Transduction Channels in Sensory Cells

Edited by
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Preface

She loves him, observes the tourist upon beholding the image of the Pharaoh and his wife in the Egyptian Museum in Cairo. And indeed, the intimate scene depicting Tutankhamun and his queen Ankhesenamun (shown on the cover of our book) confers that impression even more than 3000 years after its creation. For the sensory physiologist who recognizes Ankhesenamun's gesture as a mechanosensory gentle touch, the sensation of a hand touching a shoulder is in molecular terms no simple process. In fact, more than 20 years of hard experimental work was necessary to shed some light on the molecular steps that convert, or transduce, physical contact into an electrical signal interpretable by the nervous system. As Laura Bianchi and Monica Driscoll outline in the first chapter of this book, the path toward understanding touch was paved by a creature much less noble than Tutankhamun: the soil nematode *Caenorhabditis elegans*. Painstaking genetic analysis of the worm's response to being experimentally touched and probed with an eyelash led to identification of the transduction channels in mechanosensory neurons, known collectively now as the degenerin family of ion channels. It is these proteins that translate mechanical stimuli into electrical signals that can be processed by the sensory neurons and eventually are interpreted by the organism as a sensory experience.

In all sensory cells transduction channels show fascinating adaptations to their task of reporting sensory stimuli. Imagine this: if Ankhesenamun speaks to her husband, or when the Pharaoh listens to his musicians playing cymbals and harp, tiny protein filaments tug at the transduction channels in his inner ear to excite mechanosensory hair cells and to produce a neuronal auditory signal. Robert Fettiplace describes in his chapter the biophysical examination of these exquisitely sensitive transduction channels.

In the world of chemoreception, transduction channels appear to be as numerous as the qualities of chemical stimuli. Acid-sensing ion channels respond to the simplest of all chemicals. They are opened by protons and probably serve multiple functions in the body, including the generation of heartache when ischemia turns things sour within the myocardium. Other chemoreception modalities are more conducive to Pharaoh's bliss. In particular, the metabotropic transduction cascades of taste and smell form the molecular basis of sensory pleasures, which were so highly cherished by the ancient Egyptians that the hieroglyphic determinative for happiness was a nose. The chemoreception chapters in our book describe the state of knowledge about transduction channels in chemosensory cells. Here we meet an entire zoo of different transduction channels, including cyclic nucleotide-gated cation channels, calcium-activated chloride channels, and a channel family that plays an increasingly prominent role in sensory physiology: the transient receptor potential channels. Transient receptor potential channels mediate sensory transduction in systems as diverse as mouse pheromone receptors, insect ommatidia, and human thermoreceptors, apparently acting as one of nature's multiple-purpose transduction components.

Looking at his wife is probably what makes the Pharaoh really happy. And, indeed, the beautiful daughter of Nefertiti must have been an exceptional visual experience. Just look at how the rays of the sun seem to caress her and her husband with tiny hands of light! The old Egyptians surely had a way of representing sensory perception in art. In modern days, we have learned to understand how photoelectrical transduction works in the light-sensitive cells of the retina. Dimitri Tränkner and Benjamin Kaupp describe the pivotal role that transduction channels play in such different visual tasks as looking at stars at night or beholding bright and colorful images such as the one on the book cover. And Armin Huber explains the ingenious method that flies use to achieve high temporal resolution in vision: the formation of multimolecular signaling complexes to rapidly drive transduction channels. If you have ever wanted to know why you can rarely catch the fly that annoys you, read this chapter. It won't help you in catching the insect, but you will understand why the bug is so fast.

In the concluding chapter, Robert C. Peters and Jean-Pierre Denizot discuss a sensory modality that Tutankhamun and Ankhesenamun did not use to perceive the world: electroreception. If not the Pharaoh, another denizen of Egypt is a master of electroreception. A small fish with a long nose, the elephant nose (*Gnathonemus petersii*), finds his way through murky waters by means of emitting and perceiving electrical signals. The elephant nose is one of the best-studied weakly electric fish, and it has slowly revealed how it does it. It is fascinating to read about the sensory equipment that electric fish employ to feel their way in the dark!

Thus, the authors of this book cover many sensory modalities and explain the generation of receptor currents in a wide range of sensory cells. They address their chapters to students of biology, physiology, and medicine, as well as to scientists interested in signal transduction, sensory physiology, and perception. And who knows – even some aficionados of Egyptian archaeology may wish to know more about the Pharaoh's senses.

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The Molecular Basis of Touch Sensation as Modeled in *Caenorhabditis elegans*

Laura Bianchi and Monica Driscoll

Abstract

One of the looming mysteries in signal transduction today is the question of how mechanical signals, such as pressure or stretch, are sensed. Elegant electrophysiological studies in organisms ranging from bacteria to mammals support that mechanotransduction can be mediated by ion channels that gate in response to mechanical stimuli. Despite the importance of the molecular identification of these ion channels for elaborating mechanisms of mechanotransduction, genes encoding mechanosensitive ion channels eluded cloning efforts for a long time. Breakthroughs in the understanding of mechanosensitive channels have come from genetic analyses of touch sensation in *Caenorhabditis elegans* and *Drosophila*.

In *C. elegans,* screens for touch-insensitive mutants identified two genes, *mec-4* and *mec-10,* that encode channel subunits implicated in touch sensation and are postulated to be the core of a mechanotransducing ion channel complex. *mec-4* and *mec-10* encode proteins with similarity to subunits of the mammalian amiloride-sensitive epithelial Na^+ channel (ENaC) that mediates sodium reabsorption in the kidney and lung. *mec-4* is expressed exclusively in six neurons that laser ablation studies have identified as gentle-touch receptors, and *mec-10* is expressed in these six neurons plus two pairs of touch receptors that are thought to sense harsher touch. The same genetic screens that identified *mec-4* and *mec-10* identified other genes required for normal touch sensation in the nematode. MEC-5, a novel collagen, and MEC-9, a protein that includes multiple Kunitz-type protease inhibitor repeats and EGF repeats, are extracellular matrix proteins that may interact with MEC-4/MEC-10 channel subunits on the extracellular side of the neuron to help exert gating tension on the channel. Inside the touch receptor, a specialized cytoskeleton is assembled that features 15-protofilament microtubules composed of MEC-12 α-tubulin and MEC-7 β-tubulin subunits. This cytoskeleton may be linked to tether MEC-4/MEC-10 on the intracellular side. When a mutant hyperactivated MEC-4(d) subunit is heterologously expressed in *Xenopus* oocytes, voltage-independent Na^+ currents are produced that can be modulated in both amplitude and properties by two other proteins also identified by genetic screens as required for touch transduction: MEC-2, a stomatin-like protein, and

MEC-6, a protein that shares similarity with mammalian paraoxonases. The *C. elegans* genome encodes 28 members of the MEC-4 and MEC-10 channel family, called the degenerin family. We discuss here the global role of degenerins in mechanosensation, reporting findings on the function of three other degenerins (UNC-8, DEL-1, and UNC-105) in mechanosensitive and stretch-sensitive behaviors in the nematode, and we review studies addressing the role of mammalian homologues in touch sensation.

1.1 Introduction

The sense of touch is so profoundly important to our daily life that – when you actually think of it – the degree to which we take this sense for granted is unthinkable. We fully depend on our sense of touch to make and drink our morning coffee, to flip through the newspaper, to dress, and to move to the places where we type, phone, compute, pass paper, fold, sell, and manufacture things. Virtually no activities required for daily life (feeding, drinking, moving, protecting, communicating) can transpire without touch or mechanical sensation. Moreover, without touch sensation we would be unable to ensure the viability of our young. In addition to the obvious reasons for this, it is becoming increasingly clear that touch plays a critical role in both physical and emotional development. For example, hospitalized preterm infants show accelerated weight gain, enhanced activity, and faster development if they are gently stroked daily for 15 minutes – resulting in faster hospital discharge [5]. Despite widespread and fundamental importance, touch is the least understood of the senses, at both the cellular and molecular levels.

The sense of touch is initiated by the perception of a mechanical stimulus such as pressure and the conversion of this signal into electrical signaling. Groundbreaking electrophysiological studies characterized ion channels that could be gated in response to pressure or stretch rather than voltage changes or ligand binding [39, 41, 58]. Such channels could be identified in specialized mechanoreceptors [24, 48], yet the genes encoding mechanically gated ion channels that mediate the senses of touch and hearing eluded cloning efforts for years (some genes, such as those encoding the hearing channel, remain unidentified even to this day; see Chapter 2). Technically, this might have been predicted, as there are no known reagents that specifically associate with mechanosensitive channel subunits at high affinity that could facilitate protein isolation and there is a remarkable paucity of mechanically gated channels even in specialized mechanotransducing structures such as the vertebrate cochlea. Moreover, given that these channels are likely to be tethered to accessory proteins that exert gating tension, reconstitution in heterologous systems is extremely difficult. Although the cloning of mechanically gated MscL and MscS channels from bacteria constituted major breakthroughs in the field of mechanical signaling [39], the MscL and MscS channel classes have no clear eukaryotic homologues, and thus their identification did not facilitate an immediate revolution in our understanding of mammalian mechanotransduction.

Exciting advances in our understanding of the sense of touch have instead emerged from invertebrate genetics. Both nematode and fly mutants defective in touch sensation have facilitated the cloning of ion channels thought to act directly as mechanotransducing channels. More specifically, the DEG/ENaC Na^+ channel subunits (named for the *C. elegans* degenerins and the related mammalian epithelial amiloride-sensitive Na^+ channel) have been directly implicated in touch sensation in both invertebrates and vertebrates. Likewise, members of the transient receptor potential (TRP) channel family are mechanotransducing channels implicated in touch [84] and possibly hearing [50, 69] (see Chapter 2). Here we focus on reviewing the genetic, molecular, electrophysiological, and calcium-imaging studies conducted using the simple nematode *C. elegans* that have greatly advanced our understanding of touch sensation through the identification and the characterization of mechanically gated DEG/ENaC ion channels and accessory proteins. We discuss how these and TRP channels may work together to contribute to touch sensation and note how data from invertebrates has stimulated a successful search for analogous processes in higher organisms.

1.2 Features of the *C. elegans* Model System

The 1-mm long simple soil worm *Caenorhabditis elegans* is a facile system for experimental manipulation that features many developmental and behavioral pathways strikingly conserved between nematodes and mammals. *C. elegans* can be easily reared on an *E. coli* diet in the laboratory. This animal completes a reproductive life cycle in just 2.5 days at 25 °C, during which it progresses through embryonic development and four larval stages (L1-L4) before reaching sexual maturity. The most common sexual form is the hermaphrodite (XX), although males (X0), which can arise spontaneously by nondisjunction, can be easily propagated in the laboratory for use in genetic studies. The *C. elegans* body and eggshell are transparent so that each cell can be visualized by Normarski microscopy. In fact, the entire map of all cell divisions during the development of the animal has been constructed [44, 75]. The nervous system includes only 302 neurons, for which the pattern of synaptic connections, including circuits for specific mechanosensory behaviors, has been deduced using serial section electron microscopy [85]. Laser ablation experiments have helped define the importance of specific identified neurons in mechanosensory behaviors [14, 47].

The major advantage of using *C. elegans* as a model system for studying biological processes is that it is a powerful genetic system [8]. Mutations that affect development and behavior, including those affecting touch sensation, have been generated and mapped to specific genes. Sequence analysis of the *C. elegans* genome is complete [20], and powerful methods for generation of transgenic animals [26] and dsRNAi-mediated transcript disruption (RNAi) [27, 77] are routine.

Despite the considerable advantages that *C. elegans* offers for studying gene function in vivo, this model has had certain limitations for electrophysiological analysis of chan-

nel function, especially for channels expressed in neurons. The tiny neurons (1–2 μm diameter) are embedded in poorly accessible tissues confined in a pressurized cuticle. However, recent technical improvements established in the field have led to the development of electrophysiological methods for characterizing channel function in *C. elegans* [35]. In addition, a recently developed method for culturing *C. elegans* cells now allows routine electrophysiological recordings from neurons, muscles, and other cell types [19, 76]. Finally, sophisticated methods for monitoring intracellular calcium concentration changes during channel activity in living and behaving nematodes have been developed and have led to important findings [49, 76].

Taken together, the identification of specialized mechanosensory neurons, the cloning of genes required for mechanosensitive responses, and the study of their function both in vivo and in vitro have led to significant insight into the molecular mechanisms of mechanotransduction in *C. elegans*.

1.3 Mechanosensation Is a Major Mechanism by Which *C. elegans* Senses Its Environment

C. elegans does not have a sense of sight and must evaluate its environment primarily by chemosensation and mechanosensation (see Chapter 4). *C. elegans* can respond to a range of mechanical stimuli encountered virtually anywhere on its body. The best-characterized mechanosensitive behavior is the movement away from a gentle brush of an eyelash hair delivered to the body, generally referred to as gentle-touch sensation [13]. Other mechanosensitive behaviors include response to head-on collision with an object (the nose touch response), response to light touch to the side of the nose (head withdrawal response), response to harsh touch delivered by a metal wire, and response to tapping on the plate on which the worms are reared. The process by which males mate most likely involves touch-mediated recognition of the hermaphrodite vulva. Mechanical stimuli also impact on locomotory behaviors, foraging, feeding, egg laying, and defecation circuits.

Because the avoidance of gentle touch is the behavior most intensively investigated, here we will first focus on summarizing how the study of gentle touch has produced a detailed molecular model for a mechanically gated ion channel. Later, we will review what is known about the identities of genes that influence other mechanosensory behaviors, and we will consider emerging molecular themes in touch sensation.

1.4
Gentle Body Touch

1.4.1
The Touch Receptor Neurons

In the laboratory, *C. elegans* moves across an agar plate on its side with a readily observed sinusoidal motion. When stroked with an eyelash hair on the anterior body, the animal will reverse its direction and move backwards; if touched on the posterior body, it will move forward [13]. The neurons required for the sensation of the gentle-touch stimuli have been identified by laser ablation studies and genetic disruption. These six touch receptor neurons were initially called microtubule cells because their processes are filled with distinctive 15-protofilament microtubules. Their processes are embedded in the hypodermis adjacent to the cuticle (the worm "skin") and run longitudinally along the body wall, a distribution that enables them to more or less "cover" the touch sensory field of most of the body. Two embryonically generated PLM neurons (posterior lateral microtubule cells) are situated in the posterior body, on the right and left sides; two embryonically generated ALM neurons (anterior lateral microtubule cell) are situated in the anterior, on the right and left sides. In the first larval stage, AVM (anterior ventral microtubule cell) and PVM (posterior ventral microtubule cell) are added to the body plan. Laser ablation of individual ALMs, PLMs, and AVM established roles for these neurons in gentle touch [14]. Although PVM looks identical to the other touch neurons, it does not initiate a behavioral response to gentle touch on its own, and thus it has been postulated to modulate other behavioral circuits that can be influenced by touch [14] (Fig. 1.1A).

1.4.2
Ultrastructural Features of the Touch Receptor Neurons

1.4.2.1 Touch Cell-specific Microtubules

Touch receptor processes are filled with bundles of wide-diameter (15-protofilament, pf) microtubules that are uniquely assembled in this group of six neurons [15, 16]. Most other nematode cells include 11-pf microtubules (Fig. 1.1C). The 15-pf microtubules are required for touch receptor function: if microtubules are disrupted by the microtubule assembly inhibitor colchicine or by genetic mutations, touch sensitivity is completely lost [13, 15]. Individual microtubules are not long enough to extend from end to end of the touch neuron. Rather, single microtubules (10-20 μm long) overlap with each other to span the full length of the touch cell processes (about 400-500 μm). Interestingly, the distal microtubule end is diffusely stained and is always situated outside of the microtubule bundle, often positioned adjacent to the plasma membrane. This ultrastructural feature suggests that the oriented microtubule network might associate with plasma membrane proteins, such as the mechanosensitive ion channels that sense touch, a hypothesis that remains to be tested [16] (see discussion of mechanotransduction model below).

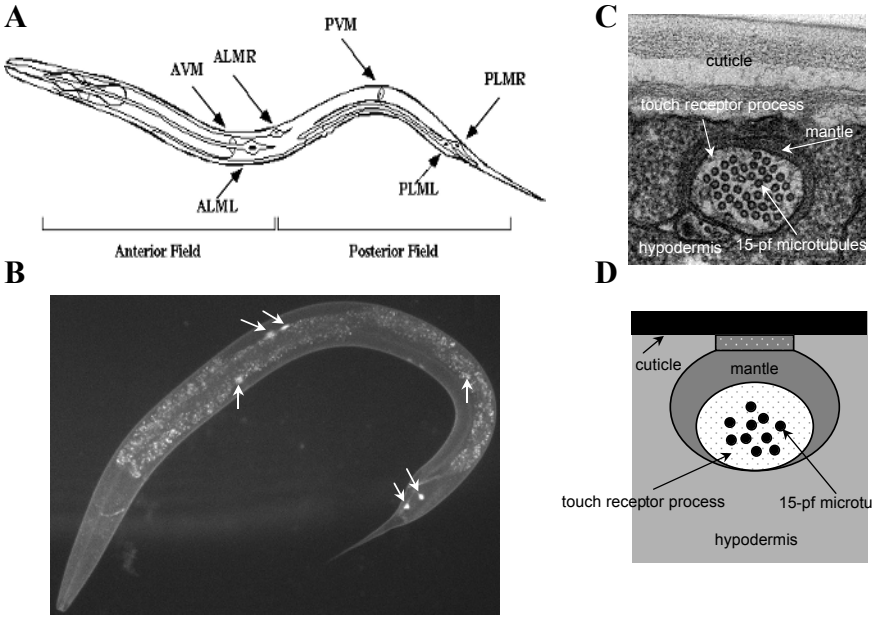

Fig. 1.1 *C. elegans* neurons that sense gentle body touch. (A) Diagram showing the position of the six neurons that in *C. elegans* sense the gentle stroke of an eyelash hair on the body; anterior body is to the left. There are two fields of touch sensitivity defined by the position of the touch neurons processes along the body axis. The ALMs and AVM sense touch to the anterior field, whereas PLMs sense touch to the posterior field. (B) Touch neurons are here visualized in a living nematode, by expression of the Green Fluorescent Protein under the control of the *mec-4* promoter, which is active exclusively in these neurons. Arrows point to touch receptor cell bodies. (C) Electron micrograph of a cross-section of a touch receptor neuron process. The touch cell process, which is surrounded by the mantle and embedded in the hypodermis, is filled with 15-pf 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 down to the touch neuron process. (D) Schematic representation of a touch receptor neuron EM cross-section, depicting its most important components. The darkly stained region, depicted here as a bar-shaded rectangle connecting the mantle and the cuticle, is the fibrous organelle (not visible in the electron micrograph). Such specializations occur periodically along the length of the touch receptor process and may serve to attach the process to the cuticle. Adapted from [78]

1.4.2.2 **The Extracellular Mantle**

Touch receptor processes are surrounded by a specialized extracellular matrix, called the mantle, which appears to help maintain the touch receptor process in close association with the cuticle [13]. Cuticular structures resembling muscle attachment sites are positioned periodically along the length of the touch receptor process in close contact with the mantle and may be sites at which the touch receptor process is fixed to the cuticle (Fig. 1.1D). Although genetic mutations support that the integrity of the mantle is critical for touch receptor function, mutations in *him-4* cause touch neurons to stray away from the cuticle, yet the mutants still sense touch [82]. Since detachment is variable in the *him-4* background, it is possible that adequate contact is maintained

for some touch sensation; alternatively, any deflection of even a "loose" mechanoreceptor neuron might be sufficient to activate the behavioral avoidance response.

1.4.3 Genetic and Molecular Analysis of Body Touch

In pioneering studies on the genetics of touch sensation, Martin Chalfie and colleagues mutagenized animals and screened their progeny for the failure to respond to the gentle brush of an eyelash hair [11, 13]. The mutants selected exhibited grossly normal locomotion and were still able to respond to the prod of a metal wire, so that defects appeared to specifically alter gentle-touch sensitivity. Hundreds of touch-insensitive mutants, many of them designed as *mec* (mechanosensory abnormal), defined several genes that contribute specifically to touch cell development and function. It should be emphasized that since the criteria for mutant isolation demanded that other aspects of nematode locomotion and harsh-touch sensation be unaffected by the mutations, genes that encode proteins used for gentle-touch sensation but also used in other locomotory activities would not have been identified in this screen. Likewise, genes that encode functionally redundant proteins would be missed. Nonetheless, the genes identified in this screen provided a major breakthrough in our understanding of the molecules needed for touch sensation.

1.4.3.1 *mec-4* and *mec-10* Ion Channel Subunits Form Na^+ Channels

mec-4 and *mec-10* loss-of-function mutants are touch-insensitive, yet their touch receptor neurons appear to develop normally and share all apparent ultrastructural features of wild-type (WT) touch receptor neurons [13]. Cloning revealed that *mec-4* and *mec-10* encode homologous proteins related to subunits of the amiloride-sensitive, voltage-independent Na^+ channel, which mediates Na^+ reabsorption in vertebrate kidney, intestine, and lung epithelia (the ENaC channel [9, 10, 12, 22, 45, 52]). The *mec-4* channel subunit is expressed only in the six touch receptor neurons (Fig. 1.1B [55]), and the *mec-10* channel subunit is expressed in the six touch receptor neurons as well as in two other neuron pairs that may mediate stretch-sensitive or harsh-touch responses (FLPL/R and PVDL/R [45]). Because the MEC-4 and MEC-10 subunits are expressed exclusively in touch neurons and are clearly needed for the function of these neurons, and because no other channel genes were identified among touch-insensitive mutants, it was proposed that the MEC-4 and MEC-10 subunits assemble in vivo to create a mechanically gated channel that responds directly to touch. Progress toward addressing this hypothesis is outlined in more detail below.

1.4.3.2 MEC-4 at the Molecular Level

There are many more *mec-4* mutations than there are *mec-10* mutations (perhaps suggesting that *mec-4* plays a more central role in gentle touch), and thus MEC-4 structure/function is better understood. MEC-4 is a 768-amino-acid membrane protein that includes two membrane-spanning domains (MSDI, MSDII; see Fig. 1.2B). The chan-

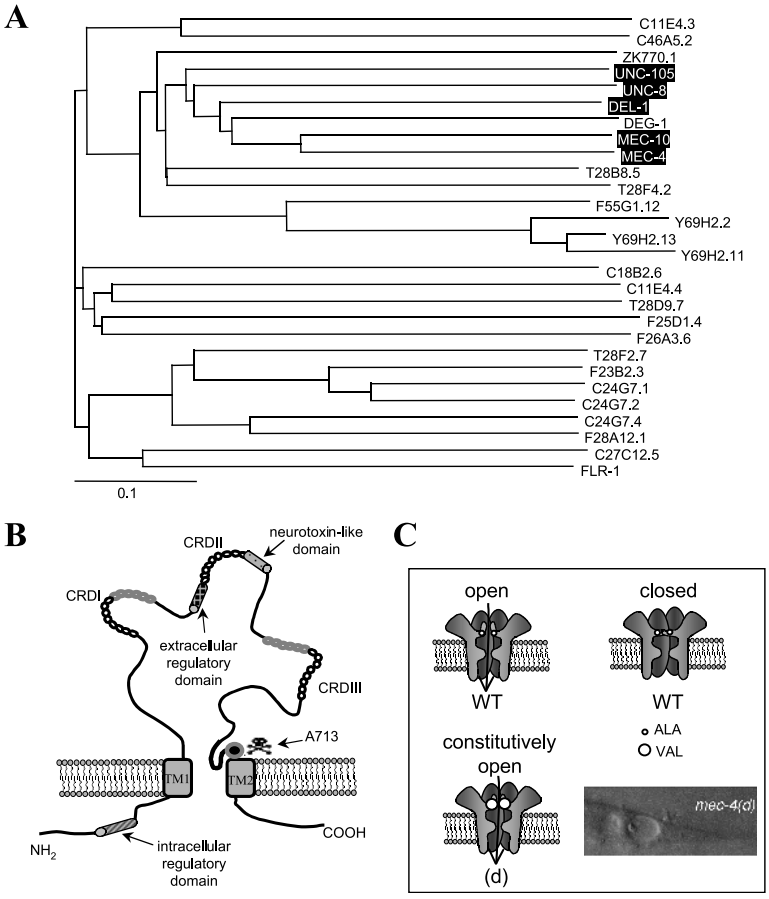

Fig. 1.2 Degenerin MEC-4 structure/function. (A) Dendrogram of the 28 degenerins encoded by the *C. elegans* genome. The 28 genes encoding postulated degenerin subunits were identified by searching the *C. elegans* database, compiled by the *C. elegans* Genome Sequencing Consortium, for predicted proteins sharing homology with known degenerins. Black background indicates the most characterized degenerins, including MEC-4. (B) Structural features of a single MEC-4 subunit (likely four subunits form a channel). The MEC-4 polypeptide spans the membrane twice, leaving the N- and C-termini in the cytosol. The second membrane-spanning domain, which is longer than required for a single transmembrane pass, may loop back in the membrane to participate in the formation of the pore. Ala713, which when replaced by a bulkier amino acid results in necrotic cell death, is indicated by the skull and crossbones icon. MEC-4 protein 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. Other important domains include the putative extracellular regulatory domain, the neurotoxin-related domain, and the intracellular regulatory domain. (C) Model for *mec-4(d)*-induced toxicity. WT MEC-4 channels are able to open and close, but MEC-4(d) channels, which encode substitutions for a conserved alanine adjacent to MSDII, are thought to be "locked" in an open conformation due to steric hindrance. This is thought to result in excessive Na^+ influx that triggers necrotic-like cell death, which manifests itself in the early stages as cell swelling (lower right panel)

nel subunit is positioned in the membrane such that relatively short N- and C-terminal domains project into the cytosol and a single large central loop extends extracellularly [52] (this is typical of all DEG/ENaC family members). The MEC-4 extracellular domain also includes three cysteine-rich domains (CRDI, CRDII, and CRDIII) and one region similar to venom neurotoxins (NTD) [79].

Understanding of structure/function relations in MEC-4 is still at an early stage, but studies on this and other members of the DEG/ENaC superfamily have highlighted three conserved regions important for function: (1) MSDII contributes to the channel pore [42]; (2) a short but highly conserved intracellular stretch adjacent to MSDI influences ion permeation and selectivity [36, 43]; and (3) the Cys-rich extracellular loop domains are important for function in some way, possibly mediating protein-protein interactions that may help tether the MEC-4 channel to the specialized extracellular matrix of the touch neuron.

An unusual type of *mec-4* mutation acts dominantly to induce swelling and neurodegeneration of the touch neurons. Substitution of large side-chain amino acids for a highly conserved Ala residue situated adjacent to channel pore MSDII (AA713; see Fig. 1.2B [22, 52]) generates MEC-4 mutant subunits (named MEC-4(d)) that induce necrotic-like death of the touch receptor neurons (Fig. 1.2C [11, 13]). The channel pore must be intact for neurodegeneration to occur [42], suggesting that ion influx is critical in the toxicity mechanism. Since large side-chain amino acids at the conserved "d" position are toxic but small ones are not, it was originally proposed that these large substitutions favor the channel-open conformation and hyperactivate ion influx [22, 42]. Indeed, the A713V substitution markedly enhances whole-cell currents when MEC-4(d) channel activity is measured in the *Xenopus* oocyte expression system [34]. Since other *C. elegans* family members (e.g., *deg-1* and *mec-10*) can be altered by analogous amino acid substitutions to induce neurodegeneration [17, 45], the *C. elegans* branch of the gene family has been named the "degenerin" family (Fig. 1.2A). A mutant variant of neuronally expressed mammalian DEG/ENaC member MDEG (ASIC2), engineered to encode a large side-chain amino acid at the corresponding position, induces swelling and death when introduced in to *Xenopus* oocytes and hamster embryonic kidney cells [83]. The small amino acid normally situated at the "DEG" site can be modified with chemical reagents only when the channel is activated, supporting that conformational changes associated with an open channel involve this residue [1].

1.4.4
The Candidate Mechanotransducing Channel is a Heteromultimeric Complex

The subunit compositions and stoichiometry of DEG/ENaC channels remain somewhat uncertain. Electrophysiological assays of the rat ENaC channel reconstituted in *Xenopus* oocytes determined that at least three homologous subunits (α-, β-, and γ-rENaC) must be co-expressed to form a channel with pharmacological properties similar to the in vivo channel [10]. Stoichiometries of four to nine subunits per ENaC channel have been supported [3, 6, 7, 21, 28, 51, 70]. Genetic interactions suggest that

MEC-4 and MEC-10, which cannot functionally complement one another and thus appear to perform distinct functions in vivo, form a heteromeric channel in touch neurons: engineered *mec-10(d)* subunit (harboring the substitution analogous to channel-activating, death-inducing MEC-4 substitution A713V) requires functional *mec-4* to be toxic [37, 45].

1.4.4.1 MEC-4 and MEC-10 Form a Functional Ion Channel

MEC-4 and MEC-10 co-assemble in *Xenopus* oocytes to form a Na^+-selective channel sensitive to the ENaC-blocking agent amiloride. This channel exhibits a high permeability to lithium, as do other members of the DEG/ENaC superfamily. Interestingly, while MEC-4 can form channels of low conductance on its own, MEC-10 is not functional when expressed alone. However, the co-introduction of the MEC-10 subunit to the MEC-4(d) channel in oocytes affects the Kd for amiloride, consistent with MEC-10 being included in the same channel as MEC-4 [34]. Still, the introduction of MEC-10 in the oocyte system does little to change most properties of the MEC-4 channel, and the "MEC-10(d)" mutant subunit cannot conduct current on its own. Thus, the MEC-4 subunit appears most critical for channel properties.

Importantly, in *Xenopus* oocytes the MEC-4/MEC-10 channel has not been demonstrated to be gated by mechanical forces (membrane stretch induced by hypotonic solutions), probably due to the lack of intracellular and extracellular proteins, normally present in vivo, that are essential for channel gating (see below). The MEC-4(d) subunit conducts much more current than the MEC-4(+) subunit (at least 10 times larger currents), consistent with the idea that the "d" substitutions next to the channel pore hyperactivate the channel. Most electrophysiological studies have therefore concentrated on the activated MEC-4(d) channel, which conducts markedly more robust current than the MEC-4(+) subunit.

1.4.4.2 MEC-2 Is a Stomatin-like Protein That May Help Tether the MEC-4/MEC-10 Channel to the Membrane Bilayer and/or the Cytoskeleton

MEC-2 May Participate in Several Protein Interactions in the Touch Channel Complex

Mechanosensitive ion channels are thought to be gated by forces exerted upon the channels via associated protein attachments. MEC-2 is a candidate protein that might help exert gating tension on the MEC-4/MEC-10 channel from the membrane and/or from the intracellular side. *mec-2* encodes a 481-amino-acid protein expressed in the touch receptor neurons and in a few additional neurons in the head [46]. There are three candidate protein interaction domains in MEC-2: (1) a cytoplasmically situated N-terminal domain (positioned between aa 42 and aa 118) needed for the localization of MEC-2::*lacZ* enzymatic activity to the touch receptor process; (2) a central domain that exhibits 65 % identity to the human red blood cell protein stomatin, an integral membrane protein that associates with the RBC cytoskeleton and affects ion balance via an unknown mechanism [74] (note that the stomatin-related domain includes a hydrophobic stretch that is membrane-associated, but most of this domain is thought to project into the cytoplasm); and (3) a C-terminal intracellular proline-rich region that