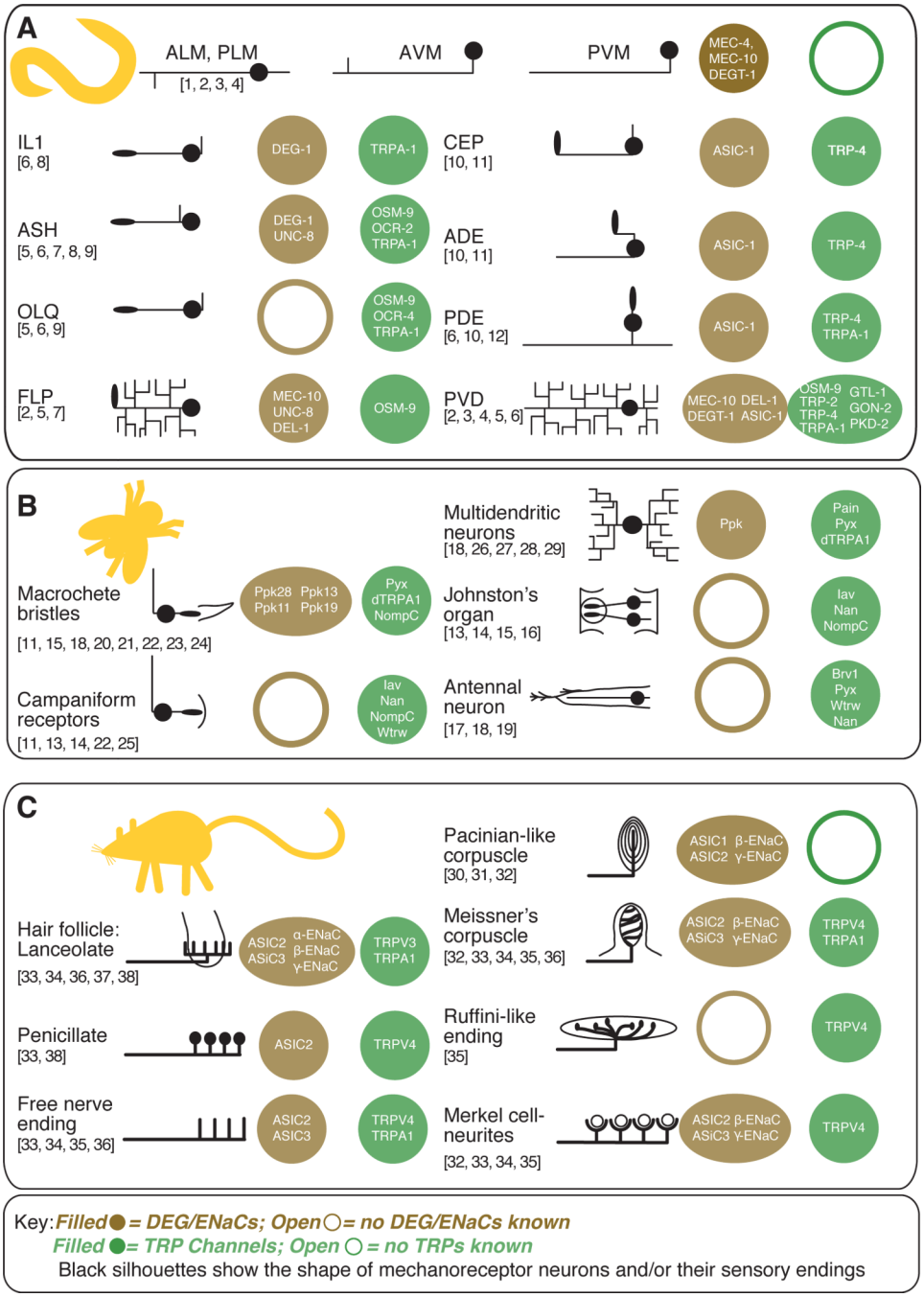

Figure 2. DEG/ENaC and TRP channel proteins co-expressed in mechanoreceptor neurons in *C. elegans* nematodes, *Drosophila melanogaster* fruitflies, and *Mus musculus* mice

This graphical table illustrates the gross morphology of entire mechanoreceptor neurons (*C. elegans*) or peripheral sensory endings (*Drosophila,* mice) and lists ion channel subunits that are expressed in each class of mechanoreceptor cell. Sources for *C. elegans* mechanoreceptor expression are listed by number above: 1) Driscoll and Chalfie, 1991, 2) Huang and Chalfie, 1994, 3) Chatzigeorgiou et al., 2010, 4) Smith et al., 2010, 5) Colbert et al., 1997, 6) Kindt et al., 2007, 7) Tavernarakis et al., 1997, 8) Hall et al., 1997, 9) Tobin et al., 2002, 10) Voglis and Tavernarakis, 2008, 11) Walker et al., 2000, 12) Li et al., 2006. *Drosophila melanogaster* sourcesare: 13) Gong et al., 2004, 14) Kim et al., 2003, 15) Lee et

al., 2010, 16) Liang et al., 2011, 17) Gallio et al., 2011, 18) Lee et al., 2005, 19) Liu et al., 2007, 20) Hamada et al., 2008, 21) Kim et al., 2010, 22) Cheng et al., 2010, 23) Chen et al., 2010, 24) Liu et al., 2003, 25) Bechstedt et al., 2010, 26) Tracey et al., 2003, 27) Zhong et al., 2012, 28) Adams et al., 1998, 29) Zhong et al., 2010). These sources establishing expression in peripheral endings in mice and humans were consulted: 30) Calavia et al., 2010, 31) Montaño et al., 2009, 32)Drummond et al., 2000, 33) García-Añoveros et al., 2001, 34) Price et al., 2001, 35) Suzuki et al., 2003b, 36) Kwan et al., 2009, 37) Price et al., 2000, 38) Fricke et al., 2000, 39) Xu et al., 2002).

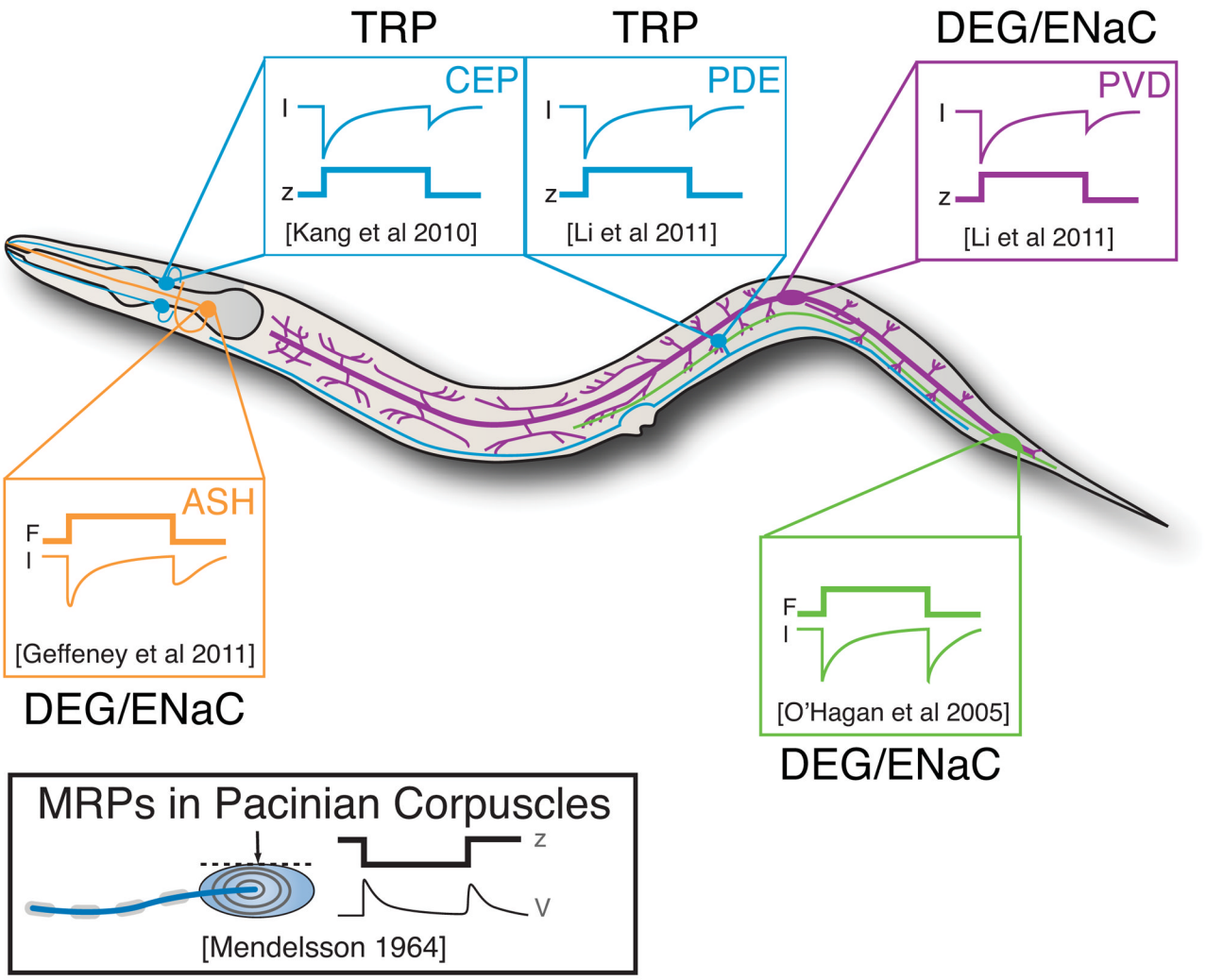

Figure 3. Mechanoreceptor currents in *C. elegans* mechanoreceptor neurons activate in response to the application and removal of mechanical stimulation

Mechanoreceptor currents have been recorded using *in vivo* whole-cell patch clamp recording and the predominant ion channel type identified by genetic dissection. Traces adapted from the following: PLM: O'Hagan et al. (2005); CEP: Kang et al. (2010); PDE, PVD: Li et al. (2011b); ASH: Geffeney et al. (2011). Displacement stimuli were applied to activate CEP, PDE, and PVD, while mechanical stimuli delivering known forces were applied to activate PLM and ASH. Receptor potentials in PLM and ASH mirror the receptor currents (Geffeney et al., 2011; O'Hagan et al., 2005) and are reminiscent of the response dynamics of Pacinian corpuscles in cats (inset, lower left).

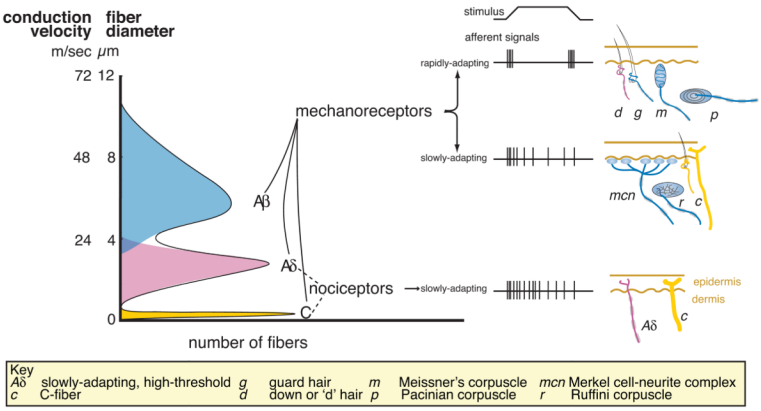

Figure 4. Classification schemes for skin mechanoreceptors in mammals

Mammalian mechanoreceptor nerve fibers are classified by according to three physiological properties: 1) the speed of action potential propagation (which depends on fiber diameter and myelination state); 2) the threshold for activation; and 3) the rate of adaptation to mechanical stimuli. The broad categories of Aβ, Aδ and C-fibers are defined by their propagation speeds where Aβ-fibers have the most rapid propagation speeds and the slender, unmyelinated C-fibers have the slowest. Most fibers in these categories share other properties. For example, C fibers have slow rates of adaptation to mechanical stimuli and many have high mechanical thresholds. In contrast, most Aβ-fibers have low mechanical thresholds and these fibers are thought to innervate light-touch receptors in the skin. The challenge is to link fiber properties to the diverse endings in the skin.